

ALTERATION OF SELF-ASSEMBLED PATTERNS BY MICROORGANISMS IN EVAPORATING DROPLETS

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By
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Alteration of self-assembled patterns by microorganisms in evaporating droplets

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August 2016

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.



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ABSTRACT

ALTERATION OF SELF-ASSEMBLED PATTERNS BY MICROORGANISMS IN EVAPORATING DROPLETS

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The science of self-organization comprises a diverse range of processes where a disordered system of components form ordered pattern or structure spontaneously without any external instruction [1]. Plentiful examples of this phenomenon appear in nature at almost all scales [2]. Over the past decades, self-assembly has become the apple of many researchers' eye by offering breakthroughs for many applications in not only physics but also chemistry, biology and material sciences [3]. Among several self-assembly methods, using evaporating droplets shines out as it provides ease and simplicity. Along with these advantages, it increases its popularity by providing the opportunity of obtaining a variety of patterns such as uniform depositions, central bumps, polygons and hexagons [4] and more famously (coffee) rings [5]. Nonetheless, most of the studies resulting in these patterns have been carried out by using Brownian particles which fluctuate randomly due to the collisions with the molecules of the surrounding fluid, while only little is known when it comes to active particles suspended in evaporating droplets. The self-propelling nature of active particles [6] permits them to explore their environment differently from Brownian particles and opens new doors in this research line. Being in the quest of understanding what will happen in the presence of active particles such as the well-studied bacteria *Escherichia coli* (*E.coli*), we investigate and explore the self-assembled patterns in evaporating droplets by using digital video microscopy. We demonstrate that the presence of *E.coli* bacteria tunes the self-assembled patterns. Moreover, we enrich the patterns by introducing salt. We show that the activity of these microorganisms has an influence on salt crystallization based on the characteristic dendritic crystals obtained

with active and motile bacteria and unaltered, regular crystals obtained with non-motile bacteria with inhibited activity. Our results suggest a simpler, faster and cheaper method in which common salt can be used as a biomarker to detect bacterial activity.



Keywords: Self-assembled patterns, evaporating droplets, salt crystals, bacteria, motility, activity.

ÖZET

BUHARLAŞAN DAMLALARDA KENDİLİĞİNDEN OLUŞAN DESENLERİN MİKROORGANİZMALAR TARAFINDAN DEĞİŞTİRİLMESİ

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Kendi kendini organize etme bilimi, parçaları dağınık olan sistemlerin dışardan bir talimat olmadan, kendiliğinden düzenli bir desen ya da yapı oluşturduğu birçok işlemi kapsar [1]. Bu fenomenin örneklerine doğada neredeyse her düzeyde rastlanabilir [2]. Son yıllarda, sadece fizikte değil kimya, biyoloji ve malzeme biliminde birçok uygulamada yenilikler sunduğu için öztoplanma (self-assembly) araştırmacıların gözbebeği haline geldi [3]. Muhtelif yöntemler arasında, buharlaşan damlaları kullanmak ise sağladığı kolaylık ve sadelik sayesinde biraz daha öne çıkmakta. Bu faydaların yanısıra, buharlaşan damlaları kullanmak eşit dağılımlı tortular, merkezi tümsekler, çokgenler, altıgenler [4] ve daha çok bilinen (kahve) hal-kaları gibi birçok deseni elde etme olanağı da sağlayarak popüleritesini arttırmakta [5]. Ama, bu desenlerin elde edildiği çoğu çalışma, aslı kaldıkları akışkanın molekülleri ile çarpışmalarından dolayı gelişigüzel hareket eden Brown parçacıkları ile yapılmakta iken söz konusu aktif parçacıklar olduğunda çok az şey bilinmekte. Aktif parçacıkların kendi kendilerine hareket edebilme doğaları sayesinde çevrelerini Brown parçacıklarından daha farklı keşfetmeleri bu araştırma çizgisinde yeni kapılar açıyor. Aktif parçacıkların bulunduğu durumlarda neler olacağını anlamanın arayışında, buharlaşan damlalalarda en çok çalışılanlardan olan *Escherichia coli* (*E. coli*) bakterilerinin bulunduğu durumlarda kendiliğinden oluşan desenleri dijital video mikroskopi ile inceleyip araştırıyoruz. *E. coli* bakterilerinin kendiliğinden oluşan desenleri değiştirdiğini gösteriyoruz. Aktif ve hareketli bakterilerle elde edilen karakteristik dallantılı kristallerden ve aktivitesi kısıtlanan hareketsiz bakterilerle elde edilen

düzgün kristallerden yola çıkarak, bu mikroorganizmaların aktivitelerinin tuzun kristalleşmesini etkilediğini gösteriyoruz. Elde ettiğimiz sonuçlar, diğer metodlara kıyasla, tuzun bakterilerin aktivitelerini gösteren bir biyoışaret olarak kullanılabileceği daha kolay, hızlı ve ucuz bir yöntem öneriyor.



Anahtar sözcükler: Kendiliğinden oluşan desenler, buharlaşan damlalar, tuz kristalleri, bakteri, hareketlilik, aktivite.

Acknowledgement

Endless thanks to my family and my little survivors...



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Chapter 1

Introduction

Aren't we all dazzled by Nature, when we see the riot of colors and perfectly formed patterns on the wings of a butterfly [7] or the beauty of the structures of the snowflakes falling from the sky [8]? The answer to this rethorical question is the proof of the success of Nature's way of creating organization and order, or in other words self-assembly. But, don't think that the examples are limited to these two; they go beyond what meets the eye starting from molecules to even galaxies [3].

Along with Nature, getting organized and ordered patterns and structures via self-assembly has also been useful for mankind's many purposes; taking inspiration from Nature's accomplishments, self-assembly principles are now being mimicked by many scientists. The diversity of the patterns has led to many different applications (Chapter 3), further increasing the interest in this research area.

Particularly, using the stains left behind by evaporating droplets containing dispersed particles or dissolved solutes has provided a rather easy way of obtaining self-assembled patterns [9]. Variety of patterns can be obtained this way depending

on several parameters. While they may lead to problems by forming unwanted ring-like structures during deposition like in the case of ink-jet printing [10, 11], they may also help us to even diagnose a disease [12, 13].

With this thesis, I study the self-assembled patterns that are obtained experimentally after the evaporation of the droplets. After going over the fundamentals of droplets and evaporation processes in Chapter 2, self-assembly is introduced in detail in Chapter 3. By linking these two concepts, evaporation-driven self-assembly which is an easy and simple example of self-assembly methods is presented as a way of obtaining organized and ordered patterns later on. All the information provided until the end of Chapter 3 paves the way towards our experiments.

Chapter 4 presents the experimental study of the self-assembly of Brownian particles in evaporating droplets by using digital video microscopy. Unlike most of the studies which have used Brownian particles alone, we will focus on the influence of active swimmers such as the well-studied bacteria *E.coli* which can move in a directed path [6] on particles. The results show that the distinctive ability of self-propelling of the bacteria tunes the self-assembled patterns.

In the pursuit of enriching self-assembly in evaporating droplets at a higher level, inducing the evaporation with salt (NaCl) helps us to step forward in Chapter 5. Comparison of the salt crystals formed after the evaporation shows that the activity of the bacteria has an influence on salt crystallization. In particular, the salt crystals show dendritic patterns in which active and moving bacteria are present in the evaporating droplet and no such patterns but bulky crystals are observed when the activity of the bacteria is inhibited. Our results show that the characteristic self-assembled patterns obtained after the evaporation of droplets containing *E.coli* bacteria can be used as an effectively low-cost tool for detecting and indicating the activity of these microbial cells.

In short, starting from such a simple concept like evaporation-driven self-assembly, we ended up having a proposal for answering a challenging question such as whether the bacteria present in a droplet are active or not by just using common salt.



Chapter 2

Fundamentals of Evaporating Droplets

2.1 Introduction

A droplet is a small amount of liquid that takes spherical form when it is suspending in a medium due to surface tension. However, this geometry changes slightly when it falls on a flat solid surface and becomes a spherical cap instead of a sphere as shown in Figure 2.1. These shapes ensure the droplet to have the minimum surface energy possible.



(a) Water droplet suspending in air



(b) Water droplets lying on a surface

Figure 2.1: Photographs of water droplets (a) suspending in air (Adapted from [14]), (b) lying on a flat surface. Although droplets form a sphere while suspending in a medium, they tend to take the form of spherical caps when they are dropped on a flat surface in order to minimize the surface-to-volume ratio.

A droplet may start to lose some of the molecules on its surface due to vaporization (see Figure 2.2), if the surrounding medium is not saturated with the liquid vapour. As the molecules of the liquid can move and collide with each other, they can transfer some of their energy to their neighbours. The energy transfer creates a variation among the liquid molecules causing some of them to increase their energy. This variation provides an opportunity to the molecules on the surface of the droplet, because they can gain enough energy to leave the droplet by changing phase from liquid to gaseous, or in other words they can evaporate as depicted in Figure 2.3.

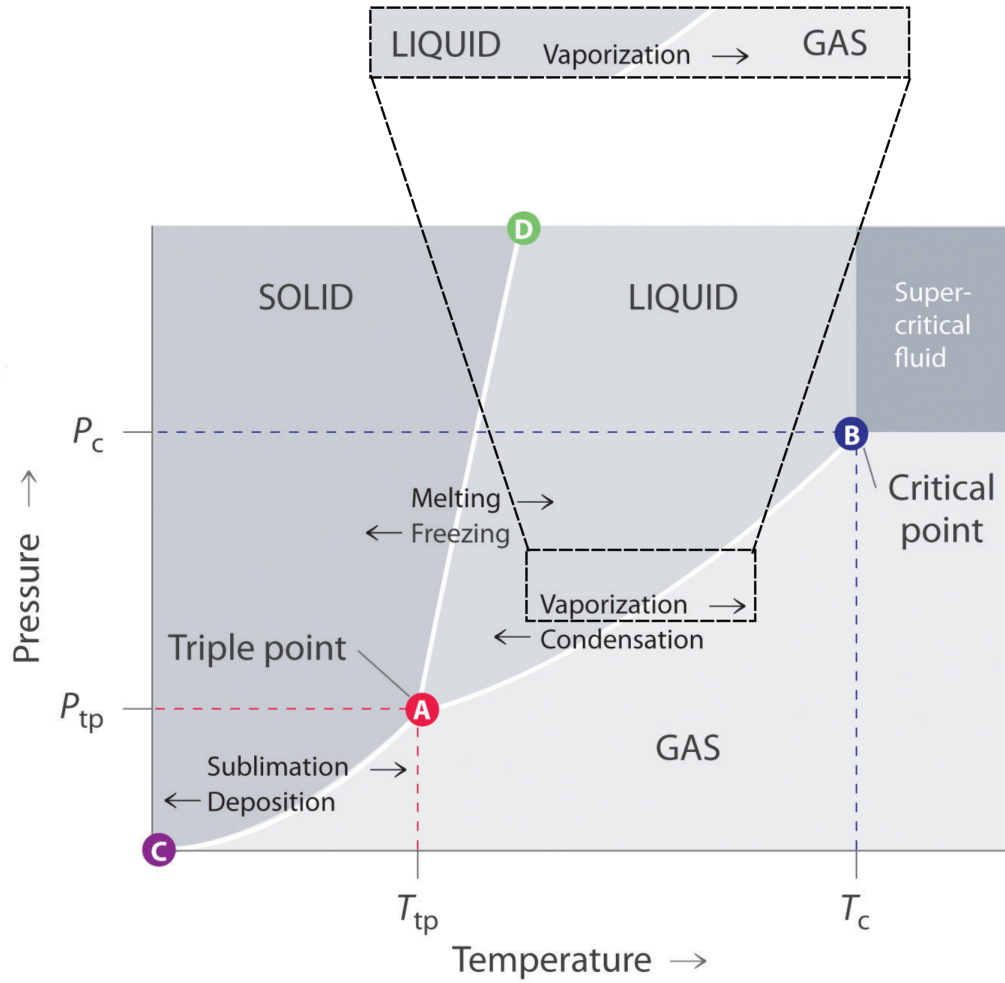


Figure 2.2: General phase diagram. Liquids can change phase and become gas by vaporization under the suitable temperature and pressure conditions. Adapted from *Averill et al. (2007)* [15].

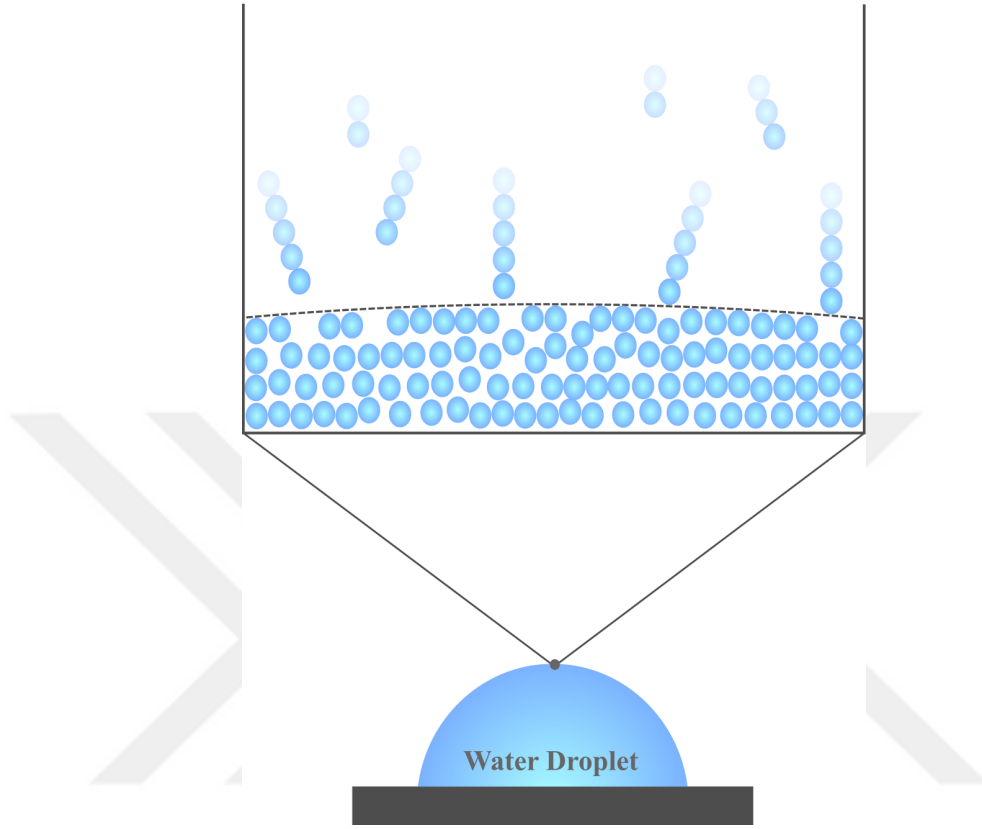


Figure 2.3: Schematic representation of the evaporation process of a water droplet lying on a solid substrate. The liquid molecules on the surface can leave the droplet when they reach a certain energy level by changing phase from liquid to gaseous, i.e they evaporate.

The study of pure droplet evaporation dates back to the 19th century and to the work of James Clerk Maxwell [16]. Maxwell assumed that the vapor concentration difference between the surface of the droplet and the surrounding medium causes the evaporation; this assumption made him believe that the evaporation process was mainly controlled by diffusion [17]. Later on, thanks to further studies, it was understood that he was essentially right about diffusion, but his study had some rough edges [9]. Now it is known that mutual heat and mass transfer between the

droplet and its surroundings cause the evaporation [18]. The heat exchange occurs due convection, conduction and radiation; the mass is transferred from the vaporizing droplet to the surrounding gaseous medium by diffusion and convection [18, 19].

In this chapter, we will first become familiar with pure droplets by starting to introduce the fundamental science behind their shapes. Then, I will define the contact angle which will help us to identify the states of wetting of the droplets and characterize the properties of the underlying substrate. I will end the chapter by describing pinning and depinning of the droplets.

2.2 Surface Tension

It is a well-known fact that liquids tend to take the shape of the container that they are poured into. In the absence of a container, on the other hand, they tend to take the form of spherical or hemispherical droplets when they get heavy enough to fall. We are all used to see the droplets in these forms. The reason behind this shape lies in the phenomenon of surface tension.

The molecules inside the droplet keep it together by applying equal attractive forces to their neighbours. As the molecules inside the droplet are surrounded by other liquid molecules, they feel the intermolecular forces in every direction and they remain under uniform influence. However, the liquid-gaseous interface causes the molecules on the surface of the droplet to have fewer liquid neighbours and this causes an imbalance in the forces. The molecules on the surface start to bend the shape of the droplet as they undergo the attractive effect of their neighbours and get pulled towards the center as there is no liquid molecule outside to balance them. This imbalance creates a tension on the surface, i.e. surface tension, causing the droplet to take a spherical shape and making it appear as if covered with a transparent elastic

membrane. This geometrical shape also permits the droplet to acquire the minimum surface area possible for a given volume [15].

Incidentally, surface tension is also why some dense objects like a steel needle or a paperclip can float on water rather than submerging as shown in Figure 2.5 as if they are carried by the so-called membrane [20].

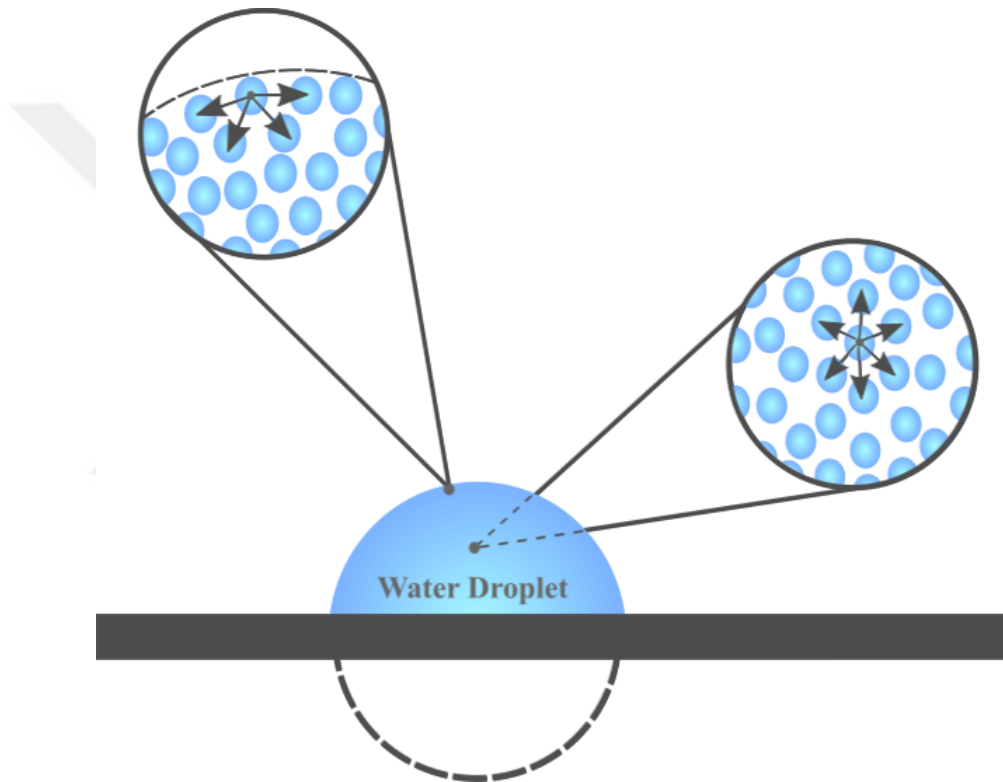


Figure 2.4: Schematic representation of a hemispherical droplet on a smooth solid substrate pointing out the reason behind its shape. The magnified portions show the intermolecular forces by focusing on the molecules inside and on the surface of the droplet. The molecules inside the droplet are balanced thanks to the attractive forces applied by the surrounding liquid molecules in each direction. However, the molecules on the surface get pulled towards the center by the imbalanced attractive forces due to the lack of the liquid molecules outside.



Figure 2.5: Effect of surface tension: Even though the density of steel is higher than the density of water, a steel needle floats on water thanks to surface tension and it looks as if the surface is covered by a transparent membrane.

2.3 Contact Angle

When a liquid droplet rests on a solid flat surface, it spreads on it until reaching equilibrium and wets a particular area depending on the properties of the underlying surface. That area is limited by the contact line of the droplet formed by the three tensions among the liquid-vapor, solid-liquid and solid-vapor interfaces as shown in Figure 2.6 [19, 21].

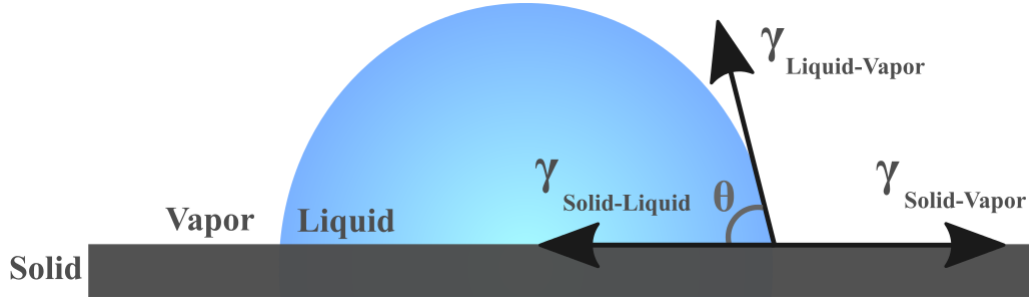


Figure 2.6: Balance of the interfacial tensions at the three-phase contact line of a droplet lying on a flat solid surface. An angle, i.e. contact angle, is formed between the solid-liquid and liquid-vapor interface.

As seen again in Figure 2.6, when the profile of the droplet resting on an ideal, smooth surface is observed, an angle defined as the contact angle is formed because of the mechanical equilibrium of the surface tensions among the interfaces of the three phases.

Thomas Young, who was the first one to describe the contact angle in 1805, derived an equation for calculating the contact angle of a droplet resting on a solid surface. The equation that also bears his name shows the relation between the cosine of that angle and the three surface tensions of the droplet as follow;

Young's Equation:

$$\gamma_{sv} = \gamma_{sl} + \gamma_{lv} \cos(\theta) \quad (2.1)$$

where θ is the contact angle, γ_{sv} is the tension between solid-vapor interface, γ_{sl} is the tension between solid-liquid interface and γ_{lv} is the tension between liquid-vapor interface [22]. This equation takes only the tensions into account, assumes that they are constant and the gravity is negligible. To put it simply, it ignores the influence of the surface topography. As a consequence of real life surfaces being far from this ideal condition, experimentally measured contact angles may not match with the

Young's contact angle [23].

Rather than using equations which requires extra terms, the contact angle can be also measured directly from the profile of the droplet by following a geometrical approach. Applying a tangent from the starting point of the contact line where liquid and solid meet along the liquid-vapor interface of a droplet lying on a surface horizontal to the ground is enough to obtain the value of contact angle as shown in Figure 2.7 [24].

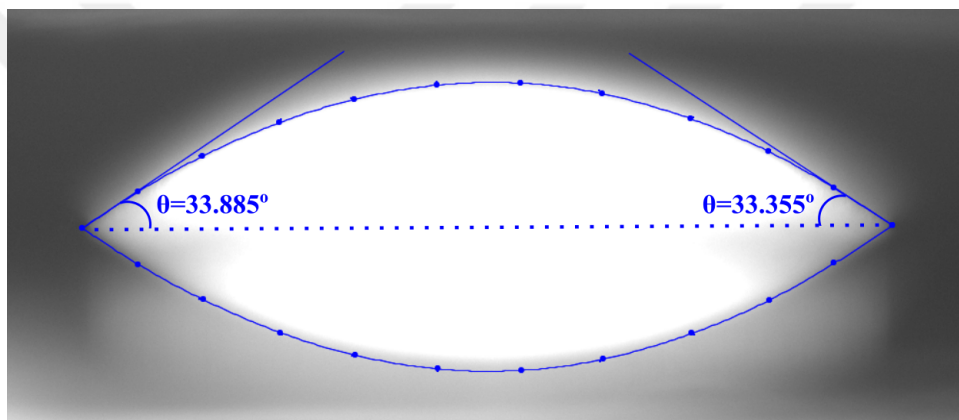


Figure 2.7: Experimentally recorded side view of a water droplet lying on a glass slide showing the contact angle θ measured by following geometrical ways.

2.4 Droplet Wetting and Pinning

The contact angle described in the previous section also helps us to determine the state of wettability of a liquid, because it tells how much the liquid spreads over the solid surface when they interact. Therefore, we can identify a droplet as it is wetting or non-wetting the surface by just looking at the measured contact angle [25, 26].

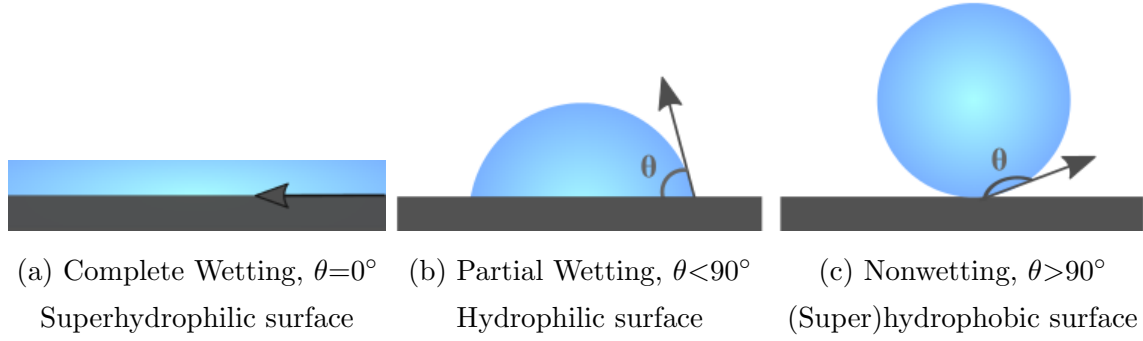


Figure 2.8: Different states of wetting of a droplet on a flat solid substrate. Contact angle changes depending on the surface properties of the underlying substrate: $\theta=0^\circ$ indicates the complete wetting of the droplet, θ becomes smaller than 90° on hydrophilic surfaces and exceeds 90° on hydrophobic surfaces.

If the contact angle is 0° , it means that the droplet wets the solid surface completely and this indicates the extreme case of a superhydrophilic surface. If the contact angle is less than 90° , the droplet wets the surface partially. In view of droplet spreading over a considerably large area, it can be said that the surface is in favor of wetting and the term ‘hydrophilic’ which means ‘water-loving’ in ancient Greek can be used to characterize it. On the other hand, if the contact angle is greater than 90° , it indicates that the contact between the liquid and the solid surface is reduced and the surface repels the liquid, hence it may be called ‘hydrophobic’ (‘water-hating’) as shown in Figure 2.8 [21, 27, 28]. Moreover, when the contact angle exceeds 150° , the surface may be defined as superhydrophobic [29].

As already explained in the previous sections, evaporation of a droplet causes a decrease in the volume of the liquid. During the evaporation process, the droplets may adapt the volume loss by decreasing the height and keeping the wetting area same. In other words, the three-phase contact line, the interface where the solid, liquid and vapor co-exist, may have a tendency to stay stationary while the volume

of the droplet decreases indicating the pinning. Additionally, the volume of the liquid may decrease also by reducing the wetting area along with decreasing the height of the droplet. In this case, the contact line also keeps moving instead of remaining static in consequence of the volume loss during the evaporation and the droplet starts to depin as depicted in Figure 2.9 [30].

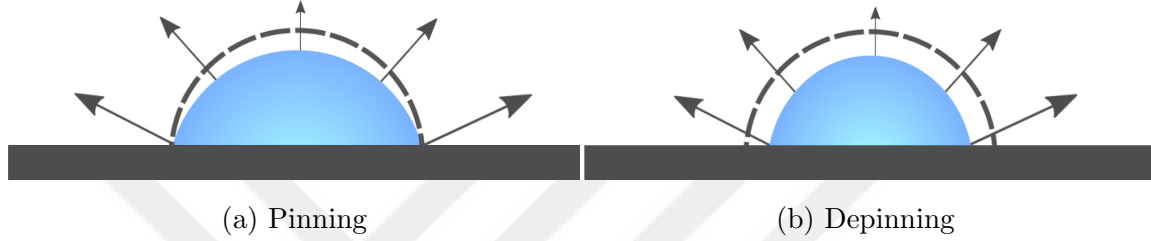


Figure 2.9: Schematic illustration of pinning and depinning of droplets on a flat solid substrate.

Although evaporation of pure droplets itself is an interesting process, dissolving solutes or dispersing particles in the droplets introduces a whole new research line, because of the fact that these evaporating droplets leaves a stain behind. The variety of the patterns after the evaporation depending on several experimental parameters (will be explained in Chapter 3) has been very promising and opened new doors to several research areas in not only physics, but also chemistry and biology [9, 31]. The ease of carrying out the experiments has also helped to increase the level of interest for the evaporation of droplets [4, 32, 33]. Hence, in the next chapter we will meet with droplets containing additives and focus on the self-assembled patterns caused by their evaporation.

Chapter 3

Self-Assembly using Evaporating Droplets

3.1 Introduction

Creating organized and ordered patterns generates its own challenge and encourages us to find simpler ways. Luckily, dissolving solutes or dispersing particles in droplets helps us to obtain a variety of patterns, because the evaporating droplets leave behind a stain and provide the opportunity of benefiting from self-assembly, i.e. a process in which disordered components of a system get organized and form ordered patterns or structures spontaneously without any external instructions [2, 3, 34]. By making use of these, self-assembled patterns can be formed on-demand.

The examples of this autonomous generation of organization can be seen at almost every scale from the double helix structure of DNA [35], the smallest genetics code box that carries most of the essence of life in all the organisms on Earth, to the

largest galaxies in the universe [3]. There are also the examples that we are used to see in daily life besides the small droplets like the flocks of birds up in the air, the schools of fish down in the ocean or the swarms of bacteria, bees or ants on Earth [2, 36] as some of them shown in Figure 3.1.

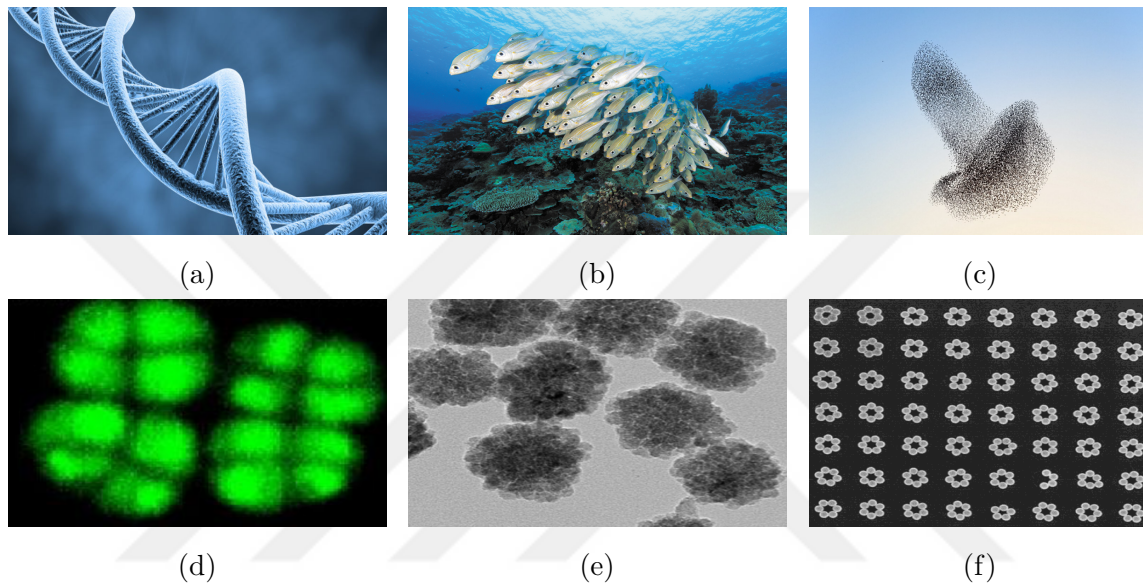


Figure 3.1: Examples of self-assembly: (a) DNA double helix, (b) a school of fish, (c) a flock of birds, (d) clusters of bacteria, (e) magnetic colloidal clusters, (f) colloidal aggregates. Adapted from [37], [38], [39], *Jena et al. (2006)* [40], *Kostopoulou et al. (2014)* [41], *Yin et al. (2001)* [42].

Not only nature but also technology takes its share from it. Over the past decade, many researchers from different disciplines have become attracted to transform this useful concept into an effecting tool with their own perspective [34]. This interest has brought fruits and now there are lots of potential and actual applications of the desired self-assembled patterns in 2D/3D structure building [43], electronics [2, 34], photonics [2, 34, 44, 45], photovoltaics [46], near field optics [47], robotics, netted systems [2], biomaterial fabrication, printing [48] and deposition [49].

This chapter will start with one of the examples of self-assembly methods, evaporation-induced self-assembly. Then, coffee-ring patterns which are one of the most well-known examples of the evaporation-induced self-assembly will be introduced and the underlying mechanisms will be explained along with the applications.

3.2 Evaporation-Induced Self-assembly

As the name suggests, evaporation-induced self-assembly refers to the self-assembly method which is derived by the evaporation process of droplets. This method provides an easier way to obtain self-assembled patterns by just dispersing some particles or dissolving some solutes in the liquid droplets. By the time the solvent evaporates, the additives inside the droplets start to get organized and they form ordered structures spontaneously when the drying process is complete.

However, one should not be fooled by the simplicity of this method to underestimate it. By playing with the parameters such as the shape of the dispersed particles [50, 51, 52, 53, 54], size of the droplet [55], size of the dispersed particles [56], type of the additives [33, 57, 58, 59, 60], motion of the particles [61, 62], the forces on the particles [4, 63], the flows [32, 64], conditions of drying [65] and the type of the substrate [66], variety of patterns (some of them shown in Figure 3.2) such as rings, uniform deposits, central bumps, multiple rings, polygons, hexagons [4], fractals [67, 68] and dendrites [60] can be obtained after the evaporation increasing the significance of the method.

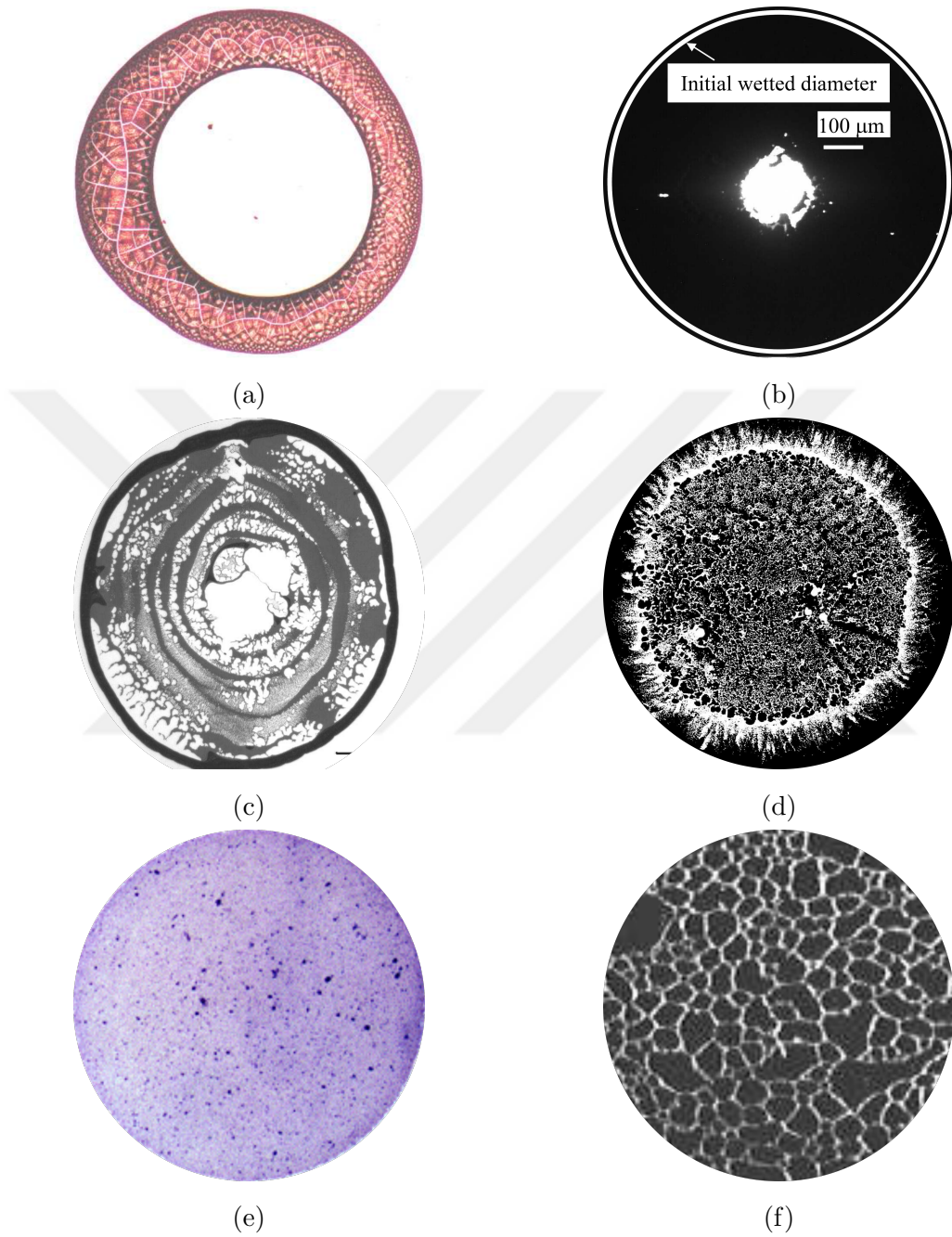


Figure 3.2: Different patterns obtained via evaporation of droplets. (a) Ring-like pattern, (b) central bump, (c) multiple rings, (d) fingering at wetting line, (e) uniform deposition, (f) hexagonal cells. Adapted from *Bhardwaj et al. (2010)* [4].

3.2.1 Coffee Ring Effect

Among all these patterns, one of them shines a bit more; the ring-like stain left behind the evaporated droplets which even the people outside from the scientific community should have noticed after a clumsiness of spilling some coffee (see Figure 3.3).

So, have you ever wondered why the edge of a spilled coffee droplet gets darker and darker as it dries out? Some scientists did.

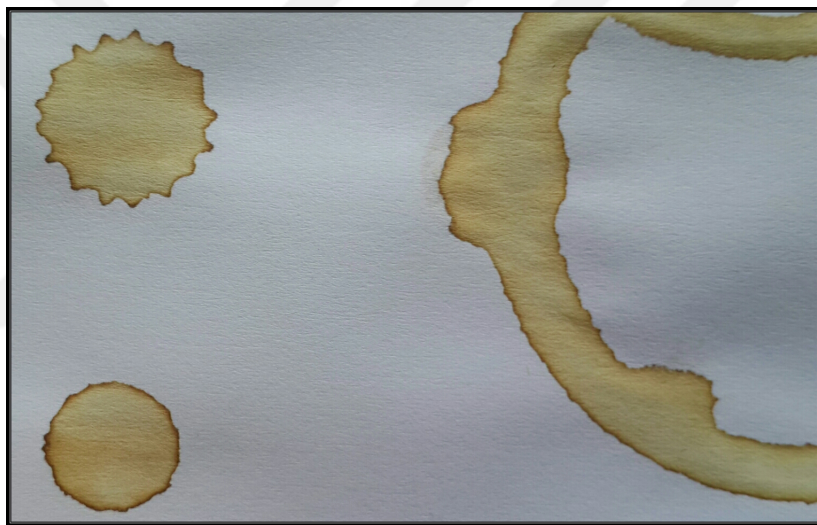


Figure 3.3: Photograph of spilled coffee stains.

Although Adachi and his co-workers touched on the tendency of the particles collecting near the edge of a droplet boundary in 1995 [69], the real explanation that lies behind the reason of the motion of the particles came from Robert D. Deegan and his colleagues two years after Adachi's team in 1997 [5]. An everyday life experience of a spilled coffee droplet made these people wonder the underlying reason for seeing a denser stain along the perimeter of the drop because of the accumulation of the particles present the liquid. They pioneered a new research area

at the University of Chicago in the United States of America by drawing attention to the fact that those stains are including (but not limited to) coffee droplets and can be also observed with droplets of aqueous solutions containing suspended spherical particles (see Figure 3.4) [33, 70].

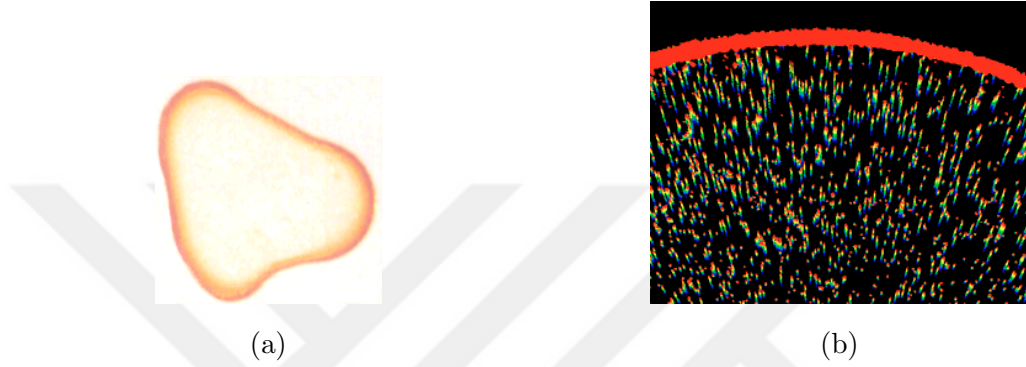


Figure 3.4: Pictures of (a) a dried coffee droplet, (b) an evaporating water droplet containing 1-micron polystyrene particles. Initially uniformly dispersed particles go towards the edge of the droplets as the solvent dries out and create a darker stain by accumulating at the edge. Adapted from *Deegan et al. (1997)* [5].

Consequently, the accumulation of migrating coffee particles at the edge of the droplet gave the name of coffee-ring to the characteristic ring-like pattern formed after the evaporation of the coffee droplet.

3.2.1.1 Mechanisms Behind the Coffee Ring Effect

Deegan and his colleagues observed the evaporation process of water droplets containing polystyrene spheres via optical microscopy and identified capillary flow as the cause of ring stains from dried liquid droplets.

When a volatile liquid droplet containing suspended particles meets the underlying

solid substrate directly and partially wets it, the edge of the droplet, which is known also as the contact line, gets pinned to the surface and remains stuck there due to the roughness of the surface. During evaporation, the radius of the droplet remains constant as the pinning of the contact line prevents any change unlike the case of a pure droplet, while the contact angle and the height of the droplet decrease.

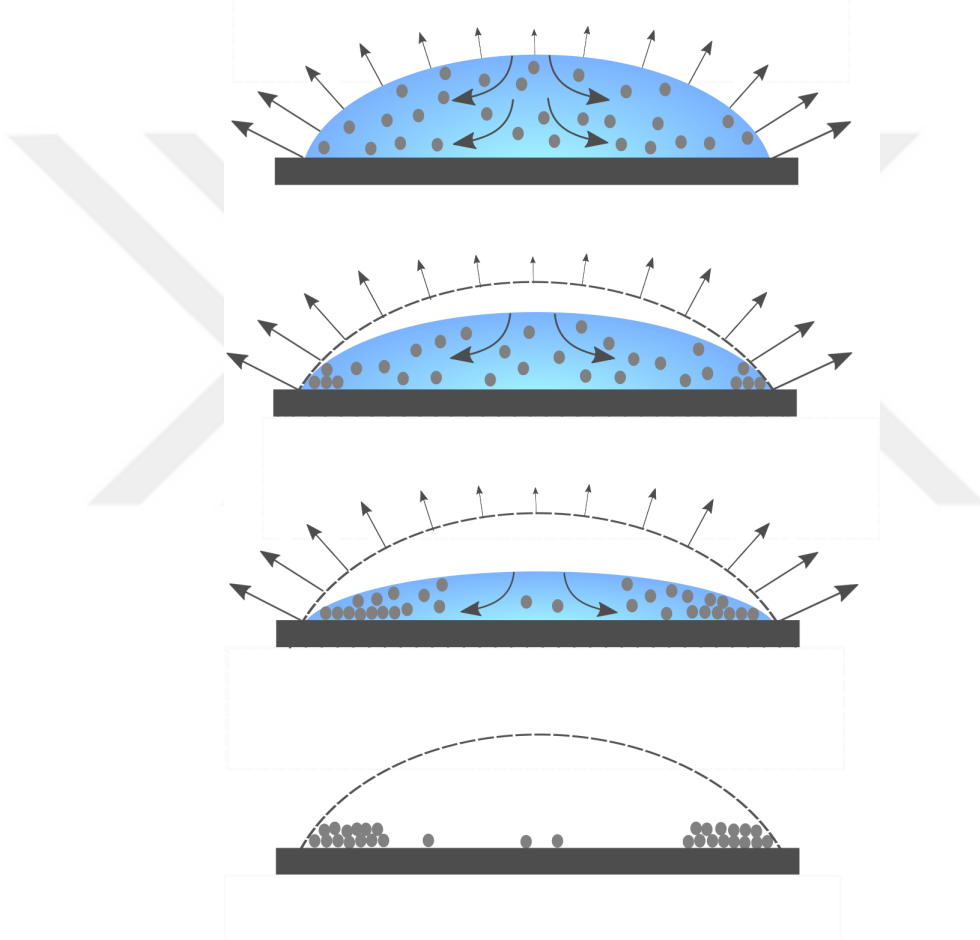


Figure 3.5: Schematic representation of the motion of the particles in an evaporating droplet effected by the outward flow. Evaporation induced flow, i.e. capillary flow, carries the particles towards the edge while also replenishing the liquid loss.

The evaporation rate at the stationary contact line becomes higher than at center of the droplet as the liquid molecules near the edge are surrounded by fewer neighbouring molecules than the ones in the center. Thus, the divergence in the evaporation rate causes more liquid loss at the edge. In order to compensate that liquid loss caused by the evaporation, more liquid is brought to the edge from the center. As a consequence of the liquid transporation, a capillary flow is induced from the center of the droplet towards the edge. This radially outward flow not only replenishes the liquid lost at the contact line, but also carries the suspended particles inside the droplet towards the edge. The driven particles populate at the perimeter of the droplet and form a concentrated ring-like structure namely coffee-ring as depicted in Figure 3.5. In short, the underlying mechanism of the ring formation phenomenon can be elucidated by firstly the pinning of the contact line and secondly the evaporation induced outward capillary flow [5, 19, 24, 32, 33, 70, 71].

3.2.2 Applications

When considering the fact that the pionnering work started with such a trivial daily life experience such as spilled coffee, the stains may seem to be insignificant. On the contrary, those patterns obtained after evaporation of droplets have a great significance in many scientific and industrial applications.

The so-called coffee-rings contribute to science with their characteristic structures. By taking advantage of particles going to the contact line of the droplet and geometrical constraint arising from contact line pinning and decreasing height of the droplet towards the contact line along with the size of the particles; scientists revealed the particle (as well as the biological entities, see Figures 3.6 and 3.7) separation capability of coffee ring effect [56, 72].

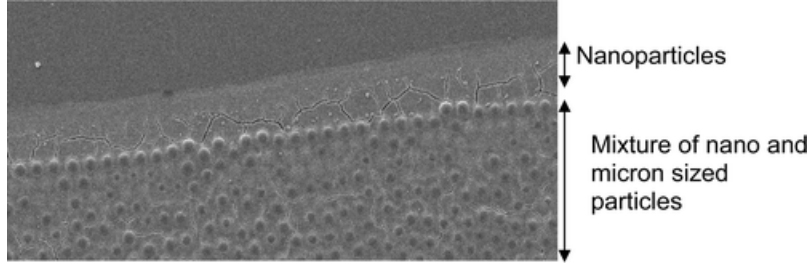


Figure 3.6: Sorting of colloidal mixtures with the help of coffee-ring effect. While the nanometric colloids go to the edge of the droplet, the geometrical constraints prevent the micrometric colloids to reach the edge. Adapted from *Monteux et al. (2011)* [72].

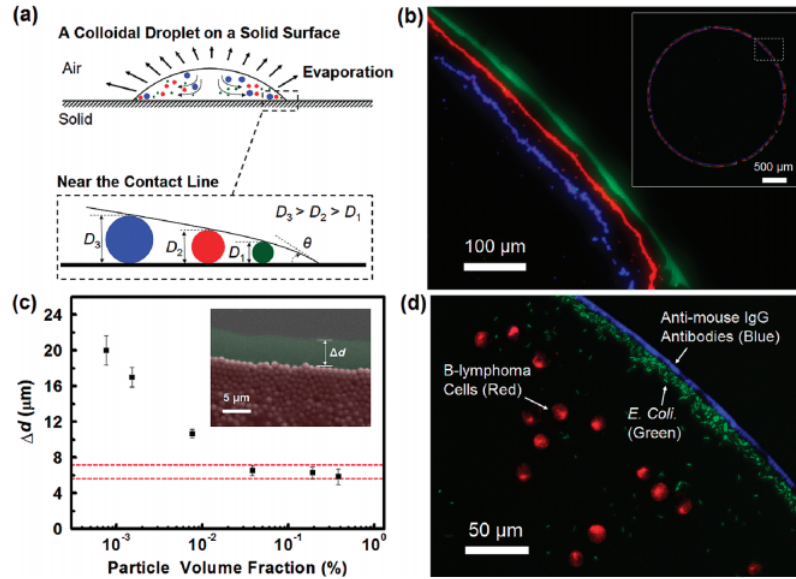


Figure 3.7: Nanochromatography with the help of the coffee-ring effect. The biological entities such as proteins, micro-organisms and mammalian cells with different sizes can be also separated by making use of the coffee-ring effect. Although the particles with the smallest diameter can reach the droplet boundary, the ones with greater diameters fail to reach and get blocked at certain places. Adapted from *Wong et al. (2011)* [56].

On the other hand, this ring-like structure can be also a real problem for researchers wanting to obtain uniform coating such as the case for ink-jet printing [10, 11].

The desire of homogeneous deposition has led the researchers to find new ways gain control over the coffee-ring effect and even overcome it. A simple suggestion for suppressing the coffee-ring effect came from Peter J. Yunker and his coworkers which was basically playing with the isotropy of the particles present in the liquid. By using ellipsoids instead of spheres, Yunker and coworkers overcame the unwanted ring-stains and succeeded to obtain uniform depositions after the evaporation as shown in Figure 3.8 [50].

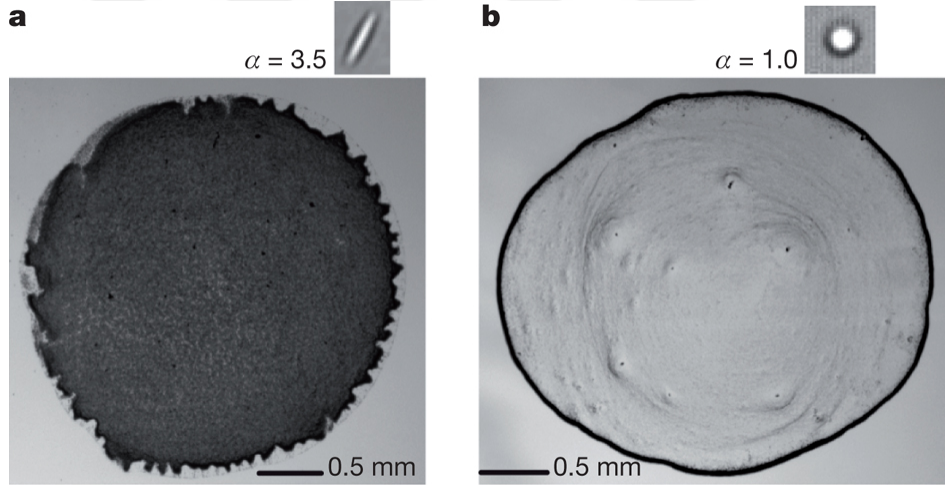


Figure 3.8: Effect of particle shape on pattern formation: Using ellipsoids instead of spheres suppresses the coffee-ring effect. Although (a) ellipsoids are distributed more uniformly after the evaporation, (b) spheres form rings at the edge of the droplet. Adapted from *Yunker et al. (2011)* [50].

Apart from this simple change which provides the opportunity of keeping the chemistry of particles and solvent the same, there are also other ways to obtain

relatively uniform coatings after the evaporation by playing with several parameters such as introducing surfactants and generating inward Marangoni flows opposing to capillary flow inside the droplets [57, 58, 73] (can be also witnessed in Section 4.3.1.2), coupling acoustics waves to the solid substrate underlying the droplet [74], using electrowetting [75, 76], heating the underlying substrate [77] and using solid substrates with different wettabilities [66].

Furthermore, pattern formation by the evaporating droplets can be of use with biological samples such as blood and serum (see Figure 3.10 and 3.11). The characteristic differences between the patterns of the samples taken from different patients have offered a fast and low-cost alternative for medical diagnostics as well [12, 13].

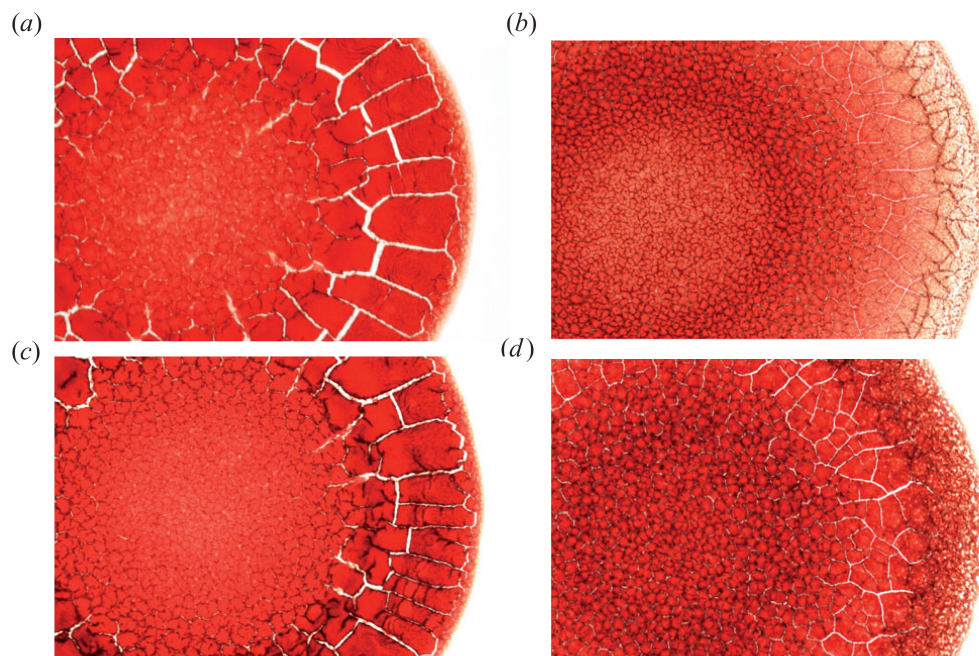


Figure 3.9: Images of blood droplets taken from different people. Four droplets of blood taken from different individuals; (a) and (c) in good health, (b) suffering from anemia, (d) suffering from hyperlipidaemia show different pattern formation after the evaporation. Adapted from *Brutin et al. (2011)* [12].

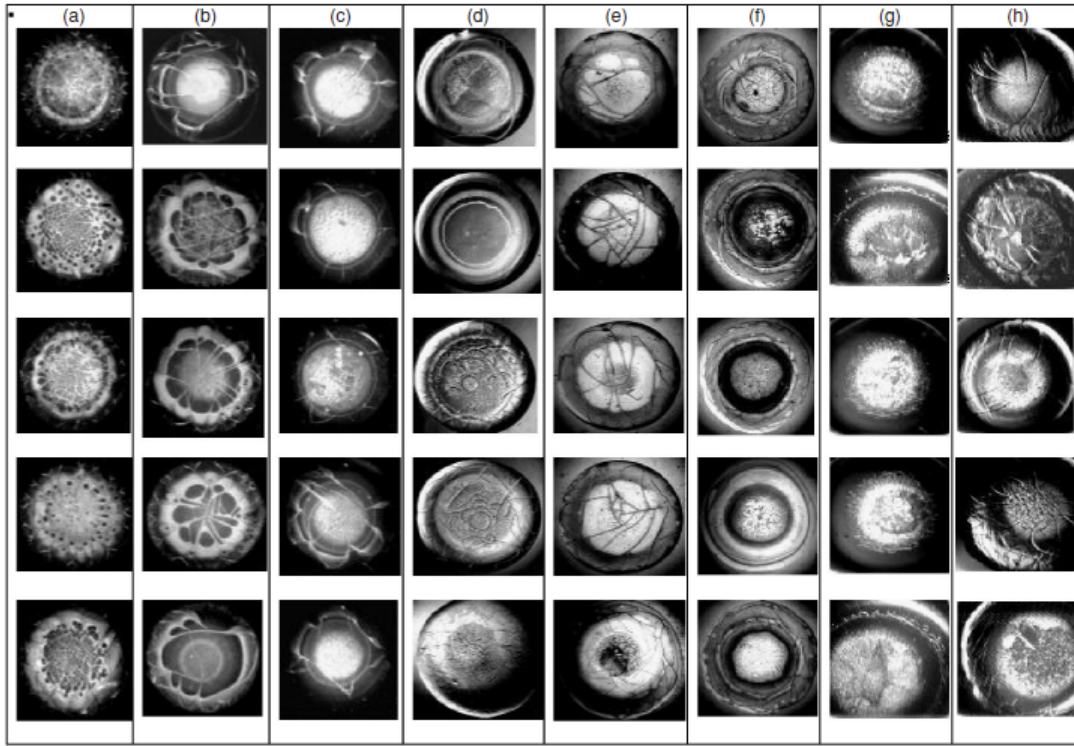


Figure 3.10: Images of serum droplets taken from different individuals suffering from a disease or a physiological disorder. (a) Control group, (b) breast cancer patients, (c) lung cancer patients, (d) people in-time delivery, (e) people in premature delivery, (f) people with threatened abortion, (h) people carrying hepatitis. Adapted from *Yakhno et al. (2005)* [13].

These are just some of the applications derived thanks to the self-assembled patterns that shows even a small evaporating droplet can be used for understanding many things. In conclusion, even though evaporating droplets may form some unwanted stains, they may turn out to be useful in many research areas.

Chapter 4

Self-Assembled Patterns in Evaporating Droplets containing Brownian Particles and Bacteria

4.1 Introduction

The variety of the applications acquired from the self-assembled patterns, the ease and the simplicity of the method have increased the interest in evaporation-driven self-assembly. However, so far most of the studies were conducted by using simple Brownian particles alone such as spherical, ellipsoidal or rod-shaped particles [5, 50, 51] which move due to thermal fluctuations, while the behavior of active particles still remains to be explored. When considering the fact that active particles have the potential to tune the self-assembled patterns with their distinctive ability of self-propelling, this topic becomes even more interesting. Here, in this chapter, we focus on the effect of the motility of the active particles, e.g. bacteria, over the

simple Brownian particles present in the evaporating droplets and examine the self-assembled patterns obtained after evaporation. Our results show that the presence of motile bacteria has an effect on the polystyrene particles in the droplets: they change the pattern formation and provide more uniform, disk-like deposition rather than a ring under controlled evaporation conditions.

4.2 Materials and Methods

4.2.1 Experimental Setup

A homemade inverted microscope (see Figure 4.1) was designed for observing the evaporation process of the droplets. The setup was built with several optical components on a stabilized optical table in order to avoid the disturbances coming from the environment.

Three different cameras which were controlled by a computer were positioned for the homemade inverted microscope accordingly so that the full view of the droplet, the side view of the droplet and the magnified view of a portion of the droplet could be observed by digital video microscopy simultaneously with the help of an incoherent white light lamp directly projected onto the droplets. The full view was recorded by using a 4X microscope objective, numerical aperture (NA) of which is 0.13 with a CMOS camera. A beam splitter was placed between the lamp and the sample holder for reflecting the illuminated light onto the CMOS camera. For the side view, another microscope objective (10x, NA=0.30) and a convex lens with a focal length of 35.0 mm were used with a monochrome CCD camera which were placed on two connected translation stages moving in x and y directions to make it easier to detect the right position of the droplet while recording. Lastly, the videos of the magnified view of a certain portion of the edge of the droplet were recorded by a CCD camera using

20x microscope objective with 0.5 NA which provided higher magnification when compared to others in order to study the behaviour of the suspended particles in the droplet. The frame rate was set to 5 fps for all the cameras during the experiments to obtain synchronized results.

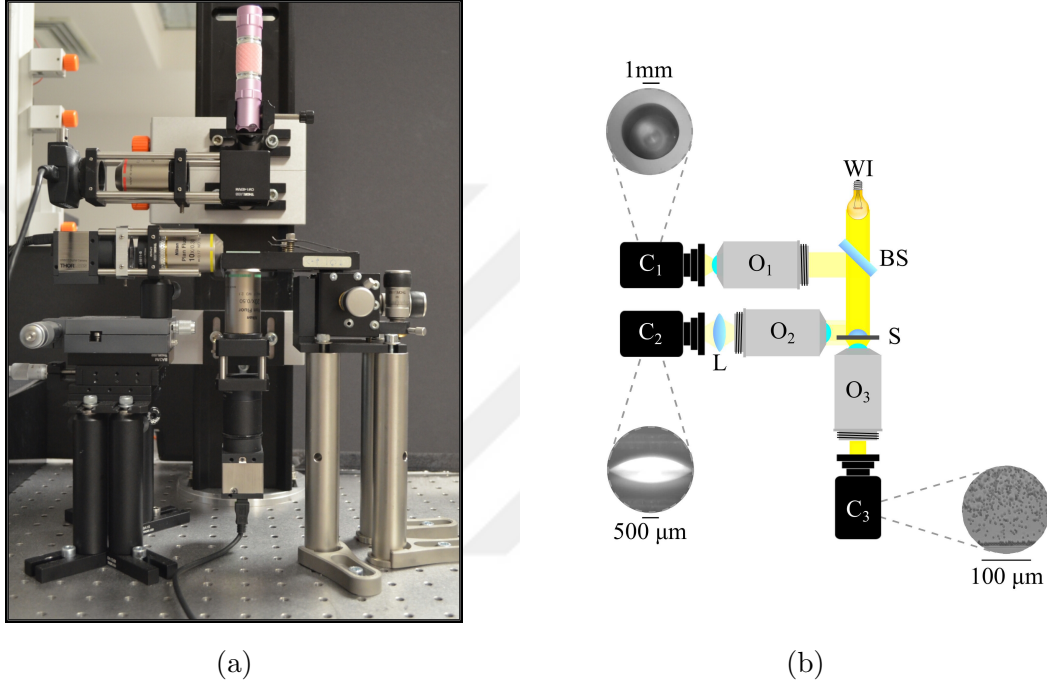


Figure 4.1: (a) Photograph, (b) schematic representation of the experimental setup: Homemade inverted microscope capable of recording full view of the droplet, side view of the droplet and magnified view of a certain portion of the droplet.

4.2.2 Cleaning Procedure for the Glass Slides

Ground edges 1 in x 3 in (25.4 mm x 76.2 mm) x 1 mm thick microscope slides (Sail Brand, China) were used throughout the experiments and all were treated with the same cleaning procedure to obtain the same surface quality for each slide. In

order to get rid of the impurities and contamination on their surfaces, the glass slides were cleaned with acetone, followed by isopropyl alcohol with the help of a folded lens cleaning tissue by wiping it across from one edge of the slide to the other. Then, they were soaked in 0.25 M of sodium hydroxide (NaOH) solution for approximately 15 minutes to make the surfaces hydrophilic and to reduce the adhesion of the particles onto the glass slides. After rinsing them with deionized water, glass slides were blow-dried with nitrogen (N_2) and became ready to be used.

4.2.3 Sample Preparation

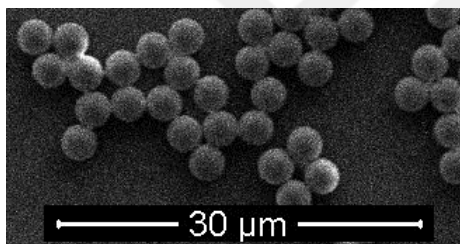


Figure 4.2: SEM image of PS particles ($3.00 \mu\text{m}$) deposited on glass slide.

The polystyrene (PS) particles (shown in Figure 4.2) with diameter of $3.00 \mu\text{m}$ were used for these samples as they were suitable for preparing colloidal suspensions. Three different colloidal suspensions were prepared by using monodisperse spherical polystyrene particles (Microparticles GmbH, Berlin, diameter $d = 3.00 \pm 0.07 \mu\text{m}$, volume fraction $\phi = 0.095$) as passive particles in deionized water, plain motility buffer and motility buffer containing *E.coli* bacteria separately.

Deionized Water Sample: $58 \mu\text{l}$ of monodisperse spherical polystyrene particles (Microparticles GmbH, Berlin, diameter $d = 3.00 \pm 0.07 \mu\text{m}$, volume fraction $\phi = 0.095$) were suspended in $942 \mu\text{l}$ of deionized water ($\phi = 0.0055$).

Motility Buffer Sample: $58 \mu\text{l}$ of monodisperse spherical polystyrene particles (Microparticles GmbH, Berlin, diameter $d = 3.00 \pm 0.07 \mu\text{m}$, volume fraction $\phi = 0.095$) were suspended in $942 \mu\text{l}$ of motility buffer having the same volume fraction as the water sample. The reason behind using motility buffer as a medium of suspension

here was to have a base for better understanding the behaviour of the passive particles in this medium as *E.coli* bacteria were already suspended in motility buffer and check whether the medium itself had any effect on the pattern formation.



Figure 4.3: Illustration of *E. coli* bacteria.
Adapted from [78]

Bacteria Sample: 58 μl of monodisperse spherical polystyrene particles (Microparticles GmbH, Berlin, diameter $d = 3.00 \pm 0.07 \mu\text{m}$, volume fraction $\phi = 0.095$) were suspended in 942 μl of motility buffer containing freshly cultured flagellated *Escherichia Coli* (*E. coli*) bacteria (single-celled, rod-shaped bacteria capable of taking up energy from the environment and move with the

help of their tail-like filament called flagella [79]) (illustration shown in Figure 4.3) to see the effect of their motility on pattern formation as they are one of the most widely studied and well-understood active swimmers.

4.2.3.1 Preparation of the Motility Buffer

The stock solution for the motility buffer was prepared by adding 10 mM monobasic potassium phosphate (KH_2PO_4) 0.01 mM ethylenediaminetetraacetic acid (EDTA), 0.002 % of Tween 20 and 10 mM of Dextrose ($\text{C}_6\text{H}_{12}\text{O}_6$) to deionized water. The resultant solution was mixed with the help of a magnetic stirrer to obtain homogeneity in the buffer. The pH of the solution was adjusted to 7.0 by putting appropriate amount of NaOH solution.

Then, the plain motility buffer was treated with tryptone as the bacteria culture was mixed with tryptone growth solution during the growth procedure (see section

4.2.4.1) in order to have the similar effect. Three separate falcon tubes with 4 ml of motility buffer were prepared. 2 ml of tryptone was added to the first falcon tube and mixed well. From the resulting solution, 2 ml of mixture was taken and added to the motility buffer to the second tube. Then, 2 ml of the new mixture obtained one step back was added to the third tube containing only motility buffer as mentioned before. By doing so, the concentration of the tryptone growth medium which was originally high in the first tube was diluted. Finally, the motility buffer treated with tryptone used throughout these experiments was obtained in the third tube.

4.2.3.2 Preparation of the Bacteria Culture

Motile *E.coli* bacteria were freshly cultured from the wild-type strain RP437 (*E. coli* Genetic Stock Center, Yale University) for each experiment (see Figure 4.4). The bacteria were inoculated in 50 ml of Tryptone Broth (TB) inside a 250 ml flask overnight, approximately 17 hours at 32°C and 180 rpm in a shaker incubator. After the saturation, overnight culture was diluted 1:100 into a 50 ml of fresh TB inside another 250 ml flask. The diluted culture was incubated for another 4 hours under the same conditions with the overnight culture incubation until its optical density at 600 nm (i.e. OD600) reaches to 0.4. 7 ml of the diluted culture was transferred into two falcon tubes and centrifuged at 2000 rpm at room temperature for 10 minutes. Once the centrifuge was complete, the bacterial pellets which were precipitated at the bottom of the falcon tube were gently collected with the help of a pipette. Then, the resultant pellets were suspended in 4 ml of motility buffer of which ingredients were given in section 4.2.3.1. The bacteria in the motility buffer was centrifuged, collected and resuspended again in motility buffer in order to get rid of the growth medium as much as possible.

4.2.4 Experimental Procedure

$0.40 \pm 0.04 \mu\text{l}$ of each colloidal solution were dropped on clean glass slides by using a micro-pipette for normal evaporation. The micro-pipette was tried to be kept as still as possible during the dropping to avoid vibration as it could cause deformation in the droplet profile.

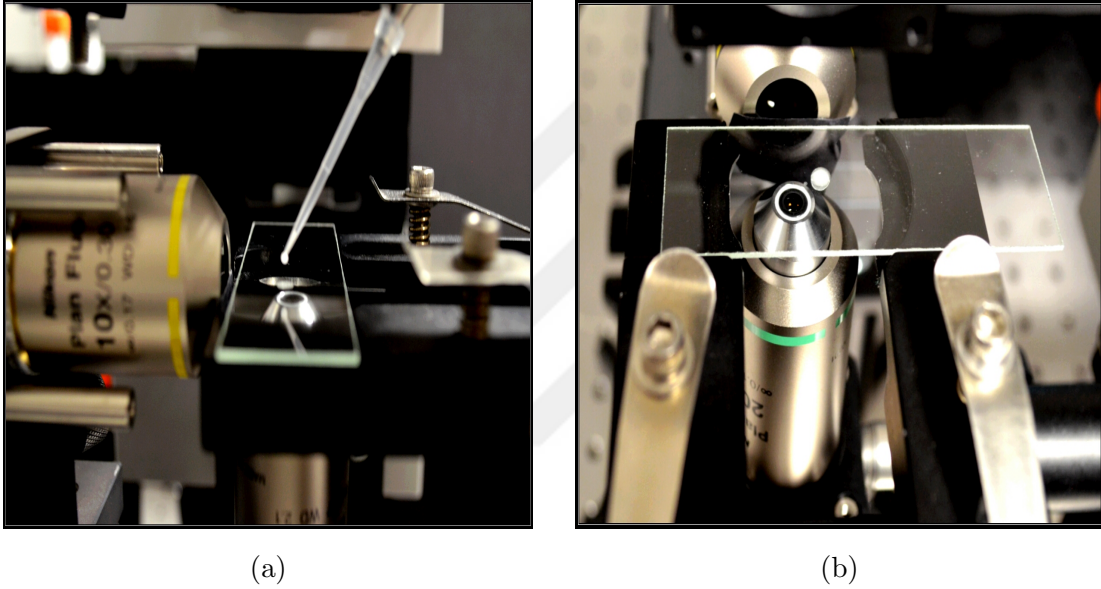


Figure 4.4: Photographic demonstration of the experimental procedure: (a) Dropping, (b) droplet on a microscope slide ready for evaporation.

Both the concentration of the solutions and the volume of the droplets were kept constant for each sample within the measurement error. Each solution was mixed well before each trial to obtain uniformity in the composition of the droplets. The temperature of the laboratory was $20 \pm 1 ^\circ\text{C}$ while performing the experiments.

4.3 Results under Normal Evaporation Conditions

Two different media of suspension were used in this section (deionized water and motility buffer). Images of the full view of the droplet was recorded both before and after the evaporation with the side view of it immediately after the dropping.

The contact angles of the droplets were calculated using the corresponding side view images with the help of droplet shape analysis method [80]. Several knots were placed manually around the droplet contour starting from the left and ending at the right contact line where liquid and solid meet by paying attention to keep approximately same distances between them. Due to the possibility of droplet being asymmetrical, two tangents were applied along the liquid-vapor interface starting from the contact lines, the contact angle was measured from both left and right and the averaged result was calculated accordingly in order to increase the accuracy. Each experiment was repeated for several times to see whether the same pattern after evaporation and the same contact angle for corresponding samples could be obtained. By doing so, reproducibility was checked and confirmed.

4.3.1 Droplets Containing only Brownian Particles

4.3.1.1 Water Droplets

0.4 μl of water droplet containing polystyrene particles was dropped on the microscope slide which was previously treated with NaOH solution and we were able to reproduce the regular coffee-rings with polystyrene particles in water droplets under normal evaporation conditions

The contact angles of left and right side of the droplet immediately after the dropping were measured as 12.37° and 12.01° correspondingly as stated in Table 4.1. The average value of the contact angle was calculated as 12.19° with 0.25 standard deviation (σ). The evaporation time of the droplets was approximately 3 minutes for these experiments under the laboratory conditions that were already specified in Section 4.2.4.

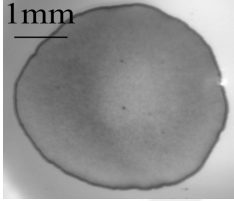
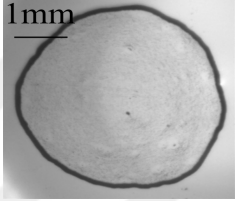
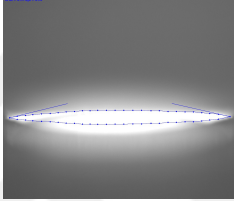
Full View of the Droplet		Side View of the Droplet	Contact Angle Values
Before Evaporation	After Evaporation		
			Left: 12.37° Right: 12.01° Average: 12.19°

Table 4.1: Images of deionized water droplet containing PS ($3 \mu\text{m}$) particles before and after normal evaporation along with the side view immediately after dropping including the values of the contact angles under normal evaporation conditions.

4.3.1.2 Motility Buffer Droplets

Unlike the standard coffee ring obtained from the previous experiments, depinning at the edge of the droplet was observed due to the change in the media of suspension (deionized water replaced with motility buffer) as shown in Table 4.2.

The contact angles were measured from the recorded image of the side view of the droplet immediately after dropping were nearly same with the average values of 20.82° and 20.21° with the average value of 20.52° and $\sigma=0.43$. The evaporation time of these droplets was approximately 4 minutes which was longer than the water sample due to increase in the viscosity caused by the change in the medium.

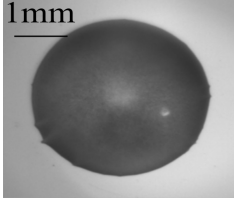
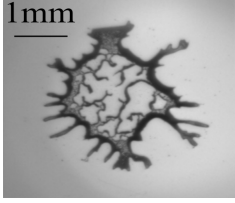
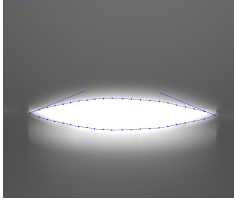
Full View of the Droplet		Side View of the Droplet	Contact Angle Values
Before Evaporation	After Evaporation		
			Left:20.82° Right:20.21° Average:20.52°

Table 4.2: Images of the motility buffer droplet containing PS ($3\ \mu\text{m}$) particles before and after normal evaporation with the side view immediately after dropping including the values of the contact angles under normal evaporation conditions.

4.3.2 Droplets Containing Brownian Particles along with Bacteria

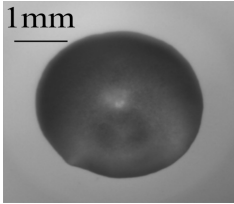
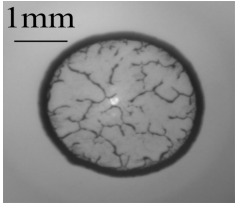
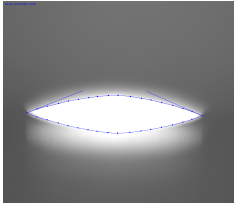
Full View of the Droplet		Side View of the Droplet	Contact Angle Values
Before Evaporation	After Evaporation		
			Left:20.79° Right:20.95° Average:20.87°

Table 4.3: Images of the motility buffer droplet containing cultured *E. coli* bacteria and PS ($3\ \mu\text{m}$) particles before and after normal evaporation with the side view immediately after dropping including the values of the contact angles under normal evaporation conditions.

The addition of the motile bacteria also changed the pattern after evaporation. The particles along with some of the bacteria started to pin at the edge of the droplet again as shown in Table 4.3.

The contact angles were measured as 20.79° and 20.95° and the average contact angle was calculated as 20.87° with $\sigma=0.11$ which were in consistent with the data obtained before from the motility buffer samples as they had the similar medium of suspension.

4.3.3 Comparison of the Results and Discussion

Due to the glass slide being hydrophilic after the NaOH treatment, the water droplets started to spread to the solid surface more easily and the shapes of the droplets were not as ideal as the other ones obtained with motility buffer. After the contact line was pinned, the particles moved towards the edge due to the capillary flow. Therefore, when the evaporation was completed, a concentrated ring (i.e. a full coffee ring) consisting of monodisperse polystyrene particles at the periphery of the water droplet where they were pinned was observed.

However, the presence of the surfactant (0.002 % of Tween 20) in the motility buffer induced Marangoni flow inside the droplet and the polystyrene particles started to slip backwards towards the center instead of pinning at the edge after being dropped [57]. The capillary flow which was forcing the particles to go outward towards the edge of the droplet was partially counteracted by the reverse surfactant induced Marangoni flow which was forcing particles to go inward towards the central area of the droplet. That's why, some particles were prevented from reaching the contact line by this flow and stayed at the central area of the droplet forming branch-like patterns inside the droplets. The difference in the pattern was shown in Table 4.4 in detail.

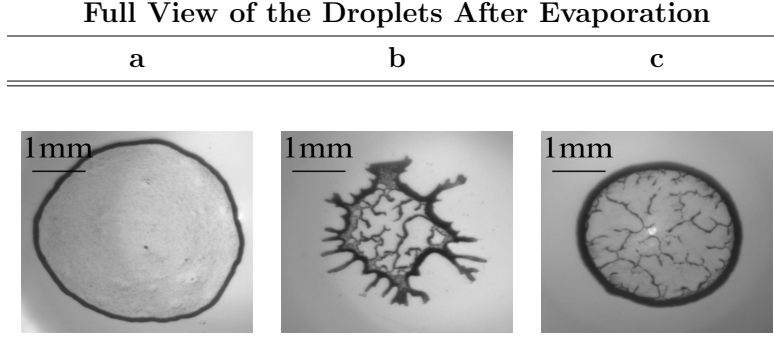
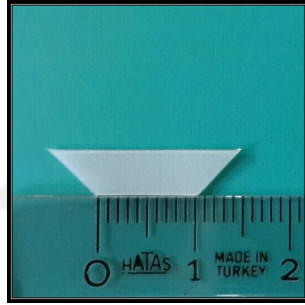


Table 4.4: Comparison of the results under normal evaporation conditions: (a) Polystyrene particles in the water droplets were carried by the capillary flow and accumulated at the edge of the droplet forming a full coffee-ring. (b) The presence of the surfactant (0.002 % of Tween 20) generated Marangoni flows causing the motility buffer droplet to depin and the particles to go towards the central area of the droplet. (c) Addition of motile bacteria further changed the pattern by causing the droplet to pin.

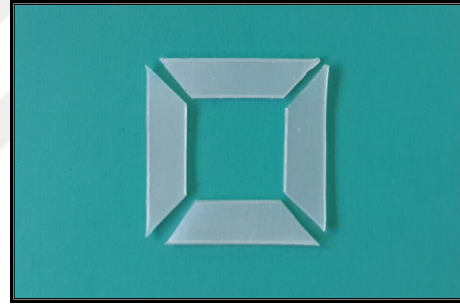
We expected to see some differences when we added motile *E.coli* bacteria in the system due the active nature of bacteria as they exhibit much more different motility behaviours than passive particles. Although they further changed the pattern by causing the particles to pin at the edge of the droplet, we couldn't observe any significant difference due to the main factor that we were focusing on, the motility of the bacteria. That's why, in order to see the real effect of the motility of the bacteria, we decided to slow down the evaporation hoping for the bacteria to be more effective on the passive particles and change the regular ring pattern.

4.4 Results under Controlled Evaporation Conditions

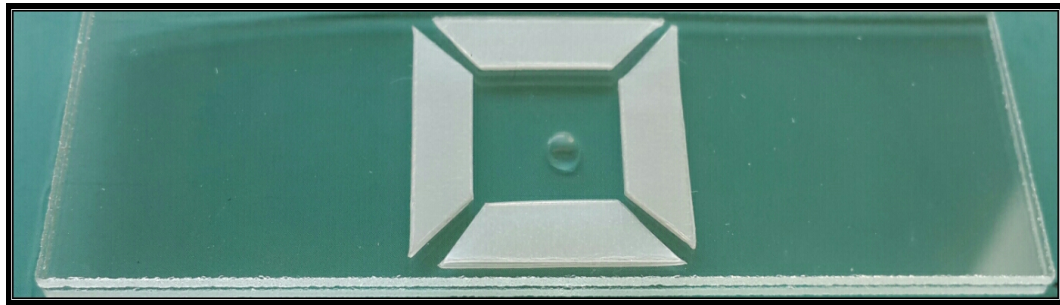
With a view to give a chance to bacteria to be more effective on the polystyrene particles present in the droplets with their distinctive ability to self-propel, we decided to repeat the experiments under controlled evaporation conditions by also considering the fact the organisms such as bacteria prefer to live in moist habitats [81]. Our aim was to decrease the evaporation rate by increasing the evaporation time and weaken the capillary flow and providing a better environment to bacteria so that we could see the effect of motility of them.



(a) Wall of the parafilm cage



(b) Complete parafilm cage



(c) Ready to use parafilm cage

Figure 4.5: Steps for building the parafilm cage for controlling the evaporation.

In order to slow down the evaporation, we put an additional glass slide on top of the first glass slide at which the colloidal solution was dropped with a confined area of approximately 1 cm x 1 cm built with parafilm as shown in Figure 4.5 in such a way that the vertical distance between the glass slides was nearly 400 μm . The walls of the cage were positioned with some space between them to control the air flow. By doing so, the duration of the evaporation was extended up to 30 minutes (nearly 4 to 5 times longer than normal evaporation condition) with this so called cage.

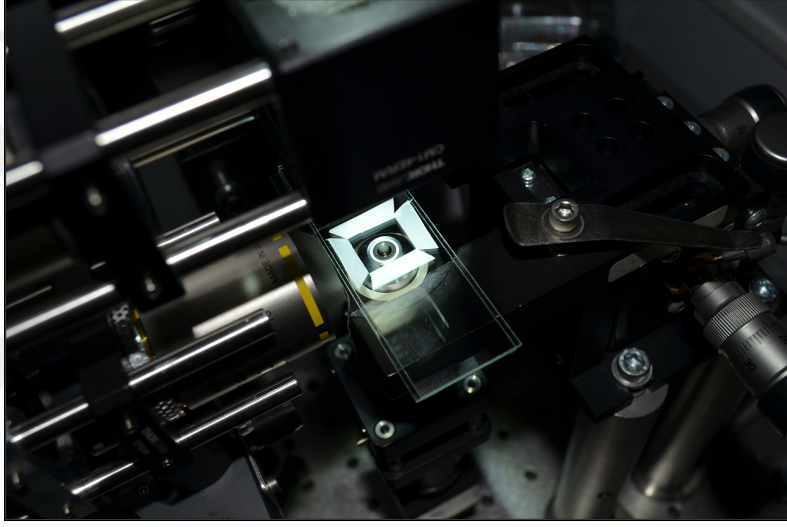


Figure 4.6: Confined droplet ready for controlled evaporation.

4.4.1 Droplets Containing only Brownian Particles

The same steps and recipes for preparing the samples with PS particles in deionized water and motility buffer were followed. The contact angles were measured by using the corresponding side view of the droplets which were recorded before placing the parafilm cage on top right after the dropping.

4.4.1.1 Water Droplets

Regular ring-like structure were obtained after slow evaporation as shown in Table 4.5. The contact angles for the left and right side of the droplet were measured as 12.71° and 12.38° with the average value of 12.55° ($\sigma=0.23$) from the snapshot of the side view of the droplet showing consistence with the previous experiments for water samples.

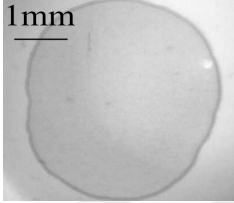
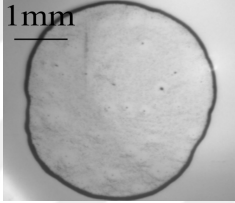
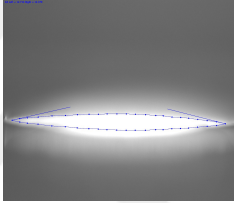
Full View of the Droplet		Side View of the Droplet	Contact Angle Values
Before Evaporation	After Evaporation		
			Left: 12.71° Right: 12.38° Average: 12.55°

Table 4.5: Images of deionized water droplet containing PS ($3\text{ }\mu\text{m}$) particles before and after slow evaporation with the side view immediately after dropping including the values of the contact angles under controlled evaporation conditions.

4.4.1.2 Motility Buffer Droplets

As we used the same motility buffer which had surfactants in it for controlled evaporation experiments as well, we observed the same behaviour in these droplets. The particles started to slip backwards instead of pinning at the edge and formed the branch-like patterns again.

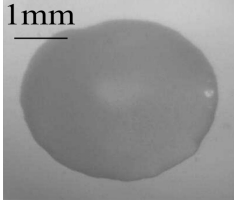
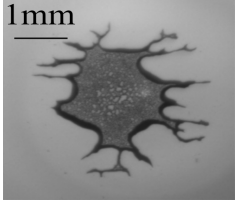
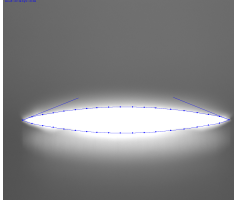
Full View of the Droplet		Side View of the Droplet	Contact Angle Values
Before Evaporation	After Evaporation		
			Left:20.14° Right:20.38° Average:20.26°

Table 4.6: Images of the motility buffer droplet containing PS ($3\text{ }\mu\text{m}$) particles before and after slow evaporation with the side view immediately after dropping including the values of the contact angles under controlled evaporation conditions.

The contact angles for the left and right side of the droplet were measured as 20.14° and 20.38° with the average value of 20.26° ($\sigma=0.16$ which were consistent with the previous results obtained under normal evaporation conditions.

4.4.2 Droplets Containing Brownian Particles along with Bacteria

As can be seen in Table 4.7, the motility of the bacteria has been proven to be effective on the pattern formation.

The contact angles for the left and right side of the droplet were measured as 20.35° and 20.39° with the average value of 20.37° ($\sigma=0.02$) which were also consistent with the experiments for normal evaporation.

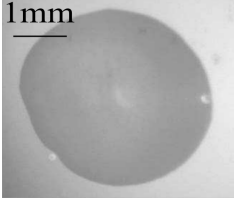
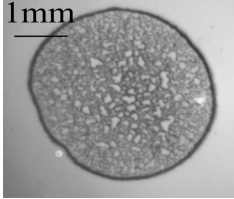
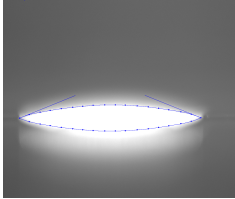
Full View of the Droplet		Side View of the Droplet	Contact Angle Values
Before Evaporation	After Evaporation		
			Left:20.35° Right:20.39° Average:20.37°

Table 4.7: Images of the motility buffer droplet containing cultured *E.coli* bacteria and PS ($3\ \mu\text{m}$) particles before and after slow evaporation with the side view immediately after dropping including the values of the contact angles under controlled evaporation conditions.

4.4.3 Comparison of the Results

As there was no factor to be affected by the evaporation rate like the presence of bacteria, slowing down the evaporation process did not change the pattern and a regular coffee-ring was obtained after evaporation except the fact that capillary flow becoming lighter and causing less particles to accumulate at the edge.

Slowing down the evaporation caused the capillary flow generated inside the droplet to weaken. As the flow weakened, less particles were carried towards the edge of the droplet during evaporation. Therefore, the coffee ring that was obtained after the slow evaporation was much thinner than the one obtained after normal evaporation because less particles accumulated at the edge after slow evaporation due to the weakened flow. This comparison can be seen in the magnified images of the edge of the water droplets containing PS particles in Figure 4.8.

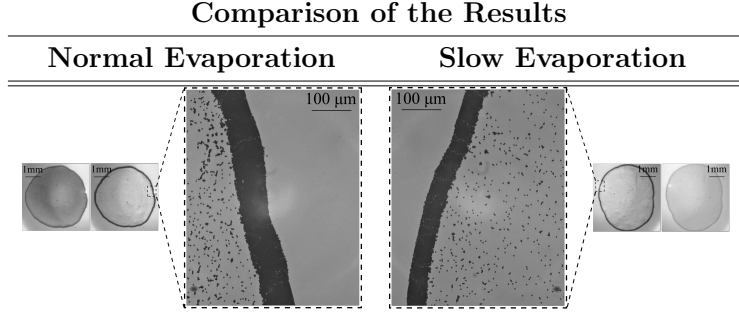


Table 4.8: Comparison of the results obtained with the PS particles in water droplets after both normal and controlled evaporation conditions with the corresponding magnified images of certain portion of their edges showing the difference in the thickness of the coffee rings caused by the increase in the evaporation time.

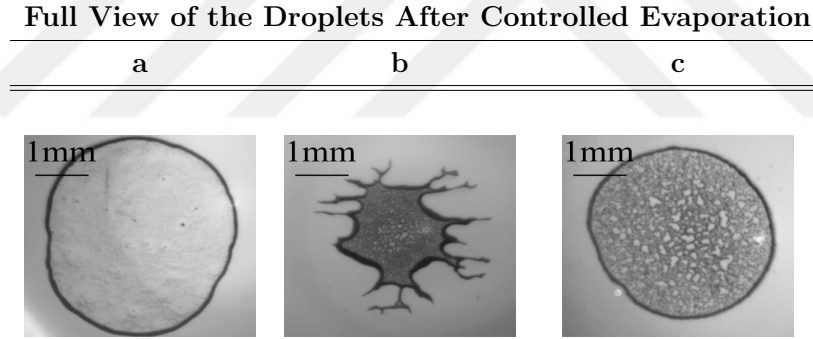


Table 4.9: Comparison of the results under controlled evaporation conditions. (a) Regular ring-like structure (thinner) were reproduced from the water droplets containing polystyrene particles. (b) The presence of the surfactant (0.002 % of Tween 20) generated Marangoni flows causing the motility buffer droplet to depin and the particles to slip towards the central area of the droplet. The central area of the droplet was denser due to the increase in the evaporation time. (c) Addition of motile bacteria not only caused the particles to pin at the edge of the droplet, but also further changed the pattern and formed a disk-like deposition instead of a ring.

As we used the same motility buffer which had surfactants in it for the slow evaporation experiments as well, we observed the same behaviour in the droplets. The particles started to depin and when the evaporation was completed after approximately half an hour later, the particles formed the same branch-like patterns except the difference that the central area of the droplet was denser from the ones which were obtained under normal evaporation conditions due to the increase of the evaporation time and the decrease in the evaporation rate.

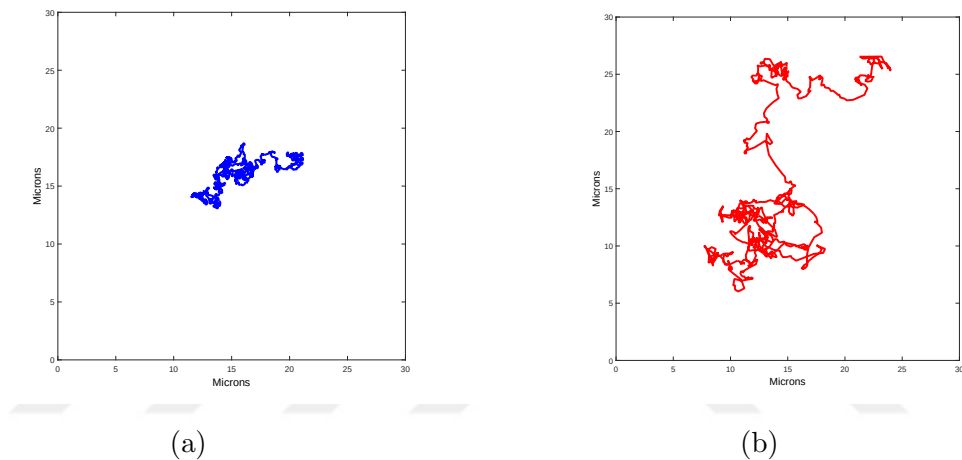


Figure 4.7: Experimentally obtained trajectories of a PS ($3.00\ \mu\text{m}$) particle suspended in (a) water, (b) active bath consisting of motile *E. coli* bacteria. The particles follow different trajectories with and without the influence of motion of the bacteria. While they can undergo Brownian motion when they are alone, they become active in the presence of motile bacteria.

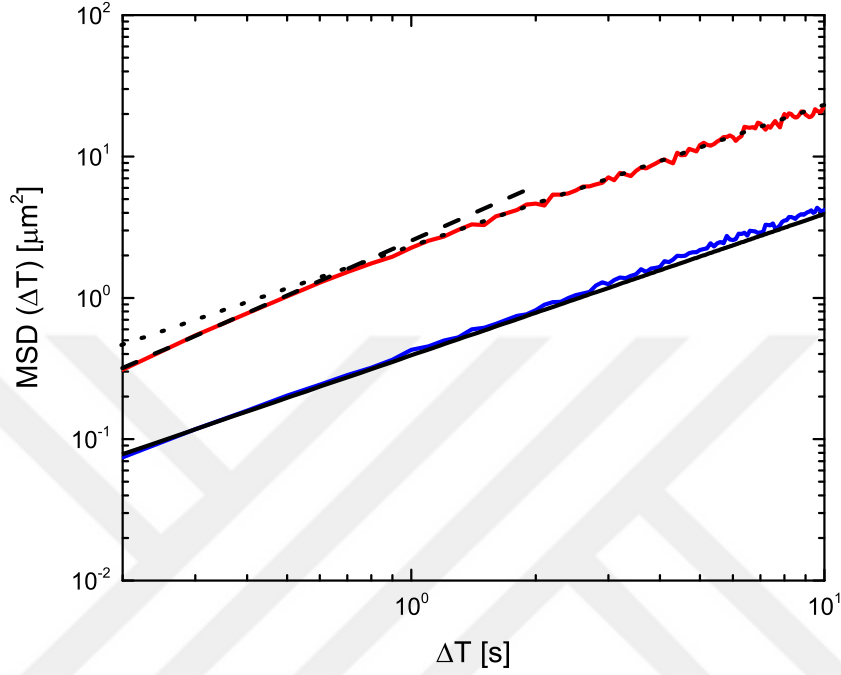


Figure 4.8: Comparison of the Mean Square Distances (MSD) of a PS ($3.00 \mu\text{m}$) particle suspended in water (blue line) and in active bath consisting of motile *E. coli* bacteria (red line). While the slope of the MSD of the PS particles in active bath differs from the case without bacteria exhibiting super diffusivity at short time scales (slope >1) and enhanced Brownian motion later, PS particles exhibit Brownian motion alone (slope=1) [82].

When considering the fact that motile bacteria cause the passive particle to become active, as can be understood from the experimentally obtained trajectories shown in Figure 4.7, we have decided to slow down the evaporation to provide more time to bacteria for showing their effect on the PS particles. Unlike the data obtained under normal evaporation conditions, bacteria were proven to be more effective when

we increased the interaction time between the bacteria and particles.

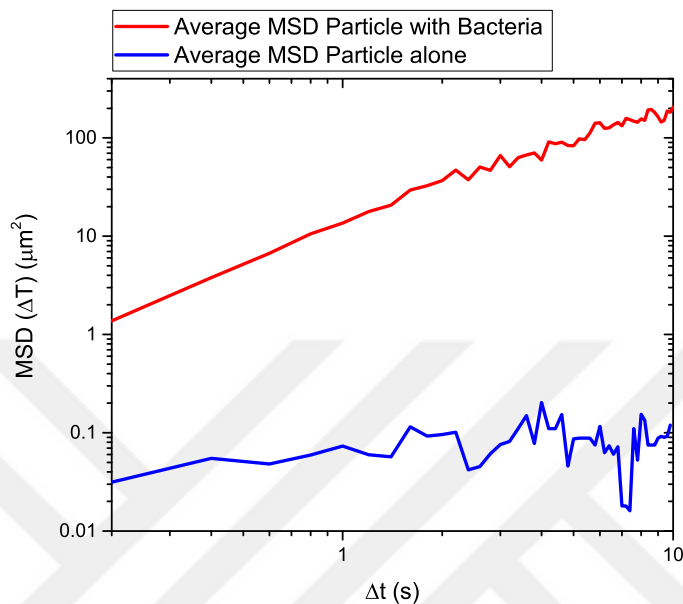


Figure 4.9: MSDs of the PS particles after they reached to the droplet boundary with and without the influence of the bacteria. Although the particles got stuck after reaching the edge when they were alone, they became active thanks to the addition of bacteria and kept moving even at the border.

Calculated MSDs of the polystyrene particles after they reached the droplet boundary both with and without the influence of the bacteria confirmed that the presence of the bacteria changed the motion of the passive particles. The particles got stuck at the edge when they were alone. However, when the bacteria were present, the particles which were passive in the beginning became active and kept moving even after reaching the boundary as shown in Figure 4.8. Thus, they moved not only under the influence of capillary flows, but also under the effect of the motion due to the presence of the bacteria. As the evaporation slowed down, the moving bacteria caused some of the particles to overcome the capillary flow that was trying

to lead them to the edge. Consequently, the particles were distributed also through the central area of the drop. Hence, the regular coffee ring pattern was altered.

4.5 Conclusion

Slowing down the evaporation rate was a good opportunity for the bacteria to show their capability as active swimmers on passive particles. However, we didn't observe much of a difference under normal evaporation conditions due to the limited time of interaction between bacteria and PS particles. As we didn't give enough time to bacteria for being effective on the PS particles, they were also carried towards the edge of the droplet along with them forming not much of a different pattern. By taking the control of the evaporation process, the strength of the flow was decreased and the motility of the bacteria became more important. They not only became effective on PS particles by turning them into active particles, but also changed the pattern formation. The particles which became active due to the presence of the bacteria were distributed over the whole droplet during the evaporation process. In the end, a coffee-disk rather than a regular ring was obtained. In the light of these data obtained, we came to the conclusion that the presence of active particles in the droplets have the ability to effect the motion of the passive particles present in the droplets and they can alter the pattern formation after slow evaporation.

Chapter 5

Self-Assembled Patterns in Evaporating Saline Droplets containing Brownian Particles and Bacteria

5.1 Introduction

In the light of the fact that additives and the change in the interactions between the dispersed particles caused by additives have a potential to tune the patterns in the droplets, we decided to spice the evaporation process up by using common salt (NaCl) and see what happens. Further in this chapter, we experimentally report the salt crystal structures that we obtain after the evaporation of saline droplets containing simple Brownian particles and bacteria alone using digital video microscopy. By comparing the crystals obtained from saline droplets with Brownian particles/bacteria

and without Brownian particles/bacteria, we show that the activity of these microorganisms have an influence on salt crystallization. In particular, we demonstrate that salt crystals showed dendritic patterns when alive bacteria are present in the evaporating droplet and no such patterns but bulky crystals are observed when alive bacteria are replaced with the inactivated ones. Our results suggest a simpler, faster and cheaper method in which common salt can be used as a biomarker to detect bacterial activity.

5.2 Materials and Methods

5.2.1 Experimental Setup

The same homemade inverted microscope was used for this chapter except the fact that the camera recording the magnified portion of the droplet was replaced with a new CMOS camera and the distance between the 20x objective and new CMOS camera was increased to have better resolution.

5.2.2 Cleaning Procedure for the Glass Slides

Cut edges 76 x 26 x 1 mm microscope slides (Marienfeld, Germany) were used throughout the experiments in this chapter and all were treated with the same cleaning procedure to obtain the same surface quality for each slide. The impurities and contamination on the surface were eliminated by wiping from one edge of the slide to the other with a folded lens cleaning tissue dipped in acetone and isopropyl alcohol respectively. After rinsing them with deionized water, glass slides were blow-dried with nitrogen (N_2) and became ready to be used.

5.2.3 Sample Preparation with/without Brownian Particles

Saline Droplets of Deionized Water: 26.25 μl of monodisperse spherical polystyrene particles (Microparticles GmbH, Berlin, diameter $d = 3.00 \pm 0.07 \mu\text{m}$, volume fraction $\phi = 0.095$) were suspended in 0.5/0.4/0.3/0.2/0.1/0.0 M of saline solutions (volume fraction $\phi = 0.002$).

Saline Droplets of Motility Buffer: 26.25 μl of monodisperse spherical polystyrene particles (Microparticles GmbH, Berlin, diameter $d = 3.00 \pm 0.07 \mu\text{m}$, volume fraction $\phi = 0.095$) were suspended in 0.5/0.4/0.3/0.2/0.1/0.0 M of saline solutions in motility buffer with same volume fraction (see Section 4.2.3 for the reason).

5.2.4 Experimental Procedure

0.60 $\pm$ 0.06 μl of each colloidal solution were dropped on clean glass slides by using a micro-pipette for evaporation. All the precautions regarding not causing deformations in the droplet profile, homogeneity of the solutions, uniformity of the concentrations and the constancy in the volume of the drops were taken into consideration here as well. The temperature of the laboratory was $19 \pm 1 \text{ }^\circ\text{C}$ with a relative humidity of % 40 ± 5 while performing the experiments.

5.3 Experimental Results

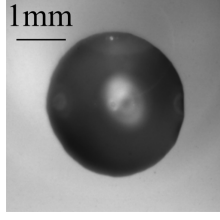
5.3.1 Saline Droplets with and without Brownian Particles

As already explained before, in the absence of salt, regular coffee-rings were obtained with particles as shown in Figure 5.1.

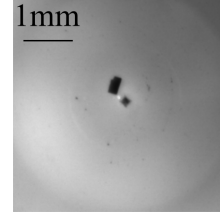


Figure 5.1: Images of water droplets containing polystyrene particles both before and after evaporation. Regular ring-like structures are obtained in the absence of salt (see Section 3.2.1)

However, the saline droplets leave different stains. Unlike the water droplets containing colloidal particles, saline droplets depinned during evaporation and formed no rings. The solvent loss due to evaporation caused salt saturation to increase. At some point, when the droplets reached a supersaturation level, salt present in the evaporating droplets started to crystallize. An example of this progress is shown in Figure 5.2 with the top views of 0.1 M of saline droplet before and after evaporation.



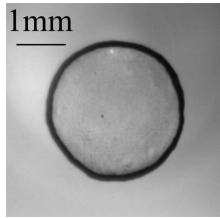
(a) Before evaporation



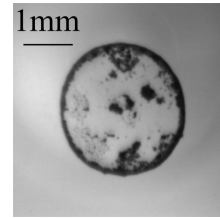
(b) After evaporation

Figure 5.2: Images of a saline droplet before and after evaporation. Dissolving salt results in crystallization due to the loss of solvent followed by supersaturation unlike the droplets containing suspended colloidal particles which forms regular ring-like structures.

In view of the fact that interactions between particles can change the pattern formation in evaporating droplets, we induced the evaporation process of the droplets containing polystyrene particles by adding common salt (i.e. NaCl). As a combination of both cases, we observed pinning of the particles and crystallization of salt with these droplets. In addition, the resultant pattern changed. While we obtained regular coffee-rings in the absence of salt, we observed clusters of particles in the central area of the droplet along with the accumulation at the edge as shown in Figure 5.3.



(a) In the absence of salt.



(b) In the presence of salt.

Figure 5.3: Images of (a) water droplet, (b) saline droplet containing polystyrene particles after evaporation. Addition of salt (0.1 M) changes the regular ring-like structure obtained after evaporation.

When the evaporation process was induced with salt, the polystyrene particles that are negative charge-stabilized colloids started to aggregate. The salt causes a decrease in the Debye-Hückel screening length which results in a decrease also in the repulsive barrier between the particles as they approach to each other [83, 84, 85]. When the barrier gets weak enough, they start to stick and form irregular clusters as shown in the experimentally obtained Figure 5.4. Some of these clusters formed during evaporation stayed in the central area of the droplet and determined the resulting pattern.

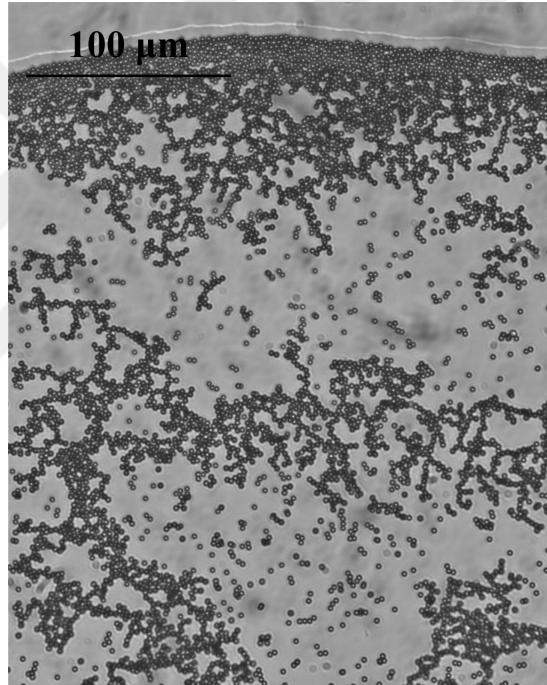


Figure 5.4: Salt (0.1 M) induced aggregation of polystyrene particles: Negatively charged particles start to aggregate due to the presence of salt and form clusters.

Starting from the least amount of salt (0.1 M), we gradually increased the salt concentration in water and buffer droplets (due to the same concern that was already pointed out in Section 4.2.3) up to 0.5 M and studied the patterns in order to compare

the case when there is no particles in the droplets and when there are particles. Although the droplets containing particles pinned at the contact line of the droplet (similar slipping behaviour of particles observed in buffer, check Section 4.3.3)), pure saline droplets depinned due to the absence of particles and increasing the salt concentration resulted in bigger crystals. By comparing these cases, we came to the conclusion that addition of salt changes the regular ring formation. Although we could still see a ring at the periphery of the droplet, we also saw clusters of particles in the central area of the droplet.



Saline Water Droplets with PS Particles

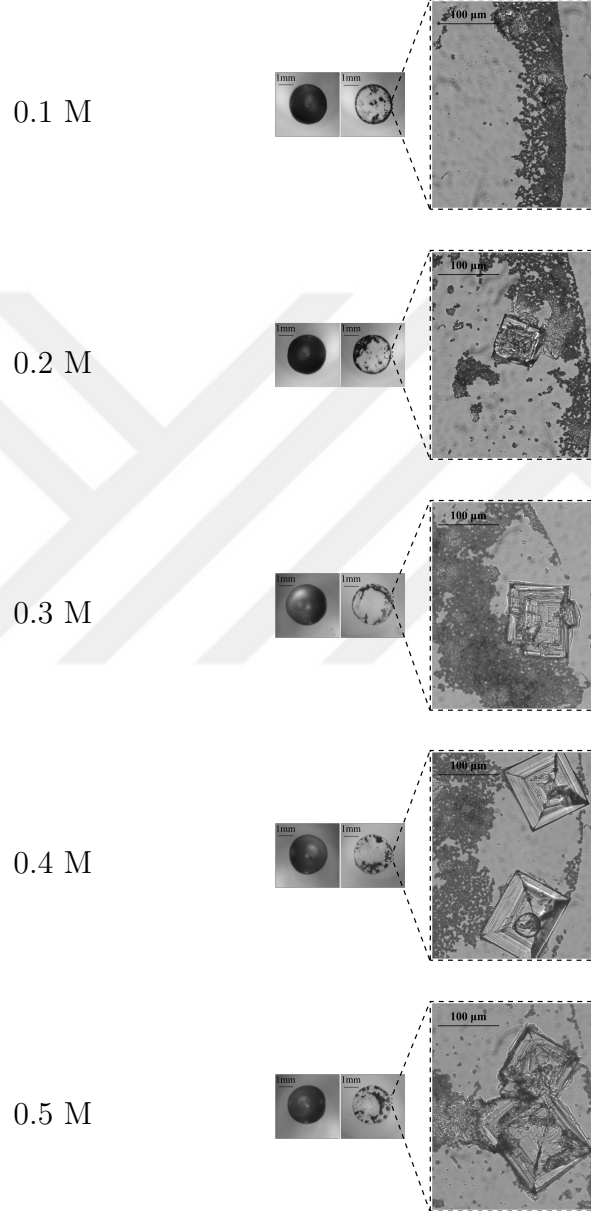


Table 5.1: Images of saline droplets of water with different salt concentrations containing PS particles before and after evaporation along with the magnified portion showing the salt crystals. Regular crystals are observed in the saline droplets containing PS particles.

Saline Water Droplets without Particles

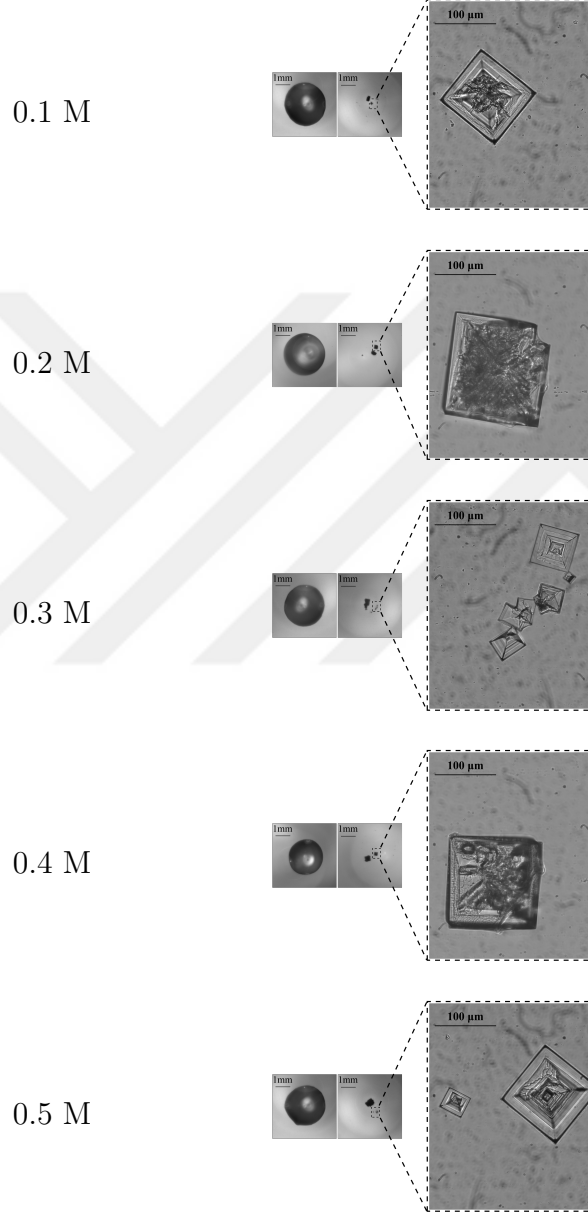


Table 5.2: Images of saline droplets of water with different salt concentrations containing PS particles before and after evaporation along with the magnified portion showing the salt crystals. Regular crystals are observed in the saline droplets.

Saline Buffer Droplets with PS Particles

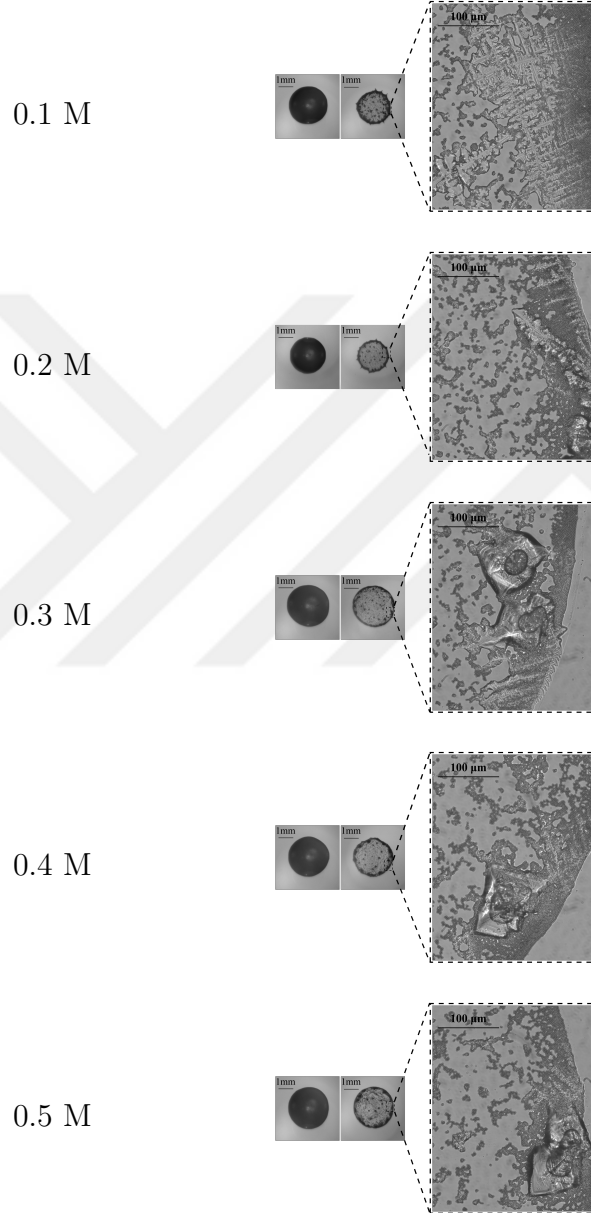


Table 5.3: Images of saline droplets of buffer with different salt concentrations before and after evaporation along with the magnified portion showing the salt crystals. The motility buffer changes the salt crystallization and causes dendritic crystals at low salt concentrations.

Saline Buffer Droplets without Particles

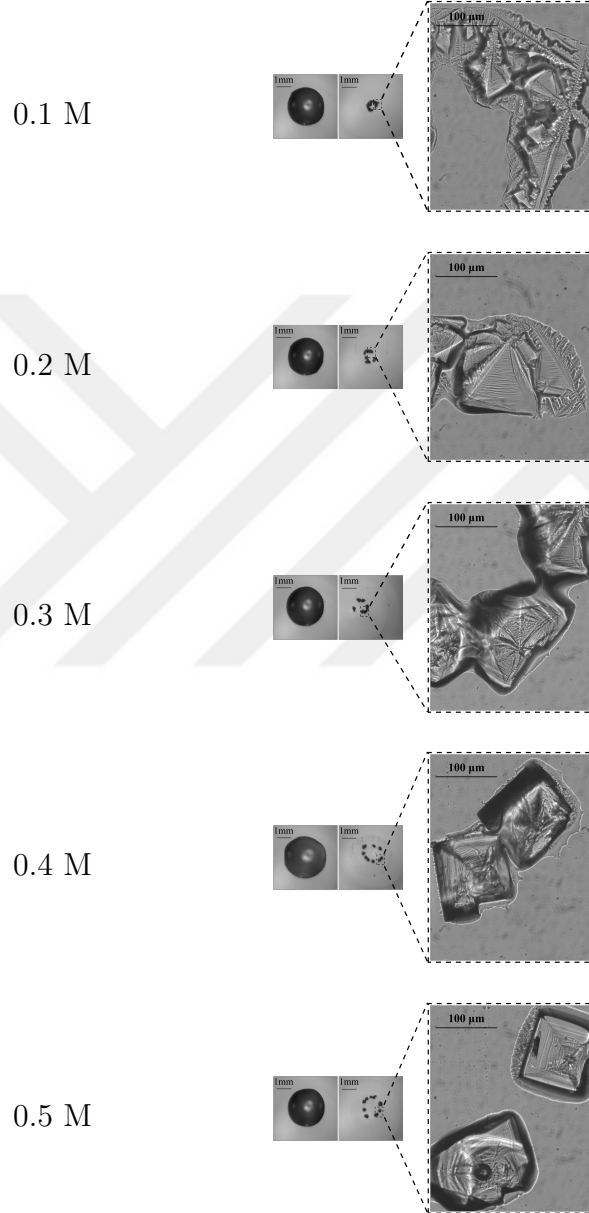


Table 5.4: Images of saline droplets of buffer with different concentrations before and after evaporation along with the magnified portion showing the salt crystals. The motility buffer changes the salt crystallization and causes dendritic crystals at low salt concentrations.

5.3.1.1 Comparison of the Results

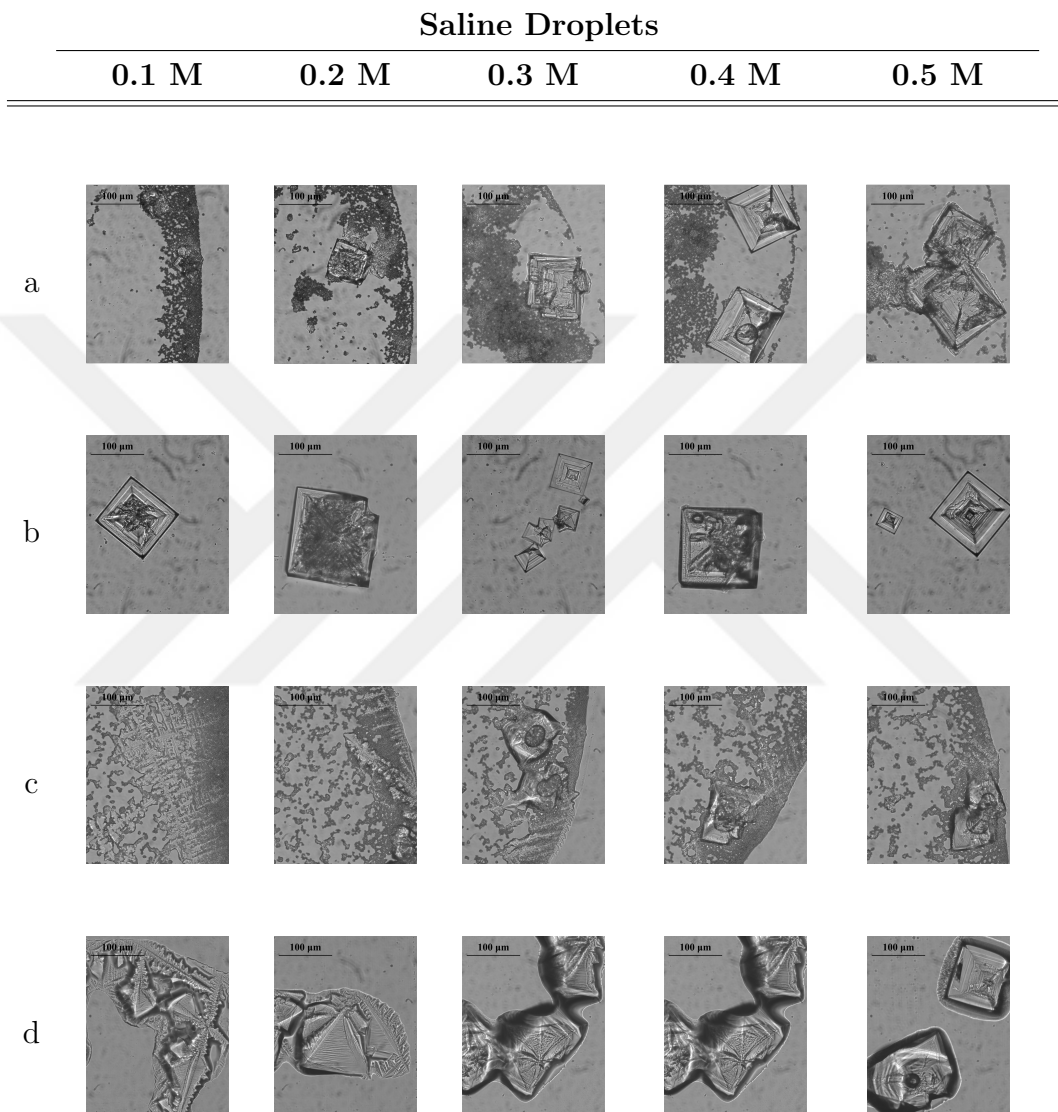


Table 5.5: Images of the crystals formed in saline water droplets (a) with PS particles, (b) without particles; saline buffer droplets (c) with PS particles, (d) without particles. Polystyrene particles had no significant effect on crystal formation, but the presence of motility buffer changes the crystallization.

The crystals formed in pure saline droplets were big and bulky as typical and comparing them with the ones obtained from the droplets containing particles helped us to deduce that the presence of polystyrene particles had no significant effect on crystal formation as shown in Figure 5.5.

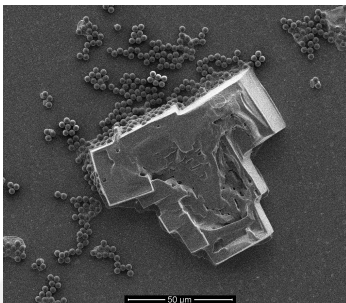


Figure 5.5: SEM image of a portion of a dried saline droplet containing PS particles. The salt present in the droplet starts to crystallize due to supersaturation caused by the solvent loss during evaporation. PS particles have no particular influence on salt crystallization.

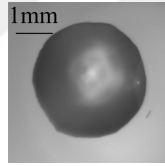
However, buffer droplets behaved differently. The growing crystals formed dendritic patterns rather than regular salt crystals due to the ingredients of the buffer and, increasing the salt concentration, reduced the dendrite formation. Interestingly, at the highest salt concentration with pure buffer droplets which is 0.5 M, grown crystals formed coffee-ring just like the case with the droplets containing particles.

5.3.2 Saline Droplets with Bacteria

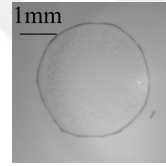
Following the same steps as the previous chapter, we wanted to see the effect of the motion of the particles on pattern formation and used bacteria instead of polystyrene particles in the droplets.

However, as we know that the motility buffer itself causes alteration in crystal formation, seeing the same dendritic pattern also with the bacteria couldn't help us to understand the actual effect of it on crystal growth. In order to eliminate the crystal alteration caused by the motility buffer itself, we decided to suspend the bacteria in water instead of buffer even though we were aware of the inconvenience of not providing a favorable environment for the bacteria.

Similar as before, salt crystals started to get bigger as the concentration increased. More dendrites were obtained at the lowest salt concentration (0.1 M). We observed that the motility of the bacteria decreased with the increasing salt concentration. By putting together the observations of decreasing motility and crystals forming less dendrites at high salt concentrations, we had a clue of motility altering salt crystals. Thus, we searched for a way to get rid of the motility ability of the bacteria. Using sodium azide offered an opportunity by inhibiting the activity of the bacteria [86].



(a) Before evaporation



(b) After evaporation

Figure 5.6: Images of a water droplet containing non-motile *E. coli* bacteria with inhibited activity before and after evaporation. Regular ring-like structures are obtained with the non-motile *E. coli* bacteria with inhibited activity. Bacteria which lose the ability to move become passive and accumulate at the contact line of the droplet just like the case with polystyrene particles (see Figure 5.2) and form rings in the absence of salt.

No crystal formation was observed in the absence of salt with the droplets containing bacteria in general. Particularly, the non-motile bacteria formed regular ring-like structures same as the case with polystyrene particles before as shown in Figure 5.6.

Saline Droplets with Bacteria

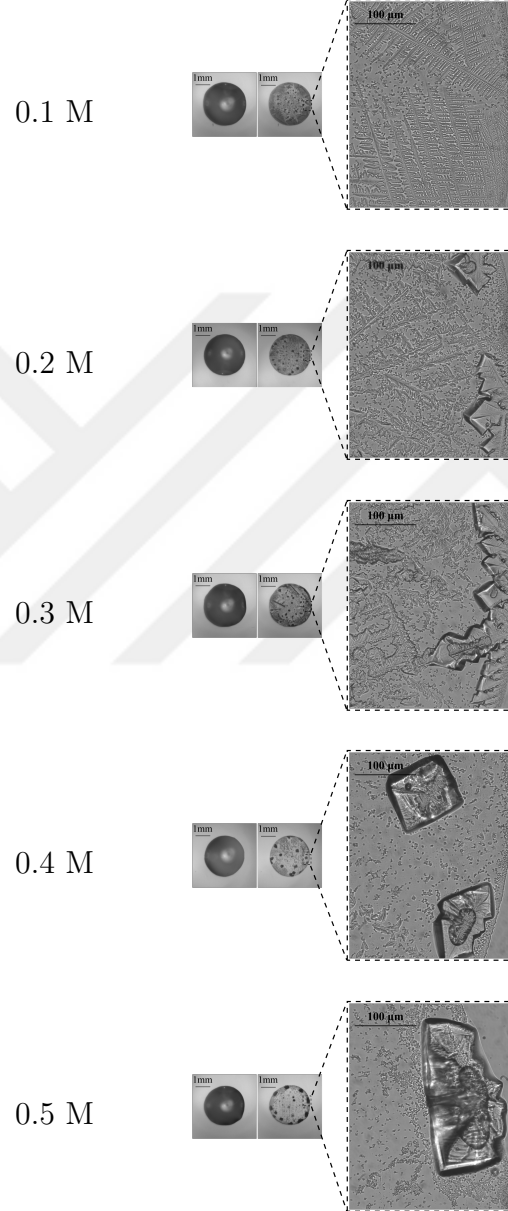


Table 5.6: Images of saline droplets different concentrations containing **freshly cultured motile *E. coli* bacteria** suspended in motility buffer before and after evaporation along with the magnified portion showing the salt crystals. Bacteria suspended in motility buffer changes the salt crystallization.

Saline Droplets with Bacteria

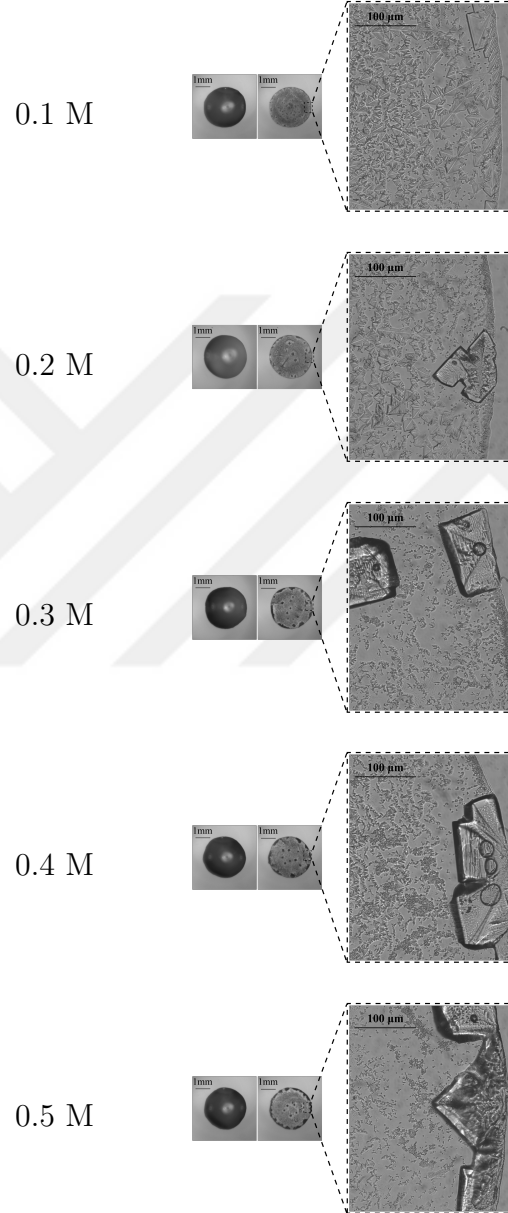


Table 5.7: Images of saline droplets different concentrations containing **freshly cultured motile *E. coli* bacteria** suspended in water before and after evaporation along with the magnified portion showing the salt crystals. The presence of the bacteria alters the crystal formation and causes dendritic patterns.

Bacteria suspended in water also gave the similar results. The salt crystals were altered by the bacteria in a dendritic way and dendrite formation reduced with the increasing salt concentration.

As 0.1 M was the concentration at which we observed the most alteration and it provided a relatively convenient environment for bacteria, we started to study with 0.1 M of salt for the rest of the experiments.

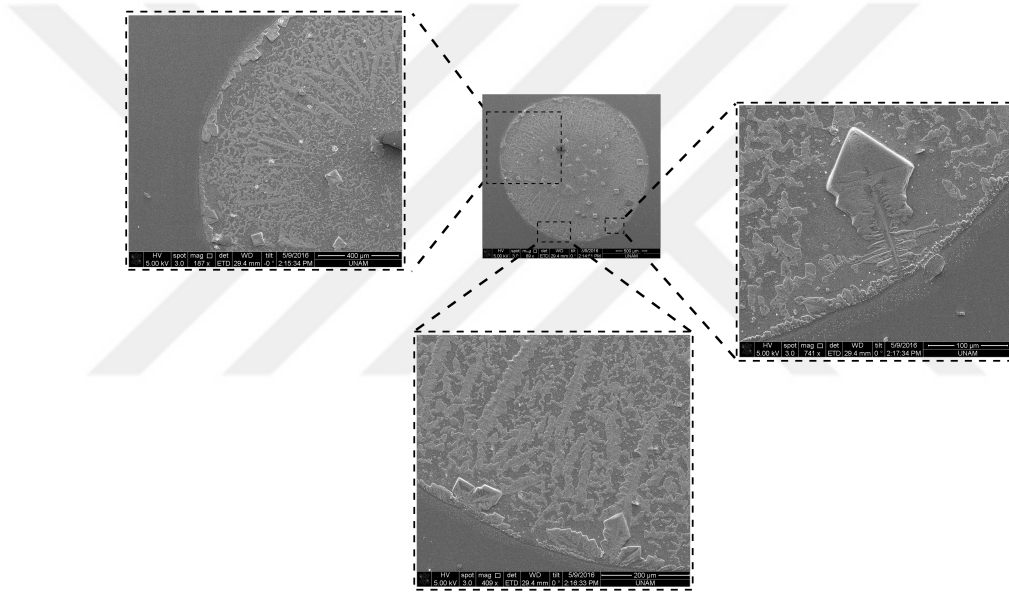


Figure 5.7: SEM images of a dried saline (0.1 M) droplet containing **freshly cultured motile *E. coli* bacteria** suspended in water. The presence of the bacteria alters the crystal formation and causes dendritic patterns.

The bacteria which became non-motile after the sodium azide treatment could not alter the crystal formation and regular crystals were obtained after the evaporation.

Saline Droplets with Bacteria

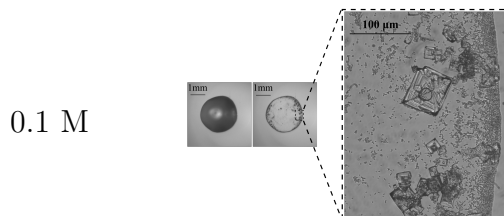


Table 5.8: Images of 0.1 M saline droplets containing **sodium azide treated *E. coli* bacteria** suspended in water before and after evaporation along with the magnified portion showing the salt crystals. When the activity and motility of the bacteria are annihilated with sodium azide, they start to behave just like passive particles and no longer be able to alter the formation.

5.4 Comparison of the Results and Discussion

During the study of saline droplets, we examined the crystals obtained after the evaporation. We started the experiments by using PS particles in the salt induced droplets. Although we observed a difference in the final pattern formation as we changed the regular ring-like structure, observing the similar salt crystals with and without PS particles in the droplets helped us to conclude that PS particles did not have a significant effect on crystal formation. Moreover, when we replaced the PS particles with freshly cultured motile *E. coli* bacteria by taking account of motility being an effective factor on pattern formation, we noticed quite a difference. The living and moving bacteria joined the salt crystallization and formed different self-assembled patterns. They altered the crystallization and form dendrites instead of regular crystals. Observing differences in motility of the bacteria and dendrites with different salt concentrations led us to the motility as a factor effective on also the

crystallization.

Thus, motility of the bacteria became the first factor that we wanted to check whether it was the main effect on this alteration. Then, we used sodium azide to inhibit the motility of the bacteria. Interestingly, the bacteria which became non-motile after the treatment could no longer alter the crystallization and regular crystals (like in the case with and without PS particles) were obtained after evaporation confirming our guess. However, we were ignoring the fact that we were also inhibiting the activity of the bacteria while using sodium azide, not just motility. We will confirm the real explanation lying under the salt crystallization alteration by genetically modifying these bacteria in such a way that they will still keep their activity but lose their motility. The experiments which will be conducted with these genetically modified non-motile bacteria will help us understand whether the motility or the activity is the main effective reason on the alteration.

Saline Droplets of Water

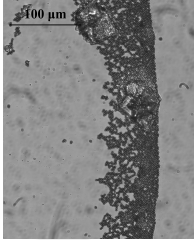
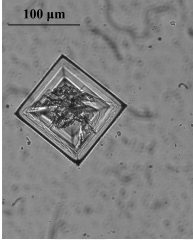
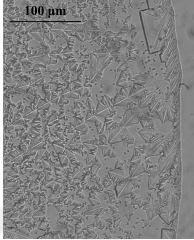
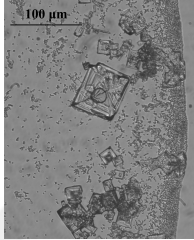
	a	b	c	d
0.1 M				

Table 5.9: Images of the crystals formed in 0.1 M of saline water droplets containing (a) PS particles, (b) no particles, (c) freshly cultured motile *E. coli* bacteria, (d) sodium azide treated *E. coli* bacteria. The presence of motile *E. coli* bacteria alters the salt crystals, causes dendritic patterns rather than regular crystals obtained with passive (non-living) particles. When the activity and motility of the bacteria are annihilated with sodium azide, they start to behave just like passive particles and no longer be able to alter the formation.

Chapter 6

Conclusion

Although they are one of the oldest forms of life on Earth, bacteria were ignored for so long because of their teeny-tiny size that prevents us from seeing them with the naked eye. Once again, science proved the ones that thought the importance of a thing lies on its magnitude and held the promise of fascinating us at all scales. The lack of understanding caused by this ignorance started to vanish day after day and the relationship between human and these microscopic beings has improved gradually [87].

Now, we all welcome the bacteria of which helpful hand we feel in the dairy products [88, 89], in our body [90] and even in medicine [91], but unfortunately they are not about to sprout wings. They may fool us with their simple cell structure, but considering the problems that they cause, they have a huge potential of damaging public health and environmental safety [92, 93, 94]. Even though we cannot see them with the naked eye, we know that they are present in almost every habitat which increases the probability of causing discomfort, disease and even death. So, much as we can not survive without some, we also cannot survive with some!

The fear of the invisible, the potential danger and the challenge of differentiating good and bad [95, 96] led people choose the easy path and focus more on getting rid of them by killing with the antibacterial products rather than understanding whether they are really active and pose any threat or not.

In order to find an answer for this fascinating problem and detect bacterial activity, here we propose an alternative method based on evaporation driven self-assembly for deciding on whether the bacteria present in the droplets are active or not by just comparing the crystal structures that we obtained after inducing the evaporation process with common salt. Our method will provide a simpler, cheaper and faster alternative for detection of bacterial activity by using common salt as a biomarker.

By taking advantage of this self-assembly process, we found a way that will help us to deduce the matter of bacterial activity. Like many other researchers from different disciplines, we were also attracted to transform this useful concept into an effective tool. Starting with one of the simplest ways of self-assembly, evaporation driven self-assembly, we spiced the process up and focused on the self-assembled patterns obtained after the salt induced evaporation of droplets containing suspended particles. The fact that bacteria have the potential to tune the self-assembled patterns with their distinctive ability of self propelling opened a new door in our research.

Comparison of the salt crystals obtained from pure saline droplet and saline droplet containing isotropic passive polystyrene particles with the ones obtained from the saline droplet containing living bacteria showed incontrovertible differences proving that the presence of the living bacteria have the ability to alter the salt crystallization.

First factor that should be checked was the motility of the bacteria based on the results obtained with different salt concentrations in Chapter 5 and also the previous results obtained in Chapter 4. So, we decided to find a way to get rid of the motility

of the bacteria by using sodium azide. We repeated the experiments with these non-motile, also inactive, bacteria and observed quite a decrease in the alteration of salt crystals. Unfortunately, underestimating these little creatures and ignoring that their activity was also inhibited along with the motility misguided us to the conclusion of motility being the real effect on the alteration. Putting together our results and studies conducted by Gómez [97], we propose the activity of the bacteria as the main reason for the alteration.

Hereafter to confirm our hypothesis, we will try to knock out the gene of the bacteria that is responsible for the motility and genetically modify them for our purposes. We will repeat all the experiments by also using this mutant bacteria which will be active but non-motile. Based on the results that will be obtained with these bacteria will help us to deduce the real factor effective on the alteration.

Bibliography

- [1] J. A. Pelesko, *Self assembly: the science of things that put themselves together*. CRC Press, 2007.
- [2] G. M. Whitesides and B. Grzybowski, “Self-assembly at all scales,” *Science*, vol. 295, no. 5564, pp. 2418–2421, 2002.
- [3] G. M. Whitesides and M. Boncheva, “Beyond molecules: Self-assembly of mesoscopic and macroscopic components,” *Proceedings of the National Academy of Sciences*, vol. 99, no. 8, pp. 4769–4774, 2002.
- [4] R. Bhardwaj, X. Fang, P. Somasundaran, and D. Attinger, “Self-assembly of colloidal particles from evaporating droplets: role of dlvo interactions and proposition of a phase diagram,” *Langmuir*, vol. 26, no. 11, pp. 7833–7842, 2010.
- [5] R. D. Deegan, O. Bakajin, T. F. Dupont, G. Huber, S. R. Nagel, and T. A. Witten, “Capillary flow as the cause of ring stains from dried liquid drops,” *Nature*, vol. 389, no. 6653, pp. 827–829, 1997.
- [6] G. Volpe, S. Gigan, and G. Volpe, “Simulation of the active brownian motion of a microswimmer,” *American Journal of Physics*, vol. 82, no. 7, pp. 659–664, 2014.
- [7] V. Saranathan, C. O. Osuji, S. G. Mochrie, H. Noh, S. Narayanan, A. Sandy, E. R. Dufresne, and R. O. Prum, “Structure, function, and self-assembly of

- single network gyroid (i4132) photonic crystals in butterfly wing scales,” *Proceedings of the National Academy of Sciences*, vol. 107, no. 26, pp. 11676–11681, 2010.
- [8] K. Libbrecht, “The snowflake designer,” *Nature*, vol. 480, pp. 453–454, 2011.
- [9] K. Sefiane, “Patterns from drying drops,” *Advances in colloid and interface science*, vol. 206, pp. 372–381, 2014.
- [10] J. Park and J. Moon, “Control of colloidal particle deposit patterns within picoliter droplets ejected by ink-jet printing,” *Langmuir*, vol. 22, no. 8, pp. 3506–3513, 2006.
- [11] J. Perelaer, P. J. Smith, C. E. Hendriks, A. M. van den Berg, and U. S. Schubert, “The preferential deposition of silica micro-particles at the boundary of inkjet printed droplets,” *Soft Matter*, vol. 4, no. 5, pp. 1072–1078, 2008.
- [12] D. Brutin, B. Sobac, B. Loquet, and J. Sampaol, “Pattern formation in drying drops of blood,” *Journal of fluid mechanics*, vol. 667, pp. 85–95, 2011.
- [13] T. A. Yakhno, V. G. Yakhno, A. G. Sanin, O. A. Sanina, A. S. Pelyushenko, N. A. Egorova, I. G. Terentiev, S. V. Smetanina, O. V. Korochkina, and E. V. Yashukova, “The informative-capacity phenomenon of drying drops,” *IEEE engineering in medicine and biology magazine*, vol. 24, no. 2, pp. 96–104, 2005.
- [14] http://math.mit.edu/~bush/?page_id=4841. Online: Accessed on June 15, 2016.
- [15] P. E. B. Averill, *General Chemistry: Principles, Patterns and Applications: Unique Properties of Liquids*. San Francisco: Benjamin Cummings, 1 ed., 2007.
- [16] N. A. Fuchs, *Evaporation and droplet growth in gaseous media*. Pergamon Press: London, 1959.

- [17] C. T. Crowe, J. D. Schwarzkopf, M. Sommerfeld, and Y. Tsuji, *Multiphase flows with droplets and particles*. CRC press, 2011.
- [18] S. Tonini and G. Cossali, “An analytical model of liquid drop evaporation in gaseous environment,” *International Journal of Thermal Sciences*, vol. 57, pp. 45–53, 2012.
- [19] H. Y. Erbil, “Evaporation of pure liquid sessile and spherical suspended drops: A review,” *Advances in Colloid and Interface Science*, vol. 170, no. 1, pp. 67–86, 2012.
- [20] B. R. Munson, D. F. Young, T. H. Okiishi, and W. W. Huebsch, *Fundamentals of fluid mechanics*. Don Fowley, 6 ed., 2009.
- [21] Y. Yuan and T. R. Lee, “Contact angle and wetting properties,” in *Surface science techniques*, pp. 3–34, Springer, 2013.
- [22] T. Young, “An essay on the cohesion of fluids,” *Philosophical Transactions of the Royal Society of London*, vol. 95, pp. 65–87, 1805.
- [23] D. Y. Kwok and A. W. Neumann, “Contact angle measurement and contact angle interpretation,” *Advances in colloid and interface science*, vol. 81, no. 3, pp. 167–249, 1999.
- [24] H. Hu and R. G. Larson, “Evaporation of a sessile droplet on a substrate,” *The Journal of Physical Chemistry B*, vol. 106, no. 6, pp. 1334–1344, 2002.
- [25] Z. Guo, W. Liu, and B.-L. Su, “Superhydrophobic surfaces: from natural to biomimetic to functional,” *Journal of colloid and interface science*, vol. 353, no. 2, pp. 335–355, 2011.
- [26] O. Carrier and D. Bonn, “Liquid spreading,” in *Droplet wetting and evaporation: From pure to complex fluids*, pp. 3–13, Elsevier, 2015.

- [27] R. N. Wenzel, “Resistance of solid surfaces to wetting by water,” *Industrial & Engineering Chemistry*, vol. 28, no. 8, pp. 988–994, 1936.
- [28] D. Bonn, J. Eggers, J. Indekeu, J. Meunier, and E. Rolley, “Wetting and spreading,” *Reviews of modern physics*, vol. 81, no. 2, p. 739, 2009.
- [29] X. Zhang, F. Shi, J. Niu, Y. Jiang, and Z. Wang, “Superhydrophobic surfaces: from structural control to functional application,” *Journal of Materials Chemistry*, vol. 18, no. 6, pp. 621–633, 2008.
- [30] M. Shanahan and K. Sefiane, “Kinetics of triple line motion during evaporation,” *Contact angle, wettability and adhesion*, vol. 6, pp. 19–31, 2009.
- [31] D. Brutin and F. Carle, “Droplets of colloids,” *Droplet Wetting and Evaporation: From Pure to Complex Fluids*, p. 279, 2015.
- [32] B. J. Fischer, “Particle convection in an evaporating colloidal droplet,” *Langmuir*, vol. 18, no. 1, pp. 60–67, 2002.
- [33] R. D. Deegan, “Pattern formation in drying drops,” *Physical review E*, vol. 61, no. 1, p. 475, 2000.
- [34] M. Boncheva and G. M. Whitesides, “Making things by self-assembly,” *MRS bulletin*, vol. 30, no. 10, pp. 736–742, 2005.
- [35] D. Philp and J. F. Stoddart, “Self-assembly in natural and unnatural systems,” *Angewandte Chemie International Edition in English*, vol. 35, no. 11, pp. 1154–1196, 1996.
- [36] W. Zhang, “Selforganizology: A science that deals with self-organization,” *Network Biology*, vol. 3, no. 1, p. 1, 2013.
- [37] <http://io9.gizmodo.com/dnas-double-helix-discovery-turns-60-how-monumental-i-494023216>, note = Online: Accessed on June 12, 2016,.

- [38] <http://www.nature.com/nature/journal/v506/n7487/full/506134a.html>. Online: Accessed on June 12, 2016.
- [39] <http://www.dailymail.co.uk/news/article-21440811>. Online: Accessed on June 12, 2016.
- [40] S. S. Jena, H. M. Joshi, K. Sabareesh, B. Tata, and T. Rao, “Dynamics of deinococcus radiodurans under controlled growth conditions,” *Biophysical journal*, vol. 91, no. 7, pp. 2699–2707, 2006.
- [41] A. Kostopoulou, S. K. Velu, K. Thangavel, F. Orsini, K. Brintakis, S. Psycharakis, A. Ranella, L. Bordonali, A. Lappas, and A. Lascialfari, “Colloidal assemblies of oriented maghemite nanocrystals and their nmr relaxometric properties,” *Dalton Transactions*, vol. 43, no. 22, pp. 8395–8404, 2014.
- [42] Y. Yin, Y. Lu, B. Gates, and Y. Xia, “Template-assisted self-assembly: a practical route to complex aggregates of monodispersed colloids with well-defined sizes, shapes, and structures,” *Journal of the American Chemical Society*, vol. 123, no. 36, pp. 8718–8729, 2001.
- [43] M. Boncheva, D. A. Bruzewicz, and G. M. Whitesides, “Millimeter-scale self-assembly and its applications,” *Pure and Applied Chemistry*, vol. 75, no. 5, pp. 621–630, 2003.
- [44] Y. Xia, B. Gates, and Z.-Y. Li, “Self-assembly approaches to three-dimensional photonic crystals,” *Advanced Materials*, vol. 13, no. 6, pp. 409–413, 2001.
- [45] Y. Xia, B. Gates, Y. Yin, and Y. Lu, “Monodispersed colloidal spheres: old materials with new applications,” *Advanced Materials*, vol. 12, no. 10, pp. 693–713, 2000.
- [46] L. Schmidt-Mende, A. Fechtenkötter, K. Müllen, E. Moons, R. H. Friend, and J. MacKenzie, “Self-organized discotic liquid crystals for high-efficiency organic photovoltaics,” *Science*, vol. 293, no. 5532, pp. 1119–1122, 2001.

- [47] M.-H. Wu and G. M. Whitesides, “Fabrication of arrays of two-dimensional micropatterns using microspheres as lenses for projection photolithography,” *Applied physics letters*, vol. 78, no. 16, pp. 2273–2275, 2001.
- [48] S. Zhang, “Fabrication of novel biomaterials through molecular self-assembly,” *Nature biotechnology*, vol. 21, no. 10, pp. 1171–1178, 2003.
- [49] C. J. Brinker, Y. Lu, A. Sellinger, H. Fan, *et al.*, “Evaporation-induced self-assembly: nanostructures made easy,” *Advanced materials*, vol. 11, no. 7, pp. 579–585, 1999.
- [50] P. J. Yunker, T. Still, M. A. Lohr, and A. Yodh, “Suppression of the coffee-ring effect by shape-dependent capillary interactions,” *Nature*, vol. 476, no. 7360, pp. 308–311, 2011.
- [51] V. R. Dugyala and M. G. Basavaraj, “Evaporation of sessile drops containing colloidal rods: Coffee-ring and order–disorder transition,” *The Journal of Physical Chemistry B*, vol. 119, no. 9, pp. 3860–3867, 2015.
- [52] P. J. Yunker, M. Gratale, M. A. Lohr, T. Still, T. C. Lubensky, and A. G. Yodh, “Influence of particle shape on bending rigidity of colloidal monolayer membranes and particle deposition during droplet evaporation in confined geometries,” *Physical review letters*, vol. 108, no. 22, p. 228303, 2012.
- [53] P. J. Yunker, M. A. Lohr, T. Still, A. Borodin, D. J. Durian, and A. G. Yodh, “Effects of particle shape on growth dynamics at edges of evaporating drops of colloidal suspensions,” *Physical review letters*, vol. 110, no. 3, p. 035501, 2013.
- [54] P. Yunker, A. Yodh, and T. Still, “Colloidal shape effects in evaporating drops,” *Physics of Complex Colloids*, vol. 184, p. 447, 2013.
- [55] X. Shen, C.-M. Ho, and T.-S. Wong, “Minimal size of coffee ring structure,” *The Journal of Physical Chemistry B*, vol. 114, no. 16, pp. 5269–5274, 2010.

- [56] T.-S. Wong, T.-H. Chen, X. Shen, and C.-M. Ho, “Nanochromatography driven by the coffee ring effect,” *Analytical chemistry*, vol. 83, no. 6, pp. 1871–1873, 2011.
- [57] T. Still, P. J. Yunker, and A. G. Yodh, “Surfactant-induced marangoni eddies alter the coffee-rings of evaporating colloidal drops,” *Langmuir*, vol. 28, no. 11, pp. 4984–4988, 2012.
- [58] T. Kajiyama, W. Kobayashi, T. Okuzono, and M. Doi, “Controlling the drying and film formation processes of polymer solution droplets with addition of small amount of surfactants,” *The Journal of Physical Chemistry B*, vol. 113, no. 47, pp. 15460–15466, 2009.
- [59] L. Cui, J. Zhang, X. Zhang, L. Huang, Z. Wang, Y. Li, H. Gao, S. Zhu, T. Wang, and B. Yang, “Suppression of the coffee ring effect by hydrosoluble polymer additives,” *ACS applied materials & interfaces*, vol. 4, no. 5, pp. 2775–2780, 2012.
- [60] D. Kaya, V. Belyi, and M. Muthukumar, “Pattern formation in drying droplets of polyelectrolyte and salt,” *The Journal of chemical physics*, vol. 133, no. 11, p. 114905, 2010.
- [61] T. Kasyap, D. L. Koch, and M. Wu, “Bacterial collective motion near the contact line of an evaporating sessile drop,” *Physics of Fluids (1994-present)*, vol. 26, no. 11, p. 111703, 2014.
- [62] T. T. Nellimoottil, P. N. Rao, S. S. Ghosh, and A. Chattopadhyay, “Evaporation-induced patterns from droplets containing motile and nonmotile bacteria,” *Langmuir*, vol. 23, no. 17, pp. 8655–8658, 2007.
- [63] B. M. Weon and J. H. Je, “Capillary force repels coffee-ring effect,” *Physical review E*, vol. 82, no. 1, p. 015305, 2010.

- [64] H. Hu and R. G. Larson, “Marangoni effect reverses coffee-ring depositions,” *The Journal of Physical Chemistry B*, vol. 110, no. 14, pp. 7090–7094, 2006.
- [65] V. H. Chhasatia, A. S. Joshi, and Y. Sun, “Effect of relative humidity on contact angle and particle deposition morphology of an evaporating colloidal drop,” *Applied Physics Letters*, vol. 97, no. 23, p. 231909, 2010.
- [66] F. Fan and K. J. Stebe, “Assembly of colloidal particles by evaporation on surfaces with patterned hydrophobicity,” *Langmuir*, vol. 20, no. 8, pp. 3062–3067, 2004.
- [67] P. Takhistov and H.-C. Chang, “Complex stain morphologies,” *Industrial & engineering chemistry research*, vol. 41, no. 25, pp. 6256–6269, 2002.
- [68] S. Paria, R. G. Chaudhuri, and N. N. Jason, “Self-assembly of colloidal sulfur particles on a glass surface from evaporating sessile drops: influence of different salts,” *New Journal of Chemistry*, vol. 38, no. 12, pp. 5943–5951, 2014.
- [69] E. Adachi, A. S. Dimitrov, and K. Nagayama, “Stripe patterns formed on a glass surface during droplet evaporation,” *Langmuir*, vol. 11, no. 4, pp. 1057–1060, 1995.
- [70] R. D. Deegan, O. Bakajin, T. F. Dupont, G. Huber, S. R. Nagel, and T. A. Witten, “Contact line deposits in an evaporating drop,” *Physical review E*, vol. 62, no. 1, p. 756, 2000.
- [71] H. Hu and R. G. Larson, “Analysis of the microfluid flow in an evaporating sessile droplet,” *Langmuir*, vol. 21, no. 9, pp. 3963–3971, 2005.
- [72] C. Monteux and F. Lequeux, “Packing and sorting colloids at the contact line of a drying drop,” *Langmuir*, vol. 27, no. 6, pp. 2917–2922, 2011.
- [73] M. Anyfantakis, Z. Geng, M. Morel, S. Rudiuk, and D. Baigl, “Modulation of the coffee-ring effect in particle/surfactant mixtures: the importance of particle–interface interactions,” *Langmuir*, vol. 31, no. 14, pp. 4113–4120, 2015.

- [74] D. Mampallil, J. Reboud, R. Wilson, D. Wylie, D. R. Klug, and J. M. Cooper, “Acoustic suppression of the coffee-ring effect,” *Soft matter*, vol. 11, no. 36, pp. 7207–7213, 2015.
- [75] H. B. Eral, D. M. Augustine, M. H. Duits, and F. Mugele, “Suppressing the coffee stain effect: how to control colloidal self-assembly in evaporating drops using electrowetting,” *Soft Matter*, vol. 7, no. 10, pp. 4954–4958, 2011.
- [76] D. Mampallil, H. Eral, D. van den Ende, and F. Mugele, “Control of evaporating complex fluids through electrowetting,” *Soft Matter*, vol. 8, no. 41, pp. 10614–10617, 2012.
- [77] Y. Li, C. Lv, Z. Li, D. Quéré, and Q. Zheng, “From coffee rings to coffee eyes,” *Soft matter*, vol. 11, no. 23, pp. 4669–4673, 2015.
- [78] <http://physics.aps.org/synopsis-for/10.1103/PhysRevLett.115.028301>. Online: Accessed on June 22, 2016.
- [79] H. C. Berg, “Motile behavior of bacteria,” *Physics Today*, vol. 53, pp. 24–29, 2000.
- [80] http://mmrc.caltech.edu/Gnometeer/drop_analysis/drop_analysis.pdf. Online: Accessed on June 22, 2016.
- [81] E. W. Dunklin and T. T. Puck, “The lethal effect of relative humidity on airborne bacteria,” *The Journal of experimental medicine*, vol. 87, no. 2, pp. 87–101, 1948.
- [82] E. Pince, S. K. Velu, A. Callegari, P. Elahi, S. Gigan, G. Volpe, and G. Volpe, “Disorder-mediated crowd control in an active matter system,” *Nature communications*, vol. 7, 2016.
- [83] J. Stankiewicz, M. A. C. Vílchez, and R. H. Alvarez, “Two-dimensional aggregation of polystyrene latex particles,” *Physical Review E*, vol. 47, no. 4, p. 2663, 1993.

- [84] J. Burns, S. Biggs, Y. Yan, and G. Jameson, “A comparison of the fractal properties of salt-aggregated and polymer-flocculated colloidal particles,” in *Trends in Colloid and Interface Science XII*, pp. 70–75, Springer, 1998.
- [85] A. L. Kjøniksen, F. Joabsson, K. Thuresson, and B. Nyström, “Salt-induced aggregation of polystyrene latex particles in aqueous solutions of a hydrophobically modified nonionic cellulose derivative and its unmodified analogue,” *The Journal of Physical Chemistry B*, vol. 103, no. 45, pp. 9818–9825, 1999.
- [86] H. C. Lichstein and M. H. Soule, “Studies of the effect of sodium azide on microbic growth and respiration: I. the action of sodium azide on microbic growth,” *Journal of bacteriology*, vol. 47, no. 3, p. 221, 1944.
- [87] K. A. Bisset, *The cytology and life-history of bacteria*. Baltimore : Williams and Wilkens, 1955.
- [88] M. Saarela, G. Mogensen, R. Fonden, J. Mättö, and T. Mattila-Sandholm, “Probiotic bacteria: safety, functional and technological properties,” *Journal of biotechnology*, vol. 84, no. 3, pp. 197–215, 2000.
- [89] N. Shah, “Probiotic bacteria: selective enumeration and survival in dairy foods,” *Journal of dairy science*, vol. 83, no. 4, pp. 894–907, 2000.
- [90] G. O. Canny and B. A. McCormick, “Bacteria in the intestine, helpful residents or enemies from within?,” *Infection and immunity*, vol. 76, no. 8, pp. 3360–3373, 2008.
- [91] P. Singleton *et al.*, *Bacteria in biology, biotechnology and medicine*. No. Ed. 6, John Wiley & Sons, 2004.
- [92] S. Edberg, E. Rice, R. Karlin, and M. Allen, “Escherichia coli: the best biological drinking water indicator for public health protection,” *Journal of Applied Microbiology*, vol. 88, no. S1, 2000.

- [93] G. Bogosian and E. V. Bourneuf, “A matter of bacterial life and death,” *EMBO reports*, vol. 2, no. 9, pp. 770–774, 2001.
- [94] J. Keer and L. Birch, “Molecular methods for the assessment of bacterial viability,” *Journal of Microbiological Methods*, vol. 53, no. 2, pp. 175–183, 2003.
- [95] M. L. Snyder and H. C. Lichstein, “Sodium azide as an inhibiting substance for gram-negative bacteria,” *Journal of Infectious Diseases*, vol. 67, no. 2, pp. 113–115, 1940.
- [96] P. V. Jones, A. F. DeMichele, L. Kemp, and M. A. Hayes, “Differentiation of escherichia coli serotypes using dc gradient insulator dielectrophoresis,” *Analytical and bioanalytical chemistry*, vol. 406, no. 1, pp. 183–192, 2014.
- [97] J. M. Gómez Gómez, J. Medina, D. Hochberg, E. Mateo-Martí, J. Martínez-Frías, and F. Rull, “Drying bacterial biosaline patterns capable of vital reanimation upon rehydration: novel hibernating biomineralogical life formations,” *Astrobiology*, vol. 14, no. 7, pp. 589–602, 2014.