Combined effects of pump and capillary pressures for the filling of microchannel networks: the difficulty of synchronization.

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ABSTRACT

Most of the time biotechnological devices comprise multiple reaction chambers, in order to achieve simultaneously as many reactions as possible. Parallelization requires the precise filling and loading of these chambers, and the best synchronization is searched for. De-synchronization triggers the formation of air bubbles and leads to anomalous functioning of the device.

It has been observed that synchronized filling of microchannels and fluidic networks is often an experimental challenge. In fact, this experimental difficulty directly stems from the conceptual approach of the design of the network. In this work we theoretically investigate the filling of networks, driven by the injection pressure of a pump. The additional effect of capillary forces is also taken into account. Experimental results are compared with the theoretical model. Rules for better synchronization are proposed.

Keywords: Network, synchronization, pump pressure, pressure drop, capillary pressure, Laplace pressure.

1 INTRODUCTION

Reaction microchambers are basis features of many biotechnological devices. Most of the time biotechnological devices comprise multiple reaction chambers, in order to achieve simultaneously as many reactions as possible in order to enhance the potentialities of the device.

The examples are many in the literature. They go from DNA sequencing where massively parallel DNA sequencing is revolutionizing genomics research [1], to gene amplification by polymerase chain reactions (PCR or LAMP) [2], to cell culture [3], and to biochemical reactions [4].

These chambers are usually connected to an input port and an output port by a fluidic network. Parallelization requires the precise filling and loading of these chambers, and the best synchronization is searched for. De-synchronization triggers the formation of air bubbles and leads to anomalous functioning of the device. In the case of PCR/LAMP amplification, trapped bubbles will expand during the thermal cycles or the isothermal heating, chasing the liquids, and preventing the amplification [5]. Air bubbles are also a considerable drawback for multi-wells cell culture [6]. Air bubbles trapping at the inlet of a network has been theoretically investigated by Bruus [7], but this analysis only concerns the network entrance and not the entity of the network.

An obvious solution to synchronize flows in networks is the use of valves controlling locally the filling of each of the channels of the network. Such a solution requires the use of a large number of valves and complicates substantially the conception, fabrication, cost and use of these devices.

Hence an approach to reduce de-synchronization by passive means is of great interest. Indeed, it has been observed that the synchronized filling of microchannels—and more generally of fluidic networks—is often an experimental challenge. In fact, this experimental difficulty directly stems from the conceptual approach of the design of the network. A physical analysis of the causes of de-synchronization is then needed, taking into account that pump pressure and capillary pressure both contribute to the filling behavior.

Note the circulation of fluids in fully filled networks has been largely investigated, but our concern is the filling of the devices [8-10]. So far there has been few reports on the filling of networks in the literature. Kim and coworkers have investigated the capillary filling of parallel channels [11]. Numerical approach of the filling of a single micro-

well under the conjugate effects of pump pressure and wall wettability has been reported by Tseng and colleagues [12], but a comprehensive approach of the synchronized filling of networks under the conjugate action of pump pressure and wettability forces is still missing.

In this work we first theoretically investigate the filling behavior of networks, using pump-driven flows. The effect of capillary forces is also included in the model. Experimental results—obtained in a massively parallel RT-qPCR (real time quantitative polymerase chain reaction) chambers device [2]—are compared to the predictions of the theoretical model. Finally rules for better synchronization are proposed.

2 THEORETICAL APPROACH

Consider an initially empty microchannel, plugged to a pump. The pump forces liquid into the channel (figure 1A). The liquid interface advances inside the channel with an average velocity noted *V*.

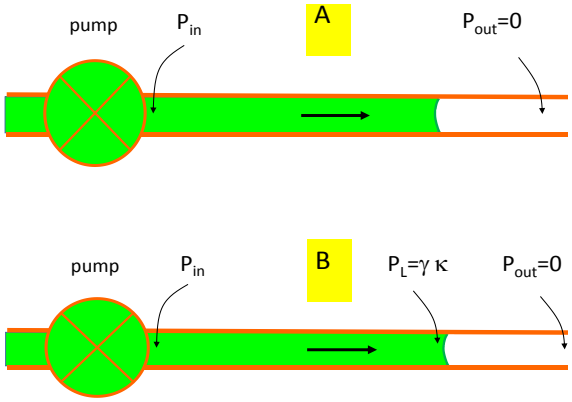

Fig.1. Sketch of the flooding of a channel using a pump: top, no capillary effect; bottom, with capillary effects.

Let us recall first that the pressure drop in a microchannel for a laminar flow is given by the general relation

$$\Delta P = RLQ \,, \tag{1}$$

where P is the pressure, L the channel length, R the hydraulic resistance per unit length, and Q the volumic flow rate. Poiseuille and Hagen have given an expression for the resistance R in the case of cylindrical tubes [13]; later, Shah and London have derived an expression for the hydraulic resistance for rectangular duct derived from a Fourier series expression [14]. There are now tables where the hydraulic resistances are listed for many different channel cross sections [7,10,15,16]. Relation (1) assumes an established flow. Let us consider (1) differently, from a transient point of view, and base our reasoning by considering that the transient flow is always established approximately everywhere. This approximation is valid except at the very front of the advancing flow.

With this assumption, relation (1) can be reinterpreted as

$$P_{in} = RzS\,\frac{dz}{dt}\,, \tag{2}$$

where z is the penetration distance and S the cross-sectional area. Integration of (2) yields

$$z = \sqrt{\frac{2\,P_{in}}{R\,S}\,t}\,. \tag{3}$$

Now, if we take into account the Laplace pressure (figure 1B), we must replace P_{in} by

$$P_{in} + \frac{\gamma\, p\, cos\theta}{S} = P_{in} + \frac{4\,\gamma\, cos\theta}{D_H} = RzS\,\frac{dz}{dt}\,, \tag{4}$$

where γ is the surface tension, p the wetted perimeter and D_H the hydraulic diameter. Integration of (4) yields

$$z = \sqrt{\frac{2\left(P_{in}+\frac{4\,\gamma\,cos\theta}{D_H}\right)}{R\,S}\,t}\,. \tag{5}$$

The time taken by the fluid to fill a channel length L is then different if one considers equation (3) or (5).

$$\tau_1 = \frac{RSL^2}{2\,P_{in}} \neq \tau_2 = \frac{RSL^2}{2\left(P_{in}+\frac{4\,\gamma\cos\theta}{D_H}\right)}\,. \tag{6}$$

The filling time is smaller in the case of hydrophilic walls ($\cos\theta > 0$), compared to hydrophobic walls ($\cos\theta < 0$). Note that if no pressure is applied (P_{in}=0)—which is the case of spontaneous capillary flow—the penetration distance indicated by (5) for a cylindrical tube of radius r reduces to the Lucas-Washburn-Rideal law [17,18]

$$z = \sqrt{\frac{r\,\gamma\cos\theta}{2\,\mu}}\,\sqrt{t}\,. \tag{7}$$

3 CASE OF A PIECEWISE CONSTANT CROSS SECTION CHANNEL

The same approach can be followed for a piecewise-constant cross-section channel, such as that shown in figure 2. Relation (1) becomes

$$P_{in} + \frac{4\gamma\cos\theta}{D_H} = \left(\sum_i R_i L_i\right) Q \ , \tag{8}$$

and relation (4) becomes

$$P_{in} + \frac{4\gamma\cos\theta}{D_H} = \left[\sum_{i=1}^{n-1} R_i L_i + R_n\left(z - \sum_{i=1}^{n-1} L_i\right)\right] S_n \frac{dz}{dt} \ . \tag{9}$$

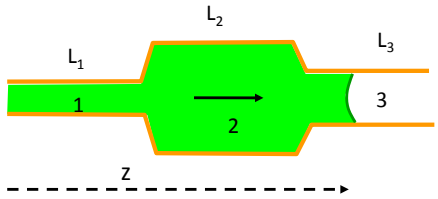

Fig.2. Sketch a piecewise constant cross section channel

Integration of (9) yields the relation between the penetration distance z and the elapsed time t

$$t - \sum_{i=1}^{n-1}\tau_i = \frac{S_n}{\left(P_{in} + \frac{4\gamma\cos\theta}{D_{H,n}}\right)}\left[\sum_{i=1}^{n-1} R_i L_i\left(z - \sum_{i=1}^{n-1} L_i\right) + \frac{R_n}{2}\left(z - \sum_{i=1}^{n-1} L_i\right)^2\right], \tag{10}$$

where the times τ_i are the times for the flow to totally fill the channel i. Note that the hydraulic diameter in (10) is that of the section where the interface advances (n). The time lapse τ_n taken for the flow to totally fill the nth channel is then

$$\tau_n = \frac{S_n L_n}{\left(P_{in} + \frac{4\gamma\cos\theta}{D_{H,n}}\right)}\left[\sum_{i=1}^{n-1} R_i L_i + \frac{R_n}{2} L_n\right]. \tag{11}$$

A first conclusion can be drawn: if the inlet pressure P_{in} is large compared to the capilary pressure, the filling time is inversely proportional to the inlet pressure P_{in}. In the case where P_{in} =0, (11) reduces to the relation derived in [19] for a purely capillary flow.

A second conclusion can be drawn: the filling time depends on the "history" of the flow. For example the two similar channels of figure 3, with sections in reverse order, do not have the same filling time. According to (10), the filling times τ_1 and τ_2 are

$$\tau_{1,2} = \tau_1 + \tau_2 = \left\{\frac{L_1 S_1}{\left(P_{in} + \frac{4\gamma\cos\theta}{D_{H,1}}\right)}\frac{R_1}{2} L_1 + \frac{L_2 S_2}{\left(P_{in} + \frac{4\gamma\cos\theta}{D_{H,2}}\right)}\left[R_1 L_1 + \frac{R_2}{2} L_2\right]\right\} \tag{12}$$

and

$$\tau_{2,1} = \tau_2^* + \tau_1^* = \left\{\frac{L_2 S_2}{\left(P_{in} + \frac{4\gamma\cos\theta}{D_{H,2}}\right)}\frac{R_2}{2} L_2 + \frac{L_1 S_1}{\left(P_{in} + \frac{4\gamma\cos\theta}{D_{H,1}}\right)}\left[R_2 L_2 + \frac{R_1}{2} L_1\right]\right\} \tag{13}$$

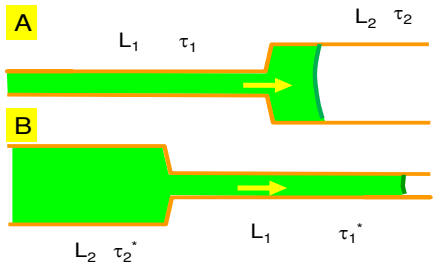

Fig.3. Sketch of two similar piecewise-constant cross-section channels, showing the different filling velocities.

The time difference between the two configurations is

$$\Delta t_{1,2} = \frac{L_1 L_2}{P_{in}}\left(\frac{S_2 R_1}{1 + \frac{1}{\Re_2}} - \frac{S_1 R_2}{1 + \frac{1}{\Re_1}}\right), \tag{14}$$

where the ratios $\Re_i = P_{in}/(4\gamma\cos\theta/D_{H,i})$ represent the relative effect of the pressure and capillary forces. Synchronization requires the minimization of (14).

4 EXAMPLE OF A PCR NETWORK

The development of the theory for a network is quite complicated. However, the preceding theoretical development already produces results extrapolable to networks. Consider the case of a network for performing parallel PCRs, shown in figure 4 [2,20,21].

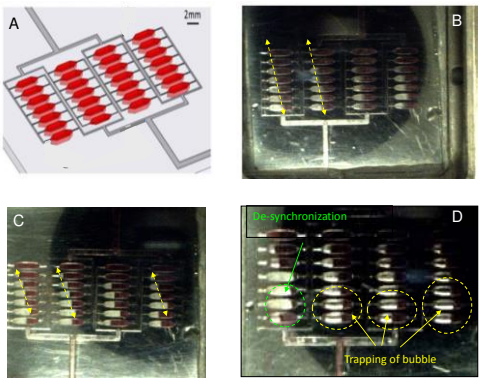

Fig.4. A:schematic of the device; B: high P_{in} =1 bar; C: moderate P_{in}= 300mbars ; D: small P_{in}= 60 mbars.

It can be shown that the Laplace pressure is $P_{cap} \approx 0.2/D_H$ ~ 1500 Pa. In the case of high pump pressure (P_{in} =1 bar, $\Re \approx 67$), figure 4.B shows that the network is nearly synchronized. In the case of moderate pump pressure (P_{in} =300 mbars, $\Re \approx 20$), figure 4.C shows that the network is partly synchronized. In the case of low pump pressure (P_{in} =60 mbars, $\Re \approx 4$), figure 4.D shows that the network is totally desynchronized, with bubbles appearing in the chambers.

5 CONCLUSION

In this work, a theoretical analysis of the filling of a simple fluidic network has been performed. An expression for the travel time in channels comprising different cross sectional areas has been derived and the de-synchronization time between two channels has been determined. The model includes both the effects of the injection pump pressure and the capillary pressure.

It is shown that the de-synchronization time is inversely proportional to the injection pressure, and proportional to the dimensions of the microchambers, and to the connection lengths. In the case of low injection pressure, wettability effects considerably affect the synchronization.

In conclusion, synchronized filling of a fluidic network is difficult to achieve. This work shows that three solutions can improve the synchronization of the filling: first, applying a large injection pump pressure at the inlet is very advantageous. Second, the reduction of the size of the microchambers improves the synchronization of the filling of the network. Finally, an optimized symmetry of the channels on both sides of the large reaction chambers contributes to the reduction of de-synchronization.

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