Journal of Microencapsulation, 2012; 29(2): 147–155

ISSN 0265-2048 print/ISSN 1464-5246 online
DOI: 10.3109/02652048.2011.635220

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Sealing liquid-filled pectinate capsules with a shellac coating

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Abstract

Liquid-filled pectinate capsules have a large potential for the controlled and site-specific delivery of liquid drugs. Earlier studies have shown that pure pectinate capsules can store drugs only for a few minutes. Here, we show that the retention time can be extended to several hours by coating the capsules with the natural resin shellac. A bilberry extract containing anthocyanins with promising therapeutic properties was used as model drug to characterize the permeability of the capsules by *in vitro* drug release measurements. Characterizing the structure of the double-layered capsule membranes by NMR microscopy, we optimized the capsule production by adjusting the pH-value in the coating process and the gelation time of the pectinate hydrogel layer. A comparison of the layer thicknesses with drug release measurements reveals that capsules with the thinnest shellac layers provide the best entrapment. Additional squeezing experiments show that the shellac layer makes the capsules also mechanically more stable.

Keywords: microencapsulation, coating, gastrointestinal, hydrogels, *in vitro* release, controlled release

Introduction

Capsules consisting of natural polysaccharide hydrogels such as alginates or pectins are frequently used for the encapsulation and transportation of drugs and for the transplantation of cells (Murano, 1998; De Vos et al., 2006; Champagne and Fustier, 2007). The encapsulation protects the content of the capsules against outer influences like enzymes of the gastrointestinal (GI) tract and provides the possibility of controlled and site-specific release. The functionality crucially depends on the permeability of the capsules as well as on their mechanical and chemical stability. In order to decrease the permeability and to improve the stability of the capsules, they can be coated with additional layers.

This study investigates the influence of an external shellac coating on liquid-filled pectinate capsules on their permeability and mechanical structure. The capsules were prepared in different ways and the relationship between the preparation, the structural composition of the capsule membrane, the mechanical stability and the permeability was observed by NMR microscopy, squeezing capsule experiments and drug release measurements, respectively.

The inner membrane layer of the studied capsules consists of the polysaccharide pectin which can be extracted from the cell walls of higher plants (Sriamornsak, 2003). Because of its biodegradability, it has been used as thickening agent, gelling agent, colloidal stabilizer and transport matrix for drugs in the food and pharmaceutical industries. Low-esterified pectins form three-dimensional ionotropic gels with divalent cations. Here, we use this property to produce liquid-filled spherical capsules with a membrane of gelled pectinate. While these capsules have been shown to be useful (Liu et al., 2003; Leick et al., 2011), the permeability of these membranes is too high for many applications (Ferreira et al., 2009).

Here, we show how the permeability of these capsules can be reduced significantly by coating the polysaccharide membranes with an additional layer. Typically used coatings are chitosan, poly-l-lysine or shellac (Lim and Sun, 1980; Chiou et al., 2001; Ravi et al., 2008a). Shellac, a physiologically harmless and biodegradable resin secreted by

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(Received 1 Jun 2011; accepted 7 Oct 2011)
http://www.informahealthcare.com/mnc

the female lac bug (*kerria lacca*), precipitates in acidic environments (Pfenning, 1996; Phan et al., 2008). Due to its various properties (e.g. thermoplasticity, cohesiveness, insulating ability, pH-dependent water-solubility), it is commonly used as an enteric coating material in pharmaceutical applications as well as an additive for coatings in the food industry (Chang et al., 1990). Aleuritic acid, shellolic acid and jalaric acid are the main components of shellac (Pearnchob et al., 2003). In previous studies, it was used to coat pectin matrix tablets which have recently been used for transporting drugs to the colon (Ravi et al., 2008b) or as an additive to form pectinate-shellac composite capsules with improved mechanical stability (Leick et al., 2011).

Targeted delivery of drugs to the lower GI tract is advantageous if they are susceptible to chemical and enzymatic degradation in the upper GI tract (Singh, 2007). Examples for such sensitive drugs are the anthocyanins. Anthocyanins are water soluble plant pigments which are responsible for the blue, purple and red colour of many plant tissues (Prior and Wu, 2006). They have been shown to be strong antioxidants, and may exert a wide range of health benefits through antioxidant or other mechanisms (Prior, 2004). Studies have also shown that anthocyanins may play a role in cancer prevention if their uptake after consumption can be enhanced (Wang and Stoner, 2008). The application of anthocyanins by shellac-coated capsules may provide a solution for enhancing the uptake if it can be ensured that the content of the capsules is protected from the gastric juice and that the loss of the content due to diffusion through the membranes is limited for the time until the capsules reach the intestine.

Here, we quantify the retention of the drug in differently coated and non-coated pectinate capsules by UV–Vis absorption spectroscopy. Measuring the drug release as a function of time allows us to characterize their permeability. We correlate the permeability of the membrane with the structure by imaging the capsules with NMR microscopy (μMRI; MRI, magnetic resonance imaging). This technique offers a non-invasive visualization of the capsules and measurements of the thickness of the membrane layers. The different layers are distinguishable in NMR-images due to different relaxation times. In earlier studies, NMR microscopy has been used to measure the size and the membrane thickness of alginate as well as pectinate capsules and to visualize poly-l-lysine layers on alginate microbeads (Zimmermann et al., 2003; Constantinidis, 2007; Leick et al., 2010).

Another aspect which is correlated with the structure of the membranes is the mechanical stability of the capsules. The stability was investigated by so-called squeezing capsule experiments, in which compression curves of the different capsules were obtained. A combination of the results from the squeezing capsule experiments with the results of the NMR microscopy studies made it possible to determine the two-dimensional surface Young's modulus of the capsule membranes.

Materials and methods

Materials

The used amidated pectin (Pectin amid AU-L 027/09) was provided by Herbstreith & Fox KG, Neuenburg, Germany. The aqueous ammoniacal shellac solution (SSB Aquagold, 25 wt% solid content, pH ≈ 7.5) for the external coating was provided by SSB Stroever Schellack Bremen, Germany. $CaCl_2 \cdot 2H_2O$ and anhydrous glycerol (≥99 vol%) were purchased from Merck Chemicals, Darmstadt, Germany. The bilberry extract 25% (product number 600761) was provided by Kaden Biochemicals, Hamburg, Germany. All chemicals were used without further purification.

Capsule preparation

Calcium pectinate capsules filled with a liquid core were prepared by extrusion in a simple one-step process. For the preparation of all capsule types, an aqueous amidated pectin solution of 0.8 wt% was used. The cross-linking solution consisted of calcium chloride dissolved in an aqueous bilberry extract solution. Furthermore, an aqueous ammoniacal shellac solution was used to create an external coating around the calcium pectinate capsules.

In detail, for the preparation of the cross-linking solution, 2.0 wt% $CaCl_2 \cdot 2H_2O$ salt and 20 wt% bilberry extract were dissolved in 100 mL of double distilled water. This solution was stirred for at least 10 min to dissolve the extract to the greatest extent possible. Then this mixture was centrifuged for 30 min for removing the non-soluble extract particles. After that, the supernatant solution was diluted with the same amount of anhydrous glycerol, which was added to improve the mechanical properties as well as the density for facilitating the dropping in. The resulting cross-linking solution contained 50 vol% glycerol and 1.0 wt% calcium chloride. A pH-value of 3 was adjusted by adding some drops of hydrochloric acid.

In the capsule production process, first the cross-linking solution was added drop-wise by means of a high-precision metering syringe into a cylindrical glass filled with 30 mL of an aqueous 0.8 wt% pectin solution. A capsule membrane was formed instantly around each droplet once the two liquids came into contact. Because the calcium cation has a smaller size than the polymer molecules, it can diffuse into the pectin solution and this leads to cross-linking processes in which intermolecular cross-links are formed between the divalent calcium ions and the negatively charged carboxyl groups of the amidated pectin molecules (Sriamornsak and Nunthanid, 1998). Induced by these diffusion processes, the gel membrane grows along the flux direction of the calcium ions (Blandino et al., 1999). In order to avoid the aggregation of the prepared capsules, the pectin solution was constantly moved by a magnetic stirrer. A dropping height of 2 cm was used to ensure that nearly spherical capsules were formed. Experimental tests showed that for larger distances, the drops were destroyed when they came into contact with the surface of the pectin

solution. If the dropping height was reduced to less than 2 cm, the resulting capsules showed distortion wedges. After gelation times of 30 s or 3 min, the capsules were separated, filtered and washed with double distilled water.

Two additional steps were necessary to apply an external shellac coating. First, after isolating the capsules, they were transferred into an aqueous solution containing 2.0 wt% $CaCl_2 \cdot 2H_2O$, with the aim of stabilizing the gel membranes and completing the polymerization process (Chai et al., 2004). In order to investigate the diffusional and mechanical properties of the acidic precipitating shellac coatings, depending on the pH-value, this calcium chloride solution was adjusted to different pH-values (pH = 1, 2 and 3) by adding hydrochloric acid. To achieve a homogeneous pH-value throughout the capsule by diffusion of the hydrogen ions, we left them in the acidic medium for 3.5 min.

In the last step, after the capsule separation and filtration, the pH adjusted capsules were transferred into the coating solution, containing 20 wt% aqueous ammoniacal shellac solution and 5.0 wt% anhydrous glycerol, used as softening agent for the shellac, to apply a shellac coating around the calcium pectinate capsules. The diffusion of the positively charged hydrogen ions from the capsules induces the precipitation of the shellac and therefore the formation of a shellac coating. After a residence time of 10 min in the coating solution, the capsules were separated, filtered, washed with double distilled water and transferred into a 2.0 wt% $CaCl_2 \cdot 2H_2O$ solution adjusted to the associated pH-value (pH = 1, 2 and 3) for storage.

The produced liquid-filled and shellac-coated pectinate capsules exhibit diameters of approximately 2.4 mm and entire membrane thicknesses in the range of 150 μm up to 340 μm depending on the pH-value and gelation time.

The encapsulation efficiency, which is defined as the amount of anthocyanins in the capsules after the entire preparation process divided by the amount of anthocyanins in the cross-linking solution, is 97–99% for non-coated and 82–84% for coated capsules.

NMR microscopy

The membrane thicknesses and sizes of different shellac-coated pectinate capsules were determined by NMR microscopy. This non-invasive method gives the possibility to obtain a two-dimensional image of the relaxation time-weighted spin density of a slice through the sample by applying pulsed magnetic field gradients (Callaghan, 1993). The physical principles used in NMR microscopy are the same as in MRI. The higher static magnetic fields and the stronger pulsed magnetic field gradients result in greatly enhanced resolution of the NMR microscopy compared to MRI, thus allowing us to use it for characterizing capsules. We used a modified spin-echo imaging sequence (Hsu et al., 1995) for acquiring T_2-weighted images of the capsules on a 14 T Varian CMX Infinity Plus 600 spectrometer, which is equipped with a Resonance Research BFG-73/45-100 MK 2 gradient unit and a 10 mm inner diameter Bruker microimaging probe.

About five capsules produced in the same process with equal parameters were placed on top of each other in an NMR-tube with an outer diameter of 5 mm. The tube also included the storage solution of the capsules confined at the top and bottom by susceptibility plugs to avoid imaging artefacts. A conventional multi-slice technique which scans the k-space of different slices within the repetition time was used for parallel acquisition of one image slice of each capsule.

The images were acquired with a field of view of $6 \times 6\,mm^2$ and a slice thickness of approximately 150 μm. Using 256 phase-encoding steps with an acquisition length of 256 points led to a resolution of $23 \times 23\,\mu m^2$. Sixteen scans were accumulated for improving the signal-to-noise ratio. The repetition time was adjusted to 5 s, which resulted in a total duration of the experiment of about 6 h. In order to achieve an optimal contrast between the shellac layer, the pectinate layer and the bulk water, the echo time was adjusted individually for each sample in the range 15–90 ms. The greyscale NMR-images were plotted and the thickness of each membrane layer was measured at several positions to obtain the average layer thickness for each capsule. Since five capsules with the same production parameters were measured together, it was possible to determine the mean value and standard deviation of the thicknesses. Additionally, the capsule diameters were also measured.

Drug release measurements

In order to characterize the release kinetics of the encapsulated anthocyanins, we used UV–Vis absorption spectroscopy to measure their concentration outside of the capsules as a function of time. The pH-differential method described below is a rapid and easy procedure to quantify monomeric anthocyanins, even in the presence of polymerized degraded pigments (Giusti and Wrolstad, 2001). It relies on measuring the absorbance of anthocyanin solutions at two different pH-values, which correspond to different structures of the anthocyanin chromophore. The coloured oxonium form predominates at pH 1.0 while the colourless hemiketal form appears at pH 4.5. The absorption peak of the anthocyanin cyanidin-3-glycoside, the main component of the used bilberry extract, is reached at the wavelength $\lambda = 510$ nm.

For the measurement process, two buffer solutions with the required pH-values have to be prepared; a 0.025 M KCl buffer adjusted to pH 1.0 and a 0.4 M sodium acetate buffer adjusted to pH 4.5. After the capsule preparation, the release measurement was initiated and the capsules were kept in 2 wt% $CaCl_2$ solution at the desired pH-value of 1, 2 or 3. At different times, 200 μL were taken out of this storage solution and diluted with 2.8 mL (dilution factor DF = 15) of the respective buffer solution in the measuring cell. After the sample preparation and a waiting period of 10 min (establishment of equilibrium), the whole visible spectra

was recorded from $\lambda = 350$–750 nm because in addition to the absorption peak of the measured anthocyanin (cyd-3-glu) at $\lambda = 510$ nm, the absorbance value at $\lambda = 700$ nm is also required. The pH-differential absorbance A of the diluted sample was calculated according to

$$A = (A_{510} - A_{700})_{\mathrm{pH}=1.0} - (A_{510} - A_{700})_{\mathrm{pH}=4.5}. \quad (1)$$

The monomeric anthocyanin pigment concentration MAP can be calculated by the equation

$$\mathrm{MAP} = \frac{(A \times MW \times DF \times 1000)}{\varepsilon}, \quad (2)$$

where MW describes the molecular weight of cyd-3-glu (449.2 g/mol) and ε is the molar absorptivity ($\varepsilon = 26\,900$ L/mol for a path length of 1 cm; Jurd and Asen, 1966). The graphical representation of the released anthocyanin pigment concentration against time allows us to compare the anthocyanin release of the encapsulated bilberry extract from the different capsule systems.

Squeezing capsule experiments

The mechanical stability of coated and non-coated capsules was investigated by squeezing them between two parallel plates (Sun and Zhang, 2002; Carin et al., 2003). An Advanced Rheometric Expansion System from TA Instruments with plate/plate geometry was used for performing these measurements. The upper titanium plate had a diameter of 25 mm. The resulting normal force was measured by a force rebalance transducer with normal force (FRTN1) with a measuring range between 2 mN and 20 N.

The capsules were compressed corresponding to a gap change from 2.5 to 0.01 mm during 360 s with a logarithmically decreasing compression rate. During the compression, the gap and the normal force at the upper plate were measured simultaneously. The measurements were performed with five capsules produced under same conditions in order to obtain a mean value. By plotting the normal force versus the gap, it is possible to obtain qualitative differences of the mechanical capsule stability (Risso and Carin, 2004; Rachik et al., 2006). By a quantitative analysis of these data, it is also possible to calculate the surface Young's modulus.

Results and discussion

Kinetics of drug release

Drug release kinetics were obtained by UV–Vis absorption measurements for differently prepared capsules. In Figure 1, we plotted the relative amount of released anthocyanins against the time elapsed after capsule preparation. The y-axis was calibrated by measuring the anthocyanin concentration of a solution of crushed capsules. Figure 1(a) shows drug release curves for non-coated pectinate capsules, which were stored at different

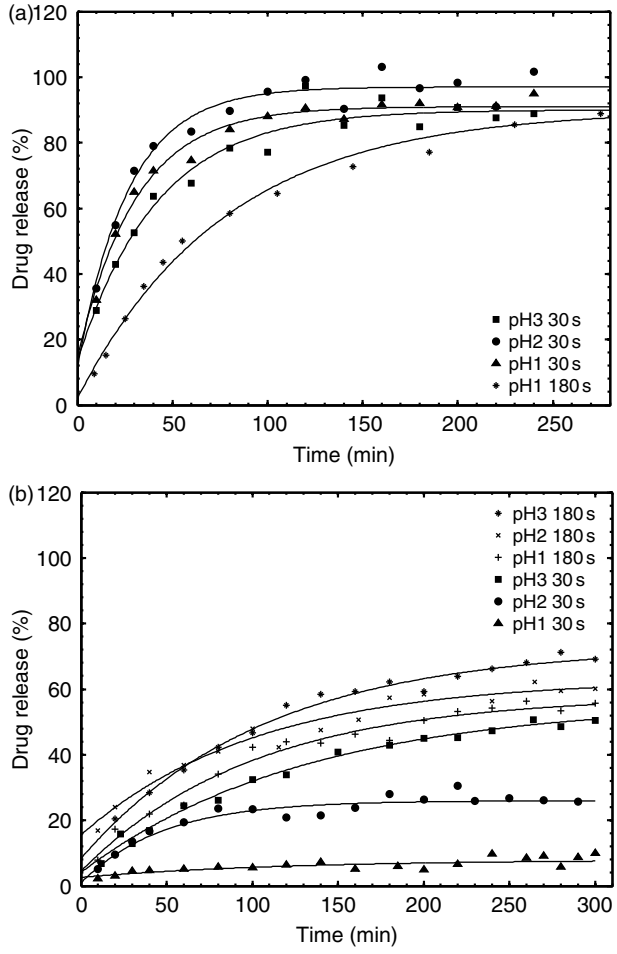

Figure 1. Drug release curves obtained by UV-Vis absorption spectroscopy with a measurement precision of 3% for non-coated pectinate capsules (a) and for shellac-coated capsules (b) at different pH-values and pectinate gelation times. The solid lines represent the results of non-linear least squares fits of the experimental data to Equation (3).

pH-values and produced with different gelation times. The capsules were stored at different pH-values to allow comparison with the coated capsules, which were also stored and prepared at different pH-values. The amount of released anthocyanins rises quickly in the first 2 h for all non-coated capsules. For capsules with a gelation time of 30 s, the drug release reaches 80–100% after 2 h. Differences of the drug release between the different pH-values can be neglected. Increasing the gelation time to 3 min leads to slightly slower drug release. After 2 h, about 70% of the encapsulated extract was released. As we show below, this retarded release is due to thicker pectinate membranes. The observation that equilibrium drug release values are below 100% can be explained by adsorption and solution processes of the anthocyanin molecules within the hydrogel membranes.

Further enlargement of the pectinate membrane thickness in order to improve the diffusive resistance is not feasible since the membrane thickness is limited by the diffusion-controlled gelation process. Thus, a suitable way of improving the diffusive resistance is to seal the capsules with an additional coating.

Drug release curves for shellac-coated capsules with pectinate gelation times of 30 s and 3 min can be found in Figure 1(b). Compared to the non-coated capsules, the rate of the drug release as well as the total amount of released anthocyanins at equilibrium is significantly reduced. The permeability of capsules with a gelation time of 30 s strongly depends on the pH-value. Decreasing the pH-value leads to a decreased permeability. The best retention was found for capsules that were coated at pH 1. These capsules only released about 5% of the encapsulated extract after 2 h.

The structure of the shellac layer depends on the pH-value because lower pH-values enhance the precipitation of shellac. An increased density or thickness of the shellac coating at low pH-values can explain the improved diffusional characteristics of the low-pH capsules. The pH-dependent differences of the permeability are less distinctive for capsules with a gelation time of 3 min. However, the tendency for higher permeability at higher pH-values is also observable for capsules produced with the longer gelation time.

It is also remarkable that the permeability of coated capsules is higher with a gelation time of 3 min, in contrast to the uncoated capsules, where the short gelation-time capsules show higher permeability. We conclude that the different release kinetics of the coated capsules arise from differences in the shellac layer, and not from differences in the pectinate layer.

For a quantitative analysis of the drug release kinetics, the obtained data can be analysed with respect to Equation (3), which describes the diffusion of a solute out of a homogeneous sphere (Crank, 1979). For a diffusion constant D_m, the concentration c of the solute in the volume outside the capsule with radius r after time t is

$$c(t) = c(\text{eq.}) \cdot \left(1 - \sum_{n=1}^{\infty} \frac{6\alpha(1+\alpha)}{9 + 9\alpha + \alpha^2 q_n^2} \cdot \exp\left(-\frac{D_m q_n^2}{r^2} \cdot t\right)\right). \tag{3}$$

In this equation, α denotes the effective volume ratio

$$\alpha = K_m \frac{V_b}{V_c}, \tag{4}$$

where V_b is the bulk volume and V_c is the capsule volume. The partition coefficient K_m is the ratio of anthocyanin concentration of the capsule and the bulk in equilibrium. The parameters q_n in Equation (3) represent the positive and non-zero solutions of Equation (5):

$$\tan q_n = \frac{3q_n}{3 + \alpha q_n^2}. \tag{5}$$

For $\alpha \gg 1$, which is valid in our system, Equation (5) is solved approximately by

$$q_n = n \cdot \pi. \tag{6}$$

A non-linear least squares fit of the experimental data to Equation (3) leads to the diffusion constant D_m and the equilibrium anthocyanin concentration in the bulk solution c(eq.). The diffusion constant D_m combines the diffusion processes in the membrane and in the liquid core of the capsules. The results of the fitting procedure are summarized in Table 1. The diffusion constants for non-coated capsules with a gelation time of 30 s are similar to the diffusion constant of the anthocyanins in water ($D = (7.4 \pm 0.3) \cdot 10^{-11}\text{m}^2/\text{s}$), which we measured by pulsed field gradient NMR (Stilbs, 1987). The diffusion coefficients and the equilibrium concentrations significantly decrease with the coating, depending on the pH-value, as described in the qualitative analysis before.

Table 1. Results of non-linear least squares fits of Equation (3) to the drug release curves in Figure 1 with the average diffusion constants D_m and equilibrium concentrations c(eq.) as parameters.

Capsule type	D_m (10^{-11} m^2/s)	c(eq.) (%)
Uncoated 3 min pH3	1.9 ± 0.4	90 ± 2
Uncoated 30 s pH3	4.8 ± 0.3	90 ± 2
Uncoated 30 s pH2	8.1 ± 0.3	97 ± 2
Uncoated 30 s pH1	6.6 ± 0.2	91 ± 1
Coated 3 min pH3	2.5 ± 0.2	73 ± 2
Coated 3 min pH2	1.6 ± 0.2	63 ± 2
Coated 3 min pH1	1.4 ± 0.4	58 ± 2
Coated 30 s pH3	1.1 ± 0.4	56 ± 2
Coated 30 s pH2	4.1 ± 0.9	26 ± 1
Coated 30 s pH1	0.9 ± 0.3	8 ± 2

In order to analyse the reasons of the different drug release kinetics, it is useful to consider the differences in the structure of the capsules.

Layer thickness studied by NMR microscopy

For the non-invasive visual characterization of the different membrane layers and for the measurements of the layer thickness, NMR microscopy was used to obtain images of the capsules. Figure 2 shows two greyscale NMR-images of 150 μm thick slices through the capsules contained in a vertically oriented NMR-tube (4.3 mm inner diameter). The brightness indicates the signal amplitude in each pixel. The signal amplitude is proportional to the density of hydrogen atoms and depends on their relaxation times, which vary with the mobility of the molecules. The bright areas in the images indicate free bulk water (capsule storage solution) inside and outside of the capsules, whereas the dark areas indicate either the capsule membranes or the air outside of the NMR-tube. The two different layers of the capsule membranes can be easily distinguished from each other as well as from the bulk water. This is due to the fact that the membrane materials pectinate and shellac yield different signal amplitudes in the images because of different relaxation times and hydrogen densities. The signal amplitude of the inner pectinate layer is higher than that of the outer (shellac) layer. This can be explained by the fact that pectinate is a hydrogel, consisting mainly of water, and thus giving a strong signal, whereas shellac consists of larger molecules with a lower hydrogen density and also lower molecular mobility. Free water inside and outside of the capsules gives the strongest signal. Compared to

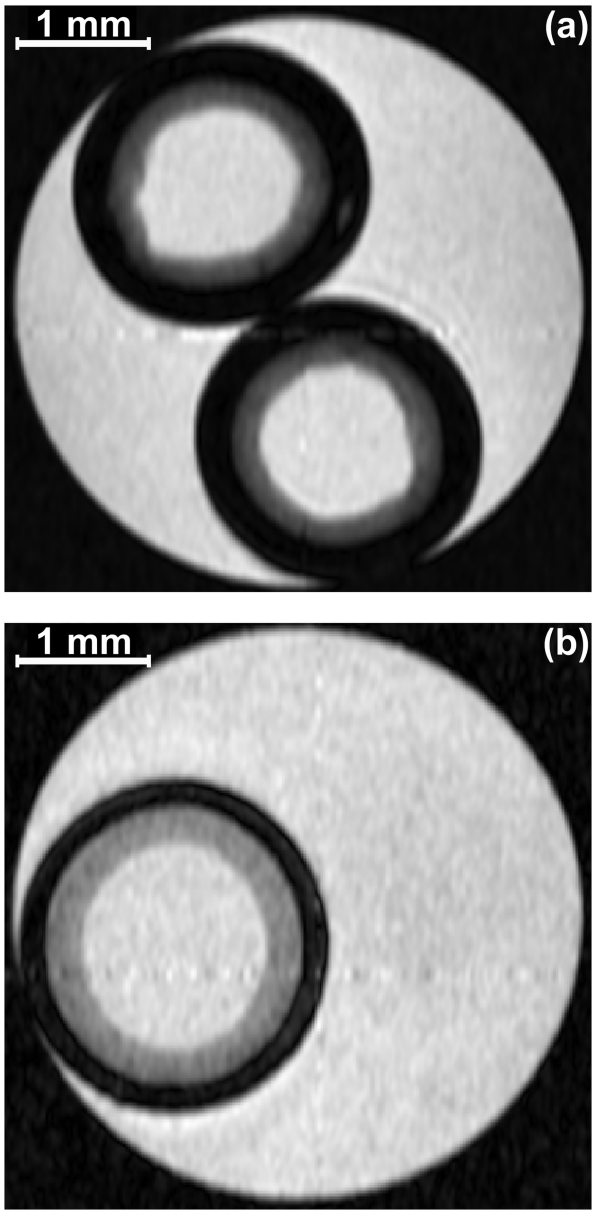

Figure 2. NMR-images of shellac-coated capsules with 3 min gelation time at pH = 1 (a) and at pH = 3 (b).

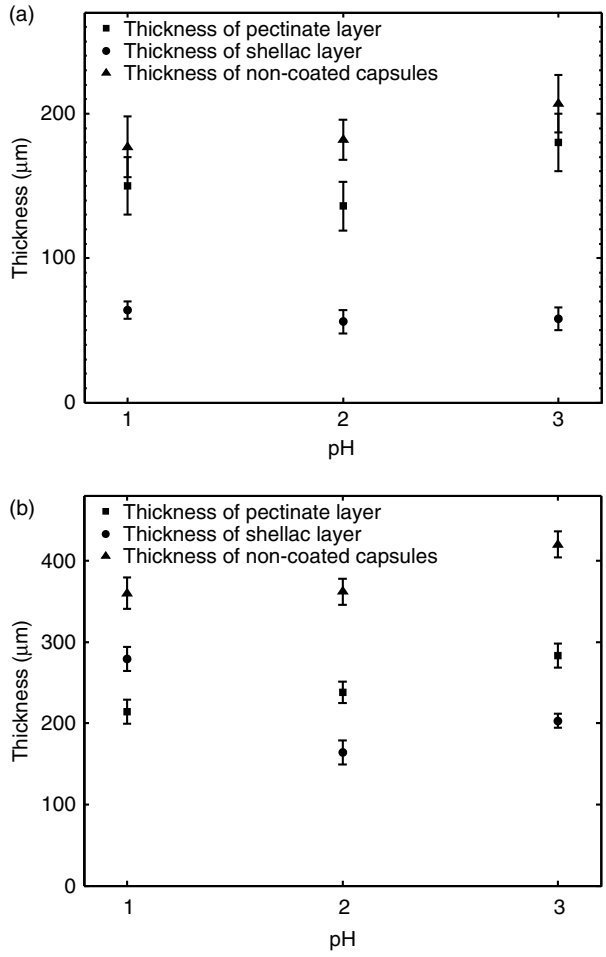

Figure 3. Thicknesses of shellac and pectinate layers for coated and non-coated capsules in dependence on pH-value with 30 s gelation time (a) and 3 min gelation time (b).

the water in the pectinate hydrogel, the water molecules of the bulk are much more mobile leading to the strongest signals.

The capsules shown in Figure 2 were produced with a gelation time of 3 min at a pH-value of 1 (Figure 2(a)) and 3 (Figure 2(b)). The shellac coating is easily identified as the dark black ring outside of the intermediate grey ring, which represents the pectinate membrane. These images clearly show that the shellac layer is thicker at a pH of 1 than at a pH of 3.

The thickness of each membrane layer was measured as a function of the pH-value and the gelation time of the pectinate layer. Figure 3(a) illustrates the measured thicknesses of coated and non-coated capsules with a pectinate gelation time of 30 s, whereas Figure 3(b) shows the same for capsules with a gelation time of 3 min. Regarding the pectinate thicknesses of coated and uncoated capsules, longer gelation times lead to significantly increased thickness. This is in good agreement with diffusion limited membrane growth, which is expected for the formation of cross-linked hydrogel membranes (Blandino et al., 1999).

The thicknesses of the pectinate layers increase with increasing pH-value within the experimental uncertainty. This fits to the observation that the volume of alginate-hydrogels, which is very similar to pectinate hydrogels, also rises at increased pH-values (Moe et al., 1993). It is interesting to note that all coated capsules have thinner pectinate layers than the uncoated capsules. A possible reason could be a shrinking of the pectinate hydrogel during the coating process. This shrinking might be induced by a depletion of cross-linked calcium ions in the pectinate hydrogel as a consequence of chemical reactions between the calcium ions and the shellac.

The thickness of the shellac layers does not vary with the pH-value for gelation times of 30 s, but for gelation times of 3 min, where the thickest layers were observed at a pH-value of 1. However, capsules with a pectinate gelation time of 3 min possess about four to five times thicker shellac layers in comparison to capsules with 30 s gelation time.

These differences of the shellac thickness depending on the gelation times may be explained by the differences in the pectinate layer thicknesses. Since thicker pectinate layers lead to thicker shellac layers, we assume that the diffusion of protons from the capsule to the shellac solution during the coating process is retarded by thicker pectinate membranes. The formation of the shellac layers should depend on the rate at which the pH-value at the surface of the pectinate layers changes. If the pH-value changes fast, the shellac precipitates instantaneously at the pectinate surface and forms a shellac layer that prevents the further diffusion of protons out of the capsule. A slower change of the pH due to thicker pectinate layers therefore leads to a slower formation of the coating, which results in an increased thickness of the shellac layer. Another aspect which could describe the differences in the thickness of the shellac layers is linked to the total amount of H^+-ions in the capsules. This total amount includes the quantity of H^+-ions in the liquid cores (adjusted by the pH-value) as well as in the pectinate hydrogel of the capsule membranes. A higher total amount of H^+-ions in capsules with thicker pectinate membranes possibly leads to the formation of thicker shellac layers.

Comparing these results to the results of the drug release measurements, the thinnest shellac layers have the best ability to hinder the anthocyanins from diffusional release out of the capsules. Thus, we assume that the thinnest shellac layers also have a higher density and therefore an increased diffusional resistance. We also assume that the density of the shellac coating depends on the pH-value. This assumption could explain the differences in the drug release kinetics depending on the pH-value.

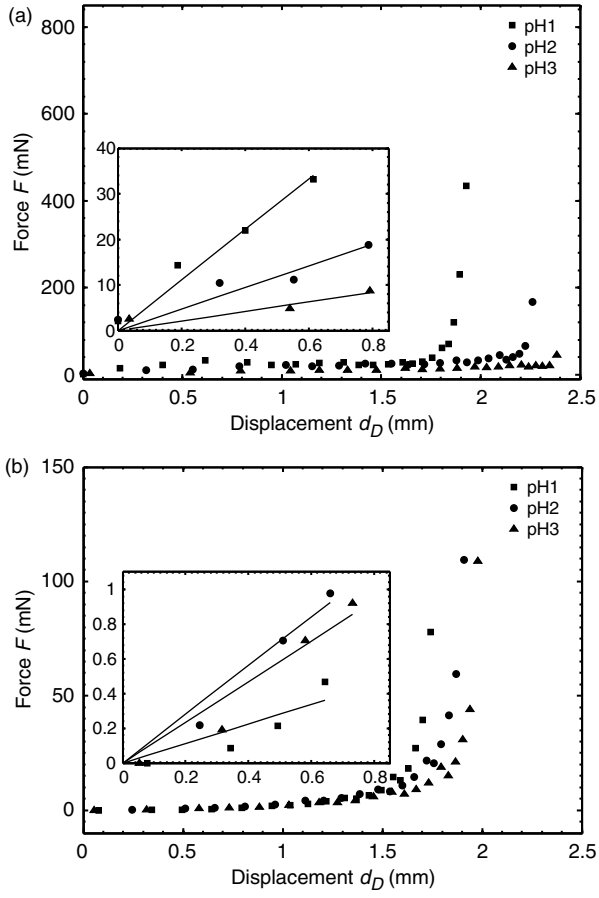

Figure 4. Deformation curves of coated (a) and non-coated (b) pectinate capsules with 30 s gelation time depending on the pH-value. The inset graph shows a magnification of the data which was used for the linear regression to calculate the surface Young's moduli.

Squeezing capsule measurements

When capsules are used as an oral drug carrier, they are subject to mechanical forces in the digestive tract, which deform the capsules. These deformations might cause the shellac coating of the capsules to spall and thus lead to an unwanted early release of the ingredients. To avoid such damaging of the capsules, it is necessary to produce them with a sufficient mechanical stability which remains to be established.

The mechanical stability of coated and non-coated capsules was studied in squeezing capsule experiments. With this method, it is possible to explore qualitative and quantitative differences in the deformational properties of the capsule membranes. Figure 4 shows typical results of such measurements in terms of force–displacement curves. For small displacements of the capsule pole, the measured normal force is a linear function of the displacement. When the displacement reaches values in the same order of magnitude as the diameter of the capsules, the slopes of the deformation curves significantly increase since the capsules are completely destroyed and the plates of the squeezing apparatus are nearly pressing against each other.

By performing a linear fit to the data in the regime of small deformations (shown in the subplots with a magnification of the force range), the two-dimensional surface Young's modulus E_S can be calculated from the relation

$$F = \frac{4E_S d}{a\sqrt{3(1-\nu_S^2)}} d_D. \tag{7}$$

In this equation, d denotes the membrane thickness, a is the radius of the capsule, d_D is the displacement of the capsule pole, F is the measured force and ν_S is the surface Poisson ratio for which we assumed a value of $-2/3$ which was obtained for similar capsules in a previous study (Leick et al., 2011). It should be noted that Young's moduli which we obtained are only a first-order approximation since Equation (7) is only applicable to small deformations of capsules with a thin shell under point loading and can only be used for comparing different capsules.

Figure 5 summarizes the measured surface Young's moduli. It is obvious that coating the pure pectinate capsules with shellac increases Young's modulus and therefore the mechanical stability. So the coated capsules need a higher force for equal deformations than uncoated capsules.

Capsules with a gelation time of 30 s and therefore thinner shellac layers show higher Young's moduli than

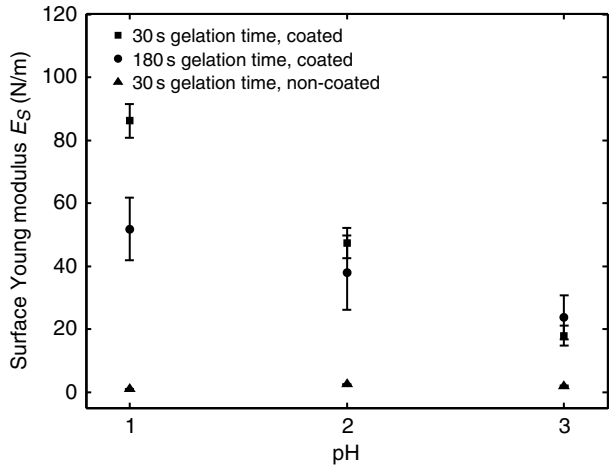

Figure 5. Surface Young's modulus of coated and uncoated pectinate capsules calculated by squeezing capsule data.

capsules with thicker shellac layers. This indicates that thin shellac layers posses a higher density since higher density should lead to increased stability.

Conclusion

In conclusion, we have studied the permeability, structure and deformability of shellac-coated pectinate capsules. In particular, the influence of the pH during the coating process, the gelation time of the pectinate layers and the coating itself on these properties were investigated.

By observing the time-dependent release of the anthocyanins from an extract of bilberries, we could show that shellac-coated pectinate capsules show a retarded release of this model drug, compared with pure polysaccharide capsules investigated in other studies (Ferreira et al., 2009). By coating pure pectinate capsules, the amount of drug released after 2 h could be reduced from ≈100% to ≈5%. Our results show that the coating achieves a good sealing of the capsules. The quality of the sealing increases with decreasing pH and is better for capsules with a pectinate gelation time of 30 s than for 3 min.

We could also show that differences in the structural composition of the capsule membranes can be investigated by NMR microscopy. With this technique, we measured the influence of the pH-value and the gelation time on the thickness of the pectinate and shellac layers. We observed that the thickness of the shellac layers strongly depends on the thickness of the pectinate layers, which can be adjusted by the gelation time. A thicker pectinate layer leads to a thicker shellac layer. The pH-value used in the coating process of the capsules has no significant influence on the shellac thickness.

A combination of the NMR imaging results with the results of the drug release measurements shows that capsules with the thinnest shellac layers have the best ability of retarding the release of encapsulated anthocyanins. Thus, we assume that the density of the shellac coating is higher for capsules with a thinner pectinate layer.

The mechanical stability of the differently prepared capsules was investigated by squeezing them and measuring the resulting normal force. With these experiments, we could show that the coating increases the stability of the capsules. Increasing the mechanical stability is important since the capsules should not be damaged in the gastric tract by shear forces from gastric motions.

In further investigations, the drug release should be investigated under simulated GI conditions for evaluating whether shellac-coated hydrogel capsules can transport liquid drugs in a sufficient concentration to the intestine and how the altered chemical environments influence the structural composition of the capsules.

Acknowledgements

We acknowledge financial support by the German Research Foundation (DFG-Project RE 681/19-1).

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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