I. INTERFACIAL RHEOMETRY

Figure 1: **a:** We performed a long term membrane formation for a single case (0.1% w/w chitosan, 0.1% w/w PFacid, \( f = 0.5 \) Hz, \( \gamma = 0.03\% \)) in order to perform a strain sweep on a thick membrane and define the limits of linear elastic regime. **B:** We observed that for long term formed membranes (>24 hours) the linear elastic regime was limited to surprisingly low values of strain (<0.1 % ). The direct microscopic observation of the membrane decorated with tracing particles during the strain sweep experiments showed that this highly non-linear behaviour was caused by wrinkling instability of the membrane. The deformation of \( \approx 20 \% \) lead to the rapid decline in \( G_i' \). The direct observation showed that it was caused by the loss of connectivity between the membrane and the bicone.
Figure 2: Strain sweep of the chitosan/oil interface. The test was performed with the bicone geometry ($f = 0.5$ Hz). The aqueous phase contained 0.1% w/w chitosan. PFacid was not added to the oil phase. The result illustrates the steady state chitosan/oil interface without the complexation. **Note:** while the stable values of interfacial viscoelastic modulus $G_i^*$ are obtained, without the solid membrane forming at the interface the Boussinesq number is very low ($Bo=0.242$ in this case). Thus this result is qualitative and served as a reference line.
II. DYNAMIC LIGHT SCATTERING

Figure 3: Two distinct cases demonstrating the changes in chitosan/PFacid membrane DLS signature. The average characteristic relaxation time of chitosan solution (0.1% w/w) was \( \tau = 0.88 \) s. The relaxation time of fully formed membrane after 10 hours of complexation was 3488.4 s. Note, that DLS signature of the chitosan solution, being a fast process, was acquired using the PM as a receiver, while the DLS signature of the membrane was acquired with fast camera.

III. ATOMIC FORCE MICROSCOPY
Fit: $y = 133x^{0.5}$
Graphical Abstract

**Structural characterization of the interfacial self-assembly of polyelectrolytes**

Revaz Chachanidze, Kaili Xie, Hanna Massaad, Denis Roux, Marc Leonetti, Clément de Loubens
Structural characterization of the interfacial self-assembly of polyelectrolytes

Revaz Chachanidze\textsuperscript{a,*}, Kaili Xie\textsuperscript{a,b}, Hanna Massaad\textsuperscript{a}, Denis Roux\textsuperscript{a}, Marc Leonetti\textsuperscript{a,c} and Clément de Loubens\textsuperscript{a}

\textsuperscript{a}Univ. Grenoble Alpes, CNRS, Grenoble INP, LRP, 38000 Grenoble, France
\textsuperscript{b}Univ. Bordeaux, CNRS LOMA UMR 5798, Talence F-33405, France
\textsuperscript{c}Univ. Aix-Marseille, CNRS, CINaM, Marseille, France

\textbf{ABSTRACT}

Controlling the assembly of colloids at liquid-liquid interfaces offers new ways to fabricate soft materials with specific physical properties. However, little is known of the relationships between the kinetics of interfacial assembly, structural and rheological properties of such interfaces. We studied the kinetics of the assembly of two oppositely charged polyelectrolytes using a multi-scale approach. Soft interfaces were formed from the complexation at water-oil interface of chitosan, a polysaccharide carrying positively charged groups, and a fatty acid exhibiting negative charges. The growth kinetics of the membrane was followed by interfacial rheometry and space- and time-resolved dynamic light scattering. This set of techniques revealed that the interfacial complexation was a multi-step process. At short time-scale, the interface was fluid and made of heterogeneous patches. At a 'gelation' time, the surface elastic modulus and the correlation between speckles increased sharply meaning that the patches percolated. Confocal and electron microscopy confirmed this picture, and revealed that the basic brick of the membrane was sub-micrometric aggregates of polyelectrolytes.

1. Introduction

Since pioneering observations by Ramdsen [1] and Pickering [2] regarding the stabilization of emulsions and foams by colloidal particles trapped at interface, the interfacial assembly of colloids has seen growing interest from scientific communities. It opens the way to the fabrication of materials with specific physical properties such as films, capsules or structured liquids by using interfaces as scaffolds [3] as well as understanding some physiological functions [4]. These materials can be produced by droplet formation [5] or 3D-printing of liquid-liquid interfaces [6]. The main driving mechanism behind the self-assembly of colloids at interface is the process of minimization of interfacial energy, which can be tuned by an external stimulus [7] or by controlling the interactions between the particles [8]. As a result of this assembly, the interface can have a solid-like or liquid-like behaviour. One striking example is the possibility to design mechanically pH-responsive and self-healing microcapsules by interfacial assembly of polymer-polymer coacervates [9], which open the way to \textit{in-situ} reconfigurable structured liquid interfaces.

Building materials based on interfacial assembly of colloids with tuneable properties (e.g. microencapsulation) requires understanding the interplay between the properties of the colloids, the kinetics of interfacial assembly and the resulting properties. For interfaces covered by model nanoparticles [10], the structure of the interface changes with the increasing surface coverage, from a fractal network of aggregates to a heterogeneous structure with voids, to a gel with dense clusters and eventually a densely-packed system [11, 12]. Consequently, viscoelasticity and yield points of these interfaces are controlled by the surface coverage, interparticle interactions and external field forces [12, 13].

H-bond acceptor and donor polymers have also been used to cover water-oil interfaces by interfacial complexation of both polymers [9]. For these systems, the elasticity is controlled by the type and strength of physical interactions [14]. Dupré de Baubigny \textit{et al.} [15] investigated the kinetics of membrane growth on long time scales (> 1,000 s) and identified a diffusion limited process. However, the authors were surprised to observe that the process was faster when polymer molar mass increased. They related this observation to the description of the structure of the membrane as a gel-like porous network, with a pore size much smaller than the radius of the diffusing polymer chains. As a result, the diffusion process should be hindered by the entropic barrier. Another possible approach stabilising interfaces...
Interfacial self-assembly of polyelectrolytes

2. Materials and methods

2.1. Materials

Chitosan powder with medium molecular weight and 75-85% deacetylation was purchased from Sigma-Aldrich. The anionic surfactant used to complex the chitosan at water-oil interface was phosphatidic fatty acid (PFacid). It was comprised of a commercially available lecithin known as lecithin YN (Palsgaard 4448, food-grade, E442, Palsgaard). In mass, the phosphatidic acids were 55% w/w, neutral triglycerides 40% and ammonium salts 5%; see [17] for details. The molecular structures of both polyelectrolytes are given in Figure 2-A. Sodium hydroxide (1 mol/L) was purchased from VWR. The oil-soluble fluorescent dye, Hostasol Yellow 3G (HY-3G), was acquired from Clariant. Rapeseed oil (from *Brassica rapa*), hydrochloric acid (36.5-38.0 %, BioReagent, for molecular biology) and cyclohexane (anhydrous, 99.5%) were obtained from Sigma-Aldrich. Deionized water (resistivity > 18 MΩ.cm) was produced from a USB camera

Figure 1: Inerfacial rheometry by means of IRS. (A) Schematic representation of the bicone rheological cell used to probe the interfacial properties of chitosan / PFacid membrane. The interface was seeded with microparticles to visualize the velocity field of the interface with an immersed camera. (B) PTV at the water-oil interface, the color gradient shows the average velocity of tracers decreasing further when moving away from bicone.

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Figure 2: Representative sketch of multi-speckles Dynamic Light Scattering. (A) Chitosan / PFacid membrane was formed at oil-water interface in a cylindrical container. The interface was illuminated by a laser beam set to propagate inside the membrane. The light scattered at 90° was reflected by a polarization holding mirror and collected with a lens onto the camera sensor. (B) Example of an image taken by the CCD camera. In the center, the laser illumination path is clearly visible, showing the speckles. The red rectangle in the middle shows the part of the image used for auto-correlation calculus. (C) Stack representation of the 20th sub-ROI obtained by sequencing the red rectangle in B in equisized small images. (D) Intensity correlation function $g_2 - 1$ as a function of the lag time $\tau$ and the sub-ROI $z$.

Millipore Filter water system. CellMask™ Deep Red Plasma membrane stain was obtained from ThermoFisher. All chemicals and solvents used in this study were commercially available and used as received unless stated otherwise.

The aqueous solution was obtained by dissolving chitosan powder in Millipore water and carefully adjusting the pH with hydrochloric acid (1 mol/L) at 3.0 to obtain a solution of 0.1 % w/w. The chitosan solution was then filtered to remove undissolved particles through Minisart syringe-filters (pore size 5.0 mm). The viscosity of the 0.1 % chitosan solution was 8 mPa-s.

The 1 % w/w stock solution of PFacid was obtained by dissolving lecithin YN overnight in rapeseed oil (carefully stirred at 35°C ). Undissolved particles were removed by centrifugation at x1000g for one hour. The solution was diluted with rapeseed oil to obtain a concentration of PFacid ranging from 0.1 to 1 % w/w. The viscosity of these solutions was 62.6 mPa·s at 23 ± 1°C.

2.2. Interfacial rheometry

An interfacial rheological study of a flat film of chitosan / PFacid complex was performed with a bicone geometry using a commercially available solution (Figure 1), which is an appropriate approach for interfaces with high moduli and viscosities [26]. The Interfacial Rheology System (IRS , Anton Paar, Austria) was mounted on the Modular Compact Rheometer MCR 501 (Anton Paar, Austria) after being thoroughly washed with ethanol and Milli-Q water. For the interfacial measurements, the bicone geometry was positioned at the height $H_1 = 19.5$ cm from the bottom of the measuring cell after the zero-gap was established. Then the cell was filled with the aqueous phase until the normal force acting on the geometry was not adjusted to zero point in order to position the edge of the bicone geometry exactly at the interface. Next, the oil phase was gently added over the aqueous phase up to the total height $H = 40$ cm. Every measurement was performed in 3-4 minutes after two phases were brought into contact. All oscillatory measurements were performed for at least five time periods per data point. All the measurements were conducted at room temperature (23 ± 1°C).

The interfacial viscoelastic properties of the chitosan / PFacid membrane in oscillatory motion are described by
the frequency-dependant complex linear viscoelastic modulus $G_i^*$,

$$ G_i^*(\omega) = G_i'(\omega) + iG_i''(\omega) $$

where $G_i'$ and $G_i''$ are the components of the interfacial complex modulus (two-dimensional elastic modulus and loss modulus, respectively). It is related to the the complex interfacial viscosity $\eta_i^*$ as [26]

$$ G_i^*(\omega) = i\omega \eta_i^*(\omega) = -i\omega \eta_i''(\omega) + i\omega \eta_i'(\omega) $$

where $\eta_i''(\omega)$ is the out-of-phase shear viscosity and $\eta_i'(\omega)$ is the the dynamic interfacial shear viscosity. The contributions of the interfacial and bulk components to the torque appearing on the bicone geometry during its motion were compared through the non-dimensional parameter, the Boussinesq number ($Bo$)

$$ Bo(\omega) = \frac{\eta_i'(\omega) - i\eta_i''(\omega)}{a(\eta_b^{(1)} + \eta_b^{(2)})} $$

where $\eta_b$ is the bulk viscosity (superscripts denote upper and lower fluid respectively) and $a$ is the characteristic length scale that depends on the measuring system. As usual, the interfacial flow was considered to be decoupled from the bulk. In that case, the interfacial shear viscosity is calculated by [27]:

$$ \eta = \frac{M - \frac{8}{3} \pi R^3 \eta_b^{(1)} + \eta_b^{(2)}}{4\pi R^2 \Omega} $$

where $\Omega$ is the angular velocity (Figure 1 A). This expression is only relevant for the $Bo \rightarrow \infty$. For low and intermediate $Bo$ a complete analysis must be used, since the influence of the bulk phases becomes important [27]. In our experiments the interfacial response was decoupled from the bulk one by using the Anton Paar application software.

### 2.3. Particle tracking velocimetry

The displacements and velocity field on the oil-water interface during rheometric experiments were quantified through particle tracking velocimetry (PTV). For this purpose, the water-oil interface was decorated at low coverage with polyethylene microspheres (63-75 \( \mu \)m Cospheric LLC, USA) used as tracers. Less than 0.01% w/w of particle powder was added to 100 ml of oil phase and mixed thoroughly with a magnetic stirrer overnight. This volume of oil containing tracers was further used for rheological experiments as described above in the Section 2.2. The USB microscope (A1 USB Digital Microscope, Andonstar) was immersed in the oil phase during rheological experiments in IRS in order to visualize the displacement of microspheres under the shear flow. The image sequences were recorded at 20 frames per second and post-processed with a custom written particle tracking routine (MATLAB, MathWorks).

### 2.4. Dynamic light scattering

The dynamic evolution of the structure of the membrane was measured by space resolved Dynamic Light Scattering (DLS) at constant temperature $T$=22\(^\circ\)C. A sketch of the custom-built DLS set-up is shown in Figure 2. The oil-water interface, which later became a membrane, was illuminated by a vertically polarized laser beam produced by a single-mode laser (MSLIII, CNI, China, $\lambda$ = 532 nm). The laser beam had a diameter of 2 mm and was shaped by a combination of two lenses with focal lengths $f_1$ =200 mm and $f_2$ =-25.4 mm. The coherent light was scattered by forming solid matter at the oil-water interface. Only the light scattered at 90\(^\circ\) was collected, after reflection onto a non-polarized mirror. Focusing the laser beam on the interface was a complicated technical task, as the oil-water interface formed a concave-convex meniscus depending on the wettability of the cylinder. However the chitosan/PF acid complexation leading to the membrane formation resulted in a drastic decline in interfacial tension causing the interface to flatten. This led to the interface displacement along y-axis and consequently signal loss. In order to minimise this effect, all measurements were performed using a large custom-made glass cylindrical container positioned vertically.
and sealed underneath with a flat sheet of glass. The dimensions of the reservoir rendered the interface displacement negligible and the precise control of the sample volume ensured the tangential contact between the interface and the laser beam throughout the experiments.

In order to follow the structural evolution of the membrane, the scattered intensity was collected either with a CCD camera (acA640-100gm, Basler, Germany) or with a photomultiplier (SPCM-AQR-13, excilis Technologies, USA). When the camera was used, a lens with a focal length of $f_l = 150$ mm allowed the image of the scattering volume to form onto the CCD sensor. A diaphragm placed in the focal plane of the lens was set in order to optimize the size of the speckles to the pixel size of the camera [28]. For fast processes, the photomultiplier associated with a correlator (Flex03-LQ, Correlator.com, USA) was used to widen the dynamic range of acquisition to include lag times as small as $10^{-6}$ s.

Our approach enabled nondestructive probing of the interfacial membrane evolution with both spacial and temporal resolution, as long as the characteristic relaxation time of the studied system allows signal detection with a digital camera. The scattered light detected by CCD camera created the image of a coherence area known as speckle (Figure 2 B). The red rectangle in the center of the image represents the Region Of Interest (ROI), only this part of the image has been used for analysis. This area was sequenced into 20 sub-ROI (Figure 2 C). The individual time autocorrelation function of the scattered intensity $g_2(\tau) - 1$ was computed for each sub-ROI.

$$g_2(\tau, z) - 1 = \frac{\langle I^z(t)I^z(t+\tau) \rangle_t}{\langle I^z(t)^2 \rangle_t} - 1$$

where $I^z(t)$ is the intensity collected within $z^{th}$ sub-ROI and $\langle \ldots \rangle_t$ denotes averaging over time. Figure 2 D shows the result of the intensity correlation function as a function of the sub-ROI $z$ and the lag time $\tau$.

We also used the Time Resolved Correlation scheme (TRC) which allows DLS investigation of heterogeneous dynamics, as introduced by [29, 30, 31]. Analogously to $g_2(\tau) - 1$, the correlation degree $c_I(t, \tau, z)$ was calculated individually for each sub-ROI

$$c_I(t, \tau, z) = \frac{\langle I^z_p(t)I^z_p(t+\tau) \rangle_p}{\langle I^z_p(t) \rangle_p \langle I^z_p(t+\tau) \rangle_p} - 1$$

where $I^z_p(t)$ is the intensity measured at time $t$ for the $p^{th}$ pixel of an image within $z^{th}$ sub-ROI and $\langle \ldots \rangle_p$ denotes averaging over pixels.

2.5. Microscopy

Scanning electron microscopy (SEM) was used to characterize the morphology of chitosan / PFacid membrane at short complexation time. The membranes were grown on the surface of chitosan drops suspended in oil phase which contained PFacid. Once the required complexation time was achieved the droplets were washed in large quantities of cyclohexane (for more details see [17], [21]) in order to remove the oil with the residues of anionic surfactant. The chitosan droplets encapsulated with the membrane were placed on a cover slip and dried at room temperature. Dried chitosan / PFacid membrane were observed by scanning electron microscopy (SEM). Samples were coated with Au/Pd in a Baltec MED-020 sputter coater and observed in secondary electron mode in a Thermo Scientific Quanta 250 microscope equipped with a field emission gun and operating at 2.5 kV.

Confocal microscopy was used to characterize the morphology of chitosan / PFacid membrane at long complexation time. Analogously to the SEM characterization described above, the chitosan droplets were injected into oil phase containing anionic surfactant. Wet (no cyclohexane washing) chitosan / PFacid membrane were observed with Leica TCS SP8 scanning point confocal microscope equipped with a ×63 water immersion objective and in-plane image resolution 0.36 $\mu$m/px.

3. Results and discussion

3.1. Interfacial rheology of chitosan / PFacid membrane

We analysed the kinetics of formation of the membrane with a time sweep experiment at constant amplitude ($\dot{\gamma} = 0.03\%$) and frequency ($f = 0.5$ Hz). The choice of these parameters was justified by the need to keep the deformation...
within the linear viscoelastic regime while maintaining the torque as high as possible (see Figure SI 1). However, as explained below, the kinetics depended on the applied strain. Figure 3-A depicts the evolution of $G''(t)$ and $G''(t)$ over time for different concentrations of PFacid. As a control, a pure water-oil interface without membrane formation was also quantified (see Figure SI 2), which showed a constant $G''(t)$ of $\sim 10^{-3}$ N/m whereas $G''(t)$ was null. In the early stage of membrane formation ($t < 1 \text{ min}$ for 1% w/w PFacid and 10 min for 0.1% w/w PFacid), $G''(t)$ was almost constant and close to the system without PFacid. $G''(t)$ was out of the measurement sensitivity. In this regime, the interface manifested purely liquid-like properties. However, within a few minutes, a slow increment in $G''(t)$ was accompanied by a rapid growth of $G''(t)$. The interfacial storage modulus $G''(t)$ quickly overcame $G''(t)$, manifesting the prevalence of solid-like properties. After ten hours of complexation, both interfacial moduli increased on a roughly linearly basis. The atomic force microscopy measurements (see Supporting material) indicate that the chitosan/PFacid film thickness scales with time as $\sim t^{0.5}$. Considering that the long term linear development of the interfacial moduli suggests a nonlinear relation between interfacial moduli and the membrane thickness.

The time for which the interfacial elasticity $G''(t)$ growth abruptly, increased with the concentration of PFacid, Figure 3-A. Following the initial rapid transition, the growth rate of $G''(t)$ slowed down and increased linearly over time. The growth rate of interfacial elastic modulus also scaled on a linear basis with the concentration of PFacid, Figure 3-B.

To gain insight into the mechanisms at play in the early moments of membrane formation, creep experiments on the forming membrane were coupled to visualisation of the deformation of the interface by PTV (Figure 4). In these creep experiments, the bicone geometry was put into motion at fixed torque values and the deformation was measured. As the membrane was forming, the shear strain increased gradually until the geometry was brought to arrest. The evolution of the deformation varied with the applied torque, Figure 4-A. In analogy with percolation of particle laden interfaces [10], we termed the time at which the strain rate was null, the percolation time. The percolation time increased on a roughly a linear basis with the applied torque, Figure 4-B. Thus, the percolation process of the interface was coupled to the interfacial shear rate. The water-oil interface was decorated at low coverage with polyethylene microspheres ($\sim 70 \mu m$) used as tracing particles. The radial velocity profile $v$ of the interface was parabolic during the first few minutes of reaction, as expected for liquid interfaces. The spatio-temporal evolution of the velocity shows that the geometry slow-down was associated with the flattening of the velocity profile, Figure 4-C.D. Macroscopically, we observed that the shear rate tended towards zero in regions closed from the geometry ($r = 0$ and $R$). However, the velocity distribution was strongly heterogeneous before the arrest of the geometry. In fact, a closer look at the interface showed a constant formation and rupture of the membrane. We observed small patch-like sheet membranes that grew all over the interface and accumulated close to the geometry (see Supporting video). When the amount of membrane pieces was high enough to fully cover the interface, the interface jammed and stopped the motion of the geometry. This result fitted with non-reactive particle laden-interface for which domains of packed particles create elastic interfaces. When

**Figure 3**: Macroscopic study of the interfacial rheological properties of a chitosan / PFacid membrane. (A) Typical time-dependant evolution of interfacial elastic $G''(t)$ and viscous $G''(t)$ shear moduli at different concentrations of PFacid. $f = 0.5 \text{ Hz, } \gamma = 0.03\% \text{ Chitosan } 0.1\% \text{ w/w}$ (B) Following a long-term linear regime of membrane, the growth rate of interfacial elastic modulus $G''(t)$, as a function of anionic surfactant concentration roughly follows a linear law.
these domains start to break-up, a transition to viscous-like behavior was observed [32]. Finally, we concluded that the complexation of chitosan with PFacid is a two-step process. At short time scale, the interface has a macroscopic rheological behaviour which is characteristic of liquid interfaces, but the interface is strongly heterogeneous and is composed of solid patches. At a critical time, called the percolation time, the patches pave the interface and percolated, which is macroscopically characterized by a sudden increase of the interfacial elastic modulus $G'$ [32]. At long time scales, the thickness of the membrane grew, as $G'$ increased, by diffusion of one of the polyelectrolytes inside the membrane [15, 17].

3.2. Dynamic light scattering experiments

In order to shed light on the structural evolution of the interface formation, we employed DLS and TRC analysis [29, 30, 33], see Figure 2 for the experimental set-up. The interface was formed at the water-oil interface in a cylindrical reservoir via complexation between 0.1% w/w chitosan and 1% w/w PFacid, Figure 2. During the first 2 s, $C_f(r = 0, t)$ fluctuated randomly around a steady value of $\approx 10^{-2}$, Figure 5. Such behaviour corresponds to a Brownian system [29, 31], meaning that the displacement of particles between any two frames was on average the same, independently of the complexation time $t$. Beyond 2 s, the resolved correlation function drastically increased and gained half a decade in 100 s, meaning that the degree of correlation in the sample increased. This behaviour indicated the formation of a
Figure 5: Time Resolved Correlation (TRC) at 0 lag time ($\tau$) of building interface of the 20 ROIs. Straights lines are eye-guides and the circle at line interception indicates the starting time of the membrane gelation.

Figure 6: (A) Auto-correlation function of the interface computed from the mean time of TRC as at different lag times for an ROI as a function of the time from 0 to 240s. (B) Evolution of the relaxation times of the forming interface within different sub-ROI computed for two separate experiments at identical conditions. The colored areas separating two experiments serve as guiding lines.

More interestingly, DLS measurements were also resolved in space, which gave us insight into the formation of patches observed during rheological characterisation. The results of spatial analysis are depicted in Figure 6-B, where each dot corresponds to an ROI of $56\mu m \times 56\mu m$. Initially, the relaxation time at the interface was the same as that of the chitosan. As the chitosan / PFacid complexation took place and a solid matter started to appear at the interface, the relaxation time increased. At the different complexation times considered here, the relaxation times differed by nearly one decade between different ROI. It indicated high dynamic heterogeneity in the interface complexation. This
was consistent with the observation of patches during the interfacial rheological measurement. We concluded that interfacial complexation of both polyelectrolytes is a spatially heterogeneous process.

3.3. Scanning electron and confocal microscopy

The morphology of chitosan / PFacid membranes formed at relatively short time scales was observed by Scanning electron microscopy (SEM). In order to minimise harsh manipulations with fragile membranes, chitosan / PFacid membranes were grown on a surface of water droplets in oil phase containing chitosan and PFacid respectively, for more details see [21]. The complexation reaction was stopped by a gentle washing in large quantities of cyclohexane. After that, the droplets now enclosed by a solid membrane were placed on the glass substrate and dried prior the SEM imaging.

Figure 7 demonstrates the morphology of the chitosan / PFacid formed at 0.5 and 2 min of complexation time. The short time formed membrane was characterized by an important heterogeneity of its structure (Figure 7 A). The membrane appeared to be formed out of a large number of non-connected patches of 1-5 μm. Additionally, large non-circular holes up to 10 μm were found in the membrane. At 2 minutes of complexation the interface appeared to be fully formed, except for the presence of large circular holes (Figure 7 B). Figure 7 C shows the transversal view of the membrane, which was characterized by sub-micrometric aggregates.

The confocal imaging was carried out on the thick membrane after 48h of complexation in order to reveal the internal structure of the interface. Water droplets containing 0.1 % w/w concentration of chitosan were injected into the oil phase containing 0.1 % w/w concentration of PFacid. Water-soluble fluorescent dye with high lipid affinity (CellMask™ Deep Red plasma membrane stain, Invitrogen™) was added to the aqueous phase. This dye has little to no fluorescence in a free form and is only fluorescent once it is "anchored" to lipids. Figure 7 depicts the results of confocal imaging. These images show that the membrane was composed of nano-metric inclusions. Figure 7-right shows the concentration gradient of these inclusions from the oil phase towards the aqueous phase. As the fluorescent dye anchored to lipids, we deduced that this gradient of light intensity corresponds to a gradient of PFacid.

This set of microscopy images consolidated the idea that membrane formation was due to the percolation of individual patches. When the patches were able to form a percolated network, large holes were present. These results were reminiscent of interfaces covered by model nanoparticles which form heterogeneous structures with voids for low particle surface coverage [12, 11, 10]. The basic bricks are sub-micrometric aggregates of polyelectrolytes. On long time scales, the membrane was fully covered of these aggregates. In the thickness of the membrane, there was a negative gradient of these aggregates from the oil phase to the water phase. This last result supports the idea that the growth of the membrane was limited by the diffusion through a gel-like porous network of PFacid on long time scales, as described for H-bond donor / acceptor polymers [15].

4. Conclusion

Membrane formation based on the complexation between chitosan and short chain fatty acid has been used as a model for interfacial self-assembly of poly-electrolytes. A multi-scale approach was used in order to perform a characterisation of membrane formation and morphology.

The rheological properties of the forming membrane at macroscopic scale were probed by a combination of interfacial rheometry using the bicone geometry and particle tracking velocimetry of the interface. On short time scales, the interface had a fluid-like behaviour and was composed of non-connected solid patches. The elastic behaviour emerged when the patches percolated, similarly to a particle laden interface with an increasing surface coverage [13, 12, 10]. On long time scales, the surface elasticity increased almost linearly with time and concentration of the short chain fatty acid. This regime was reminiscent of a membrane growth limited by the diffusion of the smallest entity of both complexing molecules, as observed for polymer membranes obtained by h-bonding [9, 14, 15]. This finding was supported by confocal imaging showing a concentration gradient of short chain fatty acid through the membrane.

The membrane formation is a two step process. First the interfacial reaction takes place and is governed by the reactivity of the interacting species. Then the reaction takes place in the membrane ans is limited by the diffusion through the interfacial membrane. We employed several techniques in order the characterize these processes at different scales. At microscopic scales, we characterized the structure of the interface by space- and time- resolved DLS, used up to now in bulk for gels [33, 31, 29]. The method demonstrated that membrane formation was strongly heterogeneous in space and confirmed that was a two step process. On short time-scale, the speckles were strongly non-correlated, meaning that the interface had a fluid-like behaviour. On long time scales, the correlation of the speckles increased,
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Figure 7: SEM images of dried chitosan / PFacid membrane. (A) CH / PFacid membrane after 30s of complexation. The interface was made of individual patches of 1-50 μm size (p), as well as large holes (h). (B) CH / PFacid membrane after 2 min of complexation. The interface was homogeneous, with the exception of circular holes (h). (C) View in the thickness of the membrane after 2 min of complexation. The membrane showed a granular structure.

as a sign of the gelation of the interface. The strong spatial heterogeneity was consistent with a process of membrane growth due to the formation of nuclei and then their percolation to form a gel. The employed method allows the dynamic and non-destructive measurement of the forming film without stopping the reaction.

Some of the other types of interfacial polymerization reactions that proved to be very interesting for industrial applications (such as polyamide, polyurethane, polyurea and etc. membranes) were studied extensively [34]. For these reaction types the membrane growth was confirmed to take place in organic phase. We however observed the smaller anionic surfactant to diffuse through the membrane into the aqueous phase. SEM and confocal imaging confirmed the presence of microscopic patches and showed also that the basic bricks were sub-micrometric aggregates of polyelectrolytes. When the interface was fully covered with these aggregates, the membrane grew in its thickness by a diffusion-like process of the short chain fatty acid. This finding was supported by the growth of the elastic modulus with the concentration of PFacid and the gradient of PFacid in the thickness of the membrane.

Finally, this multi-scale experimental approach created a robust overarching picture of the interfacial complexation of polyelectrolytes. At short time scales, the growth is limited by the formation of "nuclei" which form patches. This work brings new elements on interfacial complexation that should prove useful to control the properties of liquid-liquid interfaces for the design of new materials, such as microcapsules or structured liquids.

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Figure 8: Confocal images of wet Chitosan / PFacid membrane after 48h of complexation. The membrane was marked by a fluorescent dye with lipid affinity (see text for details). Left: A piece of a membrane laying flat on a glass substrate showing a granularly patterned structure. Right: A horizontal confocal slice of a labeled membrane.

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6. Author contributions

Revaz Chachanidze: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft, Visualization Kailie Xie: Conceptualization, Methodology, Investigation, Writing - Review & Editing Hanna Masasad: Methodology, Formal analysis, Investigation, Writing - Original Draft, Visualization Denis Roux: Methodology, Formal analysis, Writing - Original Draft, Supervision Marc Leonetti: Conceptualization, Methodology, Writing - Original Draft, Supervision Clément de Loubens: Conceptualization, Methodology, Writing - Original Draft, Supervision

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