ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

CONCENTRATION AND DETECTION OF BACTERIA WITH COMBINED AC ELECTROKINETIC AND IMPEDANCE ANALYSIS IN MICROFLUDIC SYSTEMS

M.Sc. THESIS

Kadriye ÖLMEZ

Nanoscience and Nanoengineering Department

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ISTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

MİKROAKIŞKANLARDA AC ELEKTROKİNETİK TEKNİKLERLE EMPEDANS TABANLI BAKTERİ ALGILAMASI

YÜKSEK LİSANS TEZİ

Kadriye ÖLMEZ
(513141017)

Nanobilim ve Nanomühendislik Anabilim Dalı

Nanobilim ve Nanomühendislik Programı

Tez Danışmanı: Doç.Dr.Hüseyin KIZIL

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Kadriye Ölmez, a M.Sc. student of ITU **Graduate School of Science Engineering and Technology** student ID 513141017, successfully defended the thesis entitled "**Concentration And Detection of Bacteria with Combined AC Electrokinetic and Impedance Analysis in Microfludic Systems** "which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below

Thesis Advisor : **Assoc. Prof. Dr. Hüseyin KIZIL**
İstanbul Technical University

Jury Members : **Prof. Dr. Esra Özkan ZAYİM**
Istanbul Technical University

Prof. Dr. Alper KİRAZ
Koç University

Date of Submission : 2 May 2018
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To my familiy,

FOREWORD

I would like thank my supervisor Assoc. Prof. Dr. Hüseyin Kızıl for his kind support and his guide. That was a good oppurtunity work his under supervisor. The experience I achieved will lead my future work. I also thank to my lab friends especially Emre Altınağaç working with them, conducting experince together was both enjoyfull and instructive. Finally but not last sincereley thanks to my dear familiy for their help and support me every decision I make.

June 2018

Kadriye Ölmez
(Biomedical Engineer)

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ABBREVIATIONS

AC	**:** Alternating Current
EP	**:** Electrophoresis
DEP	**:** Dielectrophoresis
p-DEP	**:** positive- Dielectrophoresis
n-DEP	**:** negative- Dielectrophoresis
EOF	**:** Electroosmotic force
MEMS	**:** Microelectro Mechanical Systems
EDL	**:** Electrical Double Layer
CM	**:** Classius-Mossoti
DNA	**:** Deoxyribonucleic acid
UV	**:** Ultraviolet
PVD	**:** Physical Vapor Deposition
PDMS	**:** Polydimethylsiloxane
CNT	**:** Carbon Nano Tube
cfu	**:** Colony Forming Unit

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CONCENTRATION AND DETECTION OF BACTERIA WITH COMBINED AC ELECTROKINETIC AND IMPEDANCE ANALYSIS IN MICROFLUIDIC SYSTEMS

SUMMARY

Microfluidic systems for pathogen detection is a enabling and emerging technology since it is cost affective, fast response and accurate results are obtained. Conventional bacteria detection tecniques are time consuming, labour, expensive and also require more sample that increase contamination risk. Electrokinetic tecniques combined with impedance analysis is an effectice way to detect bacteria with the advancement in micromachinig technology which leads new insight into microfluidic systems. Manipulation of bacteria cells, viruses, cancerous cell etc. under ac electrokinetic field provide a micro/nano hand in microfluidic channel via translational movement, attracting or reppelling behaviour in channels to electric field.

Bacteria related diseases is a common and costly public health problem around the world. Detection time and detection at low concentration level of bacteria are important parameters to study to improve. Impedance analysis combined with electrokinetic forces are effective method and important analytical tool for detection, concentration, seperation and trapping of bacteria cell. Rapid and early detection of bacteria is crtical in blood, food, water for diagnostic and control purposes. Concentration and focusing bacteria at same chip are performed by ac electrokinetic tecniques and detected by impedance analysis.

Concentrating bacteria is an critical step in order to enhance sensitivity of detection limit to be able to sense the existance of bacteria population. For label free detection geometrical architecture of system is important for detection limit in term of use electric field effectively. At this point a variety of geometrically confine region are created to find better design. The role of focusing region is faciliate to concentrate bacteria to by transporting to spesific region which is called detection region. The contribution of focusing region is shown experimentally. Dielectrophoretic and electroosmotic forces are useful electrokinetic ways to manipulate bioparticle cells. p-DEP forces are used to focus and concentrate bacteria at focusing region while impedenace spectrometry is performed at detection region to analyse detection of bacteria with Electrochemical impedance analyser.

E.coli NCTC (13167) and Enterobacter aerogenes bacteria cells are used in this study. The response of bacteria cells to external electric field depend on their size, shape, internal structure, electrical conducitivity and permittivity. Since each bacteria has charecteristic electrical properties, their response to dielectrophoretic forces will be different for same frequency and in same medium. This different response can be used for seperation of different type bacteria as well as can be used discriminate

bacteria based on different dielectric properties with analysing impedance spectroscopy.

Every bacteria has characterisitc electrical properties that will be useful for detection with help of impedance analysis. These difference electrical properties of bacteria may use to identify them without using any antibody which incerase complexity of system and the cost. To enhance the electrical signal some optimization of geometrical structure ofelectrodes and microfludic channel is employed to increase detection limit.

In microfluidic channel the effect of p-Dep are seen on the edge of electrodes where the electric field intensity is relatively high than the centre of electrode. Once bacteria cells are gathered at middle of channel, they are drived to detection region by hydrodynamic drag forces since inertial forces are insigficant at such low dimension. At detection region concentrated bacteria is analysed with change in impedance. The flow rate is another factor that change electrical impedance the more flow rate the more larger change in impedance response is obtained. At high frequency impedance response that are measured at detection region dominated resistivity of solution. In another saying the effect of bacteria cell is insgificant. In order to observe electrical property of bacteria low frequency is applied since most bacteria are polarizable in electric field and at low frequency bacetria cells tend more polarizable than fluid in microfluidic channel.

Bacteria is a prokaryotic cell which has a conducting cell wall and insulating plasma membrane surround the cytoplasma and divided to gram negative and gram positive bacteria according to difference in the cell walls. Cell wall cover the insulating cytoplasmic membrane. Cell wall in gram negative bacteria consist of outer membrane and periplasmic space. Periplasmic space include a thin peptidoglycan layer which is main component of cell wall. AC electric field that apply bacteria cell alternate between low impedance region (cell wall) and high impedance region (lipid membranes). Bacteria cell has main two high impedance lipid regions are outer membrane in cell wall which existed only in negative gram bacteria and inner membrane (cytoplasmic, plasmic membrane). Altough these two structure are consist of double layer phospholipid layers outer membrane has lipopolysaccharide component which carry negative charges and responsible for negative charge and hydrophilic surfaces which make its permittivity greater than inner membrane’s.

For each bacteria same amount of cells are conducted and the impedance responses are compared. Approximately 500 cfu/ml, 1000 cfu/ml, 5000 cfu/ml, 20000 cfu/ml are conducted for each bacteria at same medium for time and frequency dependent measurements. In order to see the contrubition of dielectrophoretic force 3.2 μm size polystyrene particles are conducted in 0.00025 S/m distile water. At this point expreiments performed with dep and without dep. In the medium of distile water p-dep is observed at the range of between 100 Hz-5 Mhz ferquency at focusing region. Results indicate that the impedance decreases with dep forces which increase sensitivity of impedance based biosensor. Another parameters that increase sentivity is narrow detection electrodes and decrease channel height. Particles concentrated at the edge of electrodes at p-dep while at n-dep concentrated on the center of electrode than drive to detection region for impredance analysis. The impedance change at detection region decreases with increasing frequency applied. Results show that after approximately 100 kHz the impedance response is purely resistive and independent

of number of bacteria. The detection limit for E.coli bacteria and Enterobacter aerogenes is determined as 500 cfu/ml.

MİKROAKIŞKANLARDA AC ELEKTROKİNETİK TEKNİKLERLE EMPEDANS TABANLI BAKTERİ ALGILAMASI

ÖZET

Mikroakışkan sistemler patojen algılamada hızlı ve doğru sonuç alma yönünden maliyeti düşük, yeni ve gelişen bir teknolojidir. Elektrokinetik tekniklerin empedans analizi ile birlikte bakteri tespiti mikroelektromekanik sistemlerdeki yeni gelişmelerle birlikte etkili bir yöntemdir. Bakterilerin, virüslerin ve kanser hücreleri ve benzerlerinin mikrokanallarda elektrokinetik kuvvet ile hücrelere döndürme, hücreleri elektrik alanından uzaklaştırma yada elektrik alanına yakınlaşırma gibi mekanik bir bileşen olmadan yönlendirilmelerin yapılması mümkündür.

Bakteri kaynaklı hastalıklar dünya genelinde çok yaygın olup tespiti maliyetlidir. Tespit zamanı ve düşük sayıda algılanması geliştrilmesi gereken önemli parametrelerdir. Mikro teknolojideki gelişmeler elektriksel kuvvetin makro ölçülerden mikro ölçülere uygulanmasını mümkün kılmıştır. Elektrokinetik kuvvetler empedans analizi ile birlikte bakteri hücrelerinin ayrıştırılması, konsantrasyonu ve algılanmasında etkili ve efektif bir yöntem olup kanda, gıdada ve suda erken tespiti kritiktir. Bakteri konsantrasyonun arttırılması algılamada hassasiyetin arttırılması için önemli bir adımdır. Herhangi bir ajan kullanılmadan oluşturulan bu sistemlerde kullanılan geometrik yapılar elektriksel alanının etkili bir şekilde kullanma yönünden önemli bir unsurdur. Bu aşamada bir çok geometrik model nümerik ve deneysel olarak denenmiş optimum model bulunmaya çalışılmıştır.

Dielektroforetik ve elektroforetik kuvvetler biyoparçacıkların manipulasyonunda etkili elektrokinetik tekniklerdir. Bu çalışmada E.coli NCTC(13167), Enterobacter aerogenes bakterileri kullanılmıştır. Bakterilerin elektriksel alana tepkisi, bakterilen büyüklüğüne, şekline, hücre ve sıvının iletkenlik ve geçirgenliğine ve uygulanan frekansın büyüklüğüne bağlıdır. Bakterilerin dilektroforetik kuvvetere karşı tepkisi birbirinden farklı olacağı için, dielektoforetik kuvvetler hücrenin diğer hücrelerden ayrıştırılmasına, belli bir bölgede hapsetme veya algılama gibi bir çok amaç için kullanılmaktadır.

Dielektroforetik kuvvet, hücreler çevresini saran sıvıdan daha fazla polarize olma durumunda göre daha yoğun olan elektriksel kuvvete doğru çekilirler ve bu kuvvet pozitif dielektoforetik kuvvet olarak adlanıdırlır. Hücrelerin daha az polarize olma durumunda zayıf elektriksel alana doğru itilir ve negatif dilektroforetik kuvvet olarak adlandırılır. Mikroakışkan kanallarda p-Dep in etkisi elektriksel alanın daha yüksek olduğu elektrot kenarlarında gözlenir. Bakteriler kanalın orta bölgesinde konumlandırılır ve hidrodinamik kuvvetin etkisiyle algılama bölgesine sürüklenir. Algılama bölgesinde uygulanan 1 kHz ile 500 Khz aralığında bakteri konstantrasyonuyla değişen empedans değişimleri ile bakteri algılaması yapılır. Akış hızı empedansı değiştiren diğer bir etmendir. Daha hızlı akan bir akışta daha büyük empedans değişimleri elde edilir. Yüksek frekanslarda ölçülen empedans cevabı büyük oranda sistemin direnç özelliğinden kaynaklanır bakterilerin etkisi görülmez.

Bakterilerin etkisinin görülebilmesi için düşük frekanslar uygulanmalıdır. Düşük frekanslarda bakteriler daha çok polarize eğilimini gösterirler.
Bakteri hücreleri prokoryatik hücreler olup duvar yapılarındaki farklılıklardan dolayı gram negatif ve gram pozitif bakteri olarak ikiye ayrılırlar. Genel olarak her iki bakteri türünde sitoplazmayı çevreleyen yalıtkan sitoplazmik zar ve iletken olan ince hücre duvarı tabakası bulunmaktadır. Gram negatif bakterilerde hücre duvarı dış bir zar ve periplazmik aralıktan oluşmuştur. Periplasmik aralıkta bulunan peptidoglikan tabakası gram negatif bakterilerde ince iken gram pozitif bakterilerde daha kalın olup bu tabaka bakterilen en iletken tabakasıdır. Bakterilere uygulanan elektriksel kuvvet düşük empedans bölgesi (hücre duvarı) yüksek empedans bölgesi (sitoplazmik zar, dış zar) arasında değişir. Bakterilerde bulunan iki yüksek empedans bölgesinden olan hücre duvarını çevreleyen çift fosfolipit tabakasından oluşan dış zar sadece gram negatif bakterilerde bulunmaktadır. Bu yağ tabakasından dolayı uygulanan düşük frekanslar bu tabakayı geçememektedir. Öte yandan yüksek frekanslarda ise elde edilen empedans değerleri ise bakteri konstrasyonundan bağımsız olup sistemin direnç değerlerini göstermektedir. Her bakterinin karakterisitik elektriksel özelliği bulunmaktadır ve bu özellik empedans analizi ile bakterileri algılamada kullanılabilir. Bakteriler arasındaki bu farklı elektriksel özellikler sistemin karmaşıklığını ve maliyetini arttıracak olan herhangi bir antikor kullanılmadan bakterileri algılama ve tanımlamada kullanılabilir. Bu elektriksel sinyalin güçlendirilmesinde kullanılan mikroakışkan kanallardaki geometik yapılar ve CNT gibi malzemelerin kullanılması algılama limitini yükseltecektir.

Üretilen sensör konsantasyon ve algılama bölgesi olmak üzere iki bölümden oluşmaktadır. Dielektroforetik kuvvet kullanılarak bakteri konsantrasyonu için birbirine geçmiş tarak şeklindeki paralel elektrotlar, 45° C eğimli paralel elektrotlar, asimetrik elektrotlar ve balık sırtı şeklinde elektrolar üretilmiştir. Üretilen bu elektrotların genişliği 15 mikron, aralarında uzaklıklık da 10 mikron olacak şekilde tasarlanmıştır. Söz konusu tasarımlar comsol multipysic 5.2 yazılımı kullanılarak her bir tasarım için dielektroforetik kuvvetleri simüle edilmiştir. Konsantre edilmiş bakterilerin ikinci bölgede empedans analizi ile algılamasını yapacak birbirine geçmiş tarak şeklindeki paralel elektrotlar üretilmiştir. Sensörün üretiminde öncelikle cam atlıkları temizlemek için KOH çözeltisi kullanılarak, camlar 10 dakika boyunca ultrasonik banyo cihazında bekletip saf su ile banyo edildikten sonra, aseton çözeltisinde 10 dakika boyunca bekletilmiş sırasıyla alkol ve saf su ile banyo edilip kurutulmuştur. Temizlenen camlar spin kaplama cihazı ile AZ9260 fotoresist kullanılarak 5 mikron yüksekliğinde fotoresist ile kaplandı. Farklı geometrilerde üretilen maske kullanılarak istenen tasarım litografi tekniği ile cam altlıklar üzerine aktarıldı sonrasında fiziksel buhar yöntemi ile 200 nm yüksekliğinde Titanyum katmanı oluşturulup aseton solüsyonunda çözdürülüp istenilen tasarımlar cam altlıklarda oluşturulmuştur. Bakterilerin akışı için 25 mikron yüksekliğinde mikrokanallar pdms malzemesi kullanılarak oluşturuldu. Pdms 10:1 oranında katılaşıtırıcısı ile birlikte kullanılarak, kabarcıkları vakum ortamında alınmış ve 90^0 C de 20 dakika boyunca hot plate cihazında bekletilmiştir. Elde edilen pdms, kanal tasarımının bulunduğu 25 mikron yüksekliğinde SU-8 kaplı altlık üzerine dökülüp kanal tasarımı ve yüksekliği pdms tabakasına aktarılmıştır. Pdms tabakası ve titanyum biriktirilmiş cam altlık plazma bonding yöntemi ile birleştirişmiş, 45°C de hot plate üzerinde 10 dakika bekletilmiştir.

İki bakteri içinde aynı konsantrede farklı miktarlarda empedans ölçümleri alındı. Sırasıyla 500, 1000, 5000, 20000 cfu/ml konsantrelerinde aynı sıvıda zamana bağlı ve frekansa bağlı empedans ölçümleri alındı. Empadans analizinde dielektroforetik kuvvetin etkisini görmek için 3.2 mikron büyüklüğündeki polyestren partikülleri ile 0.00025 S/m iletkenliğindeki distile su kullanıldı. Konsantrasyon bölgesindeki elektrotlara dep uygulayarak ve uygulamayarak alınan sonuçlarda dep uygulanan deneylerde daha büyük empedans değişimi görüldü. Dilektororetik kuvvet sensör hassasiyetini arttıran önemli bir etmendir. Distile suda p-dep 100 Hz-5 Mhz arası frekans aralığında görüldü. Tespit edilmesi istenen sıvının iletkenliğine göre gerekli frekans ayarlanark hem p-dep hem de n-dep ile konstrasyon yapmak mümkündür. P-dep te partiküller elektrot kenarlarında odaklanırken n-dep ile elektrot merkezinde odaklanırlar. Algılama bölgesinde uygulanan frekans arrtıkça empedanstaki değişim azalmaktadır, yaklaşık 100 kHz den sonra uygulanan deneylerde empedans cevapı tamamıyle dirence bağlı olarak değişmekte ve bu bölgedeki bakteri sayısından bağımsız hale gelmektedir. E.coli ve Enterobacter Aerogenes ile yapılan deneyler sonucu sensörün algılama limiti 500 cfu/ml konsantrasyonudur.

1. INTRODUCTION

Microfluidic systems or lab-on- chip is expanded scope of miniaturized total chemical analysis systems which based on performing of fluidic devices in micro/nano scale volumes **[1]**. Researchs on microfluidic devices and their fabrication with micromechanics technology back on 1970s which firstly start with gas chromotatograph and ink jet printer nozzles than continue with flow sensors, valves to complex microfluidic systems for chemical analysis and numerical simulation became used for more complex structures and systems **[2]**.Microfluidic system is a set of technology that handle fluids in geometrically constrained to a small area typically larger than 1μm and less than 1 mm **[3]** manipulation and contol of fluid, suspended particles and cells in a confined small area.

Bacteria related diseases is a common and costly public health problem around the world. Detection time and detection at low concentration level of bacteria are important parameters to study to improve. Impedance analysis is a effective method and important analytical tool for detecting, concentration, seperation and trapping of bacteria cell **[4-16]** that shown in table 1.1.

Incorporation of MEMS into microfluidic devices offer oppurtunities to utilize new detection mechanisms and high level of functionality in chemical and biological applications which strongly depend on advancement in MEMS technology.

Behaviour of fluid at micro scale is quite different than macro scale. Reynold number is ratio inertia to viscous force and it is a dimensionless number to determine the flow regime whether it is laminar or turbulent. When Reynold number in microfluidic device is smaller than 1 the regime is assumed as laminar

$$Re = \frac{pvD}{\mu} \qquad \textbf{(1.1)}$$

where p is the density of fluid, v is the characteristic velocity of the fluid, μ is the viscosity of fluid, and D_h is the diameter of hydraulic. When viscous forces are dominant the regime is considered as a laminar flow and when inertial forces are dominant the regime is considered as a turbulent flow.

Newton's law is inertia force equal sum total of all forces on fluid parcel. One side of newtons law is sum total of actual forces acting on fluid parcel. The other side mass times acceleration is assumed to be a kind of virtual force which is referred to as inertia force. If inertia force is sum total of all forces including the viscous forces(as per newton's law), inertia force must be greater than viscous forces. It implies Reynolds number which is ratio inertia to viscous force must always be greater than 1. But Reynolds number can be less than 1 because forces are vectors and if two vectors add together magnitude of resultant vector may be smaller or larger than any of vectors being added **[2], [27].**

1.1 Electrokinetic Force

Electrokinetic is a force that manipulate fluids (charge or polarizable) and suspended particles by appliying electrostatic force on a charged or polarizable fluids and particles without any mechanical component **[1], [17], [18].** With advancement in micromachining technology scaling down of electric field become possible to micro/nano scale thus electrokinetic forces in microfluidic channels become dominant effect to manipulate particles compare to mechanical based microfludic channels. Fabricating micro/nano mechanical part at this scale both difficult and it is not efective as much as electrokinetic phenomena **[3].** Electrokinetic forces are divided to electrophoresis, dielectrophoresis, electroosmotic and electrothermal subgroups.

1.1.1 Electrical Double Layer

Solid surface in aquous ionic solution generally has a thin layer that negatively or positevely charged as a result of electrostatic interaction by attraction of counterions and repelling of co-ions. This formed thin layer called electric double layer that consist of stern and diffuse layers. Due to strong electric in stern layer, electrons can not move but electons in diffuse layer are free to move. The electric potential that

arises from diffuse layer construct one of electrokinetic forces which is electrosmotic force **[3].**

The thickness of electric double layer is determined by Debye length which depends on the bulk concentration and the ionic solution shown in Figue 1.1.

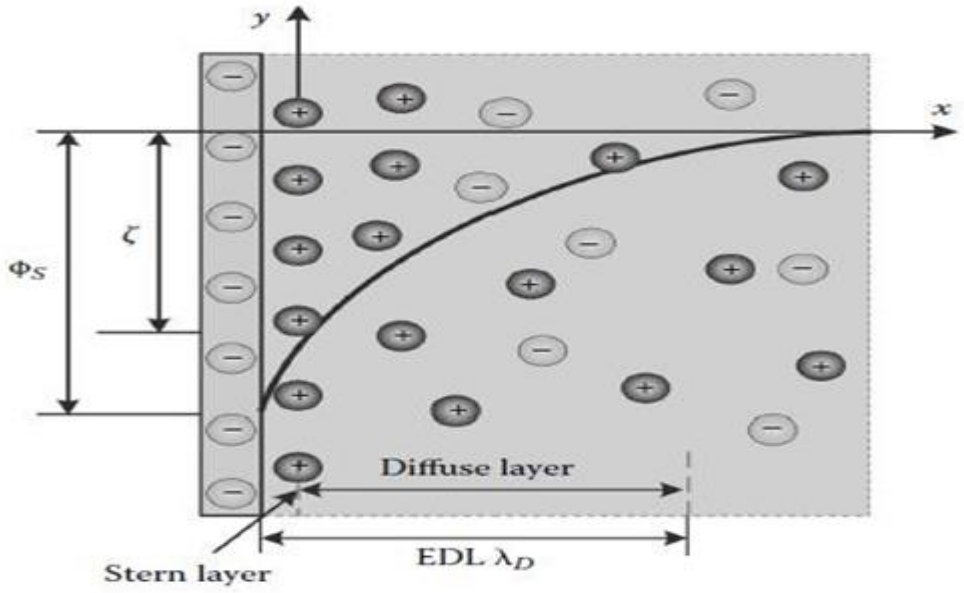


Figure 1.1: Negatively charged EDL **[3].**

1.1.2 Electroosmotic Force

EO phenomena is interaction between electrical double layer and electrolytes by applying electric field thus motion flow is created by pulling fluid along the channel for purpose of particle trapping, purification and sorting. Characteristic length of EDL is given by Debye Length. Debye layer: electric double layer occur due to interaction between an electrolyte and charged solid surface. EO forces mainly depend on conductivity of medium, diffusion coefficient and permittivity **[3]** Electroosmotic forces are formulated as;

$$F = E \sum F_{Z_i C_i} = -\varepsilon_0 \epsilon_f \nabla^2 \Phi E \quad \textbf{(1.2)}$$

Where E external electric field.Therefore fluid motion is governed by Navier-Stoke equation.

$$p = \left(\frac{\partial u}{\partial t} + u.\nabla u\right) = -\nabla p + \mu \nabla^2 u - \varepsilon_0 \varepsilon_f \nabla^2 \Phi E \quad \textbf{(1.3)}$$

where ρ is the fluid density; **u** is the fluid velocity; *p* is the pressure; and μ is the fluid dynamic viscosity that shown in Figure 1.2.

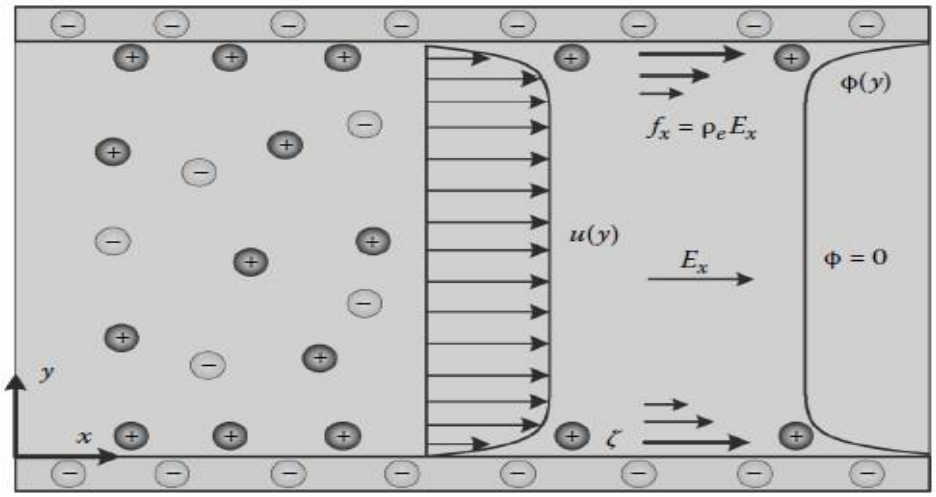


Figure 1.2: The fluid motion is governed by the modified Navier–Stokes equation **[3]**

1.1.3 Electrophoresis

Electrophoresis is the motion of charged particle under electric field in a sationary liquid. Particle will move either to the cathode or to the anode depend on the sign of surface charge of particle. Electrophoresis is widely used in seperation of particles and mathematical models have been developed to understand the nature of this phenomena in detail. The difference between electrosomotic flow and electrophoretic flow is: The channel wall (solid) in EOF is stationary, fluids moves under electric field shown in Figure 1.3.

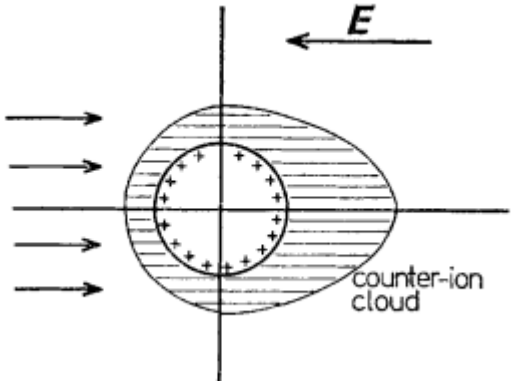


Figure 1.3: Motion of charged particle under electric field **[41]**

1.1.4 Dielectrophoresis

DEP is the motion of polarizable of particle under non-uniform electric field **[3], [5].** Dielectrophoresis phenomena first used by Pohl. The phenomena occurs due to the interaction of induced dipoles with electric fields and can be used to exhibit motions such as repulsion, attraction and rotation by adjusting dynamic electric field. Relative polarizability of the particle with respect to surrounding medium means DEP doesnt depend on the charge of particle **[3]**

The dielectrophoretic force is given by:

$$F_{DEP} = \frac{1}{4}\vartheta Re[\alpha]\nabla E^2{}_{\text{rms}} \quad \textbf{(1.4)}$$

The averaged time Alternating current (AC) DEP force on a spherical particle of radius r is given by;

$$F_{DEP} = 2\pi r^3 \varepsilon_0\, \varepsilon_f Re[K(w)]\nabla|E_{rms}|^2 \quad \textbf{(1.5)}$$

Where ϑ is volume and r is the radius op particle of ineterest, ε_0 is relative permittivty, ε_f is permittivity of fluid, [K(w)] is Clausius–Mossotti factor, $\nabla|E_{rms}|^2$ is electric field gradient **[18].**

Re[K(w)] represents the real part of the Clausius–Mossotti factor determine the behaviour of particle whether toward to strong electric field which named positive dielectrophoresis or away from from electric field which is negative dielectrophoresis

Re[K(w)] represents the real part of the Clausius–Mossotti factor expressed by;

$$K(w) = \frac{\varepsilon^*{}_p - \varepsilon^*{}_f}{\varepsilon^*{}_p + 2\varepsilon^*{}_f} \quad \textbf{(1.6)}$$

Where ε_p and ε_f are the complex permittivity of particle and fluid respectively.

Complex permittivty is given by:,

$$\varepsilon^* = \varepsilon - \frac{j\sigma}{2\pi f} \quad \textbf{(1.7)}$$

Where f is frequency of applied electric field, ε and σ is permittivity and conductivity of particle or fluid.

While the real part of Clausius–Mossotti factor determine attract-repell motion pf particle the imaginary part of the Clausius–Mossotti factor represent the rotation motion of particle

Using of DEP enhance the signal magnitutude and reduce the detection time. If a particle moves toward the high electric field it is positive DEP, if particle repelled to lower region it is negative DEP shown in Figure 1.4 and Figure 1.5.

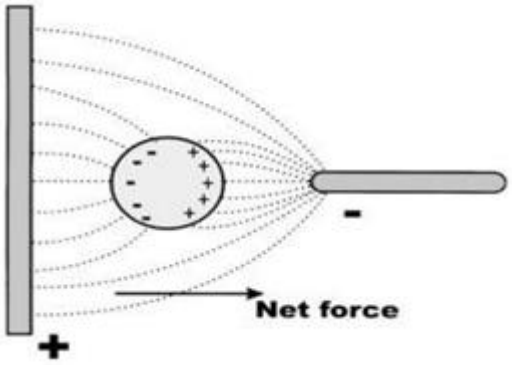


Figure 1.4 : p-DEP forces

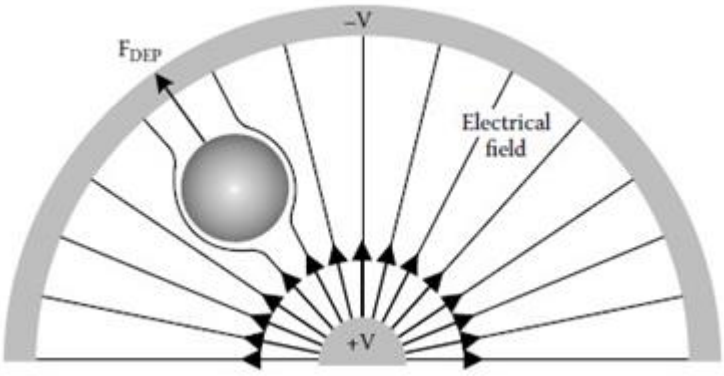


Figure 1.5 : n-DEP forces

1.1.5 Electrothermal Forces

Electrothermal effects are seen when high electric field applied as a result large power generated inside channels which calculated by Joule law for power per unit volume:

$$W = \sigma E^2 \qquad \textbf{(1.8)}$$

As can be seen from formula generated power highly depend on conductivity of medium and non-uniform electric field. Such large power generated at this micro/nano scale increase temperature rapidly. In order to calculate temperature energy balance equation needed to solve which is:

$$p_m c_p v.\nabla T + p_m c_p \frac{\partial T}{\partial t} = k\nabla^2 T + \sigma E^2 \quad \textbf{(1.9)}$$

Where c_p is specific heat capacity, v is velocity T is temperature, k is the thermal conductivity and σ is the conductivity of the fluid **[17], [20].**

Electrothermal effects are seen generally at high conductive mediums and are effectice tools for mixing or micropumps purposes. Electrothermal forces cause joule heating that may interrupt the flow regime by introducing bubbles **[33], [34].**

As a result:Both AC Electrothermal and Electroosmosis useful for micropumping and micromixing purposes

Both AC Electroosmosis and Ac electrophoresis is used for particle seperation, trapping, purification purposes

AC Electrophoresis allows for seperation of different species based on unique electrophoretic mobilities **[3]**

2. BACTERIAL CELLS

2.1 Structure of Bacteria

Bacteria is a prokaryotic cells which has a conducting cell wall and insulating plasma membrane surround the cytoplasma. Cell wall cover the insulating cytoplasmic membrane.

Cell wall in gram negative bacteria consist of outer membrane and periplasmic space. Periplasmic space include a thin peptidoglycan layer which is main component of cell wall (Figure 2.1 and Figure 2.2). This part of bacteria reported as more conductive layer as about 2.2-2.3 S/m **[35]**. This high conductivity of this layer in low conductive solution is due to fixed mobile charges in cell wall.

AC electric field that apply bacteria cell alternate between low impedance region (cortex,core) and high impedance region (lipid membranes). Bacteria cell has main two high impedance lipid regions are outer membrane in cell wall which existed only in negative gram bacteria and inner membrane (cytoplasmic, plasmic membrane). Altough these two structure are consist of double layer phospholipid layers outer membrane has lipopolysaccharide component which carry negative charges and responsible for negative charge and hydrophilic surfaces which make its permittivity greater than inner membrane's. Due to this lipid layer structures low frequncies are not expected to alternate across this layers. On the other hand low frequency dispersion of E. coli cell suspensions is not related to the potential difference across the plasma membrane but to the ion clouds outside and/or inside the cell wall containing charged residues **[36].**

Different proteins, surface proteins, amino acids, polysaccharide and dipicolinic acid compositions in bacteria cells are critical parameters to evaluate discrimination between bacteria and which gives distinct electrical properties of each individual bacteria.

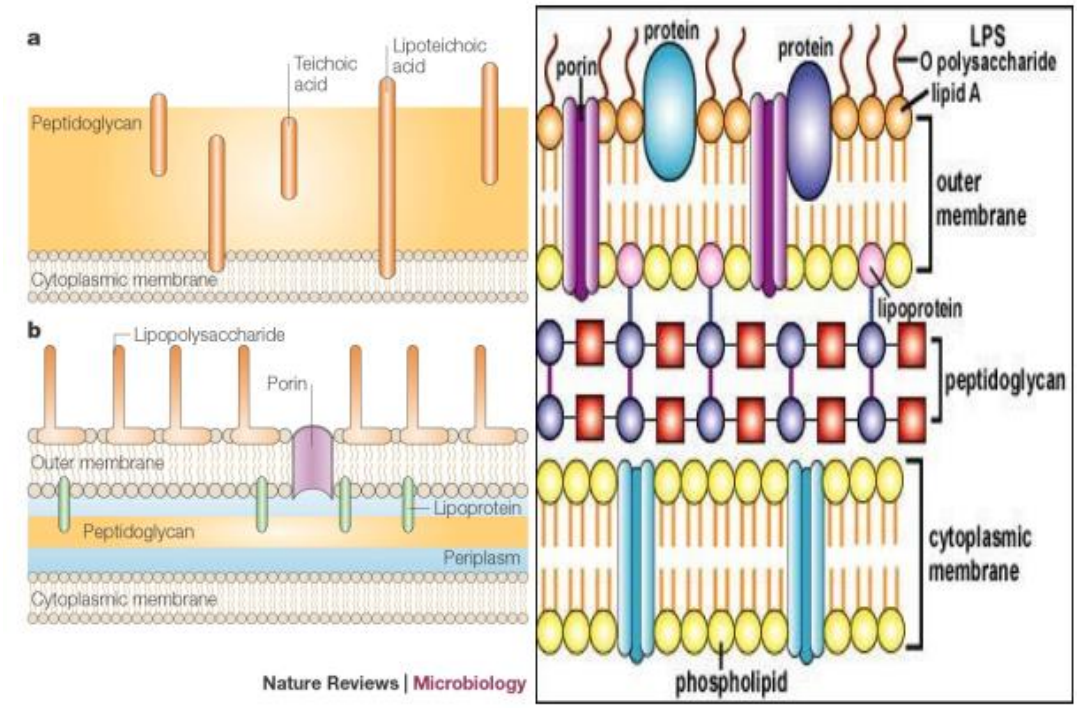


Figure 2.1: Chemical cell structure of a) Gram positive bacteria b) Gram negative bacteria

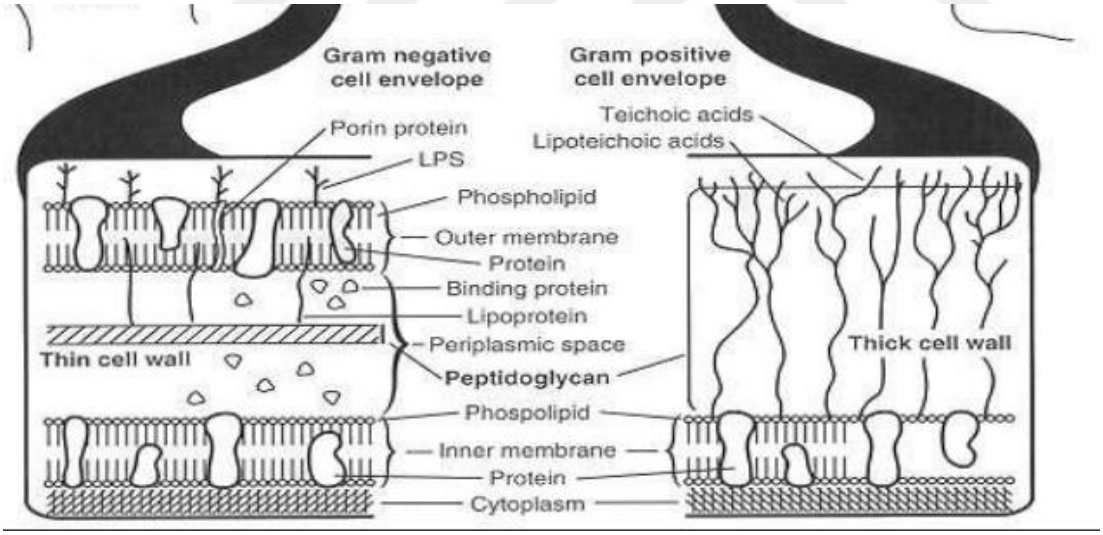


Figure 2.2: Schematic view cell structure of (left) gram negative bacteria (right) gram positive bacteria

2.2 Modelling of E.coli

2.2.1 Single shell spherical model

E.coli modeled as a spheroid covered with cytoplamic membrane. At this model membrane capacitance and the cytoplasmic conductivity is determined but insufficient to determine frequency dependent conductivity and permittivity of cell suspensions and the other electrical properties of cell components **[35]**.

2.2.2 Two shell spherical model

E.coli modeled as a elipsoid covered with two shell that correspond to cytoplasmic membrane (protoplasm) and cell wall **[37], [38].**

High conductivity of cell wall is demonstrated at low conductive solution at low frequency **[37].**

2.2.3 Three shell spherical model

In this model cell wall more detailed since it consist of outer membrane and periplasmic space. Three shell correspond to outer membrane, periplasmic space, cytoplasmic membrane (inner membrane) that shown in Figure 2.3 **[35]** and these three layers have different electrical properties

The frequency dependence of dielectric constant and conductivity of E.coli for four electrical properties are;

1.Conductivity of cell wall (outer membrane and periplasmic space) (Peptidoglycan, lipoprotein (consist of 57 aminoacide))

2.Dielectric constant of cell membrane (Lipid bilayer structure)

3,4.Dielectric constant and conductivity of cytoplasm (Semi electrolyte solution) shown in table 2.1 and 2.2.

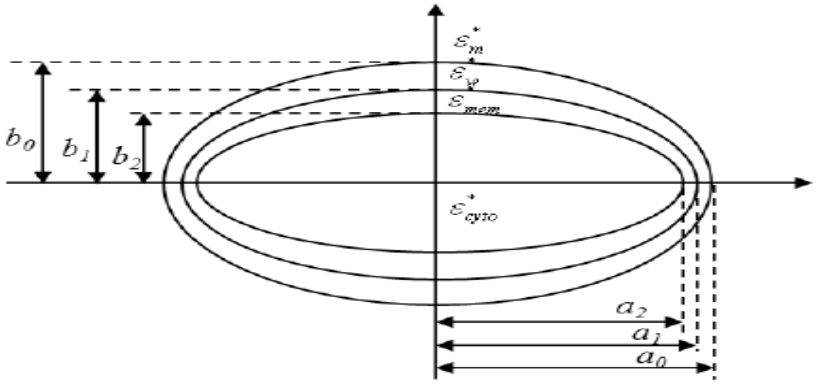


Figure 2.3: Three shell modelled of gram negative E.coli bacteria

Table 2.1. The comparison of the phase parameters estimated with the three-shell ellipsoidal model at various conductivities of the outer membrane

κ_{om} (S/m^{-1})	P	ε_{om}	ε_{im}	ε_{cp}	κ_{cp} (S/m^{-1})	κ_{pp} (S/m^{-1})
0	0.083±0.014	10.0±0.7	5.5±0.3	108±5	0.22±0.03	3.2±0.2
10^{-6}	0.083±0.014	10.0±0.7	5.5±0.3	108±5	0.22±0.03	3.2±0.2
10^{-5}	0.083±0.014	10.2±0.7	5.5±0.3	108±5	0.22±0.03	3.2±0.2
10^{-4}	0.085±0.014	12.1±0.8	5.5±0.3	108±5	0.22±0.03	3.2±0.2
10^{-3}	0.101±0.016	34±2	4.9±0.2	108±5	0.22±0.02	3.3±0.2

Table 2.2. Electrical properties of two shell modelled of gram negative bacteria

	Conductivity (S/m)	**Relative permittivity**	**Size (µm)**
Cytoplasm	$\sigma_{cyto} = 0.19$	$\varepsilon_{cyto} = 61$	$2a_2 = 1.09$ $2b_2 = 2c_2 = 1.09$
Membrane	$\sigma_{mem} = 5*10^{-8}$	$\varepsilon_{mem} = 10.8$	$2a_1 = 1.09$ $2b_1 = 2c_1 = 0.64$
Cell Wall	$\sigma_{wall} = 0.68$	$\varepsilon_{wall} = 60$	$2a_0 = 1.09$ $2b_0 = 2c_0 = 0.68$

.

3. IMPEDANCE BASED DETECTION

A number of detection systems are reported for electrochemical and biological analysis such as electrochemical, piezoelectric, thermometric, magnetic and optical **[22]** Electrochemical based detection is one of the most effective method since more sensitive, less time consuming and easy implementation to electric systems. Electrochemical based detection mainly consist of amperometry, impedimetry, potantiometry tecniques.

Impedance is the complex ratio of the voltage to the current in an alternating/direct current circuit **[24]** Non invasive method for counting, identifying and monitoring cells **[23]** Generally frequency dependent a small voltage is applied and the electric current response is measured after that the impedeance of system can be measured from given formula:

$$Z(w) = \frac{U(w)}{I(w)} = Z_{RE} + jZ_{IM} \quad \textbf{(3.1)}$$

The magnitude (IZI) and phase angle (θ) of the complex impedance are :

$$|Z| = \sqrt{(Z_{RE})^2 + (Z_{IM})^2} \quad \textbf{(3.2)}$$

$$\theta = \arctan(\frac{Z_{IM}}{Z_{RE}}) \quad \textbf{(3.3)}$$

The impedeance of each particle and total impedeance of system is:

$$\frac{1}{|Z|} = \frac{1}{|Z_1|} + \frac{1}{Z_2} \quad \textbf{(3.4a)}$$

$$|Z_1| = \sqrt{{R_{sol}}^2 + \frac{1}{(\pi f C_{dt})^2}} \quad \textbf{(3.4b)}$$

$$|Z_2| = \frac{1}{2\pi f C_{de}} \quad \textbf{(3.4c)}$$

where IZI is the total impedance, IZI_1 is the impedance of R_{sol}, R_{sol} is the resistance of solution, IZI_2 is the impedance of C_{dt}, C_{dt} is capacitance of double layer, C_{de} is the dielectric capacitance of solution and f is the excitation frequency **[25]**

Impedeance based detection is related with electrical properties of targeted particle (Figure 3.1 and 3.2). Each particle has a distinct and inherent electrical property **[28].**

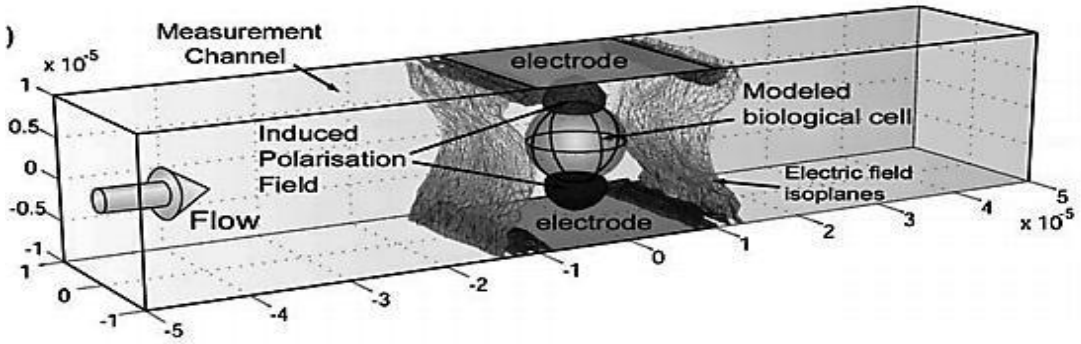


Figure 3.1: Impedance analysis of single cell between paralel electrodes

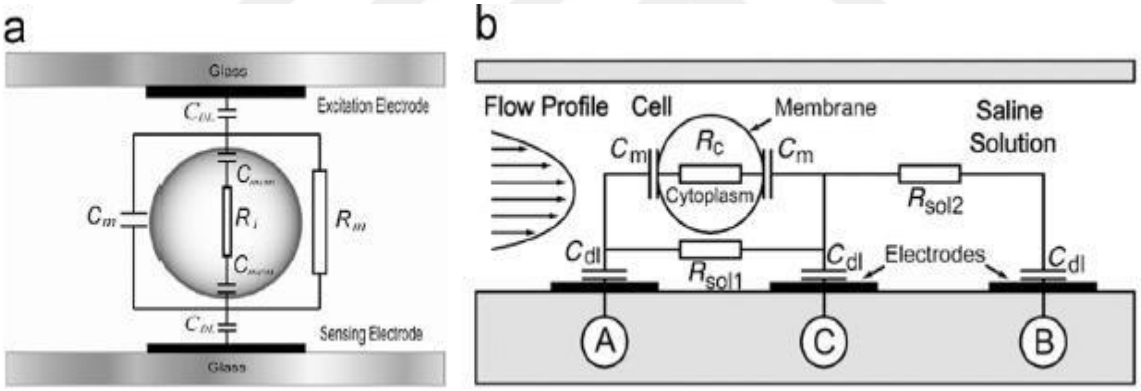


Figure 3.2: Equivalent electrical circuit of microfludic channel with suspended particle

Impedeance based detection is effectice method to detect bacteria as well as viruses, antibody, DNA etc. Detection of single cell is also possible, **[26]** and used to cell counting, identification **[23], [28]** and study of behaviour of cells **[25]**

3.1 Electrical Equivalent Circuit Model

Analysis of electrical equivalent circuit indicates that at high frequency impedance change dominated by resistivity of solution thus the effect of bacteria cell is negligible. In order to

observe electrical property of bacteria, low frequency is need to applied since most bacteria are polarizable in electric field and at low frequency, baceteria cells tend more polarizable than fluid in microfluidic channel

At low frequencies (< 10 kHz) the impedance response is dominated by capacitive impedance (mainly Cdl). At high frequency (≥ 100 kHz) the effects of capacitive component is negligible and reponse becomes purely resistive.

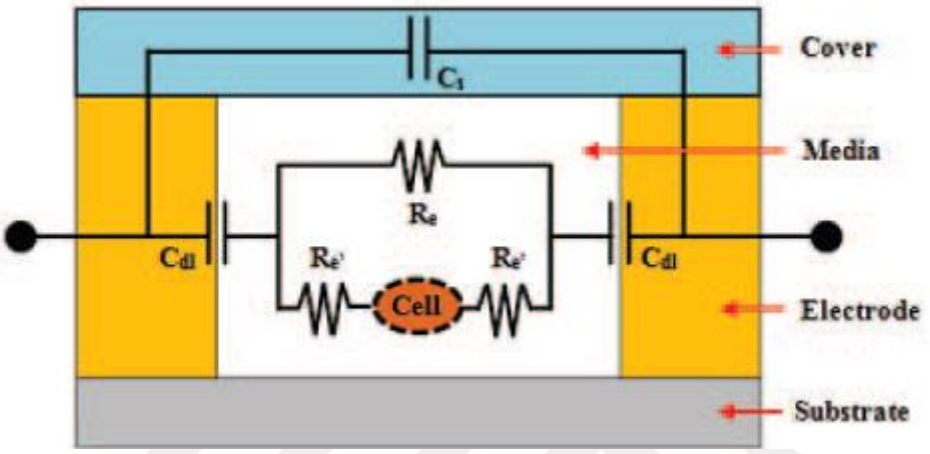


Figure 3.3: Electrical Equivalent Circuit Model **[39].**

Parameters that prevent accurate measurement of biophyiscal properties of biological cells are: Electrical double layer capacitance, high impedeance of electrodes, stray capacitance **[25].** Insulater based systems are obviate this problems but require more electric field gradient which may increase unwanted joule heating problem that will disturb flow regime.

With larger surface area and signal amplification capability of nano-materials, the high aspect ratio of microfluidic devices may result in increased sensitivity and low limit of detection. To increase magnitude of DEP force without increasing electrothermal effects is possible by increasing number of gaps between electrodes, decrease height of channel and employing asyymetric electrodes. Use of CNT at microfluidic chip offer some important advantages such as: High surface volume to ratio provide high absorptivity of particle,very high electrical conductivity (sensitive to small conductance change) and high thermal conductivity. **[29-32].**

Table 3.Literaure Review

Author	Electrode Shape	Principle	Performance	Bacteria	Bio-affinity element	Buffer	Conductivity	Frequency/voltage	Flow Rate	Concentration
Kim et al (2015)	Interdigitated electrode	DEP-IA	Concentration, Trapping and Detection of bacteria	E.coli (ATCC8739)	no element	reverse osmosis water and phosphate-buffered saline	1.5 S/m for Pbs and 0.0086S/m for RO water	10-100 kHz for pDep	1500 µL/h	300 CFU/mL
Hamada et al (2013)	Interdigitated Cr electrode	EPA-DEPIM-IA	Concentration and detection of bacteria	E.coli(K-12 NBRC3301)	no element	Manital Solution	0.1 mS/m	1 kHz and 100 kHz for Ndep and Pdep respectively	0.27 m/s	NA
Enegl et al (2013)	Pt electrode (polyacrylamide gel)	Free flow EP (supress EOF)	Concentration and trapping of bacteria	E. coli of the strains XL1-blue and K12	no element	sodium borate	250 µS/cm	230 V/m	15 µl/min	$5x10^4$ CFU/ml
Wang et al (2013)	Pt electrode function as a bridge	EOF-EP	Detection and quantification of E.coli	E.coli (DH5a)	no element	Phosphate buffered saline	1.5 S/m	80 volt	NA	1×10^4 1×10^6 5×10^6 $1x10^7$ cells/ml
Tan et al (2011)	Pt wire electrodes	NA	Detection of bacteria	E.coli(O157:H7) and Staphylococcus aureus	Nanoporous Al membrane with GMPS for SAM layer and anti-E. coli O157:H7 antibody	Phosphate buffered saline	1.5 S/m	1 Hz to 100 kHz/ 50 mVpp	NA	100 CFU/ml

Author	Electrode Shape	Principle	Performance	Bacteria	Bio-affinity element	Buffer	Conductivity	Frequency/voltage	Flow Rate	Concentration
Lu et al (2008)	Interdigitated Ti/Au electrode	IA	Single E.coli detection	E.coli (JM109)	Mercaptoundecanoic acide for SAM layer-Bovin Seum Albumine-Immunogloubin G (IgG)	DI water	NA	100 Hz to 10 Mhz 0.1 to 1 Volt	NA	NA
Kovarik et al (2008)	No electrode	EP-DEP and Hydrostatic flow	Concentration and trapping of bacteria	Caulobacter crescentus (CB15vhfsDABdsred)	no element	Phosphate with 0.1% Triton X-100 for micro and nano spheres and M2g buffer solution for bacteria	1.6 mS/cm for spheres and 4.3 mS/cm for bacteria	10 to 100 V dc to 100 kHz	68.8 (+/- 20.9) µm/s	NA
Bao and Lu (2008)	No electrode	NA	Physical traping and lysis of bacteria	E.coli	no element	NA	NA	NA	1 µl /min	$1x10^7$ cell/ml
Boehm et al (2007)	Platinum/Platinum black electrode probes in chambers	IA	Selectivity of E.coli from M. Catarrhalis and identification of E.coli bacteria	E.coli and M. Catarrhalis	Ploy clonal BL21(DE3) IgG antibody for E.coli and Mab 3F5-5E5 for M. Catarrhalis	Phosphate buffered saline	NA	50 sinusoid Hz	NA	9×10^6 CFUmL 2×10^7 CFUmL−1 1×10^8 CFUmL−1
Ashwin et al (2007)	Paralell gold electrodes	EP (uniform electric field)	Continous Concentration, Trapping of bacteria and virus	E. coli (DH5a) Pseudomonas sp. (ATCC 10145), Salmonella Newport	no element	reclaimed water	1.3 µS/cm	1.0 and 1.25 V	2 mL /h	10^6 cells/ml
Gau et al (2001)	Au/Cr electrode array	Amperometric detection	Identification of E.coli	E.coli (rRNA)	Streptavidin-ssDNA	NA	NA	NA	NA	10^4 CFUmL−1

4. DESIGN AND SIMULATION

Non-uniform electric field induced net force in dielectric particle which toward either to the high electric field region or low electric field region depend on polarizability of particles according to surrounding medium. By adjusting the applied frequency and voltage, Clausius-Mossotti factor theoretically takes negative or postive values at range between +1 and -0.5 **[42]**

In laminar flow (where Reynold number is much smaller than 1) , hydrodynamic drag forces on a particle is linearly proportional to the velocity of particle and is opposite motion to particle motion through fluid which can be described as an energy dissipating frictional force.

Microfluidic platforms for concentration and detection of bacteria consist of mainly two part region which is focusing and detection region. Focusing region concentrate bacteria and transport them to detection region. Concentration time dramatically decreased the detection time and is a prestep for detection of bacteria. Second region of biochip detected bacteria by measuring impedance changes. Intensity of impedance depend on number of bacteria concentrated at detection region. High throughput results depend on employing effective geometrical design. At this point paralel electrodes, parallel asyymetric electrodes, tilted ramp down electrodes are designed.

Dielectrophoretic force is proportional to gradient of electric field. Gradient of electric field is simulated for each design. Asymmetric interdigitated and herringbone electrodes in Figure 4.2 and 4.6 has highest dielectrophoretic force than other design that shown in Figure 4.3, 4.4 and 4.5. Figure 4.7 and 4.8 shows the distrubition of electric potential and gradient electric field. In Figure 4.9 and 4.10 altough same frequency and voltage is applied due to different conductive is used p-dep and n-dep has seen. In Figure 4.9 fluid has less conductive than in Figure 4.10.

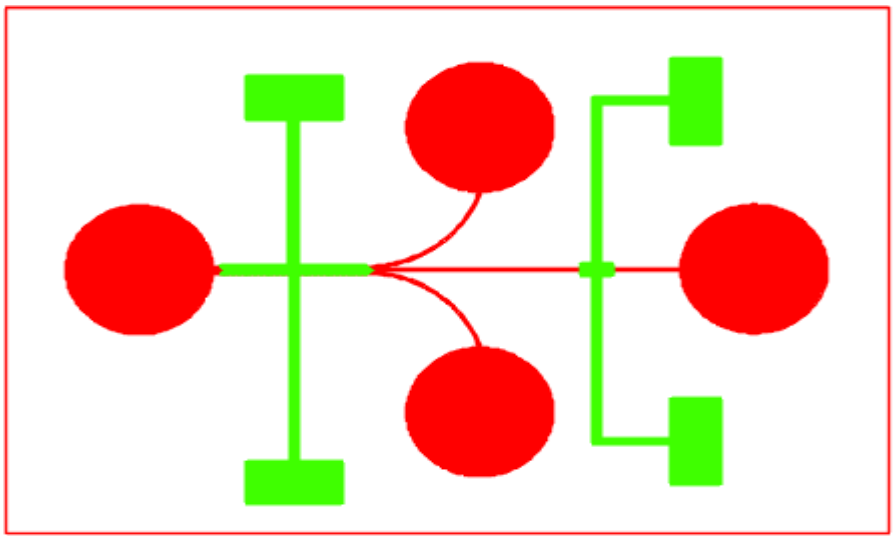
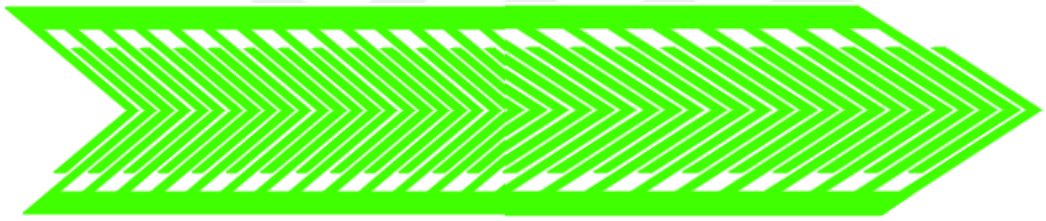


Figure 4.1: Sketch of detection of impedance sensor

4.1 Focusing Region Electrodes



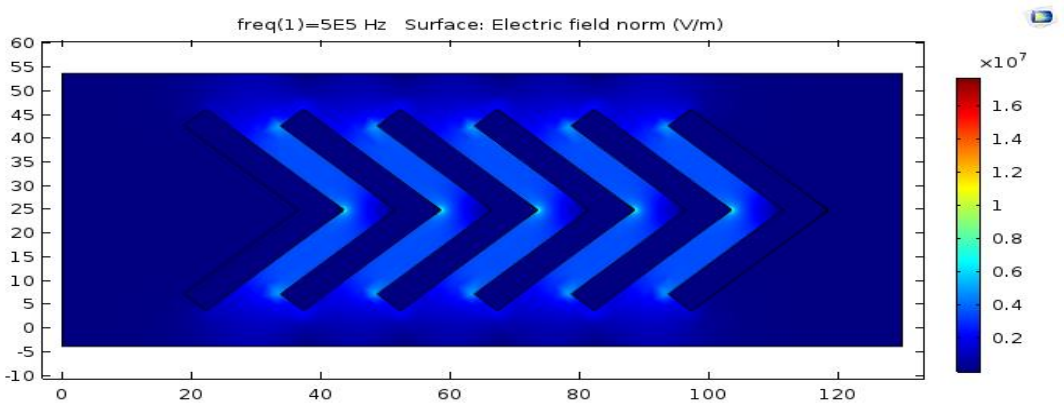
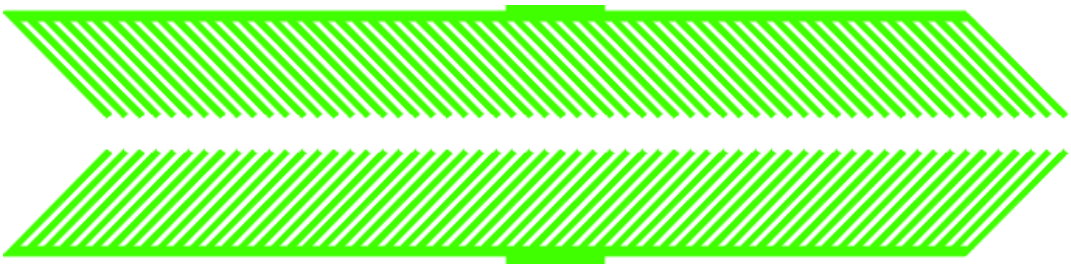


Figure 4.2: Focusing region 1: Tilted interdigitated electrodes



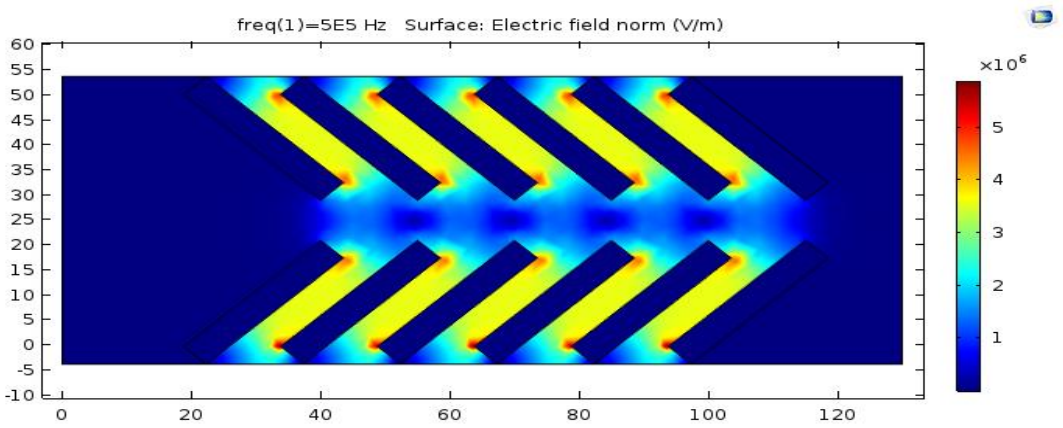
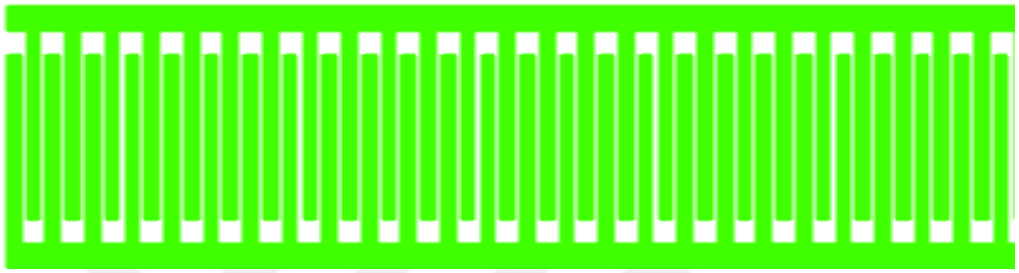


Figure 4.3: Focusing region 2: 45° tilted electrodes



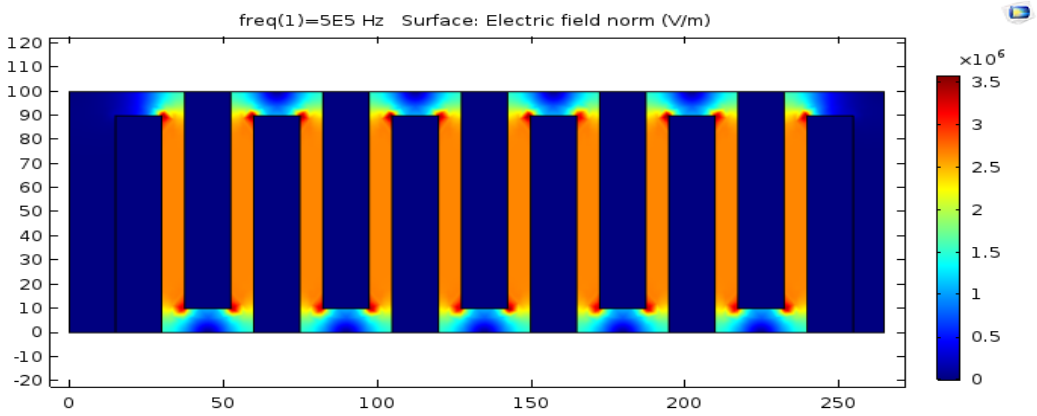


Figure 4.4: Focusing region 3: Interdigitated parallel electrodes

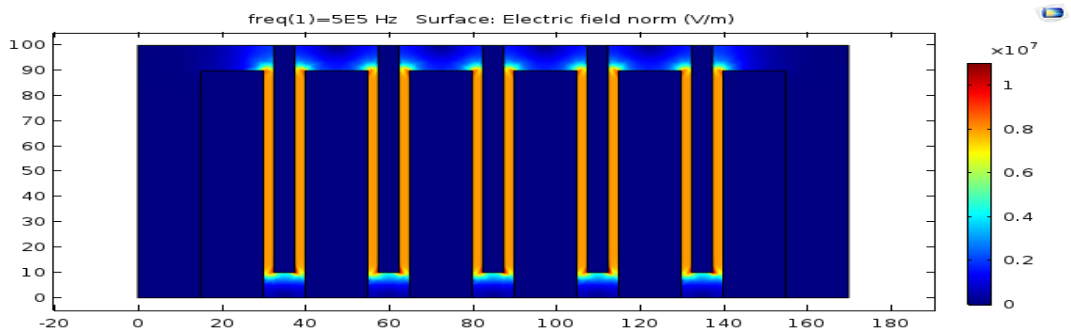


Figure 4.5: Focusing region 4: Interdigitated asymmetric parallel electrodes

4.3 Detection Region Electrode

Detection region electrodes consist of one and two detection regions. The second electrodes are narrower to increase impedance sensitivity.

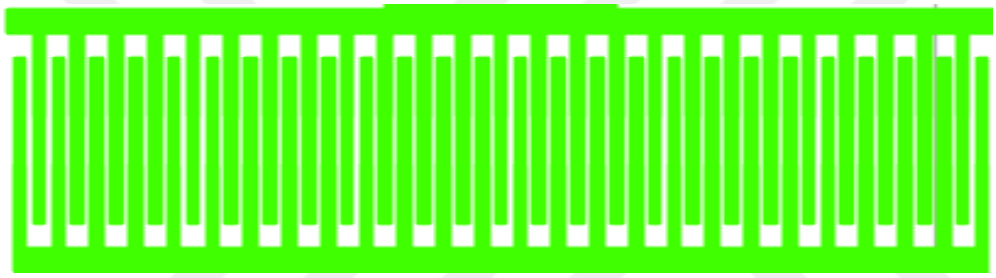


Figure 4.6: Interdigitated parallel electrodes

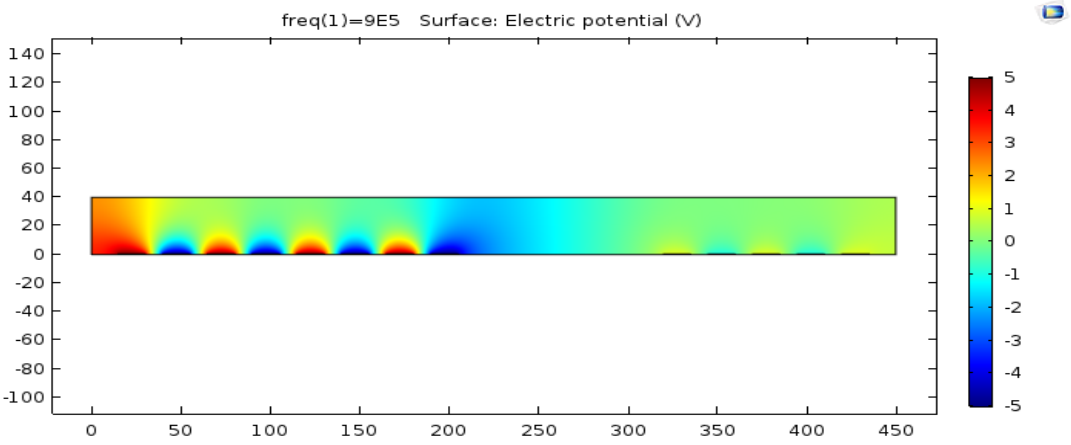


Figure 4.7: Side view of distrubition of electric potential

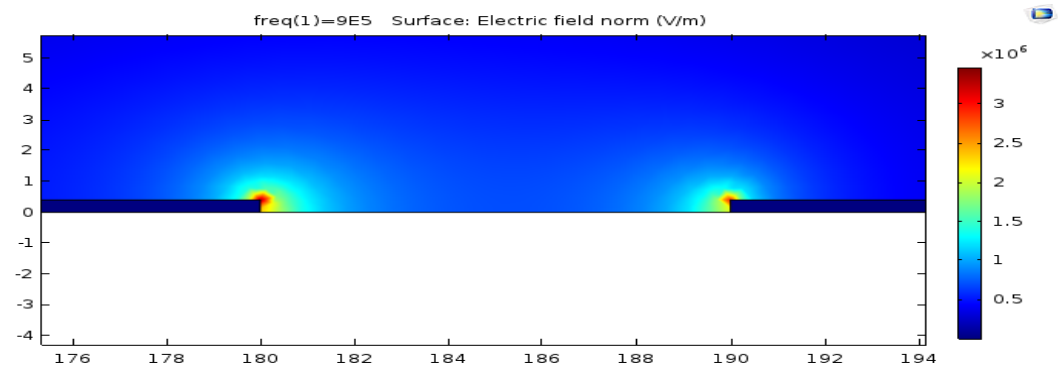


Figure 4.8: Side view of distrubition of gradient electric field

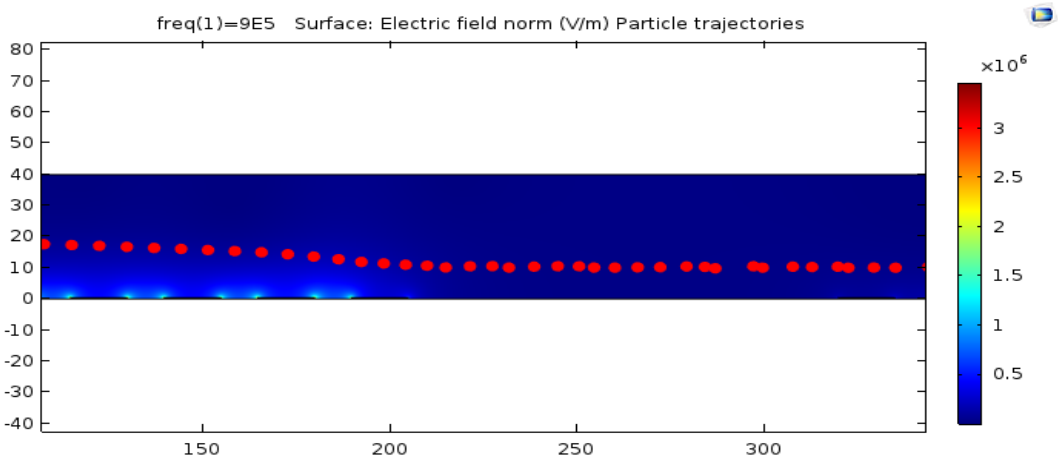


Figure 4.9: Particle trajectories under p-DEP force

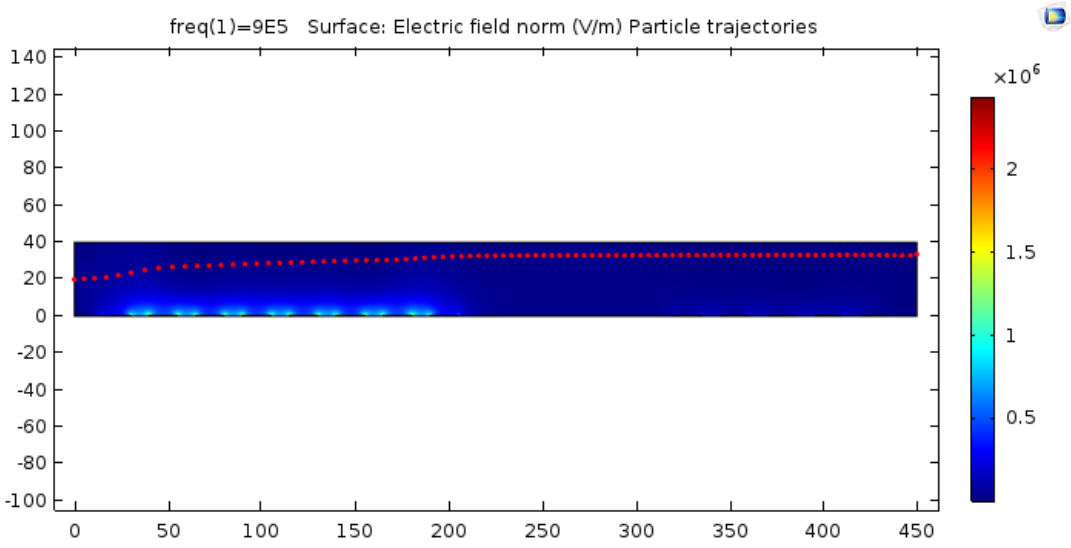


Figure 4.10: Particle trajectories under n-DEP force

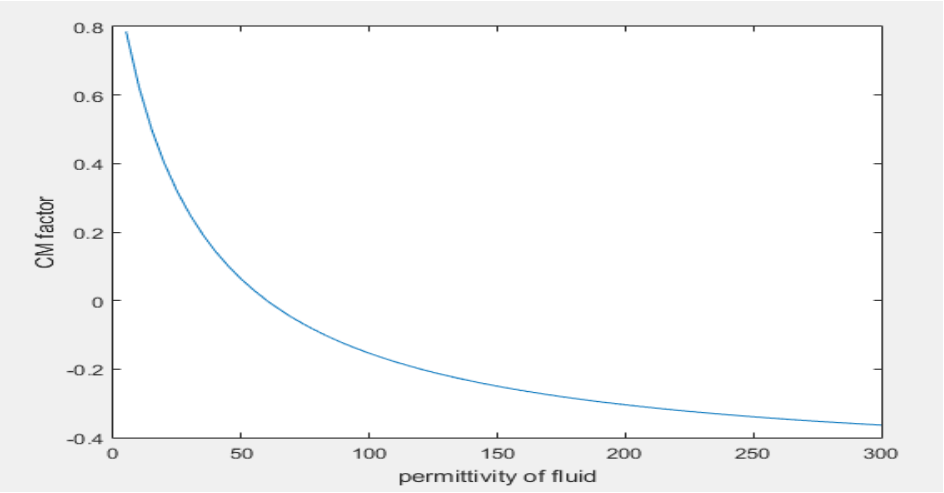


Figure 4.11: Theoretical prediction of Clausius–Mossotti factor

In Figure 4.11 CM factor takes negative values while permittivity of fluids increase which result as negative DEP

4.4.Channel Design

Channel height and weight are two important parameters to enhance sensitivity. Channel height kept at 25 μm. The channel has 300 μm weight along focusing region than divided to three channells shown in Figure 4.1. The reasons for that are to enhance enter of bacteria to focusing region than directed to narrower detection region. The other advantage is bulk fluid and other substances flow away.

5. FABRICATION AND EXPERIMENTAL RESULTS

5.1 Fabrication

The impedance based biosensor was fabricated on glass substrate using series of photolitography, plasma vapor deposition, plasma bonding, lift off processes. The cross section view of microfluidic device shown in figure produced with microfluidic channel and electrodes are embedded in. The channel's weight and height are 300 µm, 25 µm respectively.

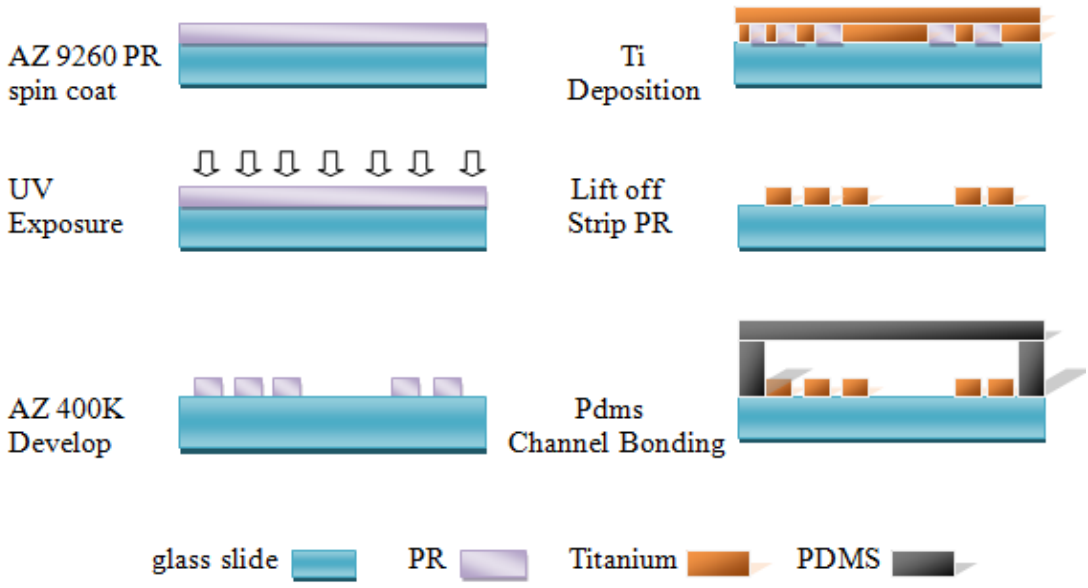


Figure 5.1: Device fabrication flow diagram

Geometrical design for electrodes and channels drawed with AutoCAD (2016) program. Mask designs written at Heidelberg DWL mask writer instrument.

Glass slides firslty washed with KOH (potassium hydroxide) solution in ultrasonic bath for ten minutes followed with another ten minute in acetone solutions to remove any dust or particles and provide good adhesion on glass. AZ9260 phoresist spin coated on glass substrate, exposed to UV light than developed at AZ 400K 1:4

Photolitograpy proccess consist of photoresist and mask that permits exposure of only defined regions to the incident radiation. The mask is typically made of soda lime or quartz glass which transparent to UV light. The pattern of interest is formed on thin Chromium or gold metal on glass mask.

Mask polarity can be designed to either by allowing UV light pass thruough patterned region which called dark field or pass through outside of pattern which is called clear field. Photoresist is a polymer material whose properties change when exposed to incident radiation. There are two different photoresist material which are positivi PR and negative PR. Positive PR is removed in developer solution when exposed to UV light while megative phoresist get hardened and areas that doesnt expose to light is removed. At this study a dark field chromium mask with positive photoresist material is used to obtain pattern.

After UV exposure glass substares placed in a vacuum chamber to deposit Titanium. PVD typically used to deposit metals, semiconductors and some insulating films. Films ranging in thickness from 10 angstrom to several microns can be deposited. The material to be deposited placed in a vacumm chamber than convert to gas phase. The vapor phase molucules land on glass substrate (target) to form desired thickness. Plasma vapor deposition is used as a deposition tecnique. PVD has two basic evaporation approaches which are thermal evaporation and electron beam evaporation. In electron beam evaporation the material heated and melted using high energy electron beam. High temperature can be achieved either e-beam thus a variety of materials can be deposited includes some insulator such as oxides and glass. Since the crucible is not heated as much there is less contamination risk according to thermal evaporation.Electron beam evaporation is used to form 400 nm Ti thickness. Photoresist stripped away by lift off tecnique in acetone solution thus desired geometrical design fabricated.

SU-8 negative photoresist and polydimethylsiloxane (PDMS) are used to fabricate microfluidic channels. SU-8 negative phtoresist is spin coated on silicon wafer to form 25 micron thickness. PDMS is a slicon polymer used with curing agent (10:1). The bubble formation is removed in vacuum chamber than pdms mixture poured onto photoresist formed silicon wafer. Silicon wafer-pdms placed on a hot plate at 90 ^{0}C for 20 minutes after that pdms peel away from Silicon wafer. Molding pdms

and Ti deposited glass substrate bonded using plasma celaner. Bonded pdms-glass sensor placed on a hot plate at 45 ^{0}C for 10 minutes.

5.2 Experimental Results

Polystyrene particles conducted in distile water with dielectophoretic force and without it and with two different concentration. In Figure 5.2 with dep 1X two times less concentrate than with dep 2X in Figure 5.3. Resuts show that impedance change both depend on number of dielectric particles and dielectrophoretic force. Impedance decreases with dep force and with number of particle as seen in figure 5.4. p-Dep is observed at 5 Mhz and 4 V.

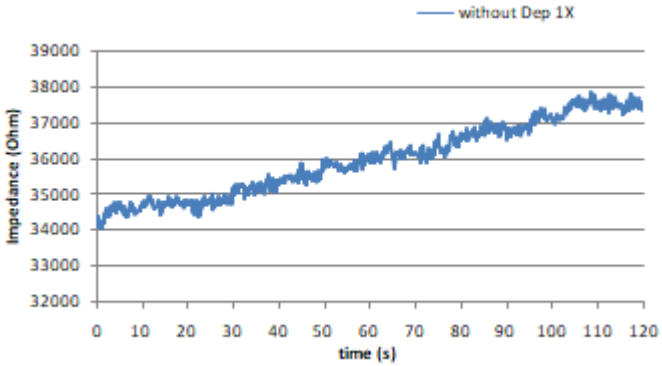


Figure 5.2: Impedance graph of polyestrene particles

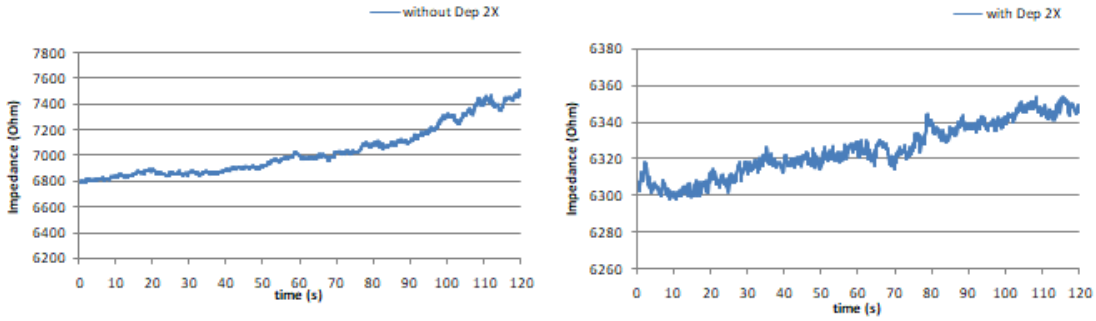


Figure 5.3: Impedance graph of polyestrene particles (two times more concentrate)

Figure 5.2 and Figure 5.3 impedance magnitude show that impedance decreases with high concentration and dielectrophoretic force.

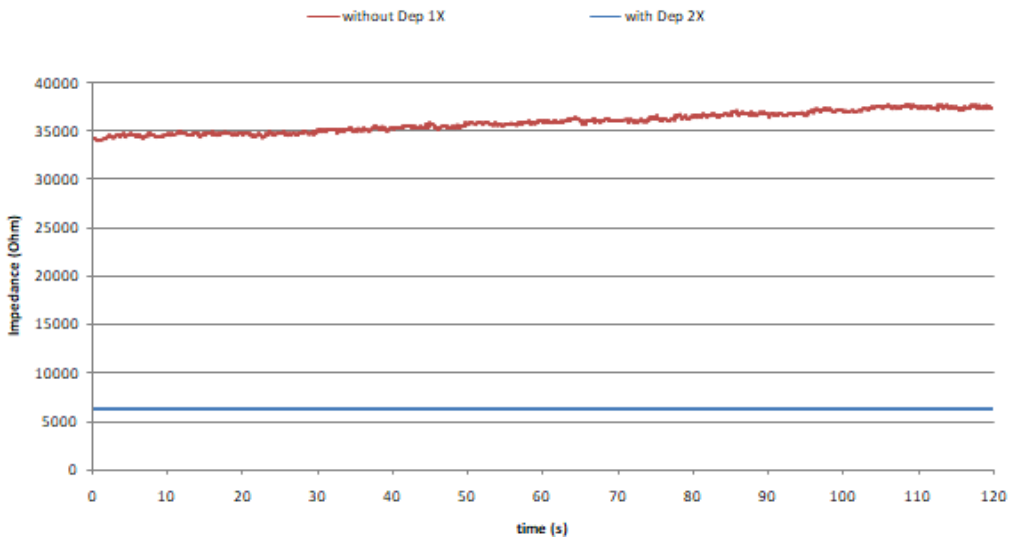


Figure 5.4: Impedance difference between without dep 1X and without dep 2X

In Figure 5.4 impedance magnitude get decrease with increasing concentration of particles

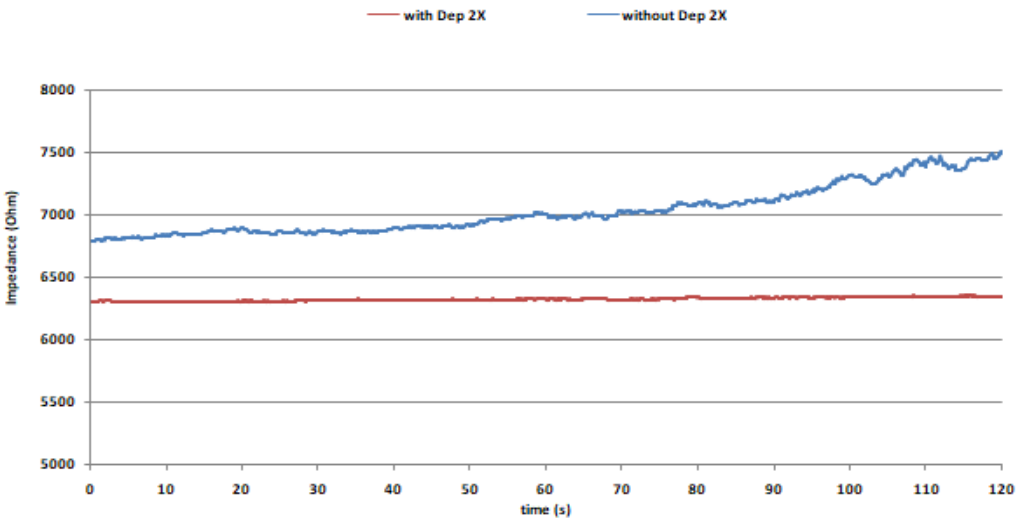


Figure 5.5: Impedance difference between with dep 2X and without dep 2X

In Figure 5.5 shows the comparision between without dep than with dep altough the same amount of particles are condcuted, impedance get decreases with dielectrophoretic force which increase the sensitivity of the sensor.

E.coli and Enterobacter Aerogenes bacterias are conducted in distile water with approximately 500 cfu/ml, 1000 cfu/ml, 5000 cfu/ml, 20000 cfu/ml concentration.

Impedance response is measured with time dependent for E.coli bacteria and frequency dependent for Enterobcater aerogenes bacteria.

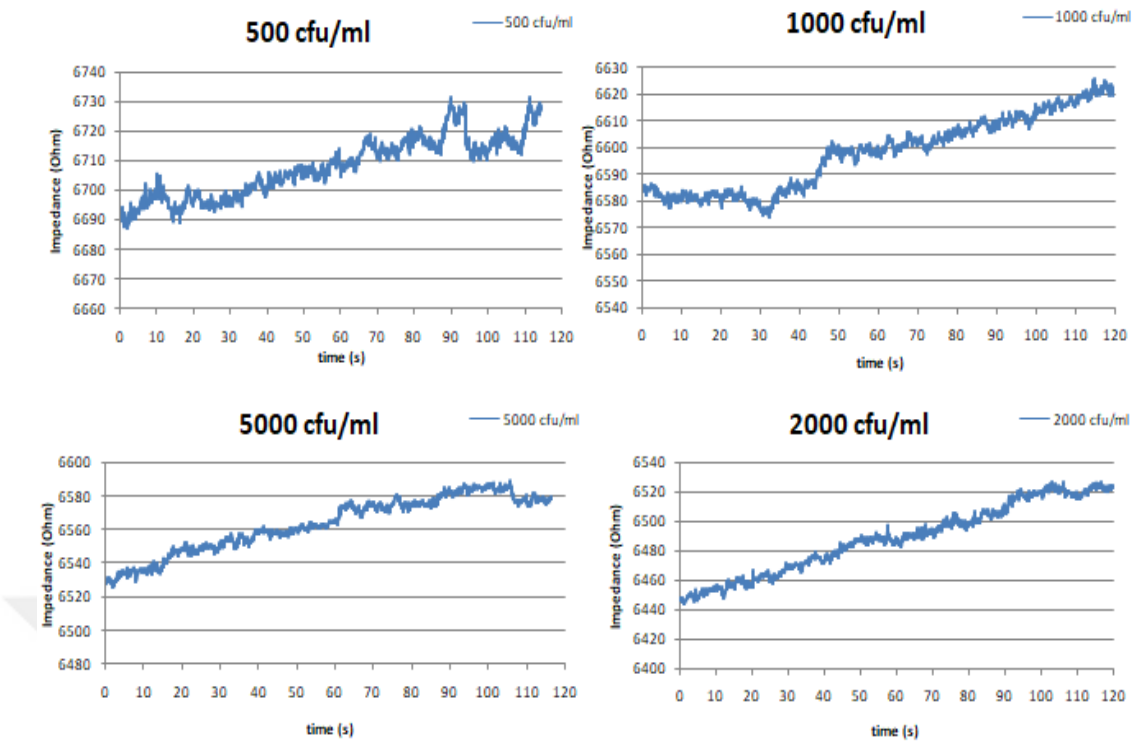


Figure 5.6: Impedance of 500 cfu/ml, 1000 cfu/ml, 5000 cfu/ml and 20000 cfu/ml e.coli bacteria

Dielectrophoretic force is applied at focusing region electrode at 5 Mhz and 4 V. Since distilated water has too low conductive value p-dep is seen in range between 10 kHz and 5 Mhz. Bacteria focused and concentrated at second impedance region. Impedance get decreases with high concentration and dielectrophoretic force is shown in Figure 5.6 and 5.7.

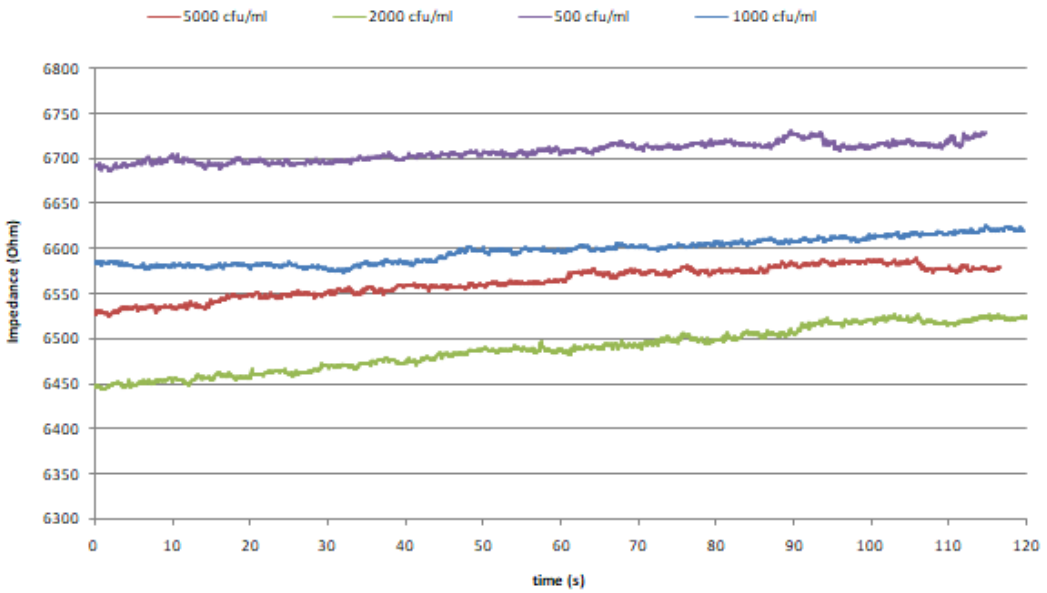


Figure 5.7: The impedance comparision between 500, 1000, 5000,20000 cfu/ml of e.coli respectively

In Figure 5.8 The impedance response of Enterobacter aerogenes bacteria with different four concentrations is measured freqeuncy dependent. Result shows that impedance get decreaes with increasing frequency. Figure 5.9 shows the comparision between different concentration of bacteria.

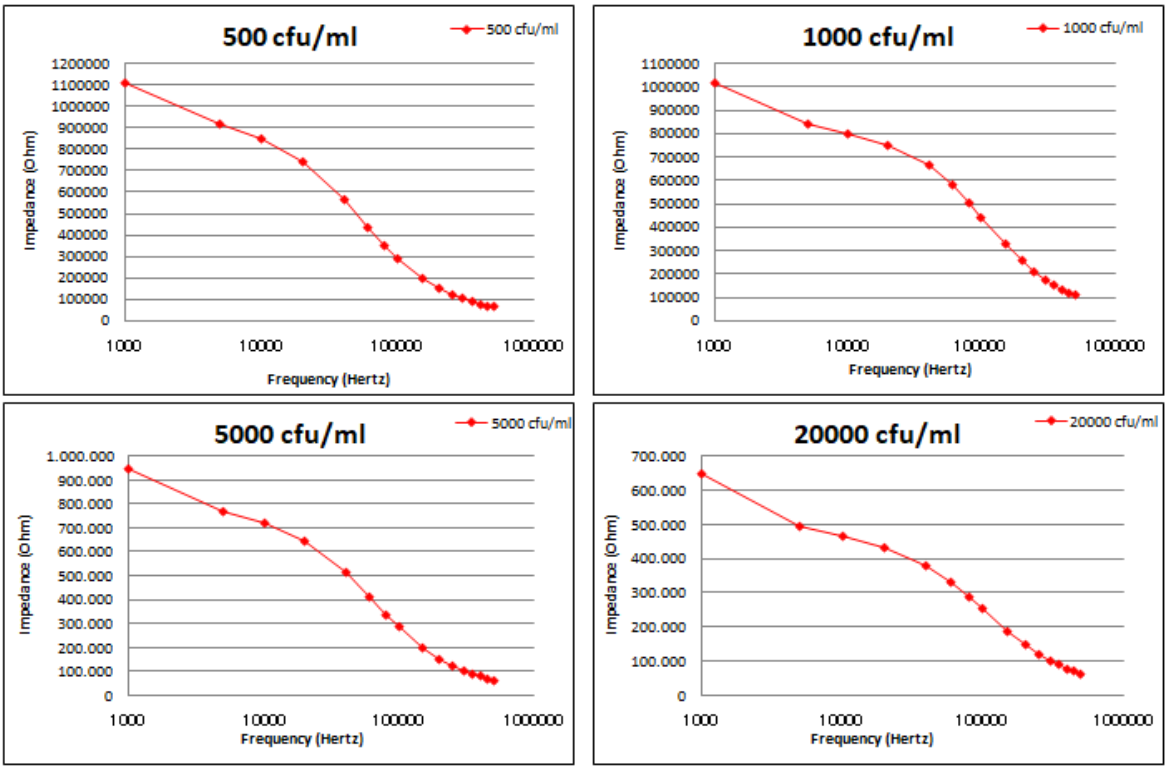


Figure 5.8: Impedance of 500 cfu/ml, 1000 cfu/ml, 5000 cfu/ml and 20000 cfu/ml of enterobacter aerogenes bacteria

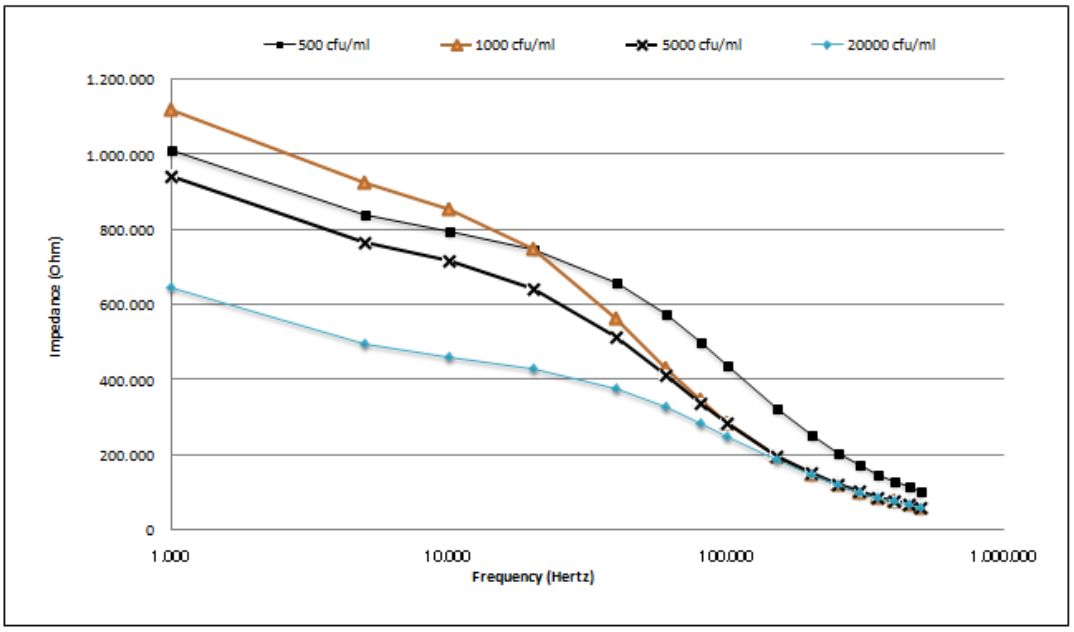


Figure 5.9:The impedance comparision between 500, 1000, 5000,20000 cfu/ml of Entrobacter Aerogenes bacteria

6. CONCLUSION

Impedance based biosensor fabricated for detection of bacteria. For each bacteria same amount of cells are conducted and the impedance responses are compared. Approximately 500 cfu/ml, 1000 cfu/ml, 5000 cfu/ml and 20000 cfu/ml are conducted for each bacteria at same medium and same frequency. The detection limit for this sensor is determined as 500 cfu/ml for both E.coli and Enterobacter aerogenes bacteria. The effect of dielectrophoretic force demonstrated via polystyrene particle. Some optimization is made to increase sensitivity such as narrow to detection electrodes and decrease the channel height. Results show that impedance decrease with increasing frequency, number of bacteria and dielectrophoretic force.

Bacteria related diseases is a common and costly public health problem around the world. Detection time and detection at low concentration level of bacteria are important parameters to study to improve. Impedance analysis combined with electrokinetic forces are effective method and important analytical tool for detection, concentration, seperation and trapping of bacteria cell. Concentrating bacteria is an critical step in order to enhance sensitivity of detection limit to be able to sense the existance of bacteria population. For label free detection geometrical architecture of system is important for detection limit in term of use electric field effectively. Every bacteria has characterisitc electrical properties that will be useful for detection with help of impedance analysis. These difference electrical properties of bacteria may use to identify them without using any antibody in future.

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CURRICULUM VITAE

PHOTO

Name Surname **: Kadriye ÖLMEZ**

Place and Date of Birth **: Hakkari/ 24.01.1991**

E-Mail **: olmezka@itu.edu.tr**

EDUCATION

- **B.Sc.** **:** 2014, Erciyes University, Biomedical Engineering
- **B.Sc.** **:**2012**,** Lublin University of Technology, Electrical Engineering and Computer Science (Erasmus Programme)

PROFESSIONAL EXPERIENCE AND REWARDS:

- **2015-2017**: Biomedical Engineer, Clinical Engineeing Department, Presidency of Public Hospital Services, Afyon.
- **2017-** :Chief Biomedical Engineer, Clinical Engineeing Department, Presidency of Public Hospital Services, Istanbul (ongoing)