Highly deformable nanofilaments in flow

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Abstract. Experimental analysis of hydrogel nanofilaments conveyed by flow is conducted to help in understanding physical phenomena responsible for transport properties and shape deformations of long bio-objects, like DNA or proteins. Investigated hydrogel nanofilaments exhibit typical macromolecules-like behavior, as spontaneous conformational changes and cross-flow migration. Results of the experiments indicate critical role of thermal fluctuations behavior of single filaments.

1. Introduction

Predicting behavior of deformable objects carried by the flowing fluid is necessary for understanding underlying physics of fibrous suspensions and transport properties of biological macromolecules. Evaluating conformational flexibility of long biomolecules and polymers suspended in fluid permits to determine their structure and mechanical properties. However, the intriguing and important interplay between polymer conformation and fluid dynamics remains still unrevealed. Its knowledge is crucial for understanding their dynamics, transport properties, and complex behavior like folding - unfolding sequences. Despite decades of theoretical studies, the fundamental dynamics of such systems remains a mystery. In addition there is still lack of experimental investigations to validate assumptions of the theoretical and numerical models. Experimental studies on molecules are difficult and rather inaccurate, mainly due to the necessary compromise between spatial and temporary resolution of optical methods at nanoscales. To systematically investigate the influence of flexible objects on their interaction with given flows and the resulting macroscopic properties, a synthetic experimental model of such nano-objects could be useful. It can help in future designing of objects transported by body fluids in order to perform targeted drugs release or as model object for biomolecules, which observation and analysis is difficult due to their small size. Introduction of microscale experimental model allows for precise optical measurements and the use of simple hydrodynamic models that give possibility for the description of dynamics of elastic bio-objects such as DNA or protein.

Theoretical assumptions, especially in the field of polymer physics, use coarse-grained models to study the folding process of nucleic acids and proteins. Most existing models describing the dynamics and properties of flexible objects use complex systems of interconnected identical spherical particles (worm-like chain) that illustrates the two particles configuration of two ends of the polymer which form the filament connected by a spring [1-5]. The possibility to describe this phenomenon on molecular scale is still limited to very small length and time scales. In these scales it is very difficult (or impossible) to find answers to basic questions about the potential effects of interactions or complex hydrodynamic behavior of long biological molecules. Additionally, so far available experimental data are not sufficiently precise to verify numerical models. Most of them are focused on experimental studies of the dynamics of DNA molecules [6] or other bio-objects like actin filaments [7], not accurate enough for detailed analysis.

Migration of fibers or other long objects in the Poiseuille flow is one of the fundamental problems of modern rheology with important impact on a variety of biological, medical and industrial contexts, such as Brownian dynamics of proteins, DNA or biological polymers, cell movement or drugs delivery. Simulations conducted for this type of long objects confirmed that stiff fibers tend to be accumulated near the wall and are slightly bended. Flexible fibers undergo large deformations and accumulate far from the wall [3-5]. Analysis of cross flow migration of micro sized objects conveyed by flow of viscous fluid in a tube have attracted considerable efforts, for example drag reduction observed for flow of blood in capillaries [8]. Experimental studies performed for solid spherical particles [9] and droplets [10,11] confirmed presence of their radial migration, however its theoretical interpretation still remains far from being completed. Despite the common wisdom on reversibility of creeping flow, groups of suspended particles or single deformable objects conveyed by Poiseuille flow may experience lift force even for negligible inertial effects. The effect depends on several factors, beside wall and velocity profile interactions. Also object deformations influence the direction of migration and the equilibrium position that is finally reached in a tube [12-14]. In case of long deformable objects like molecules and filaments, the cross-flow migration problem becomes quite complex, additionally conformational changes, tumbling and their elongation could influence it.
There are very little experimental data documenting details of the migration for long deformable nanoobjects. To the best of our knowledge, the present study offers unique possibility to evaluate effects of object deformability on its behavior in a channel flow. The knowledge gained may help to validate existing modeling attempts [3-5], perform cell sorting, improve DNA sequencing tools, and interpret actin or fibrin flow induced conformational changes.
For the purpose of our studies we have developed a new method for fabrication of highly deformable hydrogel filaments with the typical diameter of 100 nm and contour length ranging from a single micrometer to millimeters [15]. These objects are characterized by similar mechanical properties (ratio of persistence length to contour length) as DNA molecules, which supports the possibility of using them as model objects of biomolecules. An important issue is to understand hydrodynamic confinement effects. By analyzing performance of long filaments interacting with microchannel walls it will be possible to resolve effects of hydrodynamic screening, dramatically changing Stokesian description of such flow [16]. Understanding the relationship between the filaments behavior and flow properties opens the possibility of designing nano-objects capable to travel in crowded body fluids in order to selectively delivery drugs into the target area or to reach specific local tissues with the aim of helping in their regeneration.

2. Materials and Methods

2.1. Materials used

Poly(L-lactide-co-caprolactone) (PLCL), chloroform ($CHCl_3$), N,N-dimethylformamide (DMF), Bovine Serum Albumin conjugated with fluoresceine (BSA-FITC), N,N-isopropylacrylamide (NIPAAm), N,N'-methylene bisacrylamide (BIS-AAm), ammonium persulfate (APS), N,N,N',N'-tetramethylethylenediamine (TEMED).
Permanent epoxy negativ photoresist Su-8 2075, Developer mr-Dev, Silicon wafer, Sylgard 184 Silicone elastomer KIT polydimethylsiloxane (PDMS).

2.2. Preparation of flexible hydrogel nanofilaments

In first step, core-shell nanofibers were produced using coaxial electrospinning technique [17]. The shell is formed by PLCL that is produced using a polymer solution in DMF and $CHCl_3$ in proportion 1:1:9 (w/w). To prepare core solution we used 10 wt.% NIPAAm/BIS-AAm dissolved in deionized water (mass ratio of NIPAAm to BIS-AAm was 37.5:1). The core solution includes also components, which initiate the polymerization reaction (10 μl APS and 1 μl TEMED for 1ml polymer solution), and fluorescent markers enabling material imaging (BSA-FITC). In order to release the nanofilament constituting the core of electrospun nanofiber, a suitable solvent, e.g. DMF, dissolved its shell. This

process should not destroy the structure of the hydrogel nanofilaments. More details about experimental procedure of preparing hydrogel nanofilaments are given in our previous paper [15].

2.3. Nanofilaments in pulsating flow

The fluorescent hydrogel nanofilaments were imaged through a fluorescent microscope (Leica AM TIRF MC) using mercury lamp as a light source. Single nano-objects were observed using a 20x 0.40 DRY objective lens. The movement of nanofilaments was recorded using a high-gain EM-CCD camera (C9100-2, Hamamatsu) with typical framing time of 10Hz.

The experiment is based on the observations of hydrogel nanofilaments with length varying from a few of micrometers to tens micrometers, conveyed in the channel under the influence of pulsating fluid motion generated by two syringe pumps (Nemesis, Celoni GmbH). The length of the microchannel, which was used to analyze filaments flow, is 500 μm, channel width is 200 μm, and its depth is 60 μm. Figure 1 presents schematic of the described measuring system. Observations were made perpendicularly to the plane in which the objects were analyzed. The controlling software allowed the synchronized operations of the pumps in both directions. Flow rate was set to be below 100 μl/s. The presence of the objects in the analyzed area was checked by varying position of the observation, limiting area of interest to the central cross section of the channel. Only clearly distinguishable objects, remaining in focal plane and exhibiting deformations within 2-D plane of observation were taken into account (Fig. 1). For this purpose, constancy of apparent contour length of visualized filaments is used as a control parameter. Changes of observed projected filament contour length are interpreted as out of plane deformations and such sequence is not analyzed.

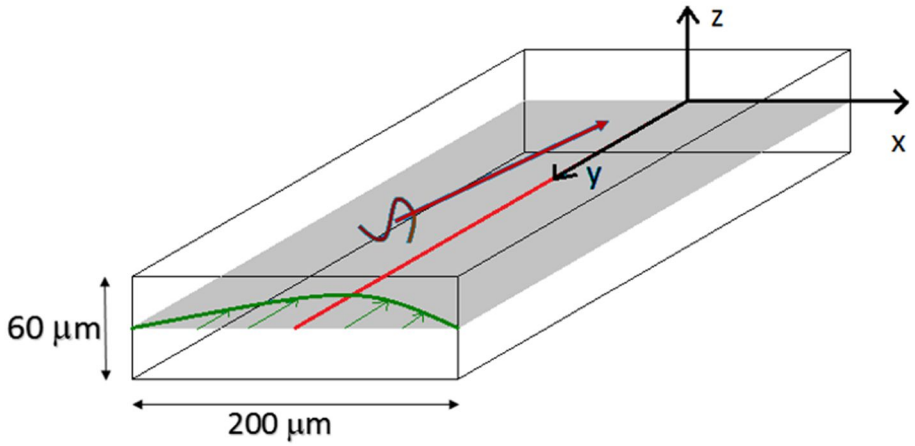


Figure 1. Schematic of the measuring system for nanofilaments conveyed by pulsatile flow. Gray marked is the analyzed area. Red line represents microchannels center.

3. Results

3.1. Deformation dynamic of flexible nanofilaments

Images of nanofilaments are recorded at constant framing rate, set depending on the flow velocity. Usually one experimental sequence consisted of nearly thousand images with resolution of 1M pixels. Selected sequences of images were used to localize filament, analyze its shape, and to describe their deformation using specially developed Matlab software [15]. Mechanical properties of the analyzed filament (Young modulus) were evaluated with help of experimentally determined persistence length. Table 1 collects typical data for filaments observed in the experiments. We may find that their mechanical properties resemble that of long biomolecules.

Table 1. Typical experimental parameters: mean flow velocity (V_f) and flow Reynolds number (Re); filament contour length (L), radius (R), persistence length (Lp), and Young modulus (E). Reynolds number is based on the channel width (200 μm).

No	V_f [μm/s]	L [μm]	R [nm]	Re	Lp [μm]	E [kPa]
1	59.02	71.91	81	$1.51 \cdot 10^{-2}$	17.62	2.17
2	77.78	72.87	81	$1.98 \cdot 10^{-2}$	12.38	1.53
3	39.65	34.49	45	$1.01 \cdot 10^{-2}$	3.44	4.45
4	68.87	54.31	45	$1.76 \cdot 10^{-2}$	7.51	9.72
5	250.43	75.58	45	$6.39 \cdot 10^{-2}$	9.56	12.37
6	199.64	82.90	81	$5.09 \cdot 10^{-2}$	14.34	1.77
7	124.24	32.12	81	$3.17 \cdot 10^{-2}$	25.26	3.12
8	134.70	29.62	81	$3.44 \cdot 10^{-2}$	2.89	0.35
9	119.20	26.84	81	$3.04 \cdot 10^{-2}$	3.75	0.46
10	59.02	71.91	81	$1.51 \cdot 10^{-2}$	17.62	2.17
11	77.78	72.87	81	$1.98 \cdot 10^{-2}$	12.38	1.53

Our experiments indicated that mobility of hydrogel filaments strongly depends on their length. It is also well visible that long filaments exhibit high bending flexibility (Fig. 2). It is interesting to note that despite negligibility of ionic interactions, bending followed by characteristic coiling and knots formation is observed. For long filaments their conformational changes are dominated over translational or rotational diffusion. Classical description of diffusion of long molecules using 'so called' hydrodynamic diameter appears in such case to be very far from the physical phenomena. For short filaments (10–20 μm) translational and rotational diffusion coefficients could be evaluated from their Brownian motion, and such filaments tend to rotate, apparently similar to elongated spheroids (Table 2). Thermal fluctuations of the filament shape observed in quiescent fluid are indicators of their mechanical properties [15]. These data can be used to evaluate interactions of deformable objects with pulsatile flow in the channel, as it is done in the next step.

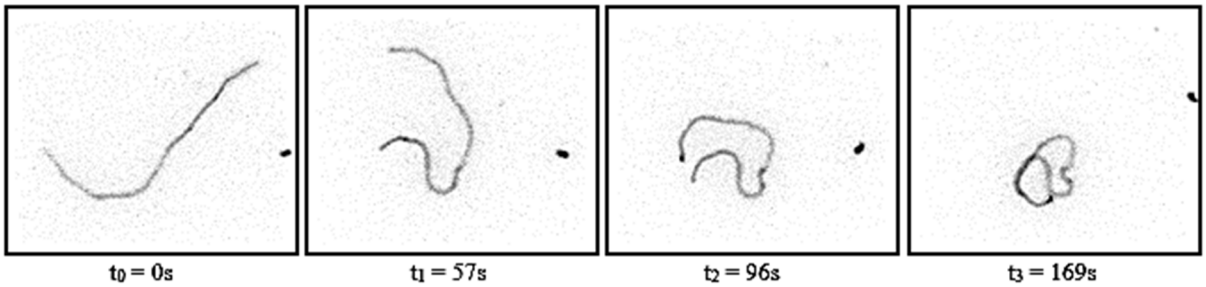
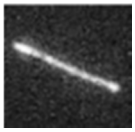
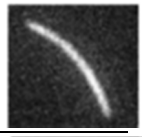
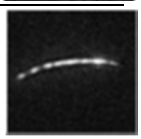


Figure 2. Sequence of images (negatives) for a single and long flexible nanofilament suspended in water, bending under influence of Brownian motion (R= 90 nm, L= 60 μm); image height 35 μm.

Table 2. Comparison between experimental and theoretical diffusion coefficients for selected nanofilaments: L – contour length; 2R – diameter of filament; D_a, D_b – translational diffusion coefficients; D_r – rotational diffusion coefficient; Theoretical diffusion values obtained modeling filaments geometry as that of elongated droplet [15]. Vm – migration velocity calculated for filament approximated by elongated droplet.

	L/2R	D_a (m^2)	D_b (m^2)	D_r (rad^2)	L (μm)	Vm [m/s]	Motion	Shape
exper.	29.8	0.059	0.140	0.0029	14.9	$2.1 \cdot 10^{-8}$	Rotation	
theor.		0.189	0.121	0.0051				
exper.	31.2	0.093	0.067	0.0025	15.6	$4.3 \cdot 10^{-8}$	Rotation	
theor.		0.182	0.116	0.0045				
exper.	43.0	0.052	0.035	0.0006	21.5	$1.3 \cdot 10^{-7}$	Bending	
theor.		0.144	0.090	0.0019				
exper.	48.4	0.112	0.105	0.0026	24.2	$6.7 \cdot 10^{-9}$	Bending	
theor.		0.132	0.082	0.0013				

Furthermore we can observe rotation and bending for filaments in pulsating flow. Short filaments move along the channel and rotate while long filaments have the tendency to coil and form knots. This effect remains prevalent for objects conveyed by Poiseuille flow (Figure 3).

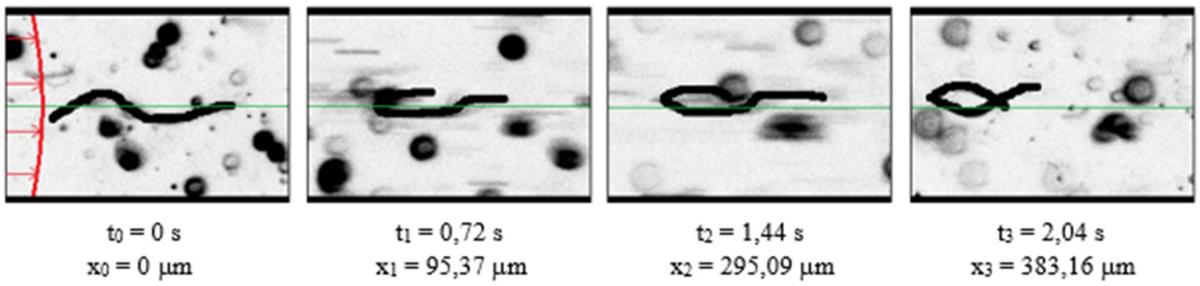


Figure 3. Sequence of images for a single flexible nanofilament conveyed by Poiseuille flow (no. 6 in Table 1), green line represents the center of the channel, t – time stamp, x – distance traveled from the channel entry (picture frame moves with the object), image height 77 μm; negative of the images shown.

Comparison of the measured fluid velocity (by nanoparticles tracing) and that of the filament center of mass observed during pulsatile flow shows slight differences in their waveforms (Figure 4). The differences result from changes in conformation of the analyzed filament as it is moved along the channel conveyed by pulsatile flow, as well as from the relative *"slip"* velocity characteristic for droplets conveyed by Poiseuille flow [13].

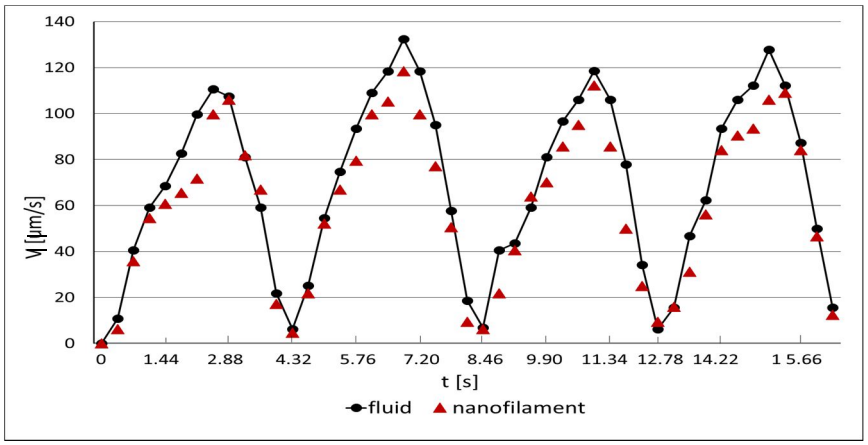


Figure 4. Absolute value of the fluid velocity and velocity of the filament (no 11 in Table 1) conveyed in the channel by the pulsatile flow; filament velocity calculated for center of mass.

3.2. Cross-flow migration

The analysis of the nanofilaments behavior in a pulsating fluid flow allowed observing migration of the objects into the center of microchannel, present in majority of cases. The observations of cross flow migration were carried out for the longest possible time in which the tested filament remained in the field of view of the camera, usually for 5 to 10 minutes. We have determined the variation of the filament distance from the centerline of the microchannel, evaluated for its center of mass. In some cases, we also have determined the distance of both ends of the object from the center of a microchannel (Figure 5). The typical duration of the imposed velocity pulse was two seconds.

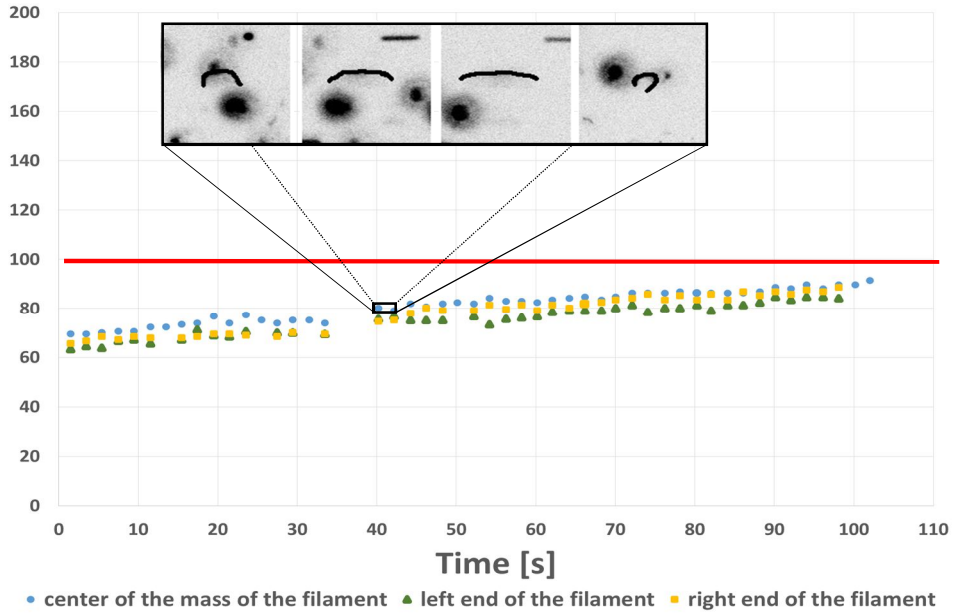


Figure 5. Cross-flow migration of nanofilament into the center of channel. Red line represents the center of the microchannel. Distance from the side wall in µm. Inserts show typical images of filaments during single velocity pulse.

Approximate value of the migration velocity for filament with geometry like elongated droplet (Vm) was calculated from the following equation [11] applying parameters of a typical hydrogel filament.

$$Vm = -2 \cdot \left(\frac{a}{d}\right)^3 \cdot \frac{\upsilon_m \cdot \mu}{\gamma} \frac{\upsilon_m \cdot \bar{x}}{(1+\lambda)^2 \cdot (2+3\lambda)} \cdot \left[\frac{16+19\lambda}{42 \cdot (2+3\lambda) \cdot (4+\lambda)} \cdot (13 - 36\lambda - 73\lambda^2 + 24\lambda^3) + \frac{10+11\lambda}{105} \cdot (8 - \lambda + 3\lambda^2)\right]$$

In our analysis, for the calculation of the objects hydrodynamic radius, the experimental and theoretical diffusion coefficients (Table 2) were used to obtain value of physical radius to be set in the formula. Hence data set in in the formula are: a = 0.25 μm; d = 100 μm – half of width of channel; υ_m = 0.0001 m/s - flow velocity at the channel axis; $\bar{x}$ = x/d = 0.1 – distance from the channel center line; λ = 21 - viscosity ratio of dispersed phase to viscosity of continuous phase (μ = 7.98 · 10^{-4} kg/ms); interfacial tension γ = 0.07 kg/s^2.

Migration velocity strongly depends on the viscosity ratio between material of the suspended object (droplet) and medium. In this estimation we took λ equal 21. It is only an approximate value for such type of materials. For rheological liquid, like hydrogel, its viscosity varies with shear rate and it has to be measured. It will be performed in the next stage of work.

Average value of the migration velocity of hydrogel nanofilaments evaluated in our experiments during pulsatile flow is around 10^{-7} m/s. We may observe that migration velocities of elongated droplets evaluated for the same flow parameters (Table 2) have similar value. It can be assumed that migration velocity of short filaments with geometry approximated by an elongated droplet is relatively well described by existing models for droplets [13].

Analyzing the migration curves of the center of mass and the two ends of the filament we may find that for most of these waveforms there is an overlap (Figure 5). However, during a single pulse the nanofilaments stretches and bends. Small deviations of the migration curves are probably associated with bending of the elongated objects during pulsatile flow.

We also observed cases where the test filament moved with carried liquid parallel to the walls of the channel all the time during the observation - without cross-flow migration (Figure 6 a). Therefore, we conducted additional test to evaluate impact of the flow velocity on the migration of filaments. Preliminary results show that the occurrence of nanofilaments migration in the analyzed channel geometry does not depend on the applied liquid flow velocity, at least in the small range of the investigated variation (doubled velocity). Further experiments will allow for verification of the occurring dependence. However, it seems that the conformation of the filament can affect the migration rate of the object (Figure 6 b). It is in line with theoretical predictions [5].

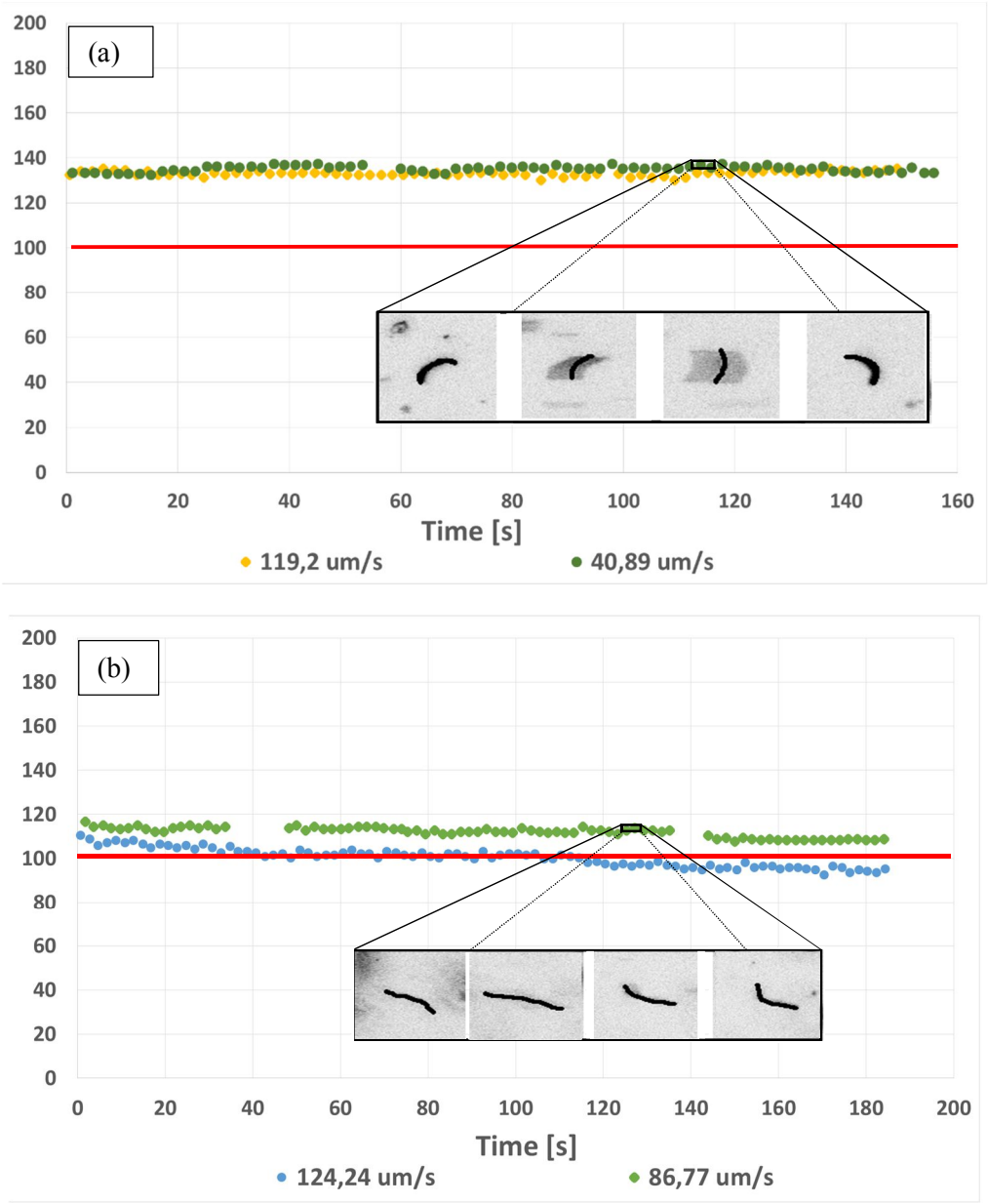


Figure 6. Effect of the flow velocity on the behavior of nanofilament conveyed in microchannel: (a) absence of migration; (b) presence of migration for less deformed filaments. Red line represents the center of the microchannel. Distance from the side wall is given in μm.

There are number of parameters that affect the rate and direction of migration for flexible filaments. Just to list the most important factors, they are: the average velocity of fluid flow (which translates directly to the speed of the filament during pulsatile flow), contour length and persistence length, length to diameter of filament ratio, viscosity of medium, Reynolds number, and position inside the microchannel (distance from the center of the channel). Knowing the contribution of individual parameters on the dynamics of filaments we should be able to control the behavior of such objects in a particular environment. Probably statistical analysis performed for large number of experiments is the only way to obtain plausible average mobility values convenient to predict longitudinal and cross-flow transport properties of such objects.

4. Discussion

Presented work demonstrates possibility of using hydrogel nanofilaments to characterize dynamics of deformable objects conveyed by pulsatile flow. Depending on the length of the filaments we observed their rotation and / or bending during short sequences of time. Especially long objects have a tendency to change configuration during flow. Comparing local velocity of fluid and that of the conveyed filament we can see that bending dynamics slightly changes mean velocity of the suspended objects.
Pulsatile flow well simulates intercellular movements of fluids inside living organisms. Present study confirms the hypothesis that the hydrogel nanofilaments may be used to model transport properties of biological molecules as well as vesicles. However, it is necessary to complete the characterization of these objects behavior, to gain full support about their potential applications, particularly in biomedical fields [18].
The phenomenon of cross-flow migration during pulsatile flow inside the microchannel is strongly associated with the bending dynamics of a nanofilament [19-21]. The issue of migration is important to describe the transport of deformable macromolecules in the capillaries. In the majority of the observed cases a tendency to migration of tested objects toward the center of the channel was found. As the rate of migration depends on several factors a deeper study is necessary to determine their identification and impact necessary to have a possibility to control the nanofilaments behavior. Several studies have shown that the occurrence of rotation, bending, and knots formation for filaments may influence the presence and rate of migration across the channel. The present study is trying to confirm such relationships by comparing the results obtained with the data evaluated for filaments with geometry approximated by elongated droplet. However, this comparison does not confirm unequivocally initial hypothesis formulated on the basis of experimental data, that the tendency of the filaments to bend favors the occurrence of migration, whereas objects showing only the rotation do not migrate across the channel or such migration is imperceptibly small. These preliminary results confirm the need for a larger number of experiments, showing various properties and behavior of the filaments.

5. Conclusions

Highly deformable hydrogel nanofilaments have demonstrated the possibility to be used to study the hydrodynamic interactions of long objects. Study of the flow characteristics for suspensions of highly deformable nano-objects has fundamental importance for the understanding and prediction of the rheological properties of typical biological fluid (cytoplasm, plasma). These data can be fundamental to create biocompatible nano-objects that can become tools for the regeneration of body tissues, e.g. neural tissue. There is still open issue of hydrodynamic effects for confined polymer solutions. In our case the confinement (channel depth) is of the same order of magnitude as contour length of the filament. The equilibrium conformation of the object can be expected to change substantially, influencing both transport as well as migration velocity. How far these effects influence observed filaments dynamics is not clear at the moment.

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