Biomimetic fluid dynamics used in lab-on-a-chip microfluidic devices

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The minimization of resources has been, over the years, a much discussed topic in society, both in terms of economic resources and energy and materials. Interest has arisen in new technologies, in particular the exploitation of micro and nanotechnologies that have been playing a crucial role in our society. Thus, the dynamic behavior of blood-like fluid droplets on electrowetting hydrophobic surfaces is characterized to evaluate the use of electrostatic-controlled microfluidic devices for agents used in the future as diagnostic aids. The focus of the diagnosis will be on the study of cell deformation, whereby the deformation of the particles that make up the fluids are analyzed in detail. Aqueous xanthan gum solutions were analyzed to mimic non-Newtonian blood behavior, while PMMA particle aqueous solutions and a semi-rigid particulate aqueous surfactant solution intend to mimic the cells. The fluid dynamic response to electrostatic actuation was evaluated in drops, quantifying the contact angle variation and the temporal evolution of the spreading diameter. The results show that the variation of gum and particle concentration and / or size does not influence the fluid dynamic response to actuation. Surfactant solutions have the worst response to actuation due to their low surface tension. The deformation of the particles was evaluated in microchannels with hyperbolic contractions that allowed a reproducible and controlled deformation, being characterized by the deformation index and the velocity along the microchannel. PMMA particles were more rigid and had a more consistent behavior in terms of both the deformation index and the velocity along the microchannel. However, the surfactant particles exhibit a deformation behavior closer to that of the actual cells.

Keywords: wettability, electrowetting, biomimetic fluid, microfluidic devices, deformable microparticles.

1.Introduction

Microfluidic devices have become one of the most promising new tools for clinical diagnosis and treatment of various chronic diseases, leading to their development [1]. In recent years, these devices have been explored [2], notably in the handling of small biological samples in microelectromechanical systems (MEMS), which are capable of performing all laboratory work such as sample preparation and manipulation, separation, reactions for analysis and detection, these devices being called lab-on-a-chip (LOC) [3, 4, 5, 6, 7].

These devices are applicable in many areas, but it is in the area of health and bioengineering that their application has fostered the most interest in the scientific community, particulary in the miniaturization of systems for clinical and biochemical analysis [8]. This technology, LOC, offers new perspectives for routine chemical analysis, bioassays, healthcare, and diagnostic devices, including noninvasive and early cancer detection [9]. There are several strands where these devices are applied. For example, there is a strong interest in using this technology to investigate blood flow, improve knowledge of healthy and pathological blood behavior, or improve medical examinations and develop better devices for broader monitoring of health parameters in point-of-care (POC) [10]. Also because of their flexibility, simplicity, portability and

automation, they are becoming increasingly important today, due to the limited resources both on energy and materials, their use requires [11], especially in developing countries [12].

Although functional, most of these devices are based on a mivrochannel configuration, which presents a number of problems due to the fact that they use pumps, sensors, valves and other complex auxiliary elements which can lead to degassing microbubbles as it is difficult to control multiple reagents simultaneously with a complex network of connected channels, even using microvalve architectures [9]. As an alternative to this microchannel configuration, an interest has arisen in systems that use micro droplets for handling and analyzing biological samples. Due to the impact of these technologies and studies, it is possible to separate, transport and mix microdrops along programmable path surfaces [13], reducing the need for auxiliary equipment due to the surface tension dominance in relation to pressure and dissipation forces. Hence, this configuration allows the transport of samples in micro drops only by changing the wettability at the drop-surface interface, without resorting to any mechanical contact, *i.e.* by electrostatic actuation [14]. This process of changing wettability is called electrowetting and allows moving, transporting, mixing and separating drops of volume in the order of micro or nanoliters quickly and efficiently, with relatively low power consumption [15]. This configuration favors, for example, point-of-care diagnosis, which in developing countries is an asset for carrying out biological analyzes under extreme conditions. The high mortality rate in these countries depends not only on the lack of treatment but also on the timely diagnosis of the disease, as in some regions it is practically impossible to perform these analysis [16, 17, 18, 19, 20]. Analysis of the chemical composition of, for example, physiological fluids (blood, urine) has been heavily explored by electrostatic action through the transport of fluid drops on the chip for cell examination [20].

Microfabricated devices can be used to develop and improve understanding of blood flow behavior and its hematological populations by separating and classifying cell subtypes. Separation techniques are an important application of microfluidic devices that play an important role in biomedical testing [21, 22, 23, 24]. Manipulation of real blood is often difficult due to ethical, economic and contamination issues, especially for laboratory work involving pathological blood.

In this context it is important to develop biomimetic fluids. These fluids are also important for the development of microfluidic devices designed for the project, both for transport and diagnostic, depending on the understanding of the fluid's characteristics and flow. The literature compiles the behavior of different blood-like fluids, to later allow for a more accessible understanding of the behavior of the blood itself, or even other physiological fluids, through the electrostatic performance and the study of microchannel transformation. However, it would be more interesting to develop a blood diagnostic at the viscosity level such as size and display capabilities, ability to simultaneously mimic rheological behavior of real blood and its multiphase heterogeneous nature, reaching the rheological Hemo response of different populations hematologic blood vessels. In this context, the present work intends to contribute to the development of analogous fluids, to evaluate their behavior and their potential use in microfluidic devices for electrostatically driven diagnosis.

2.Materials and Methods

This work evaluates the behavior of various biomimetic fluids (blood analogs) characterizing in detail the deformation behavior of the particles that constitute them. On the other hand, in the context of the work it is also important to evaluate its dynamic response to electrostatic actuation in order to verify if they can be handled in the microfluidic devices to be developed.

2.1. Experimental installation *Perspex* chamber

Except for surface tension and particle deformation, all other tests were performed inside a Perspex chamber, supported by an optical tensiometer (THETA from Attention) and with an adjustable volume drop generation system. The overall dimensions of the chamber are 55x80x90mm^3, with two quartz windows to reduce optical distortion in the measurements obtained and minimize the associated error. The images captured by the charge-coupled device camera have a size of 512x512 pixel and the optical

system has a spatial resolution of 21 µm/pixel. The entire drop generation and contact angle measurement system is automatically controlled by the computer via One Attension Software.

However, for the characterization of the scattering diameter a high-speed chamber (*Phantom v4.2* da *Vision Research Inc*.) is required since the characteristic scale of the droplet scattering time during electrostatic actuation is of the order of milliseconds. The captured images have a resolution of 512X512 pixel and are recorded at a sample rate of 2200 frames/s. The spatial resolution ranges from 30 to 33 µm/pixel depending on the experiment. The temporal resolution is 0.45 ms. The area where the camera captures images is illuminated with an LED light. For the analysis of electrostatic actuation, a tungsten electrode and a copper cylinder are used (Figure 1).

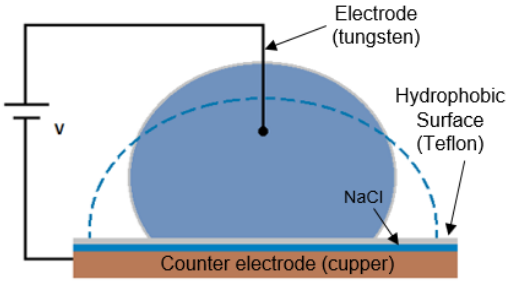


Figure 1: Experimental installation of electrostatic actuation.

The tungsten electrode and cylinder are connected to a direct current power supply (Sorensen DCR600-.75B).

2.2. Experimental installation particle deformation

Particle deformation tests were performed in hyperbolic contraction PDMS microchannels (Figure 2).

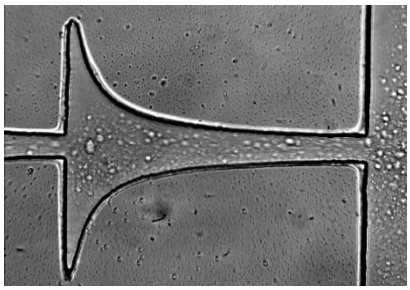


Figure 2: Microchannel geometry viewed under the optical microscope (Enlarge image).

Fluid flows through the channels being injected by a syringe pump containing the fluid to be analyzed. The pump and the device with channels are connected by Teflon tubes.

An inverted microscope (IX71, Olympus) is used to capture microchannel flow images through the high-speed camera (Fastcam SA3, Photron, USA). Images are recorded at a sample rate of 4000 frames/s with a dimension of 640x640 pixel. The spatial resolution is 1,185 µm/pixel and the temporal resolution is 0.25 ms. Since particle flow velocities are high and early particles left a trail, the shutter speed ratio had to be increased to 1/20000 which minimized particle trail.

2.3. Preparation of the solutions and characterization of their physical-chemical properties

In this work, 3 different aqueous solution species are used: a solution with a xanthan gum in 4 different concentrations (0,05wt%, 0,10wt%, 0,15wt% and 0,35wt%), according to the work of Moita et al. (2017), a PMMA solution with three different particle sizes (6 µm, 10 µm and 20 µm), with a concentration of 1wt% and finally a surfactant solution (Thermo Fisher Brij40). When mixed with water the surfactant precipitates to form semi-rigid particles of different sizes. Thus, in addition to the unfiltered solution, the use of 3 solutions was also considered, one filtered with 20 µm, another filtered with 10 µm and another without filter, also with 1wt% concentration.

The gum solution was chosen because of its non-Newtonian rheological behavior that is like that of blood. However, as this gum formed a homogeneity, *ie* it contained no particles that could mimic the behavior of cells, it was decided to study solutions with spherical PMMA particles (rigid) and compare with the analog (deformable particle surfactant).

All solutions were characterized in terms of their specific mass, ρ, surface tension, γ_{LV} and dynamic viscosity. Surface tension was measured on the optical tensiometer using the method of the pendant drop. These tests were performed inside the Perspex chamber, previously saturated at 20°C ± 3°C.

The following table presents the properties of the fluids under study. As they

are aqueous solutions their values are close

Dielectric coating	Teflon (PTFE)	PDMS	SU8 resist	Si_3N_4
Dielectric strength [kV/mm]	60	21,2	440	500
Dielectric constant	2,1	2,3-2,8	3	7,5
Typical coating thickness [µm]	25-50	38	2-15	0,15

to the values of distilled water. Thus, the dynamic viscosity was considered approximately constant and equal to that of water at 20 ° C, ie 1,000x10^{-3} Ns/$(m)^2$ [25].

Table 1: Physical properties of the solutions studied in this work and of distilled water. [1]Value taken from Ramos [26]. [2]Values taken from Moita et al. [27].

Solutions	Density ρ [g/cm^3]	Surface tension γ_{SL} [mN/m]
Water	0,998 [1]	73 ± 0,4
Gum 0,05wt%	0,996 [2]	73 ± 0,2
Gum 0,10wt%	0,997 [2]	70 ± 0,5
Gum 0,15wt%	0,997 [2]	72 ± 0,2
Gum 0,35wt%	0,997 [2]	73 ± 1,1
PMMA 6micro	0,998	77 ± 6,6
PMMA 10micro	0,998	77 ± 4,1
PMMA 20micro	0,998	82 ± 5,6
Surfactant filtered(10micro)	0,998	28 ± 0,2
Surfactant filtered(20micro)	0,998	33 ± 0,9
Surfactant without filter	0,998	28 ± 1,4

2.4. Surface characterization and selection

The application of electrowetting systems is due to the existence of an insulating dielectric layer, which prevents direct contact between the conductive drop and the substrate and allows the application of higher voltages without electrolysis.

Table 2 designates the properties of dielectric materials. The material used for electrostatic actuation application is Teflon (PTFE) with a thickness of ≈10 µm. However, the microchannel material that is used in this work is PDMS, because it is not expensive, is easy to work in microfabrication and has interesting characteristics in terms of the possibility of electrostatic actuation.

Table 2: Properties of dielectric surfaces.

3.Results and Discussion

The results are presented in three distinct phases: the first phase considers the characterization of the solutions under study, in terms of wettability with regard to the dielectric material that makes up the microfluidic devices, that are being developed. Later, in the second phase, the analysis of the fluid response to the electrostatic actuation is performed, observing the static angles and the scattering diameter. After this analysis, the solutions which are the most relevant to characterize, in relation to particle deformation (3rd phase), are chosen from amongst all the solutions under study.

3.1. Wettability characterization of biomimetic fluids

Characterization of contact angles is very important when studying a fluid at wettability level. It can be understood from both the phenomena and the macroscopic point of view that the fact that the surface is hydrophobic or superhydrophobic should facilitate electrowetting droplet movement, allowing greater scattering during actuation and greater retraction after actuation to the same applied stress relative to a hydrophilic surface.

Static contact angles without electrostatic actuation were measured for the Teflon surface for the solutions used in this work, and for distilled water that is used as the reference fluid. The static angles of the PMMA and Surfactant solution on a PDMS surface were also measured as these will be the solutions to be used in the study of particle deformation. Only quasi-static angles were measured for PMMA solutions since surfactant solutions have a low surface tension, initial equilibrium angles are very low, quasi-static angles could not be measured by the method used.

Table 3 shows that solutions with lower static contact angles correspond to lower surface tension values, such as Surfactant. The following table (Table 4) compares the contact angle of PMMA and surfactant solutions with two different surfaces, PDMS

and Teflon (solutions used in particle deformation).

Table 3: Equilibrium static contact angles measured for different solutions on the Teflon surface. [3]Value taken from Laurencia [28].

Teflon Surface	Contact angle (°)
Water	112 ± 5 [3]
Gum X0.05	94 ± 11,8
Gum X0.35	85 ± 8,5
PMMA 6micro	93 ± 3,3
PMMA 10micro	92 ± 5,5
PMMA 20micro	92 ± 1,4
Surfactant filtered(10micro)	46 ± 7,3
Surfactant filtered(20micro)	56 ± 6,5
Surfactant without filter	53 ± 5,5

Table 4: Static contact angle of PMMA solution measured on PDMS and Teflon surfaces.

Solutions	Contact angle (°)	
	PDMS	Teflon
PMMA 6micro	75 ± 4,4	93 ± 3,3
PMMA 10micro	90 ± 2,8	92 ± 5,5
PMMA 20micro	95 ± 5,2	92 ± 1,4
Surfactant filtered(10micro)	50 ± 6,3	46 ± 7,3
Surfactant filtered(20micro)	52 ± 3,8	52 ± 6,5
Surfactant without filter	56 ± 4,5	53 ± 5,5

Although Teflon is considered a hydrophobic surface, for surfactant solutions this surface contradicts its usual behavior, this is because solutions with this substance have a low surface tension and therefore a lower static equilibrium angle than expected for this solution, *i.e.*, this surface with this solution does not have the same wettability regime. Regarding to the other solutions, the teflon surface behaves as expected, being hydrophobic. It can also be concluded that for the same solution, but with different concentrations (such as gums) or different particle size, its static angle is independent of the concentration or even the size of the particles that make up the solution.

With respect to Table 4, there is a discrepancy of the PMMA solution with 6 µm particles on the PDMS surface, as it has a lower contact angle than expected, which may be justified by the fact that there is some surface impurity, or the surface has a certain heterogeneity. Surfactant has a usual behavior on both surfaces and the contact angle does not differ significantly from surface to surface nor solution to solution.

The contact angle measurement is not enough to characterize the surfaces as to their hydrophobicity, it is also necessary to evaluate the hysteresis value, *i.e.* the difference between the forward and reverse angle. Henceforth, the hysteresis value for PMMA solutions on the two surfaces under study was evaluated. However, it was not possible to perform quasi-static angle tests of Surfactant solutions, as they had a very low surface tension.

Table 5: Hysteresis for PMMA solutions on different surfaces under study (PDMS and Teflon).

PMMA	Hysteresis PDMS (°)	Hysteresis Teflon (°)
6micro	79,069	67,776
10micro	83,330	70,226
20micro	77,885	81,860

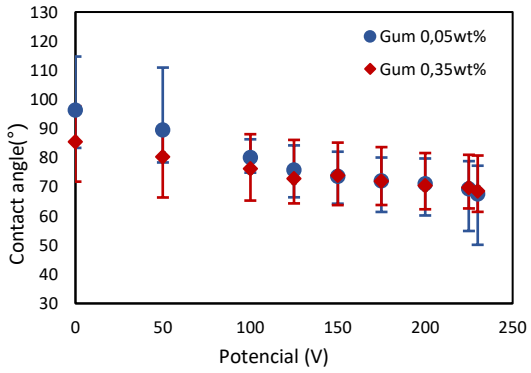
The hysteresis value on these two surfaces is large in any PMMA solution, which means that for higher hysteresis values it implies a greater adhesion of the droplets to these substrates and therefore a greater energy dissipation at the contact line, resulting in less drop retraction when the application of a tension is interrupted. It is also concluded that there is no significant difference in the various particle sizes.

3.2. Characterization of biomimetic fluid droplet response to electrostatic actuation

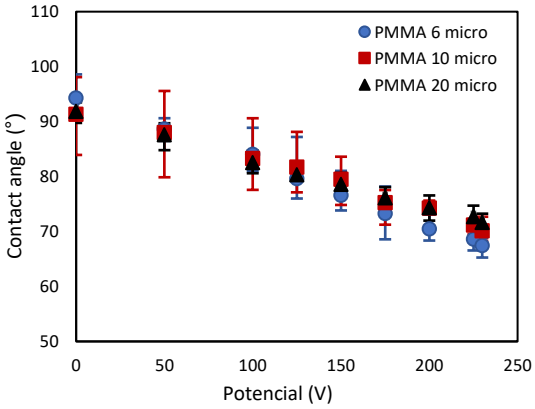
To evaluate the response of the assessed fluids to electrostatic performance, tests were performed to quantify the contact angle variation with the applied voltage and the temporal evolution of the droplet diameter during the application of different voltages.

Figure 3 represents the change in contact angle of the electrostatic actuation of the 2 gum solutions, 3 PMMA solutions and 3 Surfactant solutions deposited on a Teflon surface. The contact angle decreases as consecutively higher voltage values V are applied, which is qualitatively in agreement with the Young-Lippmann equation. Nevertheless, this variation becomes less evident for voltages from 200V, where, through the graph, there is a tendency for

static angle saturation to occur from this voltage on. These results agree with those reported by Mata [29].



(a)



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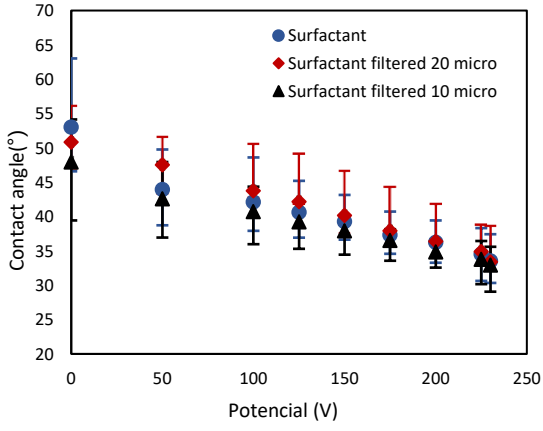
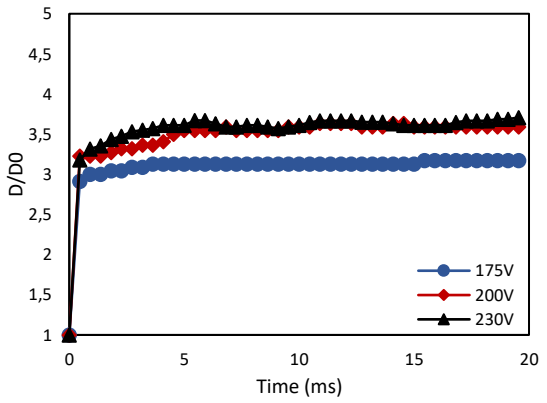


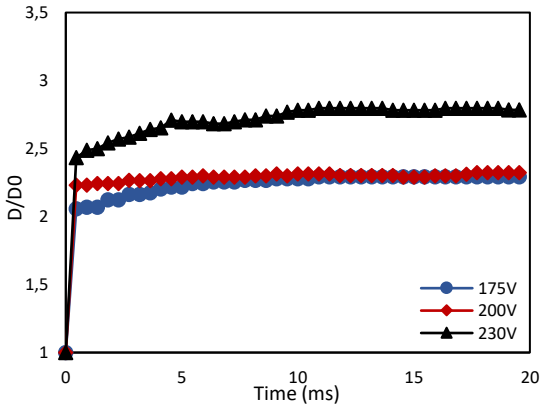
(c)

Figure 3: Contact angles for electrostatic actuation of solutions: (a) gums, (b) PMMA and (c) surfactant.

The analysis from the dynamic point of view is presented in figure 4, which represents the temporal evolution of the scattering diameter resulting from the electrostatic actuation with different voltages. The initial moment corresponds to the moment immediately before the electrostatic actuation, $t = 0\,ms$. Maximum drop diameter tends to increase with increasing applied stress. Nonetheless, in this solution when there is an increase in the spreading diameter, there is no retraction thereafter, that is, when it reaches the maximum spreading diameter the droplet maintains that same diameter, with no indentation of the contact line, when there is no voltage applied anymore. This strong irreversibility must therefore be associated with the high hysteresis previously measured with Teflon that is not overcome by electrical force.



(a)



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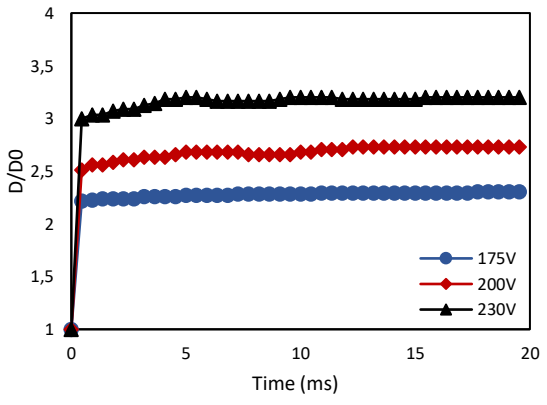


(c)

It can be concluded that although the particle sizes of PMMA solutions are different, there is no significant difference in the results obtained, having approximately the same behavior between these solutions, both from a static and a dynamic point of view. Regarding the surfactant solution, although it contains particles and is used in the study of particle deformation, since its surface tension is low, it was not possible to obtain the behavior of its scattering diameter.

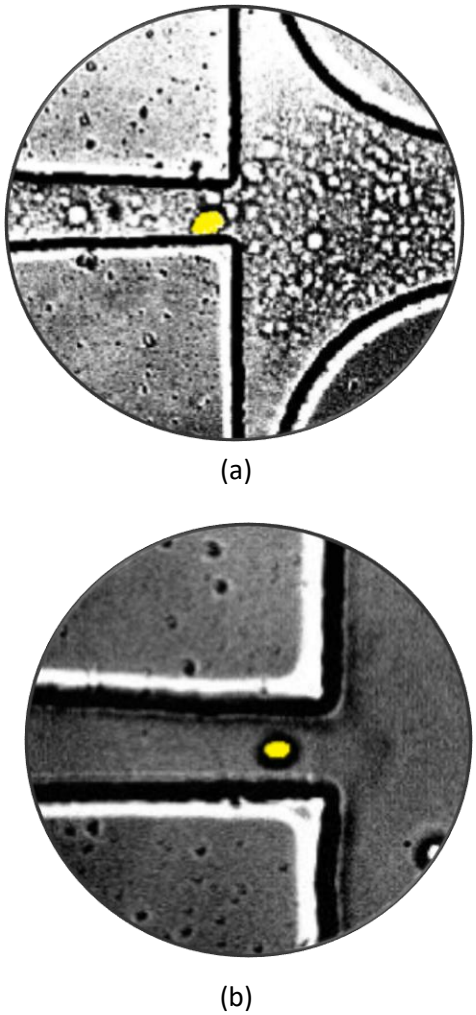
Figure 4: Spreading diameter of PMMA solutions: (a) 6micro, (b) 10micro and (c) 20micro.

3.3. Behavior of biomimetic fluids in microchannel flow: Particle deformation

Particle deformation in microchannels is also an important method for assessing fluid, through fluid particle deformation it is possible to characterize whether the particle has any anomalies, for example red cell stiffness variations are associated with various pathologies. Thus, among the analogous fluids that were characterized in terms of their response to electrostatic action, only 2 solutions were selected, one of PMMA (10 μm) particles and one of surfactant (filtered 20 μm) that were tested in the hyperbolic contraction microchannels. These dimensions were selected because they approximate the reals cell sizes (with the size of the red blood cell ~8 μm) and also because of camera resolution and channel size, smaller particles were difficult to see, while larger particles generated frequent clogging of the microchannels that obstructed the trials.

Figure 5 shows that the particle has its maximum deformation in the microchannel, where it has the maximum narrowing. It is also noticeable that PMMA particles are stiffer than surfactant particles, PMMA particles have a better contour and have virtually the same (spherical) shape along the microchannel, as opposed to surfactant particles, which are only partially spherical. at the beginning of the microchannel, however, when they reach the hyperbolic zone, they tend to lengthen along the flow axis.

The deformation is more reproducible in PMMA particles as can be seen from the series 1, 2 and 3 of the PMMA graphs where they have a maximum deformation index in the narrowest zone of the channels. Predictably, the velocity is maximum in the microchannel strait (minimum area), as the flow rate is constant, the velocity is maximum.



(a)

(b)

Figure 5: Images of microchannels illustrating deformation, (a) surfactant and (b) PMMA.

That said, the deformation index is maximum when the velocity is maximum, that is, in the narrowest section of the microchannel. Using graphics, it is particularly noticeable the good reproducibility of the tests and that the peak of deformation coincides with the maximum particle velocity.

Surfactant particles are less rigid, *i.e*. have a more erratic deformation behavior and it is less reproducible, also, the relationship of particle deformation to velocity is also less obvious because when deforming it also offers another type of main flow resistance, making it more difficult to relate deformation to velocity.

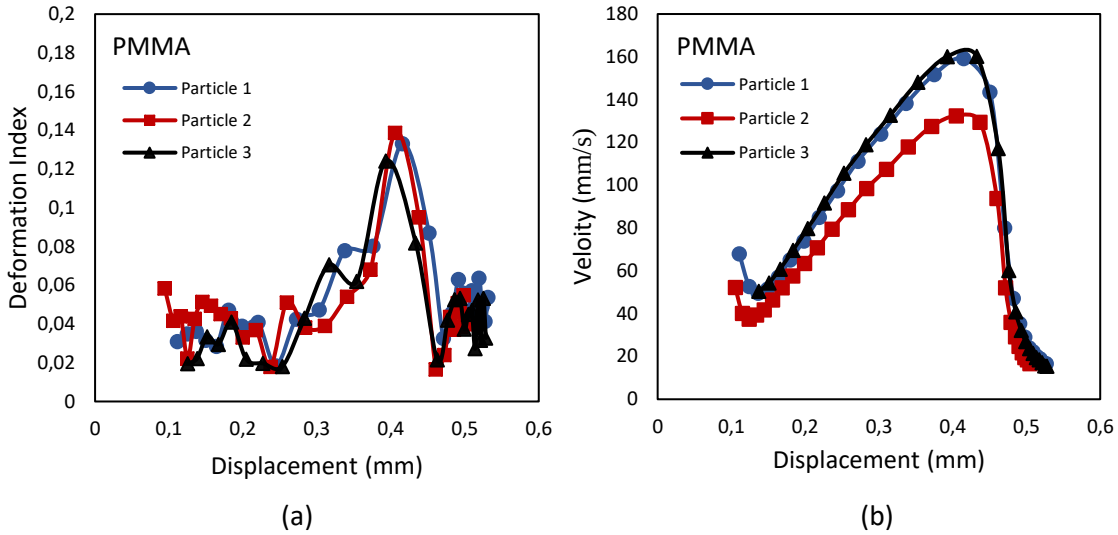


Figure 6: PMMA particle deformation graphs, (a) particle deformation index as a function of channel displacement, and (b) particle velocity as a function of channel displacement.

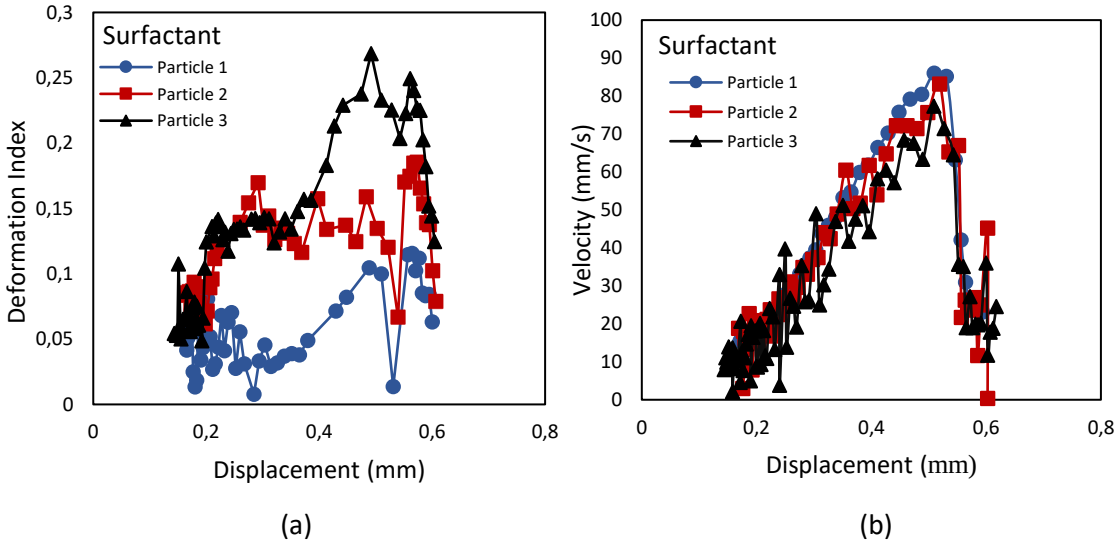


Figure 7: Surfactant particle deformation graphs, (a) particle deformation index as a function of channel displacement, and (b) particle velocity as a function of channel displacement.

It is important to consider the deformation of the real cells and to compare them with the study fluids, in order to verify whether the deformation of the study fluids is similar or not to the real cells. For this purpose, the maximum value of the deformation of each particle was considered as shown in the following figure 8, however, the deformation index of the red globes was taken from Pinho [30].

PMMA particles exhibit a more rigid behavior, being able to deform less than surfactant particles and less than real red blood cells. Although these solutions under study do not have the deformation index very close to the real cells, from the work developed, it can be considered that in the case of surfactant particles as semi-rigid particles, they should continue to be the object of study, since, they are the closest to the real cells (healthy red blood cells). Notwithstanding, surfactant particles are more difficult to correlate in terms of flow in the microchannel.

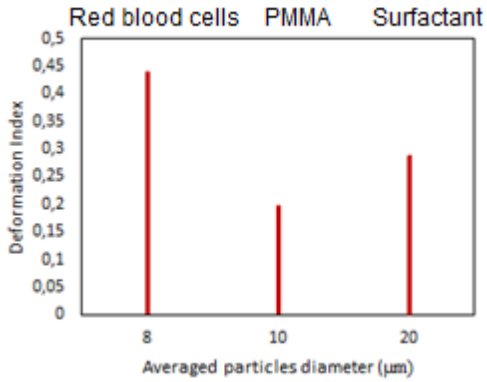


Figure 8: Maximum deformation index obtained in each solution under study and of the real cells [30] with the average particle diameter.

4.Conclusions

In this work the behavior of 3 types of biomimetic fluids, were evaluated, namely aqueous xanthan gum solutions, to mimic non-Newtonian blood viscosity, aqueous solutions containing spherical polymer particles (PMMA-poly(methyl methacrylate)) and solutions with a surfactant which, in contact with water precipitates, forming semirigid particles that mimic cells (red blood cells, whose deformation is closely related to various pathologies).

In terms of the response to electrostatic actuation, it was found that the increase of gum and / or particle concentration, as well as the variation of particle size did not affect the fluid response to electrostatic actuation. This result shows that droplet movement is controlled essentially by wettability and surface tension, with viscous forces playing a secondary role. Referring to the surfactant solution, there is consistent behavior for the different solutions on all surfaces tested, *i.e.* the static contact angles are in the same range of values, being lower when compared to PMMA solutions, due to its surface tension being significantly lower. This reduced response has consequences on the spreading of surfactant solution droplets, which, under the tested wetting conditions, presented minimal spreading under action, regardless of the applied stress values.

For the characterization of the particle deformation of the tested analog fluids, the 10 µm diameter PMMA particle solution and the 20 µm filter filtered surfactant solution were analyzed. These solutions were selected because of their analogy with the dimensions of the actual cells to mimic and to minimize channel clogging problems that occurred with the use of larger particles and which made it impossible to use multiple channels. The results obtained show an approximately linear evolution of the deformation index along the microchannel, with an evident peak in the deformation index for the PMMA solution that coincides with the maximum narrow of the microchannel, where the particle velocity is maximum. For the particles present in the surfactant solution, this relationship is not so evident as there is a greater discrepancy of the results, this is due to the fact that these particles are less rigid than the PMMA.

Both particles can be used in cell deformation studies as blood analogs, and their use in electrostatic controlled microfluidic devices is feasible. However, surfactant solutions, although they present larger irregularities, are the ones that best characterize the red blood cells.

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