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Biopolymers-Lipid Hybrid Cubosome for Delivery of Acemannan †

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Abstract: In the last decades, the pharmaceutic industry has shown great interest in new products for drug delivery, since the studies with drug nanocarriers evidenced the application potential of these systems. A relatively new strategy for nano drug deliver is the use of cubosomes, which is a nanoparticle with crystalline structure formed by lipid bilayer created for instance with monoolein lipid and Pluronic F127 as a stabilizer. In our studies we develop a cubosome containing biopolymers-shell for the delivery of acemannan as a bioactive extracted from aloe vera, which has immunomodulation properties, The cubosome was produced using monoolein and Pluronic F127, adding aqueous solutions of chitosan-*N*-arginine, alginate and acemannan. The nanopartilces were studied by means of dynamic light scattering, zeta potential and isothermal titration calorimetry to evaluate the thermodynamic interaction of the hybrid cubosomes with liposomes produced with POPG, as a model cell membrane, in different pH conditions. The encapsulation percentage and delivery profiles of acemannan were besides accessed through spectrophotometry. The encapsulation of acemannan was highly effective and delivery was attenuated and sustained, further suggesting the potential of the hybrid cubosome as a bioactive delivery system. The interaction of the hybrid cubosome with liposomes, unveiled by thermodynamic results, was favored in two different pHs (2.5 and 7.4), evidencing that the binding of the hybrid cubosomes with the model membrane present different physicochemical characteristics depending on pH, which play a role in the enthalpic and entropic contributions during the interaction. Overall, the results indicate potential of the hybrid cubosomes for oral administration of acemannan.

Keywords: acemannan; aloe vera; cubosomes; bioactive; delivery

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1. Introduction

The study of nanocarriers has been increasing in the last decades for multiple purposes in medicine and pharmaceutic fields, aiming new treatments with improved bioavailability and flexibility characteristics. In this context, drug carriers with different responses in regard for instance to the pH of the aimed biological media are produced with biopolymers, lipids or peptides bearing properties of self-assembly in aqueous medium and encapsulating drugs or bioactives, through different physicochemical techniques [1,2]. Chitosan and alginate are biopolymers considered in the pharmaceutic industry and widely used for creation of gels, micro and nano particles and biofilm to be applied in the biomedical field, for instance to enhance skin repair where properties of chitosan provide 3D scaffolds structure helping the maintenance and cell grown. In addition, in the nanocarriers field, chitosan can aid in the interaction between the drug carrier and the biological target showing biocompatible properties [3]. Moreover, there are lipid nanocarriers focused on the progressive and sustained delivery of bioactives in the treatment of

chronic diseases such as inflammation where the lipid component is the lipid monoolein, providing a three-dimensional structure as a cubic chiral phase of lm3m symmetry, attributed to spontaneous crystallization [4]. Similarly, a nanoparticle of cubic phase Pn3m [5] that shapes the organization of the nanochannel network with drug delivery capability, provides interaction with the target and can slowly deliver the carried drug [6]. In these nanoparticles, the internal crystalline structure contains a hydrophobic region in the acyl region of lipid bilayers plus a hydrophilic region in the nanochannels of the lattice, providing a relatively high surface area for the encapsulation of various drugs and bioactives. Furthermore, the binding of polyelectrolytes on the lipid cubosome can protect the encapsulated drug for the treatment of diseases through oral administration, avoiding degradation of the drug in gastric conditions besides improving absorption in the intestine [1,6].

In our study, we develop a cubosome nanoparticle encapsulating acemannan and containing chitosan chemically modified with arginine plus alginate, both biopolymers providing a pH-responsive shell on the particles surface [7]. The structural characteristics of self-assembled lipid nanoparticles functionalized with chitosan-*N*-arginine and alginate, associated as polyelectrolyte complexes, are studied by dynamic light scattering (DLS) and zeta potential measurements and the thermodynamic interaction with liposomes as model of cell membrane was studied with isothermal titration calorimetry (ITC) in solutions of pH 2.5 and 7.4. Encapsulation and release of acemannan were studied by spectrophotometry in simulated gastric and intestinal conditions unveiling the potential of the obtained hybrid colloidal systems, comprised by lipidic cubosomes with pH-responsive biopolymers shells, for applications in oral administration.

2. Materials and Methods

2.1. Hybrid Nanoparticles and Liposomes Productions

The nanoparticles were produced using monoolein (MO) adding Pluronic F127 (PF127) diluted in chloroform that was evaporated with nitrogen gas producing a film inside the glass vessel. Acemannan (AC) was prepared apart at 20 mg/mL in purified water and 250 μL of the solution were included in the MO and PF127 film leaving at rest for 45 min. Then, previously prepared solutions of chitosan-N-arginine and alginate were included completing the final volume to 3 mL with different buffers: pH 2.5 buffer (acetate) and pH 7.4 buffer (phosphate). The mixture was vortexed for 3 min and put in ultrasonic bath for 10 min (Eco-Sonics Q3.0L, 40 kHz) repeating the procedure 10 times. Samples were left at rest for 7 days. For the production of liposomes, from 100 mg/mL of POPG in chloroform, 329 μL were put in a glass vessel and solvent was evaporated with nitrogen gas to create a film inside the glass vessel where 1 mL of the different buffers pH 2.5 and 7.4 were included for individual samples. The liposomes were produced by self-assembling using tip sonicator (10%) during 10 min in ice bath.

2.2. DLS and ITC Analysis

The samples were characterized using dynamic light scattering, DLS (Zetasizer Nano 300 ZS Malvern) obtaining hydrodynamic diameter and zeta potential. For the studies of interaction between cubosomes and liposomes, isothermal titration calorimetry, ITC, was used (MicroCal Inc. VP-ITC microcalorimeter). Samples of 10 μL cubosomes at 45 mM of MO were injected in the sample at 400 s interval in total 28 injections. The sample cell contained 1.442 mL of liposomes at 5 mM POPC in buffer pH 2.5 and 3 mM POPC in buffer pH 7.4. The results were analyzed using MicroCal software applying the single set of binding sites model [8].

2.3. Encapsulation Measurement

The concentration of acemannan encapsulated in cubosomes was obtained using the centrifugation method. A standard curve for concentration was obtained with a series of

acemannan aqueous solutions at 100%, 80%, 60%, 40%, 20%, 10% and 6% of the concentration included in the cubosomes preparation. The absorbance of each was measured by spectrophotometry at wavelength 196 nm. The hybrid cubosomes containing acemannan were put in a falcon tube with a filter (Millipore 3NMWL) and centrifugated for 1 h (5000 rmp, 25 °C). The filtered was collected and measured in spectrophotometry at 196 nm. The percent of encapsulated acemannan was obtained through Beer's law:

$$A = \varepsilon bc \quad (1)$$

where A is the absorbance of the sample, ε is the molar absorptivity, b is the length of the quartz cell and c is the concentration.

2.4. Release Study

The in vitro release of acemannan from hybrid cubosomes was obtained using the dialysis method. A nitrocellulose membrane of MWCO3500 was left in water (MiliQ) for hydration. One extreme of the membrane was tied using a piece of rope and the membrane was filled with 3 mL of hybrid cubosome solution in buffer pH 2.5 or 7.4 and left suspended in a 100 mL beaker containing the equivalent buffer at 37 °C. The system was continuously stirred (100 rpm) and an aliquot of 1.5 mL was withdrawn from the beaker after 10 min, 20 min, 30 min, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h, 24 h, 26 h and 28 h and the absorbance was read as described above. The volume in the beaker was kept constant by adding the equivalent buffer solution.

3. Results and Discussions

3.1. Zeta Potential and Hydrodynamic Diameter

Table 1 shows zeta potential and size results for cubosomes produced with monoolein (MO) and PF127 and for biopolymers (POL) hybrid cubosomes carrying acemannan (AC), all in two different buffers. As shown, inclusion of biopolymers and acemannan provides changes to the nanoparticles only in pH 7.4, where large size increase and reasonable zeta potential reduction were evidenced. In pH 7.4 chitosan is predominantly neutral while alginate is negatively charged on carboxyl groups. Hence charged alginate must be responsible for the zeta potential drop. Furthermore, in absence of chitosan charges, the electrostatic interaction between both polymers [9] is weak, leading the alginate chains free to expand to the particle surface, as previously described for polyelectrolytes particles [10], resulting the characterized hydrodynamic size increase in pH 7.4. This confers a profitable pH-responsive behavior to the nanoparticles in view that size increase of mucoadhesive devices can play important role on intestinal delivery, considering that the increase of surface contact may favor the intestinal retention, thus leading to more effective absorption of the carrier and carried content.

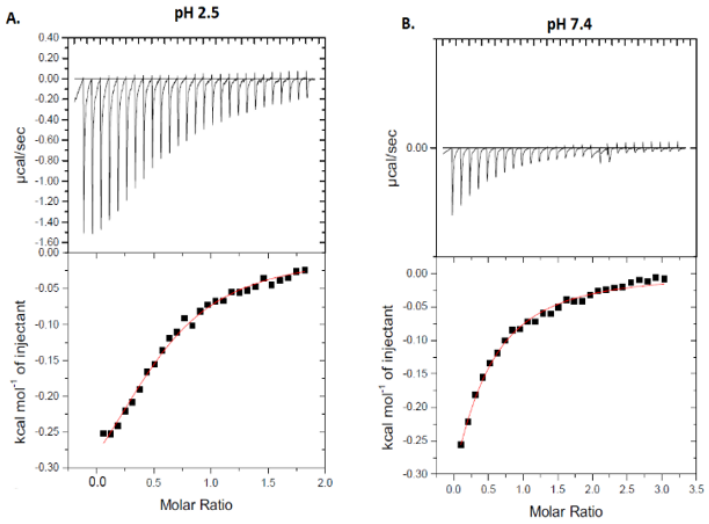
Table 1. Zeta potential, hydrodynamic diameters and polydispersity of studied samples in two pH conditions as indicated.

Sample	Zeta Potential (mV)	Hydrodynamic Diameter (nm)	PDI *
pH 2.5 MO + PF127	0.06 ± 3.5	282 ± 112	0.21
pH 7.4 MO + PF127	−0.14 ± 11.0	251 ± 92	0.17
pH 2.5 MO + PF127 + POL + AC	5.00 ± 5.9	280 ± 155	0.36
pH 7.4 MO + PF127 + POL + AC	−14.01 ± 3.8	662 ± 271	0.25

* Size polydispersity index.

3.2. Interaction with Model Membrane

The effective thermodynamic interaction between hybrid cubosomes and POPG liposomes was studied in controlled conditions through ITC (Figure 1). Hybrid nanoparticles showed a great thermodynamic interaction resulting in average enthalpy variations ΔH ~ −746 cal/mol (pH 2.5) and ΔH ~ −911 cal/mol (pH 7.4) shown in Figure 1. The number of binding sites 1/N and equilibrium constant K increased in pH 7.4 comparing to pH 2.5, indicating a highly favored interaction in pH 7.4. The negative variations in Gibbs free energy further confirm the thermodynamic interaction and the positive entropic variations may indicate disruption of liposomes in contact with cubosomes. The ITC results suggest that hybrid cubosomes may interact with cell membranes, which can improve the absorption of acemannan.



pH	2.5	pH	7.4
N	0.918 ± 0.260 sites	N	0.497 ± 0.0602 sites
K	164 ± 35 M^{-1}	K	951 ± 265 M^{-1}
ΔH	-746 ± 265 cal/mol	ΔH	-911 ± 203 cal/mol
ΔG	-5505 cal/mol	ΔG	-6672 cal/mol
TΔS	4759 cal/mol	TΔS	5760 cal/mol

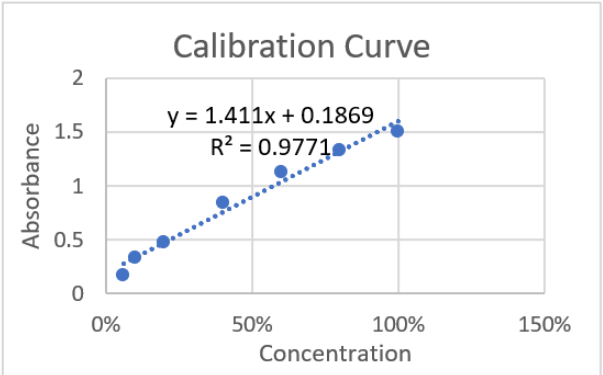
Figure 1. ITC results showing the thermodynamic interaction between hybrid cubosomes and POPG liposomes. (**A**). Buffer pH 2.5. (**B**). Buffer pH 7.4. The table shows the results obtained in applying the single set of binding sites model to the integrated heat released.

3.3. Encapsulation of Acemannan

Table 2 shows the absorbance results for different concentrations of acemannan aqueous solutions in terms of % relative to the concentration included in the hybrid cubosomes and in mM. The "Sample" corresponds to the solution obtained from the centrifugation of the same nanoparticles produced with acemannan.

Table 2. Absorbance results for acemannan aqueous solutions obtained by spectrophotometry (196 nm). The calibration curve is shown in terms of concentration percentage.

c %	c (mM)	Abs
100%	0.980	1.50
80%	0.784	1.33
60%	0.588	1.13
40%	0.392	0.84
20%	0.196	0.47
10%	0.098	0.33
6%	0.058	0.17
Sample		0.27



It is shown that absorbance of the centrifuged sample is between the absorbances corresponding to the 10 and 6% concentrations included in hybrid cubosomes preparation, indicating that at least 90% of acemannan was encapsulated in the nanoparticles. The results showed that the hybrid cubosome has high capacity of encapsulating the bioactive. The bioactive acemannan has hydrophilic characteristic and during production the self-assembly of cubosomes apparently favors the encapsulation of acemannan molecules catching the same into the particles nanochannels network [11]. The channel network features a multi-core structure, which means that the interior of the nanoparticles can accommodate bioactive concentrations in separate cores, allowing enhancement of the total concentration if comparing with micelles and liposomes, in which low encapsulation percentages are usually achieved [12]. Additionally, the properties of each molecule used to produce the hybrid cubosome, with a polymeric shell and a lipid core create the conditions to the encapsulation process, which results in increase in encapsulation percentage [13].

3.4. In Vitro Release of Acemannan

Figure 2 shows the different in vitro release properties for acemannan bioactive. In pH 2.5 the release was higher since the beginning of the experiment and it reached around 50% at 6 h. This result indicates an initial burst release followed by an attenuated release over time in the gastric condition. In pH 7.4 the release was more attenuated during the whole experiment, reaching about 70% release only at 28 h, suggesting a prolonged and sustained release in intestinal condition. Polymeric nanoparticles of pH-sensitive characteristics behave differently in each pH, presenting a tendency of structural change [14]. These properties can help the release to be greater at pH 2.5 where polymeric particles tend to expand in size, thus leaving free the nanopores of the cubosome, which is why they present a greater exchange of liquid from the exterior to the interior of the cubosome, as well as the rheological properties and the density of the macromolecules can affect the passage of transported bioactive through the entangled shell [15].

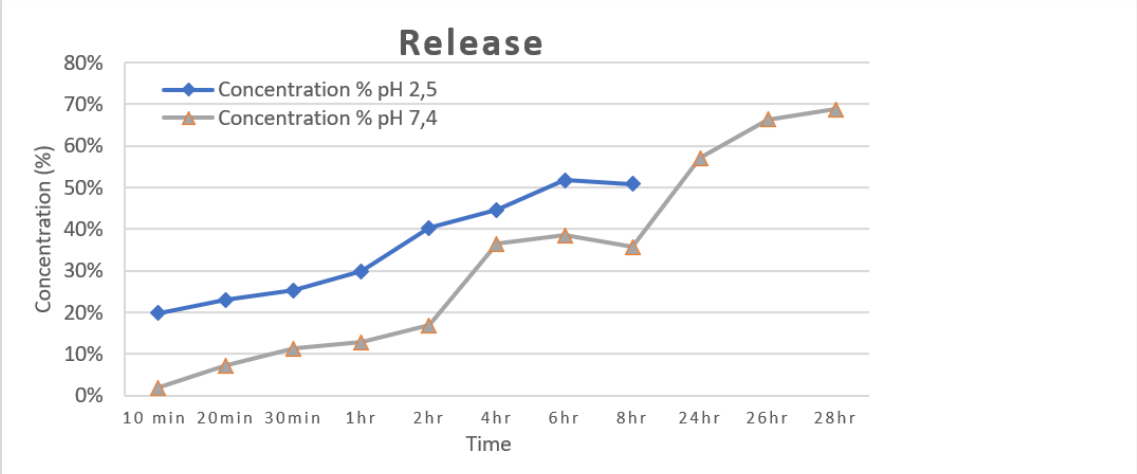


Figure 2. In vitro release of acemannan in different conditions of pH as indicated.

4. Conclusions

- The lipid-biopolymers hybrid cubosome shows high potential for encapsulation and prolonged release of acemannan besides strong interaction with cell model membrane in gastric and intestinal conditions.
- The nanoparticles showed great flexibility in different pHs.
- The variety of components used in the nanoparticles production can differently act in the biological interactions process.

- The study points out a strong perspective of the material as a drug or bioactive delivery system for oral administration.

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Conflicts of Interest: The authors declare no conflict of interest.

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