Modeling of the kinetics of capture of pathogens enhanced by a Marangoni flow

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ABSTRACT

Our work concerns a microsystem for biodiagnostics and biorecognition which we have called a "Marangoni micropump" [1]. It is aimed at the detection of specific molecules (analytes) suspended in a liquid sample (droplet). Inducing a flow inside the liquid using no moving parts or no mechanical device is particularly interesting when the liquid volume is very small. The Marangoni convective transport increases the transfer of the molecules on the detector reactive surface. In order to estimate the binding efficiency increase due to the Marangoni flow, a 2D numerical model has been set up with the Finite Element Method. The calculation is done in two steps : (1) calculation of the velocity field inside the droplet due to the Marangoni effect, (2) calculation of the concentration of the analytes using the velocity field from step (1) together with the concentration decrease by capture at the reactive surface. The first step algorithm has already been published [2], the second step is added here to complete the calculation.

Keywords: Marangoni flow, Finite Element Method, oligonucleotids, binding kinetics, oligonucleotid.

1 THE MARANGONI MICROPUMP CONCEPT

The Marangoni micropump is constituted by a suspended liquid droplet (about 30 µl) containing the analytes to be detected and a 1.76 mm^2 reactive surface (figure 1). The reactive surface is the tip of a cupper rod dipping into the droplet. The geometry is 2D axisymmetric. The standard procedure consists in the binding of the targeted analyte C to a ligand Γ immobilized on the reactive surface. When liquid is at rest, only molecular diffusion realizes the transfer of C to the reactive surface. This kind of transport is inefficient as soon as the average distance is larger than a few tens or hundreds of microns. The droplet is heated through its suspension rod and cooled at its bottom by the proximity of a cold surface. Convection inside the droplet is induced by a Marangoni effect due to the temperature gradient alongside the interface between the fluid and the surrounding gas (water vapor).

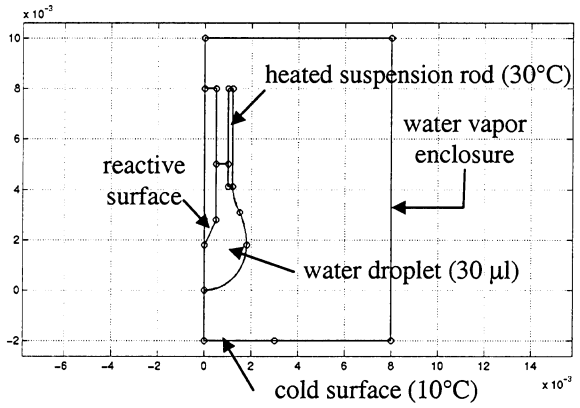

Figure 1: Scheme of the Marangoni micropump (2D axisymmetric geometry (mm)).

Generally speaking, a biodiagnostics system has two characteristic time scales : one for of the adsorption process and the other one for the transfer process to the reactive surface (convective or diffusive) [1]. The limiting mechanism of the whole system will be the one with the largest time scale. Thus, introducing the Marangoni micropump is justified only if the initially considered transfer time scale is at least as important as the adsorption time scale. Then, the convective time scale associated to the Marangoni micropump can be reduced by increasing the temperature difference applied on the droplet. The numerical model presented here is aimed at estimating exactly the gain in reaction time when introducing a Marangoni convection in connection with others process parameters.

2 THE BINDING KINETICS

In order to estimate the influence of the Marangoni flow on the kinetics of binding, one has to calculate first the concentration of the analytes C inside the droplet. This concentration is obtained by solving the usual convection-diffusion equation [1] :

$$\frac{\partial C}{\partial t} + \vec{\nabla}.(-D\ \vec{\nabla}C) + \vec{v}_f.\vec{\nabla}C = 0 \qquad (1)$$

where D is the diffusion coefficient for the analyte in the carrier liquid. The velocity field $\vec{v}_f$ contributes to the distribution of analytes in the volume. Because their concentration is small, it is assumed that the presence of the analytes does not modify the flow.
The kinetics of binding is modeled by the bimolecular approach :

$$C + \Gamma \underset{\text{Koff}}{\overset{\text{Kon}}{\rightleftarrows}} H \qquad (2)$$

where H stands for the adsorbed molecules binded to the ligands Γ. The efficiency of this hybridization reaction depends on the two reaction parameters : the coefficient of adsorption Kon and the coefficient of desorption Koff. The kinetics of hybridization can be represented by the following equation [1]

$$\frac{dH(t)}{dt} = \text{Kon}\ C(t)\ \Gamma_0 - H(t)\left[\text{Kon}\, C(t) + \text{Koff}\right] \qquad (3)$$

where C(t) is the volume concentration in analytes upon contact of the reactive surface and Γ_0 the initial concentration of molecules Γ. Mass transfer at the reactive surface is given by Fick's law

$$-D\ \vec{\nabla}C.\vec{n} = \frac{dH}{dt} \qquad (4)$$

Except for the reactive surface, the other boundaries are assumed not to capture any of the targeted free molecule; so that a Neumann boundary condition can be used on these boundaries.
Equations (1) and (3), together with the boundary conditions (4) are solved by a Finite Element Method (Femlab code [3]). The velocity field $\vec{v}_f$ in (1) is first calculated by using a vorticity-streamline-temperature formulation (ω, ψ, T) which is detailed in [1] and [2]. Kinetics of adsorption of targeted molecules C is represented by the variation of the total number NH of molecules H with time and also by the variation of the total number NC of molecules C present in the liquid volume during the reaction. Convection is expected to enhance the binding reaction ; after looking at the influence of the convective transport itself, we focus on the influence of three parameters : (i) the number of analytes versus the number of binding sites, i.e. the initial concentration in analytes versus the initial density in ligands, (ii) the initial location of the analytes in the droplet, (iii) the size of the analytes.

3 RESULTS

3.1 Velocity field calculation

The flow is induced by thermal boundary conditions on the droplet : the temperature of the suspension rod is 30°C whereas the temperature of the cold plate located below the droplet is 10°C (fig 1). Because of the volume of vapor between the cold plate and the droplet bottom, the efficient temperature difference between the hot spot and the cold spot at the gas-liquid interface is only approximately 0.3 °C Preliminary studies ([1], [2]) have shown that convective heat transfer in the vapor phase as well as vaporization/condensation at the droplet surface can be neglected. The resulting velocity field and temperature contours are shown in figure 2. Calculated maximum velocity is 8.6 mm/s and located at the upper part of the droplet surface. Figure 2 also shows the strong convective circulation inside the droplet induced by the Marangoni effect.

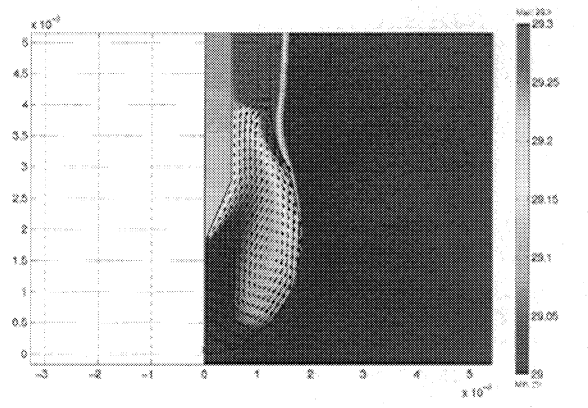

Figure 2: Velocity field and temperature contour lines in the droplet obtained with a 20 °C temperature difference between the heated rod and the cold plate.

3.2 Influence of the Marangoni convection

In this case, targeted analytes are oligonucleotides (ODN) and ligands are immobilized complementary ODN. First, we assume that the concentration in ODN is initially uniform inside the droplet (volume 30.15 µl). We analyze two different situations: (a) the initial number of oligonucleotides (NC_0) is 10 times larger than the number of immobilized ligand ($N\Gamma_0$), (b) NC_0 is 10 times smaller than $N\Gamma_0$. In case (a), the reactive surface will be saturated at the end of the reaction and there will be remaining ODN in the liquid; whereas in the case (b), ODN should disappear from the liquid (assuming Koff = 0 s^{-1}). The values of the variable coefficients used for the calculation are listed in table 1

	case (a)	case (b)
C_0 (mole/m^3)	10^{-5}	10^{-7}
Γ_0 (mole/m^2)	1,66 10^{-8}	1,66 10^{-8}
NC_0 (mole)	3.0154 10^{-13}	3.0154 10^{-15}
$N\Gamma_0$ (mole)	2.9153 10^{-14}	2.9153 10^{-14}

Table 1: Values of the coefficients for the calculation of the hybridization process.

The others model parameters are D = 10^{-10} m^2/s and Kon = 100 m^3/mole.s.
Adsorption kinetics (NH(t) curve) for case (a) is plotted in figure 3.

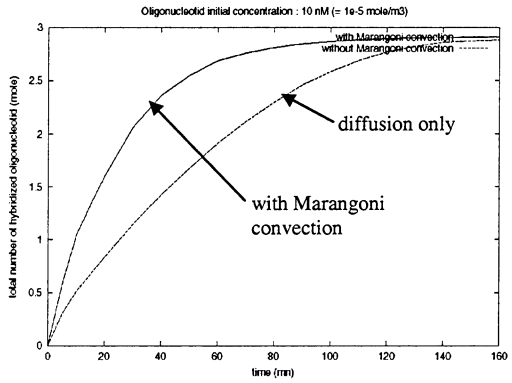

Figure 3: Binding kinetics NH(t) with or without Marangoni convection for case (a).

A permanent regime is obtained when kinetics curves approach their asymptotic value. The asymptote is reached much faster when a Marangoni convection is present (about 60 min against 140 min) showing the interest of the micropumping function.

3.3 Influence of initial concentration

Figure 4 shows the binding kinetics (NC(t) and NH(t) plots) obtained for case (a). It is observed that the permanent regime is obtained after approximately 60 min.

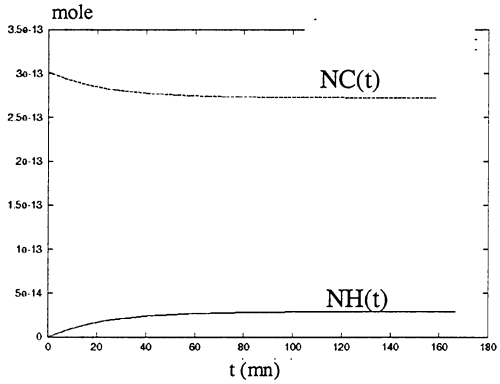

Figure 4: Binding kinetics NH(t) and concentration NC(t) vs. time for case (a).

If we do the same calculation for case (b), it takes about 300 min to reach the permanent regime (figure 5). The kinetics of adsorption is slower when the initial concentration in analytes is decreased, as could be expected from equation (3).

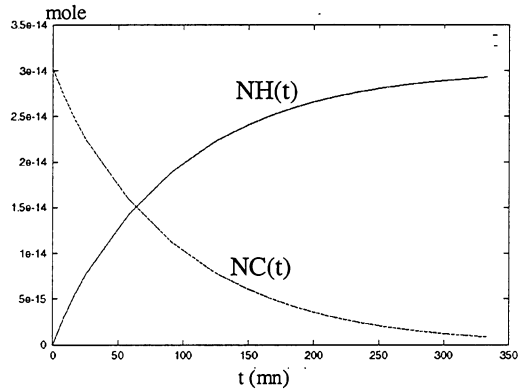

Figure 5: Binding kinetics NH(t) in the case (b).

3.4 Influence of the initial localization of the analytes

We analyze the case where the analytes are initially concentrated at a location just at the tip of the suspension rod, as shown in figure 6.

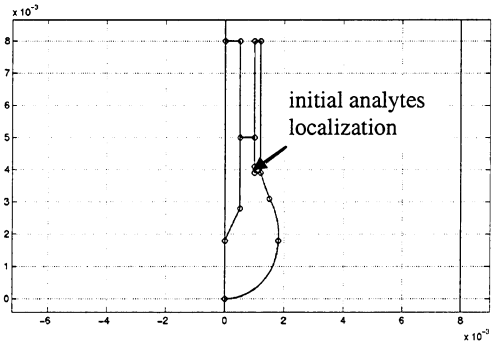

Figure 6: Scheme of the initial location of analytes.

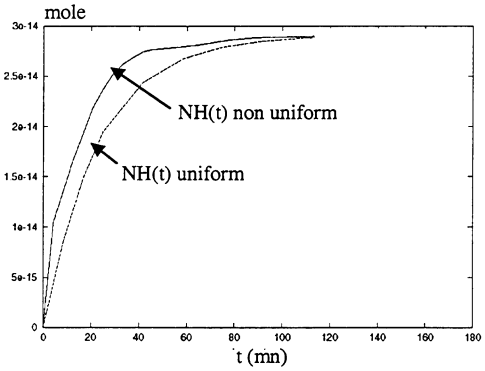

Figure 7: Binding kinetics NH(t) comparison between non uniform and uniform cases for case (a).

Kinetics of adsorption of analytes calculated with the ratio between the number of analytes to the number of ligands of case (a) is plotted in figure 7 and compared to the kinetics of an initially uniform concentration.
From the results shown in figure 7, the kinetics of binding is faster if the targets are initially located near the tip of the suspension rod; it can be correlated to the fact that the velocities are the largest near the suspension rod, resulting in a fast convection of the analytes inside the droplet. However, this does not happen in case (b) where the number of targets is small : figure 8 shows that the two kinetics are nearly the same. For case (a), the reactive surface is rapidly saturated by the targets which are present near the droplet surface. For case (b), because the reactive surface is never saturated, the targets present in the middle of the droplet need to reach the surface by diffusion transfer. This is why, for case (b), the dynamics of the reaction is not accelerated by placing the analytes at the tip of the rod.

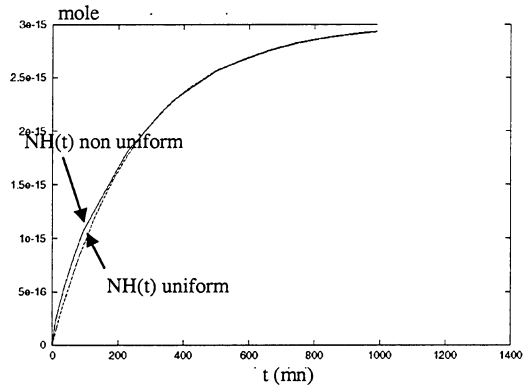

Figure 8: NH(t) kinetics comparison between non uniform and uniform cases for case (b).

3.5 Influence of the analyte size

In the model, the size of the analytes determines the value of the diffusion coefficient D through the Einstein formula

$$D = \frac{k_B T}{6\pi\mu R} \qquad (5)$$

where T is the Kelvin temperature, μ the dynamic viscosity of the carrier fluid ($Kg.m^{-1}.s^{-1}$), k_B the Boltzmann constant ($1.38\ 10^{-23}$ J/K) and R the hydraulic radius of the analyte. The reference value of D is 10^{-10} m^2/s for our ODN. A diffusion coefficient 10 times smaller ($D=10^{-11}$ m^2/s) corresponds to an analyte 10 times larger.

Considering the case (b), the limiting reaction case, and using the value $D=10^{-11}$ m^2/s, we obtain the kinetics of figure 9. Here, the acceleration of the kinetics appears clearly.

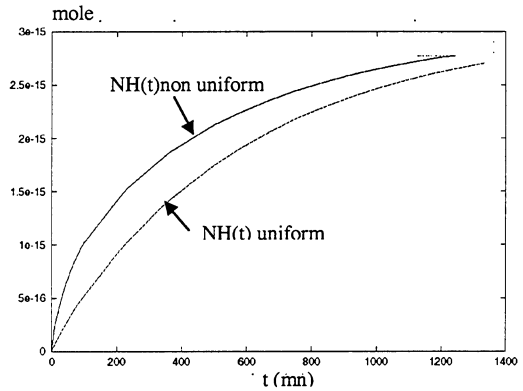

Figure 9: Binding kinetics NH(t) comparison between non uniform and uniform cases for case (b) and bigger ODN.

4 CONCLUSION

The numerical simulations of hybridization process in the presence of a Marangoni convection in a droplet geometry show that the induced convective motion accelerates the adsorption kinetics. In order to optimise the Marangoni effect on the binding of targets, we have analysed the influence of three parameters : the level of the initial targets concentration, the initial target localisation and the target size. The study of those three parameters show that binding kinetics is sensible to them. The results confirm the interest of the design of the Marangoni micropump.

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