PRL **99,** 178102 (2007) PHYSICAL REVIEW LETTERS week ending 26 OCTOBER 2007

Vesicle Dynamics in Time-Dependent Elongation Flow: Wrinkling Instability

Vasiliy Kantsler, Enrico Segre, and Victor Steinberg

Department of Physics of Complex Systems, Weizmann Institute of Science, Rehovot, 76100 Israel

(Received 30 April 2007; published 26 October 2007)

We present experimental results on the relaxation dynamics of vesicles subjected to a time-dependent elongation flow. We observed and characterized a new instability, which results in the formation of higher-order modes of the vesicle shape (wrinkles), after a switch in the direction of the velocity gradient. This surprising generation of membrane wrinkles can be explained by the appearance of a negative surface tension during the vesicle deflation, which tunes itself to alternating stress. Moreover, the formation of buds in the vesicle membrane was observed in the vicinity of the dynamical transition point.

DOI: 10.1103/PhysRevLett.99.178102 PACS numbers: 87.16.Dg, 47.60.+i, 87.17.Jj

Giant unilamellar vesicles, formed by a closed phospholipid bilayer, appear to be a well-defined simplified system for studying physical aspects of the dynamics of biological cells. Equilibrium mechanical properties of vesicles are relatively well understood. The nonequilibrium dynamics of a vesicle subjected to an external flow received intensive attention in numerous theoretical [1–5], numerical [6–9], and experimental [10–15] studies. In a shear flows a vesicle exhibits several types of motion—tank treading, tumbling, and trembling [12,13] (also called vacillating breathing [3] or swinging [9])—depending on its location in the space of the system control parameters (viscosity contrast, excess area, shear rate) [5,9,13].

There is, however, a lack of experimental observations in the regime of transient dynamics when the system undergoes a nonequilibrium relaxation toward one of its dynamically stable states. In stationary shear flows only the lowest order modes (usually just the second order modes) characterize the various dynamical states of the vesicles observed, since the energy contribution from higher-order excitation modes, if present, would be much higher. However, as pointed out recently [16], a time-dependent flow may happen to impose a negative surface tension on the membrane, which leads to the growth of the higher-order modes and to a shape instability.

Let us consider the Helfrich free energy functional in a general form [17]:

$$F = \int dA\left[\frac{\kappa}{2}h^2 + \sigma\right], \tag{1}$$

where κ is the bending rigidity of the membrane, h is the local curvature, and σ is the vesicle surface tension, which is the Lagrange multiplier corresponding to the surface area conservation constraint. If we consider for simplicity just a flat membrane, which can be parametrized by a height function $u(x, y)$, then the expansion of the functional F in the Fourier space up to second order in u gives: $F^{(2)} = \frac{1}{2}\sum_k(\kappa k^4 + \sigma k^2)|u_k|^2$. One notices that modes u_k with $k < \sqrt{|\sigma|/\kappa}$ become unstable for $\sigma < 0$, resulting in the generation of the higher-order modes becoming energetically more favorable. One possibility to experimentally realize a negative surface tension of the vesicle membrane is the use of a time-dependent flow, where the sign of the velocity gradients undergoes a fast change, under which a vesicle becomes temporarily deflated. The simplest realization of this idea is a plane elongation (hyperbolic) flow: $v_x = \dot{\epsilon}x$, $v_y = -\dot{\epsilon}y$, $v_z = 0$.

In this Letter, we present the first experimental study of giant vesicle dynamics in such time-dependent, transient plane hyperbolic flow. We study the vesicle relaxation towards a new stationary state in two cases: from an equilibrium state when the elongation flow is suddenly turned on, $\dot{\epsilon}(t) = H(t)\dot{\epsilon}_0$, where $H(t)$ is the Heaviside step function, and when the elongation flow is suddenly reversed, $\dot{\epsilon}(t) = \mathrm{sgn}(t)\dot{\epsilon}_0$, i.e., v_{xx} changes from $-\dot{\epsilon}_0$ to $\dot{\epsilon}_0$. The stationary, stretched state is known to obey $D_{\mathrm{sat}} = \sqrt{15\Delta/32\pi}$, with $\phi = \{0; \pi/2\}$ [1,4,16]. Here $D = (L - B)/(L + B)$, L and B are the large and small semiaxis of the elliptical approximation of the vesicle cross section and ϕ is the inclination angle with respect to the x axis. We assume that the membrane is impermeable on the time scale of the experiment, with the excess area $\Delta = S/R^2 - 4\pi$, where S and R are, respectively, the total surface area and the effective radius of the vesicle, defined via the volume $V = \frac{4}{3}\pi R^3$.

Measurements of the vesicle dynamics were conducted in the vicinity of the stagnation point ($v_x = v_y = v_z = 0$) via either epifluorescent or phase contrast microscopy. The flow was produced in a cross-slot microchannel of 500 μm wide and 320 μm in height manufactured in elastomer (PDMS) by soft lithography [18]. The details of the design and of the arrangement will be published elsewhere. Particle tracking velocity measurements of the flow field show that the deviation of the elongation rate $(\delta\dot{\epsilon})_{xy}/\dot{\epsilon}$ across the size of the observation window is <5%, deviations of $\dot{\epsilon}$ in the z direction on the scale of the vesicle were $(\delta\dot{\epsilon})_z/\dot{\epsilon} < 5\%$, and that the ratio of shear velocity gradient $\dot{\gamma}_z$ to $\dot{\epsilon}$ on the size of the vesicle did not exceed $(\delta\dot{\epsilon})_z/\dot{\epsilon}$. Experiments were performed in the range of velocity gra-

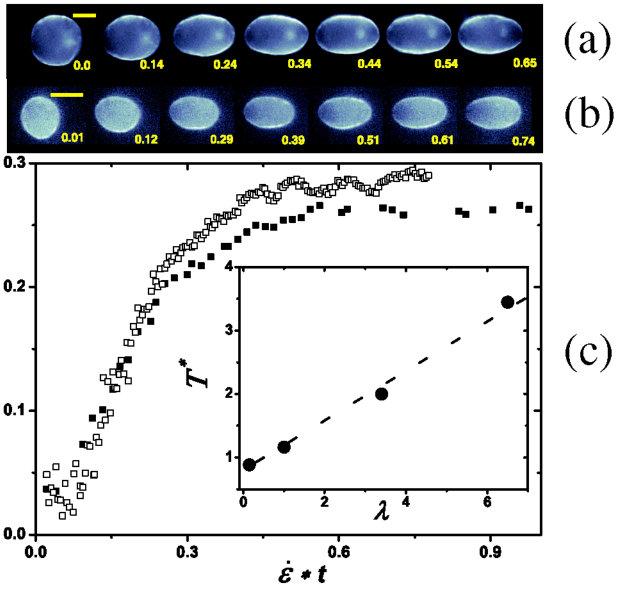

FIG. 1 (color online). Relaxation dynamics in elongation flow suddenly turned on. Snapshots of a vesicle with (a) $\lambda = 1$, $\chi = 2.2$, $\Delta = 0.5$, $\dot{\epsilon} = 0.1$, (b) $\lambda = 1$, $\chi = 4.4$, $\Delta = 0.6$, $\dot{\epsilon} = 0.9$. Numbers on the snapshots are the nondimensional times $t\dot{\epsilon}$; the scale bar is 10 μm. (c) D vs $t\dot{\epsilon}$ with ■ from data in (a), □ from data in (b). Inset: statistical average of T^* vs λ.

dients $\dot{\epsilon} = 0.05$–10. We define the dimensionless strain as $\chi = \dot{\epsilon}\eta_{\rm out}R^3/\kappa$, where $\kappa \simeq 10^{-12}$ erg for DOPC [19]. The viscosity of the fluid inside the vesicle, $\eta_{\rm in}$, can be different from the viscosity $\eta_{\rm out}$ of the surrounding fluid, and their ratio λ was varied across the experiments. The lipid solutions consisted of 85% DOPC (Sigma) and 15% NBD-PC (fluorescent lipid, Molecular Probes) dissolved in 9:1 v/v chloroform-methanol solvent (1.8 mg total lipids/ml) or DOPC in the solvent (1.5 mg/ml). The methods and conditions of the preparation of the vesicles for the experiments have been described previously [12,13,15].

The first set of the experiments was performed suddenly switching on the flow, starting after the vesicle had relaxed into an equilibrium shape. An initial growth of D, monotonic in time until saturation to $D = D_{\rm sat}$, was observed, whereas ϕ reached the stationary value either of 0 or $\pi/2$ (see Fig. 1). We characterize the process for $D \ll D_{\rm sat}$ by the linear growth time $T^* = \dot{\epsilon}[\frac{\Delta D}{\Delta t}]^{-1}$. It was found that $T^*(\chi) \approx$ const in the range of $\chi = 1$–15 for a given λ. The dependence of T^* on the viscosity contrast is found to be linear in the range $\lambda = 0.1$–7 [see inset Fig. 1(c)]. Averaging is done on the data sets of 30 to 100 points for each λ. These experimental results are consistent with the recent theoretical predictions for the same relaxation to a stationary state for $\chi > 1$. The theory too shows that the relaxation time scales linearly with $\dot{\epsilon}$ and with λ [4,5,16].

In the second set of experiments, in which the flow was suddenly switched from $v_{xx} = -\dot{\epsilon}_0$ to $v_{xx} = \dot{\epsilon}_0$ ("suddenly" essentially means that the switching time is much smaller than $\chi/\dot{\epsilon}_0$), vesicles undergo a relaxation from one stretched stationary state ($D = D_{\rm sat}$; $\phi = \pi/2$) to another one ($D = D_{\rm sat}$; $\phi = 0$). Surprisingly, we found that above some value of $\chi > \chi_c$ the vesicles develop small wavelength perturbations in the shape, which we call wrinkles [see Fig. 2(a)–2(c)].

We would like to point out that stationary wrinkles on an elastic microcapsule in shear flow have been already observed [20] and explained [21,22]. Although visually similar to the ones discussed in this Letter, such wrinkles are a distinctly different effect: their structure is stationary, while here it is transient; they appear due to permanent shear stress, here only after release and switch of elongation flow; the main reason for the appearance of an instability is extensional membrane elasticity which sustains compression there, rather than negative surface tension that tunes itself to alternating stress here.

Quantitative evaluation of the higher-order modes of the membrane shape was performed in the following way: the vesicle contour is fitted by an ellipse and the amplitude $A(\theta, \dot{\epsilon}_0 t)$ of the deviation from the elliptical fit is taken as function of the angle θ for every instant of time (see upper inset of Fig. 3). Examples of $A(\theta, \dot{\epsilon}_0 t)$ are shown in Fig. 2(d). The instantaneous Fourier transforms of the amplitudes with respect to θ define the dynamics of the spectrum $u_k(t)$. The temporal evolution of these power spectra is shown in Fig. 2(e). A difference in the development of the higher k modes for different values of χ can be clearly seen: during the transition from one steady state to another, more higher-order modes are excited for larger χ. The evolution of D is shown in Fig. 2(f) in order to precisely define the time interval where the transitional dynamics takes place.

The dependence of the average in time power spectrum of the relaxing modes, $P_k \equiv |u_k|^2$, for different values of χ, is shown in the lower inset of Fig. 3. We found that the spectra show a $P_k \propto k^{-4}$ dependence for χ less than some critical value χ_c, that is a well-known spectral decay due to thermal noise [17]. For larger χ, the spectra become rather flat at smaller k, while the higher modes still comply with the equilibrium spectral decay. From the rather sharp transition from the flat to the k^{-4} spectrum around some $k = k_{\rm thr}$, we can postulate that for $\chi > \chi_c$ modes with $k < k_{\rm thr}$ are generated dynamically via instability from thermal noise, while the higher modes are excited just thermally. We determine χ_c as the threshold above which the modes with $k \geq 3$ are excited ($k = 3$ is the first mode higher than elliptical).

To this extent, we define $k^* = \sqrt{\sum_{k=3}^{19} k^2 P_k / \sum_{k=3}^{19} P_k}$. The restriction to $k \leq 19$ is dictated by the image resolution of the smallest vesicles in the experiments. The dependence of k^* on χ, averaged over ≈ 200 data points, is shown in Fig. 3. For all $\chi < \chi_c$, k^* remains constant, since all modes in the spectrum with $k \geq 3$ obeys the same equilibrium distribution, k^{-4}. The growth of k^* starts at $\chi = \chi_c$ with $\chi_c = 6.5 \pm 0.8$, which is identified as the onset of the wrinkling instability. The dependence of k^*

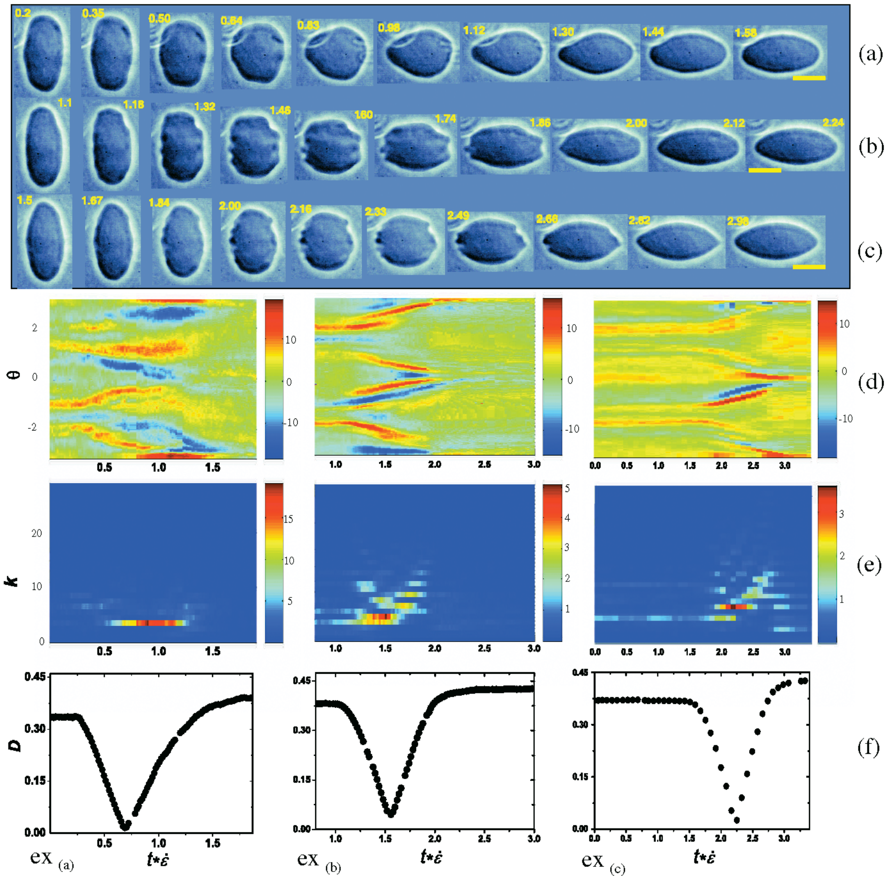

FIG. 2 (color). Wrinkling instability. Snapshots of vesicle dynamics in time-dependent elongation flow at $\lambda = 1$, $\Delta \approx 1$, and: (a) $\chi = 8.1$, (b) $\chi = 81$, (c) $\chi = 323.5$. The scale bar is 20 μm; numbers are $t\dot{\epsilon}$. Plots below the images are the data analysis for each of the cases above: (d) amplitudes $A(\theta, t)$ of higher harmonics vs θ and $t\dot{\epsilon}$ (values in color), (e) $|u_k|^2(t)$ are the instantaneous Fourier spectra of the amplitudes $A(\theta, t)$ at various $t\dot{\epsilon}$ (values in color), (f) $D(t)$ vs $t\dot{\epsilon}$. Columns ex_a, ex_b, ex_c correspond to the data presented in rows (a)–(c).

above the instability threshold is fitted by $\sim\chi^{1/4}$ in a good agreement with numerical simulations in the same range of χ [16]. All the experiments were done for $\lambda = 1$, since we did not expect critical influence of viscosity contrast on the wrinkling effect.

As we pointed out in the introduction, the generation of higher-order modes in the vesicle shape, i.e., wrinkles, would be penalized by the bending energy increase that make them improbable for a vesicle with positive surface tension. On the other hand, a recent theory predicts that a sudden switch in direction of the velocity gradient, $\dot{\epsilon}(t) = \operatorname{sgn}(t)\dot{\epsilon}_0$, produces a negative surface tension defined by a given elongation rate. Then the most unstable higher-order mode, formally defined above via unknown σ, is expressed via parameters of the problem as $k^2 \leq \beta\chi/\sqrt{\Delta}$, where β is the numerical factor [16]. Then for the first ($k > 2$) higher-order mode $k = 3$, the theory gives $\chi_c \simeq 1.2$ at $\Delta \simeq 0.6$, which corresponds to our average $\langle\Delta_i\rangle$ over the data set in the transient region. The theoretical value of χ_c is of the same order as the experimental one, and the difference can be attributed, first, to uncertainty in the value of κ taken and to rather rough estimates based on an isotropic surface tension [16].

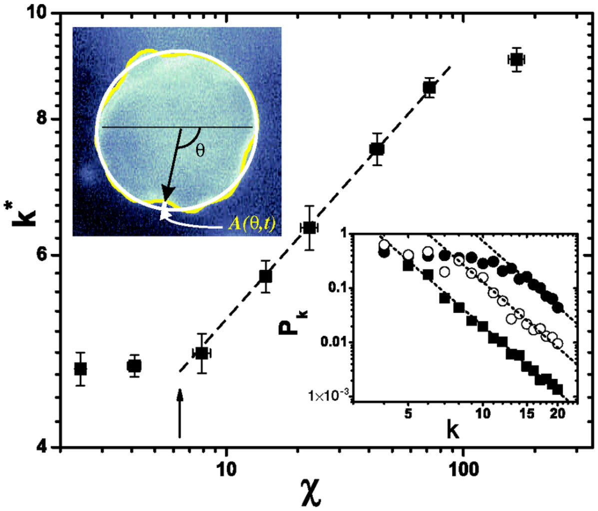

FIG. 3 (color online). k^* vs χ. The arrow defines χ_c, the onset of excitation of the mode $k = 3$. Dashed line is a fit $\sim\chi^{1/4}$ above the instability threshold. The upper inset illustrates the image analysis. Lower inset: averaged power spectrum for various χ: ■—2.6; ○—24; ●—116; dashed lines show a $\propto k^{-4}$ dependence.

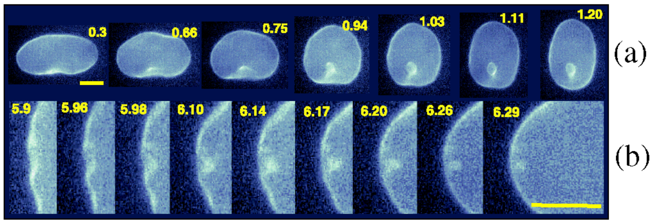

FIG. 4 (color online). Formation of buds. (a) $\chi = 6.8$, $\Delta = 0.9$; (b) $\chi = 179$, $\Delta = 0.4$. The scale bar is 10 μm; numbers are $t\dot{\epsilon}$.

Another interesting phenomenon observed is the formation of buds. These could be seen intermittently in the experiments: sometimes the vesicle surface folds to the point that it creates the enclosure of a smaller vesicle inside the main one (Fig. 4). We have not studied this phenomenon in detail, but some of its features can be described as follows: the enclosure process is irreversible, the bud scale is much smaller than the scale of the excited mode corresponding to the given χ, and the phenomenon is mostly seen in the vicinity of χ_c.

To summarize, we presented experimental results about the relaxation dynamics of vesicles in elongation flows suddenly switched on or reversed. The most surprising result of these studies is a new instability that results in the excitation of higher-order modes, i.e., wrinkles, in the membrane, during the vesicle relaxation following the reversal of the velocity gradient. This unexpected generation of wrinkles suggests that only the appearance of a negative surface tension during the vesicle deflation due to compression in the transient can explain the effect. A recent theory [16] used this physical picture to derive a criterion for the onset of the instability and the power law dependence of the average wave number of the higher-order modes as a function of χ, which are in reasonable agreement with our experiment.

We thank V. Lebedev and N. Zabusky for helpful remarks, K. Turitsyn and P. Vlahovska for enlightening communications. This work is partially supported by grants from the Israel Science Foundation, the Binational US-Israel Foundation, the Israeli Ministry of Science, Culture & Sport for Russian-Israeli collaboration, and by the Minerva Center for Nonlinear Physics of Complex Systems.

---

[1] U. Seifert, Eur. Phys. J. B **8**, 405 (1999).
[2] F. Rioual, T. Biben, and C. Misbah, Phys. Rev. E **69**, 061914 (2004).
[3] C. Misbah, Phys. Rev. Lett. **96**, 028104 (2006).
[4] P. Vlahovska and R. Gracia, Phys. Rev. E **75**, 016313 (2007).
[5] V. V. Lebedev, K. S. Turitsyn, and S. S. Vergeles, arXiv:cond-mat/0702650.
[6] M. Kraus, W. Wintz, U. Seifert, and R. Lipowsky, Phys. Rev. Lett. **77**, 3685 (1996).
[7] J. Beaucourt, F. Rioual, T. Seon, T. Biben, and C. Misbah, Phys. Rev. E **69**, 011906 (2004).
[8] H. Noguchi and G. Gompper, Phys. Rev. Lett. **93**, 258102 (2004).
[9] H. Noguchi and G. Gompper, Phys. Rev. Lett. **98**, 128103 (2007).
[10] K. de Haas, C. Bloom, D. van den Ende, M. Duits, and J. Mellema, Phys. Rev. E **56**, 7132 (1997).
[11] M. Abkarian, C. Lartigue, and A. Viallat, Phys. Rev. Lett. **88**, 068103 (2002).
[12] V. Kantsler and V. Steinberg, Phys. Rev. Lett. **95**, 258101 (2005).
[13] V. Kantsler and V. Steinberg, Phys. Rev. Lett. **96**, 036001 (2006).
[14] M. Mader, V. Vitkova, M. Abkarian, A. Viallat, and T. Podgorski, Eur. Phys. J. E **19**, 389 (2006).
[15] V. Kantsler, E. Segre, and V. Steinberg, Phys. Rev. Lett. (to be published).
[16] K. S. Turitsyn and S. S. Vergeles, arXiv:cond-mat/0705.3539v1 [Phys. Rev. Lett. (to be published)].
[17] U. Seifert, Adv. Phys. **46**, 13 (1997).
[18] Y. N. Xia and G. M. Whitesides, Annu. Rev. Mater. Sci. **28**, 153 (1998).
[19] W. Rawicz *et al.*, Biophys. J. **79**, 328 (2000).
[20] A. Walter, H. Rehage, and H. Leonard, Colloids Surf. A **183–185**, 123 (2001).
[21] E. Cerda and L. Mahadevan, Phys. Rev. Lett. **90**, 074302 (2003).
[22] R. Finken and U. Seifert, J. Phys. Condens. Matter **18**, L185 (2006).