Journal of Colloid and Interface Science xxx (2010) xxx–xxx

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

Comparison between measurements of elasticity and free amino group content of ovalbumin microcapsule membranes: Discrimination of the cross-linking degree

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ARTICLE INFO

Article history:
Received 23 September 2010
Accepted 10 November 2010
Available online xxxx

Keywords:
Mechanical properties
Inverse analysis
Microcapsule population
Microfluidic technique
Cross-linking degree

ABSTRACT

An inverse method is used to characterize the membrane mechanical behavior of liquid filled microcapsules. Cross-linked ovalbumin microcapsules are flowed and deformed into a cylindrical microchannel of comparable size. The deformed shape is compared to predictions obtained numerically when modeling a capsule under the same flow conditions. The unknown shear modulus value corresponds to the best fit. The degree of reticulation is estimated in parallel by determining the free amino groups remaining on the microcapsules after the cross-linking reaction. We characterize microcapsule populations fabricated at different reaction pH (5–8) and times (5–30 min) to study different cross-linking degrees. The capsule shear modulus and the amino groups are nearly constant with the reaction pH for the capsules fabricated after 5 min of reticulation. The shear modulus increases with the reaction time, while the NH_2 content decreases with it. A global increase in shear modulus with pH is also observed, together with an unexpected increase in NH_2 content. The study shows that the inverse method is capable of discriminating between various cross-linking degrees of microcapsules. Moreover, for this type of microcapsules, the mechanical method appears more reliable than the chemical one to obtain an estimation of their cross-linking degree.

1. Introduction

A capsule is a liquid droplet enclosed within a thin deformable membrane. Capsules are widely used in many domains such as in the pharmaceutic [23], cosmetic [12] and textile industries [17]. The membrane plays an important role, as it not only protects the internal liquid but also controls the exchanges with the external medium (by diffusion or membrane rupture). Measuring the mechanical properties of the capsule membrane therefore provides needed information to improve the design of capsules.

The elasticity of the capsule membrane is typically characterized by applying a deformation and measuring the resultant stress (or vice versa). Different experimental methods exist to deform the capsules. For millimetric capsules, compression experiments have been used [11,20,6]. The experiment consists in compressing a capsule between two parallel plates and measuring the compression force as a function of the distance between the plates. An analytical or numerical model, based on an assumed constitutive law, is then used to deduce the membrane mechanical properties. For micrometric capsules, the characterization is more difficult due to their small size. The micropipette aspiration experiment is the most used technique [5,16]. The membrane elastic properties are determined by measuring the capsule length aspirated into the micropipette under various pressures. Another technique is to use an atomic force microscope (AFM) [11,21,18] to deform the capsule under a known force. Despite the skills required by the micromanipulation, both techniques have been used with success on individual microcapsules. However, they do not enable the characterization of a capsule population.

Recently we have developed a technique to determine the shear modulus of microcapsules in batch that combines a microfluidic experimental technique with a numerical simulation [15]. Its two main advantages are the possibility to characterize an entire population and the small sample volume required for the test. Our objective is to use this technique to study the relation between the mechanical properties and the physico-chemical conditions used during the fabrication of the microcapsules. Ovalbumin membrane capsules are prepared using the interfacial cross-linking method. In this method, a cross-linking agent, i.e. terephthaloyl chloride, reacts with accessible free residues of the protein at the interface of a water-in-oil emulsion, leading to the formation of a network of crosslinked protein around the aqueous droplets of the emulsion. The free residues potentially concerned by this acylation reaction are amino groups, hydroxyl groups and carboxylic groups of the constitutive aminoacids of the protein, giving respectively amide, ester and anhydride links upon acylation with the

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0021-9797/$ - see front matter © 2010 Published by Elsevier Inc.
doi:10.1016/j.jcis.2010.11.038

cross-linking agent. Different batches of microcapsules are prepared varying the parameters controlling the reticulation, i.e. the pH and reticulation time. The inverse analysis technique is applied to characterize the different capsule populations mechanically. Chemical assays are conducted in parallel to estimate the level of reticulation. The method, developed by Edwards-Lévy et al. [9], consists in the determination of the amount of free amino groups remaining in the capsule membrane after the cross-linking reaction. It is assumed to be a good estimate of the degree of reticulation. This method has been applied to human serum albumin (HSA) capsules [9,10,1], for which the free amino group content has been found to vary globally like the inverse of the degree of cross-linking degree. We propose to apply it to ovalbumin capsules.

In Section 2, we present the techniques of fabrication of the microcapsules and of the microfluidic systems. We also describe briefly the microfluidic experiments, numerical model, inverse method and free amino group assay. The results of the characterization of the elastic properties and of the determination of the free amino group content are presented in Section 3 for various microcapsule populations. In Section 4, we discuss the relation between the physico-chemical conditions used during the microcapsule fabrication and their elastic properties, before concluding in Section 5.

2. Materials and methods

2.1. Materials

2.1.1. Microcapsule fabrication

Microcapsules are prepared using the interfacial cross-linking method already described by Edwards-Lévy et al. [9] and varying some of the preparation parameters to obtain membranes with different cross-linking degrees surrounding liquid droplets. Briefly, a 10% (w/v) ovalbumin (Sigma) solution is prepared using a phosphate buffer with various pH values (5, 5.9, 6.8, 7.4 and 8). This solution is emulsified in cyclohexane (SDF) containing 2% (w/v) sorbitan trioleate (Sigma) at a stirring speed of 1550 rpm to adjust the mean diameter of the resulting particles to about 50 μm. A 2.5% (w/v) solution of terephthaloyl chloride (Acros) in chloroform:cyclohexane (1:4 v/v) is then added to the emulsion and the cross-linking reaction is allowed to develop for various times (5 min, 15 min and 30 min). The reaction is stopped by diluting the reaction medium with cyclohexane. The microcapsules are separated from the organic phase by centrifugation, and washed successively with cyclohexane, with water containing 2% (w/v) polysorbate (Sigma) and finally thrice with pure water. The samples are kept in aqueous suspension for mechanical evaluation, or freeze-dried for amino group determination.

2.1.2. Microfluidic system fabrication

The microfluidic system consists of a glass tube (Beckman Coulter) of 75 μm internal diameter and 1.5 cm length (Fig. 1a). The tube is inserted inside another glass tube of 400 μm internal diameter and connected to the perfusion system (1.5 mm in external diameter) through a silicon pipe. As the dimension of the perfusion system is much larger than the capsule diameter, the capsules are ensured to have a spherical shape when entering the channel. The compound system is immersed in 10 ml of polydimethylsiloxane (PDMS) mixed with 10% of curing agent in a small box, 12 cm^2 in surface area. The system is degased under a pressure of the order of 10^{-3} Pa for 45 min and heated at 70 °C in an oven for 1 h. The thickness of the PDMS layer (0.5 cm) is sufficient to fix the channel system and small enough to guarantee a good image quality. There is little optical distortion as the refractive indices of PDMS (1.45), glass (1.5) and of the capsule solution (1.47) only differ by a small amount [15].

2.1.3. Suspension preparation

A suspension containing 2% of microcapsules in volume is prepared by mixing 20 μl of capsule sediment in 1 ml of 100% glycerol. Glycerol is used because of its high viscosity, miscibility with water and low toxicity. Once prepared, the suspension is used immediately to avoid osmotic effects between the capsules and the glycerol solution. The capsule concentration is considered constant during the experiment. The viscosity of the glycerol suspension slightly differs from that of pure glycerol due to the small amount of water contained in the capsule sediment. The dependency of the viscosity μ (in Pa s) of the suspension with temperature T (in °C) is measured with a Couette viscometer (Thermo Haake 1) and found to follow the regression law

$$\mu - 0.85 = -0.05(T - 20). \quad (1)$$

For each experiment, we record the room temperature and evaluate the suspension viscosity accordingly.

2.2. Experimental procedure

A syringe pump containing the capsule suspension is connected to the microfluidic system to perfuse the microchannel. The pump flow rate is varied from 0.13 ml/h to 0.54 ml/h, which induces a pressure drop along the microchannel between 2.2×10^5 and 9.13×10^5 Pa. Higher flow rates would result in the microsystem destruction by overpressure. The capsule motion in the 75 μm channel is observed with a microscope (Optika) and recorded with a high-speed camera (IF 800, Japan) at 150 frames/s and a shutter speed in the order of 10^{-3} s. The microscope is focused on the axial plane of the channel so that we obtain the cross section of the capsule in its plane of symmetry. The images are recorded in a cross-section located at 7.5 mm (i.e. 200 channel radii) from the channel entrance, where the flow is considered to be at steady state. Indeed Diaz and Barthès-Biesel [8] have shown that a typical capsule reached a steady deformed shape at distances from the entrance of the order of 5–10 tube radii.

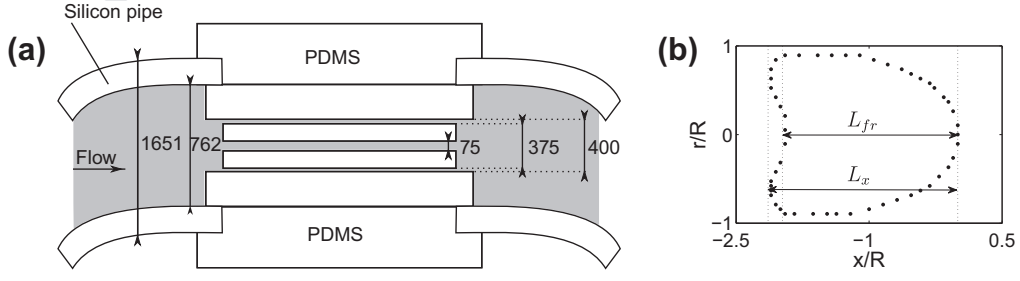

Fig. 1. (a) Schematics of the cylindrical microchannel setup to be connected to a syringe pump, following Lefebvre et al. [15]. Dimensions in μm (not to scale). (b) Characteristic geometrical quantities L_x, L_{fr} of the deformed capsule profile.

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The capsule velocity v is calculated from two successive images knowing the camera frame rate. The software ImageJ is used to extract the contour by placing 30–50 points manually along the capsule edge. The contour is split into two about the tube axis Ox. We calculate the capsule volume using the upper and lower half-profiles assuming axisymmetry and averaging the two values. From the volume, we obtain the initial capsule radius a and size ratio a/R, R being the tube radius. The contour determination is estimated to lead to a 2% error on the size ratio. We measure characteristic geometrical quantities of the experimental profile: the maximum L_x and minimum L_{fr} lengths of the capsule along the axis Ox and the area S of the meridional profile (Fig. 1b). The quantities L_x and S are obtained averaging the values for the top and bottom half-profiles.

2.3. Numerical model

2.3.1. Problem statement

The numerical model initially proposed by Quéguiner and Barthès-Biesel [19] and later improved by Diaz and Barthès-Biese [8] and Barthès-Biesel [14] is used to determine the flow of a capsule in a cylindrical channel of comparable size. The model assumptions and results are only briefly outlined; more details may be found in Lefebvre and Barthès-Biesel [14].

An initially spherical capsule of radius a is filled with an incompressible Newtonian liquid of viscosity μ_c and enclosed by an infinitely thin elastic membrane. The capsule is placed in another incompressible Newtonian liquid of viscosity μ at temperature T, flowing with mean velocity U in a cylindrical channel of radius R (Fig. 2). The flow Reynolds number is supposed to be small so that the internal and external fluid motion obeys the Stokes equations. Buoyancy effects are negligible. Consequently, the capsule is centered on the tube axis and deforms axisymetrically.

The membrane is assumed to consist of an infinitely thin sheet of hyperelastic isotropic material with surface shear modulus G_s and area dilation modulus K_s. Under the condition of axisymmetry, the principal directions of the surface deformation tensor are along the meridian and parallel curves with corresponding principal extension ratios λ_1 and λ_2, respectively. The elastic tensions in the membrane (forces per unit arc length measured in the membrane plane) have principal components T_1 and T_2 also directed along the meridian and parallel curves, respectively. Different membrane constitutive laws, relating elastic tensions to deformations, can be proposed to model thin membranes [3]. In particular, it is possible to use a law which is either strain-softening or strain-hardening under large deformation. The law must be such that it leads to a constant value of the elastic modulus for large or small deformation. In a previous study of similar ovalbumin capsules, it was shown that a strain-hardening law was not appropriate to describe the capsule membrane, whereas a neo-Hookean (NH) strain-softening law led to constant values of the membrane elastic shear modulus [15]. Consequently, we have used here a neo-Hookean law that assumes that the membrane is an infinitely thin sheet of a three-dimensional isotropic volume-incompressible material. The principal elastic tensions are expressed by [3,15]:

$$T_1 = \frac{G_s}{\lambda_1\lambda_2}\left[\lambda_1^2 - \frac{1}{(\lambda_1\lambda_2)^2}\right], \quad T_2 = \frac{G_s}{\lambda_1\lambda_2}\left[\lambda_2^2 - \frac{1}{(\lambda_1\lambda_2)^2}\right]. \tag{2}$$

The area dilation modulus is then shown to be $K_s = 3G_s$.

The problem is solved by means of a boundary integral technique, coupled to the Lagrangian tracking of the capsule interface [8,14]. The model inputs are

- The capillary number $Ca = \mu U/G_s$ that measures the ratio between the viscous and elastic forces.
- The size ratio a/R.
- The membrane constitutive law.

The model outputs are the capsule deformed profile at steady state and the velocity ratio v/U. It also provides other parameters that cannot be measured experimentally such as the pressure difference Δp between the front and back of the capsule, the elastic tension in the capsule membrane and the surface strain energy of the capsule. Since the capsule profile is axisymmetric, it is motionless at steady state. Consequently there is no internal motion at steady state and the internal viscosity does not play any role. The latter only influences the transient phases of the capsule motion.

2.3.2. Numerical results for NH law

Lefebvre [13] has shown that a critical capillary number Ca_c exists for the NH law, beyond which a capsule does not reach a steady state. We have evaluated the value of Ca_c as a function of the capsule size ratio (Fig. 3). The critical capillary number decreases when the size ratio increases. This result enforces a limit on the experimental conditions that can be tested, and specifically on the capsule velocity.

When a steady state exists, the numerical model provides values for the geometrical non-dimensionalized parameters L_x/R, L_{fr}/R, $(L_x - L_{fr})/R$, S/R^2 of the deformed capsule and for the velocity ratio v/U as a function of a/R and Ca (Fig. 4). L_x and L_{fr} increase with the capillarynumber (i.e. the deformation) except for small capsules ($a/R < 0.99$) at low capillary numbers ($Ca < 0.02$) (Fig. 4a and b). The quantity $L_x - L_{fr}$ provides information on the presence of a parachute shape with a reversed rear curvature. Fig. 4c shows that it increases with the capillary number. The most discriminating parameters are clearly L_x and $L_x - L_{fr}$, provided the size ratio is of order unity or above. Fig. 4d shows that the meridional area S/R^2 does not vary much with the capillary number and depends mainly on the size ratio. The velocity ratio v/U, shown in Fig. 4e, increases monotonically with the capillary number.

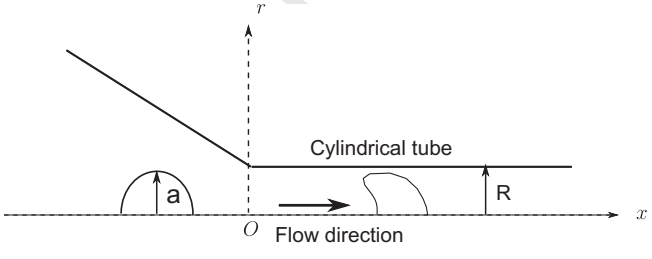

Fig. 2. Geometry used in the numerical simulation: the undeformed capsule is initially positioned in the hyperbolic entrance, before being flowed into the cylindrical channel.

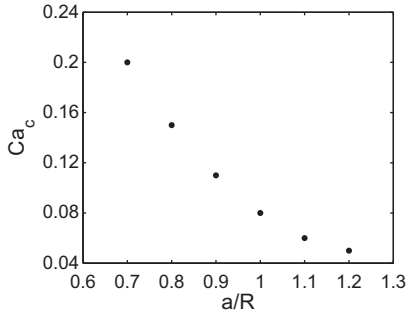

Fig. 3. Critical capillary number as a function of the capsule size ratio.

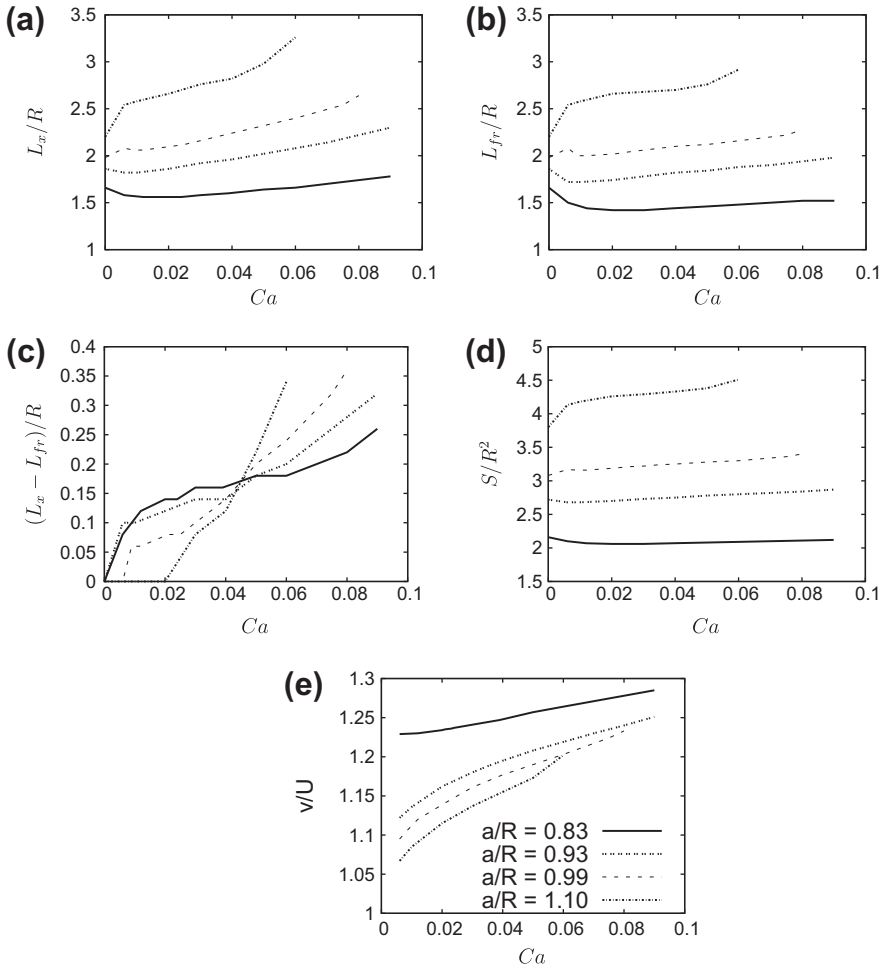

Fig. 4. Abaci of the results provided by the numerical code: evolution of the quantities L_x/R (a), L_{fr}/R (b), $(L_x - L_{fr})/R$ (c), S/R^2 (d) and v/U (e) as a function of the capillary number.

2.4. Inverse analysis procedure

The surface shear modulus of the different capsule populations is determined through inverse analysis. For an experimental value of a/R, we search the charts of Fig. 4 for the value of capillary number Ca, at which the numerical parameters L_x/R, L_{fr}/R and S/R^2 are equal to the experimental values. If two or more possible capillary numbers are found, we average the values. The fitting process is done using a small tolerance that accounts for the experimental errors in the contour determination, due to fuzziness. The tolerance is defined as the relative difference between the experimental and numerical values of the deformed capsule geometrical parameters. For example, the tolerance for the length L_x is defined by

$$\Delta(L_x/R) = |(L_x/R)_{num}/(L_x/R)_{exp} - 1| \quad (3)$$

with similar definitions for the other parameters shown in Table 1. A larger tolerance is accepted for L_{fr} than for L_x and S because of the uncertainty of the capsule profile in the concave region. The value of the capillary number Ca provides the ratio v/U using Fig. 4e and thus the flow velocity U. The shear elastic modulus is then given by $G_s = \mu U/Ca$.

Table 1
Tolerance values.

Parameters	$\Delta(a/R)$ (%)	$\Delta(L_x/R)$ (%)	$\Delta(L_{fr}/R)$ (%)	$\Delta(S/R^2)$ (%)
Errors	2	4	5	4

2.5. Determination of microcapsule free amino groups

The determination of the amino groups remaining on the microcapsules after the cross-linking reaction can be an indirect indication of the cross-linking degree of the microcapsule membranes. The assay is performed using the TNBS method, which has been described elsewhere [9], where TNBS stands for trinitrobenzenesulfonic acid. Briefly, 10 mg of microcapsule powder is incubated with an excess of TNBS (Sigma, 4 μmol/mL in 0.2 M borate buffer, pH 8). After 1 h of reaction in the dark at 40 °C, the medium is filtered. In a second step, the excess of TNBS is determined in the filtrate using an excess of valine (Aldrich, 40 μmol/mL in 1% trichloroacetic acid) and a further 1-h incubation. The resulting trinitrophenyl-valine derivative is determined spectrophotometrically at 410 nm. The values, expressed as μmol amino groups per gram of dried microcapsules, are corrected after the determination of the water content of the freeze-dried samples, using a halogen moisture analyzer (HR73, Mettler-Toledo). The mean values are calculated from four determinations: two samples are analyzed per batch and two batches of microcapsules are analyzed for each value of the varied parameters.

3. Results

3.1. Shear modulus determination

We have characterized the series of capsules fabricated at various pH and times of reticulation using the inverse analysis method

described above. For each capsule population, different size ratios and capsule velocities v are considered. Typical capsule shapes are illustrated in Fig. 5a. For a given value of a/R, the capsules experience small deformations with convex front and back at low velocities (i.e. Ca), and larger deformations with convex front and largely concave rear when the velocity is increased. Examples of the corresponding fit between the experimental and numerical capsule profiles are shown in Fig. 5b.

We first consider the results for the microcapsule populations fabricated after a time of reticulation $t_r = 5$ min. The quantitative results of the shear modulus G_s are plotted in the first column of Fig. 6 as a function of a/R for the different values of pH.

These graphs show that the capsule size ratio does not influence the mechanical properties since the measured shear modulus is found to be constant for all tested values of a/R. The mean value and standard deviation found for each pH are indicated on the graphs. The evolution of the mean shear modulus with the pH of reaction is summarized in Fig. 7a. The mean value of the shear modulus remains almost constant between pH 5 and pH 7.4. However, it increases for pH 8. The reaction pH yields a similar trend at higher times of reticulation (Fig. 7a). A sharp increase in G_s is consistently found at pH 8. For a 30 min reticulation times, the measured mean shear modulus seems to increase with the pH, but the standard deviations is also much larger. In conclusion, for a given reticulation time, the mean value of G_s is insensitive to pH for pH $\leqslant$ 7.

The data are plotted in Fig. 7b as a function of the time of reticulation to emphasize its influence on the capsule shear modulus. This shows that G_s, which is assumed to reflect the degree of cross-linking, increases with the reticulation time for all the values of pH. The inflection of the curves at pH = 5, 7.4 and 8 indicates a saturation process of the cross-linking reaction. The characteristic saturation time varies with the pH.

3.2. Free amino group determination

The content in free amino groups NH_2 has been determined using the TNBS method for all the capsule populations. Its variation with the pH of reaction is presented in Fig. 8a for various reaction times. It shows that the NH_2 content is nearly constant for a reaction time of 5 min. For longer reaction times, the amino group content of the microcapsules globally increases with the reaction pH. The standard deviations are also found to be smaller than at $t_r = 5$ min, which indicates that the pH effect is more reproducible when the reaction time increases.

The influence of the time of reticulation is more clearly observable in Fig. 8b. On the whole, the amino group content decreases with the time of reticulation, corresponding to a progressive acylation of these groups during the cross-linking reaction. The kinetics of the phenomenon seems different for a reaction pH of 8, showing a faster decrease of amino groups until 15 min and an increase between 15 and 30 min. A saturation process occurs at pH = 5, 7.4 and 8 as indicated by the inflection of the curves.

4. Discussion

The comparison of the mechanical and chemical results, shows that both quantities are constant between pH 5 and pH 7.4 for capsules fabricated after 5 min of reticulation (Figs. 7a and 8a). For this reaction time, the contact between the reactants is so short that the influence of pH cannot be detected on the degree of reticulation. This leads to identical mechanical properties for all capsules. An influence of the reaction pH is found only at pH 8, when the pH becomes clearly alkaline, favoring a rapid acylation of the functional groups of the protein. These results show that a transition occurs in the reticulation reaction depending on pH.

For longer reaction times, the discrimination between the various capsule populations becomes possible. In general the shear modulus increases with the pH, which is in agreement with the positive influence of an increase in reaction pH on the cross-linking reaction.

4.1. Influence of the reaction pH and time on membrane cross-linking

Fig. 8a shows that the free NH_2 content overall increases with the pH. This observation is surprising, as previous studies performed on cross-linked human serum albumin (HSA) microcapsules showed a decrease in amino group content when the pH increased, interpreted as an increase of cross-linking degree in the membranes [9,10,1]. In these previous studies, two groups could be distinguished in microcapsule batches as a function of reaction pH. The transition between loosely cross-linked microcapsules with smooth membranes and high NH_2 content, and highly cross-linked membranes with rugged membranes and low NH_2 content, took place between pH 7.4 and 8. Moreover, Andry and Lévy [2] showed a marked dependence of enzymatic degradation of HSA capsules on the reaction pH, capsules prepared at pH $\geqslant$ 9 showing a delayed degradation in trypsin compared to those prepared at pH $\leqslant$ 8.

Microscopic observations of the different ovalbumin microcapsules show that an increase in reaction pH leads to more spherical capsules and rougher membranes, whatever the reaction time. This observation is consistent with the previous studies on cross-linked HSA microcapsules. A raise in the reaction pH was shown to give rougher membranes and lead to a parallel decrease in free amino group content on the microcapsules. As a matter of fact, amino groups become unionized at higher pH, and are acylable only in this form. Consequently an increase in the reaction pH produces more cross-linked microcapsules.

In the present work dealing with ovalbumin microcapsules, the evolution of amino group content as a function of the reaction pH seems inconsistent with microscopic observations and with the previous studies. Another phenomenon must then account for this unexpected result. A hypothesis can be drawn, related to protein unfolding at interfaces. In the presence of an interface, proteins change their conformations to minimize the free energy, so that the polar residues are oriented towards the polar phase, and the

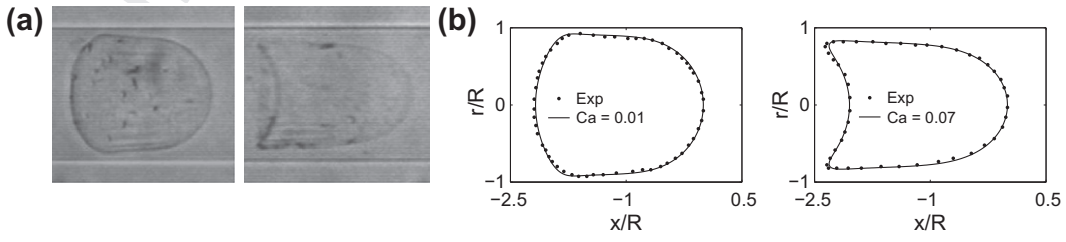

Fig. 5. Example of 2 capsules, fabricated at pH 5 with $t_r = 5$ min, flowing down the microchannel: $a/R = 1$, $v = 1.4$ mm/s (left), $a/R = 0.95$, $v = 4.8$ mm/s (right). (a) Pictures of the deformed capsule shape. (b) Corresponding superposition of the experimental (dotted line) and numerical (continuous line) profiles.

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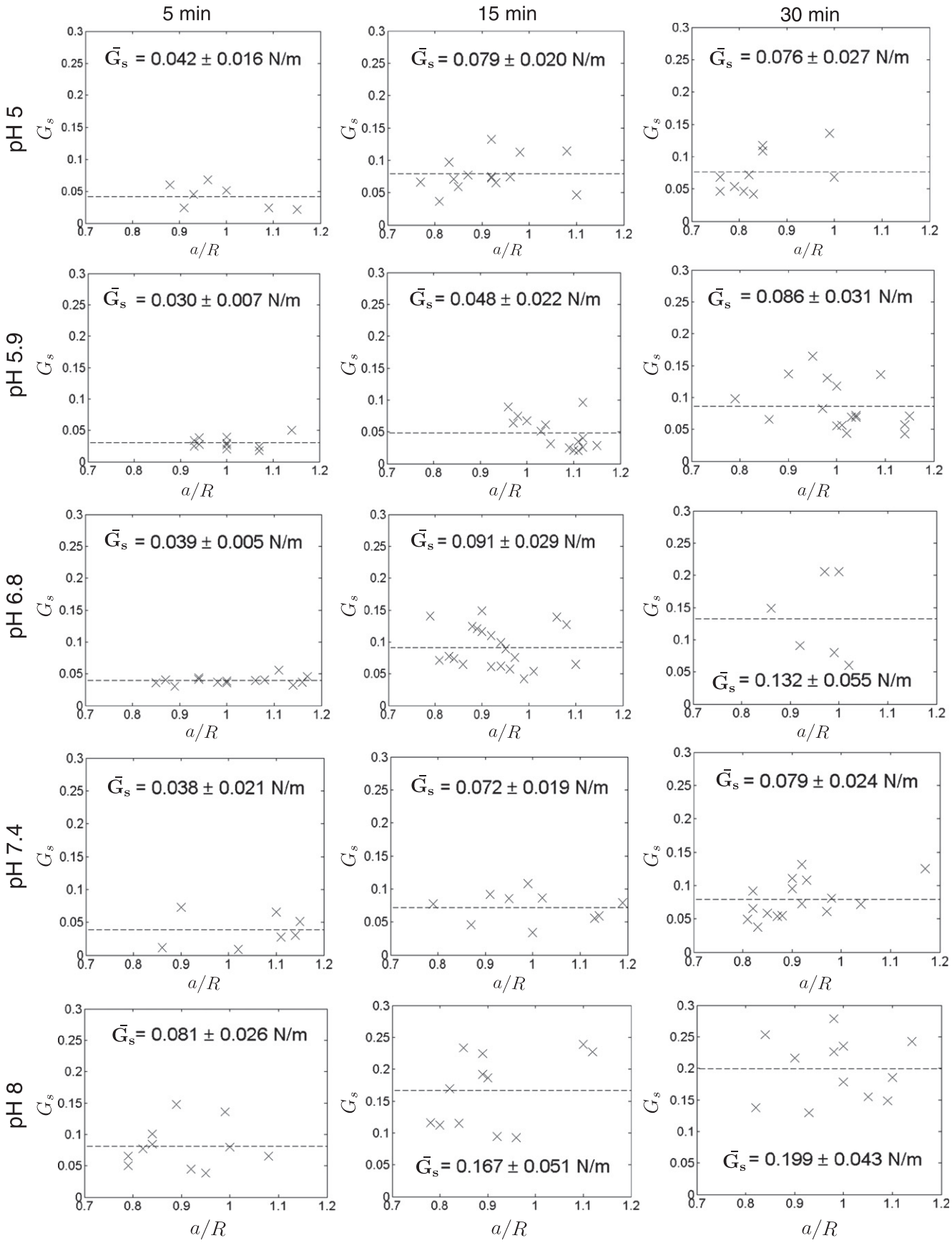

Fig. 6. Membrane shear modulus G_s of the capsules fabricated at different pH and times of reticulation as a function of the capsule size ratio. The dashed line represents the mean value of G_s, which is provided in each graph with the standard deviation.

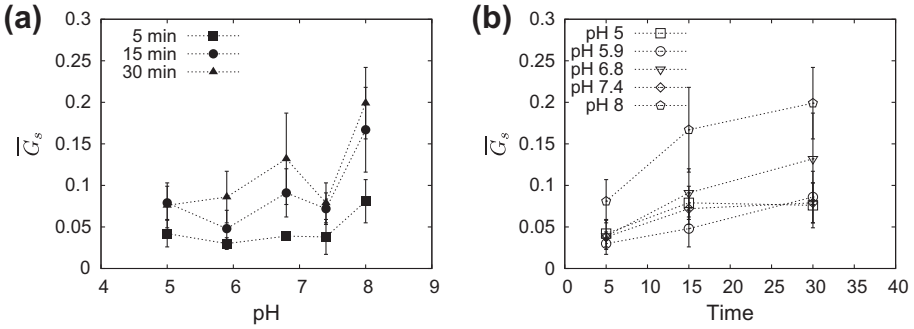

Fig. 7. Variation of the mean shear modulus of the microcapsule populations as a function of the (a) reaction pH and (b) time of reticulation.

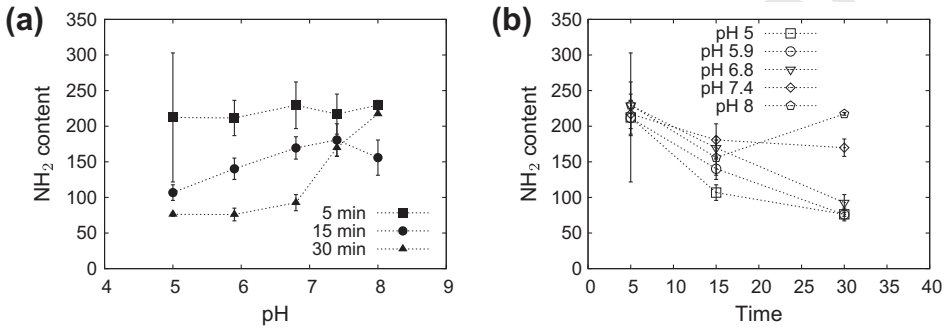

Fig. 8. Variation of the free amino group content of the microcapsule populations as a function of the (a) reaction pH and (b) time of reticulation.

non-polar residues towards the non-polar phase [22]. Protein unfolding at the interface of an emulsion then leads to exposure of previously buried groups. The number of ε-amino groups of lysine residues available for chemical reaction depends on the nature of the non-aqueous phase and on the pH [7]. This phenomenon is amplified at pH away from the isoelectric point of the protein [4].

Protein cross-linking and protein unfolding lead to opposite effects on the total number of amino groups available for the TNBS. The TNBS assays would then show the result of the two phenomena, for which both kinetics depend on the pH. This contribution of previously buried amino groups to the total number of accessible groups was not detected in the previous studies involving HSA. Ovalbumin is known to be very sensitive to surface denaturation. Furthermore HSA and ovalbumin possess close isoelectric points (HSA: 4.7; Ovalbumin: 4.9), but very different lysine contents, HSA bearing more lysine residues than ovalbumin (13 g lysine/100 g HSA; 5.7 g lysine/100 g ovalbumine). Finally, the TNBS assay applied to ovalbumin cross-linked membranes gives results that are not directly related to the degree of cross-linking of these membranes, a fortiori if the reaction pH is away from the isoelectric point of ovalbumin.

4.2. Correlation between the mechanical and physico-chemical tests

The evolutions of the mechanical and physico-chemical results with the reticulation time have opposite trends: the shear modulus increases with the reticulation time, while the free NH_2 content overall decreases with it (Figs. 7b and 8b). To see whether the two quantities follow a simple correlation, we plot the shear modulus as a function of the inverse of the free NH_2 content in Fig. 9. The graph shows that the shear modulus is approximately inversely proportional to the free amino group content for pH = 5–5.9. It proves that the inverse analysis method based on microfluidic experiments has the capability to discriminate between the various capsule behaviour. However, the correlation does not hold

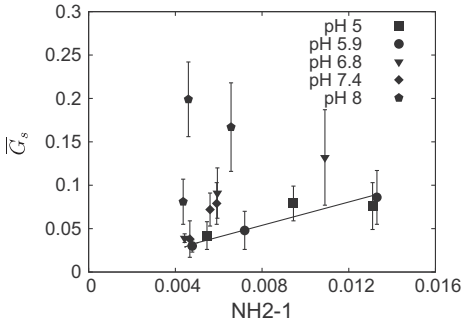

Fig. 9. Evolution of the mean shear modulus as a function of the inverse of the free amino group content for the different microcapsule populations. The straight line shows the linear correlation between $\overline{G}_s$ and the $(NH_2 \text{ content})^{-1}$ for pH < 6.

for pH ⩾ 6.8. This confirms that for this type of microcapsules the free amino group content no longer estimates the degree of reticulation at higher pH. To go further in the explanation of the present results, alternative methods to determine precisely the cross-linking degree of microcapsules need to be implemented. The determination of terephthalic residues on the membranes could be an option to quantify the cross-linking degree, but the whole experimental procedure remains to be performed.

5. Conclusion

We have used an inverse method based on a microfluidic experiment combined with a numerical model to characterize the elastic properties of different series of microcapsules with a cross-linked ovalbumin membrane. Deforming microcapsules under a shear stress offers many advantages. The microfluidic technique enables to easily manipulate micrometric capsules in batch. Entire populations of microcapsules can be characterized in order to verify the

homogeneity in mechanical properties. Furthermore a small quantity of substance is needed owing to the micrometric channel size.

We have succeeded in characterizing in batch various populations of capsules obtained by modifying the conditions of fabrication. The shear modulus and NH_2 content are found to hardly change for $5 \leqslant pH \leqslant 7.4$ for short times of reticulation. Compared to the reaction pH, the time of reticulation has a larger influence on the shear modulus. Our study shows that the shear modulus increases with the reaction time. The longer the reactants remain in contact, the higher the shear modulus.

For low pH values, an inverse correlation is found between the shear modulus and NH_2 content, the two quantities being inversely proportional. Since the NH_2 content is inversely correlated with the degree of reticulation in this pH range, the study proves that the mechanical properties are a direct consequence of the reticulation induced during the fabrication. All these results show that we can discriminate the effect of the conditions of fabrication on the membrane properties. They prove that the inversed analysis method is a useful tool for the design of new types of microcapsules and for their characterization in batch. Moreover, this method appears more useful to obtain accurate data characterizing membrane reticulation degree, in the case of unexpected secondary phenomena perturbing the interpretation of TNBS results. The TNBS test appears to fail for $pH \geqslant 7.4$ in the case of ovalbumin capsules. New tests are required to determine the cross-linking degree of the microcapsules precisely. They would enable a further investigation of all the effects of the physico-chemical conditions on the mechanical properties of the capsule membrane.

Acknowledgments

The authors gratefully acknowledge the Projet Pluri-Formations PILCAM2 at the Université de Technologie de Compiègne for providing HPC resources for calculations. We thank the Ministère de l'Éducation et de la Recherche for the PhD grant of Thi Xuan Chu. This study was supported by the Conseil Régional de Picardie through the project μ Fiec.

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