Syneresis and delayed detachment in agar plates

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Biogels made of crosslinked polymers such as proteins or polysaccharides behave as porous soft solids and store large amount of solvent. These gels undergo spontaneous aging, called syneresis that consists in the shrinkage of the gel matrix and the progressive expulsion of the solvent, which eventually leads to the gel detachment from its container. Here we report on the syneresis phenomena in agar plates that consist in Petri dishes filled with a gel mainly composed of agar. Direct observations and speckle pattern correlation analyses allow us to rationalize the delayed detachment of the gel from the sidewall of the Petri dish. The detachment time \( t' \) is mainly controlled by the gel minimum thickness \( \epsilon_{\text{min}} \) along the periphery of the plate: \( t' \) increases as a robust function of \( \epsilon_{\text{min}} \) that neither depends on the age of the gel nor on any previous mass loss. Time-resolved correlation spectroscopy reveals that the speckle decorrelation rate increases a few hours before \( t' \) and that the gel detachment can be anticipated. This work provides quantitative observables to predict the shelf life of agar plates and highlights the key role of the competition between the syneresis and the gel adhesion to the wall in the detachment process.

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I. INTRODUCTION

Biogels formed through the self-assembling of polymers such as polysaccharides or proteins are widespread in manufactured goods and biomimetic products [1, 2]. Fields of applications range from food engineering where biopolymers are used as gelling agents [3], to biotechnology where these gels commonly serve as growth media for microorganisms and as porous scaffold in tissue engineering [4, 5]. Biogels exhibit a porous microstructure made of an interconnected network that is a priori efficient to retain solvents. However, these structures are often metastable. Indeed, the constituents experience attractive interactions and biogels spontaneously rearrange and shrink on durations ranging from hours to months depending on the ambient relative humidity, leading to the progressive release of the solvent initially trapped. This phenomenon coined syneresis has been reported in biogels such as gelatin [6], polysaccharide gels [7], globular protein gels [8–10], organogels [11] and hydrogels from pNIPAM microgels [12], and more generally in colloidal gels that display weak attractive interactions [13, 14]. If the latter category of gels has been the topic of numerous studies, only a handful of paper have reported quantitative measurements on the shrinkage dynamics of biogels, while the parameters controlling the detachment of a biogel from the preparation container stands as an open issue despite its outstanding practical importance.

Here we report on the syneresis process, the detachment and the subsequent shrinking of the gel in commercial agar plates used in routine diagnostic testing as growth media for microorganisms or cells. These plates are usually incubated at constant temperature for several hours and excessive syneresis leads to the gel detachment from the sidewall of the Petri dish which makes impossible the bacterial count and invalidates the test. Such a simple issue delays each year the analysis of thousands of diagnostic and costs a fair share of money to pharmaceutical companies due to customer return. The gelling constituent of these plates is agarose, a hydrophilic colloid extracted from seaweeds and among the oldest polysaccharide used by man [7], which formation and structural properties have been thoroughly investigated over the past 40 years [15–17]. Insoluble in cold water, agar becomes soluble in boiling water and, once cooled down below 40°C, forms a thermoreversible gel that does not melt below 80°C. For concentrations above 1 % (w/w) as it is the case for agar plates, the gel is formed through a competition between a spinodal demixing process and the association of molecules in double helices [18–21]. This process leads to a fibrous network structure which final properties are strongly influenced by the agarose concentration [22], the quench temperature [23] and the quenching rate [24]. The mechanical properties of agar gels depend on both the interchain association and the nature of the solvent [25]. In the linear deformation regime, agar gels behave as soft solids and exhibit a constant and frequency-independent elastic modulus \( G' \) much larger than the viscous modulus \( G'' \) [26]. Under larger strains, agar gels display a brittle-like rupture scenario including macroscopic fractures, while considerable creep occurs under external stress before a delayed rupture [27]. Agar gels are also subject to ageing together with the spontaneous release of water. Such syneresis phenomenon is attributed to the contraction of the polymer network by a slow further aggregation of helices [28, 29] and is enhanced under external stress [7], or water-unsaturated conditions. Syneresis in agar gels has

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Here we report on the syneresis process, the detachment and the subsequent shrinking of the gel in commercial agar plates used in routine diagnostic testing as growth media for microorganisms or cells. These plates are usually incubated at constant temperature for several hours and excessive syneresis leads to the gel detachment from the sidewall of the Petri dish which makes impossible the bacterial count and invalidates the test. Such a simple issue delays each year the analysis of thousands of diagnostic and costs a fair share of money to pharmaceutical companies due to customer return. The gelling constituent of these plates is agarose, a hydrophilic colloid extracted from seaweeds and among the oldest polysaccharide used by man [7], which formation and structural properties have been thoroughly investigated over the past 40 years [15–17]. Insoluble in cold water, agar becomes soluble in boiling water and, once cooled down below 40°C, forms a thermoreversible gel that does not melt below 80°C. For concentrations above 1 % (w/w) as it is the case for agar plates, the gel is formed through a competition between a spinodal demixing process and the association of molecules in double helices [18–21]. This process leads to a fibrous network structure which final properties are strongly influenced by the agarose concentration [22], the quench temperature [23] and the quenching rate [24]. The mechanical properties of agar gels depend on both the interchain association and the nature of the solvent [25]. In the linear deformation regime, agar gels behave as soft solids and exhibit a constant and frequency-independent elastic modulus \( G' \) much larger than the viscous modulus \( G'' \) [26]. Under larger strains, agar gels display a brittle-like rupture scenario including macroscopic fractures, while considerable creep occurs under external stress before a delayed rupture [27]. Agar gels are also subject to ageing together with the spontaneous release of water. Such syneresis phenomenon is attributed to the contraction of the polymer network by a slow further aggregation of helices [28, 29] and is enhanced under external stress [7], or water-unsaturated conditions. Syneresis in agar gels has
been reported in several occasions, but the current state of art is limited to empirical laws concerning the influence of the gel composition or the total amount of exuded solvent. Syneresis in agar gels goes roughly as the inverse square root of the polymer concentration, while tuning the internal hydrophobicity of the gel by incorporating water-binding components such as sucrose, xanthan, or locust bean gum may delay and/or prevent part of the water release. Moreover, studies on syneresis mostly focus on mass loss or change in the gel shape. To our knowledge, the influence of the competition between the water release and the gel adhesion to the wall upon the gel detachment scenario from its preparation container has received very little if no attention.

In this article, we report on the syneresis and the delayed detachment dynamics of agar-based gels in plastic Petri dishes as pictured in figure 1. The detachment is named delayed as it may occur from a few hours up to 30 hours, from the moment the plates are opened and incubated at constant ambient temperature. Here, we discuss simple experimental techniques that could be easily implemented in an industrial environment, and which consist in direct visualization and time-resolved correlation spectroscopy experiments. These techniques allow us to analyze the gel dynamics before the detachment from the lateral wall of the Petri dish, and to identify the key parameters controlling the detachment time $t^*$. Surprisingly, $t^*$ does not scale as a simple function of the mass loss during the syneresis. The delay prior to the detachment is strongly correlated to the asymmetry in gel thickness along the periphery of the plate, and increases as a robust power law of the gel minimum thickness $e_{\text{min}}$. Such simple quantities constitute promising macroscopic observables to estimate and optimize the shelf life of commercial plates. Moreover, time-resolved correlation spectroscopy experiments demonstrate that the gel dynamics, which is somewhat coupled to the plastic dish deformability, increases in a systematic way when approaching $t^*$, and makes it possible to anticipate the gel detachment from a few hours.

II. MATERIALS AND METHODS

A. Agar plate samples

The samples consist in gamma-irradiated sterile agar plates (Fig. 1) commercialized by BioMérieux as microbiological growth media. Plates are made of a cylindrical plexiglas® box (diameter 8.5 cm, height 1 cm) covered with a removable lid and are partially filled with an agar gel (1.5 % w.t.) containing nutrients for bacterial growth that include peptones, sodium salts, etc.

As a first key observation, one can notice that the gel thickness is not homogeneous, especially along the edge of the Petri dish. This point is of primary importance for the dynamics of the gel detachment from the dish as will be discussed in section IIIA. Prior to any use, each plate is weighted to determine the mass $m$ of gel it contains, and the gel thickness $e(\theta)$ along the periphery of the Petri dish wall is measured by means of a webcam (Logitech HD c920) with an accuracy of ±30 µm. In particular, we record the gel minimum and maximum thicknesses, resp. $e_{\text{min}}$ and $e_{\text{max}}$ (Fig. 2). For the samples investigated here, the gel mass $m$ ranges from 21 to 27 g, while the amplitude of the thickness variations $\delta e \equiv e_{\text{max}} - e_{\text{min}}$ lies between 0.3 and 1.4 mm for a typical average thickness of about 4 mm. Both the gel weight $m$ and thickness $e(\theta)$ depend upon the casting process of the gel in the Petri dish on the production line, and therefore are not control parameters in this study. This is why we have performed experiments on a large number of plates to sample different values of $m$ and $e$. As a result of water exudation and evaporation, $m$ and $e$ progressively decrease up to the point the gel detaches from the lateral wall of the dish and retracts (Fig. 1) which marks the end of the product shelf life, as the growth media has to fill entirely the dish to be marketable. Agar plates are commercialized by batches of 10 plates wrapped together. To test the reproducibility of the results, each

FIG. 1. Commercial agar plate from a fresh batch (left) and after a 24h incubation at 25°C (right). The gel has detached from the sidewall on the right side of the dish. The scale is set by the dish diameter of 8.5 cm.

FIG. 2. Panoramic 360° sideview of a commercial agar plate ($m = 26$ g). The image is built by rotating the plate in front of a webcam and stacking consecutive vertical snapshots recorded at regular angle intervals. The representation $e(\theta)$ illustrates the variations of the gel thickness $e$ along the periphery of the Petri dish ($e_{\text{min}} = 3.58 \pm 0.03$ mm and $\delta e = e_{\text{max}} - e_{\text{min}} = 1.02 \pm 0.03$ mm). The image height is about 800 pixels and the black inscription is the plate serial number.
experiment is repeated on several independent batches, which exact number is given in the text.

B. Direct visualization

Direct visualization of the plate evolution submitted to syneresis is performed with a webcam (Logitech HD c920) used in timelapse mode with a frame rate of 1 image per minute, and placed in a programmable testing chamber (Binder MK53) that maintains a constant temperature of \((25.0 \pm 0.1) ^\circ C\) and a relative humidity of about 50%. For each batch, the 10 plates are stored at the same level inside the chamber so as to experience similar temperature and humidity conditions, while plate evolution is monitored over several hours (up to \(\sim 40\) hours).

C. Time correlation spectroscopy

The gel dynamics is monitored by speckle pattern correlation in a room thermostated at \((25.0 \pm 0.1) ^\circ C\) and a humidity level of about 50%. The optical setup pictured in figure [3] consists in a linearly polarized laser beam (He-Ne Melles Griot gas laser, 20 mW at \(\lambda =632.8 \) nm) that is expanded to 5 mm with a collimator and directed perpendicularly to the sample by means of a mirror. The light is backscattered from the center region of the plate (illuminated volume \(\sim 100 \) mm\(^3\)) and forms a speckle pattern on a low sensitivity CCD array (Webcam Phillips SPC900NC, 640×480 pixels) an example of which is represented in the inset of figure [3]. To remove the ambient light, an interference filter is placed in front of the detector and for each experiment, the angular position of the plate and the diaphragm aperture are tuned to spread the intensity range over the whole accessible grayscale of the detector, and obtain an autocorrelation radius (\(\sim\) speckle grain radius) of about 3 pixels. We have checked that the results reported here do not depend on the exact position of the enlightened volume in the sample.

As water evaporates from the gel due to the gel syneresis, the speckle pattern changes. The degree of correlation between two speckle images taken at a lag time \(\tau\) is determined by the ensemble-average intensity correlation function \(g_2(t, \tau)\) defined as follows:

\[
g_2(t, \tau) = \frac{\langle I_p(t) \cdot I_p(t+\tau) \rangle_p}{\langle I_p(t) \rangle_p \cdot \langle I_p(t+\tau) \rangle_p}
\] (1)

where \(I_p\) denotes the brightness level of pixel \(p\) and \(\langle \ldots \rangle_p\) an ensemble average over all the pixels \([35]\). The correlation function is further normalized into a function noted \(g_2^*(t, \tau)\) that verifies the condition \(g_2^*(t, \tau = 0) = 1\).

Here, the quantity \(g_2^*(t, \tau)\) is computed at the maximum frame rate of 10 Hz during the lag time \(\tau = 1\) min. For \(\tau > 1\) min, the correlation function \(g_2^*(t, \tau)\) is computed only every minute. Speckle patterns are processed in real time by means of a custom-made java plug-in with the NIH Image processing package [36], and speckle images are only saved every minute for a lag time \(\tau = 0\) to avoid storing too large amount of data [38].

Since the gel is weakly scattering the incident light, the speckle pattern is mainly produced by the interference of a light beam initially polarized, that is either reflected

![FIG. 3. Photo of the optical setup used to record the speckle pattern from the agar plate in the backscattering geometry. Inset: typical speckle pattern.](image)

![FIG. 4. (a) Lag-time temporal diagram of the intensity correlation function \(g_2^*(t, \tau)\) as a function of the lag time \(\tau\), and the experimental time \(t\). The first half of the experiment is performed with parallel polarizers (\(t < 6.3\) hours) and the second half of the experiment with crossed polarizers (\(t \geq 6.3\) hours). The detachment of the gel from the sidewall of the dish occurs over longer time at \(t = 16\) hours (data not shown). (b) Intensity correlation function \(g_2^*\) vs. the lag time \(\tau\) extracted from (a) at \(t = 4.5\) hours (parallel configuration) and at \(t = 8.5\) hours (crossed configuration) and averaged over a time window of \(\Delta t = 30\) min [yellow dotted lines in (a)]. The gel characteristics are the following: \(m = 26\) g, \(\delta_{min} = 3.74\) mm and \(\delta_e = 0.75\) mm.]
by the air/gel interface and/or by the gel/dish bottom interface. As the Petri dish is made out of a birefringent material, the polarization of the light reflected by the gel/dish bottom interface is modified, whereas the polarization of the light reflected by the air/gel interface is unchanged. Therefore, an analyzer placed in front of the detector allows us to select the photons collected by the webcam (Fig. III A) and thus to access different dynamics of the system. To illustrate this point, we have conducted a Time Resolved Correlation (TRC) experiment which first half (second half resp.) is performed in parallel (perpendicular resp.) configuration.

In the parallel configuration, the speckle results from the interference of photons coming from both the air/gel and the gel/dish bottom interface. The correlation function $\rho_s^P$ reported in figure III b decreases rapidly over a timescale $\tau_d \simeq 5$ s and further displays a periodic modulation $\tau_M$ of about 10 s that is clearly visible in the lag-time temporal dynamics of the correlation function [Fig. III a), for $t < 6.3$ h]. Both phenomena are related to the water evaporation which induces the gel thinning which in turn modifies the length of the optical path lengths. On the one hand, the rapid decorrelation is due to the change of the optical path lengths of photons that are reflected at the air/gel interface (shortest timescale). Indeed, for a constant evaporation rate $\dot{m} = 0.7$ g/hour at 25°C (see table 1 in section III A), the speckle decorrelates for a change in the optical path length of $\lambda/2$, over a duration $\tau_d$ that writes $\tau_d = \lambda \rho \pi R^2 / (4 \dot{m})$ where $\rho$ stands for the density of water ($\rho = 1000$ kg/m$^3$) and $R$ is the gel radius ($R = 4.2$ cm). The numerical application leads to $\tau_d \simeq 4.5$ s in agreement with figure III b). On the other hand, the modulation of the correlation function results from a periodic change in the optical path length difference of photons that go through the gel and are reflected at the bottom of the plate. Under this assumption, the modulation time reads $\tau_M = \lambda \rho \pi R^2 / [4(n-1)\dot{m}]$, where $n$ stands for the refractive index of the gel very close to that of water ($n \simeq 1.33$). The numerical application leads to $\tau_M \simeq 15$ s in agreement with figure III b).

In the perpendicular configuration, only the light backscattered from the bottom of the dish contributes to the far-field speckle pattern. The correlation function decreases therefore over a lag time of a few minutes, longer than in the parallel configuration [Fig. III b)]. Only a change in the path length distribution of photons reflected from the bottom plate may cause a speckle decorrelation as a result of a non affine (or non linear) deformation of either the illuminated air/gel and the gel/dish interface. The gel, which contracts as it releases water may indeed experience lateral displacements or exert stresses on the deformable Petri dish. Such effects reflect in the lag-time temporal diagram of the correlation function that displays an intermittent dynamics [Fig. III a), for $t > 6.3$ h] up to the moment gel detaches from the plastic dish (not shown in Fig. III), as further discussed in section III B. We have checked that the use of a rigid vessel (e.g. a Petri dish made of glass) strongly dampens the time fluctuations of the speckle pattern [see Fig. 1 in the supplemental material], which confirms the influence of the deformable plastic Petri dish upon the speckle dynamics. TRC therefore allows to monitor the coupled dynamics of the gel and the deformable Petri dish in perpendicular configuration and to determine the evaporation rate through the modulation of the correlation function in parallel configuration. More can be learned as discussed in section III B.

### III. RESULTS

#### A. Macroscopic approach

In a first series of experiments, a batch of 10 plates without their lids is placed in a thermoregulated chamber ($T=25^\circ$C) and left at rest. Water evaporates from the agar plates and the gels stay still for several hours before suddenly detaching from the sidewall of the Petri dish at a time noted $t^*$ [See movie 1 in the supplemental material]. After detaching, the gel shrinks and although the gel may lose up to 40 % of its initial weight during the syneresis, we observe no sign of failure or fracture at any time. Note that, pushing the experiments forward,
the gel turns into a thin and dry buckled pancake that can be rejuvenated by adding water. The gel then swells and recovers its initial cylindrical shape, occupying the whole Petri dish after a few hours (data not shown).

To identify the control parameters of the gel detachment during the early stage of the syneresis, we report in figure 6 the evolution of the gel detachment time versus the gel initial characteristics. As a key result, $t^*$ is observed to increase as a power law of the gel minimum thickness $\varepsilon_{\text{min}}$, measured along the periphery of the Petri dish [Fig. 6(a)]. This relation is robustly verified over more than 50 plates of different initial weights and mass loss history. By contrast, the detachment time is not correlated to the gel maximum thickness [Fig. 6(b)], but increases with the mass of the gel. Nonetheless, if the data obtained with a single batch can be described with a single increasing function of $m$, two gels with the same initial mass but taken from different batches may detach over very different timescales [Fig. 6(d)]. This last observation is likely related to the fact that different batches have somewhat different thermal and mechanical history. Plates may have been exposed to different storage conditions and the gels may have lost various amount of water since their production. Finally, the detachment time also appears to be correlated to the amplitude of the variations of the gel thickness quantified by $\delta e = \varepsilon_{\text{max}} - \varepsilon_{\text{min}}$, but to a lesser extent, the data being more scattered than for $t^*(\varepsilon_{\text{min}})$ [Fig. 6(c)]. One should nonetheless keep in mind that gels presenting larger variations in thickness at the periphery of the plate usually detach sooner from the Petri dish wall.

To further quantify the water release, we monitor the weight at regular intervals for Petri dishes stored in the thermoregulated chamber, and we note the gel detachment time for each dish. The experiments is repeated on six agar plates at $T=20^\circ\text{C}$. The mass loss, quantified by $\delta m \equiv m(t = 0) - m(t)$, is found to increase linearly with time, while the gel detachment is neither directly correlated to the mass loss nor affects the mass-loss rate (Fig. 6). We have repeated the experiments for two other temperatures ($T=30^\circ\text{C}$ and $40^\circ\text{C}$) comparable to the temperatures at which the agar plates are incubated during their commercial use. We obtain very similar results, and larger temperatures leads to larger loss-rates (Table I).

Last but not least, we have followed the temporal evolution of the gel thickness during the early stage of the syneresis, up to the detachment (see Fig.2 in the supplemental material). For $t \leq t^*$, the average height of the gel decreases linearly in time in agreement with the evolution of $\delta m(t)$ reported in figure 6. The thickness asymmetry $\delta e$ is observed to remain about constant up to the detachment and advected downwards while the gel average thickness diminishes. This last result strongly suggests that the friction of the gel on the sidewall of the dish plays an important role on the delayed detachment process.

### B. TRC study of the syneresis

To get deeper insights on the syneresis process, we performed a second series of diffusion light scattering experiments in crossed polarizer configuration. An agar plate is placed in the custom made optical setup described in section II C (at $t = 0$) and left at rest under the laser beam at constant temperature ($T=25^\circ\text{C}$). The speckle pattern formed by the backscattered light changes as the gel releases water. The gel dynamics is quantified by computing the intensity correlation function $g_2^\perp(t, \tau)$ over a lag time $\tau = 1 \text{ min}$, during 10 to 30 hours.

Fig. 7(a) shows for one plate the lag-time temporal diagram where the correlation data $g_2^\perp(t, \tau)$ is coded in grayscale. It is clear that $g_2^\perp(t, \tau)$ exhibits two different regimes that point toward a dramatic event at a specific timescale $t = t^*$ [red dashed line in fig. 7]. To compare this timescale to the detachment time measured in direct visualization experiments, we have carefully repeated the experiment for twenty agar plates from two batches, and the time $t^*$ determined through TRC analysis is systematically reported vs. $\varepsilon_{\text{min}}$ in figure 5. All the data points fall on the exact same power-law dependence as found

| Temperature ($^\circ\text{C}$) | $\delta m$ (g/hour) |
|-----------------------------|---------------------|
| 20                          | 0.50 ± 0.02         |
| 30                          | 0.86 ± 0.03         |
| 40                          | 1.81 ± 0.08         |

**TABLE I.** Mass loss rates $\delta m$ measured as detailed in figure 6 for three different temperatures $T$. Each value is the result of a linear fit of $\delta m(t)$ for 6 different plates, extracted from three different batches.
in section IIIA which demonstrates that the time \( t^* \) derived from TRC analysis coincides with the detachment time identified during a direct visualization experiments. Such an interpretation is further supported by an experiment performed on a plate which gel has been carefully detached from the sidewall of the dish by means of a cutter blade shortly before the start of the experiment (Fig. 8). The correlation function displays a modulated dynamics similar to the one reported for a standard gel after it has detached [Fig. 7a] for \( t > t^* \). Therefore, in figure 7 the evolution of \( g_{2,1}^{\perp}(t, \tau) \) for \( t \leq t^* \) illustrates the intermittent dynamics of the gel and the Petri dish prior to the detachment. The speckle, which decorrelates over a timescale that strongly varies from one acquisition period to the next, results both from the local displacement of the gel on the dish bottom, and from the deformation of the plastic Petri dish under the stress exerted by the gel that contracts due to the water loss. On the contrary, for \( t > t^* \), a fraction of the gel is no longer in contact with the sidewall of the Petri dish, and the lag-time temporal diagram is radically different: \( g_{2,1}^{\perp}(t, \tau) \) displays a periodic modulation of about 20 s along the lag time axis which is the signature of the progressive thinning of the gel. Note that the modulation period is slightly larger than in the case discussed in section II C. This is due to the fact that here, the gel is detached from the sidewall of the dish: the lateral shrinking of the gel partially compensates its thinning, which leads to a smaller apparent thinning-rate and thus a longer modulation time.

Let’s turn now to the gel dynamics before the detachment and have a closer look to \( g_{2,1}^{\perp}(t, \tau) \) for \( t < t^* \) [Fig. 7b]. As mentioned in section II C in crossed polarizers configuration, the speckle dynamics results from the gel displacement inside the dish which is due to the water loss, and to the deformation of the plastic Petri dish under the stress exerted by the gel, which in turn leads to the gel motion. The water release drives the speckle dynamics only at short lag times, while the dynamics becomes more complex over longer lag times [see Fig. 3 in the supplemental material]. Therefore to access the sole dynamics of the gel, we focus on the temporal evolution of \( g_{2,1}^{\perp}(t, \tau) \) for \( \tau \leq 30 \) s. We have computed for three different plates, the probability distribution function (PDF) of \( g_{2,1}^{\perp}(t, \tau) \) pictured in semi-logarithmic scale in figure 8a at \( \tau = 0.5, 2 \) and 10 s. The PDF remains nearly gaussian for short lag times \( \tau \leq 0.2 \) s, while \( g_{2,1}^{\perp}(t, \tau) \) explores smaller values for increasing values of the lag time, and the distribution \( P(g_{2,1}^{\perp}) \) develops an exponential tail [Fig. 8a]. The data to the right of the maximum are fitted by a Gaussian function which center \( \langle x \rangle \) and variance \( \sigma \) are reported in figures 8b and 8c for twenty plates. Both parameters are independent of the detachment time of the gel which demonstrates that the gel dynamics that takes place during the first hours of the syneresis is not directly related to the detachment process. This last result suggests that the width of the gaussian distribution is related to the relative motion of the gel within the dish, at local contact spots. Such small scale motion is thermally-induced and a priori reversible. Progressive water evaporation does not act as a source...
of thermal-like noise and allows gel motions of larger amplitude leading to an increase of $\sigma$ with the lag time $\tau$. In this framework, the growth of a non Gaussian tail to the distribution of $g_2^\perp$ for $\tau \geq 2$ s is related to irreversible motions of the gel relative to the dish in a few local contact areas, and that are likely to redistribute stresses through the gel [39]. The amount of these irreversible displacements, or plastic events, during the lag time $\tau$ can be assessed by computing the fraction of data points $\delta$ in the PDF $P(g_2^\perp)$ that lays outside the gaussian fit. One can see in the inset of figure III(a) that $\delta$ neither depends on the plate nor on the gel detachment time, but increases with the lag time and reaches $\sim 60\%$ at $\tau = 10$ s. This timescale should be compared to the gel dynamics after it has detached from the sidewall of the dish. The speckle indeed becomes fully decorrelated in about 10 s [see Fig. II(a)] which confirms that water evaporation can trigger irreversible displacements that are measurable over such timescale.

Finally, as the last but not the least remarkable feature of the lag-time temporal diagram pictured in figure II(a), one can see that the decorrelation rate of the speckle pattern strongly increases roughly two hours before the gel detachment. To quantify such a phenomenon, we have computed a decorrelation time defined as the lag time $\tau^*$ for which $g_2^\perp(t, \tau^*) = g_2^\perp(t, 0)/e$. The decorrelation time $\tau^*$ is plotted in figure II(b) as a function of the elapsed time $t-t^*$ for three plates with very different detachment times ranging from 6 to 14 hours. For $t^*-t \gtrsim 2.5$ h, $\tau^*$ exhibits large fluctuations around a constant mean value of about 0.7 min in agreement with the facts that the evaporation rate is constant and the gel thickness decreases linearly in time (section IIIA). For $t^*-t \lesssim 2.5$ h, $\tau^*$ decreases as a robust power-law that scales as $(t^*-t)^{0.75}$ independently of the detachment time [inset of Fig. II(b)]. Such scaling, strongly reminiscent of the finite time singularity reported for the breakup of droplets [41], could be the signature of the fission of the liquid film that separates the gel from the wall during the detachment.

Indeed, we checked by means of a homemade compression cell that small deformations of about 3-5% are sufficient to trigger the release of water from the gel. Therefore, one can easily imagine that a thin film of water may form and expand between the gel and the sidewall as a result of the progressive stress build up during water evaporation. This last point certainly deserves more investigation.

IV. DISCUSSION AND OUTLOOKS

A. Discussion

Let us now describe the syneresis dynamics as a whole. Agar plates experience syneresis which leads to the delayed detachment of the gel from the sidewall of the dish. The gel releases water that evaporates at a constant rate that is not affected by the gel detachment (Fig. II) and which value increases for increasing storage temperatures (Table I). The gel thickness decreases linearly up to the
moment it detaches from the dish at the exact angular location where the gel thickness was minimal at \( t = 0 \). This results is remarkable as it offers a simple observable to predict the detachment time of a gel and therefore the shelf life of the plate. As demonstrated by TRC experiments, the detachment process starts a few hours before the gel loses contact macroscopically with the sidewall of the dish. The gel releases water and thins, but due to the weak adhesion of the gel to the dish, the detachment is delayed. The stress inside the gel builds up which leads to an increasing amount of local contact junctions where the gel and the sidewall of the plate experience relative displacements so as to relax the stress. One could also imagine that the stress generated by the water loss could induce larger displacements always localized in the same contact areas as time goes by. However, since the fluctuations experienced by the gel before the detachment are independent of \( t^* \) (Fig. 9), it is more likely that the number of contact spots where relative motion takes place increases with time. Eventually, the gel detaches from the sidewall where the force exerted by the solid boundary is minimal, i.e. where its thickness is minimal. As a consequence, the detachment time is strongly correlated to the gel minimum thickness [Fig. 5(a)]. Our work thus puts forward the subtle interplay between the gel syneresis in open air and the gel adhesion to the dish contrary to previous contributions on syneresis which involve gels embedded in solvents of different nature and free to contract.

B. Open questions

We have shown that the dynamics of the speckle pattern formed by the light backscattered from the gel contains valuable information on the gel displacement along the Petri dish wall. Nonetheless, the link between the friction properties between the gel and the bottom of the dish, and the dynamics of the speckle pattern remains to be studied in detail. In particular, the quantitative role of the surface-roughness and wetting properties of the bottom plate on the scaling of the detachment time stands as an open issue. Future experiments will involve interferometry and Photon Correlation Imaging as described in [40] to map the gel displacement near the Petri dish lateral walls close to the detachment time.

V. CONCLUSION

We have described the slow aging dynamics taking place in agar plates incubated under constant temperature. The water release is a continuous process during which the gel eventually detaches from the sidewall of the dish, and shrinks. The detachment occurs sooner for plates that have experienced larger water loss or that display significant asymmetry with thickness variations of larger amplitude. More quantitatively, the detachment time scales as a robust function of the gel minimal thickness, which is therefore an excellent candidate to predict the shelflife of commercial plates, provided that the relation between the detachment time and the minimal gel thickness has been previously established for the type of plates and gels under scrutiny. To our knowledge, this study is among the first to use a time-resolved correlation method to probe the simultaneous effect of water exudation and gel contraction during the syneresis process. It paves the way for the use of TRC in an industrial context and more generally as a powerful tool to monitor the spontaneous or stress induced aging dynamics in poroelastic soft media.

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Note that gamma irradiation is performed after gelation to sterilize the plates. To our knowledge, there are no studies dealing with the effect of post-gelation irradiation on the mechanical properties of agar gels. Nonetheless, note that irradiation of agar prior to gelation is known to impact the gel mechanical properties by lowering the gel failure stress in comparison with non-irradiated samples.

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