4^{th} *Micro and Nano Flows Conference*
UCL, London, UK, 7-10 September 2014

Mechanical characterisation of cross-linked albumin capsule membranes

Pierre-Yves Gires[1], Dominique Barthès-Biesel[1], Eric Leclerc[1], Florence Edwards-Lévy[2], Anne-Virginie Salsac[1,*]

[*]Corresponding author: a.salsac@utc.fr
[1]Laboratoire de Biomécanique et de Bioingénierie (UMR CNRS 7338), Université de Technologie de Compiègne, France
[2]Institut de Chimie Moléculaire de Reims (UMR CNRS 7312), Université de Reims Champagne-Ardenne, Reims, France

Abstract We used a microfluidic technique in order to study the mechanical properties of cross-linked albumin capsule membranes. The capsules were produced from aqueous human serum albumin (HSA) solutions with concentrations of 15%, 20%, 25% and 30% (w/v). The radii were in the range 55-80 μm. We find that the capsules follow the neo-Hookean law, which is strain-softening and depends on one parameter, the surface shear modulus. A possible pre-inflation effect due to osmolarity is accounted for by imposing various values of pre-inflation. Small values of pre-inflation are sufficient to fit the data, which indicates that the capsule membrane is under small osmotic stress. Accounting for pre-inflation changes only slightly the surface shear elastic modulus of the membrane. Whatever the pre-inflation ratio, the shear modulus sharply increases with the HSA concentration from a low-value plateau.

Keywords: Microfluidics, capsule, cross-linked membrane, mechanical properties

1. Introduction

Capsules are droplets enclosed in a membrane with shear resistant properties. Among all their industrial and clinical applications, one can highlight the great potential they offer in bioengineering and in medicine. By encapsulating drugs, genetic material or cells, capsules have for instance found applications in drug targeted delivery (Cho et al., 2008; Demirgöz et al., 2009; Banquet et al., 2011) and in the development of bioartificial organs (Orive et al., 2004). In many applications, a strict control of the release or protection of the internal medium is required. It is possible to tune the membrane mechanical properties by changing the physico-chemical conditions during the fabrication process. A good control of the process requires not only to be able to characterise the mechanical behavior of microcapsules, but also to know how the latter depends on the physico-chemistry. Both aspects are great scientific challenges, because the objects of interest are a few tens of microns in size at the most.

One technique that enables the characterisation of an entire capsule population is the one developed by Chu et al. (2011). It consists in flowing a capsule suspension in a transparent microcapillary tube of comparable transverse dimension. The capsule mechanical properties are identified by comparing the experimental deformed shapes to the ones predicted by a mechanical model of the system. This technique is presently used to characterise the membrane elastic properties for microcapsules made of covalently cross-linked Human Serum Albumin (HSA). The objective is to study the influence of the protein concentrations on the mechanical behavior. Capsules in the size range 55-80 µm are produced through emulsification varying the HSA concentration.

2. Material and methods

2.1. Experimental procedure

The experimental procedure is similar to the

one used in Chu et al. (2011). We present briefly the experimental, numerical and identification techniques, highlighting the key points.

The capsules were prepared using an interfacial cross-linking method on an emulsion of droplets of HSA aqueous solutions. The droplets were suspended in an organic phase containing terephthaloyl chloride as cross-linking agent (Edwards-Lévy et al., 1993). Four HSA concentrations were considered: 15%, 20%, 25% and 30% (w/v). Each capsule population will be denoted HSAx, where x is the HSA concentration in % (w/v). The pH was set at 8 and the reticulation time at 30 min. The capsules were transferred into a glycerol solution and stored in it. Capsules were generally measured one month after fabrication, apart from the HSA30 capsules (3-4 months) and a few data points in the HSA15 set (8 months). After resuspension in glycerol, the capsules were injected into a cylindrical glass microchannel (radius 77 ± 2 μm, length 1.5 cm) using a syringe pump. Videos of the capsule in the middle of the channel were recorded with a high-speed camera mounted on an inverted microscope.

2.2. Viscosity measurement

As the viscosity of a mixture of water and glycerol quickly varies with the mass fraction of glycerol, when it is close to 1, special care is taken to prepare the suspensions. They consist of 50 mg of capsule sediment, resuspended in 2.5 g of technical glycerol. Mass measurements are made with a high precision balance (Sartorius), allowing a precision within ± 0.1 mg. As the capsule membranes are permeable both to water and glycerol, osmotic transfers equilibrate the glycerol mass fraction of the inner and outer liquids. The capsule suspensions are kept at rest for at least 10 min before the experiments are conducted.

The viscosity of the suspending liquid is obtained in two steps. We first estimate the mass fraction of water contained in the glycerol solution by measuring its viscosity at 20°C: the mass fraction is deduced from a linear interpolation of tabulated data of the glycerol viscosity as a function of water content (GPA, 1963). The measurement is performed with the supernatant of the capsule suspension after it has been left to sediment for at least a day. In order to calculate the viscosity of the glycerol solution at the room temperature, we then measure the temperature of a glycerol droplet previously deposited onto the microchannel. The viscosity of the suspension is adjusted by linearly interpolating tabulated data of glycerol viscosity as a function of temperature.

2.3. Acquisition of experimental data

The capsule deformed profile and the microchannel radius R are manually extracted by positioning points in the middle of the interfaces on the acquired images. Different geometric quantities, characteristic of the deformed capsule shape, are then calculated from the profile: the total length L_x, the front-to-rear axial length L_{fr}, the parachute depth $L_p = L_x - L_{fr}$, the transverse length L_r and the cross-section surface area S (Fig. 1). The initial capsule radius a is deduced from the capsule volume, which is calculated by rotating the half-profile around the channel axis of symmetry. We then calculate the geometric quantities by averaging the values obtained for the upper and lower half-profiles. Capsules are discarded from further analysis if the relative differences between the upper and lower half-profiles are larger than 2% for L_x, 1% for L_r, 3% for a and 2% for S.

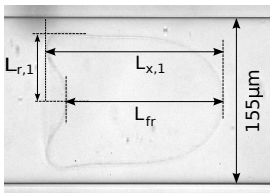

Figure 1: Capsule geometrical characteristics.

The uncertainties of measurement of the geometrical quantities are estimated on typical capsule profiles. They are obtained by extracting the internal and external borders of the dark region representing the capsule contour. Their values are provided in Table 1.

The capsule velocity V is deduced from the distance travelled by the front of the capsule

Quantity	L_x	L_{fr}	L_p	L_y	a	$\Delta S/S$	R
Uncertainty	13 px	13 px	13 px	7 px	3 px	4%	3 px

Table 1: Uncertainties over the measured values (px denotes the pixel length, equal to 0.425µm).

while it crosses the microscope observation window. In the experiments reported in this work, the velocity is in the range 0.5-7 cm/s.

2.4. Numerical model

The capsules are assumed to be spherical at rest with a membrane made of a bi-dimensional strain-softening neo-Hookean material, with a surface shear modulus G_s. Both internal and external liquids are incompressible and Newtonian, with viscosity μ. The capsules are neutrally buoyant and, when they are forced to flow in the small capillary tube, inertia is negligible. When a steady state exists, the velocity and deformed shape of a capsule only depend on the size ratio a/R and the capillary number $Ca = \mu U/G_s$, where U is the mean unperturbed velocity of the external liquid. Ca represents the ratio between viscous and elastic stresses. Axisymmetry is assumed. A possible pre-inflation is considered, characterized by $\alpha = (a - a_0)/a_0$, where a_0 is the initial radius of the capsule (Lefebvre and Barthès-Biesel, 2007).

2.5. Numerical database

The fluid-structure interactions of the capsule flowing in the microchannel are solved by means of a boundary integral formulation. The model yields the deformed profile of the capsule as a function of a/R, Ca and α. A database is then designed which gives the capsule geometric characteristics L_x, L_{fr}, L_r, $\Delta S/S$ and velocity ratio V/U as functions of a/R, Ca and α (Chu et al., 2011). Two values of pre-inflation are considered: $\alpha = 0\%$ and $\alpha = 1.5\%$. A linear interpolation is performed on the simulation points in the $(a/R, Ca)$ space for each value of α with a grid size equal to 0.001 for Ca and 0.005 for a/R.

2.6. Inverse analysis

Only capsules with a size ratio within the database range are kept in the analysis ($0.77 \leq a/R \leq 1.02$). For each analyzed capsule, we determine the ensemble of geometric and dynamic parameters $\{a/R, Ca\}$ for which the experimental and numerical values of $\{a/R, L_x/R, L_{fr}/R, L_p/R, L_r/R, S/R^2\}$ correspond when the tolerances of Table 1 are accounted for. The mean value and standard deviation of the possible shear moduli ensemble are respectively denoted G_s and σ.

At low Ca, the deformation of the capsule can be too small to be determined with any precision. We thus restrict our analysis to capsules in a flow strong enough for a rear concavity to appear. Furthermore, the parachute depth has to be higher than the lowest possible value for all the possible size ratios. At high Ca, a comparison is made between the mean capillary number and the critical capillary number Ca_c for which no steady state exists. The value of Ca_c for $\alpha = 0\%$ is given in (Chu et al., 2011), while the one for $\alpha = 1.5\%$ is estimated from the maximal values of the current database. The same values are found for the investigated a/R range. The capsule is excluded if $Ca_c - Ca < 0.02$. Finally, if the capsule cannot be fitted and its rear concavity is larger than the highest possible value in the database, the capsule is excluded, as it could be that it has not reached a steady state.

3. Results

All the selected capsule profiles can be fitted with the neo-Hookean model, with either value of the pre-inflation parameter. The number of analyzed capsules for each HSA concentration is given in Table 2. It depends on α because of the filtering process described above.

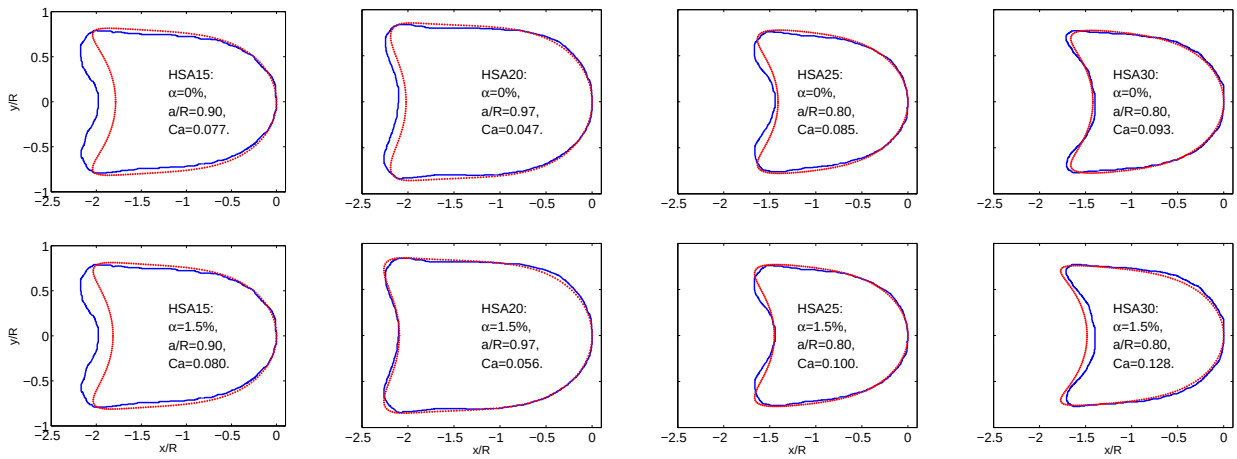

Figure 2: Comparison between measured (red) and simulated profiles (blue). Top: $\alpha = 0\%$. Bottom: $\alpha = 1.5\%$.

[HSA] (%(w/v))	15	20	25	30
$\alpha = 0\%$	12	9	8	4
$\alpha = 1.5\%$	7	9	14	5

Table 2: Number of analyzed capsules as a function of the HSA concentration and pre-inflation ratio.

Typical comparisons between experimental and numerical profiles are presented in Fig. 2 for the capsules which passed the selection with both pre-inflation ratios. The overlap between numerical and experimental profiles is within the experimental error for the HSA20, HSA25 and HSA30 capsules. Some small discrepancies of about 10 µm exist, mainly at the capsule rear (i.e. HSA25 capsules with $\alpha = 0\%$), but the experimental-numerical correspondence is overall very good. This is less verified for the HSA15 capsules. The capsule rear shape is then not well described by the numerical model. It may be due to the fact that the bending resistance, associated with the actual membrane thickness, can no longer be neglected in the case of the shear-softer HSA15 capsules.

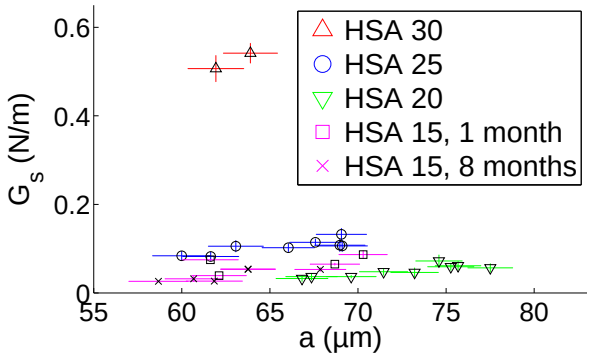

Figure 3: G_s as a function of the capsule radius a for $\alpha = 0\%$. Horizontal error bars correspond to the uncertainty on the radius measurement, while vertical error bars are defined as $\pm\sigma$.

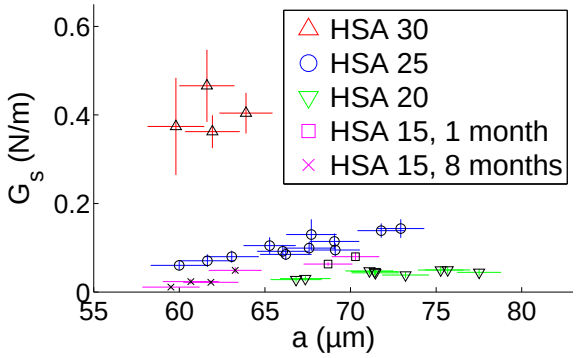

Figure 4: G_s as a function of the capsule radius a for $\alpha = 1.5\%$. Error bars: see Fig. 3.

For a given capsule, σ/G_s is an estimate of

the precision of the G_s determination. In the following analysis, we only keep capsules for which $\sigma/G_s < 1/3$. The surface shear modulus is presented in Fig. 3 ($\alpha = 0\%$) and in Fig. 4 ($\alpha = 1.5\%$) as a function of the radius for all the HSA concentrations. We find that the modulus G_s is lower for $\alpha = 1.5\%$ than for $\alpha = 0\%$. Finding a lower value with increasing pre-inflation ratios is consistent with the fact that the pre-stress strengthens the membrane. We also note a slight tendency towards an increase of G_s with the capsule size. However, since we have only considered a narrow size range in this study, we can ignore this effect and introduce a mean value $< G_s >$ for a given HSA concentration. No clear effect of storage time could be detected for HSA15.

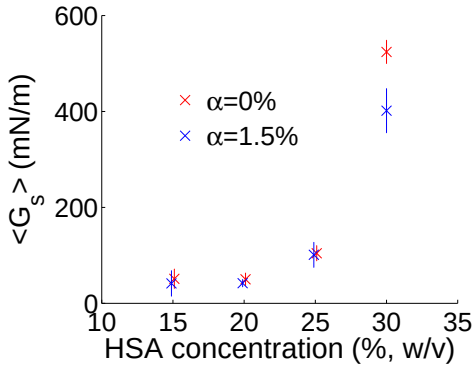

Figure 5: G_s as a function of HSA concentration. Error bars: see Fig. 3. Results are slightly shifted horizontally for the HSA15, 20 and 25 sets for clarity.

The influence of HSA concentration on $< G_s >$ is shown in Fig. 5 for both pre-inflation ratios. It is found that the membrane shear modulus increases significantly with the HSA concentration. For instance for $\alpha = 0\%$, $< G_s >$ increases by about one order of magnitude (from 51 mN/m to 524 mN/m), when the HSA concentration is increased from 15 to 30% (w/v). It is interesting to notice that the mechanical properties seem to converge towards a low value plateau, when decreasing the concentration in protein. There, however, exists a limiting HSA concentration, under which capsules can no longer be produced. For the present emulsification speed (1700 rpm), capsules could not be fabricated for a 10% (w/v) HSA concentration.

The increase in mechanical strength of the membrane with the HSA concentration could be due to two phenomena: a thickening of the capsule wall, or a tightening of its molecular structure. Direct measurements of the membrane thickness and of the covalent bonds density are needed before we can resolve this question.

4. Conclusion

The microfluidic method allowed us to characterise capsules with a cross-linked HSA membrane prepared under different physicochemical conditions. We find that capsules with a radius in the range 55-80 μm and a HSA concentration between 15% and 30% (w/v) have a membrane which can be described by a neo-Hookean model. A possible pre-inflation effect due to osmolarity is accounted for by imposing various values of pre-inflation. Small values of pre-inflation are sufficient to fit the data, which indicates that the capsule membrane adapt to osmotic stretching over long time durations. Accounting for pre-inflation changes only slightly the surface shear elastic modulus of the membrane. Whatever the pre-inflation ratio, the shear modulus sharply increases with the HSA concentration from a low-value plateau. The origin of this strengthening is currently under investigation, altogether with the capsule behavior for different radii and concentration windows.

Acknowledgements

We thank Océane Ly, Van Tuan Dang and Abbas Hamadi for their participation in the experimental characterisation. The research was funded by the French Agence Nationale de la Recherche through the CAPSHYDR grant (ANR-11-BS09-013) and Labex MS2T (ANR-11-IDEX-0004-02), by the Conseil Régional de Picardie (MODCAP grant) and by the French Ministère de la Recherche (Pilcam2 grant).

References

Banquet, S., Gomez, E., Nicol, L., Edwards-Lévy, F., Henry, J.P., Cao, R., Schapman, D., Dautreaux, B., Lallemand, F., et al., F.B., 2011. Arteriogenic therapy by itramyocardial sustained delivery of a novel growth factor combination prevents chronic heart failure. Circulation 124, 1059–1069.

Cho, K., Wang, X., Nie, S., Chen, Z., Shin, D.M., 2008. Therapeutic nanoparticles for drug delivery in cancer. Clin. Cancer Res. 14(5), 1310–1316.

Chu, T., Salsac, A.V., Leclerc, E., Barthès-Biesel, D., Wurtz, H., Edwards-Lévy, F., 2011. Comparison between measurements of elasticity and free amino group content of ovalbumin microcapsule membranes : Discrimination of the cross-linking degree. J. Colloid Interface Sci. 355, 81–88.

Demirgöz, D., Pangburn, T., Davis, K., Lee, S., Bates, F., Kokkoli, E., 2009. PR B-targeted delivery of tumor necrosis factor-a by polymersomes for the treatment of prostate cancer. Soft Matter 5, 2011–2019.

Edwards-Lévy, F., Andry, M., Lévy, M., 1993. Int. J. Pharm. 96, 85–90.

Glycerine Producer's Association, 1963. Physical properties of glycerine and its solutions. New-York.

Lefebvre, Y., Barthès-Biesel, D., 2007. Motion of a capsule in a cylindrical tube: effect of a membrane pre-stress. J. Fluid Mech. 589, 157–181.

Orive, G., Hernández, R., Gascón, A.R., Calafiore, R., Chang, T., de Vos, P., Hortelano, G., Hunkeler, D., Lacík, I., Pedraz, J., 2004. History, challenges and perspectives of cell microencapsulation. Trends in Biotechnology 22(2), 187–92.