The Effects of Micromixing Two Solutions of Two Concentrations in a Two Tier PDMS Micromixer

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Abstract. Micromixing technology has drastically advanced in the past few decades. Micromixers are one of the elements in integrated microfluidic systems for chemical, analytical chemistry, pharmaceutical, and biological applications. In this study, two tier micromixer was used to mix and dilute two solutions of similar and different concentration in order to investigate performance of micromixer’s mixing. This paper presents the fabrication of a designed micromixer using polydimethylsiloxane (PDMS) and vinyl tape methods which reduce time, cost and complexity of prototyping. The serpentine structure of the microchannels was designed to enhance both mixing and dilution. Two types of food dyes and distilled water were used to investigate the mixing performance of the micromixer followed by spectrophotometry analysis. It is observed that the single dye solution and distilled water shows better mixing performance compared to the micromixing of two dye solutions which was supported by the diffusion theory. 2.00 ml/min was the optimum flow rate that allow optimum mixing and dilution between two different concentrated liquids.

1. Introduction
Micromixing plays a significant role in many liquid-related processes such as precipitation, crystallisation, polymerisation, self-catalysis, enzymatic catalysis in chemical, pharmaceutical and biological applications [1-8]. The main purpose of microfluidic mixing is to obtain a thorough and rapid mixing of numerous samples in microscale devices. Sample mixing is accomplished by improvising the diffusion effect between different species flows [9]. Generally, micromixers are classified as passive or active, whereby in active micromixers, an external energy force is exerted to blend the sample species where in passive micromixers, microchannels configurations are specifically designed to increase the contact area as well as contact time between the liquids for better mixing [10]. For example, in [11] micromixer with zig-zag channels, elliptical barriers and short mixing length were used to investigate the mixing performance and its Reynold number and in [12], T and Y shape with longer mixing length micromixer was used to evaluate the performance of the micromixing. Although both reported designs show a higher mixing index 0.90-0.95 but fabrication require the use of cleanroom facilities.

Alternatively, we proposed a non-lithographic low-cost fabrication technique, to fabricate a PDMS based serpentine micromixer using vinyl tape as the master mould. However, vinyl template requires a
good workmanship and extra caution throughout the process. Reusability of the vinyl template dependent on stickiness of the material it is made off and clean cut of the vinyl template. In mass production, clean cut and better quality-made vinyl template may improve the durability of the vinyl template [13]. In this work, two solutions of two concentrations were used to investigate the mixing performance. The advantages of implementing micromixers in liquid processing include processing accuracy, efficiency, minimising the usage of reagents and ease the devices and fluids disposal [14, 15].

Two equations that are commonly used to govern the flow characteristics within a microfluidic device are Reynolds number and Péclet number which are simplified from the convection-diffusion equations. Reynolds number measure the relative significance between inertial and viscous forces defined as in equation 1 [14]. Meanwhile, Péclet number is the ratio of convective transport to the diffusive transport used to analyse the mass transport in gradients generators as defined in equation 2 [14].

\[
R_e = \frac{\rho UL}{\mu} \quad (1)
\]

\[
P_e = \frac{UL}{D} \quad (2)
\]

Where \( \rho \) is the fluid density, \( U \) is the mean fluid velocity, \( L \) is the characteristic length of the fluidic channel, \( \mu \) is the dynamic viscosity and \( D \) is the diffusion coefficient [14]. It is commonly known that microfluidic device has a very small Reynolds number \((R_e << 1)\) and high Péclet number \((P_e >> 1)\) indicate that the flow within the microfluidic device is always laminar [16, 17]. Diffusion of a particular solute specie in a diluent species is classified by the diffusion coefficient diffusivity, \( D \) as referred in Fick’s first and second laws [17-19]. Both laws support two general concepts on the diffusion process in the microfluidic applications [17-19]. Firstly, mixing occur from a high concentration gradient to a low concentration gradient. Second, diffusion is a time-based evolving phenomenon which creates both high and low concentration regions [17-19]. In order to quantify concentration of the mixed and diluted liquids, Beer-Lambert’s equation of absorbance \( (A) \) as in equation 3 is used to measure the light absorption of different liquid concentrations produced at the outlets [20, 21]. Due to the linear relationship, it is expected that the measured absorption increases with the increase in concentration of solute in a fluid in each outlet of the micromixer based on the spectrophotometry analysis. However, the law is not obeyed at higher concentration of solutes in a solution.

\[
\text{Absorbance, } A \propto l.c \quad (3)
\]

\[
\text{Absorbance, } A = \varepsilon l c \quad (4)
\]

Where \( \varepsilon \) is the absorbivity, \( l \) is the travel length of light and \( c \) is the concentration of solution.

The aim of this work is to fabricate PDMS micromixer and compare the micromixing performance of two solutions of similar and different concentration. Micromixing performance was analysed by measuring light absorbance of sample droplet at the four microchannel outlets. Furthermore, different flow rates were applied in order to find the optimum rate in fluid mixing.

2. Methodology

2.1. Preparation of Solutions for Liquid Mixing

Three different solutions red dye solution, yellow dye solution and distilled water were prepared. For preparation of the solution of the red dye, 42.25 mg of the red dye powder was diluted with 50 ml of distilled water to produce a solution with concentration of 0.845 mg/ml. Similar steps were repeated to prepare the yellow-dyed solution at 0.845 mg/ml. 2 ml of red and yellow dyed solution with varied concentration from 0.035 to 0.845 mg/ml were prepared to obtain the their respective peak absorbance and produce a reference graph of peak absorbance to compare the performance of mixing and dilution of the micromixer.

2.2. Fabrication of Micromixer using Vinyl Tape Method
The microfluidic mixer designed consists of two inlets with a diameter of 3.0 mm each (Figure 1). Each of the four outlets has a 4.0 mm diameter. The total length from inlets to outlets is 50.0 mm. The width of the micromixer is approximately 17.0 mm and the serpentine route is in a length of 11.0 mm with a thickness of 300 μm and 1 mm depth and width of the microchannel respectively. The serpentine routes are located at the first and second tiers of the micromixer. The vinyl tape method was used to fabricate the microfluidic mixer template designed as shown in Figure 2. The master template of the channel or pattern produced is highly economical, easy for fabrication, highly reproducible and is safe when compared with the chemical process of soft lithography [13, 22]. The micromixer channel pattern was designed using a Solidworks® 2015 x64 Edition software and vinyl adhesive template was cut using a vinyl adhesive cutting machine (Roland STIKA SV-12, Japan). The patterned adhesive tape was stuck on a petri dish and was stacked in 10 layers. Based on previous study [13], the thickness of each layer of vinyl adhesive tape were 30 μm and with the ten layers produced a total thickness (depth) of approximately 300 μm. PDMS mixture was prepared with a volume ratio of 10:1 for silicon elastomer and curing agent. Then, 16.5 ml PDMS mixture is poured into the petri dish. The PDMS mixture was vacuumed at -80 kPa in a 3-gallon vacuum chamber for one hour to remove the bubbles in the mixture. Next, the PDMS mixture was left at room temperature for four days to be polymerised. The cured PDMS was carefully peeled off from the mould and was cut into the size of glass slide at 70 mm × 20 mm. Next, cured PDMS is covalently bonded to the glass slide using PDMS gel and was left for 2 hours. The holes for the two inlets and four outlets were made using a 2 mm PDMS puncher. The holes were firmly connected using a Tygon® tubing with an outer diameter of 2 mm.

![Figure 1](image-url). (a) the schematic diagram and (b) dimensions of the micromixer (R denoted radius of outlets).
2.3. Dilution and Mixing the Two Solutions of Dye-Dye and Dye-Distilled Water.

The experimental setup to mix and dilute two solutions of similar and different solutions are as shown as in figure 3. The first experiment was to dilute and mix a solution of red dye and distilled water using the designed micromixer. The second experiment was to dilute and mix similar solutions of red and yellow dyes. Solution 1 contained pure distilled water while solution 2 contained a red-dyed solution of 0.845 mg/ml. Solutions 1 and 2 (5ml each) were withdrawn into two units of 5 ml syringe with the end of the syringe fitted with a pipette tip and a Tygon tubing of 2 mm in diameter. The tubings of the syringe which contained solutions 1 and 2 were then fitted into respective inlets 1 and 2 of the microfluidic device. Solutions 1 and 2 were then fed into a micromixer using a custom made infusion pump with a flow rate ranging from 1.00 to 4.00 ml/min. The custom-made infusion pump was allowed to pump in the solution at 1.00 ml/min until the colour intensity of the mixed solutions become visible and could be analysed using a spectrophotometry. Similar steps were repeated by varying flow rates from 2.00 to 4.00 ml/min.

The whole process was then repeated by substituting solution 2 with a yellow-dyed solution at 0.845 mg/ml. In order to obtain a proper mixing and dilution, it is important to observe for any presence of bubbles within the microchannels which should be purged out before further the mixing process. Purging the solutions into the micromixer at least three times before collecting droplet sample from the outlets can improve the spectrophotometry readings to a certain extent. Figure 3 illustrates the process of micromixing and spectrophotometry analysis. Ambient temperature of 25°C was kept constant controlled by an air conditioner throughout the processes.
Figure 3. Summary of micromixing and spectrophotometry

2.4. Spectrophotometry
The infusion pump was allowed to purge the solutions through the channels for 1 minute until the well-mixed solutions sample accumulated at four respective outlets. Sample droplets (5.0 μl) were collected from each of the four collection well using a micropipette. For each new sampling a new pipette tip was used to avoid mixing from other sample droplets. Collections of samples from the outlets must be carried out immediately when the infusion pump was stopped and placed on the microdrop plate. This is because withdrawal of samples from an outlet will cause an inflow of mixed solutions from another channel which would disrupt the concentration plate containing the samples from the four outlets contents in the outlet microchannels. The microdrop plate containing the samples from the four outlets was then placed into the microplate reader (SkanIt Go by Thermo Scientific). Six assays were labelled as Inlet_1, Inlet_2, Outlet_1, Outlet_2, Outlet_3, and Outlet_4 which represent the samples collected from the four outlets. Spectrum analysis was added in the protocol of the SkanIt Go software and spectrophotometry analysis was carried out at wavelength of between 350 nm and 800 nm. The readings from the spectrophotometry were then merged in a single graph. Similar steps were repeated to sample solution of varied concentration from 0.035 to 0.845 mg/ml to obtain respective peak absorbance to compare the performance of mixing and dilution of the micromixer.

3. Results and Discussion
Figure 4 shows the results of spectrophotometry conducted on sample solution collected from each outlet by micromixing solution of red dye and distilled water. Figure 5 shows the trend of mixed and diluted liquids from the different concentrated solution (red dye solution and yellow dye solutions) produced as the flow rate increased.
Figure 4. Experimental results of micromixing two solutions of different concentrations at a flow rate of (a) 1 ml/min (b) 2 ml/min (c) 3 ml/min (d) 4 ml/min
Figure 5. The trend of peak absorbance of light for micromixing of solution of red dye and distilled water.

Absorption of lights occurs between the wavelength of 450 and 600 nm while no absorbance was recorded from 620 nm and beyond (Figure 4). The peak absorption occurs approximately at wavelength of 520 nm while distinctive peaks were formed representing measured light absorption of samples from the inlets and the outlets which may occur since maximum absorbance of red dye occurs approximately at between 520 to 525 nm [23]. The numerical value of peak absorbance indicates the difference in concentration of solute (red dye solution) content in samplings from each outlet. The higher is the concentration of the red dye solution, the higher would be the peak absorbance value recorded from the outlets sample. When the flow rate was gradually increased from 1.00 ml/min to 4.00 ml/min, the peak value of measured light absorption at 520 nm fell closer to each other indicating that there is no differences in the concentration of liquid at the outlets (Figures 4 a-d). However, for flow rate 1.00 and 2.00 ml/min, there are distinctive peak absorption value for each droplet sample from the respective outlets. In addition, it is visible that the red colour tone produced at outlet channel gradually increases in intensity as it moved from outlet 1 to 4 (Figures 4a and 4b). From Figure 5, it is clear that flow rates of 1.00 and 2.00 ml/min produced a linear micromixing compared to the other flow rates (3.00 and 4.00 ml/min) which resulted in non-distinctive peak absorbance from the spectrophotometry reading of outlets sample. Linear increment of measured light absorption of outlets sample across outlets 1 to 4 showed a good mixing performance indicated that flow rates do affects the mixing performance of the micromixer designed and 2.00 ml/min are the optimum flow rates that support linear mixing.

Figure 6 shows the results of spectrophotometry conducted on a sample of solution collected from each outlet by micromixing solution of red dye and distilled water while Figure 7 shows the trend of mixed and diluted liquids from the different concentrated solutions produced with increasing flow rates.
Figure 6. Experimental results of micromixing two solutions of similar concentrations at flow rate of (red and yellow dyes) (a) 1 ml/min (b) 2 ml/min (c) 3 ml/min (d) 4 ml/min
Figure 7. Trend of peak absorbance of light for micromixing of two solutions with similar concentration

Absorption of lights occurs between the wavelength 360 and 600 nm while no absorbance recorded from 620 nm and beyond (Figure 6). The peak absorption occurred twice at approximately 430 and 520 nm with the peaks formed representing the amount of light absorption of samples from both the inlets and the outlets. This is because the maximum absorbance of red dye occurs at between approximately 520 and 525 nm while the maximum absorbance of the yellow dye occurred at approximately between 400 and 450 nm [23]. The peak absorbance of the outlet samples indicated clear separation of similar concentration solution that have been mixed and diluted. The numerical value of peak absorbance indicated the difference in solute concentration (red and yellow dye solutions) from each outlet. The formation of a second peak absorbance at 1.00 and 2.00 ml/min which was smaller indicated the mixing of red and yellow solutions which occurred at similar concentration (Figures 6a and 6b). When the flow rate was gradually increased (from 1.00 ml/min to 4.00 ml/min), it was observed that the peak value of the measured light absorption at 430 and 520 nm fell closer to each other indicating no differences in the concentration of the liquid at the outlets (Figures 6a-d). However, Figure 7 showed that mixing of two solutions of similar concentrations show fluctuated and flow rates unable to support a thorough micromixing.

Figure 8. Graph of concentration of solution and respective peak absorbance
Figure 8 shows graph of the peak absorbance produced by different concentration of solution. By referring to the graph, it is clearly seen that concentration produced at each outlet varies according to the respective peak absorbance. Hence, micromixer designed shows the ability of mixing and diluting two solution of different concentration to produced four output of different concentration solution as simulated in previous study [24].

Based on the findings, it was clearly seen that both flow rates and solute concentrations affect the mixing performance. It can be concluded that the optimum flow rate for two solutions of similar and different concentration to achieve mixing and dilution is 2.00 ml/min. Based on changes in colour intensity of mixed solutions in outlet microchannels and spectrophotometry, as the flow rate increased, poor mixing and dilution occurred with no obvious differences in colour tone and measured value of light absorption from droplet samples. Mixing and dilution in a passive micromixer is fully dependent on the diffusion process which occurs from region of high concentration to low concentration region until equilibrium was achieved. It is plausible that the ideal diffusion between red dye solution and distill water which produced a good mixing and dilution was due to the fact that the particles from red dye solution was able to move freely in the direction of fluid flow thereby filling in the empty spaces in the water region (Figure 9). Meanwhile, in mixture of red and yellow dye solutions of the similar concentration, particles of the dyes collide with one another, therefore taking longer time to fill in the narrow spaces in between them, resulting poor mixing and dilution.

![Figure 9. Comparison of diffusion occur between red dye solution with distilled water and red dye solution with yellow dye solution.](image)

4. Conclusion
A micromixer which could produce four different concentration gradients from a mixture of two solutions of two concentration was successfully fabricated and experimented. The optimum flow rate was 2.00 ml/min, which enabled optimum mixing and dilution to produce distinctive concentrated liquids at the four outlets indicate a proper mixing and dilution. Although at higher flow rates (3.00 and 4.00 ml/min), micromixing was still possible, it could not produce distinctive concentration gradient at the four outlets. Additionally, mixing and dilution between the two solutions of different concentration showed a better mixing performance when compared to mixing between two similar concentrations. Further research should take this points into concern when applying micromixer for mixing solution.

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