Designing and Fabrication of Lab-On-a-Chip for the Therapeutic Drug Monitoring of Erlotinib Hydrochloride

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Abstract

Microfluidics and Biosensors are two principal fields which paved the way for the inception of Lab-on-a-chip (LOC) which provides early and cost-effective disease detection, from monitoring to treatment. LOC is a device which uses very small amounts of fluid on a microchip to do certain laboratory tests. These microfluidic devices use body fluids or solutions containing cells or cell parts to diagnose diseases. The main advantages of LOCs include dramatically reduced sample size, much shorter reaction and analysis time, high throughput, automation, and portability. The potential use of LOC technology in the medical domain includes early diagnostics, therapeutic drug monitoring and personalized medicines. Currently, the anticancer drugs are prescribed on the basis of Body Surface Area (BSA), which can cause significant side effects such as chemo brain, anemia, infertility, organ failure etc. Prevailing Therapeutic Drug Monitoring (TDM) techniques such as chromatography, bioassay etc are time consuming, expensive, and need expertise. As an alternative an LOC device was designed, which helps in the continuous TDM of antineoplastic drug Erlotinib Hydrochloride. The electrode system detects homogeneously mixed Erlotinib in the blood interface and the organic solvent. Using COMSOL Multiphysics software, various passive micromixers were simulated. Their velocity and concentration profile were analyzed for studying the efficiency of mixing

Keywords: Microfluidics, Lab on a Chip, Therapeutic Drug Monitoring, COMSOL Multiphysics

1. Introduction

Cancer is a broad term which describes a disease that results when there is an uncontrolled growth and division of cells. Chemotherapy, immune therapy, hormone therapy, radiation therapy, etc. are a few of the methods used to treat cancer. In order to kill malignant cells, chemotherapy uses antineoplastic medications that target fast dividing cells [1]. Erlotinib is one of these and selectively inhibits a number of tyrosine kinase receptors linked to angiogenesis and cancer growth, for treatment of non-small cell lung cancer and advanced or metastatic pancreatic cancer [2]. Currently chemotherapeutic drugs are administered into the patient's body based on their body mass index. Due to the great heterogeneity in the absorption and metabolism of various medications due this, accumulation of drugs cause infertility, neurotoxicity, organ failure, hearing issues, etc. [3].

In order to avoid such side effects, continuous monitoring of drug concentration in patient's blood is necessary. Therapeutic drug monitoring (TDM) is the process of periodically testing a patient's bodily fluids, most frequently blood plasma, to assess the presence of a certain medicine. There are currently a number of analytical methods that can be used to do TDM, including spectrophotometry, fluorimetry, TLC, HPLC, GLC, as well as radioimmunoassay, enzyme immunoassay, etc. [4-6]. These methods have a variety of drawbacks, including a lack of accuracy and sensitivity in pharmaceutical assay methods, a lack of infrastructure in rural areas, report variations from lab to lab, a lack of training and skills for quality assurance, and the fact that they are very expensive and time-consuming [7].

As an alternative to such analytical techniques an electrochemical sensor was developed to perform real-time monitoring of the antineoplastic drug Erlotinib. In electrochemical sensors use an electrode as a transducer element when an analyte is present [8]. Some of the main electrochemical methods used include, Potentiometry, Conductometry, Amperometry and voltammetry, Coulometry, Capacitance, where responses monitored by different methods are based primarily on potential, resistance, and electrical current [9].

The term" microfluidics" describes the behaviour, exact control, and manipulation of fluids that are geometrically limited to a small scale, at which surface forces predominate over volumetric forces [10]. Homogeneous mixing of the fluids passing through the microchannel is one of the major challenges to design LOC devices. Effective mixing can be achieved using either an active mixer or a passive mixer. In passive mixer, no external energy source is required for mixing the fluids through the microchannel, which influenced by channel geometry and pressure gradient between inlet and outlet. In order to improve the mixing efficiency by different geometry of inlets channels (like T and Y shaped) and flow channel (like zig-zag, serpentine structures, and baffle arrangements) [11], then compared to parallel flow pattern which takes more time for diffusion [12][13]. And other ways comprise of splitting the inlet stream into sub streams and rejoining [14-17] and network system is a microfluidic gradient generator [18]. To introduce chaotic advection, certain static mixers repeatedly shift the direction of the channel or add ridges and barriers to the wall [19]. In active mixing external energy is used for moving the fluid, this includes di-electrophoresis, electrokinetic disturbance, pressure perturbation, magnetohydrodynamic disturbance and ultrasonic vibration [20-28]. Another design includes the ciliated and flagellated channel where the movement controlled by electrostatic actuation or magnetic induction [29-31].

A lab-on-a-chip is a miniature device that incorporates one or more laboratory-based studies, onto a single chip [32]. LOC is also known as a micro total analysis system because to the fact that it requires such a small fluid volume, frequently less than picolitres [33]. The benefits of LOC include, reduced sample volume required, quicker analysis, improved process control, cheaper manufacturing cost, large parallelization due to compactness, allowing high-throughput analysis and a safer platform for chemical, biological, and radioactive substance research [34-38]. Shorter analytical times, less reagent costs, and reduced chemical waste are other benefits of LOC devices [39]. Hence combining of microfluidics into a single chip, the instruments are continually being developed for carrying out multiple laboratory investigations at once, such as DNA sequencing, chemical synthesis, and biochemical detection [40].

Passive mixers with varying geometry can be designed and simulated using COMSOL Multiphysics software. It permits the integration and coupling of a number of physical phenomena that are described in terms of partial differential equations (PDEs) into a single model, which is used for both learning and research and development [41]. The designed LOC device can be fabricated using techniques such as photo-lithography, screen printing, inject printing, laser micromachining and imprinting [10]. This programme combines several systems to examine whether the design is practical good or not [42]. This tool's benefits include the ability to tackle complex single, couple, multi-physics problems. It includes a variety of analysis types, including stationary, time- and frequency-domain, and combine many physics in one environment with high performance processing. Simulation study required measurable results, and COMSOL allows analysis of results using a set of functions which explore a data set, and use it for further report more flexible and easily do the analysis [43].

The objective of this work is to design and simulate a passive micro-mixer with a greater mixing efficiency using COMSOL Multiphysics software for TDM.

2. Micromixers Design and Simulation Studies

2.1 Design of Micromixers

Design simulation helps manufacturers verify and validate the intended function of a product under development, as well as the manufacturability of the product. Simulation is used to evaluate the effect of process changes, new procedures and capital investment in equipment, which helps to predict the performance of a planned system, comparing alternative solutions and designs. By changing the parameters of the micro-channel inlet, the influence of the channel shape on fluid mixing was examined. The length (L), depth (D), and height (H) of the microchannels were designed as 45 mm, 0.1 mm, and 1.4 mm, respectively, for modelling and simulation. One of the inlet systems with ethanol and other with water is referred to as a binary inlet system. When the two streams came to the point of contact is where mixing begins.

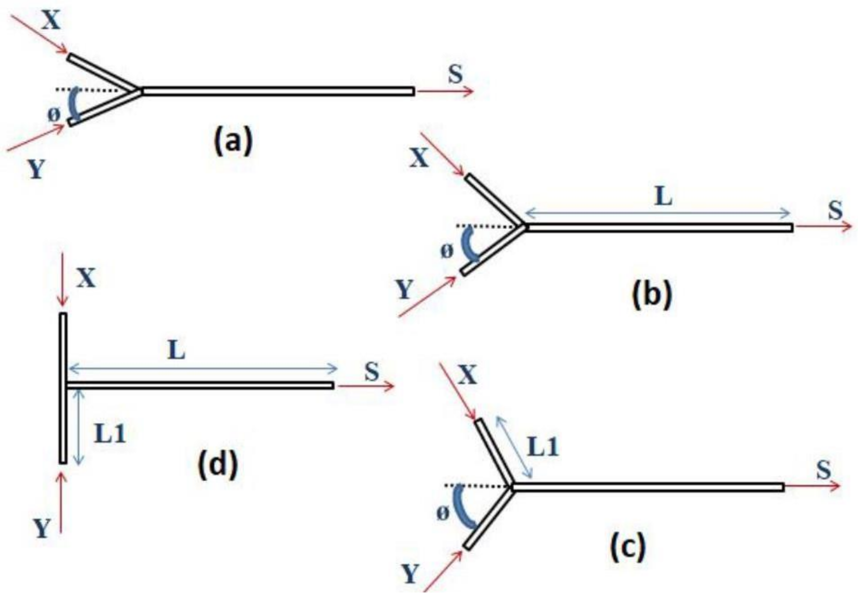

Fig. 1. Geometry of various Y channel having ‘θ ′ 30°, 45°and 60° and T-shaped Microchannel having 5mm entry length.

The simulation studies of the microchannel starts by comparing the dimensions of inlet channel which includes the angle of inclination and the entry length. And the best simulation result from the studies chosen to compare the mixing channel. In this comparison studies of mixing channel includes straight and curved channel. Fig. 1 depicts the straight channel having no obstruction with varying angle of inclination ‘θ’. Here ‘θ’ values were taken as 30°, 45°and 60° which form a Y-shaped microchannel. From the figure, inlets and outlet stream indicated by 'X', 'Y' and 'S' respectively. Straight microchannel having best simulation outcome was chosen to study concentration profiles of microchannel having entry lengths 'L1' 3mm, 4mm and 5mm to increase the diffusion rate and residence time of the fluid stream.

Microchannels with meander pattern on the flow features are shown in Fig. 2. Comparing with normal meander have path obstruction, which contribute to effective mixing of binary or other system. The structure of modified meander represented in the Fig. 3. The design of the modified meander is selected according to the dimensions of normal meander. Hence the radius of the curved part reduced to 0.1 mm, but each unit of meander is 0.6 mm width and 1.4 mm height, which is kept as constant according the normal microchannel dimensions. Same as normal meander, the modified one also have 14 meander units, where each meander consists of 15 subunits, which provides more advection to the fluid flow. Each of the subunits have size of 0.2 mm.

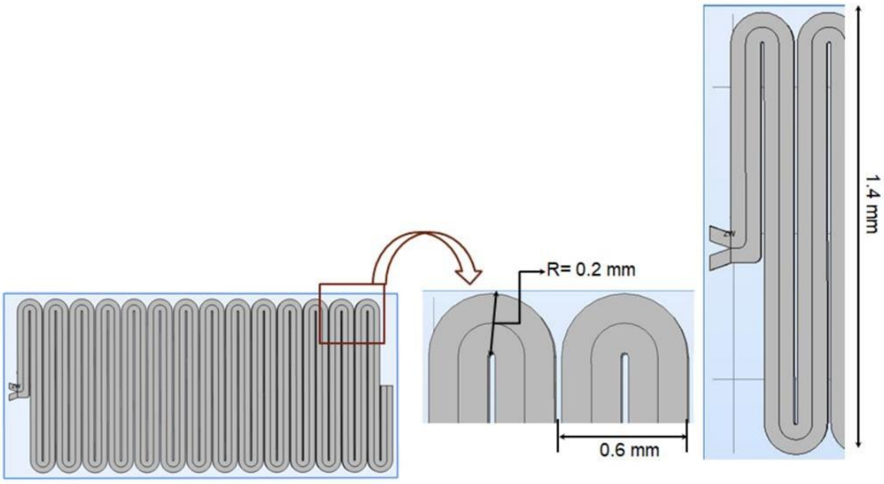

Fig. 2. Geometry of Normal meander having inlet dimensions; L1 5mm and ‘θ ′45°

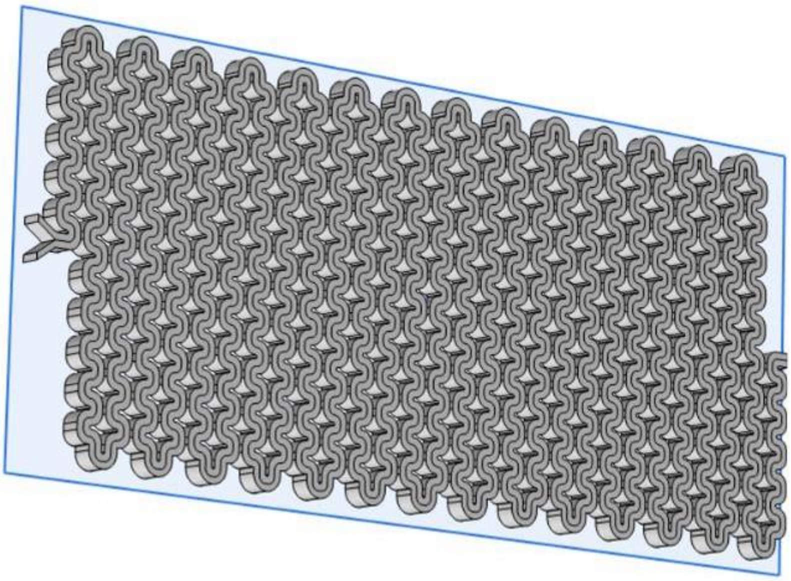

Fig. 3. Structure of Modified meander

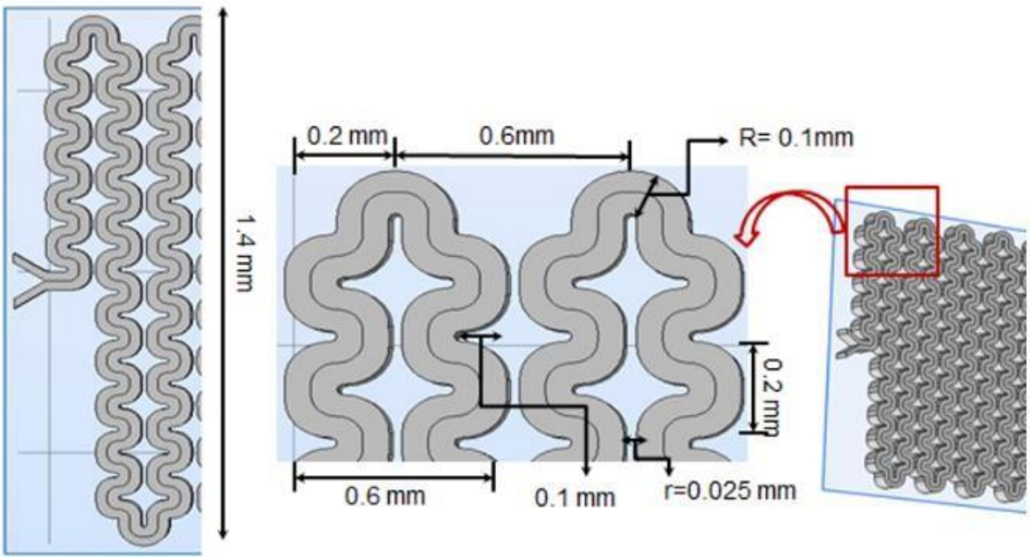

Fig. 4. Geometry of modified meander having inlet dimensions; L1 5mm and ‘θ’ 45°

2.2 Optimization of Parameters

During the simulation studies, certain parameters were kept constant which is explained in Table. 1 The inlets were given the boundary conditions of laminar inflow with an inlet pressure of 1 atm. The outlet boundary condition was specified to be laminar outflow with zero static pressure [11]. Single phase laminar flow and Transport of diluted Species physics interface was used to study the flow behaviour. For simulation study, an equimolar concentration 5 µM of primary inlet streams was considered. To each of the inlets the solvents were assigned.

Table. 1. Properties of model fluid at 20 °C

Fluid	Density (Kg m^{-3})	Viscosity (Kg m^{-1} s^{-2})	Diffusivity (m^2 s^{-1})
Water	9.998×10^2	0.9×10^{-3}	1.2×10^{-9}
Ethanol	7.89×10^2	1.2×10^{-3}	1.2×10^{-9}

2.3 Numerical Analysis

In this study, we investigated the potential of exploiting chaotic advection of different structures to improve the mixing quality of two fluids in a microchannel using the COMSOL Multi-physics software. The Laminar flow and Transported of Chemical Species Modules of the COMSOL Multiphysics software were used to model the flow fields for each structure [44].

For an incompressible Newtonian fluid, the flow fields are derived by solving the Navier-Stokes formulas of motion is:

$$\rho\nabla.u = 0 \quad (1)$$

Where, ’ρ’ is the density and ’u’ is the velocity vector.

In order to bring flow inside the channel there must be no slip along the solidwalls, a given velocity profile on the intake boundary, and a given pressure on the outflow boundary. Zero flux on solid walls and convective flux on the outlet boundary are theboundary conditions for the Convective Diffusion equation. If there is just one entrance port, there is zero concentration in one half and one concentration in the other. If the geometry has two entrance ports, the concentration in one is zero and in the other is one; the average concentration will be half. In the first method, a discontinuous concentration profile across theintake is used to solve the convection-diffusion equation in steady flow is:

$$\frac{dc}{dt} + u.\nabla c = \nabla.(D\nabla c) + R \qquad (2)$$

Here, C is the concentration of the species (mol/m^3); D is the diffusion coefficient (m^2 s^{-1}), R is the reaction rate expression for the species (mol/(m^{3}s)); u is the velocity vector (m/s). By separately resolving the Naiver - Stokes equations for the given geometry, the velocity field’s solution may be found. The convection-diffusion equation for the steady state is then solved. Although mixing a diluted chemical with another liquid in a slow, laminar flow is a challenging task, the findings revealed that for 1.0 Reynolds number, the amount of mixing depended primarily on the flow length divided by the Peclet number, across all geometries [9]. Calculating the standard deviation at sampled microchannel portions will reveal the degree of mixing. Due to its influence on the amount of time that a fluid spends in the channel, velocity is a key factor in mixing efficiency. The design with the lowest average flow velocity would suggest a longer fluid residence time in the microchannel. The average velocity at any particular cross section is equal to the flow rate divided by the cross-sectional area. The average velocity was determined from the simulation by integrating the velocity profile over all places and dividing it by the cross-sectional area [11].

2.4 Simulation Studies

It is possible to analyse the level of mixing between the various streams using the concentration plot obtained from the transport of dilute species interface by solving the convection-diffusion model, which uses the diffusion mass transfer equations to determine stream concentration at various locations. The concentration of the output stream will be constant across the cross-section of the microchannel in the case of optimum mixing. The efficiency of mixing is significantly impacted by velocity and diffusion because they change how long a fluid stays in the channel. Based on the concentration and velocity profiles from the simulation studies, the effective microchannel parameters are selected for further comparison [11].

The capacity to effectively mix various fluid components is one of the fundamental needs for microfluidic devices. The fluid flow in a pressure-driven system is laminar since the Reynolds number in microchannels is low, hence it is necessary to design the channel shapes to encourage transverse flows and mixing [44].

3. Result and Discussion

3.1 Effect of Inlet Channel Dimension

3.1.1 Angle of Inclination

From the velocity profile Fig. 5a shows that 30° have significant impact on homogeneous mixing due to greater velocity component in the direction of flow. From the values of velocity profile at starting point of mixing channel shows in Table. 1, 30° inlet stream angle has greater velocity at the center of channel that's is; 2.2217 m/s, which is far greater than other angles. This causes reduction in both residence time and effective homogeneous mixing.

Compared to the structure wise fabrication, angle 45° inlet channel inclination required intermediate area and velocity. where as in the case of 60° have lower velocity in the flow direction and maximum area utilized for the fabrication. The result has to concluded by considering both velocity and the inlet channel area. Hence the inlet stream of the fluid having angle of inclination 45° have normal velocity component in the horizontal and intermediate area provide the maximum effect in the homogeneous mixing.

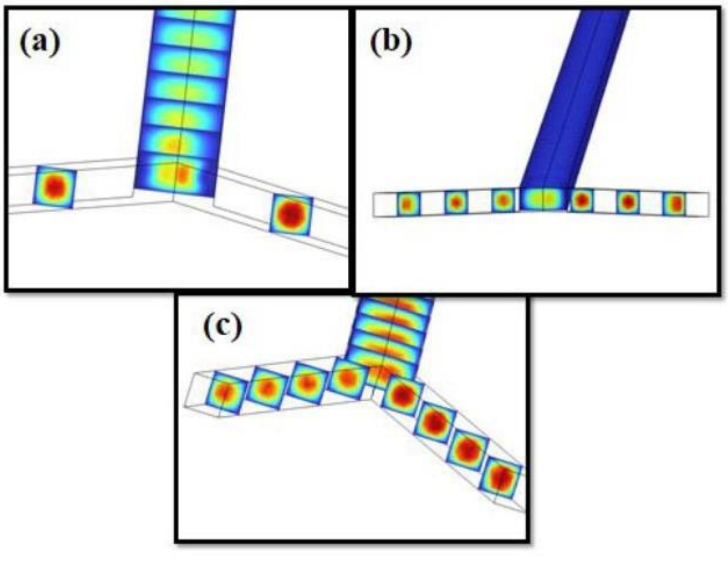

Fig. 5. Inlet velocity profile for different angles of inclination a) 30°, b) 45°and c) 60°

Table. 2 Variation in the inlet velocity with respect to angle of inclination

Angle of inclination of Inlet channel ‘θ’	**Inlet center velocity (ms^{-1})**
60°	1.6828
45°	1.8334
30°	2.2217

From the simulation results in Fig.4.1, it was observed that microchannel with an inclination of 45° has more efficient mixing. For further simulation 45° angle of inclination is chosen.

3.1.2 Entry Length

The outlet velocity profile of Y-channel having different entry length (Fig. 6). It's clearly visible that the fluid attained a uniform maximum velocity at the center and zero velocity at the boundary. Due to Frictional force, the velocity profile tends to reduce from center to near the walls. Depends on the efficiency of the fluid stream to attain the maximum velocity at the center the mixing also changes. Hence for further studies carried with straight channel with 45° inclination and various inlet entry length (L1) 3, 4 and 5 mm.

The entry length, 5mm have more effect on mixing due to increase in residence time. When residence time increase, the fluids have more contact and maximum mixing. Whereas other entry length had lesser residence compare to 5 mm. Y-channel having 3 and 4mm entry length could not attain the fully developed flow before the mixing starts, due to small length of the inlet stream. Hence the Y-channel having the L1 value 5mm have greater efficiency in mixing.

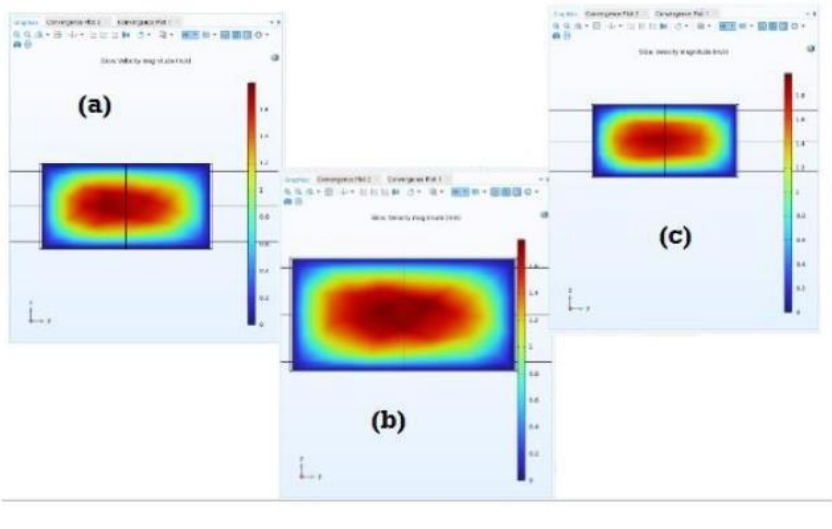

Fig. 6. Velocity profile for different entry length at an angle of inclination 45° (a) 4mm, (b) 5mm, (c) 3mm

3.1.3 Entry Length

In the Fig. 7 shows the velocity profile of the straight channel T and Y respectively as 'a' and 'b'. The T-channel can't attain the fully developed flow easily due to the change in the entry point of the inlet stream, compared to the mixing channel flow. And the inlet fluid stream has only horizontal velocity component has the flow of the stream will very less than other inlet stream shape. As well as the T-channel takes more space in the fabrication of this channel.

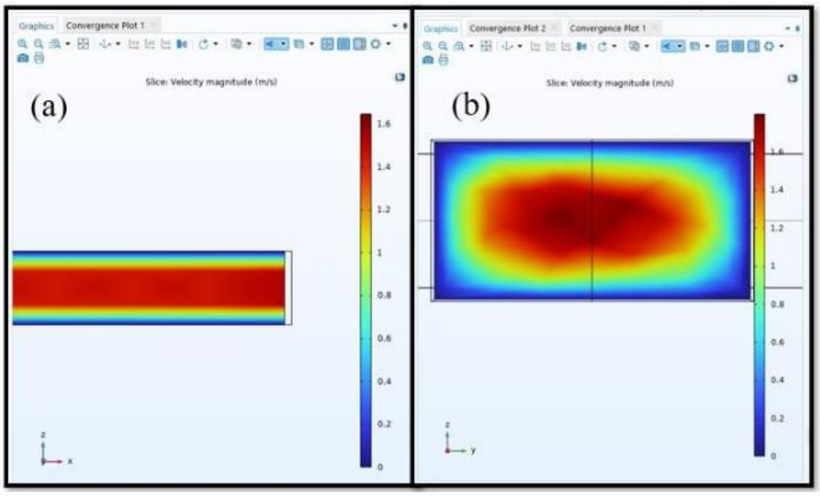

Fig. 7. Velocity profile straight channels having entry length 5mm; (a) T-channel and (b) Y-channel with angle of inclination 45°

And the Y-channel with 45° inclination have velocity component in the direction of mixing channel, hence the Y-channel inlet shape shows more mixing efficiency. So, the Y-channel with 5 mm entry length and 45° inclination was compared with the curved mixing channel. From both simulation results, the combination of inlet channel entry length 5mm and angle of inclination 45° provide more mixing by increasing the residence time. Hence this combination chosen to carried out further comparison studies.

3.2 Effects of Mixing Channel

The Fig. 8 depicts the concentration profile perpendicular to the fluid flow which varies along the y axis. From this profile it comes to a conclusion that comparing the normal straight channels the meander has more chaotic advection which led to the mixing of fluids. During the flow through the curved surface a centripetal for enact on the fluid and provide more diffusion. Changing the direction of velocity also contributed to the homogeneous mixing. Mixing efficiency of straight and curved channel was studied.

The observation of straight mixing channel has complete mixing at 40 mm and 32 mm for T and Y – channel respectively. And in case of normal meander the homogeneous mixing starts at 19mm.

When the LoC fabricated with straight channel, the homogeneous mixing attained by the binary system at far distance. This constrain can be solved by introducing a meander channel. which further increase the pathlength of the fluid flow as well as the residence of the whole system. Hence comparing the straight channels of Y and T with normal meander, latter one shows more efficiency in mixing and size reduction of overall system.

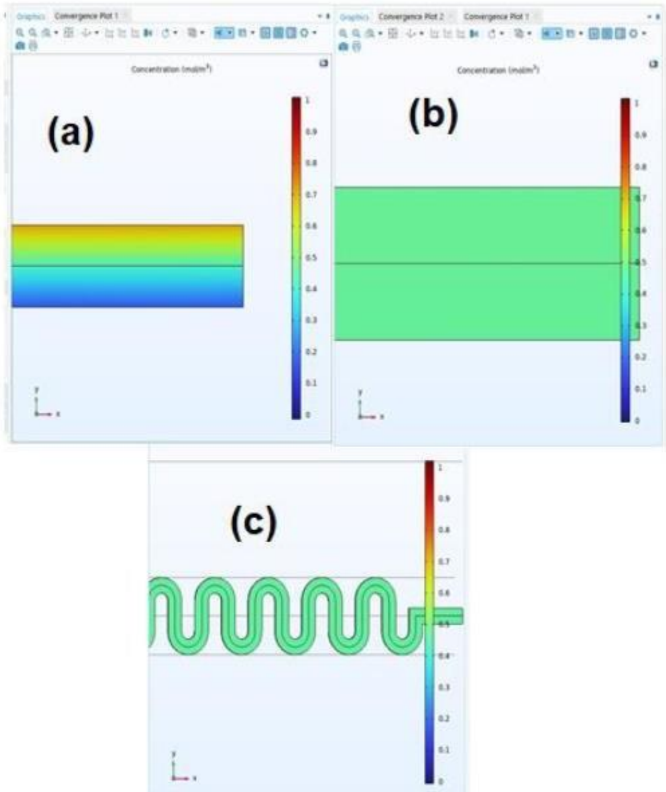

Fig. 8. XY plan Concentration profile of; a) T-channel, b) Y-channel with 30° inclination and 5mm entry length and c) normal meander

3.3 Curved Channel

The Mixing efficiency y was more observed in the modified meanders as compared to normal one. The flow path of the normal meanders added with some obstruction which designed into meander Incorporated meander. Relating to the chaotic advection of normal meander, the flow path of the fluids changing in smaller interval of time, from Fig, 9 proves that greater mixing offered by the modified meanders. Along the flow of the fluids, the streams have lesser concentration varies that mixing observed at the value of 2.5µM at cut line 30mm in different channels. Modified meander has homogeneous mixing at 10 mm.

The overall advantage of the modified meander, reduction in the size of mixing channel, provide maximum chaotic advection, increase in residence time and provide centripetal force at curved surface for mixing. Number of curved surfaces in the modified meander increasing the mixing efficiency.

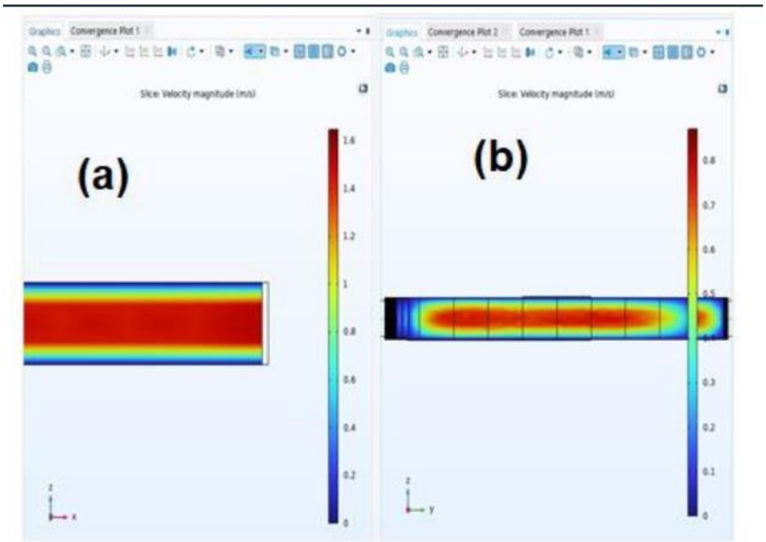

Fig. 9. YZ plan Concentration profile a) normal meander and b) modified meander

The graph depicts on the Fig. 10 shows that variation of the concentration gradient of the binary system. The concentration profile of straight channels, normal and modified meanders are obtained by introducing cut-line at 30 mm mixing channel length. Then the concentration at that particular point of each channel noted. And the standard deviation of each channel calculated and plotted this graph. According to the simulation, the homogeneous mixing having concentration of 2.5µM. and it's shows that the modified have less deviation from effective efficiency.

Table. 3 Standard deviations for the various designs

Design Analysed	**Standard Deviation (mol m^{-3})**
T-channel	0.325
Y-channel	0.210
Normal Meander	0.080
Modified Meander	0.002

Table. 4 Comparison of outlet concentration of various microchannel

Design Analysed	Outlet Concentration (mol m^{-3})
T-channel	0.38700
Y-channel with 'θ' 60°	0.42065
Y-channel with 'θ' 45°	0.46635
Y-channel with 'θ' 30°	0.47281
Normal Meander	0.47346
Modified Meander	0.49863

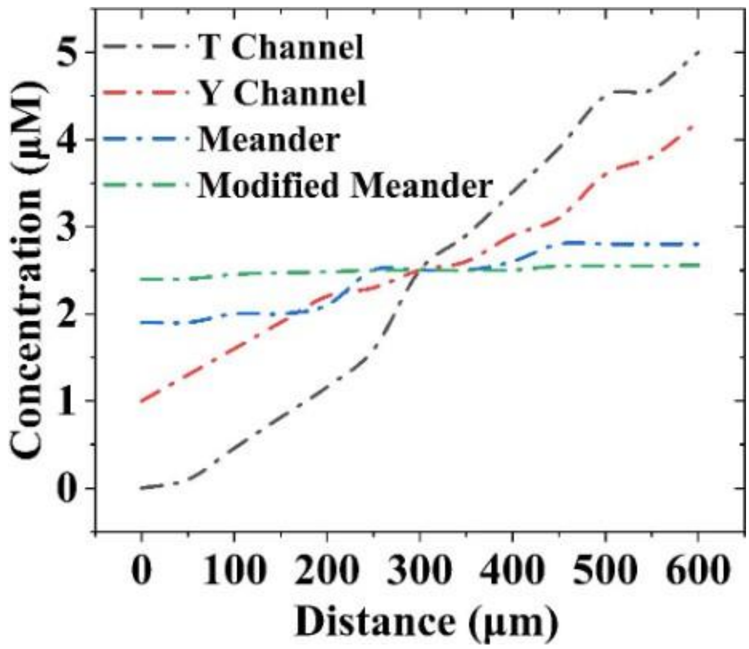

Fig. 10. Concentration profile at cut line 30mm in different geometries

The graph depicts on the Fig4.6 shows that variation of the concentration gradient of the binary system. The concentration profile of straight channels, normal and modified meanders are obtained by introducing cut-line at 30 mm mixing channel length. Then the concentration at that particular point of each channel noted. And the standard deviation of each channel calculated and plotted. According to the simulation, the homogeneous mixing having concentration of 2.5μM. and it's shows that the modified have less deviation from effective efficiency.

Sharp edges are necessary for effective mixing, which utilised to increase this force even more. However, it result in additional pressure loss and the emergence of dead zones. Sharp angles were therefore preferred over rounder designs. When the diameter of the channels is increased, the concentration gradient across them becomes less steep, which reduces mass transfer. The results of the computer simulation revealed that a decrease in angle of inclination promoted mixing in the channel.

4. Conclusion

A computational analysis of the mixing effectiveness of several channels with variable inlet angles and lengths, meanders, and modified meanders was done using the COMSOL Multiphysics software. Using Naiver-Stokes equation and convective diffusion equation of different geometries simulations were performed. By resolving the diffusion equation and concentration profile to trace particles, it is possible to see the mixing profiles of two fluids. These geometrical patterns have demonstrated the capacity to stimulate transverse flows, improving the mixing quality. Modified meander designs provide better mixing qualities, presumably as a result of the system's chaotic flow. Hence it is better to use modified meander instead of both normal meander and straight channel. This designed help to reduce the size of the channel and complexity. Modified meander has high efficiency in homogeneous mixing due to smooth curved structure, more chaotic advection, residence time and centripetal force.

So, the LoC having modified meanders microchannel is highly portable, homogeneous mixing and more accuracy.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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