

## Dissolution and mixing of flavin mononucleotide in microfluidic chips for bioassay

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**Abstract.** Dissolution and mixing of flavin mononucleotide (FMN), which activates a luminescent reaction, were considered in various designs of microfluidic chip for pollution analysis of liquid samples. The aim was to determine the velocity mode of fluid flow ensured the uniform distribution of the FMN in the reaction chamber. Simulation of concentration distribution of FMN in various designs of microfluidic chips was conducted. It was shown that the passive mixing techniques based on the constant flow rate didn't provide mixing of FMN in acceptable time (3 seconds). The most efficient mixing was achieved using variable flow rate with a gradually increasing frequency of oscillation.

### 1. Introduction

The use of microfluidic technique and bioluminescent method increases an accuracy and sensitivity of a biological assay of liquid samples on toxicity, reduces the cost of analysis and its time compared to traditional methods using experimental animals and protozoa [1, 2]. One can make conclusions about the degree of a pollution of a sample by measuring a change in luminescence intensity associated with a reduction in the catalytic activity of enzymes in the presence of pollutants [3-5]. In the considered method the bioluminescent reaction starts when flavin mononucleotide (FMN) mixed with other components of the reaction stored in gel film solved in a reaction chamber. As FMN activates the reaction of light emission [6] it is important to have an uniform concentration of FMN in all parts of the reaction chamber. For example, poor and slow mixing can lead to that the reaction won't come to its peak, and the analysis won't be possible. FMN is initially immobilized in dried form in a microfluidic chip, so various designs of microfluidic chips and velocity regimes of fluid flow were considered using a numerical simulation to provide fast dissolution and efficient mixing of FMN.



## 2. Methods

Simulation was performed by means of COMSOL Multiphysics software for solving differential equations based on finite element method. The Navier-Stokes equations were solved to calculate the velocity profile of the fluid:

$$\rho \left( \frac{\partial \mathbf{u}}{\partial t} + \mathbf{u} \cdot \nabla \mathbf{u} \right) = -\nabla p + \mu \nabla^2 \mathbf{u} \quad (1)$$

$$\nabla \cdot \mathbf{u} = 0$$

where  $\mathbf{u}$  is the flow velocity vector (m/s),  $\rho$  is the fluid density (kg/m<sup>3</sup>),  $\mu$  is the fluid viscosity (Pa·s),  $p$  is the pressure (Pa). The Fick's second law with the added convective term was used for modeling the concentration distribution:

$$\frac{\partial c}{\partial t} + \mathbf{u} \cdot \nabla c = D \nabla^2 c \quad (2)$$

where  $c$  is the bulk concentration of FMN (mol/m<sup>3</sup>),  $D = 4.8 \cdot 10^{-10}$  m<sup>2</sup>/s is its diffusion coefficient [7]. The simulation of dissolution process was based on Noyes–Whitney equation. On the boundary related to the placement of dissolved reagent the equations for the rate of dissolution process, the change of FMN boundary concentration  $c_s$  and reagent flux  $N$  were applied with the following conditions:

$$\text{if } c_s > c, \text{ then } \frac{dQ_h}{dt} = (c_s - c), \text{ else } \frac{dQ_h}{dt} = 0 \quad (3)$$

$$\text{if } Q_h > Q_s, \text{ then } \frac{dc_s}{dt} = 0, \text{ else } \frac{dc_s}{dt} = -1000 \cdot c_s \quad (4)$$

$$N = \frac{dQ_h}{dt} \quad (5)$$

where  $Q_h$  is the quantity of dissolved FMN from unit area (mol/m<sup>2</sup>),  $Q_s = 0.005$  mol/m<sup>2</sup> is initial quantity of dried FMN per unit area. As initial condition  $c_s$  is equal to saturated concentration  $c_{\text{sat}} = 200$  mol/m<sup>3</sup> [8].

The quality of mixing was determined by calculating the variance of concentration normalized by average concentration  $c_{\text{av}}$ :

$$\sigma^2 = A^{-1} \int \left( \frac{c}{c_{\text{av}}} - 1 \right)^2 dA \quad (6)$$

where  $A$  is a total area of cross-section (m<sup>2</sup>) and  $dA$  is its element.

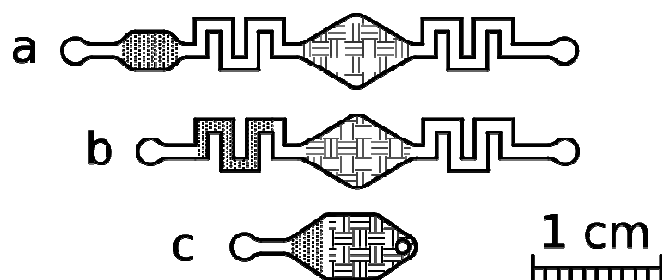
To characterize the flow the Reynolds number  $Re$  and Womersley number  $\alpha$  were evaluated:

$$Re = \frac{U \rho L}{\mu} \quad (7)$$

$$\alpha = L \left( \frac{2\pi f \rho}{\mu} \right)^{1/2} \quad (8)$$

where  $L$  is an appropriate length scale (m),  $U$  is average velocity (m/s),  $f$  is frequency of the oscillations (Hz). The Reynolds number describes the transition between laminar and turbulent flow regimes. The Womersley number characterizes the changes of velocity pattern at pulsatile flow.

During the work the designs shown in figure 1 were considered. The depth of their structures was 0.5 mm.



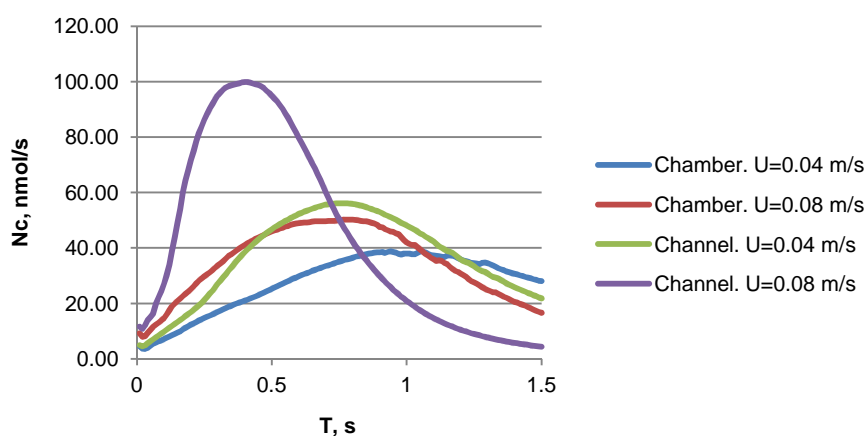
**Figure 1.** The designs of microfluidic chips: (a), (b) for passive mixing; (c) for active mixing. Stripes indicate the place of immobilization of reagents for the bioluminescent reaction, the points indicate immobilized FMN

### 3. Results and discussion

The distribution of FMN concentration was obtained for designs shown in figure 1 (a, b) during its dissolution by exposure constant fluid flow with characteristic download velocities (0.04 and 0.08 m/s). It was found that dissolution process was going rather fast and dissolution time didn't exceed 1 second. Immobilization of FMN in serpentine microchannel provided the fastest dissolution. So with the average flow velocity 0.08 m/s the 99% of reagent dissolved in 0.67 s in the channel, while in the chamber it took 0.78 s. With the velocity decreasing to 0.4 m/s the dissolution time increased by 0.11 s in serpentine channel and by 0.05 s in the chamber. The acceleration of dissolution in the serpentine channel mainly related to Dean vortices, which provided vertical convection of reagent. For this reason, the growth of flow velocity had a stronger effect on the dissolution time in the serpentine channel: it not only reduced the diffusion layer thickness, but also actively produced vortices. Checking this statement the study of dissolution in the straight channel with the same length was conducted. The dissolution time in this case was close to that for the chamber.

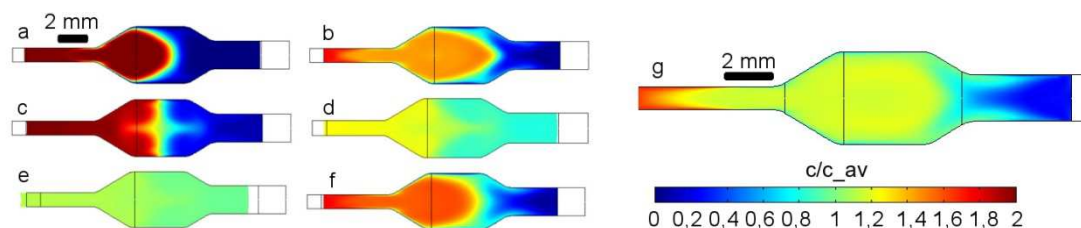
Although this scheme provided a rather rapid dissolution, efficient mixing was not achieved and reagent mainly located at the bottom of the channel with the variance down to 6.5. This was due to the initial very uneven distribution of the reagent and its position at the bottom of the channel, whereby Dean vortices drove reagent only at down part of the channel. In addition, the FMN flux through the cross-section of the channel showed significant time dependence (figure 2). There was increase in flux associated with the beginning of the dissolution and decrease caused by the completion of the dissolution process and the start of reagent washout. Thus, the passive mixing in suggested designs hasn't provided the uniformity of sample injection.

The experimental studies confirmed the simulation results that passive dissolution and mixing in considered designs don't provide required quality of reaction: kinetics of the reaction had no maximum, which made quantitative analysis impossible.



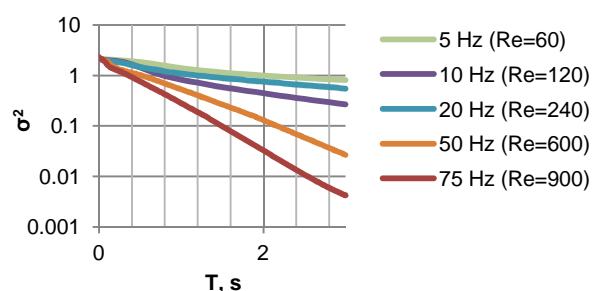
**Figure 2.** The flux of FMN  $N_c$  through the cross section of the channel during the time  $T$  for FMN immobilization in the chamber and in the serpentine channel for different average fluid velocities  $U$

The active influence on FMN mixing was conducted by using alternating flow velocity in the design shown in figure 1 (c). During analysis two-dimensional formulation was used and only FMN mixing was considered. Its initial distribution was set at FMN immobilization area marked by points in figure 1 (c). The simulation area was changed as shown in figure 3 to provide the same velocity pattern as at the 3D version.

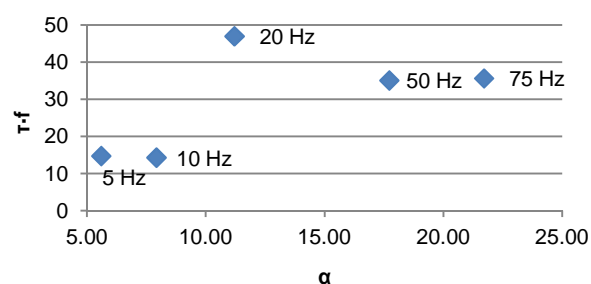


**Figure 3.** The distribution of FMN concentration after 3 seconds sinusoidal wave oscillations with frequency: (a) 5 Hz; (b) 10 Hz; (c) 20 Hz; (d) 50 Hz; (e) 75 Hz; the distribution of FMN concentration after 3 seconds rectangular wave oscillations with uniformly increasing frequency from 1 to 10 Hz and peak-to-peak amplitude: (f) 3  $\mu$ l; (g) 4  $\mu$ l.

Variance of concentration distribution of FMN was calculated for sinusoidal flow rate oscillations with peak-to-peak amplitude 3  $\mu$ l and frequencies changed from 5 to 75 Hz (figure 3 (a-e)). As the varying volume was kept constant with the change of frequency, the average velocity was increased and hence the  $Re$ , which varied from 60 to 900 proportional to frequency. These values correspond to the transitional regime between laminar and turbulent flow when vortexes are actively produced, but don't have chaotic nature. The results showed that variance value after 3 second mixing decreased from 0.81 to 0.0042 with the change of frequency from 5 to 75 Hz (figure 4). As the variance exponentially depended on time the mixing efficiency could be expressed as the time constant of the process divided by period of oscillations (or multiplied by their frequency) that shows the number of periods needing to decrease the variance of FMN concentration  $e$  times (figure 5). Thus, the maximum mixing efficiency per cycle was achieved for frequencies not exceeding 10 Hz. As can be seen, a significant deterioration in the mixing efficiency occurred when the Womersley number exceeded the threshold quantity of 10. In this case, a flow velocity profile became flatter and the vortex formed as a result of the fluid velocity direction change localized only in an expanding part of the design in contrast to the case with a low frequency when it covered the entire reaction chamber. As a result, instead of distribution all over the chamber reagent occupied only half of it. Separating the impact of  $\alpha$  and  $Re$  the additional studies were conducted. Thus, maintaining the average velocity (keeping  $Re$  constant) and increasing frequency from 10 to 20 Hz the variance was found to be 1.22 up to 3 seconds. Such reduction in mixing efficiency, which is more significant compared to constant varying volume condition, shows that Womersley number has the major impact on mixing efficiency.



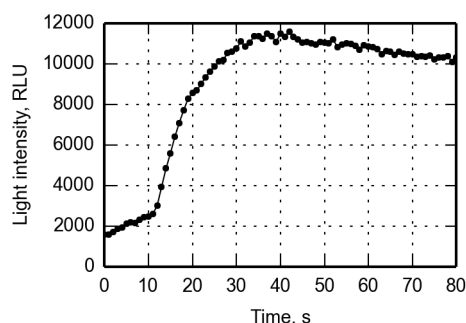
**Figure 4.** The variance of FMN concentration during sinusoidal oscillations of fluid velocity and peak-to-peak amplitude 3  $\mu$ l



**Figure 5.** The number of periods needed to decrease the variance of FMN concentration in  $e$  times (time constant  $\tau$  multiplied on oscillations frequency  $f$ )

Besides sinusoidal wave flow rates a square wave with a frequency of 10 Hz and rectangular wave with uniformly increasing frequency from 1 to 10 Hz were tested. Square wave signal with a constant frequency showed worse mixing compared to sinusoidal wave with the same period. However, the use of variable frequency provided the same variance as sinusoidal wave (figure 3 (f)). Thus, the variable frequency signal allowed increasing the mixing efficiency per one period of oscillation, since the same results was achieved with lower average frequency equal 5 Hz. This was because at low frequencies the reagent was spread over the chamber and then at higher rates was finally mixed. The variance of concentration after 3 seconds in the reaction chamber equal 0.011 was achieved using this mode with peak-to-peak amplitude 4  $\mu$ l (figure 3 (g)).

Experimental results for the dye mixing have confirmed the efficiency of selected mode with varying frequency as well as its deterioration at 20 Hz. Mixing efficiency has been also confirmed by the growth of luminescent kinetics quality. As shown in figure 6, after the active mixing was started at 10 s the reaction accelerated and light intensity reached the maximum.



**Figure 6.** Bioluminescent reaction rate before mixing at 10 s and after it

#### 4. Conclusions

The passive mixing didn't provide the uniform distribution of dissolved FMN. On the other hand, active mixing will be able to provide constant amount of FMN in the reaction chamber and a suitable mixing time less than 3 seconds. The highest mixing efficiency per cycle was achieved by varying frequency of rectangular flow rate oscillations from 1 to 10 Hz in 3 seconds which provided variance of FMN equal 0.011.

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