STUDY OF ANISOTROPIC PARTICLES IN ACTIVE BATH

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IN
PHYSICS

By
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August, 2015

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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

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Brownian (passive) particles undergo random motion due to thermal agitation in the surrounding medium. In recent years, a lot of attention has been devoted to study active Brownian particles, i.e., microscopic particles capable of self-propelling. Unlike simple passive particles, active particles feature an interplay between random fluctuations and active swimming. Thus, active particles are out of thermal equilibrium and, therefore, they explore their environment completely different from passive particles. Bacteria and other microorganisms are natural examples of active particles that take up energy from their environment and convert into directed (run) motion. Recently, there has been a lot of research progress in the realization of artificial active particles (microswimmers) due to their potential technological applications. We report the anomalous diffusion of anisotropic particles propelled by biological microswimmers (E. Coli Bacteria). The anisotropic particles of various shapes (L-shape, U-shape, cross-shape, Star-shape and Z-shape) were fabricated using soft lithography method. We study the motion (translation and rotation) of various anisotropic shaped particles in the thermal and active bath. The results are compared with isotropic (spherical) particles. We observe that shape anisotropy in particle plays a vital role when they are suspended in the bath of E-Coli cells showing different diffusion behavior as the swimming pressure of E-Coli bacteria differs in the shape anisotropy.

Keywords: Anisotropic particles, Active bath, Bio-propellers.

ÖZET

TÜRKÇE BAŞLIK

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Tez Danışmanı: Yrd. Doç. Dr. Giovanni Volpe
Ağustos, 2015

Brownian (pasif) parçacıkları termal çalkantı altında gelişigüzel hareket ederler. Son yıllarda Brownian parçacıkları üzerine çalışmalar büyük önem kazanmıştır. Bunlara örnek olarak kendi kendilerine hareket edebilen mikroskobik parçacıkları verebiliriz. Pasif parçacıkların aksine, aktif parçacıklar rastgele dalgalanmalar ve aktif yüzme arasında rol oynar. Bundan dolayı, aktif parçacıklar termal dengede değildir ve etraflarındaki ortamı pasif parçacıklardan farklı olarak keşfederler.Bakteri ve diğer mikroorganizmalar etraflarındaki enerjiyi alan ve bunu harekete çeviren doğal aktif parçacıklara örnektirler. Son zamanlarda olası teknejoji uygulamalarının farkedilmesinden dolayı yapay aktif parçacıklar (mikro yüzücüler) üzerine yapılan çalışmalarda birçok ilerleme kaydedilmiştir. Biyolojik mikroyüzücüler (E. Coli Bacterisi) tarafından itilen anizotropik parçacıkların kuraldışı difüzyonunu rapor ediyoruz. Anizotropik paçacıkları farklı şekillerde (L-şeklinde, U-şeklinde, Cross-şeklinde, Star-şeklinde ve Z-şeklinde) yumuşak litografi yöntemiyle elde ettik. termal ve aktif ortamda farklı şekillerdeki anizotropik parçacıkların hareketlerini (öteleme ve dönme) çalıştık. Sonuçları izoteropik (küresel) parçacıkların hareketleri ile karşılaştırdık. Anisotropik parçacıkların şekiileri değiştikçe, mikroyüzücülerin, E-Coli bakterilerin, parçacıklara uyguladıkları basıncın değiştiğini bu yüzden E-Coli hücreleriyle dolu bir ortam içerisine bırakılan parçacıklar için şekil anizotropisinin hayati bir önem sergilediğini gözlemledik.

Anahtar sözcükler: Anizotropik parçacıklar, Aktif ortam, Biyolojik pervaneler.

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I am grateful to Almighty Allah, the most beneficent and merciful, All embracing, all knowing who provided me with the opportunity, courage and ability to complete this research work.

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Chapter 1

Introduction

Randomness provides natures with the essence of beauty. Few examples of randomness includes leaves on tree, distribution of stars and galaxies in universe, outcomes of throwing dice, games of luck etc.[1].Among those random processes, Brownian motion is one of the most important and interesting phenomenon that exists in nature.

1.1 Brownian Motion

Brownian motion was first discovered by Robert Brown back in 1827 when he was trying to observe pollen suspended in water under the microscope[2]. He observed that pollen are moving randomly in water. Initially he thought that this random motion of pollen was due to the pollen itself as they were living entities. But later his experiments showed similar random motion even for the dead pollen as suspended particles in the water[3]. He was able to observe this motion but could not come up with idea what caused that motion. Later on , it was established that the Brownian motion was the kind of motion which was postulated in the kinetic theory of molecules for the molecular motion[4]. In 1905, Einstein came up with the exposition of the Robert Brown's observation and

formulated the theory of Brownian motion[5]. Liquid molecules move mercurially because of the thermal energy they posses inside them. Einstein proposed that the Brownian motion occur due to the collision of the suspended particles in the liquid, with the liquid molecules. Due to microscopic nature of the liquid molecules, these perpetual movements of the molecules cannot be seen with naked eyes. But to the suspended particles in the liquid, these molecules are really huge. So the suspended particle execute random Brownian motion due to the thermal fluctuations of the liquid molecules[6]. The diffusion constant calculated by Einstein is given by the equation 1.1[5].

$$D = \frac{kT}{f} \tag{1.1}$$

where kT is thermal energy, k is Boltzman constant and f is frictional coefficient of the particle. Instead of f, Einstein used K to represent frictional force of particle . As German word for force is Kraft, so Einstein used used K as notation to represent resistive force [7].

$$f = 6\pi\eta R \tag{1.2}$$

where η is viscosity of the solution and R is the radius of the spherical particle. Combining 1.1 and 1.2

$$D = \frac{kT}{6\pi\eta R} \tag{1.3}$$

Equation 1.3 is called the Stokes-Einstein diffusion coefficient. Einstein also reported equation of the diffusion of the particle in a liquid. The diffusion coefficient in equation 1.1 can be related as

$$< r^2 >= 6DT \tag{1.4}$$

where $r^2 = x^2 + y^2 + z^2$; x, y, z are the direction of the particle motion. Equation 1.4 states that a particle with diffusion coefficient D diffuses in such a way that the average 3 dimensional displacement $< r^2 >$ is proportional to time t. Equation 1.4 also called as mean square displacement. For 2 dimensional displacement, we can write

$$< r^2 >= 4DT \tag{1.5}$$

After Einstein, Smoluchowsky using different numerical coefficients obtained the same results as Einstein did. Later on , Langevin pointed out a mistake in the assumptions of Smoluchowsky and gave a corrected derivation of equations. Following all above giants of history, Jean Baptise Perrin performed experiments on Brownian motion and provided the experimental proof of the Einstein's theory of Brownian motion[8]. In his experiments, Perrin used 0.52 μ granules in a uniform emulsion suspended them in water. Temperature of sample was monitored very carefully with a thermometer as it could change the viscosity very easily. Three different trajectories of 0.52 μ granules suspended in water at a time interval of 30 seconds corresponding to 200 points is shown in Fig: 1.1 [9]. These experiments won Perrin the Nobel prize in 1926.

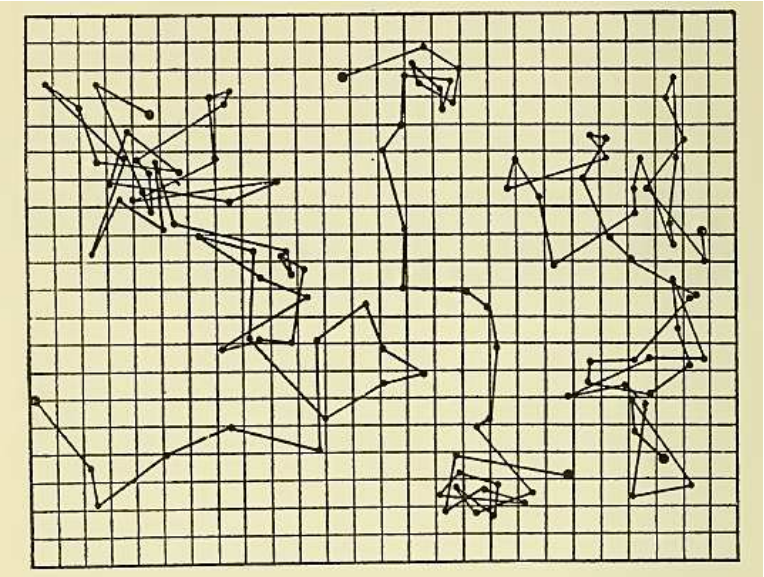


Figure 1.1: Three different trajectories of 0.52 μ granules recorded by Perrin by joining consecutive positions at an interval of 30 seconds. Reproduced from [9]

1.1.1 Properties of Brownian Motion

All above theories and discoveries led to the conclusion that Brownian motion holds the following properties.

- Brownian motion follows straight translations and rotates randomly at different angles. There is no tangents to the trajectories and there is no well defined velocity.
- With the increase of temperature, less viscosity of liquid and smaller size of the granule, Brownian motion becomes more fervent
- The motion is not dependent on the closeness of the particles to each other.
- There is no effect of the composition of the particles on the Brownian motion.
- Brownian motion is ubiquitous and perpetual.

1.2 Brownian Motion of Isotropic Colloids

As mentioned in previous section, the Stokes-Einstein diffusion coefficient of spherical or isotropic particle is shown in equation 1.3. In Fig. 1.3 the experimentally obtained trajectory and the average MSD of few 5 μm sized spherical particles are shown. The slope of the MSD corresponds to 1 which is confirmation of Brownian motion.

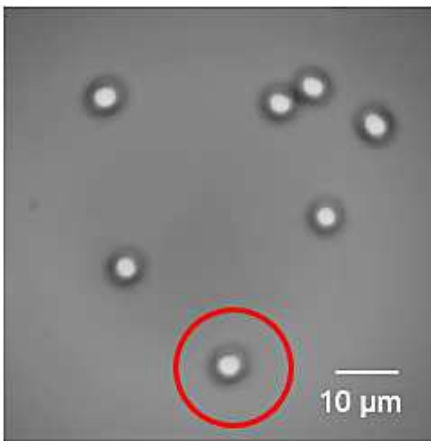
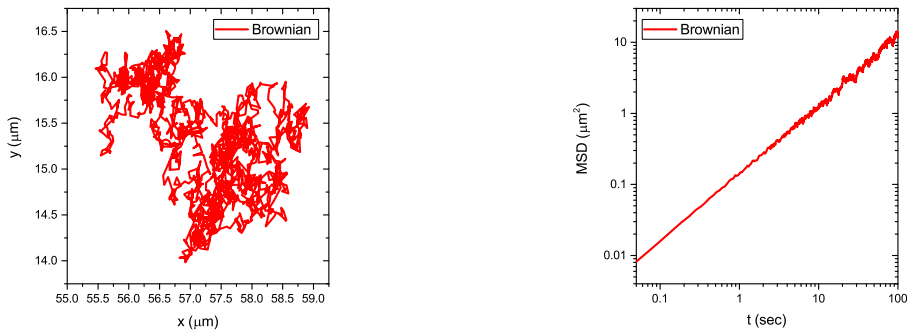


Figure 1.2: Snap shot of isotropic spherical colloids suspended in water



(a) (b)

Figure 1.3: (a) Trajectory of a particle highlighted by a circle in Fig. 1.2 (b) The average MSD of all the spherical particles as shown in Fig. 1.2

1.3 Brownian Motion of Anisotropic Colloids

In previous section the Brownian motion of particles is due to translation. In addition to translation, there exist rotational motion which is negligible for isotropic particles. Anisotropic particles show different Brownian behavior as compared to isotropic particles like spheres [10]. Their rotation plays an important role in differentiating them from isotropic particles. Rotational Brownian motion which tends to randomize particle orientation just like translation diffusion which randomize particle position. Therefore the equation 1.3 can be written as

$$D_r = \frac{kT}{f_r} \tag{1.6}$$

Ayan Chakrabarty et al showed the Brownian motion of boomerang particle in their work [11]. The SEM image of boomerang particles is shown in Fig: 1.4.

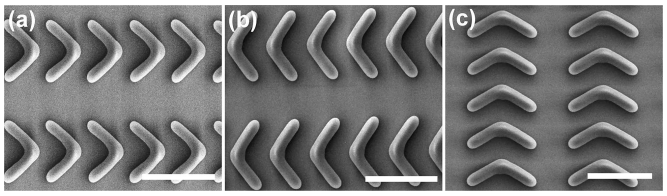


Figure 1.4: (a)SEM image of vertex angle 90^o boomerang Particle (b)SEM image of vertex angle 110^o boomerang Particle (c)SEM image of vertex angle 120^o boomerang Particle. Scale bar is 4μm. Reproduced from [11]

They showed that anisotropy of the particles can cause the MSDs to behave differently. For spherical and ellipsoid particles, the MSD grows linearly straight both for short time and long time. However in case of boomerang particles, its not like this. For short time, the MSD behave differently and then it goes linearly straight for long time. This can been seen in the Fig. 1.5

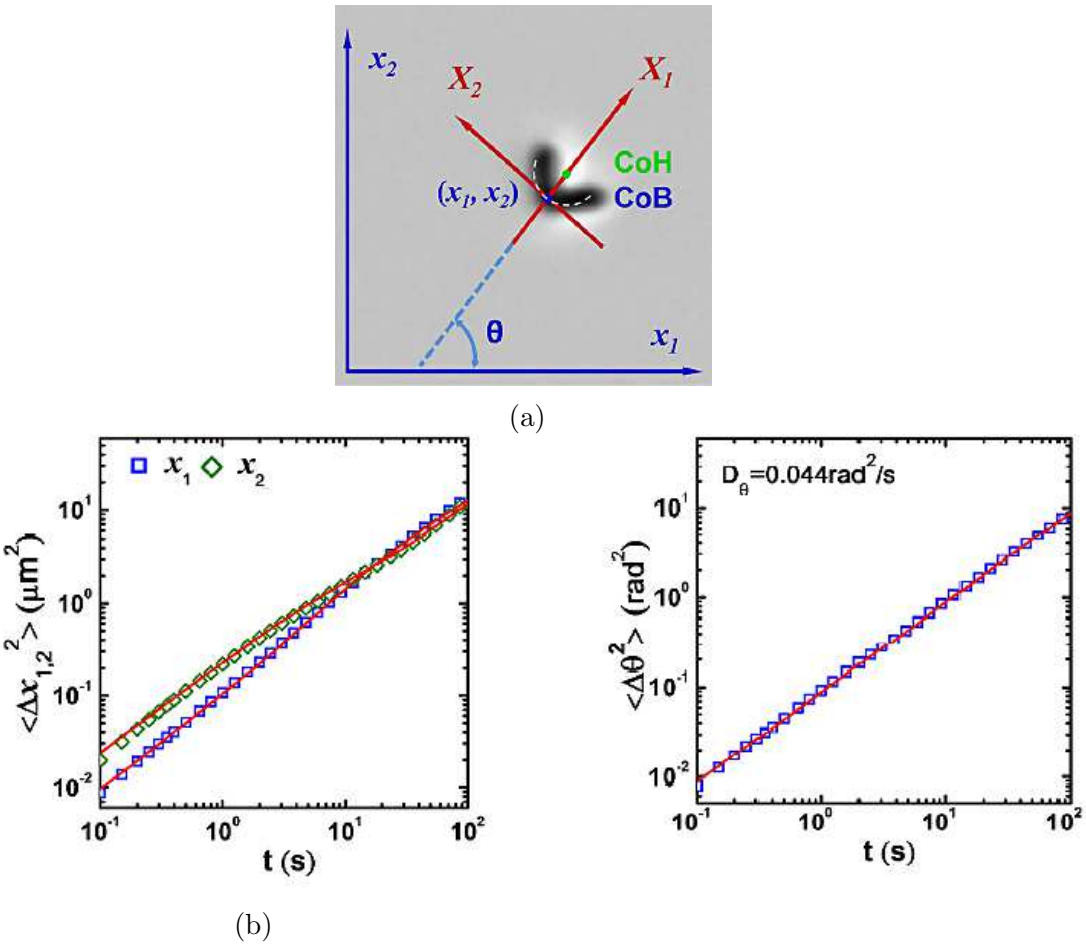


Figure 1.5: (a) Microscopic image of Boomerang particle and also schematic of frame of referance. (x_1, x_2) are lab frame and (X_1,X_2) are body frames. (b) Shows the different behavior of MSD of position at short time scale and then grows linearly in longer times. Also it shows mean squre angular discplace which grows linearly in both short and long time scale. Reproduced from [11]

1.4 Active Brownian Motion

The motion of a particle suspended in a liquid which is able to take energy from its environment and then able to convert it into directed is termed as active Brownian motion[12] [13]. Unlike passive Brownian motion where suspended particles are in thermal equilibrium with their environment, active Brownian particles can derive energy from the environment and can self propel themselves in the medium[14] [15]. As these particles become far from equilibrium with their environment, so they propel themselves as a response[16]. There are several examples of mechanisms through which active Brownian particle can propel themselves. They can be categorized as follows

- Natural active Brownian particles
- Artificial active Brownian particles

1.4.1 Natural Active Brownian Particles

There are many micro-swimmers in nature which exhibit active Brownian motion. Escherichia coli (E-coli) is a strain of bacteria which itself acts as active Brownian particle [17]. Their run and tumble motion is caused by of the rotation of their flagella. The rotation of flagella is just like reversible rotary motor and it is driven by flux of protons [18]. Fig. 1.6 shows the trajectories of bacteria moving.

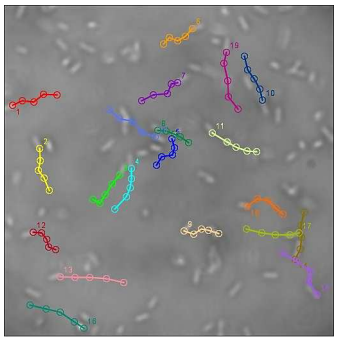


Figure 1.6: Trajectories of run motion of E-Coli bacteria

1.4.2 Artificial Active Brownian Particles

There can be many examples of artificially created active Brownian particles. Few are given below.

- Propulsion by Magnetic Field[19]
- Propulsion by chemical reaction[20] [21]
- Propulsion by self-thermophoresis[22]
- Propulsion by Bio-propellers[23]

1.4.2.1 Magnetic Field

Magnetic nano-particles have already been studied for their propulsion in medium and they can be controlled by manipulating applied magnetic field[24]. They are being used extensively in microbiology for targeted delivery of certain drugs, isolation of biomaterials etc. They are found to be used very effectively as they are non-toxic, suitable for the living organisms if they are properly synthesized and functionalized[25]. An example of propulsion by magnetic field is shown in Fig. 1.7.

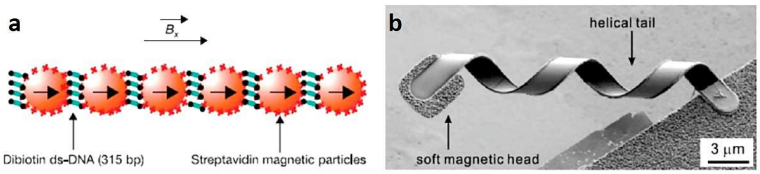


Figure 1.7: (a) Schematic of a DNA-linked flexible flagellum actuated by a magnetic field. Reproduced from [26]. (b) SEM image of an artificial bacteria flagellum with diameter of 2.8 μm

1.4.2.2 Chemical Reaction

Propulsion by chemical reaction can also be a method of creating active Brownian particles. For example, platinum coated janus particles in hydrogen per oxide (H_2O_2) enriched water can propel themselves by the chemical reaction on the surface of metal[20] [21]. Fig. 1.8 shows an example of propulsion by chemical reaction.

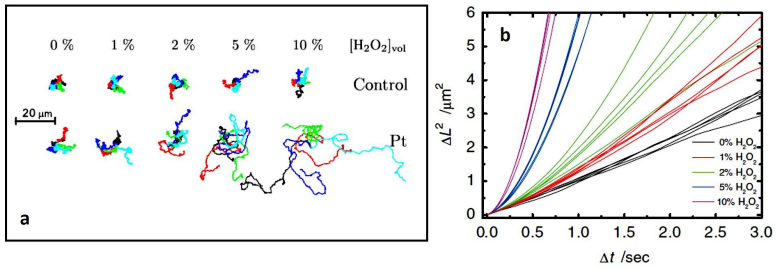
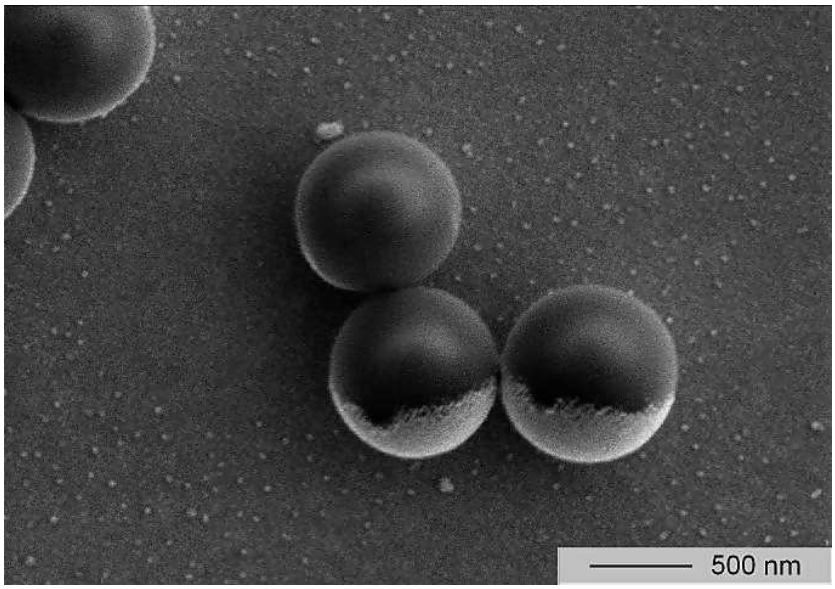


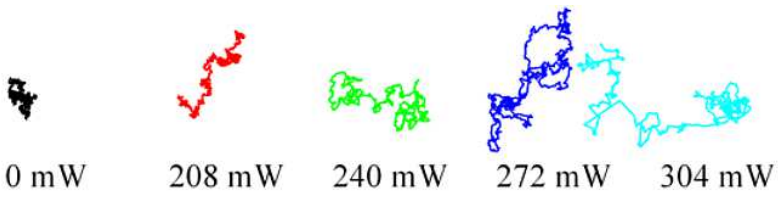
Figure 1.8: (a) Trajectories of control polystyrene particles and platinum-coated particles in water and varying solutions of hydrogen peroxide and (b) Mean squared displacement for the trajectories of platinum-coated particles corresponding to different hydrogen peroxide concentrations. Reproduced from [27]

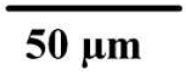
1.4.2.3 Light

Active Brownian motion can also be induced by Light. Claudio Maggi et al show that the asymmetric gear can convert light in to work by the mechanism thermocapillary effect [28]. Self-diffusiophoretic motion of particles can also be achieved by the light which converts passive particles into active particles [29] [30]. Light can also induce self-thermophoresis in janus particles[12]. Buttinoni etal. showed that Janus particles half coated with gold exhibited active Brownian motion. Janus particle coated with gold is shown in Fig:1.9 (a). Fig: 1.9 (b) shows that how the diffusion of these particles become enhanced with increasing laser power. They become active from passive with increasing power of laser.



(a)





(b)

Figure 1.9: (a) STEM image of Janus particles coated with gold (b)Trajectories of particles with increasing laser power. Reproduced from [12]

1.4.2.4 Bio-propellers

Bio-propellers can also be used to achieve active Brownian motion of particles. Wu et al showed that beads of size upto 10 μm undergo superdiffusion in bacterial bath[31]. I reproduces and performed the experiments with 5μm silicon particles in an active bath where RP437 E-Coli bacteria strain are present as bio-propellers. The motion of E. Coli is itself active Brownian motion induced by chemo-taxis[32]. These E. Coli induce active Brownian motion in Passive particles. The trajectory and MSD of the particles is shown in Fig:1.11 (a) and (b) respectively. Fig:1.10 shows the isotropic colloids in active bath.

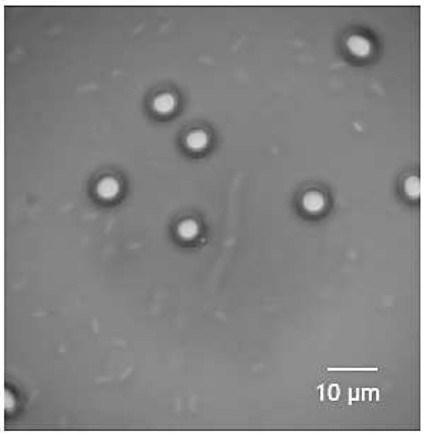
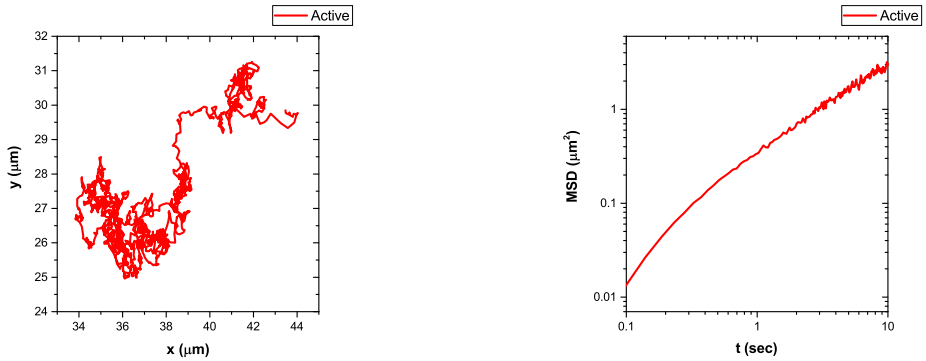


Figure 1.10: Isotropic spherical colloids suspended in active bath



(a) (b)

Figure 1.11: (a) Trajectory of Spherical colloids in active medium (b)MSD of the isotropic colloids as a function of time. Short time super diffusion is observed and it grows linearly in longer time scale.

1.5 Thesis Layout

Brownian (passive) particles undergo random motion due to thermal agitation in the surrounding medium. In recent years, a lot of attention has been devoted to study active Brownian particles, i.e., microscopic particles capable of self-propelling. Unlike simple passive particles, active particles feature an interplay between random fluctuations and active swimming. Thus, active particles are out of thermal equilibrium and, therefore, they explore their environment completely different from passive particles. Bacteria and other microorganisms are natural examples of active particles that take up energy from their environment and convert into directed (run) motion. Recently, there has been a lot of research progress in the realization of artificial active particles (microswimmers) due to their potential technological applications. Some of the driving mechanisms that have been proposed and implemented to realize artificial microswimmers include: propulsion using magnetic field (DNA-linked magnetic particles), self-thermophoresis (Janus particles), chemical reaction (platinum coated polystyrene particles in hydrogen peroxide), and biological mechanisms (passive particles along with swimming bacteria). Among them, special attention is focused on biological mechanisms after the demonstration of (non-pathogenic) bacterial species capable of targeting diseased tissues and successfully deliver plasmid DNA, proteins and other therapeutic agents into mammalian cells; these active particles can be employed for technological applications such as targeted delivery of therapeutic and imaging cargos.

In this thesis, we report the anomalous diffusion of anisotropic particles propelled by biological mechanisms (passive anisotropic particles along with swimming bacteria) was not studied so far. In particular, we will use living, motile non-pathogenic *Escherichia coli* Bacteria along with anisotropic particles. The anisotropic particles of various shapes (L-shape, U-shape, cross-shape, Star-shape and Z-shape) were fabricated using soft lithography method. We study the translation motion and the rotational orientation of various anisotropic shaped particles with and without E. coli bacteria using digital video microscopy. Diffusion of these fabricated anisotropic is studied in both thermal and active bath. The

results are also compared with isotropic spherical particle. It is established that which particles shows the most enhanced diffusion in active bath (with bacteria) as compared to other particles. The angular orientation is also studied as a function of time in both thermal and active bath and it is shown which particle shows the highest rotation in active bath.

Chapter 2

Experimental Methods

2.1 Fabrication of Anisotropic Particles

Various shapes of anisotropic particles (L-shape, U-shape, Z-shape, Cross shape and Star shape) are fabricated using soft photolithography method. The step by step fabrication process involved is described in the upcoming section.

2.1.1 Substrate Preparation

Cleaning of Silicon wafer is done by dipping the wafer in piranha etch solution. Hydrogen per oxide (H_2O_2) is added slowly to concentrated Hydrosulfuric acid (H_2SO_4) in the ratio of 1:3 in order to obtain piranha etch solution. While adding hydrogen per oxide to the sulfuric acid, the beaker is shaken gently for uniform mixing. The reaction of hydrosulphuric acid with hydrogen per oxide is highly exothermic and it needs to be handled with great care. Hydrogen per oxide should always be added to the hydrosulfuric acid not vice-versa. Silicon wafer is left in piranha etch solution for about 5 to 10 minutes. This process removes all the impurities and dust particles from the wafer. Later, silicon wafer is taken out of the piranha etch solution and washed with deionized water. Then,

silicon wafer is blow-dried with nitrogen (N_2) and further silicon wafer is washed with Isopropanol and blow-dried with Nitrogen (N_2) again. Finally, the silicon wafer is put on the hotplate at 65 ^0C for 30 seconds to remove any residue of isopropanol by evaporation. Let the silicon wafer to cool down till it reaches room temperature. Now silicon wafer is ready for the next step.

2.1.2 Coating of Omnicoat

The cleaned silicon wafer is spin coated with Omnicoat. Omnicoat will act as a sacrificial layer in the end where it dissolves in remover PG to give easy lift-off of the desired particles from the silicon surface. Omnicoat is coated on the silicon wafer in two step spin coating. Spin coating of omnicoat is done @500 rpm for 5 second at 100 acceleration immediately followed by 2500rpm for 30 seconds at 100 acceleration. In order to achieve uniform omnicoat coating, silicon wafer should be placed well centered on the vacuum chuck of spin coater. After spin coating of Omnicoat, wafer is placed on hotplate at 200 ^0C for 1 minute in order to remove extra solvent. After this, wafer is allowed to cool down to room temperature before proceeding to next step.

2.1.3 Coating of SU8 (Photoresist)

Silicon wafer is again centrally mounted on the spin coater for the uniform coating of SU8-2005 photo-resist. SU8-2005 is also coated in 2 step coating. First 500rpm at 100 acceleration for 5 seconds immediately followed by the 4000rpm at 300 acceleration for 35 seconds. This step ensures how thick our end particles will be. According to Michrochem SU8-2005 data sheet, 4000rpm should result a 5 micron thickness of photo-resist on the surface of silicon wafer.

2.1.4 Soft Bake

After spin coating of SU8-2005, the wafer is placed on hot plate at 95 ^0C for 3 minutes. This step also defines the thickness of the particles according to Michrochem SU8-2005 data sheet which corresponds to 5 microns thickness for 3 minutes of soft bake time. After 3 minutes, the substrate was removed from hotplate and let it cool down to room temperature. If wrinkles were found on the coated resist, then heat the substrate for a few more minutes and again let it cool down to room temperature. Repeat the heating and cooling process until uniform coating is observed.

2.1.5 Exposure

After the soft bake procedure, the substrate is placed on the mask aligner for the UV exposure. 95 mj/cm^2 exposure energy dose is applied to achieve 5 micron thick particles. The UV exposed area on the substrate will generate patterns after post exposure baking procedure.

2.1.6 Post Exposure Bake (PEB)

After exposure, the substrate is heated on hot plate for 2 minutes at 95 ^0C. This increases the cross link between structures of the particles. The particles structure starts to become visible on the substrate with in 5 to 15 seconds of post exposure bake. After PEB, substrate is allowed to cool down to room temperature before proceeding to next step.

2.1.7 Development

Now the substrate is placed in the SU8 developer for about 1 minute. The petri dish containing SU8 developer is gently agitated to remove all excess SU8-2005

from the substrate. The developer washes all the SU8-2005 other than the exposed SU8-2005 which became hard and nicely crossed linked after PEB. After 1 minute, the substrate is taken out of developer and washed with isopropanol followed by blow-drying of with Nitrogen (N_2). If after drying, a while film appears on the substrate then it is sign of underdevelopment. Let the substrate be in the developer again for a few more seconds and wash again with isopropanol followed by blow-drying with Nitrogen (N_2). Repeat the process until the white film vanishes completely. The 100x magnified images of various shape particles over the substrate after development is shown in Fig. 2.1.

2.1.8 Lift Off

Next step is to lift the particles off the substrate surface. To lift the particles from the substrate, we use Remover PG solvent which is also a product of Michrochem. The Remover PG dissolves the sacrificial layer (which is Omnicoat) off the substrate to give easy lift of the particles from the substrate surface.In our case, 3ml of Remover PG is taken in a small petri dish and the substrate containing developed particles is left immersed into Remover PG. This petri dish containing the substrate is then put in the sonicator and substrate is sonicated at 35 ^0C. After this particles become suspended in the 3ml of Remover PG. Then this 3ml of Remover PG is taken in two 1.5ml eppendorf tubes [33].

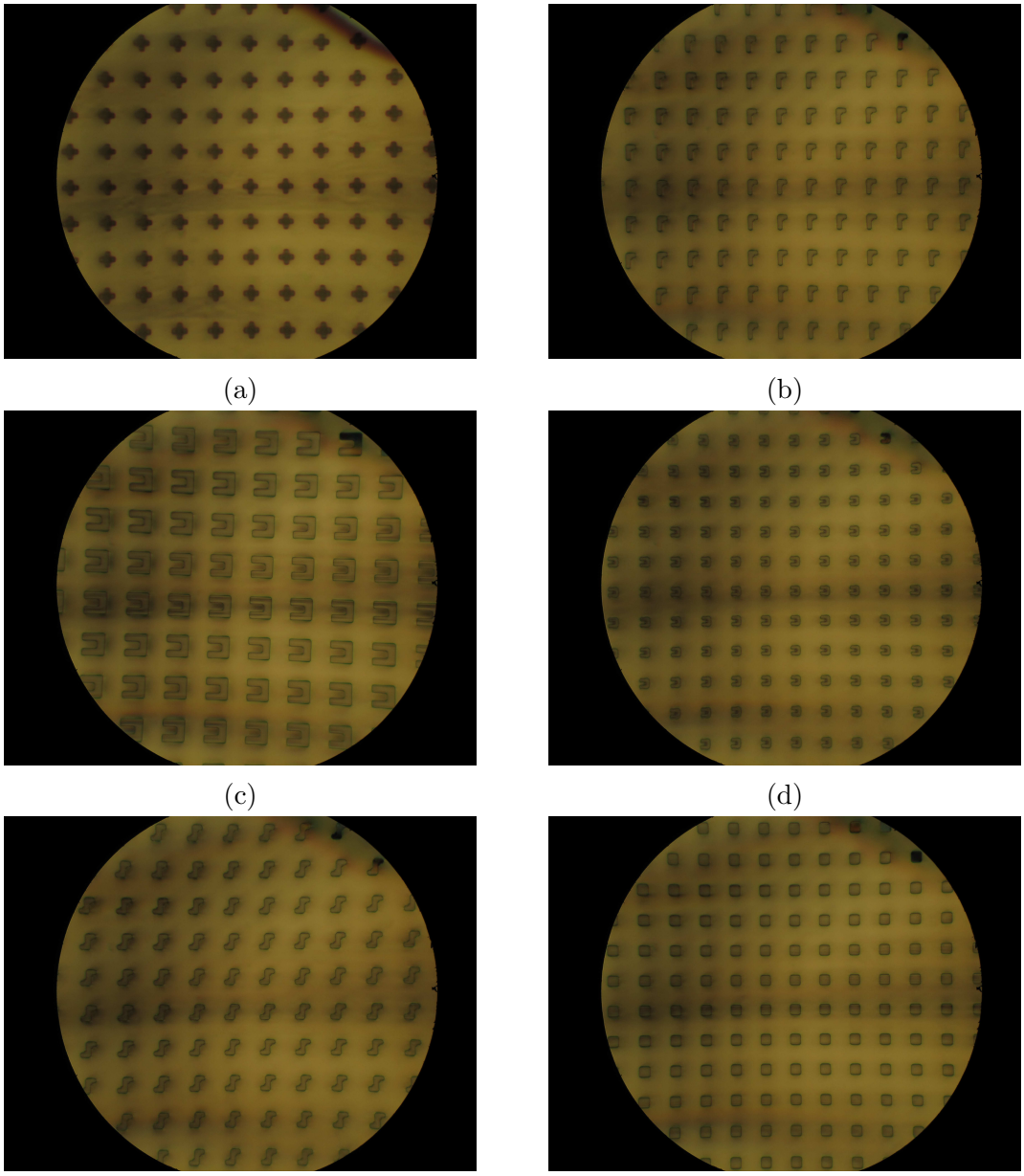


Figure 2.1: snap shot of (a) Cross Shape (b)L-shape (c) U-shape(Big) (d) U-shape (small) (e) Z-shape (f) Square shape particles seen clearly on the substrate after development

2.1.9 Resuspension in Water

The 1.5ml appendorf tubes containing suspended particles in Remover PG are then centrifuged at 16000g RCF (Relative Centrifugal Force) value for 3 minutes which forces all the particles in the appendorf tube to settle down at the bottom. The supernatant is then removed leaving the particles at the bottom of the appendorf tube. Fresh 1.5ml Remover PG is added to the appendorf tubes to re-suspend the particles again and repeat the same procedure. Again the supernatant is discarded and this time 1.5ml deionized water is added to the appendorf tubes. Centrifugation is performed again at the same values and after supernatant is discarded. This cleaning process with deionized water is done 4 times to get rid of any remains of Remover PG. Finally, the particles are re-suspended in 1.5ml of deionized water. Thus the whole procedure is followed to fabricate various particle shapes.

2.2 RP-437 E-Coli Bacteria Preparation protocol

E-Coli bacteria wild strain RP437 is purchased from Yale University Bacteria stock center. The preparation of motile bacteria culture involves certain steps which are described below.

2.2.1 Motility Buffer

Motility buffer for RP437 is prepared in 100ml of deionized water. Potassium Phosphate monobasic 10mM is added to the water which is equivalent to 136.46mg in 100ml of water. Then 0.1mM Na-EDTA is added to the water. It is very hard to have such low molar concentration by directly adding Na-EDTA. So 10mM stock solution of Na-EDTA is prepared in 100ml of water by adding 0.372gm of Na-EDTA. From this stock solution, 1ml is added to the mobility

buffer which is equivalent to 0.1mM concentration. 2μm of tween-20 is added to the solution which acts as a surfactant. This solution is mixed with magnetic stirrer and the PH of the solution is adjusted to 7.0 by adding appropriate amount of NaOH. Finally, 10mM of dextrose i.e 180.16mg is added to the solution. This mobility buffer is filtered in 50ml falcon tube and can be stored at room temperature.

2.2.2 Growth Medium

Trypton growth medium is prepared in which RP437 E-coli is allowed to grow over night. In 250ml flask, 50ml de-ionized water is taken. The volume of flask should be 5 times more than the amount of water for the proper aeration. .500gm of tryptone is added to the water along with .250gm of NaCl. Then this mixture is mixed vigorously to have uniform mixing of tryptone. After this, the growth medium is put for autoclave in order to kill all microorganisms if there exist any in water.

2.2.3 Tryptone Agar Preparation

Agar plates are used to grow bacterial colonies over them. 1gm of trypton, 1gm of NaCl and 1.5 of Agar is added to 100ml of deionized water and then vigorously mixed in order to dissolve tryptone completely. Then the resulted solution is autoclaved. After the solution is autoclave, pour the solution in petri dishes and leave them undisturbed for 3 to 4 hours. During these hours, the autoclaved solution will be solidified.

2.2.4 Streaking RP437 over Agar Plates

Liquid culture of RP437 bacteria is taken from -80^0C and it is streaked onto the sterile solidified agar plates using loop hole sticks. Then these agar plates are

placed in incubator for almost 20 hours at 32.5 ^0C suitable for growing bacterial colonies in overnight. If after 20 hours, there appears different colors in the colonies, then this is indication of some contamination and process is repeated again in order to have uncontaminated samples.

2.2.5 Preparing Bacteria Culture for Propelling Passive Particles

Single colony of bacteria is picked from the agar plate containing different RP437 bacteria colonies. This is done by sterile toothpicks and these sterile toothpicks are put into tryptone growth medium. Growth medium containing bacteria toothpicks is then put in incubator at 32.5 ^0C and constantly shaken at 180rpm for 17 hours over night. After 17 hours, this growth medium is diluted into a fresh growth medium in a ratio of 1:100. 500ml from overnight culture is transferred to fresh growth and put back for incubation at 32.5 ^0C and 180rpm until they reach to an optical density of .40 @600nm wavelength. This is normally achieved in 4 hours and 15 minutes. After this, 7ml of growth medium containing bacteria is transferred into a 50ml falcon tube and centrifuged at 2000rpm for 10 minutes. After 10 minutes, bacteria pellets are concentrated at the bottom of falcon tube. The bacteria pellets are collected gently from the bottom of falcon tube along with some amount of tryptone growth medium. These pellets are gently released in 4ml mobility buffer and centrifuged again at 2000rpm for 10 minutes. This cleaning process is done 3 times. The only purpose of this process is to reduce the tryptone growth medium concentration in the final falcon tube. Final culture obtained after 3 times cleaning processes is used for experimental purposes [34].

2.3 Sample Cell Preparation

2.3.1 Cleaning

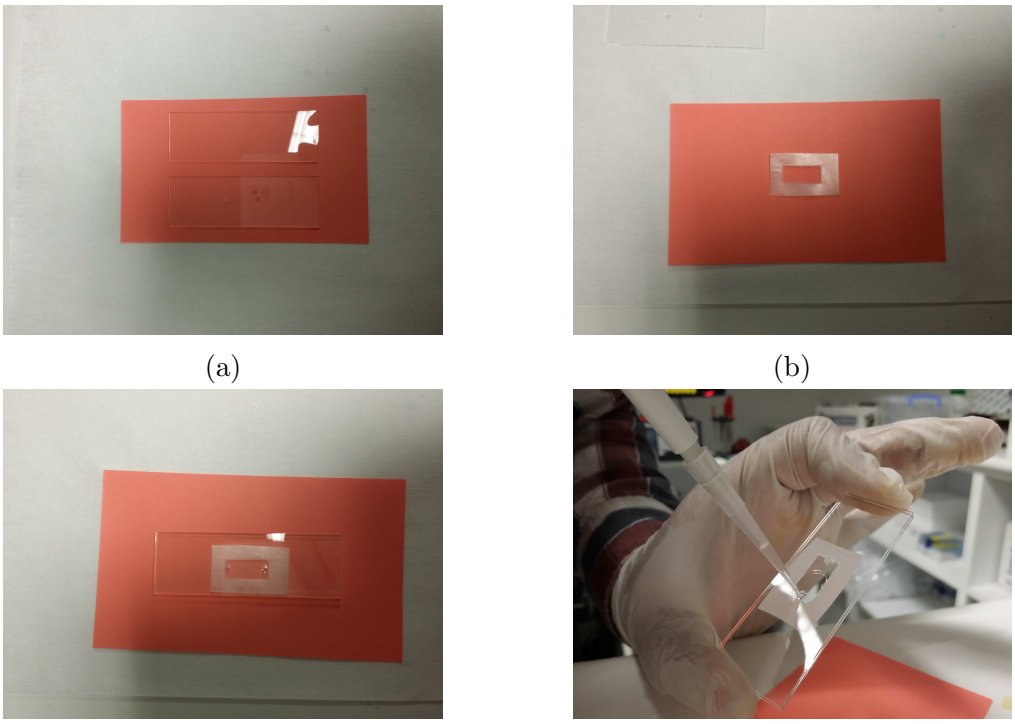
Two glass slides are used to prepare the sample cell. In one of the glass slides two holes are drilled apart from each other will act as inlet and outlet of the sample cell. Both glass slides are first cleaned with acetone followed by isopropanol. After this, the glass slides are left immersed in the .25M NaOH solution for 20 to 30 minutes to make the surface of glass hydrophilic. After this both glass slides are washed with deionized water and blow-dried with nitrogen.

2.3.2 Creating Cell

A parafilm of size 3.8x3.8cm^2 is taken and folded twice so that it is folded in 4 layers. The 4 layer folded parafilm is placed in the glass slide in which two holes were drilled. The holes position is marked on the parafilm. Then I cut the parafilm across the markings in rectangular shape to make rectangular channel in the parafilm. Then this parafilm is placed between the two glass slides such that it does not cover the holes on the drilled slide. Then these slides are placed on a hot plate at 90^0C. When the parafilm is melted, I remove the glass slide from hotplate and let it cool down to room temperature. Parafilm solidifies as it cool down, sticking to the both glass slides firmly. Now there is a cell created in which sample can be inserted from one hole of the drilled glass.

2.3.3 Sample Preparation and Filling in Cell

Particles solution is shaken well in 1.5ml appendorf tube so that particles are equally distributed in the water and not settled down at the bottom. For Free diffusion experiment, $100\mu l$ of particle solution is inserted in the sample cell with the help of Pipette. Sample should be inserted in the cell very carefully so that no air bubble is created with in the cell. For experiment with bacteria, $200\mu l$ of particle solution is taken in new appendorf tube along with $200\mu l$ of bacteria culture solution. Filling in the sample cell is just like I described above.



(a) (b)

(c) (d)

Figure 2.2: (a) Glass slides (b)Parafilm cutting to make cell (c)Cell created after heating on hotplate (d) Sample filling

2.4 Experimental Setup

The schematic diagram of the experimental setup is shown in Fig: 2.3. White light flashes on the sample which is used to illuminate the sample. A 20x objective

is used for magnification. A deflecting mirror is used to direct light coming from objective towards CCD monochrome camera. CCD camera is controlled by the computer for digital microscopy.

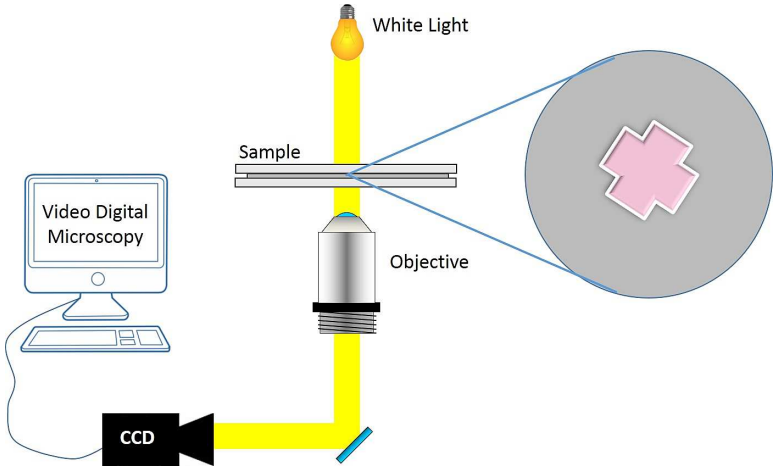


Figure 2.3: Schematic of experimental setup

2.5 Data Collection and Data Analysis

Particles after being inserted into the sample cells are studied under home made inverted microscope. White light is used for illumination. Dynamics of particles are video recorded by IDS CCD monochrome camera. Resolution(Mpix) is 5.20 and resolution(hxv) is 2448x2048. Image size in the video is 512x512 pixels^2. Nikon 20x objective with numerical 0.5 is used in the microscope. The videos are recorded at 50 pixel clock and 19.71 fps(frames per seconds). The recorded videos are then analyzed with LabView code which tracks the position and orientation of the particle in each frame. MSD (Mean square displacement),Trajectories and orientation of particles is obtained using Mathematica code.

Chapter 3

Results and Discussion

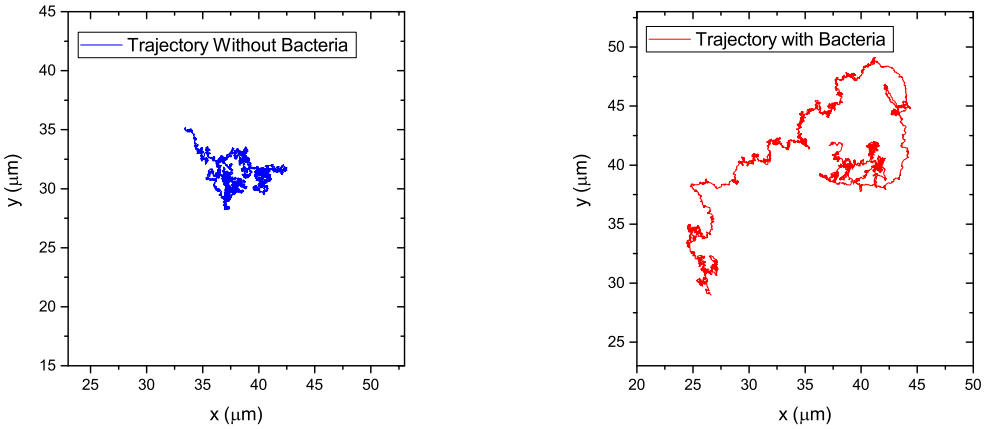
3.1 L-shape particles

The diffusion of L-shape particles is studied using digital video microscopy. The trajectories of the particles are obtained by tracking the position of particle in each frame. Fig: 3.1 (a) and (b) shows the trajectory of the particles with and with out bacteria. It has been observed that the particle in active bath explored more than in the case of passive particle revealing that particles are self propelled by the bacterial activity. In order to understand the dynamics of L-shape particles with and without bacteria, we quantified the obtained trajectories by their mean square displacement(MSD). Fig: 3.2 (a) shows comparison of MSDs with and with out bacteria. The blue curve corresponds to non-bacteria case. The slope of the MSD curve will determine the dynamics of the particle. In the case of particles without bacteria, the observed slope is ≈ 1 confirming they exhibit similar Brownian like motion as predicted by Einstein theory. While in the case of L-shape particles with bacteria what we observed is completely different due to their self propelling behavior. The obtained MSD (red curve)shows a slope greater than 1 and they exhibit super diffusion. At short time scale, it behaves almost similar to passive case. However at longer time scale L-shape particle shows super diffusion due to presence of bacterial activity. Due to anisotropic nature

of the particle we anticipate the angular orientation of the particle in addition to translational motion. Thus orientation of the particle is also analyzed for both bacteria and non-bacteria cases. We noticed two different type of particles with respect to orientation.

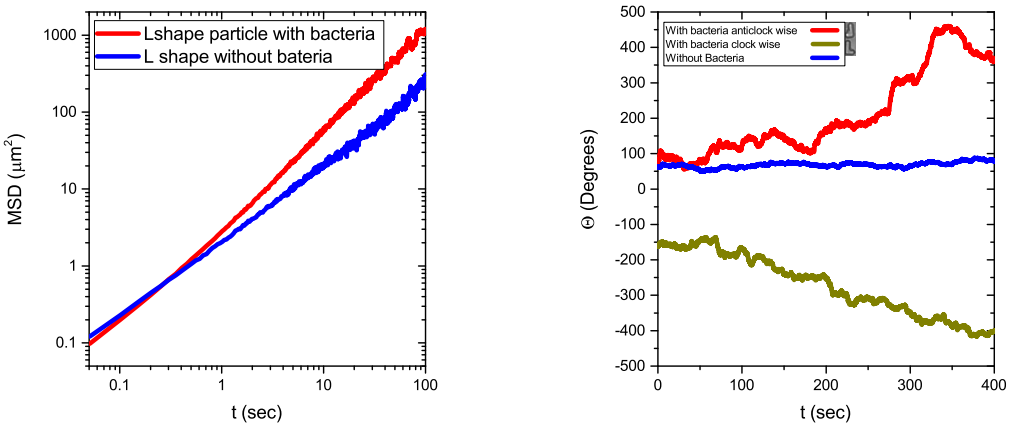
- Clockwise rotating particle
- Anti-clockwise rotating particle

Orientation of both clockwise and anti-clockwise particles with bacteria and with out bacteria is shown in the Fig.3.2 (b). The blue line corresponds to non-bacteria case. It can be seen clearly that the particle in passive medium shows very little variation in angular orientation. The red and yellow lines shows angular orientation with bacteria. The graph shows clearly that with bacteria particles show more rotation as compared to passive case.



(a) (b)

Figure 3.1: (a) L-shape Particles Trajectory without Bacteria showing free brownian diffusion (b)L-shape particles Trajectory with Bacteria which shows more diffusivity as compared to passive case



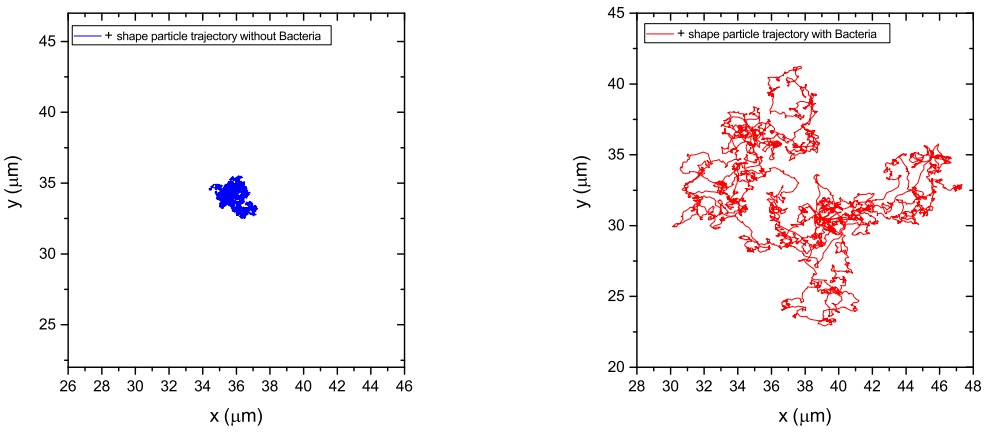
(a) (b)

Figure 3.2: (a) MSD Comparison of L-shape particles with and without bacteria. Without bacteria MSD has slope 1 indicating free Brownian diffusion. However with bactria MSD has slope greater than 1 indicating super diffusion (b)Comparison of orientation of L-shape particles with and without bacteria which shows that particle rotate more freely in active bath as compared to passive case.

3.2 Cross Shape particles

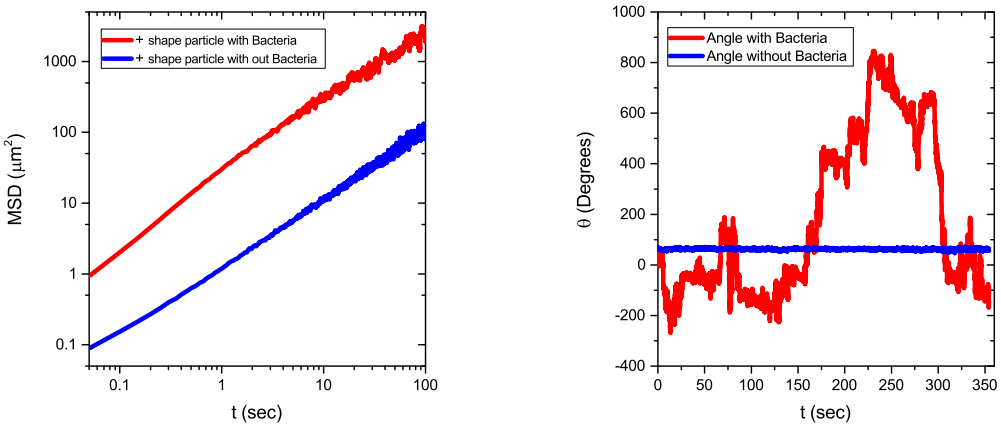
In this section, we present the dynamics of cross-shape particles using digital video microscopy. The trajectories of the particles are obtained by tracking the position of particle in each frame just like we did for L-shape particles. Fig: 3.3 (a) and (b) shows the trajectory of the particles with and with out bacteria. Again one can notice that the particle in active bath explored more than in the case of passive particle revealing that particles are self propelled by the bacterial activity. The obtained trajectories of cross-shape particle with and without bacteria are quantified by mean square displacement(MSD). Fig: 3.4 (a) shows the comparison of MSDs with and with out bacteria. The blue curve corresponds to non-bacteria case whose MSD behave differently at short time scale. While at long time scale, the observed slope is ≈ 1. Similar behavior has been observed for anisotropic particles exhibiting free Brownian motion. In the case of Cross-shape

particles with bacteria, we observed again different behavior due to their self propelling property. The obtained MSD (red curve)shows a slope greater than 1 in short times exhibiting super diffusion and in longer time scale MSD grows linearly with a slope 1 exhibiting enhanced Brownian diffusion. This observation is similar to spherical particles diffusion in active medium as shown in Fig.1.11 (b). The angular orientation of the cross shaped particle with bacteria and with out bacteria is shown in the Fig.3.4 (b). The blue line corresponds to non-bacteria case. It can be clearly noticed that the particle in passive medium shows very little variation in angular orientation. While the red line shows angular orientation with bacteria Which shows clearly that their rotation is enhanced when compared to passive case.



(a) (b)

Figure 3.3: (a) Cross-shape Particles Trajectory without Bacteria showing free brownian diffusion (b)Cross-shape particles Trajectory with Bacteria which shows more diffusivity as compared to passive case



(a) (b)

Figure 3.4: (a) MSD Comparison of Cross-shape particles with and without bacteria. Without bacteria MSD behave differently and short time scale then increase linerely in longer time scale having slope 1 indicating free Brownian diffusion. However with bactria MSD has slope greater than 1 indicating super diffusion (b)Comparison of orientation of Cross-shape particles with and without bacteria which shows that particle rotate more freely in active bath as compared to passive case.

3.3 Z-shape Particle

This section focuses on the diffusion of Z-shape particles. The trajectories of the particles are obtained by tracking the position of particle in each frame. Fig: 3.5 (a) and (b) shows the trajectory of the Z-shape particles with and with out bacteria. Likewise in L-shape and cross-shape particles, the Z-shape particle in active bath explored more than in the case of passive particle confirming their propulsion due to bacterial activity. We quantified the obtained trajectories by their mean square displacement(MSD) and Fig: 3.5 (c) shows MSDs comparison of Z-shape particles with and with out bacteria. The blue curve corresponds to non-bacteria case whose MSD behave differently in short time scale (slope $\neq 1$). At long time scale, their slope is ≈ 1 confirming the Brownian nature of motion. While in the case of Z-shape particles with bacteria (red curve) we observed a slope greater than 1 in both short and long time scale exhibiting super diffusion.

The angular orientation of the Z-shape particle for both bacteria and non-bacteria cases. Angular orientation with bacteria and with out bacteria is shown in the Fig.3.5 (d). The blue line corresponds to non-bacteria case and the red line shows angular orientation with bacteria. The plot shows clearly that with bacteria, the particles show more rotation as compared to passive case.

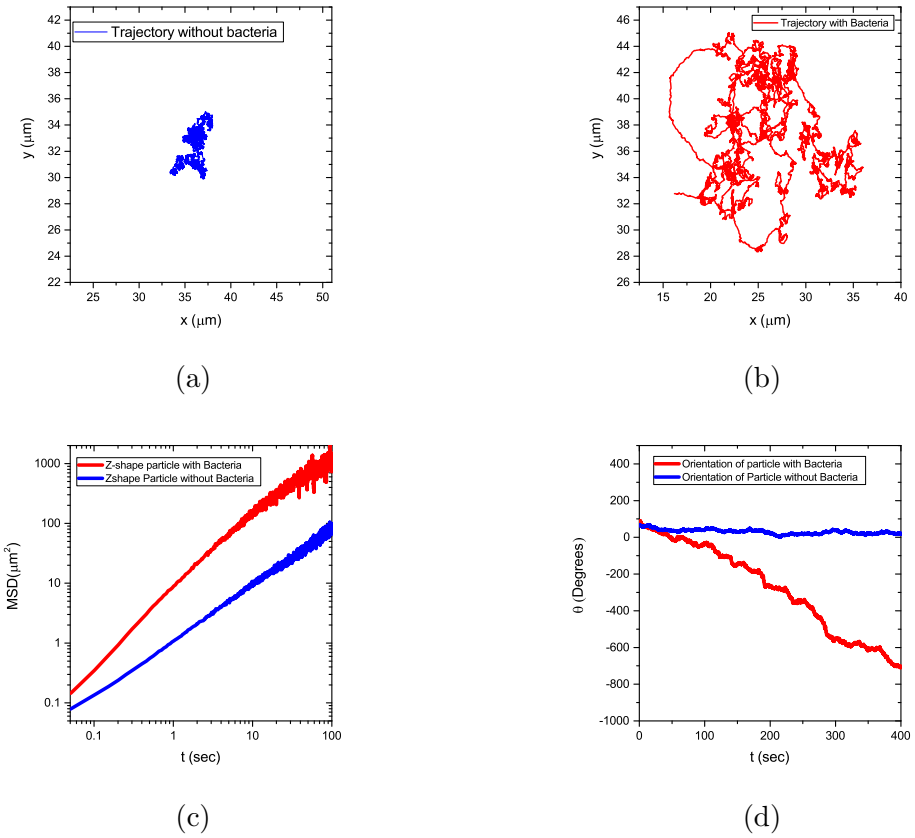
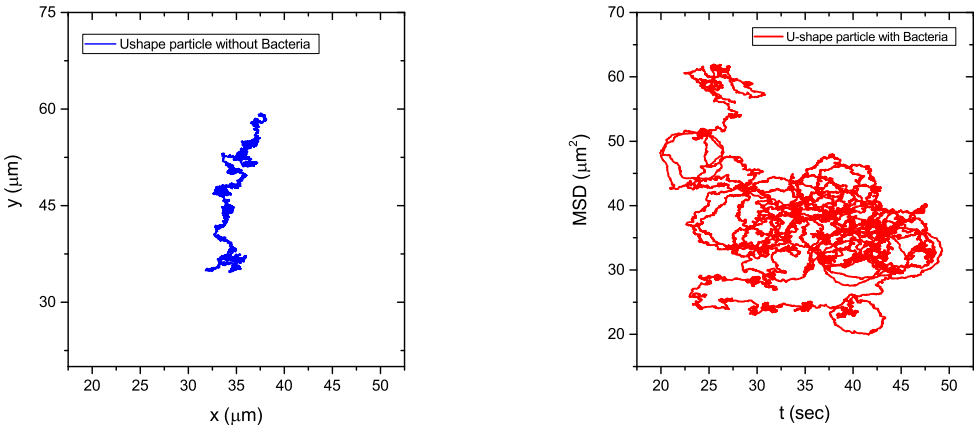


Figure 3.5: (a) Z-shape Particles Trajectory without Bacteria showing free brownian diffusion (b)Z-shape particles Trajectory with Bacteria which shows more diffusivity as compared to passive case (b) MSD Comparison of Z-shape particles with and without bacteria. Without bacteria MSD behave differently and short time scale then increase linerely in longer time scale having slope 1 indicating free Brownian diffusion. However with bactria MSD has slope greater than 1 indicating super diffusion (d)Comparison of orientation of Z-shape particles with and without bacteria which shows that particle rotates more in active bath as compared to passive case.

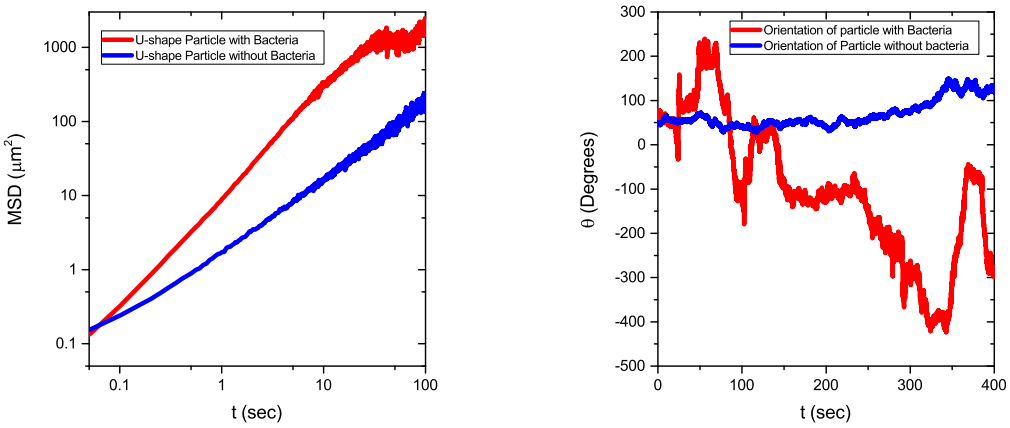
3.4 U-shape Particle

In this section we will report the diffusion of U-shape particles studied by digital video microscopy. In Fig: 3.6 (a) and (b) the trajectory of the particles with and with out bacteria are shown. It has been observed that the particle in active bath explored more than in the case of passive particle confirming the self propulsion by the bacterial activity. Like other particles, the obtained trajectories are quantified by their mean square displacement(MSD). Fig: 3.7 (a) shows comparison of MSDs with and with out bacteria. The blue curve corresponds to non-bacteria case whose MSD shows different bahavior in short time scale. In long time scale, the observed slope is ≈ 1 just like other anisotropic particles discussed in previous sections. While for case of U-shape particles with bacteria (red curve) we observed the slope of obtained MSD greater than 1 in all time scales. The Orientation of the particle is analyzed for both bacteria and non-bacteria cases that are shown in the Fig.3.7 (b). The blue line corresponds to non-bacteria case and the red line shows angular orientation in the presence of bacteria. The graph shows clearly that with bacteria, the particles show more rotation as compared to passive case.



(a) (b)

Figure 3.6: (a) U-shape Particles Trajectory without Bacteria showing free brownian diffusion (b)U-shape particles Trajectory with Bacteria which shows more diffusivity as compared to passive case



(a) (b)

Figure 3.7: (a) MSD Comparison of U-shape particles with and without bacteria. Without bacteria MSD behave differently and short time scale then increase linerely in longer time scale having slope 1 indicating free Brownian diffusion. However with bactria MSD has slope greater than 1 indicating super diffusion (b) Comparison of orientation of U-shape particles with and without bacteria which shows that particle rotates more in active bath as compared to passive case.

3.5 Star-shape Particle

In this section, we will study the diffusion of Star-shape particles using digital video microscopy. In Fig: 3.8 (a) and (b) we show the trajectory of the particles with and with out bacteria. We observed that both the trajectories seem similar to each other, however their MSDs shown in Fig: 3.8 (c) shows some difference between them. In the case of particles without bacteria just like previously discussed particles, MSD (blue curve) behave differently in short time scale (slope $\neq$ 1). In long time scale, the observed slope is $\approx$ 1 exhibiting free Brownian motion. While in the case of Star-shape particles with bacteria what we observed is that the MSD (red curve) has almost same slope as non-bacterial case showing only enhanced Brownian diffusion instead of super diffusion. The angular orientation is analyzed for particle both in bacteria and non-bacteria cases. As shown in

Fig.3.8 (d) the blue line corresponds to non-bacteria case and the red line corresponding to the particles with bacteria. The graph shows clearly that with bacteria particles show more rotation as compared to passive case.

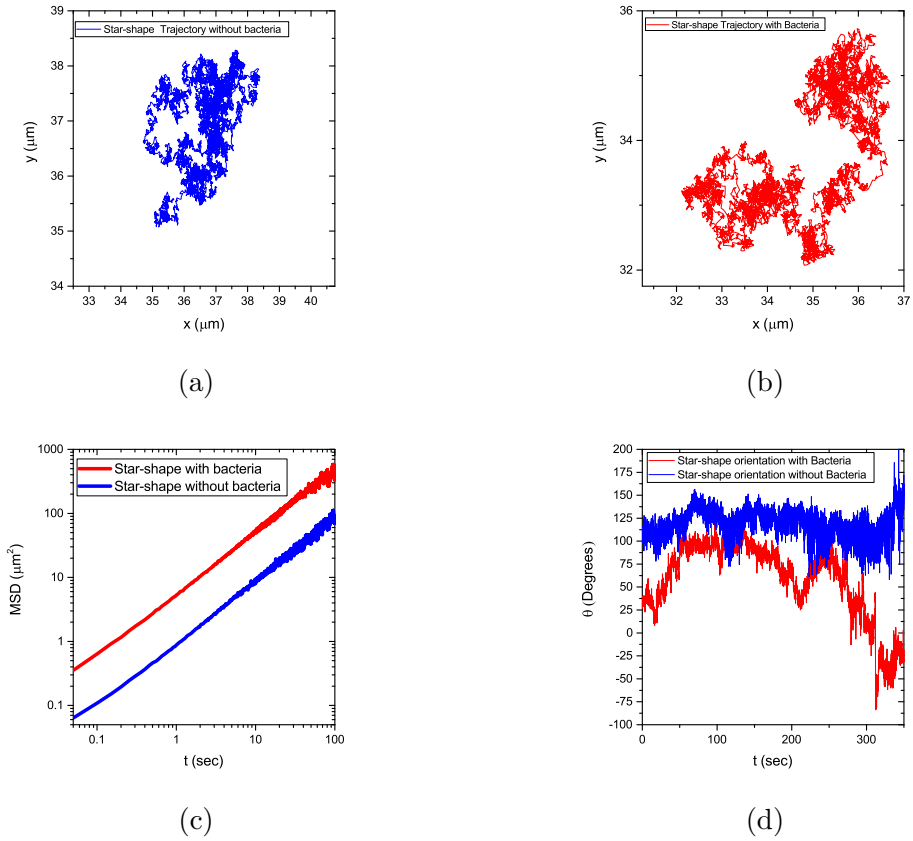


Figure 3.8: (a) Star-shape Particles Trajectory without Bacteria showing free brownian diffusion (b)particles Trajectory with Bacteria which shows enhanced Brownian diffusion instead of super diffuaion (b) MSD Comparison of Star-shape particles with and without bacteria. Without bacteria MSD behave differently and short time scale then increase linerely in longer time scale having slope 1 indicating free Brownian diffusion. With bactria MSD has similar slope only exhibiting enhanced Brownian diffusion (d) Comparison of orientation of Star-shape particles with and without bacteria which shows that particle rotates more in active bath as compared to passive case.

Chapter 4

Conclusion

So far in the previous section, we shown different behavior of anisotropic particles in active bath. In Fig. 4.1, we show the comparison of the MSDs obtained for various anisotropic particles (L , U , Z , Star , Cross) along with the isotropic spherical particle MSD. We are aware of that for the simplest case of spherical particle whose MSD slope equal to 1 confirming Brownian motion. Our experiment on anisotropic particles reveal that the MSDs at long time shows slope ≈ 1 similar as observed for spherical particles. How ever they show different behavior at short time due to the anisotropic nature [11].

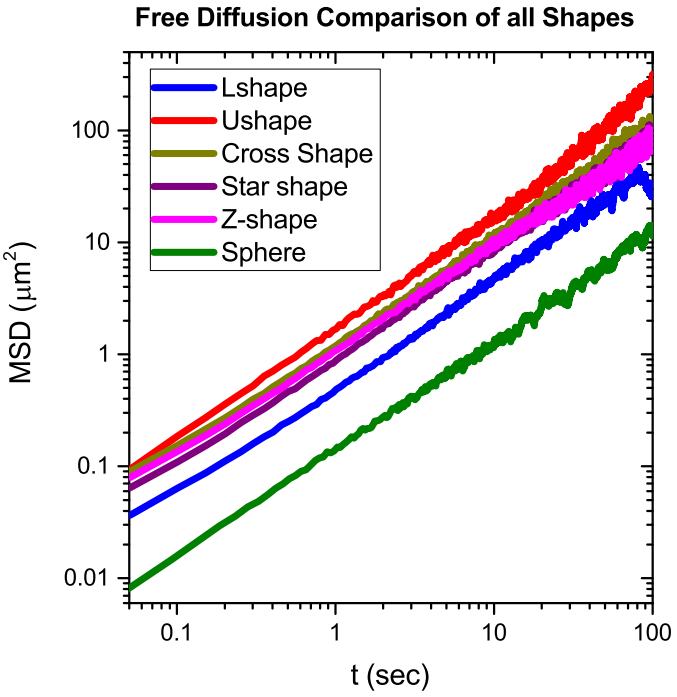


Figure 4.1: MSD Comparison for passive case

Apart from the behavior of various anisotropic particles in thermal bath, we observe interesting features of anomalous diffusion when these particles were in active bath (with bacteria). In Fig. 4.2 the comparison of MSDs obtained for various anisotropic particles in active bath are shown along with the isotropic spherical particle in active bath. In comparison between different anisotropic shape particles, The cross shape particle show similar behavior to the spherical particles in active bath showing short time super diffusion and long time enhanced Brownian diffusion. The other particles (L , U , Z) show different behavior than cross-shape particles. All the particles show super diffusion at both short and long time scale. The slopes of L-shape, Z-shape and U-shapes particles are 3.5, 4.4 and 5 respectively which shows the super diffusive nature of the particles. As U-Shape particle has the slope greater than all other particles. So U-shape particle is the most super diffusive particle in active bath.

The star-shape particle, however showed completely different behavior as compared to all other. Cross-shape particle showed only enhanced Brownian diffusion in the active bath which is because of its symmetry and anisotropy. The diffusion behavior of all the particles can be summarized in the table 4.1.

Particle Shape	Short Time Super diffusion	Long Time Enhance Diffusion	Short Time and Long time Super Diffusion
Spherical	✓	✓	
Cross	✓	✓	
L-shape			✓
U-shape			✓
Z-shape			✓
Star		✓	

Table 4.1: Diffusion behavior of particles in active bath in short and long time scale

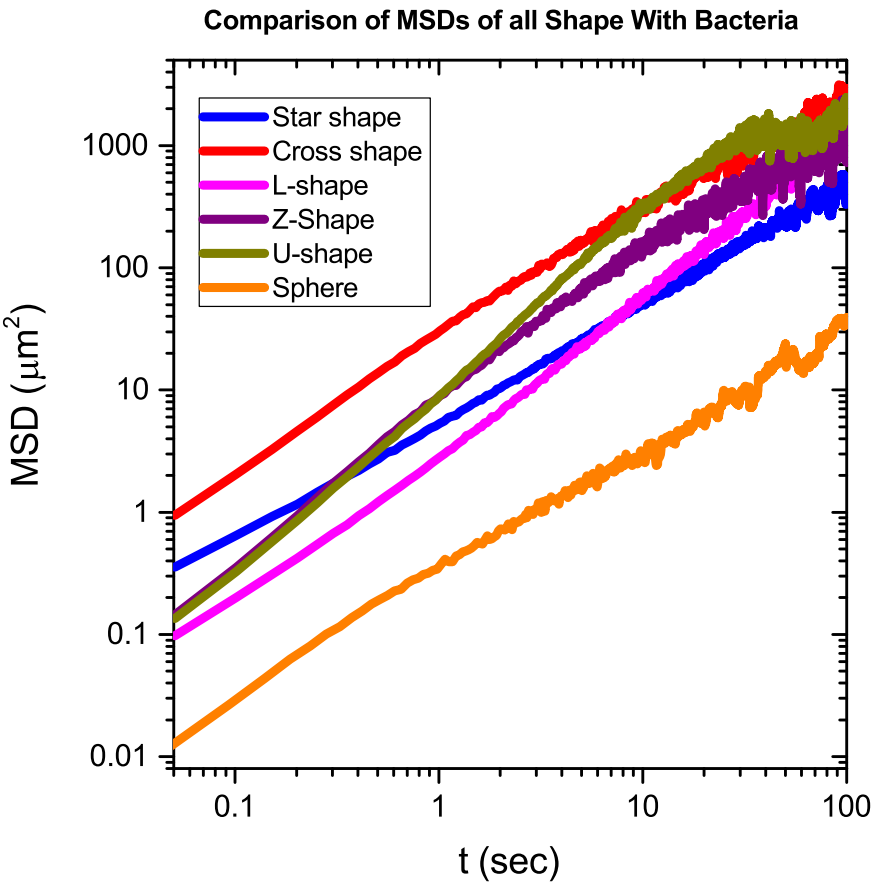


Figure 4.2: Comparison of MSDs of the particles with bacteria

Comparison of angular orientation for both particles in active and thermal bath is given in the Fig. 4.4 and 4.3. In the case of particles in thermal bath, we observe very little angular variation and translation motion is dominated. In active bath, we observe much enhances angular variation and particles tends to rotate and move simultaneously.

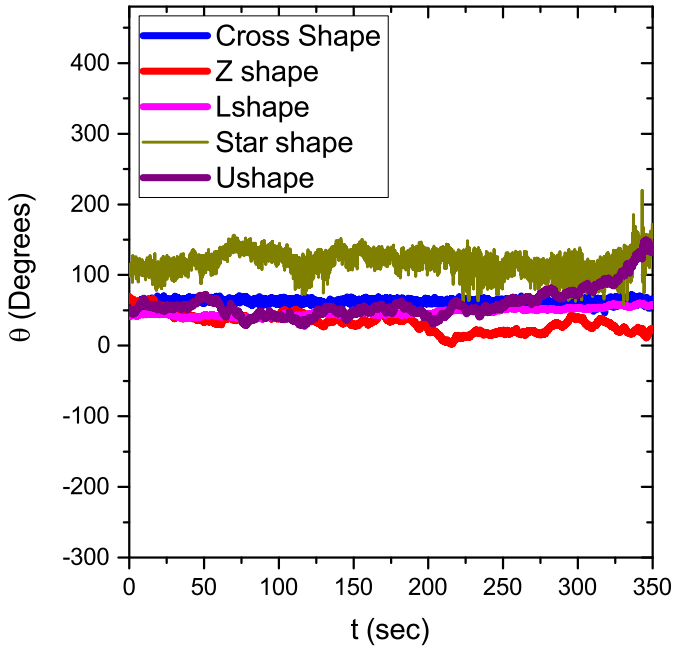


Figure 4.3: Comparison of angular orientation of the particles with out bacteria

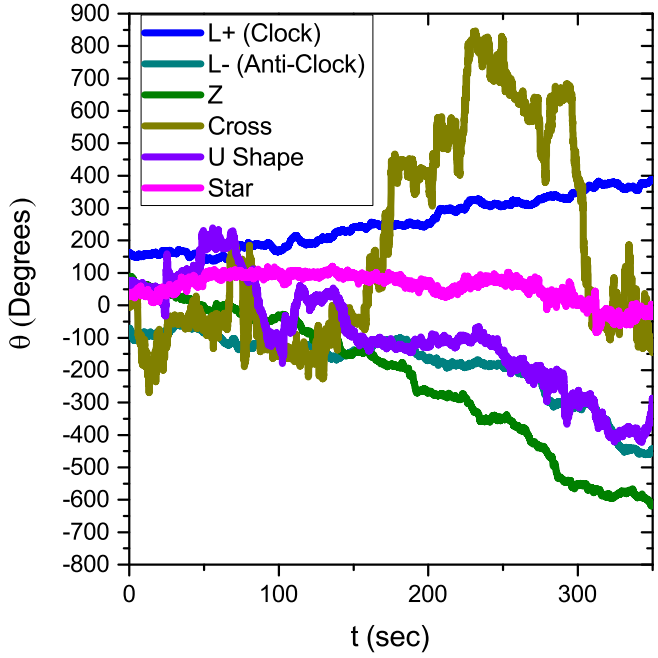


Figure 4.4: Comparison of angular orientation of the particles with bacteria

To summarise, we fabricated various shape anisotropic particles (L, U, cross, star and Z) using soft softlithography and studied their motion dynamics in thermal, active bath and compared with that of spherical particles (isotropic). In thermal bath, all the anisotropic particles show Brownian motion at longer times $\gg 0.3$ s similar to isotropic particles. For short times $\ll 0.3$ s they show different behaviour due to the anisotropic nature of the particles. Interesting motion dynamics features i.e., active Brownian motion were observed, when these various anisotropic particles were suspended in the bath of E. coli cells. The particles display super diffusion behaviour due to the collisions with the E. coli cells. In addition, we observed that the shape anisotropy in particles play an important role in their motion mechanisms. The swim pressure of E. coli cells vary with the particle shape anisotropy and thus their motion dynamics were altered. Further, we also investigated their rotational orientation in addition to translation motion due to their anisotropic nature. Among various shaped particles, U-shape particles were promising in performing translation motion than others as their MSD slope$\approx$5. With respect to angular rotation, our results suggest that cross-shape particles perform highest rotation than other anisotropic particles. Our study can be extended to guide one or predict or even design a new shape of particles whose dynamics can be controlled in active bath. As controlling the motion dynamics is quite challenging and it is important for many potential applications such as target delivery, bioremediation and even in lab-on-chips.Therfore, it is worthy to study further in detail in order to control their dynamics to make them suitable for applications.

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