Compression of a capsule: Mechanical laws of membranes with negligible bending stiffness

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The compression of a capsule between two plates is considered. The problem is solved numerically for a capsule made of an incompressible liquid drop surrounded by a thin elastic membrane which has a negligible bending stiffness. Numerical results are provided for three different mechanical laws of the membrane. By considering elastic moduli independent of the deformation, we show that the isotropic dilation plays the major role. In particular, an asymptotic behavior independent of the shear modulus is found for large deformations. For more complex models, the deformation limits beyond which the variation of elastic moduli starts to play a role are examined. The results indicate that the distinction between the different models requires a careful inspection of both small and large deformations. The theoretical predictions are compared with experimental results. For millimetric capsules with membranes made of covalently linked human serum albumin and alginate, the best agreement is obtained by considering that the elastic moduli are independent of the deformation and range from 0.1 to 4 N/m.

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I. INTRODUCTION

Capsules consist of a liquid internal medium surrounded by a deformable membrane. This general definition applies to both living cells and synthetic capsules that are used in many industrial applications including pharmacology and cosmetics. The role of the capsules is to transport, protect and control the release of the encapsulated substance. The membrane properties are thus of major practical interest: osmotic properties govern the mass transfer through the membrane [1] and mechanical properties control the capacity of capsule deformation [2]. However, their determination is a challenging problem, since the membrane is available only in the form of capsules and not as large sheets of material suitable for classical experimental tests.

Several experimental techniques were introduced for the determination of the mechanical properties of the membrane: the compression between two plates was first applied to urchin eggs [3]; the sucking into micropipette [4] and the inflation by osmotic pressure [5] were first used for the characterization of red blood cells. They are now commonly used. Whatever the experimental techniques considered, the determination of the mechanical properties involves three steps: (i) Measurement of the relationship between the capsule shape and the force responsible for the deformation; (ii) evaluation of the same relationship by postulation of a mechanical model for the membrane; (iii) determination of the model parameters by comparison between theoretical predictions and experiments.

The present study focuses on the compression technique which is now used for the characterization of many kinds of capsules from micrometric living cells to millimetric artificial capsules: [6–11]. We will only consider membranes made of a sheet of a three-dimensional material which is thin enough to neglect the bending. Note that membranes which are made of a few layers of molecules, as those of living cells, generally have a non-negligible bending stiffness due to their complex molecular network. For example, the model due to Helfrich [12] for phospholipidic vesicles is based on curvature energy (for recent developments see [13,14] and references therein). Here, our objective is to investigate the capability of the compression experiment to distinguish between different mechanical constitutive laws for a membrane with negligible bending stiffness. Section II is devoted to a brief presentation of the different available models. In Sec. III, we solve numerically the compression problem for three classic models and a wide range of parameters and we also derive asymptotic laws for small and large deformations. In Sec. IV, the theoretical predictions are compared with experiments for millimetric capsules with membranes made of covalently linked human serum albumin and alginate.

II. MEMBRANE MECHANICAL LAWS

The thin membrane of the capsule can be considered either as a two-dimensional or a three-dimensional continuous material. When the membrane thickness is small compared to the capsule radius but large compared to the size of the molecules, it is possible to describe the membrane as a sheet of a three-dimensional continuous material. This allows the use of classical mechanical laws and to directly address the role of the membrane thickness [9]. On the other hand, considering the structure and the forming process of most of the membranes, it is unlikely that the properties of the material in the plane of the membrane are the same as those in the normal direction. For this reason, the two-dimensional de-

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scription is preferred here. Mechanical behaviour of the material is then characterized by the constitutive law that relates the tensions inside the membrane to the deformations it experiences. It is necessary to distinguish the deformations in the plane of the membrane which cause tangential tensions from membrane curvatures that generate tangential bending moments. By assuming that the two-dimensional description is obtained by integration of the three-dimensional stresses over the membrane cross section, the magnitude, η, of the ratio between bending and membrane-expansion effects is given by

$$\eta = \frac{\epsilon^2 C^2}{\alpha}, \tag{1}$$

where ϵ, C and α are respectively the membrane thickness, curvature and relative expansion. Several authors have considered the effect of membrane bending stiffness (see [15,16] and references therein). Here we will focus on situations where the membrane thickness is small enough so that η is negligible.

Evans and Skalak [17] established the general expression for an isotropic homogeneous purely-elastic two-dimensional material with negligible bending stiffness. Noting λ_1 and λ_2 as the principal extension ratios, the deformation is fully characterised by the two independent invariants $\alpha=\lambda_1\lambda_2-1$ and $\beta=\frac{1}{2}(\lambda_1/\lambda_2+\lambda_2/\lambda_1)-1$. In the principal axes, the first component of the tension is

$$T_1 = \underbrace{K\alpha}_{\text{isotropic part}} + \underbrace{\mu \frac{\lambda_1^2-\lambda_2^2}{2\lambda_1^2\lambda_2^2}}_{\text{deviatoric part}}, \tag{2}$$

the second component is obtained by exchanging subscripts 1 and 2. The area dilation modulus K and the area shear modulus μ are functions of the two invariants α and β. These two functions, $K(\alpha,\beta)$ and $\mu(\alpha,\beta)$, define the mechanical constitutive law of the material. In the limit of small deformations, $e_1=1/2(\lambda_1^2-1)\ll 1$and $e_2=1/2(\lambda_2^2-1)\ll 1$, Eq. (2) becomes

$$T_1 = K_0(e_1+e_2) + \mu_0(e_1-e_2), \tag{3}$$

where K_0 and μ_0 are the limits of the functions K and μ when α and β tend towards zero. Equation (3) is the two-dimensional Hooke law, in which the Young modulus is $E=4K_0\mu_0/(K_0+\mu_0)$ and the Poisson ratio $v=(K_0-\mu_0)/(K_0+\mu_0)$.

The simplest constitutive law is obtained by considering that the two elastic moduli are independent of the deformation, $K=K_0$ and $\mu=\mu_0$. For historical reasons, this law (hereinafter noted ES), is not often used. On the other hand, the two-dimensional Mooney-Rivlin law (MR), which has been derived by considering an infinite thin sheet of incompressible elastomer, is commonly used,

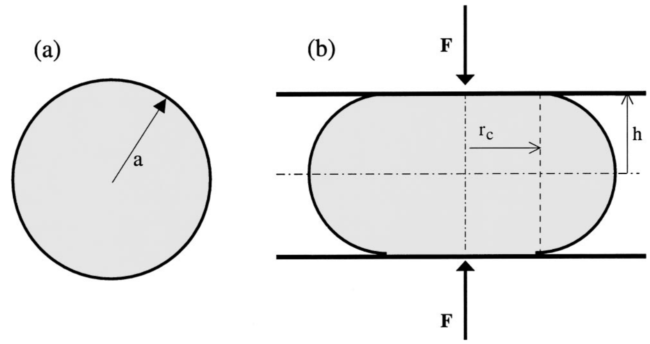

FIG. 1. Schematic of the compression experiment. (a) Initial shape; (b) during compression.

$$T_1 = \frac{G^{MR}}{\lambda_1\lambda_2}\left(\lambda_1^2 - \frac{1}{\lambda_1^2\lambda_2^2}\right)(1+\Psi' + \lambda_2^2\Psi'). \tag{4}$$

G^{MR} is a surface elastic modulus and Ψ' a non-dimensional parameter ranging from 0 to 1. The corresponding expressions of K and μ are complex and their limit in small deformations are $K_0=3G^{MR}$ and $\mu_0=G^{MR}$. Another usual law is that proposed by Skalak, Tözeren, Zarda, and Chien [18] for the description of the red blood cell (STZC):

$$T_1 = G^{STZC}\left(\frac{\lambda_1}{\lambda_2}(\lambda_1^2-1) + C^{STZC}\lambda_1\lambda_2(\lambda_1^2\lambda_2^2-1)\right). \tag{5}$$

The expressions of K and μ are again very complex, their limits in small deformations are $K_0=G^{STZC}(1+2C^{STZC})$ and $\mu_0=G^{STZC}$.

III. THEORETICAL AND NUMERICAL RESULTS

We consider the mechanical equilibrium of a capsule compressed between two rigid plates separated by a distance $2h$ and subjected to a force F (Fig. 1). The capsule is made of a thin impermeable elastic membrane that separates two incompressible Newtonian liquids. Initially, the capsule is a sphere of radius a. During compression, the membrane can be decomposed in two parts. The first is in contact with the plates and constituted of two disks of radius r_c. The other part is curved and balances the pressure difference, $\Delta P=F/\pi r_c^2$, that exists between the inner and outer fluids. Since the mechanical constitutive law of the membrane is given by Eq. (2), the problem depends on two nondimensional parameters, $\xi=(1-h/a)$ and $\zeta=\mu_0/K_0$, and two nondimensional functions, $\tilde{K}=K/K_0$ and $\tilde{\mu}=\mu/\mu_0$. For a given membrane rheology, i.e., for given functions $\tilde{K}$ and $\tilde{\mu}$, the nondimensional force exerted on the two plates can be written as

$$F^* = \frac{F}{aK_0} = f(\xi,\zeta). \tag{6}$$

In the limit of small deformations, the mechanical equilibrium can be written on the initial spherical shape, $T_1=T_2=\alpha\Delta P/2$. The noncontact region is thus a truncated sphere of radius R terminated by the two disks of radius r_c. The volume conservation law then imposes that R

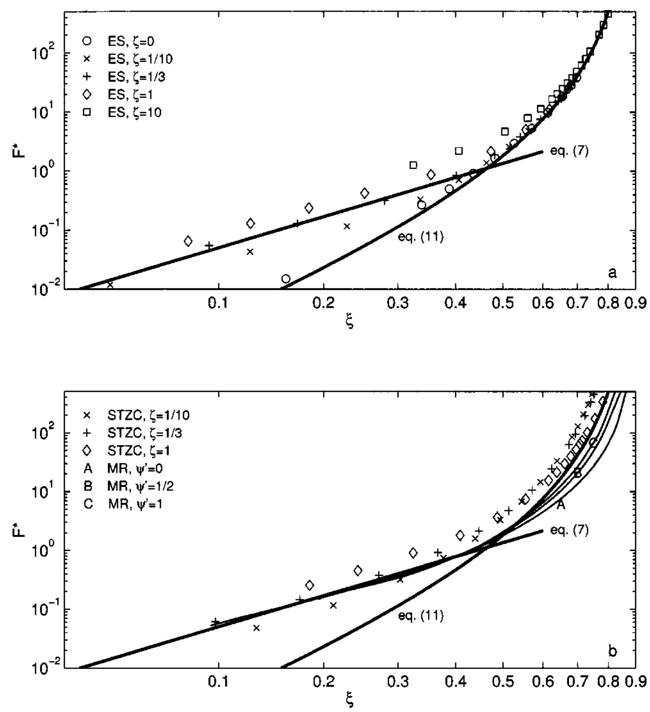

FIG. 2. Numerical results. (a) ES model; (b) STZC and MR models.

$=a\sqrt{1+a^2F^*/(2\pi r_c^2)}$ and we finally obtain at the first nonvanishing order in ξ:

$$F^* = 2\pi\xi^3. \tag{7}$$

For finite deformations, we solved the problem numericaly by using the method described in [6] and [7]. Here, 40 grid points were used to describe the contact region, 300 for the noncontact region, and the error on the capsule volume was always less than 10^{-3}. The results for the three models, ES, MR and STZC, are shown in Fig. 2.

Figure 2(a) shows the results obtained with the ES model for values of the modulus ratio ζ ranging from 0 to 10. First of all, one notes that the asymptotic behavior for large deformations is independent of ζ. The meridian section of the noncontact region tends to become a semicircle of radius h. Assuming this shape, the capsule volume and area are

$$V = 2\pi h r_c^2 + \pi^2 h^2 r_c + \frac{4}{3}\pi h^3, \tag{8}$$

$$A = 4\pi h^2 + 2\pi r_c^2 + 2\pi^2 r_c h. \tag{9}$$

For the volume to be conserved r_c is given by

$$r_c = \frac{1}{12}([96a^3/h + (9\pi^2 - 96)h^2]^{1/2} - 3\pi h). \tag{10}$$

By assuming uniform area variation and negligible shear, we finally obtain

$$F^* = \pi\left(\frac{A}{4\pi a^2} - 1\right)\frac{r_c^2}{ah}. \tag{11}$$

Figure 2(a) confirms that Eq. (11) provides the correct asymptotic behavior for large deformations. Smaller the shear modulus, sooner the asymptotic behavior is reached. In addition, for ζ between $1/10$ and 1, the numerical results tend towards Eq. (7) for small deformations. The effect of the shear modulus is then restricted to the intermediate range which connects the two asymptotic behaviors. For $\zeta=1/3$ this intermediate range even disappears. For ζ smaller than $1/10$ or larger than 1, the range of deformations investigated did not allow to confirm the small deformations relationship (7).

Let us go back to the assumption of negligible bending stiffness. The membrane curvature, which is of order $1/h$, is not bounded when h becomes small. It is therefore necessary to check whether the relative magnitude of bending effects, η, can remain small when the deformations become very large. Assuming $h \ll a$, one obtains from Eqs. (9) and (10) that $\alpha \sim a/h$. Injecting this result in Eq. (1) yields

$$\eta \sim \frac{\epsilon}{a}\frac{\epsilon}{h}. \tag{12}$$

Since $\epsilon/h \leqslant 1$, the condition $\epsilon/a \ll 1$ is sufficient to ensure that bending effects are negligible over the whole range of deformations.

Figure 2(b) shows the results obtained with MR and STZC models. For small to moderate deformations ($\xi \leqslant 0.4$), the results are similar to those of the ES model. But at large deformations, MR and STZC do not tend towards a unique asymptotical behavior. For $\zeta=1$, the behavior of the STZC model is close to Eq. (11) and the resistance it opposes to the deformation increases when ζ decreases. Contrarily, the resistance of the MR model is always less than that of the ES model and increases as Ψ' increases.

The present work deals with a capsule which is initially at rest: there is no pressure difference between the inner and the outer fluids prior to the compression. Let us consider for an instant the case of capsule which is initially inflated. Its initial radius is then λ_s times larger than its radius at rest. Assuming that $(\lambda_s - 1)$ is small, the small-deformation relationship (7) becomes

$$F^* = 2\pi\left(\frac{4}{3}(\lambda_s - 1)\xi + \xi^3\right). \tag{13}$$

Figure 3 compares this analytical expression with numerical results obtained for $\zeta=1/3$ by using the MR model. (Note that the choice of the mechanical model has no importance since we only consider small deformations.) Equation (13) is a good approximation up to $\xi=0.3$ for $\lambda_s \leqslant 1.02$. The presence of the linear term, $(\lambda_s - 1)\xi$, suggests that a possible initial inflation can be detected from the initial slope of relationship (6). Note that the present analysis is not valid for an intially underinflated capsule ($\lambda_s < 1$). In this case, the initial shape would be nonspherical and show concave parts.

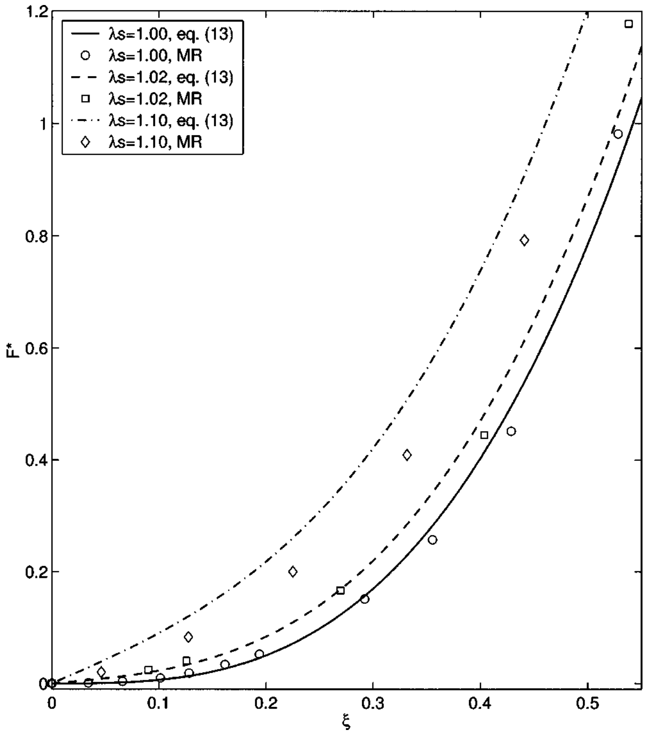

FIG. 3. Numerical results: effect of initial inflation.

The present results show that the determination of the membrane mechanical properties requires observation of a wide range of deformations. Weak deformations are independent of the mechanical model considered and should hence permit determination of K_0 and μ_0. Concerning the MR model, the present calculations showed that, for $\xi \leqslant 0.55$, the results are independent of Ψ', similar to those of the ES model with $\zeta = 1/3$, and well predicted by Eq. (7). For the STZC model, the discrepancies with the ES model start to be significant from $\xi = 0.4$. On the other hand, large deformations are sensitive to the mechanical model considered. In case the parameters of the model were previously determined from weak deformations, large deformations should therefore permit to discriminate between the different possible constitutive laws. However, we may anticipate from Fig. 2 that if one tried to adjust the model parameters by considering only large deformations, many constitutive laws could seem to agree with the experimental results. Consequently, the discrimination between the different mechanical models requires to focus on both the weak and large deformation regimes. Owing to the very rapid increase of the force with the deformation (starting with ξ^3 and getting higher and higher as the deformation increases), the use of the logarithmic representation is strongly recommended.

IV. EXPERIMENTS

Our purpose is now to apply the previous theoretical analysis to the determination of the mechanical properties of real capsules. The present capsules were kindly provided to us by F. Edwards-Lévy of the Faculté de Pharmacie de Reims. Their membrane is made of covalently linked human serum albumin (HSA) and alginate (Lévy and Edwards-Lévy [19] and Edwards-Lévy and Lévy [8]). Calcium-alginate gel beads coated with a HSA-alginate membrane were originally designed for medical applications such as hepatocyte encapsulation for bioartificial liver [20] or encapsulation of genetically modified cells for AIDS treatment [21]. In this study, HSA-alginate capsules were prepared according to the procedure described in [8]. Then, the gel core of the coated beads was reliquified by Na citrate in order to obtain capsules with a liquid core surrounded by a membrane made of cross-linked HSA and alginate.

Ten capsules with radii ranging from 1.50 to 1.95 mm have been tested (Table I). Capsules of set 1 belong to a first batch and have a membrane thickness of approximately 20 μm. Capsules of sets 2a and 2b belong to second batch and have a membrane thickness of approximately 30 μm. Before being used, all capsules were conserved in their original 9 g/1 NaCl aqueous solution at a temperature of 5 °C. Capsules of sets 1 and 2a were directly tested in the compression apparatus whereas capsules of set 2b had a more complex history: after 12 months, they were immersed in silicon oil (Rhodorsil 47V1000) and used for tests in Poiseuille flow inside a tube of 4 mm diameter; they were finally put back into the original solution before the compression test. During the compression tests all the capsules were immersed in a 9 g/1 NaCl aqueous solution. The experimental setup used for capsule compression has been described in [11] where capsules of the same nature were studied. These capsules nevertheless differ from the present by their thickness, age and history. It is therefore not possible to make direct quantitative comparisons with the result of this previous study. In applications, capsules can be used during a long time and immersed in various solutions. For that reason, we decided to test capsules which differ by their age and history.

TABLE I. Area dilation modulus measured for the ten capsules. (The accuracy in the determination of K_0 is of ±5 %.)

Capsule		1	2	3	4	5	6	7	8	9	10
Set		1	1	1	2a	2a	2a	2a	2a	2b	2b
Age (month)		0.1	6	6	0.5	3.5	3.5	13	13	13	13
Radius (mm)		1.64	1.56	1.63	1.56	1.93	1.66	1.67	1.64	1.62	1.58
	ES	3.10	0.84	0.89	2.25	1.70	1.85	0.41	0.16	0.62	0.53
K_0 (N/m)	MR	4.00	1.35	1.44	3.25	2.40	2.50	0.59	0.26	0.97	0.84
	STZC	2.00	0.65	0.68	1.60	1.20	1.25	0.29	0.12	0.46	0.40

It is important to stress that our objective is not to study in detail the aging process of the membrane material but to check whether the compression experiment is relevant to determine the mechanical properties of the membrane at different stages of the capsule life.

The reproducibility of the results was ensured by performing the tests several times. In each case the membrane material remained within the elastic domain. We showed in a previous work [1] that the initial concentration of the capsule inner liquid results from a Donnan equilibrium. This implies that the capsule can be sligthly overinflated but not underinflated ($1<\lambda_s\leqslant 1.05$). Here we observed that, in all cases, the measured force initialy increases with ξ^n for at least $n\geqslant 3$. Owing to Eq. (13), we shall consequently assume that the initial inflation is negligible for the present capsules. The measurement accuracy is $\pm 10^{-4}$ N for the compression force and ± 1 μm for the plate displacement. This allows reliable measurements from $\xi=0.4$ for the most rigid capsules and from $\xi=0.6$ for the less rigid ones. Since the results of the three models differ for $\xi\geqslant 0.4$, it is not possible to measure accurately K_0 and ζ from weak deformations without postulating any mechanical model. In [11], the analysis of the experiments was based on the determination of the average values of the elastic moduli which provided, over the whole range of deformation available, the best agreement between measurements and numerical predictions. This method privileged the largest deformations and did not permit to distinguish, for the capsules considered, between the ES and the STZC model. In the present study, we will try to distinguish between the different constitutive laws by a careful inspection of the different ranges of deformation, as suggested by the theoretical results presented above.

We start with the analysis of large deformations. For each experiment, we adjusted the value of K_0 in order to match as well as possible the asymptotic behavior of the ES model. We obtain a good agreement with the values of K_0 given in Table I. The ES model seems thus able to correctly represent the present membranes. But note that if all the capsule membranes had the same ratio ζ, all the experimental curves would match as well. For that reason, we also determined the values of K_0 that gave the best agreement between the experiments and the two other models. We fixed $\zeta=1$ for STZC and $\Psi'=1$ for MR because these were the values which minimized the difference with the ES model (see Fig. 2). The values of K_0 obtained for MR ranged from 1.3 to 1.55 times those of the ES model. For STZC, they ranged from 0.6 to 0.78 times ES values. Since the uncertainty in the determination of K_0 due to the fitting procedure is of ± 2 %, the discrepancies between the three models are significant. Using the values of K_0 provided in Table I, it is thus possible to obtain, for large deformation ($\xi\geqslant 0.75$), a reasonably good agreement between the experiments and each of the three models. For a given a model, the values of K_0 are obtained with an accuracy of ± 5 % when both the fitting and experimental errors are taken into account.

We now consider the smallest deformations available. Figure 4 compares predictions of the ES model with three representative capsules. It can be seen that the experimental curves separate for $\xi\leqslant 0.70$, indicating different modulus ratios ζ. By varying ζ from $1/3$ to 1, the model ES is indeed

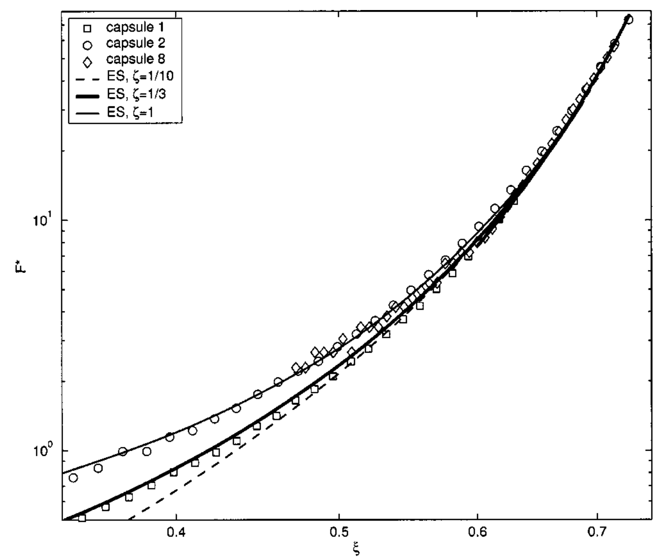

FIG. 4. Experimental results: comparisons with the ES model.

capable of reproducing well all the experimental results. The two other models are considered in Fig. 5. For a given value of K_0, the MR model cannot reproduce correctly the results for both small and large deformations since the value of ζ is fixed at $1/3$ by constitution. For the STZC model, the problem is due to the fact that we fixed $\zeta=1$ in order to correctly reproduce the large deformations. When the ES model predicts that ζ is close to $1/3$ (capsule 1), STZC fails as soon as ξ is less than 0.6 [Fig. 5(a)]. When the ES model predicts that ζ is close to unity the distinction between ES and STZC model is less visible [Figs. 5(a) and 5(c)]. For $\zeta=1$, indeed, the prediction of STZC and ES models superimposed at large deformation provided the value of K_0 is adjusted correctly (see Fig. 2). That is probably the reason why no distinction could be found in [11] between the ES and STZC models,

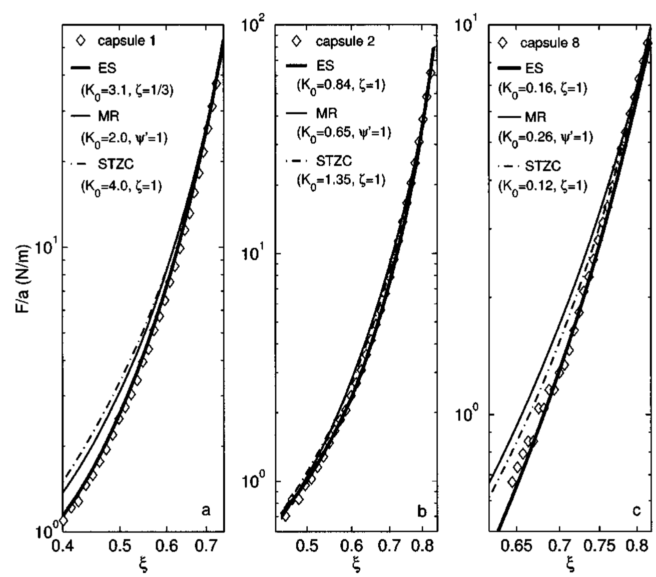

FIG. 5. Experimental results: comparisons with the three models. (a) Capsule 1, (b) capsule 2, and (c) capsule 8.

since ζ was close to unity for all the capsules considered. Here (capsule 2 and 8), the STZC model matches the experiments well for small ($\xi \leqslant 0.6$) and very large deformations ($\xi \geqslant 0.75$). For capsule 2 the very small differences observed in the intermediate range of deformation are not sufficient for invalidating the STZC model. But for capsule 8, it can be concluded that the STZC model fails in the intermediate range.

Careful inspection of the whole range of deformations available seems therefore to exclude the MR and STZC models. On the other hand, the ES model predicts well the behaviors of all the present capsules. The accuracy in the determination of K_0 is fairly good (± 5 %) as it is measured from large deformations. The determination of ζ is less accurate and depends on the lowest values of ξ available. For capsule 1, the experimental curve is obtained for a range that intersects the domains of validity of both Eqs. (7) and (11); the agreement with the numerical prediction for $\zeta = 1/3$ is thus significant. On the contrary, since only deformations larger than 0.65 are available for capsule 8 [Fig. 5(c)], the value of ζ cannot be accurately determined. It is nevertheless possible to conclude that ζ is in all cases between 1/3 and 1.

The comparisons between the different sets of capsules show that the membrane properties evolve with time. We ignore the mechanisms that are responsible for this aging process but we have observed that the value of K_0 of capsules belonging to the same batch decreased with time (this point has been confirmed from other tests concerning 35 capsules in Poiseuille flow [22]). The solution in which the capsules are conserved is also important since the elastic moduli of capsules that have the same age may differ significantly depending on whether the capsule have been immersed in silicon oil or not (see results for capsules 7,8 from set 2a and 9,10 from set 2b). But the interesting point is that the ES model is able to describe the mechanics of young as well as old capsules (up to 13 months) although the molecular structure of the membrane is probably significantly altered.

V. CONCLUDING REMARKS

We have simulated the compression of a capsule between two plates by means of numerical calculations. We have considered three membrane mechanical constitutive laws. As shown by Barthès-Biesel, Diaz and Dhenin [2], a rigorous way to compare the different models is to relate the different moduli involved in their definitions in the limit of small deformations. In this limit, all the constitutive laws indeed degenerate into the linear expression (3), which involves only K_0 and μ_0. We considered first the ES model which is appropriate for investigating the respective roles of these two moduli since it assumes that the surface dilation modulus, K, and shear modulus, μ, are independent of the deformations. The finding of asymptotic behaviors independent of μ suggests that the compression experiments privileges isotropic dilation compared to shear. We then analyzed the two classic models, MR and STZC, for which K and μ depend on the deformations in a complex manner. The results show that the compression experiments allow the discrimination of the three models provided a wide range of deformations was investigated.

We have applied the previous results to the experimental determination of the mechanical properties of particular capsules. Despite the lack of accurracy in the domain of small deformations, it has been possible to reach valuable conclusions. The MR and the STZC models failed to reproduce the experimental results in the whole range of deformations investigated. (Note that the values of K_0 obtained strongly depended on the model considered.) On the other hand, the ES model was in agreement with all the experimental results. Since the isotropic dilation plays a dominant role in compression experiments, it would be inaccurate to conclude that ES model is able to describe these membranes for any kind of deformation. It thus seems more reasonable to conclude that (i) the surface dilation modulus can be considered independent of the deformation in the range of area variations investigated ($\alpha \leqslant 1.5$); (ii) the surface shear modulus lies between $1/3K$ and K. It is remarkable that the simplest constitutive law, which assumes constant elastic moduli, is able to model real capsules. Consequently the analytical asymptotic laws derived here can be used for the determination of K without the need of a full numerical simulation. Another interesting finding concerns the aging process of capsules conserved in their initial NaCl solution. Since it causes the decrease of K without altering the constitutive law of the membrane, it can constitute a simple way to obtain a wide range of elastic moduli from a single batch of capsules.

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