

The background of the cover is a vibrant orange-to-yellow gradient. Overlaid on this is a complex, white circuit board pattern consisting of numerous interconnected lines and nodes, resembling a printed circuit board (PCB) layout. The lines vary in thickness and direction, creating a sense of depth and technical precision.

GRINREY

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Mathematical Modelling of the Voltage Required for Separation of Cell-like Particles

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ABSTRACT

Microfluidic devices separate the cells by using dielectrophoresis, magnetic, acoustic, or other techniques. This study focuses on a voltage required in a microfluidic device that uses dielectrophoretic force to separate the cell-like particles based on their size difference. Numerical simulation shows that the particles separate above a certain minimum voltage and do not separate above a certain maximum voltage, which defines the particle separation range. This voltage requirement depends on cell and buffer solution parameters. This study uses data-sets generated using numerical simulation by varying these parameters. Detailed analysis of these data-sets gave the significant parameters, which decide the voltage requirement, as the size of the two cell-like particles and viscosity of the buffer solution. A mathematical model is developed using regression analysis, to calculate the voltage requirement, considering particle size ranging between 2 μm to 30 μm and viscosity ranging from 0.8cp to 4cp. The voltage predicted from the mathematical model is compared with the values obtained from the numerical simulation. The RMS error obtained for the entire data-set is 1.4412%. The mathematical model thus predicts the required voltage with good accuracy and will be helpful in healthcare, which needs frequent screening or testing of samples for early diagnosis of HIV, cancer, malaria, and many such applications.

Keywords: Dielectrophoresis, Electric voltage, Mathematical modeling, Microfluidics, Separation.

1. INTRODUCTION

In today's world, the main concern of the researcher is to diagnose the disease for better health care. The clinical research is done by liquid biopsy, i.e. blood test which requires various complicated instruments with the large volume of blood as compared to micro and nanoliters [1]. There are many places where lab facilities are absent or it may be difficult to set up a proper lab, thus one can have a microfluidics platform for blood fractionation and sample preparation to perform the liquid biopsy. The main advantage of using these Lab-on-Chip phenomena for cell trapping and plasma analysis is that low blood volume is required. Results can be obtained in short duration because of its high integration, miniaturization and automation which helps it to diagnose the disease at an early stage [2, 3].

Various research activities have been carried out in the field of cell separation based on their cell dimensions and properties such as the magnetic properties of cells, adhesive properties, dielectric properties, etc. The cells can be separated by using two techniques i.e. by active technique or by passive technique. In active technique, the external field is used which include electric forces, acoustic forces, magnetic forces and mechanical forces, whereas the passive technique includes inertia forces, sorting due to adhesion, pinched flow, filtration and many more [4]. This study focuses on the voltage required in the separation of cells by using dielectric properties of the cells. It can be done with the help of dielectrophoresis as this force allows moving and separating the polarized particle in the non-uniform electric field. Dielectrophoresis (DEP) is derived from the electrophoresis technique in which there is no need to charge the particles [5]. It can move the particles in the DC or AC electric field by polarizing it in the non-uniform electric field without changing their biological properties and which does not damage the cells. In previous research, the dielectrophoresis has successfully been used for separating cancerous cells, infected blood cells and other blood components such as erythrocyte and leukocytes for diagnosing various illnesses [6, 7].

Pommer et al [8] have used a two-stage device for the separation of platelets from diluted whole blood in a microfluidic channel by using dielectrophoresis. The cell sorting has been performed based on the cell sizes. Park [9] has designed a device based on electrokinetics and electric field for the separation of pathogenic bacterial cells from the whole blood cells by using continuous dielectrophoresis. Piacentini [10] has used the microfluidic device for the separation of platelets from red blood cells and white blood cells by using dielectrophoresis field flow fractionation and hydrodynamic focusing by using a low applied voltage. Ali [11] has developed the numerical model for the

separation of red blood cells, white blood cells and platelets with the application of low voltage. It was used to count the number of cells separated and it also gave the different outlet design for efficient blood separation.

A. Dabighi [12] has made a microfluidic device used for separating tumor cells based on their physical properties by using dielectrophoresis force. R. Derkshian [13] has used the approach of the DEP force to separate the three polystyrene particles with minimum electric potential. Y. Zhang [14] have used dielectrophoresis microfluidic chips for the separation of WBCs, RBCs and platelets.

The objective of this mathematical modelling is to generate the equation to calculate the voltage required to separate the particles ranging between $2\mu\text{m}$ to $30\mu\text{m}$. This study uses the properties of dielectrophoresis field flow fractionation for the study of the different electrical and physical properties of the cells.

2. THEORETICAL BACKGROUND

This section explains the theory related to the cells, electrical properties of the cells and their separation techniques from which dielectrophoresis is used in this study.

2.1 Theory of cell

The cells have different electrical properties such as its permittivity, conductivity, etc. All these electrical properties arise from the physical properties of the membrane and the ion channels in the membrane [10]. The cell membrane consists of a bilayer which acts as a dielectric medium between the salt solution and the cytoplasm, therefore the cell membrane acts as a capacitor. The membrane thickness ranges approximately between 7 to 10nm [15] and the healthy cells range between 2 to 15 μm .

2.2 Theory of Dielectrophoresis

The field of dielectrophoresis was first studied by Herbert Pohe in the 1950s whereas it was published in 1978 [16]. Dielectrophoresis is a force which is used to move and thus separate the polarized particles or cells in the non-uniform electric field. As the advancement in the field of microfabrication and the lab on chip phenomenon have led to the development of dielectrophoresis [17]. The dielectrophoretic force arises when the polarized particle is placed in a non-uniform electric field and it is given by equation (1) [17]:

$$F_{DEP} = 2\pi\epsilon_o\epsilon_m r^3 \text{Re}[K(\omega)]\nabla(E)^2 \quad (1)$$

The factor $\text{Re}[k(\omega)]$ depends on the frequency and the complex permittivity of the particle and the medium as it is given by equation (2):

$$K(\omega) = \frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_p^* + 2\epsilon_m^*} \quad (2)$$

Depending upon these properties, this factor can be positive or negative which gives rise to two types of dielectrophoresis i.e. if $\text{Re}[K(\omega)]$ is negative then it gives rise to negative dielectrophoresis (nDEP) and when $\text{Re}[K(\omega)]$ is positive then it is positive dielectrophoresis (pDEP). Positive DEP occurs when the particle is more polarized than the medium in which it is surrounded i.e. $\epsilon_p \gg \epsilon_m$ hence the particle will get attracted towards the area having high electric field whereas negative DEP occurs when the particle is less polarized than the medium in which they are surrounded i.e. $\epsilon_p \ll \epsilon_m$.

Hence, the particle will move towards a low electric field. Positive DEP is effective for the medium having low conductivities therefore, the medium with high conductivities of $>1\text{S/m}$ are suitable for negative DEP. Thus, in this study, we will be using negative DEP whose medium permittivity is much more than the permittivity of the particle. The field flow fractionation method has been used for the separation of the particle as it uses a low voltage to generate negative DEP force. It is a technique in which the force is applied perpendicular to the direction of flow to separate the particle which is present in the medium.

3. NUMERICAL SIMULATION AND PARAMETER STUDY

In this section, we have discussed the numerical simulation of the model and the number of parameters. These are used in the separation of two particles which are referred to as the diameter of one particle (D_1) and diameter of another particle (D_2). Thus, a mathematical model is proposed which is used to separate the particles with an application of low voltage. This paper aims to model an equation of voltage required for the separation of particles of particular sizes which is done by using DEP forces as they are directly proportional to the size of the particles. Hence, particles can be separated by the same force with the variation in the voltage.

3.1 Numerical Simulation

In this paper, the 2D geometrical model of the microfluidic device is used for the separation of particles based on their size with the use of dielectrophoretic field-flow fractionation. This study uses the experimental model described by Piacentini and Renaud [10] which is used for the separation of two particles. The left side of the geometry has two inlet sections, from the upper inlet the blood is injected whereas from the lower inlet the buffer solution (PBS) is

injected. The length of both the channels is $200\mu\text{m}$ and the width of the channel is $40\mu\text{m}$. The electric voltages are applied in the separation region. All the electrodes have voltages of the same magnitude but with opposite polarity. The length of the electrode array of the geometry is $520\mu\text{m}$ as shown in Fig. 1. The right side of the geometry has the outlet section which has two outlet channels for the collection of two different particles whose lengths are $200\mu\text{m}$ and the angle between them is 90° . Thus, the total length of the channel is approximately equal to $802.8427\mu\text{m}$.

As per the study for the separation of cells, the simulation is performed in the COMSOL Multiphysics software as shown in Fig. 2 for the decided geometry. The values of the parameters used in the simulation are given in Table 1.

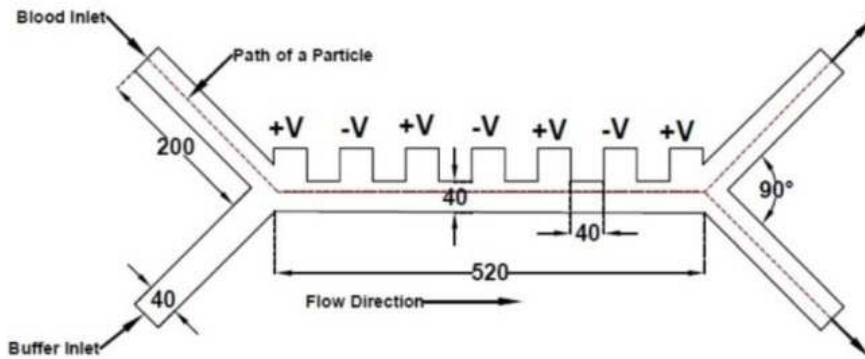


Fig. 1. Geometry of Micro-channel

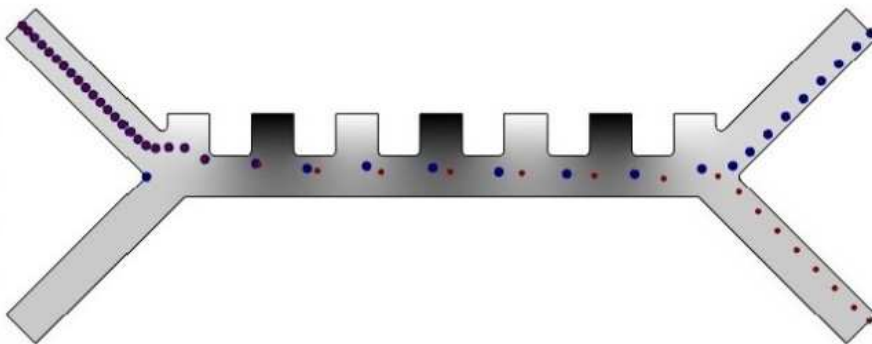


Fig. 2. Model for Numerical Simulation

Table 1. Parameter values for numerical simulation

| Sr. No. | Parameters | Value | Unit |
|---------|-----------------------------------------------------|-------|-------------------|
| 1 | Fluid Conductivity | 1 | S/m |
| 2 | Fluid Permittivity | 80 | |
| 3 | Fluid Density | 1080 | kg/m ³ |
| 4 | Fluid Dynamic Viscosity | 1 | cP |
| 5 | Particle Density of RBCs and Platelets | 1050 | kg/m ³ |
| 6 | Range of diameter D ₁ and D ₂ | 2-30 | μm |
| 7 | Conductivity of particle | 0.25 | S/m |
| 8 | Permittivity of particle shell | 6 | |
| 9 | Relative Permittivity of particle | 100 | |
| 10 | Conductivity of particle shell | 1e -6 | S/m |
| 11 | Range of particle shell thickness | 6-10 | nm |
| 12 | Frequency | 100 | kHz |

Various simulations are performed for the same set of values used in Table 2 by varying the magnitude of the voltages applied in the separation region. Thus, by varying the voltages it was observed that for the separation of two given particles there is the lowest voltage below which they will not separate as shown in Fig. 3. Further by increasing the voltage, it was observed that there is the highest voltage above which particle will not separate as shown in Fig. 4.

In Fig. 3 it is seen that the particles in the lower channel touch the left side of the wall. Hence, if we apply lower voltage the particles will move in the upper channel and do not separate. Therefore, it can be said that this is the minimum voltage which is required for the separation of two given cell-like particles, below which both the particles will move in the upper channel. Similarly, in Fig.4 it is observed that the particles in the upper channel touch the right side of the wall. Hence, if we apply higher voltage the particle will move in the lower channel and do not separate. Therefore, it can be said that this is the maximum voltage which is required for the separation of two cell-like particles, beyond which both the particles will move in the lower channel. Hence, it is clear that there is a range from the lowest voltage (LV) to the highest voltage (HV) for the same size of particle separation.

Table 2. Dynamic Viscosity of different solutions

| Sr. No. | Solution | Dynamic Viscosity |
|---------|--------------|-------------------|
| 1 | Water | 1cP |
| 2 | Blood | 3-4 cP [19] |
| 3 | PBS Buffer | 1±0.05cP [20] |
| 4 | Other Buffer | 0.8-1.5 cP |

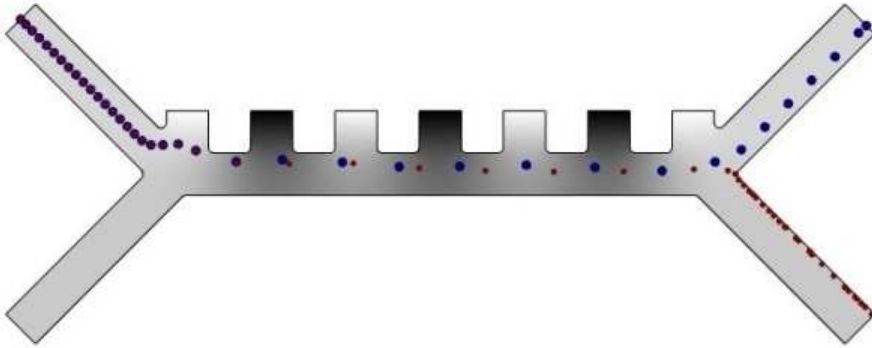


Fig. 3. Lowest voltage for the separation of particles

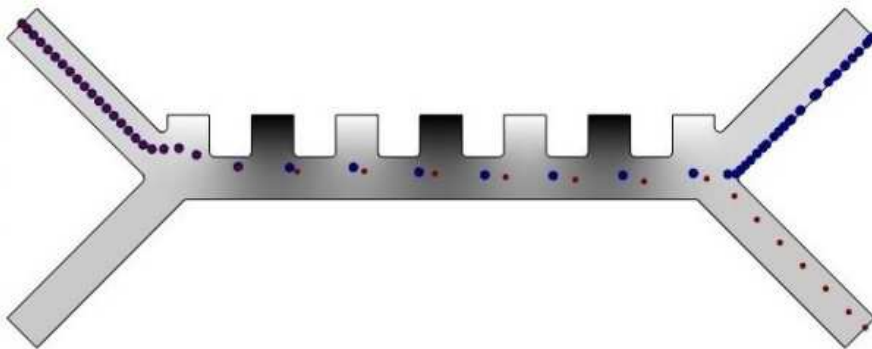


Fig. 4. Highest voltage for the separation of particles

3.2 Parameters Study

This section consists of the study of different parameters which are significantly used in the separation of particles. The parameters are classified as the buffer parameters, cell parameters and the parameters of the cell membrane. The study of a given parameter is done by keeping all other parameters constant for the given two particle size. Thus, in this section, these parameters are plotted against the lowest and the highest voltages required in the separation as discussed in section 3.1.

3.2.1 Buffer parameters

These are classified into three different parameters, i.e. fluid conductivity, fluid density and fluid viscosity. The variation of each parameter is studied against the lowest and the highest voltages by keeping the rest values to be constant. Thus the following graphs are obtained as shown in Fig. 5. In Fig. 5a, it is observed that the voltage decreases with the increase in the conductivity and

becomes constant after 500mS/m. The conductivity of the buffer solution ranges approximately between 1500 to 2000mS/m [18]. Thus, from the graph, it is clear that the conductivity remains constant for the given range. Therefore, conductivity does not contribute to the separation of particles. In Fig. 5b, it is observed that there is no change in the voltage with the increases in the fluid density which ranges between 1000 to 1100 kg/m³ [18]. Thus, it does not affect the separation of particles. Fig. 5c shows the variation of viscosity with the voltage. It is observed that with the increase in the viscosity the voltage shows the power trend as shown in the equation (3). From the literature, it is studied that the range of dynamic viscosity for different solutions varies as given in Table 2. In this study, the range of viscosity from 0.8cp to 4cp is considered.

$$V \propto \mu^{1/2} \tag{3}$$

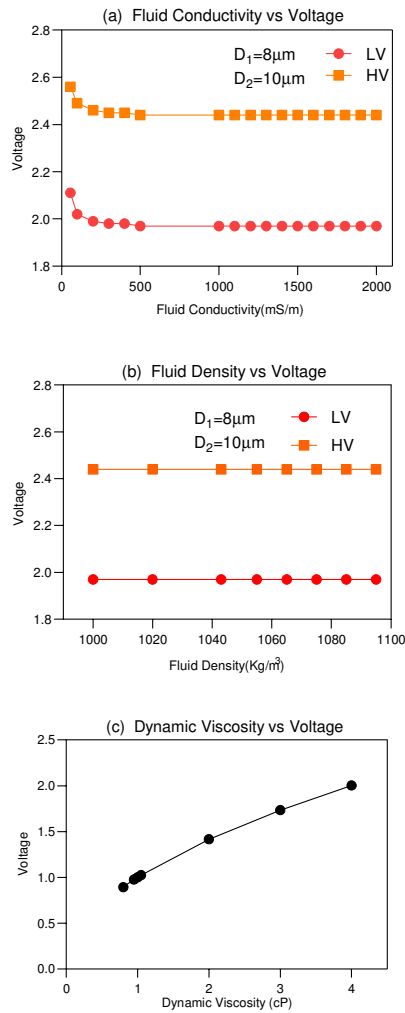


Fig. 5. Buffer Parameters vs Voltage

3.2.2 Parameters of cell membrane

These are again classified into three different parameters, i.e. shell conductivity, shell permittivity and shell thickness which are plotted against the lowest and the highest voltage as shown in the Fig. 6. In Fig. 6a, it is observed that the slope of the line is almost negligible. Thus, it can be treated as a constant voltage with the increase in the conductivity of the shell and that does not affect the separation of particles. In Fig. 6b, it is observed that the slope is almost equal to zero along the permittivity and in Fig. 6c, the voltage remains constant along the shell thickness therefore it does not play any role in the separation of particles.

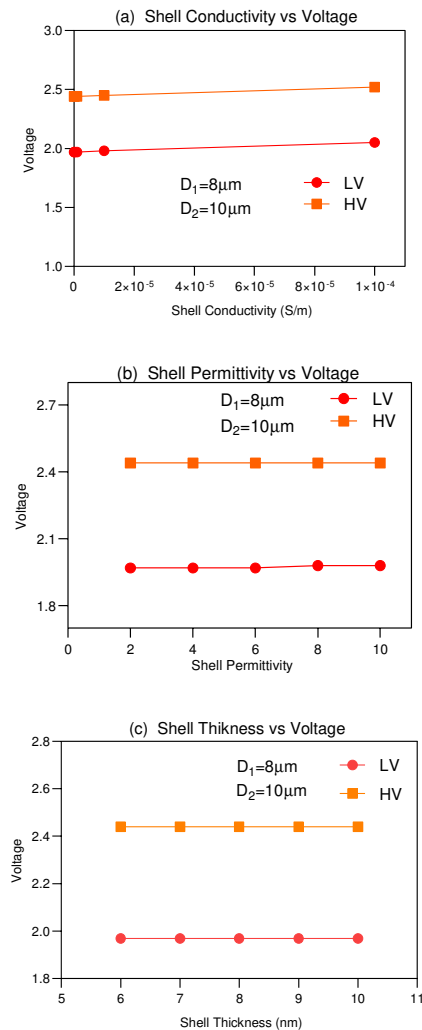


Fig. 6. Parameters of cell membrane vs voltage

3.2.3 Particle properties

This consist study of three different parameters, i.e. particle conductivity, particle density and particle permittivity which are plotted against the highest and the lowest voltage as shown in Fig. 7.

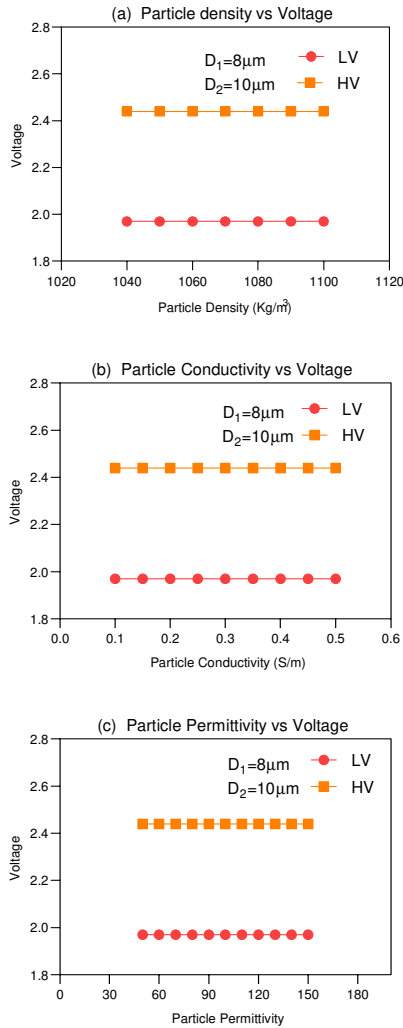


Fig. 7. Particle Properties vs Voltage

In Fig. 7a, 7b, 7c, it is observed that the variation between the particle density, conductivity and permittivity versus the applied voltage is observed to be constant. Hence, it does not contribute to the separation of the particles.

3.2.4 Particle dimensions

This gives the variation between the size of the particles with the applied voltages by keeping all other parameters constant.

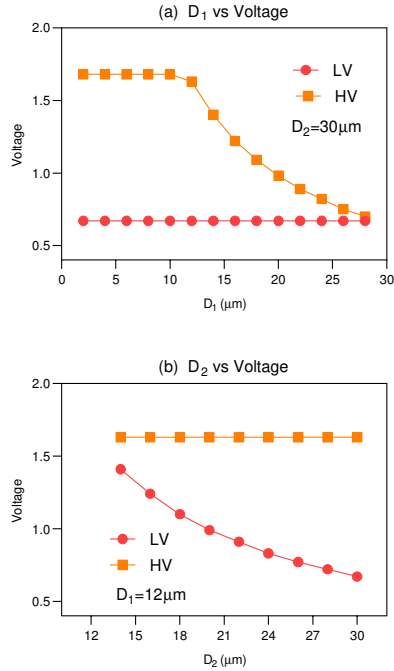


Fig. 8. Particle Dimensions vs Voltage

Thus, the graph obtained for different dimensions is shown in Fig. 8. In Fig. 8a, it is observed that the highest voltage varies with the increase in the diameter D_1 whereas the lowest voltage remains constant with an increase in D_1 by keeping D_2 constant. In Fig. 8b, it is observed that the lowest voltage varies with the increase in the diameter D_2 whereas the highest voltage remains constant with the increase in D_2 by keeping D_1 constant.

4. MATHEMATICAL MODELING AND VALIDATION

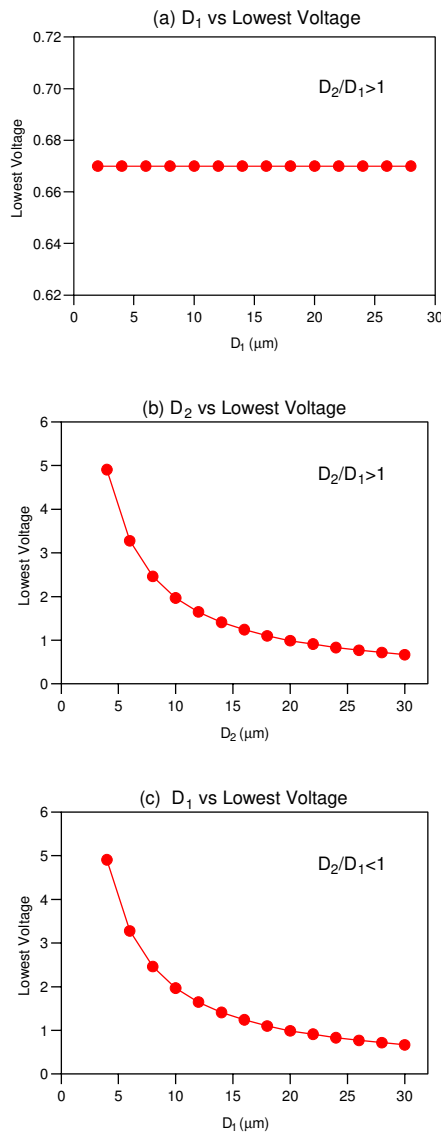
In this section, the mathematical equation is modelled for the electric voltage required in the separation of given two particles. From section 3.2 it is clear that the voltage depends upon the two main parameters which are particle dimensions and the fluid viscosity. Thus, in the latter part of this section, these parameters are validated with the values obtained by the mathematical model and the results are plotted as shown.

4.1. Mathematical Modeling

In this section the mathematical equation is modelled for the voltage which depends on D_1 , D_2 and the dynamic viscosity of the fluid. As explained in section 3.1 the given two particles are separated between the lowest and the highest voltage. Thus, the effect of the lowest and the highest voltage is studied with the increase in the size of the particle dimensions.

4.1.1 Study of lowest voltage

The study for the lowest voltage is classified into two parts i.e. $D_2/D_1 > 1$ and $D_2/D_1 < 1$ where the diameters are varied from $2\mu\text{m}$ to $30\mu\text{m}$. Thus from the Fig. 9a it is observed that for the ratio of $D_2/D_1 > 1$ the lowest voltage remains constant with the increase in the size of D_1 . Whereas from the Fig. 9b it is observed that the lowest voltage decreases as D_2 increases from $2\mu\text{m}$ to $30\mu\text{m}$. Thus, it can be said that the lowest voltage is dependent only on D_2 and D_1 does not contribute to the same. For the ratio $D_2/D_1 < 1$ voltage decreases with the increase in D_1 and remains constant along with D_2 as shown in the Fig. 9c, 9d without the change in the coefficients.



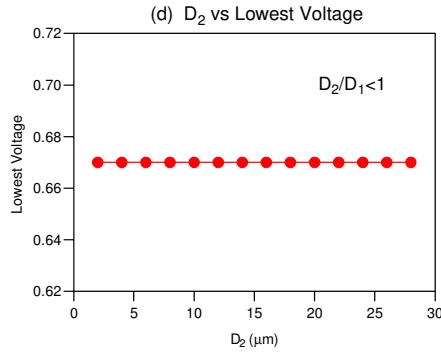


Fig. 9. Particle size vs Lowest voltage

4.1.2 Study of highest voltage

This study is again classified into two parts i.e. $D_2/D_1 > 1$ and $D_2/D_1 < 1$. It is observed from the simulation that within this ratio it is further divided into another two parts i.e. for the ratio of $D_2/D_1 > 1$ it is divided as $D_2/D_1 < 2.5$ and $D_2/D_1 > 2.5$. This is because it is observed that as D_2 exceeds 2.5 times D_1 it gets stuck to the lower wall of the separation region. Hence, the particle cannot move further in the channel as shown in Fig. 10.

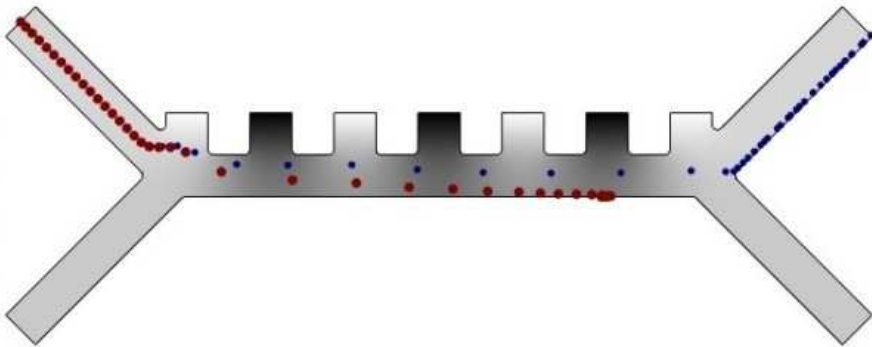


Fig. 10. Highest voltage for ratio $D_2/D_1 > 2.5$

Thus, to separate the particle it is needed to apply the lower voltage and due to it the trend of the line changes above this ratio. It is observed that the highest voltage for the ratio of $D_2/D_1 < 2.5$ the separation depends on D_2 and remains constant along D_1 as shown in Fig. 11c and Fig. 11d. Similarly for the ratio $D_2/D_1 < 1$ will be classified as $D_1/D_2 < 2.5$ and $D_1/D_2 > 2.5$. For the ratio $D_1/D_2 < 2.5$ the highest voltage remains constant along D_1 and varies with the increase of D_2 . Whereas for $D_1/D_2 > 2.5$ the voltage depends on D_1 and remains constant along with D_2 .

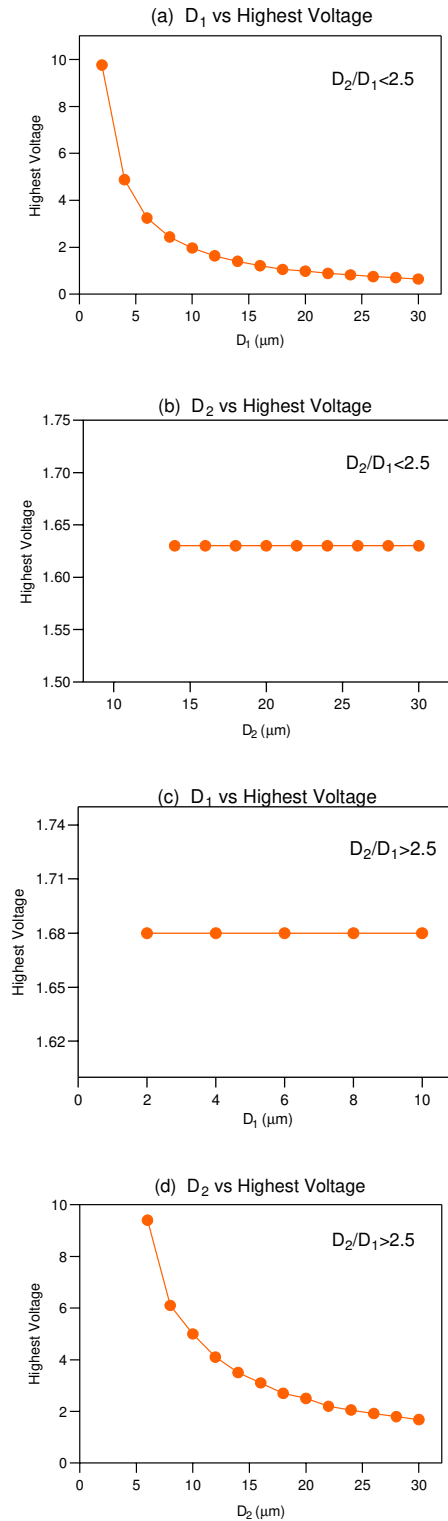


Fig. 11. Particle size vs Highest voltage

From the simulation done in section 3.1 and Fig 3 and 4 shows that the highest and the lowest voltages applied for the separation of any given two particles are very close to the wall.

This may destroy or rupture the cell thus to avoid this situation the average of this voltage can be applied to separate the particle at the particular voltage. Thus, the average voltage is given by equation (4) and whose lowest and highest voltages are given in Table 3. Thus, by using equation (4) one can separate the two given particles between the range of 2 μ m to 30 μ m at a particular voltage.

$$V = \left(\frac{V_L + V_H}{2} \right) \mu^{1/2} \quad (4)$$

Table 3. Lowest and Highest Voltages

| Voltage | $D_2/D_1 > 1$ | | $D_2/D_1 < 1$ | |
|---------------------------------------------------------------------------------------------------------|--------------------|------------------|--------------------|------------------|
| V_L | $a_1 D_2^{-a_2}$ | | $a_1 D_1^{-a_2}$ | |
| V_H | $D_2/D_1 \leq 2.5$ | $D_2/D_1 > 2.5$ | $D_1/D_2 \leq 2.5$ | $D_1/D_2 > 2.5$ |
| | $b_1 D_1^{-b_2}$ | $c_1 D_2^{-c_2}$ | $b_1 D_2^{-b_2}$ | $c_1 D_1^{-c_2}$ |
| Where: $a_1 = 19.100$; $a_2 = 0.985$; $b_1 = 19.384$; $b_2 = 0.996$; $c_1 = 50.695$; $c_2 = 1.003$ | | | | |

4.2. Validation

In this section, the results obtained from the numerical simulation are compared with the results obtained by the mathematical model in section 4.1. Hence, their root mean square error is calculated for these two results by considering a different range of particle dimensions and the dynamic viscosity of the fluid.

The Fig. 12 shows the validation of results between D_2 versus required average voltage for different diameters of D_1 with their root mean square (RMS) error which is given in table 4.

Table 4. Validation of results for particle dimensions

| Fig.13 | Size of D_1 (μ m) | RMS error (%) | Fig.13 | Size of D_1 (μ m) | RMS error (%) |
|--------|--------------------------|---------------|--------|--------------------------|---------------|
| a | 2 | 2.6959 | c | 18 | 1.0656 |
| b | 10 | 0.2879 | d | 26 | 0.3008 |

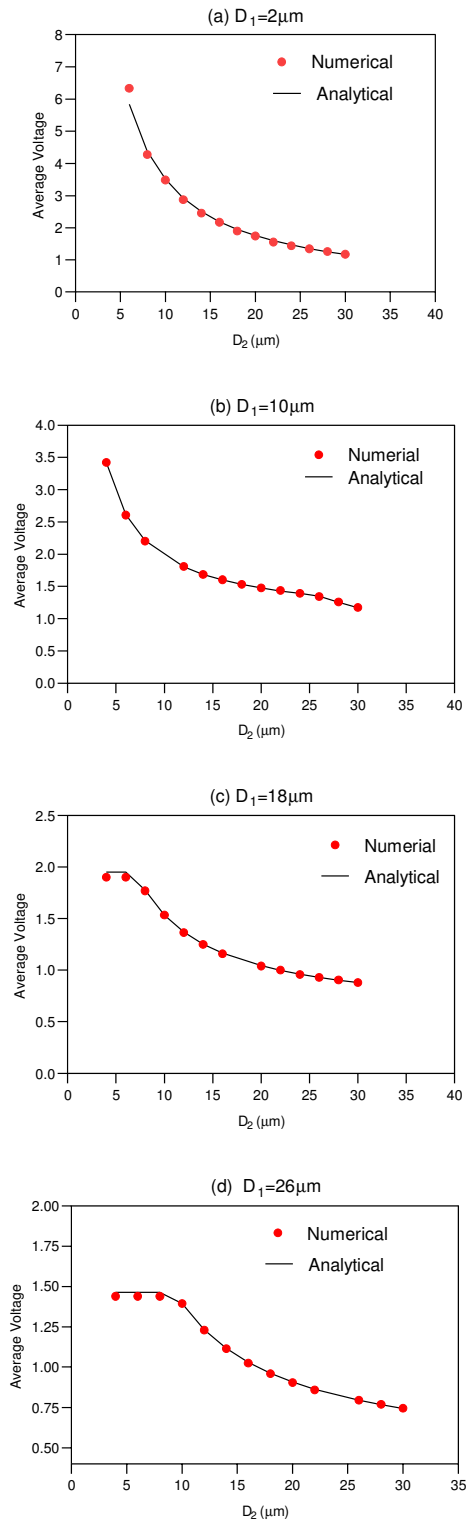


Fig. 12. D_2 vs Average voltage

The numerical and the analytical values for the fluid viscosity against the voltage are plotted in the Fig. 13 and it is observed that the root mean square error is equal to 0.1495%, which is approximately equal to the numerical values. The RMS error calculated for all the 1450 combinations of data-sets is found to be 1.4412%. Hence, it can be seen that the values calculated from the mathematical model are almost equal to the values obtained from the numerical data. Hence, the model prepared to calculate the voltages is approximately correct as it shows good accuracy.

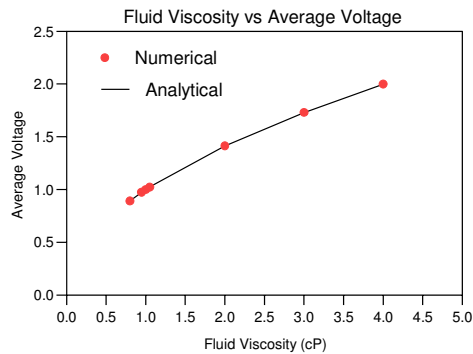


Fig. 13. Fluid viscosity vs Voltage

5. CONCLUSION

Microfluidics is used for separation of cell-like particles required for diagnostic purposes. This study focuses to detect the voltage required for separating two cell-like particles. From the numerical simulation, it is observed that the particles separate above a certain voltage and on further increasing the voltage, it is observed that the particles do not separate above a certain voltage value. Thus, there exists the voltage range in which the particle separates. It is also observed that at the lowest and the highest voltage values the cell-like particles touch the wall of the channel which may damage the cell. Thus, it is recommended to apply an average voltage which will prevent the cell from sticking the channel walls. Hence, a mathematical model is prepared to calculate the average voltage required in the separation of two cell-like particles.

The parameters, such as the cell geometry, cell properties and buffer solution properties are studied in detail to study their contribution to the required voltage. It is observed that the size (diameter) of the two cell-like particles and the dynamic viscosity of the buffer solution are the significant parameters affecting the values of voltage required in the cell separation. Other properties such as density, permittivity and conductivity of cell particles and its membrane do not contribute to the voltage required for separation of the cell-like particles.

This model considers the particle size (diameter) ranging from $2\mu\text{m}$ to $30\mu\text{m}$ which include both healthy and unhealthy cells and the dynamic viscosity ranging between 0.8cp to 4cp .

Further, the results obtained from the numerical simulation are compared with the values of the voltages predicted from the formulated mathematical model. Whereas the RMS error for the entire data-set of size variation is observed to be 1.4412% . Thus, the model predicts the voltage required in the separation of two cell-like particles with good accuracy. It is recommended that the developed model can be used for finding voltage required using the channel geometry considered for this study.

NOMENCLATURE

| | |
|------------------------|-------------------------------------------------------------------|
| $\text{Re}[K(\omega)]$ | : Claussi Mossotti Factor |
| r | : Radius of the sphere |
| $\nabla(E)^2$ | : Gradient of magnitude of a square of the electric field |
| ϵ_o | : Vacuum Permittivity= $8.85 \times 10^{-8}\mu\text{F}/\text{cm}$ |
| ϵ_m | : Medium Permittivity |
| ϵ_p | : Particle Permittivity |
| ϵ_p^* | : Complex permittivity of particle |
| ϵ_m^* | : Complex permittivity of medium |
| D_1 | : Diameter of particle 1 |
| D_2 | : Diameter of particle 2 |
| μ | : Fluid Dynamic Viscosity |

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