Microfluidics with on-line dynamic light scattering for size measurements

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We present a detailed investigation on the feasibility of on-line dynamic light scattering measurements of colloidal sizes in a pressure-driven microfluidic flow. We review some theoretical arguments showing that such experiments are difficult to perform due to the Poiseuille flow that induces interferences of different Doppler shifts. Such a theoretical approach is however very useful to figure out the range of parameters where on-line size measurements are possible. We then build a dynamic light scattering setup around a microfluidic chip that enables us to estimate the size of Brownian scatterers flowing in PDMS-based microchannels, thus validating experimentally the theoretical estimations. We finally present a microfluidic chip that can mix two reactants in ≈200 ms, and allows size measurements using dynamic light scattering at about 300 ms after complete mixing. Two applications are presented: the continuous monitoring of the viscosity of a two-fluid mixture, and the electrostatic co-assembly of oppositely charged nanoparticles and block copolymers.

I. Introduction

Accessing the early stage of reaction kinetics is challenging for many applications such as the synthesis of nanoparticles, the binding of proteins and the formation of vesicles. Stopped-flow techniques coupled to analytical measurements permit in most cases a millisecond temporal resolution, as recently demonstrated by probing the nucleation and growth of gold nanoparticles using small-angle X-ray scattering for instance. Microfluidics is emerging as a promising candidate for such experiments since small time scales can be probed continuously with minute amounts of sample, as shown by measurements of fast chemical reactions kinetics, biomolecules folding, chromophore structural changes, or even cationic exchange of nanoparticles. Moreover, the control of transport phenomena in such miniaturized systems and the development of complex labs on chip, offer new interesting possibilities such as the high-throughput screening at the nanolitre scale.

Among the existing techniques combined with microfluidics, one finds a variety of tools such as X-ray scattering, spectroscopy (UV, Raman and fluorescence), but Dynamic Light Scattering (DLS) has rarely been implemented until recently. Dynamic light scattering is however a common tool for measuring diffusion coefficients of scattering particles subject to Brownian motion, reducing DLS to shear rate measurements. DLS therefore seems unadapted to microfluidics since high shear rates are often involved in miniaturized pressure-driven flows.

In the following, we present a thorough investigation of DLS combined with microfluidics, in order to obtain the narrow range of parameters for which size measurements of Brownian particles flowing in a microchannel are yet possible. We first review some theoretical issues concerning namely DLS in simple shear flows, and we extend these results to the case of Poiseuille microfluidic flows. This approach allows us to estimate the range of flow rates for which size measurements can be performed using DLS in a pressure-driven microfluidic flow. We then confirm experimentally such results using calibrated nanoparticles injected at various flow rates in a PDMS-based microchannel.

In a second stage, we develop a microfluidic chip that can mix two reactants down to 200 ms and permits continuous size measurements using DLS at a time scale down to ≈300 ms after complete mixing. We validate this device thanks to continuous viscosity measurements of a two-fluid mixture, and we finally present a preliminary investigation on the formation of electrostatic coacervates in a system of coated cerium oxide nanoparticles and polyelectrolyte-neutral block copolymers.

II. Theory: Dynamic light scattering in a microfluidic flow

A. Dynamic light scattering in a simple shear flow

Fig. 1 sketches a classical dynamic light scattering setup. The sample is illuminated with a coherent laser light of wavelength $\lambda$, and the scattered light is collected at an angle $\theta$ by a photomultiplier tube PMT. The scattering volume $V$ is defined by the intersection of the incident beam with the image of the field diaphragm $D_i$ through the collecting lens $L$. The aperture diaphragm $D_o$ reduces the range of collected angles, and consequently controls the contrast of the device. The auto-correlation
function \( C(\tau) = \langle i(t) i(t+\tau) \rangle \) of the scattered intensity \( i(t) \) is obtained by an electronic autocorrelator as a function of the time lag \( \tau \).

For small fluctuations of the dielectric permittivity \( \delta \varepsilon (r, t) \), the electric field scattered at angle \( \theta \) is given by \( E_s (q, t) \sim \delta \varepsilon (q, t) \), with

\[
\delta \varepsilon (q, t) = \int_V d^3 r \, e^{i q \cdot r} \delta \varepsilon (r, t),
\]

and \( q \) is the scattering vector defined by \( q = k_s - k_a \), of magnitude \( q = (4 \pi n / \lambda) \sin(\theta / 2) \), \( n \) is the refractive index of the sample. The fluctuations of the scattered intensity \( i(t) \sim \| E_s (q, t) \|^2 \) correspond to the spatio-temporal fluctuations of the dielectric permittivity at length scale \( q^{-1} \). In most cases, \( \delta \varepsilon (r, t) \) is a gaussian random variable and

\[
C(\tau) = 1 + C_i g^i(\tau),
\]

where \( g^i(\tau) \) is the heterodyne correlation function

\[
g^i(\tau) = \langle \delta \varepsilon (q, t) \delta \varepsilon^* (q, t+\tau) \rangle,
\]

and \( C_i \) is the device contrast.\(^{15} \)

In the case of isotropic Brownian particles, a classical model for the fluctuations of the permittivity is\(^ {15} \)

\[
\delta \varepsilon (r, t) = \Sigma \delta (r - r_i(t)),
\]

where \( \delta \) is the Dirac distribution and \( r_i(t) \) are the positions of particles \( i \) at time \( t \) in the scattering volume. For independent and identical scatterers, the heterodyne correlation function becomes

\[
g^i(\tau) = \int_V d^3 r_0 \langle e^{-i q \cdot (r(t) - r_0)} \rangle
\]

\[
= \int_V d^3 r_0 \tilde{\rho}(q, \tau, r_0),
\]

where \( \tilde{\rho}(q, \tau, r_0) \) is the Fourier transform of \( \rho(r, t, r_0) \), the probability to find a particle at \( r(t) \) when initially at \( r_0 \) for \( t = 0 \).

In the case of a simple shear flow as sketched in Fig. 1

\[
r(t) = r_0 + \gamma \tau e_z,
\]

previous theoretical works have shown that \( \tilde{\rho}(q, \tau, r_0) \) is given by:\(^ {16,18} \)

\[
\tilde{\rho}(q, \tau, r_0) = \exp \left( -D q^2 \tau - i q \cdot v(r_0) \tau \right) \times \exp \left( -D \left( \frac{q z_0^2}{3} \tau^3 - D q z_0^2 \tau^2 \right) \right),
\]

where \( D \) is the diffusion coefficient of the scatterers. The first term involves the classical diffusion decorrelation time \( \tau_d = 1 / (D q^2) \) and the Doppler frequency shift \( q \cdot v(r_0) \) of the scatterers. The second term corresponds to the coupling between diffusion and shear flow and presents two characteristic times.

When the transit time \( \tau_t = b \nu_0 \) of the particles through the scattering volume \( V \) is long compared to the other decorrelation times, \( g^i(\tau) \) can be approximated by

\[
g^i(\tau) = \int_V d^3 r \tilde{\rho}(q, \tau, r_0)
\]

\[
= \exp \left( -D q^2 \tau - i q \cdot v_0 \tau \right) \times \int_V d^3 r \exp \left( -i q \cdot z \tau \right).
\]

Indeed, the last term involving the interferences of the different Doppler shifts in the scattering volume \( V \), presents a decorrelation time \( \tau_\gamma = 1 / q \nu_0 \) of the particles through the scattering volume \( V \), which is in most cases shorter than the characteristic times due to the coupling between shear flow and diffusion [see Eq. (7)\(^ {11} \)]. This term corresponds to the spatial coherence of the scattered light which is modified by the shear flow.\(^ {18} \)

In order to measure the size of the scatterers, the decorrelation time \( \tau_d \) due to the Brownian motion has to be smaller than the shear rate decorrelation \( \tau_\gamma \), i.e. \( q \gamma \nu_0 \ll D q^2 \). Basically, this condition is fulfilled for a small size \( a \) of the scatterer or a narrow range of possible angles. Note also that these estimations only hold for long transit times \( \tau_t = b \nu_0 \) of the scatterers compared to the other decorrelation times, i.e. the mean velocity \( \nu_0 \) in the scattering volume has to satisfy \( \nu_0 < D q b \). For typical values given above, \( \nu_0 \) has to be smaller than 1 cm/s.

The decorrelation time \( \tau_\gamma \) due to the modified spatial coherence of the scattered light makes the measurements of the diffusion coefficients of scatterers in a shear flow difficult. On the other hand, DLS can also be a powerful tool to access the local shear rate \( \gamma \).\(^ {17,19} \) Different strategies were developed to overcome the above difficulties, and perform DLS in flows with moderate shear, as for instance two crossing laser beams methods,\(^ {22} \) or the use of reference scatterers.\(^ {25} \)
Microfluidic pressure-driven flows present a different configuration as discussed here, since velocity profiles involve a continuous range of shear rates. We thus have to modify the present analysis to take into account the Poiseuille flow, and to estimate the range of possible angles for such measurements.

B. Dynamic light scattering in a microchannel

Fig. 2 displays a classical DLS experiment performed in a microchannel of width \( w \) and height \( h \). As discussed above, the scattering wavevector should be as orthogonal as possible to the velocity field, i.e. \( \mathbf{q} \cdot \mathbf{r} = 0 \), in order to avoid the decorrelation due to the shear rate. A simple way is to illuminate the microchannel and to collect the scattered light in the \( x \) - \( y \) plane, as depicted in Fig. 2. The incident beam and the optics of collection define a scattering volume \( V \) which is located inside the microchannel, with the characteristic dimensions \( w_i \) and \( a = w_i / \sin(\theta) \). For \( w_i < w \) and for a relatively large aspect ratio \( w_i / h \), significant shear rates in the scattering volume are only present in the \( y \)-direction, and the velocity profile can be approximated by \( v(y) = v_0 \left( 1 - \left( 2y/h \right)^2 \right) \). To our knowledge, the exact form of the probability \( p(r, t, r_0) \), in the case of a Poiseuille flow does not follow a simple calculation, and it is consequently difficult to estimate the expected correlation function of Brownian scatterers in such flows. However, solutes and more specifically colloids, are convected at high Pécelet numbers in classical microfluidic devices. The scatterers thus do not explore the entire Poiseuille flow during their transit time in the scattering volume, but only a small part of typical extension \( l_y^2 \approx D w_i / v_0 \), where the shear rate can be safely assumed as uniform. Indeed, for a typical experiment \( w_i = 75 \) \( \mu \)m, \( v_0 = 1 \) mm/s, and thus \( l_y \) is less than 1 \( \mu \)m for colloids with \( D \approx 10^{-12} \) m\(^2\)/s. In such a case, we will neglect as above the coupling between the local shear rate \( \gamma(r_0) \) and the diffusion of the scatterers [see Eq. (7)]. \( g^3(\tau) \) can now be approximated as:

\[
g^3(\tau) = \exp \left( -Dq^2 \tau - i \mathbf{q} \cdot \mathbf{r}_0 \tau \right) \times 
\int_V dy \exp \left[ -i \mathbf{q} \cdot \mathbf{v}_0 (2y/h)^2 \tau \right]. \tag{10}
\]

The last term, corresponding to the interferences of the different Doppler shifts in the scattering volume due to the Poiseuille flow, depends only on the mean shear rate \( v_y / h \) and on the dimensions of \( V \).

It is now possible to compute the expected correlation function for a given geometry of the scattering volume \( V \). We assume that \( V \) is well-centered in the channel and displays a gaussian shape \( \exp(-2(y/a)^2) \) along the \( y \)-direction, and that \( \mathbf{q} \) is not strictly orthogonal to the velocity with a typical mismatch i.e. \( q_y = q \phi \) with \( \phi = 1^\circ \). The maximal shear rate in the scattering volume is given by \( \dot{\gamma} = 4 v_y a / h^2 \), and can be significantly small for a large ratio \( h/a \).

Fig. 3 displays the corresponding calculated \( C(\tau) \) in the case of a microchannel of height \( h = 500 \) \( \mu \)m, for \( a = 175 \) \( \mu \)m, for colloids in water with \( D = 10^{-12} \) m\(^2\)/s, \( \theta = 40^\circ \) with \( \lambda = 514 \) nm, and for several velocities \( v_0 \). Two different regimes occur. Below a critical velocity \( v_c \approx 4 \) mm/s, all the correlation functions collapse and are dominated by the Brownian contribution of the scatterers, i.e. \( C(\tau) \approx 1 + \exp(-2Dq^2 \tau) \). For \( v_0 \gg v_c \), the decorrelations due to the shear rates dominate, and the characteristic times \( \tau_c \), defined as \( C(\tau_c) = 1.5 \) scale as \( v_0^{-1} \). The maximal shear rate in \( V \) at this critical value is about \( \dot{\gamma} \approx 4 v_y a / h^2 \approx 10 \) s\(^{-1} \). As in the previous discussion on simple shear flows, DLS measurements are feasible as soon as shear rates are below \( \approx 10 \) s\(^{-1} \) in \( V \), and for a mismatch \( \phi \) that does not exceed 1\(^\circ\). Note that the value of the critical velocity \( v_c \) strongly depends on the mismatch \( \phi \) but also on the size of the measured colloids, and on the exact shape of the scattering volume. The values discussed above may be easily obtained in a typical microfluidic experiment, and our rough estimations indicate that DLS measurements may be performed for a given range of flow rates as verified experimentally in the next section.

III. Experimental validation of microfluidics with on-line DLS

Fig. 4 presents our DLS setup built around a microfluidic chip. An argon laser beam (\( \lambda = 514 \) nm) is directed through the microfluidic chip, and focussed inside the channel through the lens \( L \) (focal length 5 cm). The microfluidic devices are manufactured using standard soft lithography techniques, and...
consist of PDMS microchannel networks sealed with glass slides. The chip is placed at the focal length of lens L in the \( \mathbf{e}_x - \mathbf{e}_y \) plane. Alignment of the incident beam is easily performed so as to have the incident wavevector \( \mathbf{k}_i \) almost orthogonal to the microfluidic chip, and then \( \mathbf{k}_i \cdot \mathbf{r} = 0 \).

The spatial filter \( \mathbf{SF}_1 \), composed of a microscope lens connected to the single mode optical fiber \( f_1 \) (core diameter \( d = 4 \, \text{µm} \), IDIL), collects the electric field scattered at the angle \( \theta_a \) by the sample in the microchannel. The scattered light is then directed to the photomultiplier tube PMT, and the correlation functions \( C(\tau) = \langle h(\tau)h(\tau + \tau) \rangle \) are obtained using an electronic autocorrelator (Brookhaven Instruments, BI-9000AT). Single mode fibers allow a large flexibility to choose the angle \( \theta_a \), and also permit to obtain correlation functions with a contrast \( C_n \) close to 1, as only one single coherence area is collected. A

The accurate alignment of the spatial filter \( \mathbf{SF}_1 \) to collect the scattered light originating exactly from the microchannel and get \( \mathbf{q} \) in the \( x-y \) plane minimizing \( \mathbf{q} \cdot \mathbf{r} \), may be an almost impossible task. To overcome such difficulties, we use the following trick to directly localize the scattering volume \( V \). Light from the incident laser beam can be split using the beam splitter BS, and directed into the spatial filter \( \mathbf{SF}_2 \) (see Fig. 4). The incident light then emerges from the spatial filter \( \mathbf{SF}_1 \) thanks to the single mode fiber \( f_2 \) and the coupling device C (IDIL). It is therefore possible to get a direct visualization of the scattered beam, and so to visualize the scattering volume \( V \) at the intersection with the incident beam. This trick enables to place \( V \) easily in the center of the microchannel with a precision of \( \approx 50 \, \mu\text{m} \), and also to align the scattered beam as orthogonal as possible to the microfluidic chip. We estimate that a careful alignment gives \( \mathbf{q} \cdot \mathbf{e}_z = q\phi \) with \( \phi \approx 1^\circ \).

**A. Dimensions of the scattering volume and refraction effects**

The inserts of Fig. 4 sketch the scattering volume \( V \) and the path of the scattered beam in the PDMS-glass chip. The scattered beam can be directly visualized using the trick mentioned above, since light can propagate in the optical fiber \( f_2 \) and emerge from \( f_1 \). Because the microfluidic chip is made of a channel network molded in a PDMS slab and sealed with a glass slide, refraction of the scattered beam has to be taken into account. The apparent scattering angle \( \theta_a \) and the real angle of scattering \( \theta \) are related by \( n \sin(\theta) = \sin(\theta_a) \) where \( n \) is the refractive index of the fluid flowing in the microfluidic chip. Scattering angles above \( \theta = \arcsin(1/n) \approx 50^\circ \) cannot consequently be obtained due to an internal reflection at the glass slide interface in the case of aqueous solutions (\( n = 1.33 \)).

The scattering volume \( V \) is determined by the intersection of the incident and scattered beams and their characteristic dimensions are roughly given by \( w_s \) and \( a \). In order to minimize the decorrelations due to the Poiseuille flow, \( a \) has to be as small as possible since shear rates are only relevant in the \( y \)-direction. Again, due to the refraction effects, the size \( a \) is geometrically related to \( w_s \) and \( w_i \) by

\[
 a = [w_s/\cos(\theta_a) + w_i]/\tan(\theta),
\]

and varies non-linearly with the imposed scattering angle \( \theta_a \). The widths \( w_s \) and \( w_i \) are estimated by measuring the transmitted intensity for different heights of a sharp blade moving perpendicularly across them. The lens L, the spatial filter \( \mathbf{SF}_1 \), and the distance between \( \mathbf{SF}_1 \) and the microfluidic chip, are optimized to obtain an incident and a scattered beam with small and almost constant widths at the position of the microfluidic chip. With the blade method described above, we found that the two beams are both well-described by gaussian shapes \( \exp(-2r^2/n^2) \) of widths \( w_s \approx 75 \, \mu\text{m} \) and \( w_i \approx 60 \, \mu\text{m} \) respectively. For these values, a minimal size \( a \approx 175 \, \mu\text{m} \) of the scattering volume is obtained according to Eq. (11) for \( \theta_a \approx 60^\circ \), and thus for \( \theta \approx 40^\circ \).

All the above considerations underline the experimental difficulties to implement DLS measurements in a microfluidic chip. First, only small angles can be investigated without any refractive index matching between the collecting fiber and the chip (\( \theta < 50^\circ \) in the case of aqueous solutions, \( n = 1.33 \)). Secondly, the dimension of the scattering volume across the microchannel, \( a \approx 175 \, \mu\text{m} \) for our setup, has to be small compared to the microchannel height \( h \) to minimize the decorrelation due to the shear flow, but also to avoid the possible scattering due to some irregularities at the walls. Finally, measurements of the size of Brownian scatterers flowing in the channel can only be performed at small flow rates as previously explained theoretically.

**B. Experimental validation for Brownian scatterers**

We performed DLS measurements with the setup described previously, with aqueous solutions of monodisperse calibrated nanoparticles (Invitrogen polystyrene, radii \( R = 30, 85, 235 \, \text{nm} \)) in PDMS-based microfluidic channels of section \( 500 \times 500 \, \mu\text{m}^2 \). Fig. 5(a) shows the measured correlation functions for various flow rates ranging in a logarithmic way from \( Q = 100 \, \mu\text{L}/\text{hr} \) up to \( 50 \, \text{mL}/\text{hr} \) (the corresponding range of maximal velocities \( v_0 \) is \( 0.1-100 \, \text{mm/s} \)), and for the calibrated beads of size \( R = 235 \, \text{nm} \). All the correlation functions are normalized according to \( C_n = (C(\tau) - 1)/C_0 \) for comparison. For flow rates below \( 800 \, \mu\text{L}/\text{hr} \), and thus for \( v_0 < 1.8 \, \text{mm/s} \), all the correlation functions collapse and display a single exponential decay as shown in the insert of
IV. Towards a microfluidic chip for on-line DLS measurements at small time scales

We demonstrated above that DLS measurements can be performed continuously in a pressure-driven flow within a microfluidic channel with a relatively large height, and for moderate flow rates that depend on the size of the investigated colloids. In our experimental device, it is typically possible to measure sizes up to \( \approx 300 \text{ nm} \) at a flow rate of \( Q \approx 2 \text{ mL/hr} \) in a microchannel of cross-section \( h \times w = 500 \times 500 \mu \text{m}^2 \).

Similar DLS measurements were performed recently by Chastek et al. using optical fibers directly inserted in a microfluidic device to follow the assembly of block copolymer micelles.\(^{13,14}\) Such in situ and continuous measurements were performed at small flow rates in large channels, and thus at small shear rates (channel cross-section \( 790 \times 790 \mu \text{m}^2 \), flow rate \( 500 \mu \text{L/hr} \)). To access higher velocities and thus smaller time scales, Feng et al. modified a stopped-flow apparatus to rapidly mix two reactants and perform continuous DLS measurements at the output of the mixing chamber.\(^{27}\) These successful experiments were carefully done at small shear rates in the measurement cell, thus limiting the first accessible time scales to a few seconds. Such devices however permit to investigate structural and conformational changes of biomolecules at time scales down to \( \approx 1 \text{ s} \).\(^{27,28}\)

In the following, we present how it is possible to exploit the benefits of microfabrication techniques to engineer a microfluidic chip that rapidly mixes two reactants and allows to perform continuously DLS measurements at time scales down to several hundreds of ms.

A. Design of the microfluidic chip, and operating conditions

As shown previously, simple DLS measurements are only possible at small shear rates, and so for microchannels with a large height. Mixing in such devices is therefore an important issue, since the distance along the channel that is required for complete mixing to occur is \( L_m = \frac{U h^3}{D} \), where \( U \) is the mean velocity in the main channel. For example, for small molecules in water (\( D \approx 10^{-9} \text{ m}^2/\text{s} \)) and in a \( 500 \times 500 \mu \text{m}^2 \) microchannel, \( L_m \) may reach prohibitively large values (\( L_m \approx 0.5 \text{ m} \) for \( Q \approx 2 \text{ mL/hr} \)).

It is therefore necessary to engineer on the same microfluidic chip, both a micromixer and a larger microchannel for DLS measurements. Fig. 6 displays the design of such a microfluidic chip which overcomes the above difficulties. More precisely, Fig. 6 shows a SU-8 mold with three different levels manufactured using standard soft lithography techniques. It first consists in a Y-shaped microchannel of height 40 \( \mu \text{m} \), that permits to drive two reactants in a 1 cm long microchannel of width \( w = 200 \mu \text{m} \). Chaotic mixing in such a laminar flow is performed using grooves of height 20 \( \mu \text{m} \) engineered on the main channel, according to the design investigated in depth by Stroock et al.\(^{29}\) Finally a large channel of width 500 \( \mu \text{m} \) and height 700 \( \mu \text{m} \) is added using a third level of photolithography at the output of the micromixer. DLS measurements may be performed safely in this observation window up to large imposed flow rates.

Mixing is achieved according to the strategy developed by Stroock et al.\(^{29}\) Following their terminology, we engineered a pattern of asymmetric herringbones (20 \( \mu \text{m} \) high) on the floor...
of the main channel. This pattern corresponds to 4 cycles of mixing, each mixing cycle is composed of two sequential regions of 6 ridges. According to ref. 29, this geometry can mix efficiently two reactants using chaotic advection up to large Péclet numbers $Pe = Uw/D$ ($U$ is the mean velocity in the mixing channel). We performed simple mixing experiments between pure water and fluorescent dyes in water (fluorescein and fluorescent nanoparticles, Invitrogen) injected at various imposed flow rates. These experiments confirm qualitatively the results found by Stroock et al.$^{29}$ the critical Péclet number for a complete mixing is about $Pe \approx 10^5$ at a downstream distance of 1 cm. Moreover, these experiments reveal that for flow rates above $Q > 5$ mL/hr, inertial effects occur at the entrance of the observation window with the appearance of two symmetric eddies. Such hydrodynamic recirculations appear at a Reynolds number $Re$ close to 1 ($Re = \rho U h/\eta$, $h = 700 \mu$m) in the measurement channel, and prevent us to work at such high flow rates.

To provide a complete mixing, and to avoid any inertial effects, we always work in the following with flow rates below $Q = 2$ mL/hr. Typically, at $Q = 2$ mL/hr, the mean velocity in the main channel is $U \approx 5$ cm/s, the transit time before entering the large microchannel is about 200 ms, and the micromixer permits an efficient mixing of small molecules in water ($for D = 10^{-9}$ m$^2$/s, $Pe \approx 10^3$). DLS measurements are performed in the large channel at an apparent angle $\theta_a = 60^\circ$. The scattering volume $V$ is centered in the flow as detailed previously, its main dimensions are $a = 175 \mu$m and $w_l = 75 \mu$m (see Fig. 4), and $V$ is placed at $\approx 1$ mm from the entrance of the cell. The maximal velocity $v_0$ in the chamber is about 3.3 mm/s for $Q = 2$ mL/hr, and one can therefore estimate that the transit time for the fluid streams coming out of the mixing chamber to the scattering volume is about 300 ms at this flow rate. Our device thus permits to mix two reactants in about 200 ms at a flow rate $Q = 2$ mL/hr (for solutes $D = 10^{-9}$ m$^2$/s), and to make continuous size measurements using DLS 300 ms after mixing.

As DLS measurements are performed in the chip under flow, the scattering volume is continuously fed with the colloids of interest. Measuring several correlation functions at a given position in the channel and for given flow rates thus enables to enhance the scattered intensity statistics and thus compensate the small intensities due to the small scattering volumes involved in the setup presented here.

In the following we first present a validation of this device through continuous viscosity measurements of water/glycerol mixtures using DLS, and we finally show an application around the formation of electrostatic coacervate complexes after the destabilization of a salted solution upon dilution.$^{30,31}$

### B. Application to viscosity measurements

To validate the previous setup and demonstrate its possibilities, we chose to perform the following simple experiments. Water and a water/glycerol mixture of volumic fraction $\phi_0 = 42.6\%$ in glycerol are injected at a constant total flow rate $Q = 200 \mu$L/hr in the Y-shaped device. Both solutions are seeded with monodisperse calibrated nanoparticles ($R = 30$ nm, Invitrogen). Correlation functions are measured in the wide channel as described previously at an apparent angle $\theta_a = 57^\circ$, for different ratios $Q_w/Q_g$, with $Q_w$ and $Q_g$ the water and the water/glycerol mixture flow rates respectively. For a total flow rate $Q = 200 \mu$L/hr, and for the range of investigated viscosities, the two solutions are efficiently mixed before entering the measurement cell, and the correlation functions are not dominated by the shear.

Fig. 7(a) presents different normalized correlation functions $C_n(\tau)$ obtained at various ratios $Q_w/Q_g$, and therefore for different glycerol concentrations $\phi_g = \phi_0 Q_g/Q_w$. The correlation functions are normalized according to $C_n(\tau) = (C(\tau) - 1)/C_s$ for comparison, and are all well-described by single exponential $C_n(\tau) = \exp(-\tau/\tau_c)$ with a characteristic time $\tau_c$ that evolves with the glycerol concentration $\phi_g$.

Fig. 7(b) displays the characteristic time $\tau_c$ against the imposed concentration $\phi_g$. These $\tau_c$ are related to the diffusion coefficients $D_g$ for different flow rate ratios, and thus for different glycerol concentrations $\phi_g$. The solid line corresponds to calculations from the literature values that take into account the viscosity variation with $\phi_g$. 

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**Fig. 6** SU-8 mold of the microfluidic chip. The main channel is 200 $\mu$m wide, and 40 $\mu$m high. The grooves are 20 $\mu$m high and designed according to ref. 29 to permit a rapid mixing (typically in 200 ms at a total flow rate $Q = 2$ mL/hr for solutes with $D \approx 10^{-9}$ m$^2$/s). The DLS measurements are performed at the output of the mixer in a larger microchannel (3 mm long, 500 $\mu$m wide and 700 $\mu$m high).

**Fig. 7** (a) Normalized correlation functions of calibrated nanoparticles ($R = 30$ nm) for different flow rate ratios $Q_w/Q_g$ and $Q_g + Q_w = 200 \mu$L/hr. Insert: log $C_s$ vs. $\tau$, the correlation functions are single exponentials $C_s = \exp(-\tau/\tau_c)$. (b) Characteristic times $\tau_c$ for the different flow rate ratios, and thus for different glycerol concentrations $\phi_g$. The solid line corresponds to calculations from the literature values that take into account the viscosity variation with $\phi_g$.
of the scatterers according to \( \tau_c = (2Dq_p)^{-1} \), where \( q \) is given by \( q = (4\pi n/\lambda) \sin(\theta/2) \), and \( \theta \) is related to \( \theta_p \) by \( \sin(\theta_p) = n \sin(\theta) \) to take into account refraction effects. Our measurements are direct estimations of the viscosity of the different water/glycerol mixtures, since \( D \) evolves according to the Stokes-Einstein relation \( D = k_B T/(6\pi n \eta R) \), where \( \eta \) is the viscosity of the mixture. Variations of \( \tau_c \) therefore correspond to variations of the viscosity \( \eta \) of the mixture.

However, slight variations of the refractive index \( n \) of the solution occur upon variations of the flow rate ratio, due to the glycerol concentration. All the values of \( n \) are well-tabulated, and it is thus easy to compute the real scattering angle \( \theta \) for all the investigated ratios, and so to relate exactly the measured \( \tau_c \) to the viscosity of the solution. The solid curve displayed in Fig. 7(b) corresponds to the theoretical values of \( \tau_c \) calculated from literature data and the Stokes-Einstein relation, and shows a good agreement with our experimental data.

All these measurements were done in about 30 min, and less than 100 \( \mu \)L of solution were used to measure the viscosity of ten different dilutions, consequently demonstrating the high-throughput possibilities offered by such techniques to investigate the viscosity of complex mixtures.

C. Application to phase diagram screening

For the design of artificially intelligent devices, fabrication and control of materials at the nanometer scale with chemical and physical attributes has attracted much attention over the last decade. In particular, the combination of polymers and nanoparticles has opened pathways for the engineering of novel hybrid structures combining the advantageous properties of both the organic and inorganic moieties.

So far, the formation of nanoparticle/polymer electrostatic complex cocavertes with finite sizes was initiated through the simple mixing of the two stock solutions containing the oppositely charged species according to certain empirical rules. Recently, an alternate approach in which both building-blocks are not initially interacting with each other in the aqueous solution was put forward. This process, inspired by molecular biology, was originally developed for in vitro reconstitutions of chromatin, the DNA/histones macromolecular substance that forms the chromosomes of our cells, and consists of two steps. In the first step the components are mixed together in a high ionic strength aqueous solution. The salt screens the electrostatic interactions between the individual components preventing them from forming aggregates. Their interaction is then turned off. In the second step, the salt is removed progressively by dialysis or by dilution effectively triggering the self-assembly process. The electrostatic interaction is then turned back on in a controlled manner.

Anionic poly(acrylic acid) coated cerium oxide nanoparticles (CeO\(_2\)-PAA\(_{2K}\)) and cationic-neutral poly-trimethylammonium ethylacrylate methylsulfate-b-poly(acrylamide) block copolymers (PTEA\(_{11K}\)-b-PAM\(_{30K}\)) were co-assembled using this approach generating core-shell complexes with typical sizes around 100 nm.

To study such electrostatically co-assembled systems, it is crucial to determine the critical salt concentration (or ionic strength) at which the attractive electrostatic interaction between oppositely charged species is totally screened or turned off in the solution. In order to obtain a complete phase diagram, DLS measurements are classically performed on the salted solution at different ionic strengths during the dilution process; a repetitive, tedious and material intensive procedure when realized manually. To overcome these inconveniences, we performed continuous DLS measurements of this reactive system within the observation window of our multilevel chip described further up (see Fig. 6). In the two inlets, deionised water and a mixture of CeO\(_2\)-PAA\(_{2K}\)/PTEA\(_{11K}\)-b-PAM\(_{30K}\)/NH\(_4\)Cl (0.044%/wt./0.056%/wt./0.6 M) were both introduced at constant flow rates \( Q_w \) and \( Q_m \) respectively. The ratio \( Q_m/Q_w \) controls the mass fraction of colloids \( \phi_p \), and the overall salt concentration (or ionic strength \( I \)) of the homogeneous reactive solution in the observation window after complete mixing. When the NH\(_4\)Cl concentration exceeds 0.4 M in the mixed solution, both components are not interacting anymore and the system is stable. Below this critical concentration, the electrostatically screened copolymer/nanoparticle system undergoes an abrupt transition between an unassociated and a cluster state. The effect of the water dilution through the variation of the flow rate ratio \( Q_m/Q_w \) may thus trigger the formation of the copolymer/nanoparticle core-shell complexes.

Fig. 8 shows the scattered intensity \( I_s \) measured at \( \theta = 40^\circ \) using the setup described previously (\( \theta_p = 60^\circ \)) for various \( Q_m/Q_w \) ratios keeping the total flow rate constant at \( Q = 300 \mu \)L/hr. The mass fractions of colloids \( \phi_p \) are then given by \( \phi_p = \phi_0 Q_w/Q \) where \( \phi_0 = 0.1\%\text{wt.} \). With the experimental conditions described above, complete mixing is achieved \( \approx 1.2 \) s after the injection of the two solutions since the diffusion coefficients of the nanoparticles and the copolymers are roughly \( D = 4 \times 10^{-11} \) m\(^2\)/s respectively, with corresponding Péclet numbers below \( 5 \times 10^4 \) at this particular flow rate. DLS measurements are performed

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**Fig. 8** (●-left axis) Scattered intensity \( I_s \) (arbitrary units) vs. \( \phi_p \) and \( I \) the ionic strength in the solution. (■-right axis) Corresponding diameters estimated from the measured correlation functions. For \( \phi_p < 0.07\% \) (\( I < 0.4 \) M), the sample scatters light significantly and well-defined correlation functions are measured, as shown by a typical one in the insert. Error bars account for the standard deviation over five consecutive measurements. The gray area indicates the domain for which no coacervates are formed upon dilution, and shows the abrupt transition that occurs at \( \phi_p \approx 0.07\% \) (\( I \approx 0.4 \) M).
in the observation window as explained previously. For \( \phi_p > 0.07\% \) or \( I > 0.4 \) M, the scattered intensity is very weak, and no particular characteristic times can be estimated from the measured correlation functions. Both the nanoparticles and the copolymers do not scatter enough at the investigated concentration to be measured by our setup. At \( \phi_p \approx 0.07\% \) i.e. \( I \approx 0.4 \) M, the scattered intensity increases sharply, and well-defined correlation functions \( C(\tau) \) are measured as shown in the insert of Fig. 8. These \( C(\tau) \) are well-fitted using the single exponential relation \( C(\tau) = 1 + C_s \exp(-\tau/\tau_c) \) indicating likely monodisperse scatterers. Using the Stokes-Einstein relation, the measured \( \tau_c \) gives a characteristic size of 100 nm in diameter for the complexes, corresponding more likely to the coacervates observed at longer time scales using simple mixing and conventional DLS measurements.\(^{20,37} \) For \( \phi_p < 0.07\% \), the scattered intensity decreases well with dilution and gives rise to identical correlation functions. This result suggests that the size of the coacervates does not evolve upon dilution, and that we probably only observe the dilution of the scattering coacervates.

Our microfluidic chip permits rapid screening using minute amounts of sample, the formation of electrostatic coacervates (\( \approx 200 \mu L \) of the CeO\(_2\)-PAA/PTEA-PAM/NH\(_4\)Cl solution in less than 1 hr for the studied case); it is a very promising tool to explore complex phase diagrams efficiently.

V. Conclusion

In this work, on-line dynamic light scattering measurements of colloidal sizes in a pressure-driven microfluidic flow have been demonstrated. Theoretical arguments were given to underline the difficulties to perform such measurements under flow in a microchannel, due to the Poiseuille flow that induces a shear-dependent decorrelation term. We however highlight a range of parameters for which DLS measurements are feasible in spite of the Poiseuille flow in relatively large microchannels. A specific dynamic light scattering setup has been built around microfluidic PDMS-based chips. This setup permits to estimate the size of Brownian scatterers flowing in microchannels, thus validating experimentally the theoretical estimations. Finally, we developed a microfluidic chip that can mix two reactants down to 200 ms, and allows size measurements using DLS down to about 300 ms after complete mixing. Two applications of such a device are presented: continuous measurements of the viscosity of a two-fluid mixture, and the formation of electrostatic coacervates in a solution of oppositely charged nanoparticles and block copolymers.

This miniaturized device dedicated to DLS measurements is a promising tool to study dynamics at the subsecond timescale. Thanks to the opportunities offered by microfluidics (small amounts of reactants, control of transport phenomena, ability to rapidly screen numerous experimental conditions), high-throughput data collection can be also achieved. This tool could be particularly interesting for investigating nanoparticles synthesis, phase transition or more generally fast dynamics in soft matter systems.

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References

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