Polymer coated mucoadhesive liposomes intended for the management of xerostomia

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Abstract

The aim of this work was to prepare and test different pharmaceutical formulations in respect of their potential in relieving dry mouth symptom. Since many of the products available on the market provide only temporary relief to the patients, there is need for new formulations able to retain on the oral mucosa. The prolonged moisture protection could be achieved by combining mucoadhesive materials, such as polymers containing hydrogen bonding groups, with vesicles capable of releasing hydration medium from the inner compartment. In this study three different types of liposomes (positively, negatively and neutrally charged) were coated with five different types of polymers: low-methoxylated pectin (LM-pectin), high-methoxylated pectin (HM-pectin), alginate, chitosan and hydrophobically modified ethyl hydroxyethyl cellulose (HM-EHEC). The particle size and the zeta potential of the obtained carriers were tested by measuring dynamic light scattering (DLS) and electrophoretic mobility.
Later on, selected positively charged liposomes were deposited on a negatively charged mica surface and depicted by atomic force microscopy (AFM). The water sorption properties of polymers, uncoated liposomes and polymer-coated liposomes were studied by the means of dynamic vapor sorption (DVS). The experiments were performed within the relative humidity range RH = 95 – 0 – 95 %, at 35°C. It was found that coating the liposomes with polymers significantly increased the water sorption capacity of the formulations, making them an attractive choice for hydration of the oral mucosa.

**Keywords:** xerostomia, oral mucosa, mucoadhesive polymers, liposomes, water sorption properties

1. **Introduction**

The presence of saliva is one of the most important prerequisites for maintaining a healthy oral environment\(^1\). However, up to 30 % of the population is subjected to decreased or absent saliva flow due to taken medications, cancer treatment, systemic diseases or salivary gland dysfunction\(^2,3\). An uncomfortable feeling of dryness in the oral cavity is often accompanied by an increased occurrence of dental caries, tooth erosion, and fungal infections. Products for the treatment of xerostomia available on the market can be divided in two categories: saliva stimulants (for patients with functioning salivary glands) or saliva substitutes (in case of severe damage to the salivary glands). Treatments of xerostomia are mostly based on local drug delivery to the oral cavity, and as such must comply several conditions, i.e. lack of bitterness, easiness of administration, and high patient compliance\(^4\). Even though there are many formulations that provide temporary relief from dry mouth, their residence time in the oral cavity is limited due to the swallowing reflex, speech and mastication\(^5\). Therefore, there is a need for a formulation to be able to adhere to the oral mucosa and provide prolonged moisture protection.
Mucoadhesion is defined as the adherence of a material to the mucosal surface lasting longer than mucus turnover (clearance time, typically minutes to hours). It is a complex process that involves several types of binding mechanisms, such as electrostatic interactions, interactions with hydrophilic functional groups, or binding to the specific receptor sites in mucin\(^5, 6\). Mucoadhesive polymers are flexible-backbone macromolecules containing hydrogen bonding groups, capable of developing interactions with the glycoproteins present in the mucin. During the contact with a mucous membrane the polymers swell and therefore expose maximum number of adhesive sites, which enables interdiffusion and interpenetration of polymer chains and mucin network\(^7, 8\). There are many polymeric materials that have been identified as mucoadhesive, e.g. poly(acrylic acid) PAA, poly(vinyl alcohol) PVA, chitosans, pectins, alginates, cellulose derivatives, hyaluronic acids and thiomers\(^8-11\). Polyanions are often used since they demonstrate stronger mucoadhesive properties than nonionic materials and lower toxicity than some of polycations. However, positively charged chitosan is widely studied as a mucoadhesive agent for systemic delivery, due to its ability to open tight junctions between epithelial cells and thus enabling efficient mucus permeation\(^12\).

Liposomes are nano-sized spherical vesicles with an aqueous core enclosed by lipid bilayers. They are extensively used as carriers of therapeutically active agents, since they provide reservoir for the controlled release of a load\(^13, 14\). The long-term stability of liposomes can be immensely improved by coating them with various polymers\(^15, 16\). Combination of mucoadhesive polymers with liposomes gives therefore an unique opportunity to improve the residence time of a formulation on the oral mucosa, as well as provide prolonged moisture protection.

The aim of this study was to identify/develop a formulation that would combine advantages of prolonged mucoadhesion with high water sorption capacity. Thirteen different formulations (polymers, liposomes and polymer-coated liposomes) were included in the study. The
mucoadhesive properties and biocompatibility of these formulations were previously studied on HT29-MTX mucus-producing cells and described elsewhere\textsuperscript{17}. The water sorption properties of formulations were studied with a gravimetric method (dynamic vapor sorption – DVS). The obtained sorption kinetics and isotherms were used to study the characteristics of water adsorption and desorption in the formulations.

2. Materials and methods

2.1. Materials

Phosphatidylcholine from soybean lecithin (Soya-PC, $M_W = 787$ Da, > 98% phosphatidylcholine) was a kind gift from Lipoid GmbH (Ludwigshafen, Germany). The cationic lipid dioleoyl trimethylammoniumpropane (DOTAP), anionic phosphatidylglycerol (Egg-PG), and fluorescent lipid 1-oleoyl-2-{6-[(7-nitro-2-1,3-benzoazadizol-4-yl)amino]hexanoyl}-sn-glycero-3-phospholipid (NBD-PC) were purchased from Avanti Polar Lipids, Inc. (Alabaster, USA). High-methoxylated pectin (HM-pectin, Genu® pectin 150 USA-SAG, DM = 70\%, $M_W = 1.1 \times 10^5$ Da) and low-methoxylated pectin (LM-pectin, Genu® pectin LM12CG-Z, DM = 34.8 \%, $M_W = 7.6 \times 10^4$ Da) were obtained from CPKelco (Großenbrode, Germany). Hydrophobically modified ethyl hydroxyethyl cellulose (HM-EHEC, $M_W = 2.5 \times 10^5$ Da) was a gift from AkzoNobel Chemicals AS (Stenungsund, Sweden). Chitosan (Protasan UPCL 213, Novamatrix, DD = 83 \%, $M_W = 3.1 \times 10^5$ Da) and sodium alginate (Protanale LF 10/60, $M_W = 1.47 \times 10^5$ Da) was a gift from from FMC Biopolymer AS (Sandvika, Norway). Sodium dihydrogen phosphate monohydrate, disodium hydrogen phosphate dihydrate and chloroform of analytical grade were purchased from Merck (Darmstadt, Germany). Potassium sulfate was obtained from Sigma-Aldrich (St. Louis, USA).

2.2. Preparation of liposomes
Liposomes were prepared according to the thin film method\textsuperscript{18}. First, a solution of phospholipid components in chloroform was prepared in order to obtain a homogeneous mixture. Later on, chloroform was removed in a rotary evaporator (Heidolph W 2001 rotavapor, Heidolph Instruments GmbH & Co. KG, Kelheim, Germany) and the subsequent lipid films were dried under vacuum (Christ Alpha 2-4 freeze drier, Christ, Osterode am Harz, Germany) overnight. The lipid cake was hydrated with phosphate buffer solution (5 mM, pH 6.8) and gently stirred from time to time during 2 hours storage at room temperature. The resulting solution of multilamellar vesicles was then extruded (Lipex extruder, Lipex Biomembranes Inc., Vancouver, Canada) through two-stacked polycarbonate membranes with pore size of 200 nm (Nucleopore\textsuperscript{®}, Costar Corp., Cambridge, USA), in order to obtain unilamellar liposomes. The final concentration of the lipid in the samples was equal to 3 mM. Liposomal formulations were stored in a glass container layered with nitrogen in refrigerator before further use.

2.3. Coating the liposomes with polymers

Commercially available polymers were additionally purified (except chitosan) in a three-step process including centrifugation, dialysis and freeze–drying of the solutions as described elsewhere\textsuperscript{16}. The solutions were prepared by dissolving the polymers in phosphate buffer (5 mM, pH 6.8), at a concentration of 0.125\% (w/v). The mixtures were then allowed to stir overnight at a room temperature and filtered through 2 µm polycarbonate membrane (Nucleopore\textsuperscript{®}, Costar Corp., Cambridge, USA), in order to minimize the presence of contaminating particles. Later on, the liposomes were added to the polymer solutions in 1:4 ratios, under magnetic stirring and in a dropwise manner by use of Watson-Marlow peristaltic pump. Table 1 summarizes the composition of all liposomal formulations.
<table>
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<th>Sample abbreviation</th>
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<th>Type of coating</th>
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</table>

**Table 1.** The composition, type of coating and charge of the liposomal formulations.

2.4. Characterization of liposomes

2.4.1. Particle size and zeta potential measurements

Dynamic light scattering and microelectrophoretic measurements were carried out in order to determine the hydrodynamic diameter (size) and electrophoretic mobility (zeta potential) of the liposomes, using Zetasizer Nano Series (Malvern Instruments Ltd., Worcestershire, UK). The measurements were performed at 25°C and with 173° backscatter angle. The samples were measured in a triplicate and the resulting values were counted as an average from three subsequent runs with 10 or 20 measurements each (for size and zeta potential, respectively).

2.4.2. Atomic Force Microscopy measurements
Positively charged, uncoated liposomes and chitosan coated liposomes were deposited on freshly cleaved mica surface, bearing negative charge. The samples were left for air-drying until the next day. AFM imaging was performed with NanoWizard® instrument (JPK Instruments, Berlin, Germany). The intermittent contact mode images were obtained in the air, using ultrasharp silicon cantilevers (NSC35/AIBS, MicroMash, Spain). The images were recorded at the scan rate of 1 Hz for the three randomly chosen places. The images were flattened using an algorithm provided with the instrument.

2.5. Characterization of water sorption properties

2.5.1. Preparation of samples

100 µl of solution (polymers, liposomes or polymer coated liposomes) was placed onto previously weighted aluminum sample pans (Perkin-Elmer, Boston, US). Later on, the samples were kept at room temperature (25°C) and at relative humidity of 97 %. In order to ensure constant level of humidity, a closed dessicator filled with saturated solution of potassium sulfate was used for storing the samples. The samples were kept in a container until no more liquid phase could be seen with a bare eye, which took approximately 1 – 4 weeks for different types of samples.

2.5.2. Dynamic Vapor Sorption measurements

The water sorption properties of the samples were measured with a gravimetric instrument (DVS, Surface Measurement Systems Ltd., UK), using a sensitive method developed specifically for measurements of the liposomes. The sample pan was placed on a hang-down wire connected to the microbalance in a closed chamber, in which the desired level of relative humidity is established by mixing water vapor with a flow of nitrogen. During the measurements, the temperature was held constant at 35°C and the starting relative humidity was set to 95 %. The sample mass readings during desorption experiment were recorded at
relative humidity values changing stepwise (10 % at the time) until 15 %, and afterwards at 7.5 % and 0 %. Later on, the adsorption experiment was performed at the same RH values. The duration of each stage was variable: mass equilibrium criteria was defined as \( \frac{dm}{dt} = 0.0008 \, \text{mg/min} \), and stability duration was set up to last at least 60 min. Once the state of equilibrium was reached, the relative humidity was programmed to change stepwise. In the case of samples that could not fill a mass equilibrium requirement, time limit of a stage was set to 600 minutes in order to ensure effectiveness of the analysis. The sample mass readings were recorded every minute.

3. Results and Discussion

Figures 1 and 2 present the size and the zeta potential of the obtained formulations, respectively. The size of the liposomes without polymer coating was within the range of 135 – 174 nm, as shown in Figure 1. The polydispersity indexes (\( \leq 0.12 \)) indicate that all the solutions were monodisperse. Coating the liposomes with polymers resulted in size increase of all the formulations. Alginate and LM-pectin coated liposomes were quite similar in size (~250 nm), while HM-pectin, chitosan and HM-EHEC coated liposomes were above 400 nm. The size distribution of all the polymer coated liposomes was broader than the uncoated liposomes, but with the exception of chitosan-coated liposomes the PDI index did not exceed 0.25. The uncoated liposomes exhibited positive (ZP ~ 38 mV), negative (ZP ~ -52 mV) and nearly neutral charge (ZP ~ -1.8 mV). Coating the positively charged liposomes with negatively charged polymers (LM-pectin, HM-pectin and alginate) resulted in reversal of the zeta potential to negative values, which together with size increase confirms the formation of polymer coating. Similarly, the negatively charged liposomes exhibited positive charge after coating them with chitosan. In both cases, the driving force for the polymer adsorption was the strong electrostatic interactions between oppositely charged species. In case of HM-EHEC
coated liposomes, the main controlling factor in the adsorption process was most probably hydrogen bonding and/or hydrophobic force\textsuperscript{20}.

**Figure 1.** Size of the liposomes before and after coating with the polymers. The labels represent values of the polydispersity index (PDI). The error bars are the standard deviation from three measurements.
Figure 2. Zeta potential of the liposomes before and after coating with the polymers. The error bars represent standard deviation from three measurements.

Representative AFM images of positively charged, uncoated liposomes (pos LP) and chitosan coated liposomes (chit cLP) are shown in Figure 3. The imaging of chit cLP revealed the presence of large, round aggregates together with population of smaller particles, which could be some residual chitosan particles. The bridging of liposomes could explain the bigger size and the higher PDI index obtained by DLS. The uncoated liposomes present on the mica surface occurred in two different forms; round, intact liposomes with average diameter well corresponding with DLS measurements (~140 nm), as well as collapsed and flattened structures damaged due to the air-drying step prior to the imaging. Interestingly, chit cLP were not so flattened as posLP (liposome height 51 nm against 16 nm), which could be related to the additional stabilization coming from the polymer coating.
Figure 3. AFM height and phase images of chitosan coated liposomes (a, b) and positively charged, uncoated liposomes (c, d). Each micrograph has a size of 5 x 5 µm, and z scale is equal to 51 nm/16 nm (for a and c, respectively), or 31 deg/11 deg (for b and d).
b)
Figure 4. Kinetic curve of water sorption for (a) alginate and (b) alginate-coated liposomes. The dashed upper curve represents steps in the relative humidity, while the solid lower curve denotes for changes in sample mass related to the water content.

Figure 4 presents an exemplary data obtained from two different gravimetric experiments, in which desorption and adsorption of water in alginate and alginate-coated liposomes was studied. The kinetic curves describe changes in the mass of water (per mg of dry material) occurring due to the stepwise changes in relative humidity RH, going from 95 % down to 0 % and then increasing again to 95 %. As expected, a decline in relative humidity resulted in decreased water content in the sample, while increase in relative humidity was followed with increased water content. The changes in water content were most pronounced in the initial stages of desorption and the final stages of adsorption, that is for 95 and 85 % RH. As shown in Fig. 4a, the alginate water sorption experiment lasted ca. 50 h, and the desorption stage was 2 h longer than the adsorption stage. Particularly at 85 % RH, the equilibrium mass for the adsorption was reached almost three times faster than for the desorption. However, for many stages (55 %, 35 %, 25 %, 15 %), the desorption time was shorter than adsorption. Interestingly, for the alginate coated liposomes (Fig. 4b) the equilibrium mass for the adsorption was reached faster than for the desorption with only few exceptions. That effect is favorable and might be related to slow water release from the alginate coated liposomes in the desorption process, and fast material regeneration in the adsorption stage. The unexpected increase in water content during the desorption stage at 95 % RH might be related to the fact that when the sample was moved from the dessicator into the experimental chamber, quick water loss might have occurred and therefore, the liposomes re-adsorbed water after closing the chamber. Therefore, the desorption stage at 95 % RH was not taken into account while comparing these two materials. Similar experiments were performed for all the polymers, bare
liposomes and polymer-coated liposomes. In order to preserve the clarity of the paper, the data is not shown here.

The equilibrium values of water content for all the formulations studied were used for plotting water sorption isotherms. As an example, Table 2 summarizes the equilibrium values of moisture content for alginate; positive, uncoated liposomes (pos LP); and alginate coated liposomes (alg cLP).
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Table 2. The equilibrium values of adsorption capacity (AC) for alginate, positive, uncoated liposomes (pos LP), and alginate coated liposomes (alg cLP).

It is worth noticing that the moisture content of the uncoated, positive liposomes was at all times lower than for alginate, during both desorption and adsorption experiments. After coating the liposomes with alginate, however, the values of moisture content were substantially altered. Coated liposomes demonstrated higher water content than bare liposomes within all the range of studied humidities, doubling the water content at 95 % RH in the adsorption experiment. Alginate coated liposomes displayed higher water content than alginate between 95 – 65 % RH in the desorption stage, same (or very similar) water content between 5 – 35 % RH in the adsorption stage, and higher water content from 45 % RH until the end of the adsorption stage. Taking into account that the humidity values in dry mouth oscillate around 50-80 % RH, it is clearly demonstrated that water sorption properties of the alginate coated liposomes are superior to bare liposomes and polymers within the mentioned humidity range.

A modified Brunauer-Emmet-Teller (BET) model considering a limited number of adsorbed layers was applied to fit the adsorption isotherms to the experimental data:  

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

(1)

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

The obtained adsorption isotherms are presented in Figure 5.

a)
Figure 5. Water sorption isotherms of (a) positively charged liposomes, alginate and alginate coated liposomes, (b) positively charged liposomes, pectins and pectin coated liposomes, (c)
negatively charged liposomes, chitosan and chitosan-coated liposomes, and (d) neutral liposomes, HM-EHEC and HM-EHEC coated liposomes. The points denote equilibrium values of adsorption capacity for uncoated liposomes (filled square), polymers (filled circle and rhombus), and polymer coated liposomes (empty circle and rhombus). The solid and dashed lines represent theoretical curves fitting according to Eq. (1).

Figure 5a demonstrates the differences in water sorption behavior of positively charged liposomes, alginate and alginate coated liposomes, as described in details above. As presented in Figure 5b, the uncoated, positive liposomes adsorbed more water than LM-pectin and HM-pectin only at 95 % RH, while pectin coated liposomes demonstrated higher water content than bare liposomes within all the range of studied humidities. Starting from 65 and 75 % RH (for LM-pectin and HM-pectin, respectively), water sorption properties of the coated liposomes were superior to the polymers as well. The maximum water sorption capacity of pectin coated liposomes was at least twice as high as the water sorption capacity for LM-pectin and HM-pectin at 95 % RH.

The behavior of negatively charged liposomes (Fig. 5c) between 5 – 35 % RH was quite similar to chitosan, while starting from 45 % RH the water content of chitosan was superior to the one of liposomes. Surprisingly, chitosan coated liposomes displayed lower water content than bare liposomes within 5-35 % RH, but performed better at 45 % and higher humidities, reaching a very high value of 2.1 mg water/mg of material at 95 % RH (three times higher than uncoated liposomes and 2.5 times higher than chitosan itself).

The neutral liposomes (Fig. 5d) displayed higher water sorption capacity than HM-EHEC within 5 – 95 % RH (maximum sorption capacity 1.5 higher than the polymer). HM-EHEC coated liposomes performed better than the bare liposomes at 25 % RH and higher humidities, improving the water sorption capacity from 1.2 mg water/mg of material to 2.0 mg/mg.
All the formulations studied have either type II or type III adsorption isotherms according to IUPAC classification\textsuperscript{22}. Type II isotherms are characteristic for systems with an initial monolayer, followed by multilayer adsorption at higher relative humidities. The relatively flat intermediate region corresponds to the amount of water necessary for the monolayer formation. In type III isotherms, adsorption forces are repulsive and the interactions between water and the material are weak. The uptake is facilitated at higher RH values, when water interacts with already adsorbed water layers rather than with the adsorbent surface. Most of the polymers and uncoated liposomes represented type II of isotherm, with the exception of HM-EHEC and neutral liposomes. Interestingly, all the liposomes coated with polymers represented type III of isotherm. One can conclude that a careful analysis of the isotherms behavior allows to anticipate optimal water sorption properties of the materials.

Figure 6 summarizes the water sorption capacities of all the studied materials at 95 % RH. The sorption capacity of the uncoated liposomes enhanced in the following order: neg LP < pos LP < neu LP. The higher content of soya-phosphatidylcholine (99 mol % for neutral liposomes, compared to 89 mol % for others) resulted in the higher sorption capacity. Negatively charged liposomes, containing 10 % of egg-PG, adsorbed less water than positively charged liposomes with 10 % of DOTAP as a second main lipid. The water sorption capacity of the polymers is dependent on many variables, such as molecular weight, temperature, crystallinity degree, or the presence and amount of functional groups\textsuperscript{23}. For example, the rate of water sorption in chitosan has been found to be higher for lower deacetylation degree\textsuperscript{24}. Therefore, it might be difficult to predict the behavior of very different polymeric materials. In general, water sorption capacity of the polymers increased in the following order: LM-pectin < HM-pectin < chitosan < HM-EHEC < alginate. HM-pectin displayed higher water sorption capacity than LM-pectin due to the higher content of methoxyl groups (70 to 35 %, respectively), which represent hydrophilic sites for water
adsorption. Chitosan with high deacetylation degree (DD = 83 %) demonstrated an intermediate water sorption capacity of 0.8 mg/mg, which was only slightly lower than the one of HM-EHEC (0.82 mg/mg). Alginate is known for its very high ability to adsorb and retain water and commonly used in wound dressing industry\textsuperscript{25}; the water sorption capacity was the highest among the studied polymers and reached 1.2 mg/mg. Water sorption capacity of the polymer coated liposomes was the lowest for LM-pectin coated liposomes (1.11 mg/mg), followed by HM-pectin coated liposomes (1.64 mg/mg), quite similar for alginate and HM-EHEC coated liposomes (1.91 and 1.99, respectively), and the highest for chitosan coated liposomes (2.1 mg/ml). It was found that the higher molecular weight of polymer used for coating, the higher was water sorption capacity of the obtained liposomes.

Figure 6. Summary of water sorption capacity of all the studied materials, including polymers, bare liposomes and polymer coated liposomes (RH = 95 %).
Interestingly, the polymer coated liposomes demonstrated three different types of behavior regarding the total value of water sorption capacity. In the case of alginate and HM-EHEC coated liposomes, the final water sorption capacity was approximately a sum of water sorption capacities coming from the polymer and the uncoated liposomes. The water sorption capacity of LM-pectin coated liposomes was slightly lower than a sum of water sorption capacities of bare polymer and positively charged liposomes. Finally, coating the positively charged liposomes with HM-pectin and negatively charged liposomes with chitosan resulted in a synergistic effect; the final water sorption capacity of the coated liposomes was higher than it could be expected from simple sum of its parts. A synergy was particularly pronounced for chitosan coated liposomes, whose water sorption capacity reached 2.1 mg/mg, which is 1.4 times higher than what could be estimated from summing up water sorption capacities of the components. Above all, coating the liposomes with polymers significantly improved the water sorption capacity of the formulations, with no exception.

4. Conclusions

This work has presented a comprehensive study of the water sorption properties of polymers, liposomes and polymer coated liposomes, intended for hydration of the oral mucosa. Among the polymers, negatively charged alginate displayed the highest water sorption capacity. The water sorption properties of neutral liposomes, composed mainly of soya phosphatidylcholine, were superior compared to positively and negatively charged liposomes. In all the studied cases, coating the liposomes with polymers prominently improved the water sorption capacity of the formulations. The water sorption capacity of the polymer coated liposomes increased in the following order: LM cLP < HM cLP < alg cLP < HM-EHEC cLP < chit cLP. In view of high mucoadhesions and mucosal biocompatibility demonstrated by alg cLP in another study on HT29-MTX mucus-producing cells (17), it can be concluded that the alginate coated liposomes could be an excellent choice for prolonged moisture protection in the oral cavity.
Chitosan coated liposomes, with the highest water sorption capacity among the studied formulations, and high mucoadhesion to the mucus-producing cells are another possible selection for relieving dry mouth symptoms. The important drawback of chitosan coated liposomes was decreased mucosal biocompatibility, which could be related to its high deacetylation degree (83 %). Hence, use of chitosan with lower content of amino groups might be of interest, as it could further increase the rate of water sorption in the formulation and decrease the toxicity to the mucosa.

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Abbreviations

DLS, dynamic light scattering; AFM, atomic force microscopy; DVS, dynamic vapor sorption; RH, relative humidity; pos LP, positively charged uncoated liposomes; LMpect cLP, LM-pectin coated liposomes; HMpect cLP, HM-pectin coated liposomes; alg cLP, alginate coated liposomes; neg LP, negatively charged uncoated liposomes; chit cLP, chitosan coated liposomes; neu LP, neutral uncoated liposomes; HM-EHEC cLP, HM-EHEC coated liposomes; ZP, zeta potential; PDI, polydispersity index; AC, adsorption capacity.

References


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