



Pharmaceutical Nanotechnology

Water sorption properties of HM-pectin and liposomes intended to alleviate dry mouth

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ABSTRACT

Pharmaceutical formulations intended for treatment of xerostomia (dry mouth) should be able to keep the oral mucosa hydrated for a prolonged period of time. The products already existing on the market contain water-soluble polymers, however their ability to moisturize the oral mucosa for a longer period of time seems limited. In this paper the sorption properties of water vapor of high-methoxylated pectin (HM-pectin, a hydrophilic biopolymer) and phosphatidylcholine-based (Soya-PC) liposomes have been studied and compared using a gravimetric method. The kinetics of water desorption and sorption have been recorded over the relative humidity range RH = 95–0–95%, at 35 °C. The obtained isotherms were found to be well described by the n-layer Brunauer-Emmet-Teller (BET) adsorption model. The water isotherms on HM-pectin were Type II (IUPAC), while water isotherms on liposomes were Type III. The maximum water sorption capacity of liposomes (1.2 mg water per mg of adsorbent at 95% RH) was found to be twice as high as for pectin. Due to the slower water release from the liposomes, as well as their high water sorption capacity, they seem to have great potential in relieving the symptoms of dry mouth syndrome.

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1. Introduction

The oral cavity is frequently used for both mucosal and transmucosal drug administration as it allows bypassing the gastrointestinal tract and hence avoiding the enzymatic degradation of an active pharmaceutical ingredient (Madsen et al., 2013). Drug delivery systems intended for local treatment of the oral cavity need to fulfill several requirements, such as high patient compliance, lack of taste and easiness of administration (Joshi and Petereit, 2013). Different studies indicate that up to 30% of the adult population is affected by xerostomia (dry mouth) (Guggenheimer and Moore, 2003). Xerostomia is defined as a subjective feeling of dryness in the oral cavity, resulting from decreased or absent saliva flow. Decreased secretion of saliva can be due to salivary gland dysfunction, cancer treatment to the head and neck region, or side effect of many medications (Alimi, 2015). Treatments of xerostomia are mainly based on local (mucosal) drug delivery to the oral cavity and are expected to either stimulate or

substitute the secretion of saliva. Salivary constituents are mainly composed of hydrophilic polymers, such as xanthan gum or carboxymethyl cellulose, which tend to swell in a liquid and have the ability to interact with the mucus layer present in the oral cavity (Momm et al., 2005). However, the efficacy of the formulations is quite limited and there is need for products that will keep mucosa hydrated for a prolonged period of time (Hearnden et al., 2012). From this point of view, bioadhesive drug delivery systems – such as liposomes and polymeric particles – can contribute to prolonged therapeutical effect.

Liposomes are spherical vesicles with an inner aqueous compartment surrounded by one or more lipid bilayers. Due to the presence of two different domains they can be used for encapsulation of both hydrophilic and lipophilic drugs, incorporated in the core or in the lipid membrane, respectively. Liposomes have been widely investigated for both intravenous and cutaneous use and have shown promising potential in cancer treatment, antimicrobial therapy, enzyme and hormone therapy, as well as adjuvants for vaccines and vectors for gene transfer (Gan et al., 2013; Damitz and Anuj, 2015; Kroon et al., 2014; Davies et al., 2014). The ability of lipids to bind water together with the presence of an aqueous compartment makes liposomes attractive as humectants, promoting skin hydration

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by both (a) delivering moisture and (b) providing a barrier inhibiting water desorption from stratum corneum (Egbaria and Weiner, 1990). The liposome core is expected to act as a depot containing the hydration medium, which will leak out slowly over time and provide prolonged moisture protection. Therefore, the water sorption studies of liposomes can provide us with a new insight into the hydration capacity of drug delivery systems.

High-molecular polysaccharides have the ability of adsorbing water easily, mainly by hydrogen bonds formed with hydroxyl and amide groups present in their structure, and are therefore used as film coatings for moisture protection and taste masking (Joshi and Petereit, 2013; Mucha et al., 2005). Pectin is a representative of the polysaccharide family and a major component of the cell walls in plants. It is usually extracted from fruits and vegetables in a low-cost production process (Mohnen, 2008). Regarded as non-toxic and generally water soluble (solubility depending on the type of material and pH), pectin is widely used as a gelling agent in the food industry, as well as an excipient in pharmaceutical formulations (Tho et al., 2002; Sørensen et al., 2009). Pectins are also known for their mucoadhesive properties and as such can increase the residence time of a formulation on the oral mucosa (Klementsruud et al., 2013).

The aim of this study was to measure the adsorption properties of water vapor in Soya-PC liposomes and HM-pectin, targeting a future development of an advanced oral drug delivery system. In order to develop an optimal system, a sensitive and reliable method for measuring small variations in the water content of the samples had to be established. The water sorption properties of liposomes and HM-pectin were studied by the means of dynamic vapor sorption (DVS). The DVS unit is an ultra-sensitive microbalance capable of measuring changes in sample mass lower than 1 ppm in an atmosphere where both temperature and relative humidity can be controlled (Johnsen et al., 2011). The obtained high resolution isotherms were used to study the characteristics of water adsorption, desorption and diffusion in the samples.

2. Materials and methods

2.1. Materials

High-methoxylated pectin (Genu[®] pectin 150 USA-SAG, DM = 70%, M_w after purification = 1.1×10^5 Da) was purchased from CPKelco (Großenbrode, Germany). Phosphatidylcholine from soybean lecithin (Soya-PC, Lipoid S PC, $M_w = 787$ Da, >98% phosphatidylcholine) was a kind gift from Lipoid GmbH (Ludwigshafen, Germany). Fluorescent lipid 1-oleoyl-2-{6-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]hexanoyl}-sn-glycero-3-phosphocholine (NBD-PC) was from Avanti Polar Lipids, Inc. (Alabaster, USA). Sodium dihydrogen phosphate monohydrate and disodium hydrogen phosphate dihydrate used for the preparation of phosphate buffer were of analytical grade from Merck (Darmstadt, Germany). Potassium sulfate and water (Chromasolv[®] gradient grade, for HPLC, $\geq 99.9\%$) was purchased from Sigma-Aldrich (St. Louis, USA).

2.2. Methods

2.2.1. Polymer solutions

HM-pectin solutions were prepared by dissolving the polymer in phosphate buffer (5 mM, pH 6.8) at a concentration of 2% w/v. The mixtures were allowed to stir overnight at room temperature and then filtered through 2 μ m polycarbonate membrane (Nucleopore[®], Costar Corp., Cambridge, USA).

2.2.2. Preparation and characterization of liposomes

Liposomes were prepared by the thin film method (Nguyen et al., 2011). Both phospholipid components (Soya-PC and NBD-PC) were dissolved in chloroform in order to obtain a homogeneous mixture. Chloroform was then evaporated in a rotary evaporator (Heidolph W 2001 rotavapor, Heidolph Instruments GmbH & Co. KG, Kelheim, Germany) and the lipid films were thoroughly dried under vacuum overnight in order to remove organic residues (Christ Alpha 2–4 freeze drier, Christ, Osterode am Harz, Germany). The lipid films were hydrated with phosphate buffer solution (5 mM, pH 6.8) and gently agitated for 2 h at room temperature. The resulting solution of large, multilamellar vesicles was further downsized by extrusion (Lipex extruder, Lipex Biomembranes Inc., Vancouver, Canada) through two-stacked polycarbonate membranes with pore size of 200 nm (Nucleopore[®], Costar Corp., Cambridge, USA). The final concentration of the lipid in the samples was equal to 3 mM. The liposomes were characterized by the means of dynamic light scattering and microelectrophoresis method (Zetasizer Nano Series, Malvern Instruments Ltd., Worcestershire, UK). Size and zeta potential measurements were performed at 25 °C with 173° backscatter angle. The obtained values of the zeta potential were counted as an average value of three subsequent runs with 20 measurements each, while the size was an average value from three runs with 10 measurements. The size of the liposomes was equal to 174 nm, with polydispersity index of 0.12, and the zeta potential was of -1.6 mV.

2.2.3. Preparation of samples for water sorption experiments

100 μ l of solution for testing was applied onto previously weighted sample pans (PerkinElmer aluminum DSC sample pans, Boston, US). The samples were kept at room temperature (25 °C) in a closed desiccator filled with saturated solution of potassium sulfate, which allowed to maintain the relative humidity at a constant level of 97% (Rockland, 1960). The samples were stored in a container until no more liquid could be seen with a bare eye, which took approximately 12 and 24 days for HM-pectin and liposomes, respectively.

2.2.4. Gravimetric measurements

The water sorption properties of the samples were measured with a dynamic vapor sorption (DVS) instrument from Surface Measurement Systems Ltd., UK. The samples were suspended from a microbalance in a closed chamber, in which the desired level of relative humidity is achieved by mixing a flow of nitrogen with water vapor. The temperature was held constant at 35 °C and the starting relative humidity was set to 95%. The sample mass readings were recorded at relative humidity values changing stepwise (10% at the time) until 15%, and afterwards at 7.5% and 0%. The sorption experiment was performed at the same RH values. The duration of each stage was variable: mass equilibrium criteria was defined as $dm/dt = 0.0008$ mg/min, stability duration 60 min. Once the state of equilibrium was reached, the relative humidity was programmed to enter to the next stage. If the sample however could not achieve a mass equilibrium requirement, a maximum stage time limit was set to 600 min in order to ensure effectiveness of the analysis. The sample mass readings were recorded every minute and the values recorded before reaching equilibrium for every stage were used to calculate diffusion coefficients.

3. Results and discussion

3.1. Sorption kinetics

Fig. 1 presents the entire measured curves of water sorption (desorption and sorption) for both (a) HM-pectin and (b) Soya-PC liposomes. They are described by the dependence of adsorption

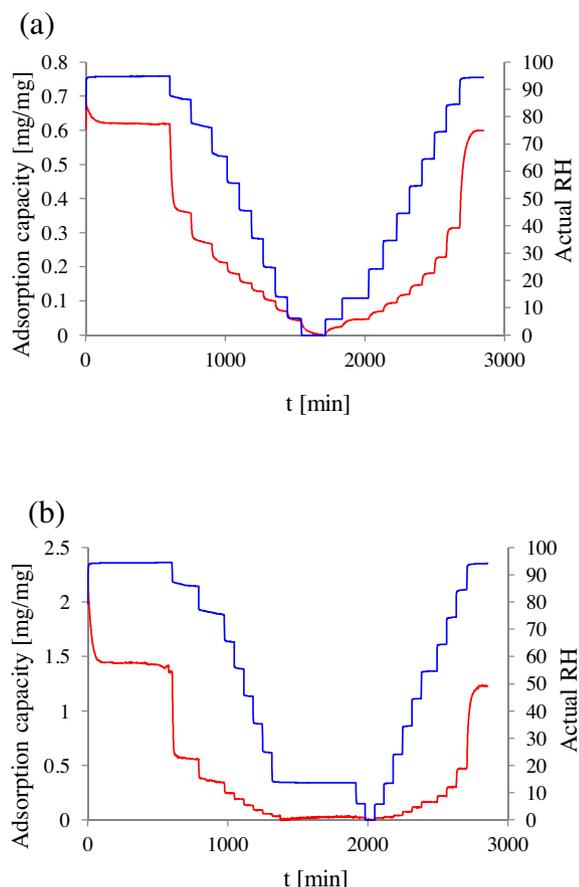


Fig. 1. Kinetic curves of water sorption for (a) HM-pectin and (b) Soya-PC liposomes. The upper blue curves in the figures represent steps in the relative humidity, while the lower red curves denote for changes in sample mass related to the moisture content. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

capacity of the material (mass of water present in the sample per 1 mg of dry material) on time and relative humidity conditions. Time duration of both experiments was similar (ca. 47 h), and for both HM-pectin and Soya-PC liposomes the desorption stage lasted longer than adsorption, which means that the time required for reaching mass equilibrium was also longer. However, the desorption time counted until the end of 0% RH stage observed in the case of liposomes was significantly longer than the one for HM-pectin (72% of experiment duration compared to 60%). That effect could be somehow connected with the slow leakage of water from the inner liposome compartment and is favourable since it offers prolonged moisture protection.

As expected during the desorption experiment, the lower relative humidity in the chamber, the lower water content in the sample is detected. On the contrary, increasing the relative humidity resulted in increased moisture adsorption and sample mass. The highest mass changes were recorded for the initial and final stages (95 and 85% RH during desorption and adsorption experiments) and they were gradually decreasing for lower humidities. Table 1 compares the equilibrium values of moisture content for HM-pectin and liposomes at different stages of relative humidity.

According to the DVS intrinsic operation manual, the actual relative humidity values can differ from the programmed theoretical humidity steps for up to 1.5% for commonly used temperatures and flows (25 °C, 200 sccm). Usually the difference between theoretical and actual humidity did not exceed 0.5%.

Table 1

The equilibrium values of moisture content for HM-pectin and liposomes at different stages of relative humidity in desorption and adsorption experiment.

Liposomes		HM-pectin	
RH (%)	Moisture content (mg/mg)	RH (%)	Moisture content (mg/mg)
94.7	1.362	94.9	0.618
85.9	0.557	86.3	0.358
75.5	0.345	76.0	0.268
65.4	0.244	65.5	0.212
55.5	0.189	55.6	0.179
45.5	0.134	45.5	0.152
35.3	0.089	35.3	0.128
24.7	0.056	24.8	0.100
13.6	0.032	13.9	0.070
5.9	0.019	6.1	0.042
0	0	0	0
5.8	0.017	5.9	0.025
13.5	0.022	13.6	0.047
24.1	0.041	24.3	0.071
34.5	0.075	34.7	0.095
44.6	0.115	44.7	0.120
54.7	0.163	54.8	0.148
64.6	0.217	64.7	0.182
74.6	0.303	74.6	0.229
84.7	0.471	84.6	0.315
94.3	1.228	94.5	0.600

However, in the case of 7.5% RH the actual humidity values were 1.6–1.7% lower than the theoretical value. That could probably be explained by the dependence of humidity conditions on temperature, as increasing the temperature (35 °C) lowers the RH.

It is worth noticing that between 95 and 55% RH the moisture content of the liposomes is higher than for HM-pectin (for both desorption and adsorption experiments). The opposite situation occurs between 45 and 0–45% RH, where HM-pectin displays higher water content than the liposomes. One can conclude that the liposomes are preferable in terms of slow water release at higher RH values, while HM-pectin has the advantage over liposomes at lower humidities. The humidity inside an oral cavity of a healthy person is maintained at around 100%. A decrease of salivary flow rate ranging from 30 to 50% would result in a sensation of dry mouth, corresponding to a humidity condition of ca. 50–80% (Scholz et al., 2008; Daves, 1987). It is therefore an advantage that a material selected for hydration of the oral mucosa performs well in humidities higher than 50%; lower values of RH could disrupt liposomal structure. Nevertheless, the maximum water sorption capacity of liposomes is twice as high as the one for pectin at 95% RH.

The liposomes are expected to gradually lose their integrity and fuse while decreasing humidity (in e.g. freeze-drying process), which leads to complete loss of entrapped water (Hoekstra et al., 1997). This change is reversible and after increase of humidity the lipids will reorganize and form liposomes again. The adsorption capacity of recovered liposomes was 10% lower than adsorption capacity of initial sample, most probably due to the fact that lipids will not organize into exactly the same structures as before. It is expected that recovered liposomes will be larger and vary in size. However, when applying the liposomes for hydration of the oral cavity, the humidity level is not expected to be lower than 50% and therefore water loss from liposomes is not complete. Since soya-PC has low phase transition temperature (<−10 °C), no phase transition will occur at the tested humidity range at 35 °C.

In a study from Einhorn-Stol et al. (2014), it was found that storage of pectin at 60 °C and 80% humidity for two weeks changed the properties of material (crystalline structure became amorphous and water uptake was reduced). It is therefore possible that the storage of pectin in dessicator at 97% humidity somehow

influenced water-pectin interactions. As for the lower humidity conditions, they should not have bigger impact on pectin stability as normally it is available as a powder.

3.2. Sorption isotherms

The experimental results for water sorption were used to model shape of isotherms according to the Brunauer-Emmet-Teller (BET) model of multilayer (n-layer) adsorption (Do, 1998):

$$\frac{q}{q_m} = \frac{CRH}{1 - RH} \frac{1 - (n + 1)RH^n + nRH^{n+1}}{1 + (C - 1)RH - CRH^{n+1}} \quad (1)$$

where q is the amount adsorbed at different relative humidities (RH), q_m is the loading corresponding to a water monolayer, C is the BET constant, and n is the maximum number of adsorption layers.

The fitting procedure was done using the residual sum of squares as an error function:

$$RSS = \sum (q_{CALC} - q_{EXP})^2 \quad (2)$$

The obtained sorption isotherms were found to be well described by the modified BET model ($RSS \leq 9.9 \times 10^{-3}$). The values of the parameters calculated for HM-pectin and Soya-PC liposomes are summarized in Table 2.

Standard BET model assumes that as the value of relative humidity increases, it is possible to adsorb an infinite number of layers. However, the surface has a finite capacity to adsorb water. It is therefore common to use modified BET model that assumes a maximum n layers that can be adsorbed onto the internal surfaces (Do, 1998). When n approaches infinity (high n value for Soya-PC liposomes), the equation reduces to the classical BET model; the higher value of n constant, the higher adsorption capacity at large relative humidities. The parameter C defines character of interactions between the adsorbent and adsorbate. If C is greater than 1 (for HM-pectin), the attractive forces between the adsorbed water molecules and the adsorbent predominate over the forces between water molecules in the gas phase (Do, 1998). When $C = 1$, the interactions between water molecules and surface are within the same range as the interactions between water molecules in the gas phase (heat of evaporation same as heat of desorption: $\Delta H_{vap} = \Delta H_{des}$).

Fig. 2(a) and (b) presents water vapor isotherms of pectin and liposomes at 35 °C. HM-pectin represents type II of adsorption, according to BET isotherm classification (Brunauer et al., 1938). It can be described by a sigmoidal curve, which results from unrestricted monolayer-multilayer adsorption behavior up to high relative humidity values. The intermediate flat region of the isotherm corresponds to easy water adsorption and formation of monolayer. Once a monolayer is created, multilayer formation and/or pore condensation occurs. Water equilibrium sorption uptake increases with increase of RH, and the increase is more significant at high RH values.

The water sorption isotherm for Soya-PC liposomes is a type III isotherm. The interactions between adsorbent and adsorbate are relatively weak; therefore, the adsorbed water molecules are clustered around the most favourable sites on the surface of

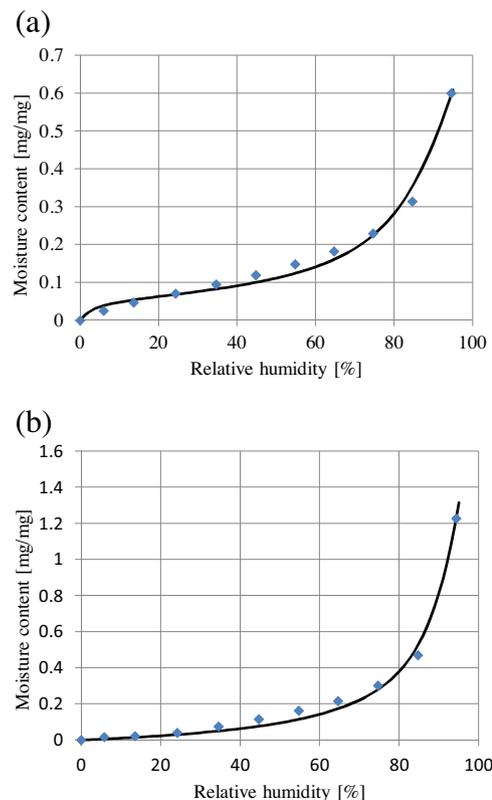


Fig. 2. HM-pectin (a) and Soya-PC liposomes (b) water sorption isotherms. The solid lines represent theoretical curves fitting, according to Eq. (1).

material. The water equilibrium sorption uptake is facilitated at higher RH values, since the water interaction with already adsorbed layer is greater than the interaction with the adsorbent surface.

3.3. Determination of diffusion coefficient

Water diffusion coefficients were calculated according to the second Fick's law describing the rate at which concentration is changing due to unsteady diffusion conditions (Ruthven, 1984). A simplified solution of this equation can be obtained with the assumption that (a) water vapor penetrates the sample unidirectionally from the plane surface and (b) diffusion coefficient does not depend on the derivative of the isotherm. The second requirement is normally fulfilled for linear isotherms (where derivative is a constant value). The obtained isotherms for liposomes and HM-pectin are not linear; however, on each of the chosen intervals of RH there is almost a linear behavior. Therefore, solution of Fick's equation is given by the following formula (Crank, 1975):

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2} \sum_{j=0}^{\infty} \frac{1}{(2j + 1)^2} \exp \left[-\frac{D\pi^2}{e^2} (2j + 1)^2 t \right] \quad (3)$$

where M_t is the water mass in time t , M_∞ is the final water mass in saturated state (at the end of each RH stage), and $M(t)/M_\infty$ is therefore an adimensional normalized amount of water adsorbed; j is an integer number, D is the diffusion coefficient, and e is the sample thickness.

The sum was computed for the first 25 terms (until $j = 24$). For each relative humidity step M_t/M_∞ is calculated as a function of time, allowing a fitting procedure where the diffusion coefficient is

Table 2

Values of the parameters for modified BET model used to describe water sorption isotherms of HM-pectin and Soya-PC liposomes.

Parameters	HM-pectin	Soya-PC liposomes
q_m (mg/mg)	0.058	0.095
C	26.272	1.000
n	25.419	42.823

the variable to be optimized. The optimization was done using the residual sum of squares as an error function:

$$RSS = \sum \left(\frac{M_t}{M_\infty} \Big|_{\text{CALC}} - \frac{M_t}{M_\infty} \Big|_{\text{EXP}} \right)^2 \quad (4)$$

Fig. 3 presents an example of the fitting of theoretical curves to the experimental data for chosen RH stages for HM-pectin (a) and Soya-PC liposomes (b).

Afterwards, the dependence of the diffusion coefficient on relative humidity was observed. Fig. 4(a) and (b) shows the obtained values of water diffusion coefficient D for HM-pectin and Soya-PC liposomes as a function of relative humidity. In both samples the diffusion coefficient increases until reaching a maximum value at relative humidities around 60–80% and then starts going down, following the tendency given by the adsorption equilibrium isotherm. At the same time, the diffusion coefficient of water vapor in the liposomes is nearly one order of magnitude higher than in the pectin, indicating that the adsorption will take place faster (see Fig. 1 for an overall picture). The opposite effect (slower desorption) is observed for liposomes, but that is strongly influenced by the shape of the adsorption isotherms presenting multilayer adsorption for higher relative humidities.

4. Conclusions

The characteristics of water adsorption, desorption and diffusion in HM-pectin and Soya-PC liposomes were studied at

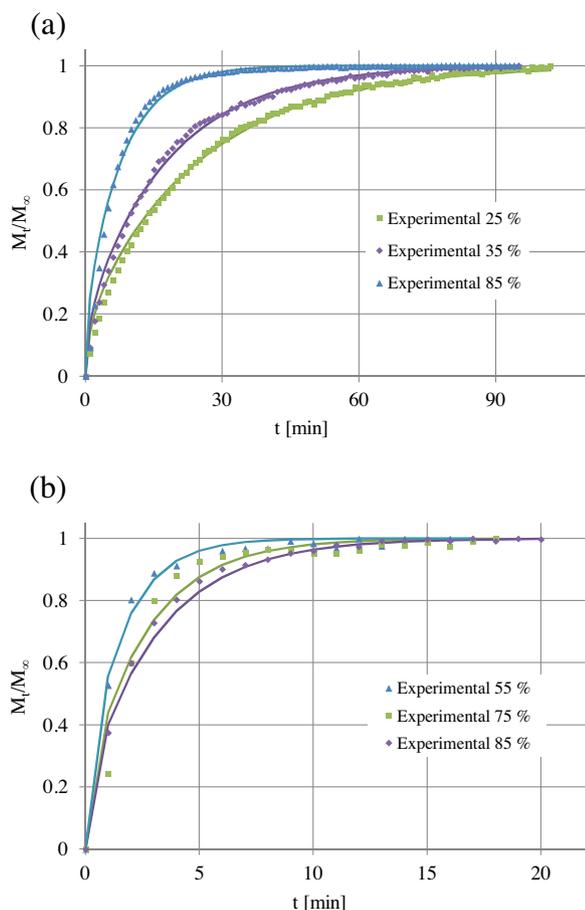


Fig. 3. Evolution of adimensional loading M_t/M_∞ as a function of adsorption time for (a) HM-pectin at three different relative humidities: 25%, 35% and 85%, and for (b) Soya-PC liposomes at 55%, 75% and 85% RH. The solid lines denote theoretical curves fitting, according to Eq. (3).

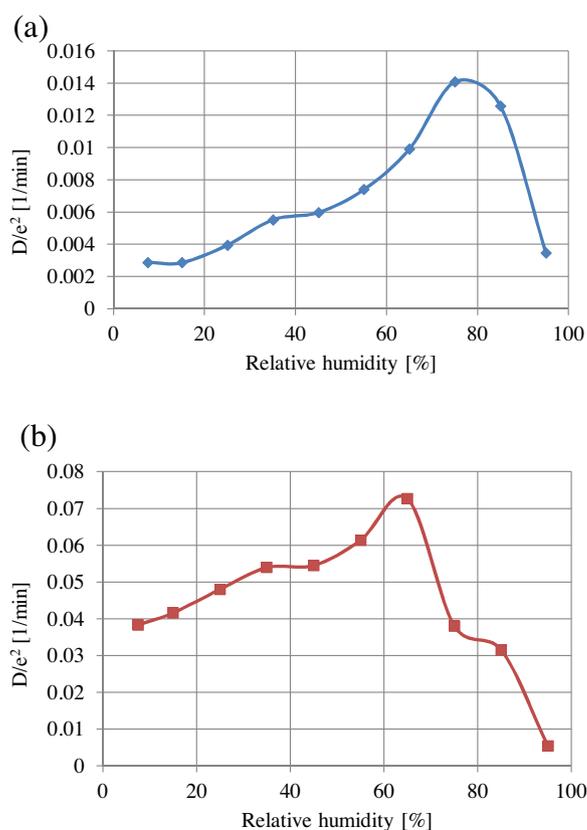


Fig. 4. Water diffusion coefficient for (a) HM-pectin and (b) Soya-PC liposomes as a function of relative humidity.

35 °C, targeting suitability of these materials for moisture protection in the oral cavity. The gravimetric method used proved to be sensitive enough to measure very small changes in the sample mass over wide relative humidity range. It was found that between 95 and 55% RH the moisture content of the liposomes was higher than the one for pectin, reaching the maximum value of 1.2 mg water/mg of material at 95% RH (twice as much as for pectin). The materials studied represented two different types of isotherm: type II for HM-pectin and type III for Soya-PC liposomes. The water diffusion in the liposomes was found to be one order of magnitude faster than in HM-pectin (0.04 1/min compared to 0.003 1/min measured at RH=7.5%). The diffusion strongly depended on the amount of water adsorbed, increasing until relative humidities around 60–80%, and decreasing thereafter. Based on this study it is possible to determine that the Soya-PC liposomes are capable of retaining water for longer period of time at higher relative humidities, making them optimal choice for hydration of mucosa in the oral cavity.

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References

- Alimi, D., 2015. Xerostomia induced by radiotherapy. *Ther. Clin. Risk Manag.* 11, 1149–1152.
- Brunauer, S., Emmet, P.H., Teller, E.J., 1938. Adsorption of gases in multimolecular layers. *Am. Chem. Soc.* 60, 309–319.
- Crank, J., 1975. *The Mathematics of Diffusion*. Clarendon Press, Oxford.
- Damitz, R., Anuj, Ch., 2015. Parenteral emulsions and liposomes to treat drug overdose. *Adv. Drug Deliv. Rev.* 90, 12–23.

- Daves, C., 1987. Physiological factors affecting salivary flow rate, oral sugar clearance, and the sensation of dry mouth in man. *J. Dent. Res.* 66, 648–653.
- Davies, L.A., Nunez-Alonso, G.A., McLachlan, G., Hyde, S.C., Gill, D.R., 2014. Aerosol delivery of DNA/liposomes to the lung for cystic fibrosis gene therapy. *Hum. Gene Ther. Clin. Dev.* 25 (2), 97–107.
- Do, D.D., 1998. *Adsorption Analysis: Equilibria and Kinetics*. Imperial College Press, London.
- Egbaria, K., Weiner, N., 1990. Liposomes as a topical drug delivery system. *Adv. Drug Deliv. Rev.* 5, 287–300.
- Einhorn-Stol, U., Benthin, A., Zimathies, A., Görke, O., Drush, S., 2014. Pectin-water interactions: comparison of different analytical methods and influence of storage. *Food Hydrocolloids* 43, 577–583.
- Gan, L., Wang, J., Jiang, M., Bartlett, H., Ouyang, D., Eperjesi, F., Liu, J., Gan, Y., 2013. Recent advances in topical ophthalmic drug delivery with lipid-based nanocarriers. *Drug Discov. Today* 18, 290–297.
- Guggenheimer, J., Moore, P.A., 2003. Xerostomia: etiology, recognition and treatment. *J. Am. Dent. Assoc.* 134, 61–69.
- Hearnden, V., Sankar, V., Hull, K., Juras, D.V., Greenberg, M., Ross Kerr, A., Lockhart, P. B., Pattoni, L.L., Porter, S., Thornbill, M.H., 2012. New developments and opportunities in oral mucosal drug delivery for local and systemic disease. *Adv. Drug Deliv. Rev.* 64, 16–28.
- Hoekstra, F.A., Wolkers, W.F., Buitink, J., Golovina, E.A., Crowe, J.H., Crowe, L.M., 1997. Membrane stabilization in the dry state. *Comp. Biochem. Physiol.* 117A, 335–341.
- Johnsen, G.K., Norlen, L., Martinsen, Ø.G., Grimnes, S., 2011. Sorption properties of the human stratum corneum. *Skin Pharmacol. Physiol.* 24, 190–198.
- Joshi, S., Petereit, H.-U., 2013. Film coatings for taste masking and moisture protection. *Int. J. Pharm.* 457, 395–406.
- Klemetsrud, T., Jonassen, H., Hiorth, M., Kjøniksen, A.-L., Smistad, G., 2013. Studies on pectin-coated liposomes and their interaction with mucin. *Colloids Surf. B* 103, 158–165.
- Kroon, J., Metselbaar, J.M., Storm, G., van der Pluijm, G., 2014. Liposomal nanomedicines in the treatment of prostate cancer. *Cancer Treat. Rev.* 40, 578–584.
- Madsen, K.D., Sander, C., Baldursdottir, S., Pedersen, A.M.L., Jacobsen, J., 2013. Development of an ex vivo retention model simulating bioadhesion in the oral cavity using human saliva and physiologically relevant irrigation media. *Int. J. Pharm.* 448, 373–381.
- Mohnen, D., 2008. Pectin structure and biosynthesis. *Curr. Opin. Plant Biol.* 11, 266–277.
- Momm, F., Volegova-Neher, N.J., Schulte-Monting, J., Guttenberger, R., 2005. Different saliva substitutes for treatment of xerostomia following radiotherapy. *Strahlenther. Onkol.* 181, 231–236.
- Mucha, M., Ludwiczak, S., Kawińska, M., 2005. Kinetics of water sorption by chitosan and its blends with poly(vinyl alcohol). *Carbohydr. Polym.* 62, 42–49.
- Nguyen, S., Alund, S.J., Hiorth, M., Kjøniksen, A.-L., Smistad, G., 2011. Studies on pectin coating of liposomes for drug delivery. *Colloids Surf. B* 88, 664–673.
- Rockland, L.B., 1960. Saturated salt solutions for static control of relative humidity between 5 and 40 C. *Anal. Chem.* 32 (10), 1375–1376.
- Ruthven, D.M., 1984. *Principles of Adsorption and Adsorption Processes*. Wiley, New York.
- Sørensen, I., Pedersen, H.L., Willats, W.G., 2009. An array of possibilities for pectin. *Carbohydr. Res.* 344 (14), 1872–1878.
- Scholz, O.A., Wolff, A., Schumacher, A., Giannola, L.I., Campisi, G., Ciach, T., Velten, T., 2008. Drug delivery from the oral cavity: focus on a novel mechatronic delivery device. *Drug Discov. Today* 13, 247–253.
- Tho, I., Sande, S.A., Kleinebudde, P., 2002. Pectinic acid, a novel excipient for production of pellets by extrusion/spheronisation: preliminary studies. *Eur. J. Pharm. Biopharm.* 54, 95–99.