Characterization of the dynamic response of biofluid droplets to electrostatic actuation

Catarina Lopes d’Oliveira Laurêncio


Abstract

This study reports the characterization of the static and dynamic wetting behavior of droplets of biofluids on hydrophobic smooth Teflon surfaces under electrostatic actuation, aiming at the development of a surface microfluidic chip for sample handling in lab-on-a-chip applications. Given the wide interest and variety of applications, protein solutions were chosen to be tested, namely BSA (Bovine Serum Albumin) and GFP (Green Fluorescence Protein).

The results show negligible effect of the concentration of the proteins in promoting droplet motion by electrowetting. Increased concentrations mainly promote a passive adsorption mechanism, which alters the local wettability of the substrate thus contributing for the initial droplet spreading and for the energy dissipation at the contact line (due to hysteresis) which in turn precludes the recoiling motion of the droplet. Laser scanning confocal microscopy performed to the substrates under droplet deposition before and after actuation confirms the existence of passive adsorption. As an alternative approach, the Teflon surfaces were coated with a perfluoralkyltrichlorosilane combined with perfluoropolyether carboxylic acid and a fluorinated solvent. The substrates become superhydrophobic with very low hysteresis, which enhances droplet response to the electrostatic actuation within small time scales. A thermographic study was performed to infer on possible temperature variations in the droplet and its evaporation during actuation. No change on the droplet temperature was observed during the actuation. Hence, significant droplet evaporation may occur by mass diffusion when the relative humidity of the environment air is lower than 70% (30% of the droplet may evaporate in 25min). This effect must be taken into account when using the transport of biosamples in microdroplets in microfluidic devices for biochemical analysis. Apart from this, the biosamples can be effectively transported inside the microdroplets under electrostatic actuation. Increasing surface temperature variation induces an increase of the spreading diameter with reduced recoiling motion, which turns droplet handling more difficult. Therefore, the chip design should be performed taking into account the results obtained at ambient temperature and for non-heated surfaces.

Keywords: electrowetting, EWOD, electrostatic actuation, BSA, GFP, adsorption, contact angle, wettability, induced spreading diameter, evaporation

Introduction

Microscale analysis is rich in assay possibilities in diagnostics and bioanalytical applications. The successful implementation of lab-on-chip devices offers a significant reduction of samples and reagents, as well as faster analysis, given the smaller diffusion distances, which also allow a more efficient control of the reactions. In these devices, the accurate control of samples transport and manipulation is a vital issue. Local modification of the surface tension is the governing mechanism of sample transport. It can be achieved by a variety of methods, but electrostatic actuation is one of the most popular, being considered as the backbone of digital microfluidics by few authors (Pollack, et al., 2011). In most of the case studies, closed configuration systems are used, which are based on continuous flows or small droplets moving in an immiscible continuous medium (Kuo, et al., 2003) (Pollack, et al., 2011). An alternative approach has recently been suggested, to use an open configuration, based on electrowetting aided by surface microfluidic, i.e., fluidic pathways using hydrophobic or superhydrophobic patterns, created on a single surface. The aim is the implementation of a single plate droplet-based transport device, driven by electrostatic modification of surface tension, for bio-analytical applications (Garza, 2011). The properties of the hydrophobic/superhydrophobic surface are vital to control the appropriate motion of the droplet. Surface properties also introduce additional complexity when modeling electrowetting, which is already a complex and unfinished task. The most classical approach describing electrowetting follows the thermodynamic balance of interfacial tensions, as introduced by Young (1805) and by Lippmann (1875),

\[
\cos \theta = \cos \theta_0 - \frac{\varepsilon_0 \varepsilon_S}{2 \varrho_0 \gamma_{SV}} (U - U_{sat})^2 \quad (1)
\]

These models consider that the decrease of the contact angle is the governing effect of electrowetting controlling droplet motion. Alternatively, electromechanical (Kuo, et al.,
2003) (Jones, 2005) and energy minimization models (Mugele & Baret, 2005) (Bahadur & Garimella, 2006) show that the energy gradient is in fact the driving effect behind electrowetting induced motion.

The contributions cited in the previous paragraphs allowed good knowledge on the basic principles of electrowetting and the EWOD – electrowetting on dielectric configuration, within a wide range of applications. However, models explaining droplet motion, which accurately account for saturation conditions, the actual effect of the electric field and of possible local modification and/or divergence of the electric fields near the contact line, double layer effects, among others (Kang, 2002) (Mugele & Baret, 2005) are still a work in progress.

Additional challenges arise when dealing with the transport of biofluids. Although several authors report the successful electrowetting-induced transport of proteins, DNA and even physiological fluids (Srinivasan, et al., 2004), the local wettability seems to be affected by the prompt and irreversible adsorption of the biomolecules, as reported for instance by (Rupp & Axmann, 2002) to occur for hydrophobic substrates, such as Teflon. Passive or active adsorption of proteins, as reported for instance by (Yoon & Garrell, 2003) are complex processes which depend on the composition of the protein, but also on the properties of the substrate, such as ionic force and composition. These phenomena are not well described yet, although they are much likely to affect the electrowetting efficacy and influence the occurrence of contact angle saturation conditions, thus limiting the transport processes.

In this context, the present work aims at characterizing the static and dynamic wetting behavior of BSA (Bovine Serum Albumin) and GFP (Green Fluorescence Protein) droplets on hydrophobic surfaces, considering the effect of several parameters that are commonly altered in sample analysis (concentration). BSA was chosen due to its hydrophobic nature and because it is very similar to the protein present in the human plasma, while GFP was selected due to its natural fluorescent properties which allow easy detection in the droplet and over the substrate.

**Experimental and methods**

**Experimental set-up**

The experimental configuration required for the tests under electrostatic actuation is directly mounted on an optical tensiometer (THETA, from Attension), so that the liquid pumping system and droplet formation can be automatically controlled by the software (One Attension) that runs the measurements at the tensiometerer. At this stage of the research, large droplets with initial diameters fixed at \( D_0 = 3.0 \pm 0.2 \) mm are used, to assure a good spatial accuracy of the measurements, which is vital for the precise description of the main fundamental quantities that are being investigated. The droplets are deposited on the hydrophobic substrate (a PTFE film) and are actuated afterwards. All the tests are performed inside a Perspex chamber, with total dimensions of 65x70x65mm\(^3\), which is saturated with the working fluid, at room temperature (20\(^{\circ}\)C±3\(^{\circ}\)C). The tests are performed under continuous monitoring of temperature and relative humidity of the surrounding air. This chamber has four quartz windows to minimize distortion which introduces errors in the image based techniques.

The electrostatic actuation is performed in a single-plate configuration, following the schematics presented in Figure 1.

![Figure 1 - Single-plate configuration schematics](image)

A 10\(\mu\)m Teflon film (Goodfellow Cambridge Ltd) is used as the dielectric hydrophobic substrate. As recommended by (Restolho, et al., 2009) a very thin film of sodium chloride was placed between the counter electrode and the dielectric to avoid the presence of an air gap.

The electrode dipped inside the droplet is a tungsten wire with 25\(\mu\)m diameter (Goodfellow Cambridge Ltd). The counter electrode is a copper cylinder. Both electrodes were connected to a Sorensen DCR600-75B power supply and DC voltage is applied. Although AC voltage is reported by some authors to lead to better performances of the electrowetting systems, namely decreasing the contact angle hysteresis and delaying the contact angle saturation (Jones, 2005) (Chen & Bonaccuroso, 2014), the mechanisms behind hysteresis and contact angle saturation in the present work seem to be more related to the wetting characteristics of the surfaces and with the properties of the solutions. Also, since the purpose of this work is to extrapolate some of these results to develop a test chip that may overcome the limiting frequencies for which the conditions for which Lippmann equation are not satisfied (Mugele & Baret, 2005) the results presented here were obtained with DC voltage.
Preparation of the solutions and characterization of their physico-chemical properties

The experiments were performed using BSA (Bovine Serum Albumin), from Sigma solutions, prepared with different concentrations, ranging between 0.15mM and 1.5mM and GFP (Green Fluorescent Protein), from Sigma.

The solutions prepared here were characterized in terms of density, viscosity and surface tension. Density, \( \rho \) was measured using a picnometer for liquids and the dynamic viscosity, \( \mu \) was measured under controlled temperature conditions, at ATS RheoSystems (a division of CANNON® Instruments, Co.). The accuracy of the data is within ±5%. Within this accuracy, the solutions were observed to have density and viscosity values very close to those of water (0.998 < \( \rho \) < 1.0059, \( \mu \approx 8.9 \times 10^{-4} \text{Pa.s} \)).

Surface tension, \( \gamma_{lv} \) was measured under controlled temperature conditions (20°C) with the optical tensiometer THETA (Atension), using the pendant drop method. The value taken for the surface tension of each liquid tested was averaged from 15 measurements. The surface tension of GFP solution was close to that of water (73.8mNm\(^{-1}\) with a standard error of the mean of 0.04). For BSA it tends to slightly decrease for larger values of concentrations, as shown in Table 1.

<table>
<thead>
<tr>
<th>BSA concentration [mM]</th>
<th>Surface tension, ( \gamma_{lv} ) [mNm(^{-1})]</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.15</td>
<td>64.8</td>
<td>0.16</td>
</tr>
<tr>
<td>0.375</td>
<td>60.3</td>
<td>0.42</td>
</tr>
<tr>
<td>0.75</td>
<td>61.0</td>
<td>0.20</td>
</tr>
<tr>
<td>1.5</td>
<td>60.3</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Measurement of the contact angles and contact diameters

The wettability is quantified by the static \( \theta_0 \) and quasi-static (advancing and receding) contact angles, measured using the optical tensiometer THETA from Atension, for all the pairs-liquid-surface considered in the present study. Distilled water is also used as a control fluid. For the characterization of the wettability, five to six consistent measures were taken for each sample using the sessile drop method. Images of the deposited droplet were taken using a monochrome video-camera coupled with a microscope. The images size is 640×480 pixels and the spatial resolution of the system for the current optical configuration is 15.6µm/pixel. The images were post-processed by a drop detection algorithm based on Young-Laplace equation (One Atension software). The accuracy of these algorithms is argued to be of the order of ± 0.1° (Cheng, et al., 1990).

For the characterization of the contact angle under electrostatic actuation, at least 6 tests are performed to obtain an average curve (contact angle vs. applied voltage). In each of them, the droplet must be deposited on a clean area of the substrate and the tests only proceed for similar values of the contact angle at 0V. The curves are constructed based on voltage increments of 25V.

Then, complementary information is given by the contact diameter of the droplet, which is obtained from high-speed visualization and post-processing. The high-speed images were taken at 2200fps using a Phantom v4.2 from Vision Research Inc., with 512x512pixels@2200fps resolution and a maximum frame rate of 90kfps. For the present optical configuration the spatial resolution is 25pixel/µm and the temporal resolution is 0.45ms.

The contact diameter is evaluated based on a home-made post-processing routine developed in Matlab. Temporal evolution of the contact diameter is presented as the average curve of 6 events, obtained at similar experimental conditions. Accuracy of the measurements is evaluated to be ±25µm.

Laser Scanning Confocal Microscopy for adsorption analysis

For qualitative evaluation of the possible adsorption of the proteins on the PTFE surfaces, simple tests were performed in which droplets of 3,0±0.2mm of GFP were deposited on the surfaces. After deposition, a sequence of tests with electrostatic actuation was performed with applied tension between 0 and 230V. Afterwards, the Teflon surface is observed on the Confocal Microscope (Leica SP8), looking for the droplet “footprint” over the substrate. A similar test was performed depositing the droplet and looking for its “footprint”, but without electrostatic actuation (the droplet remained over the surface within the same time period required for the electrostatic actuation tests to be performed). Most of the observations intended to see the footprint of the droplet as a whole, so most of the images were taken with a 4X magnification (0,10 of numerical aperture), with a pixel size of 5,42µm x 5,42µm.
Evaporation and temperature variation

Droplet evaporation has been reported to occur during electrowetting, with negative consequences in terms of modifying the concentration of the biosample (e.g. Cooney et al., 2006, Ramos et al., 2014). Evaporation may occur due to temperature increase in the droplet, during actuation or due to mass diffusion to the air. Using a time resolved thermographic camera it is possible to study the temperature evolution on the droplet deposited on the Teflon surface. The use of this camera is complex and well defined procedure steps are mandatory to achieve good measurements. A camera ONCA 4969 from Xenics is used with the following characteristics:

<table>
<thead>
<tr>
<th>Table 2- Thermography camera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Camera characteristics</td>
</tr>
<tr>
<td>Sensor: InSb (MWIR)</td>
</tr>
<tr>
<td>Spectral sensibility:</td>
</tr>
<tr>
<td>3.5 to 5 µm</td>
</tr>
<tr>
<td>Spatial resolution:</td>
</tr>
<tr>
<td>320x256 pixel</td>
</tr>
<tr>
<td>Pixel dimension:</td>
</tr>
<tr>
<td>30x30 µm</td>
</tr>
<tr>
<td>Thermal sensibility:</td>
</tr>
<tr>
<td>&lt;17 mk</td>
</tr>
<tr>
<td>Pixel Operability:</td>
</tr>
</tbody>
</table>

For the current configuration, the actual accuracy is 1 K/pixel. The images are taken with an integration time of 150 s, at a frame rate of 1100 fps. The measures are taken considering water emissivity as 0.98. The IR camera is positioned to visualize the droplet from the top, perpendicularly to the substrate in which the droplet is deposited, to minimize distortion effects.

It is worth mentioning that the accurate measurements require an evaluation of several variables, which influence the temperature of the object (droplet) that is taken by the camera. The droplet temperature is measured by means of a balance of the various radiative energy contributions that reach the camera (eq. 2), namely the surface energy emitted by the surface \( E_s \), the energy emitted by the atmosphere \( E_{\text{amb}} \) and the liquid energy \( E_l \) which can be split into two different components, the one that reaches the liquid-vapor interface and the one that is emitted by the liquid and reaches the surface liquid-solid (Figure 2).

\[
E_i(T_\text{c}) = \rho_{\text{app,amb}} E_{\text{amb}}(T_{\text{amb}}) + \rho_{\text{app,s}} E_l(T_\text{c}) + \rho_{\text{app,A}} E_s(T_\text{c}) \tag{2}
\]

Where \( \rho_{\text{app,amb}} \), \( \rho_{\text{app,A}} \) and \( \rho_{\text{app,s}} \) are the droplet attenuation and reflection, \( E_c = F_{\lambda} - \lambda \sigma T_c^4 \) the energy measured by the camera.

From this balance the camera software uses equation 3 to compute the drop temperature.

\[
T_c = \sqrt{\frac{\rho_{\text{app,amb}} E_{\text{amb}}(T_{\text{amb}}) + \rho_{\text{app,s}} E_l(T_\text{c}) + \rho_{\text{app,A}} E_s(T_\text{c})}{\rho_{\text{app,s}} \cdot \rho_{\text{app,amb}} \cdot \rho_{\text{app,A}}}} \tag{3}
\]

Additional measures were taken by heating the substrate, to evaluate on possible changes on the dynamics behavior of the droplet and its response to the electrostatic actuations for particular essays that should be performed at temperatures closer to that of the human body (Bishop, et al., 2010).

Results and discussion

Static and dynamic wetting under electrostatic actuation- Effect of the concentration

Figure 3 depicts the equilibrium contact angle obtained under electrostatic actuation \( \theta \) as a function of the applied voltage, for different concentrations of BSA and for GFP.

Looking at the evolution of \( \theta \) for the protein solutions (Figure 3), GFP and BSA show similar responses to the electrostatic actuation, although GFP seems to have a higher starting contact angle and achieves saturation also from lower voltages. Any clear effect of the concentration can be identified. Also, there is some trend indicative of saturation when the highest voltages are applied, which also depict initial lower contact angles. This may be explained by the
adsorption of the protein by the dielectric substrate, which reduces the contact angle until a constant, but still high value. This change in the wettability is irreversible, affecting the hydrophobic properties of the substrate and leading to a saturation angle scenario: the motion of the contact line is more difficult for the latest parts of the test, which correspond to the highest actuation voltage.

Figure 3 - Contact angle as a function of the applied voltage for solutions of BSA, with different concentrations, and GFP

Additional information can be obtained by observing the transient morphology of the actuated droplets, by high-speed visualization. In this case a new droplet is used for each event, to infer if the motion of the contact line is indeed being influenced by the irreversibility caused by the decrease of the contact angle. Hence, Figure 4 shows the temporal evolution of the electrostatic induced spreading diameter, $D(t)$ of BSA solutions, for the concentrations reported in Figure 3. $t=0$ corresponds to the instant when the droplet is actuated. The curves obtained here are made non-dimensional with the initial contact diameter obtained for $V=0\text{V}$, $D_0$, to account for small variations in the initial size of the droplet and in the $\theta_0$, which would be propagated along the entire temporal evolution.

Overall the temporal evolution of the spreading diameter depicted in Figure 4 is in agreement with the trends suggested in Figure 3, thus corroborating the hypothesis of adsorption of the protein, which, in turn is in line with the passive adsorption mechanism suggested by (Yoon & Garrell, 2003). Hence, concerning the temporal evolution of the contact diameter for the BSA solutions, there is a slightly larger spreading as the concentration increases up to 0.375mM and then the spreading diameter is again lower. As the concentration slightly increases, the local wettability increases. This is evident also in Figure 3, as $\theta$ were lower for the largest concentrations. This leads to favor the spreading at these faster time scales that are being considered in the analysis of the contact diameter (after tens of milliseconds the diameter is already totally stabilized). However, if the protein is indeed being adsorbed by the substrate, higher concentrations promote the adsorption and given the high irreversibility observed with this substrate (hysteresis larger than 20°) the contact angle is too low to favor a further spreading of the droplet and already near the values where the saturation starts to occur, so that the spreading diameter cannot further increase. Given that the energy dissipated at the contact line is proportional to the hysteresis, such large hysteresis precludes the recoiling motion of the droplet.

Detection of adsorbed proteins by confocal microscopy

The discussion of the previous paragraphs suggests that many of the results observed here, which lead to a saturation scenario are actually due to local modifications of the wettability due to adsorption of the proteins by the Teflon substrate. GFP was used to take advantage of
its natural fluorescent properties. Hence, droplets of 3.0±0.2mm of GFP (Green Fluorescent Protein) dissolved in water were deposited on the surfaces. After deposition, the “footprint” of the droplet is observed in the confocal microscope. A new droplet is then deposited on a clean region of the Teflon surface and a sequence of tests with electrostatic actuation is performed ranging values of applied tension between 0 and 230V. Afterwards the “footprint” of the actuated droplet is observed. It is worth nothing that all the experimental conditions are identical to those used with the BSA solutions (Figure 5).

Quantitatively the value of TCFD is higher for the footprint obtained when the droplet is actuated. However the difference is of about 1%, which is within the uncertainty of the measurements. So one may argue that there is adsorption of the protein in the Teflon, but by a passive mechanism, so it does not depend on the actuation.

Given the limitations to the motion of the droplet caused by the adsorption, which are leading to large energy dissipation at the contact line, an alternative in treating the Teflon with a superhydrophobic coating. For this purpose a commercial coating was used, named Glaco©, which is mainly a perfluoroalkyltrichlorosilane combined with perfluoropolyether carboxylic acid and a fluorinated solvent.

The analysis performed by confocal microscopy clearly shows a significant minimization of the protein adsorption on the surface coated with the Glaco© (Figure 7). Considering this result, it is worth investigating if this results indeed in an improved motion of the droplet.

Surface treatment effect on electrowetting

The variation of the contact angle with the applied voltage (Figure 8a) and evolution of the spreading diameter (Figure 8b,c) were again evaluated, for BSA solutions with extreme concentrations (0,15mM and 1,15mM) for the Teflon coated with Glaco©.
When using Glaco© as surface treatment the wettability of the substrate changes, which can be seen in the starting contact angle that raises almost 50º when comparing with Teflon without surface treatment. Other improvement is the contact angle difference between the equilibrium contact angle and the final contact angle when 230V are applied, which is about 76º for Teflon+Glaco©, much larger than the 40º observed for Teflon without surface treatment.

Using Glaco© clearly improves the dynamic response of the droplet to the actuation: the spreading diameter is considerably larger and is followed by a significant recoiling of the droplet, which can be associated to the reduction of the hysteresis and energy dissipation at the contact line, as a result of the minimization of the protein adsorption.

**Evaporative effects and temperature variation**

Previous work (Ramos et al., 2014) reported an increase in the concentration of BSA of 1.5 times after the actuation tests. By mass conservation principles, the concentration increase has to be ascribed to droplet evaporation, being indicative of a decrease in the volume of the droplet by evaporation, of approximately 30%.

Evaporation of a sessile droplet on a hydrophobic surface is not a simple process (Singh, et al., 2013). In some cases, droplet evaporation occurs due to a combined effect of mass diffusion and natural convection. However, considering the experimental conditions of the present study, it is reasonable to assume that the evaporative process is mainly governed by mass diffusion, from the surface of the droplet to the air. So the general diffusion equation is given by

$$\frac{\partial c}{\partial t} = D \nabla^2 c$$  \hspace{5cm} (4)

where \(c\) is the vapor density and \(D\) is the diffusion coefficient of vapor in the surrounding air. The surface temperature of the droplet will decrease during evaporation, but this process, as well as modifications in droplet shape are nearly three times smaller than the total evaporation time of the droplet for microliter droplets (Hu & Larson, 2002), so the diffusion mechanism can be considered to occur at a quasi-steady state (Singh, et al., 2013). The diffusion coefficient can be taken as in McHale et al. (1998) \(D=2.32\times10^{-5}\text{m}^2/\text{s}\). Assuming that vapor density at the surface \(R_s\) is at equilibrium \(c_s\), the and that the evaporation rate for a sphere in an infinite medium is given by:

$$\frac{dm}{dt} = 4\pi R_s^2 D (c_s - c_\infty)$$  \hspace{5cm} (5)

where \(c_\infty\) is the vapor density at far away from the droplet (detailed derivation can be found for instance in (Maxwell, 1890) and in (Barash, et al., 2008). Considering the analogous between electric potential and diffusive flux one may derive a more general formula for the sessile
droplet, to estimate the evaporative mass rate of the droplet pinned over a hydrophobic surface:

\[
\frac{dm}{dt} = \frac{4\pi R_0 D (c_5 - c_\infty) E m_0^{2/3} \left( \frac{C}{R_S} \right) \sin \theta_0}{2 \rho T^{2/3} \sin \theta (t)}
\]

(6)

Here, \( \theta \) is the contact angle, \( m_0 \) is the initial mass of the droplet and \( E = 3/\pi (1-\cos \theta(t))^2(2+\cos \theta(t)) \) (Singh, et al., 2013). \( C \) is the capacitance of the droplet and the values for \( C/R_S \) can be taken from (Picknett & Benson, 1977). From this estimate, 30% of the droplet deposited on Teflon (to match the contact angles) may indeed occur at relative humidity values of 70% and temperatures of 23ºC within 1500s. These results are qualitatively in agreement with those reported by (Singh, et al., 2013).

Electrowetting is not expected to alter droplet evaporation, but at the light of these results one may speculate that some local heating of the surface and consequently of the droplet may have occurred at the highest applied voltages, which might explain these results. To infer on this hypothesis, a thermography camera is used and the images of the temperature measurements processed.

Figure 9, which depicts the infrared time resolved images of the droplet obtained before and during actuation at 230 V, further show that the temperature of the droplet remains unchanged during actuation, thus rejecting any hypothesis that droplet evaporation would have occurred due local heating during actuation.

Figure 9- Thermal images of the droplet over the copper surface coated with Teflon

![Figure 9](image)

A quantitative analysis was also executed in order to perceive if there is any sharp change on the temperature during the actuation, by analyzing the data from four different droplets to two values of voltage applied during actuation. Three different temperatures were evaluated to detect possible temperature variations during the actuation time, namely the minimum (Figure 10a), the average (Figure 10b) and the maximum (Figure 10c).

![Figure 10a](image)  
**Figure 10a- Temperature variation with time a) minimum;**

![Figure 10b](image)  
**Figure 10b- Temperature variation with time b) maximum;**

![Figure 10c](image)  
**Figure 10c- Temperature variation with time c) average of the droplet**

These results clearly show that any abrupt temperature variation occurs in the droplet during actuation. The time between consecutive images (0.9ms) is short enough to capture the actuation process (occurring during 5ms) and the droplet was evaluated over 60ms. Hence, form these
results one may argue that droplet evaporation is only due to mass diffusion.

The study of the dynamic behavior of the droplet under electrostatic actuation was further extended to the situation in which the surface was heated from 23°C to 37°C and 50°C. The spreading diameters were measured at 37°C and 50°C and compared with those obtained for the non-heated surface (Figure 11).

![Figure 11](image_url)

**Figure 11-** Electrowetting induced spreading of the contact line obtained for different temperatures to a 0.15mM BSA concentration a)175V; b)220V

The results show that heating the surface leads to the increase of the spreading diameter. This behavior is mainly related to the decrease of the liquid surface tension with the temperature and does not affect the response to the electrostatic actuation, since the spreading diameter increases and there is no risk for the droplet not to touch the next electrode. Hence, the design of the chip should be performed for the case of a non-heated surface.

**Conclusions**

The present paper addresses the forced spreading of biological liquid droplets over hydrophobic surfaces under electrostatic actuation, in the context of sample handling in lab-on-a-chip applications. Given the wide interest and variety of applications, protein solutions were chosen to be tested, namely BSA (Bovine Serum Albumin) and GFP (Green Fluorescence Protein). BSA was chosen due to its hydrophobic nature and because it is very similar to the protein present in the human plasma, while GFP was selected due to its natural fluorescent properties which allow easy detection in the droplet and over the substrate.

Increased concentrations of the protein solutions do not affect the dynamic behavior of the droplet, but promote a passive adsorption mechanism, which alters the local wettability of the substrate thus contributing for high surface energy dissipation near the contact line, further limiting the spreading (and receding) motion of the droplet.

Using a superhydrophobic surface treatment that reduces the passive adsorption of the proteins improves the motion of the droplet, both by increasing the spreading diameter and promoting the recoil of the droplet. This improvement is the result of a lower hysteresis and therefore dissipation at the contact line during the motion of the droplet. Although the coating used here has provided satisfactory results, further optimization in the selection of the coating is recommended.

Infrared time resolved imaging does not show any evident local heating of the droplet during actuation, even at high applied voltages, to be associated to evaporation, which must be governed by quasi-steady mass diffusion. Theoretical prediction of droplet evaporation governed by quasi-steady mass diffusion to the surrounding air shows that significant droplet evaporation may occur by mass diffusion when the relative humidity of the environment air is lower than 70% (30% of the droplet may evaporate in 25min).

Heating the surface leads to the increase of the spreading diameter. However, this behavior is mainly related to the decrease of the liquid surface tension with the temperature and does not affect the response to the electrostatic actuation. So, the design of the chip should be performed for the case of a non-heated surface.

**Acknowledgements**

The authors are grateful to Fundação para a Ciência e Tecnologia (FCT) for supporting C. Laurêncio with a fellowship under the framework of the project RECI/EMS-SIS/0147/2012.

**References**

Bahadur, V. & Garimella, S. V., 2006. An energy-based model for electrowetting-induced droplet


