Article

Mesoscopic Modeling of Encapsulation of Capsaicin by Lecithin/Chitosan Liposomal Nanoparticles

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Abstract: Transport of hydrophobic drugs in the human body exhibits complications due to the low solubility of these compounds. With the purpose of enhancing the bioavailability and biodistribution of such drugs, recent studies have reported the use of amphiphilic molecules, such as phospholipids, for synthesis of nanoparticles or nanocapsules. Given that phospholipids can self–assemble in liposomes or micellar structures, they are ideal candidates to function as vehicles of hydrophobic molecules. In this work, we report mesoscopic simulations of nanoliposomes, constituted by lecithin and coated with a shell of chitosan. The stability of such structure and the efficiency of encapsulation of capsaicin, as well as the internal and superficial distribution of capsaicin and chitosan inside the nanoliposome were analyzed. The characterization of the system was carried out through density maps and the potentials of mean force for the lecithin–capsaicin, lecithin–chitosan and capsaicin–chitosan interactions. The results of these simulations show that chitosan is deposited on the surface of the nanoliposome, as has been reported in some experimental works. It was also observed that a nanoliposome of approximately 18 nm in diameter is stable during the simulation. The deposition behavior was found to be influenced by pattern of N-acetylation of chitosan.

Keywords: capsaicin; chitosan; lecithin; dissipative particle dynamics

1. Introduction

The advent of nanobiotechnology has seen increasing research and development in the use of bioconjugates as new therapeutic alternatives [1]. One of the main goal of these technologies is to focus on utilizing inherent structural, specific recognition or catalytic properties of biomolecules to assemble composite nanoscale materials or devices with unique or novel properties [2]. For instance, several carriers, comprised by biopolymers, macromolecules and liposomes have been used to deliver drugs *in vivo* [3]. Liposomes are microscopic vesicles formed essentially by phospholipids dispersed in water, which are amphiphilic molecules containing polar heads and hydrophobic hydrocarbon tails with the ability to self-associate spontaneously and form bilayer vesicles [4].

The use of colloidal carriers made of hydrophilic polysaccharides, i.e. chitosan (CS), has arisen as a promising alternative for improving the transport of therapeutic peptides, proteins, oligonucleotides, and plasmids across biological surfaces [5]. CS is the common name of a linear, random copolymer of β-(1–4)–linked D–glucosamine and N–acetyl–D–glucosamine whose molecular structure comprises a linear backbone linked through glycosidic bonds. CS is a hydrophilic, biocompatible, and biodegradable polymer of low toxicity. Recent reviews have highlighted the potential use of CS–based drug deliverers [6, 7]. Also, the characteristics of CS–coated liposomes and their interactions with leuprolide have been investigated by Gou et al. [8].

On the other hand, lecithin, which has two long hydrocarbon chains, is a major component of lipid bilayers of cell membranes and a natural, biological amphiphile. Lecithin is a natural lipid mixture of phospholipids and is frequently used for the preparation of various nanosystem delivery vehicles, such as microemulsions, liposomes, micelles, and nanoparticles, and is considered to be a safe and biocompatible excipient [9, 10, 11]. The potential of the various applications that have been found in lecithin/CS nanoparticles has already been reported, for instance, its potential as a mucoadhesive colloidal nanosystem for transmucosal delivery of melatonin was investigated [12] as a topical delivery system for quercetin [13]. The encapsulation of quercetin into lecithin/CS nanoparticles [14] and chitosan-coated nanocapsules [15], as well as the influence of loaded tamoxifen on the structure of lecithin/CS nanoparticles has also been investigated [16]. Other nanostructured materials that harness the interactions of lecithin phospholipid/CS have comprised electrospun nanofibers [17] and nanoporous hydrogels [18].

Capsaicin (8–methyl–N–vanillyl–6–nonenamide), a lipophilic drug, is the pungent vanilloid compound in spicy chili peppers. It is also approved as a drug for the treatment of chronic pains (e.g. arthritis, migraine, diabetic neuropathy) [19, 20] and its potential use in the treatment of urological disorders, control of satiety and obesity [21], has also been documented. Capsaicin is known to be an agonist of the transient receptor potential vanilloid 1 (TRPV1), a nonselective cation channel which is involved in the detection of body temperature and heat nociception [22]. Capsaicin has a nociceptive-blocking action that is the basis of its pharmacological use as an analgesic in persistent pathological pain states [23].

In light of the well- established pharmacological activities of capsaicin, identifying new potential nanocarriers for intracellular, transdermal and/or for intranasal delivery of capsaicin, so as to regulate the activity of its receptors in various tissues, appeals as a potential emerging therapeutic strategy. However, the handling and administration of capsaicin is not always feasible due to its pungency, cytotoxicity at high concentrations [24], and sparing solubility in water [25]. Several studies have been conducted to incorporate capsaicin into nanoformulations in an attempt to make it more compatible with aqueous physiological environments [26, 27]. For instance, capsaicin–loaded nanoemulsions stabilized with natural biopolymer such as alginate and CS has been used as a functional ingredient delivery system [28]. We have encapsulated capsaicin using oil-core CS-based nanocapsules and examined the effect on modulating its pungency [27, 29], just to mention a few studies.

Notwithstanding the aforementioned, the use of nanostructured materials or functionalized nanocarriers with biopolymers, has received a great deal of attention. Recent studies show that biopolymers, such as CS or cellulose and modifications of these, are ideal candidates for developing improved nanocapsules [30, 31], with increased efficiency in the delivery of different drugs, mainly

of the hydrophobic type [32]. These drugs have very low solubility, hence its biodistribution is very limited if there is no suitable vehicle for administration. Thus, nanocapsules represent a very good option to solve this problem. In addition, colloidal nanocapsules with an oily core and a CS shell have been investigated as potential nanocarriers for transmucosal drug delivery [33]. These systems assemble by spontaneous emulsification [34, 35] and are versatile because they can carry both lipophilic and hydrophilic macromolecules [36, 37].

Experimental work [38, 39] has shown compatibility between lecithin/CS liposomal nanoparticles as nanocarriers. Coated liposomes may subsequently be of significant interest to food and pharmaceutical industries for the improved delivery of lipophilic and hydrophilic functional components such as flavors, antioxidants, antimicrobials, and bioactives [40]. However, at the molecular level it has been difficult to elucidate, the nature of the interactions between these components to optimize the formation of nanocapsules. On the other hand, one can dispose of the methods of computational modeling that are available to study such interactions [41]. These tools have shown to be quite effective in the prediction of physicochemical and structural properties of complex systems [42, 43]. Thus, the goal of the current work was to use mesoscopic simulations through the approach of dissipative particle dynamics (DPD) to analyze and characterize nanocapsules constituted by nanoliposomes of lecithin coated with a shell of CS and its function as nanocarriers for the administration of capsaicin, which has proven applications in some therapies.

The remainder of this paper is organized as follows. The models and methods are detailed in Section 2. The results and discussion are presented in Section 3. Finally, the conclusions are drawn in Section 4. The general equations of the DPD approach, the simulation methodology, and full details of our models presented in this work can be found in the supplementary information (SI) that accompanies this paper.

2. Models and Methods

DPD is a mesoscopic technique previously shown to be successful in the prediction of equilibrium and non–equilibrium properties of soft matter systems [44, 45], which makes it suitable to study the coating of nanoliposomes by CS. The numerical simulations presented in this work were carried out in the canonical assemble (constant density and temperature), with the global density set equal to three, as is usually done. Molecules of lecithin, capsaicin and CS were derived from atomic structure in a coarse–graining molecular model, as show in Figure 1. For practical purposes, particles in the coarse–graining models are interpreted as groups of atoms instead of individual atoms. The details of this parametrization are in the SI that accompanies this document. All simulations reported here were performed with our software, SIMES [46], which is designed to run simulations completely on graphic processing units (GPUs), under the DPD framework.

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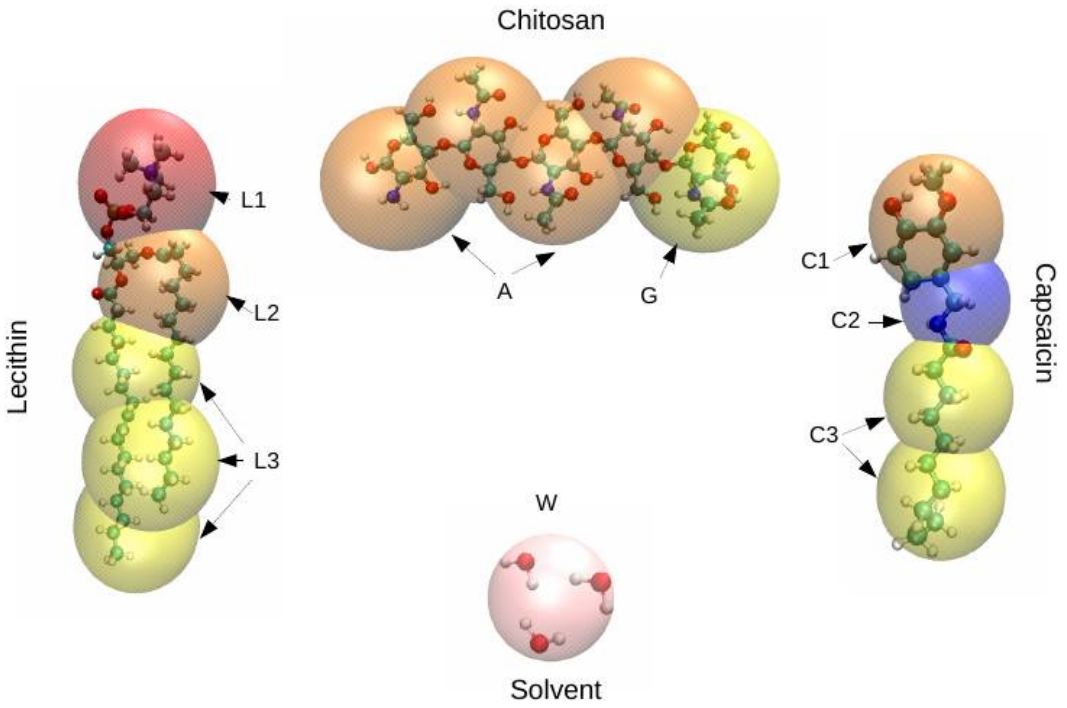

Figure 1. (Color online). Schematic representation of the coarse-grained models adopted in this work. Mesoscopic models for lecithin (left), CS (center up), water as solvent (center down) and capsaicin (right). The exact division of every functional group is presented in the SI. In overview, the molecular structure of lecithin is composed of three different beads that we have labelled as *L1*, *L2*, and *L3*, that correspond to head, neck and tail groups respectively. The same nomenclature is used for capsaicin, where the beads *C1*, *C2*, and *C3* correspond to the head, neck, and tail groups respectively. The CS model consists of two types of beads, the first bead represents the glucosamine units, which are labelled *G*, while the second bead represents the *N*–acetyl–glucosamine units, which are labelled *A*. Finally, the solvent (water) is represented by bead *W*. These Figures were prepared with VMD package [47].

3. Results and discussion

The simulations are performed starting from an initial configuration of the liposome as shown in Figure 2 (left). This liposome is found in aqueous solution together with capsaicin molecules and polymeric chains of CS, which are dispersed in a random configuration, as shown in Figure 2 (right). The simulations carried out in this work are divided into two sets: the first one consists of keeping the concentration of capsaicin fixed and exploring the behavior of the nanocapsule as a function of CS concentration in solution. The second set involves CS and we explored the influence of the degree of deacetylation (DA) by changing the polymer sequence, as is described below. The degree of deacetylation represents the proportion of glucosamine units (deacetylated monomers) in a CS polymer molecule. The DA of CS is an important characterization parameter since it influences several physicochemical properties [48, 49]. For this second case, the distribution of *N*–acetyl–D–glucosamine (GlucNA) and D–glucosamine groups (GlcN), over the CS chain affects the properties of the polymer. It also allows the appearance of cooperative effects due to the association of hydrophobic units with the concomitant effect over the properties of nanocapsules. To achieve this purpose, we explored two different sequences (S1 and S2) in the CS polymer, the first (S1) was more random that the second (S2). Both sequences maintained a degree of *N*-acetylation of 30%. The CS monomer sequences considered in the simulations were prepared as follows:

$$a)[-\mathrm{GlucNA}-[\mathrm{GlcN}]_3-\mathrm{GlucNA}-[\mathrm{GlcN}]_3-\mathrm{GlucNA}-\mathrm{GlcN}-]_5$$

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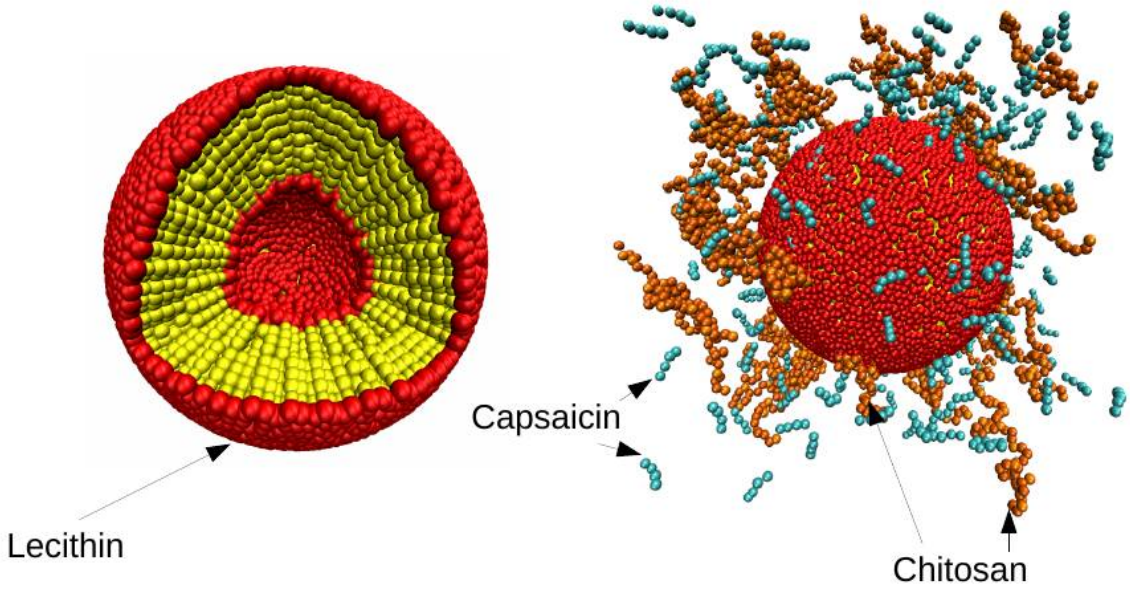
163 $b)\ [-[\mathrm{GlucNA}]_4 - [\mathrm{GlcN}]_9]_3 - [\mathrm{GlucNA}]_3 - [\mathrm{GlcN}]_8$

164 According to this nomenclature, the first sequence consists of five blocks. Each of these blocks
165 was composed of a unit of GlucNA, followed by three units of GlcN. These two sections are repeated
166 and the sequence ends with a GlucNA unit linked to GlcN. The second sequence is composed of three
167 sections. The first section consists of three blocks, which are formed by four units of GlucNA bonded
168 to nine units GlcN. The second section contains three units of GlucNA, while the third section
169 contains eight units of GlcN.



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171 **Figure 2. (Color online). Initial configuration of nanoliposome.** A snapshot of the initial
172 configuration of lecithin molecules into the structure of a liposome bilayer (left). The yellow spheres
173 represent the hydrophobic part, while the red spheres represent the hydrophilic part of the lecithin.
174 The capsaicin and CS molecules were placed in a random configuration around the lecithin (right).
175 The orange chains represent the CS polymers, while the turquoise chains represent the capsaicin
176 molecules. These Figures were prepared with the VMD package [47].

177 **Influence of chitosan concentration on lecithin**

178 Density maps corresponding to lecithin, CS and capsaicin were obtained from the simulations.
179 These density maps, mainly of lecithin, are useful to determine if the structure of the liposome was
180 affected by the presence of CS or capsaicin. First, the concentration of capsaicin in the system (250
181 molecules of capsaicin) was fixed and then the concentration of CS was changed, starting with 50
182 chains up to a total of 200 chains, in increments of 50 chains. These amounts of CS correspond to
183 concentrations of 6, 12, 18 and 24 mM respectively. The density maps of lecithin as function of CS
184 concentration are shown in Figure 3. They show the structure of the liposome, as well as the region
185 formed by the lipid membrane which is presented as the region with the highest concentration (in
186 yellow) in the four cases. From these maps it is possible to observe that the core zone shows a more
187 reddish tone, which indicates that the lipid density is lower, as expected. Density maps also show
188 that the nanoliposome is stable during 24 μs of simulation, since the lecithin molecules do not spread
189 all over the simulation box, nor do they collapse in the aqueous core to form a micelle.

190 In regard to the increase of CS in the system, Figure 3–A) shows that the structure of the
191 nanoliposome is not affected by CS; even more, the nanoliposome remains quasi spherically
192 symmetrical. Figure 3–B) shows the nanoliposome undergoes minimal alteration in its structure,
193 losing some spherical symmetry. Figure 3–C) shows a pronounced protuberance in the

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nanoliposome, located in the interval (5, 10) along the x coordinate. In this case the lipid membrane could break, thus influencing the structural stability of the nanoliposome. In Figure 3–D) a protuberance similar to that shown in Figure 3–C) occurs. Under these conditions, the liposome membrane is thicker.

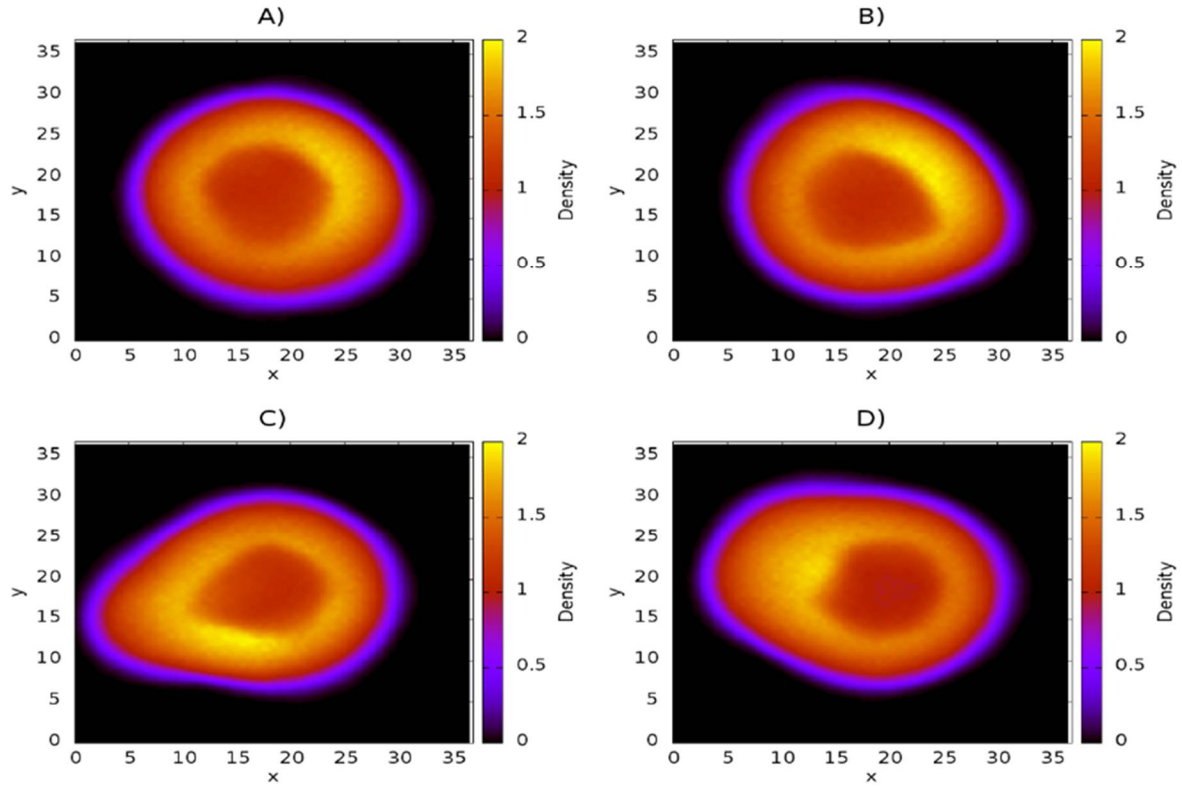

Figure 3. Density maps of lecithin on the xy plane at different concentration of CS. A) 50 chains of CS (6 mM). B) 100 chains of CS (12 mM). C) 150 chains of CS (18 mM). D) 200 chains of CS (24 mM). The scale of density bars starts at 0.0 (black regions) and reaches the maximum at 2.0 (yellow regions). All quantities are expressed in reduced DPD units.

Distribution of chitosan on liposome

The density maps corresponding to CS are shown in Figure 4. These maps show that CS is adsorbed over the surface of the nanoliposome, as has been reported in experimental work [50-52]. It is also possible to see how the surface becomes more homogeneous with increasing CS concentration. Figure 4–A) shows that the CS concentration is not sufficient to cover the surface of the nanoliposome, since the area comprising the radius of the nanoliposome exhibits regions of very low density, which indicates a deficiency of polymeric chains in the zone. Figure 4–B) shows that increasing the concentration in 50 CS chains, equivalent to a concentration of 12 mM, the nanoliposome becomes uniformly coated by the polymer. The appearance of yellow regions are indicative of an association of CS polymers. Figure 4–C) shows the distribution of CS at a concentration of 18 mM on the liposome. The regions of greater density are more pronounced than in the previous case. A greater adsorption of CS is observed too. In this case the polymer acts as a protective layer. The last case represents the adsorption of polymers of CS at a concentration of 24 mM. (Figure 4–D). Under this condition the surface of the liposome becomes almost completely coated. It is also possible to observe how an increasing concentration of CS,raises the quantity of supernatant polymer in the aqueous medium, thus indicating that a competitive association between CS–liposome and CS–CS is present. The blue regions refer to low density of CS, while the red regions refer to regions of high density of CS.

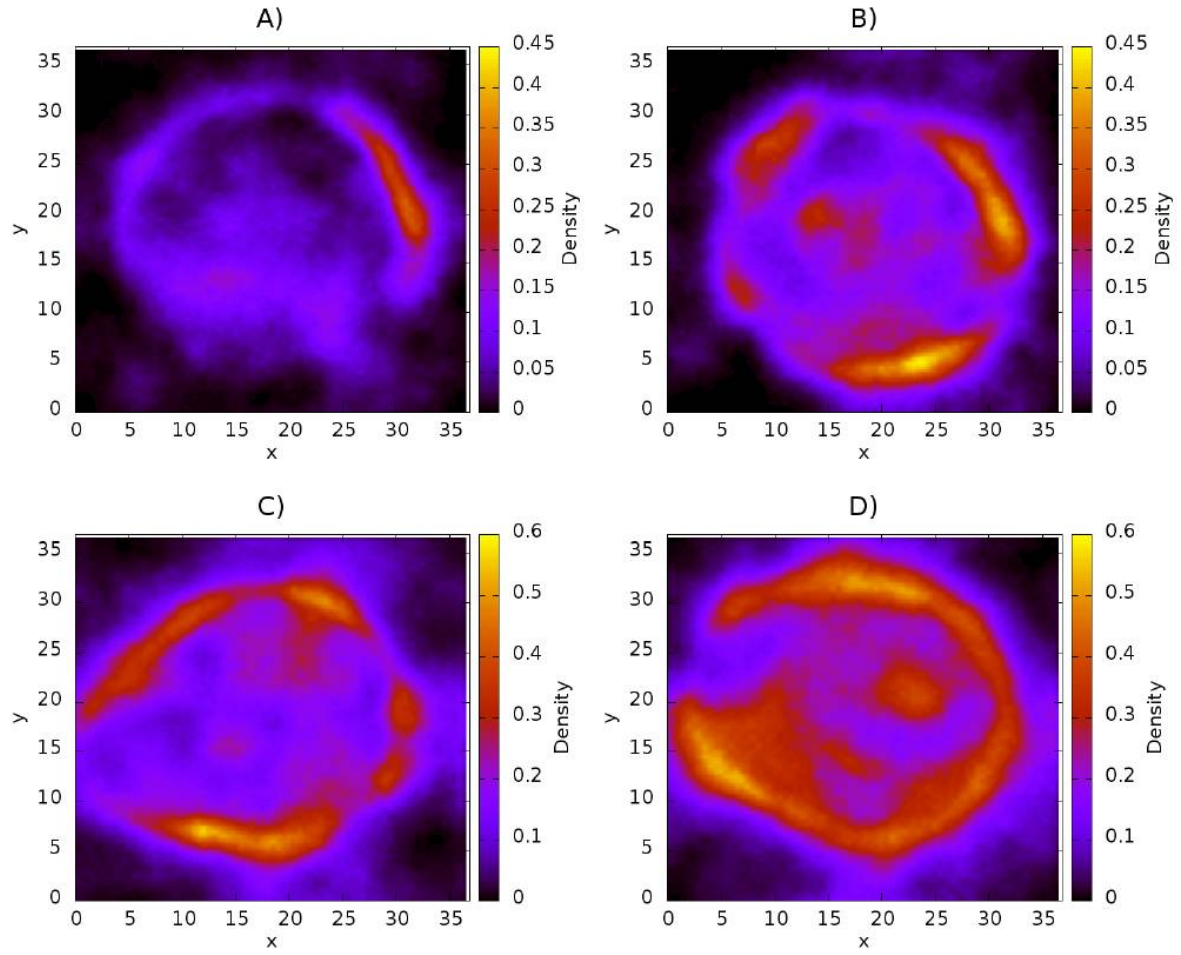

Figure 4. Distribution of CS on liposome on the xy plane. A) 50 chains of CS. B) 100 chains of CS, C) 150 chains of CS. D) 200 chains of CS. All quantities are reported in reduced DPD units.

Influence of chitosan concentration on capsaicin

Density maps corresponding to the capsaicin molecules are shown in Figure 5. These maps clearly show that capsaicin is absorbed and encapsulated in the nanoliposome. This phenomenon is not affected by the presence of the CS polymer, which indicates that interactions between capsaicin and CS are very weak in comparison to the interactions between lecithin and capsaicin. In Figures 5–A), 5–B), 5–C) and 5–D) it is possible observe how the capsaicin molecules are deposited close to interface between the nanoliposome and the aqueous medium and even in the interface with the aqueous core which suggests that capsaicin will be transported by the oil phase in the nanoliposome, thus leaving free the aqueous core with capacity to transport other hydrophilic molecules with therapeutic potential. If the capsaicin reached the core, a yellow or high density region would be observed in the center, which does not occur.

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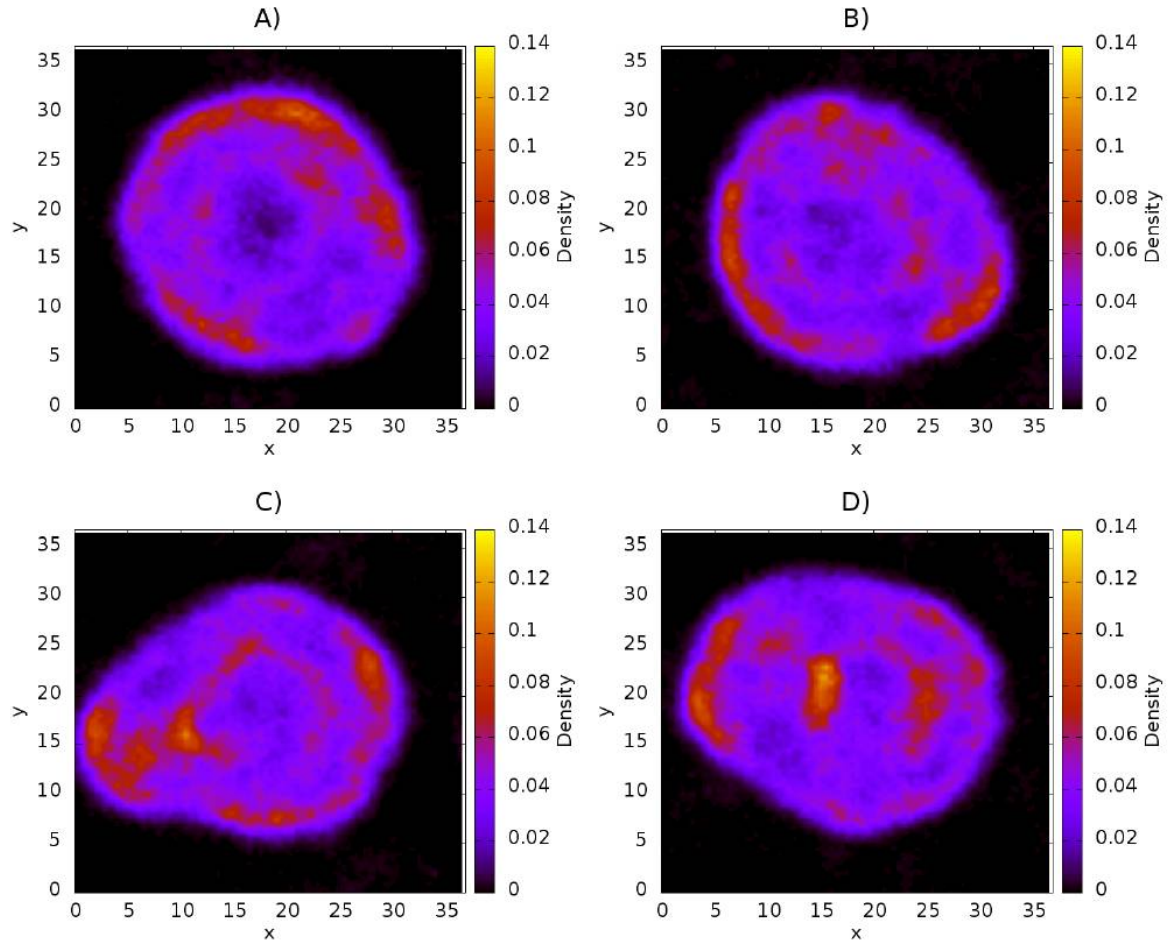

Figure 5. Influence of CS concentration on capsaicin on the xy plane at different concentrations of CS polymer. A) 50 chains of CS. B) 100 chains of CS. C) 150 chains of CS. D) 200 chains of CS. All quantities are reported in reduced DPD units.

Potential of mean force

Other properties obtained from simulations are the potentials of mean force (PMF), which are many – body interactions arising from their complex interplay beyond mean – field approximations [53]. The details about the calculation of the PMF can be found in the SI. Figure 6 shows the PMF between lecithin–CS, lecithin-capsaicin and capsaicin–CS. At higher concentrations of CS the interaction becomes weaker indicating that adsorption over the surface of the nanoliposome decreases when more CS molecules are in solution. Figure 6–B) corresponds to PMF of lecithin–capsaicin. This shows that the interaction between capsaicin and the nanoliposome is not significantly affected by the presence of CS chains, since their PMF are practically the same, making it clear that the interactions between CS and capsaicin are not the leading mechanism of nanocapsule conformation. Both Figures (Figure 6-A, 6-B) show two minimal values that are attributed to the lipid bilayer. Figure 6–C) shows the PMF between capsaicin and CS. It is evident that the attractive interactions become weaker as the quantity of CS increases. This is due to the presence of competitive adsorption which promotes the self–association between CS molecules, so that the interactions between polymer chains with the surface of nanoliposome as well as with capsaicin molecules become weaker thus causing the deposition mainly in the surface of the of the nanoliposome.

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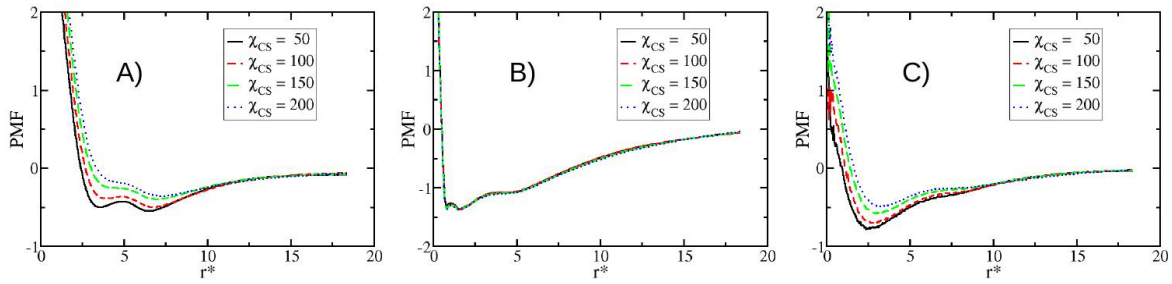

Figure 6. Potentials of mean force (PMF) for different concentration of CS as function of separation distance between mass centers of each molecule. A) lecithin–CS, B) lecithin–capsaicin, and C) capsaicin–CS. All quantities are expressed in reduced DPD units.

Modifying the sequence of chitosan

In this section we present the results obtained from the changes in the CS sequence pattern composition. The analysis presented below corresponds to a second group of simulations described at the beginning of this section. The results show that the pattern (sequence) in which the GlucNA and GlcN monomers are arranged has a clear effect on the PMF (Figure 7). We note that the PMF corresponding to CS-lecithin and CS–capsaicin indicates a favourable condition for attractiveness in the case of sequence S2 with respect to sequence S1, indicating a slight presence of cooperative effects between GlucNA units which promote the association of CS with the nanoliposome and hence with capsaicin. On the other hand, the interaction between lecithin and capsaicin is not affected or modified by these cooperative effects, so that the interaction between capsaicin and CS remains weaker than the interaction of capsaicin with lecithin.

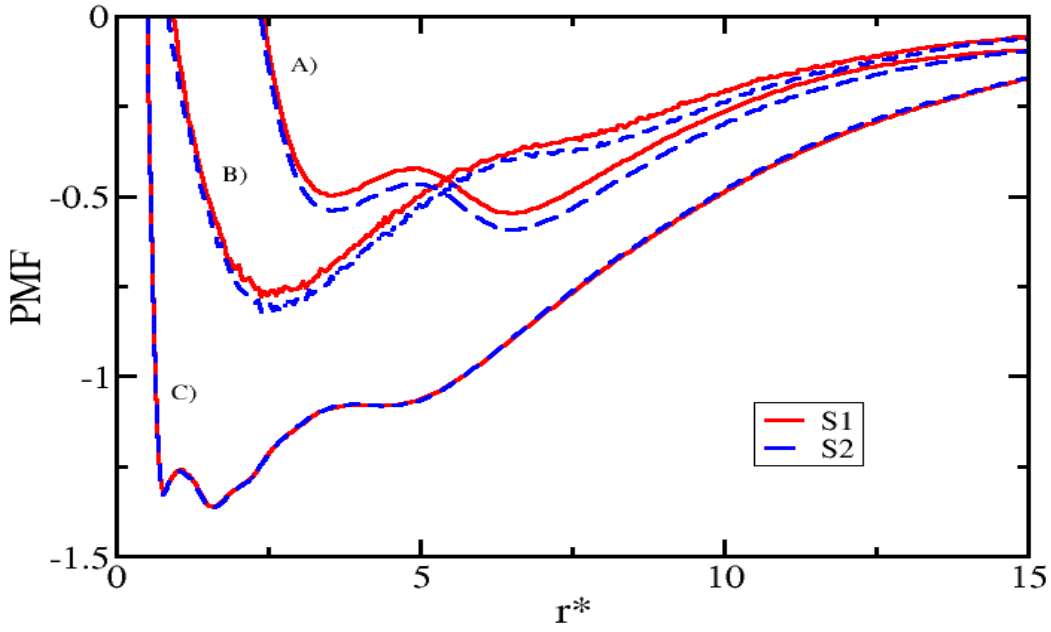

Figure 7. PMF for the two sequences of CS used in this work, S1 (red line) and S2 (blue dotted line), to concentration of CS is 6 mM. PMF for: A) lecithin–CS, B) CS–capsaicin, and C) lecithin–capsaicin. All quantities are expressed in reduced DPD units.

Mean size of nanoliposome and encapsulation efficiency (EE)

Additional properties obtained from the simulations are the size of the nanoliposome and encapsulation efficiency of capsaicin. These properties are shown in Table 1. We obtained the encapsulation efficiency (EE) and mean size from the density profiles of capsaicin and lecithin, respectively, which are shown in the SI. EE is obtained from the Equation: EE = ((CapsT-CapsF)/CapsT)x100., where CapsT is the total concentration of capsaicin in the system and CapsF is the free capsaicin in solution.

Table 1. Mean size of the nanoliposome and encapsulation efficiency (EE) as function of quantity of CS.

CS	Size (nm)	± (nm)	EE(%)	± (%)
50	17.89	0.61	96.80	0.59
100	17.95	0.46	96.27	0.69
150	18.04	0.95	96.28	0.51
200	17.90	1.00	96.71	0.61

The mean size of the simulated nanoliposome is smaller but comparable to those obtained experimentally for CS-coated oil-core nanocapsules (~ 80–250 nm) [11, 29, 50, 51]. This is an encouraging aspect of mesoscale simulation techniques, such as DPD. Additionally, the percentage of capsaicin encapsulation was very close (96%) to that reported experimentally (92%) [50].

Figure 8 shows snapshots obtained from the simulation trajectory where the system was monitored along time. It can be observed that capsaicin is being encapsulated inside of nanoliposome while CS is deposited on the surface. An animation of this simulation is added in the SI section where capsaicin encapsulation is clearly observed.

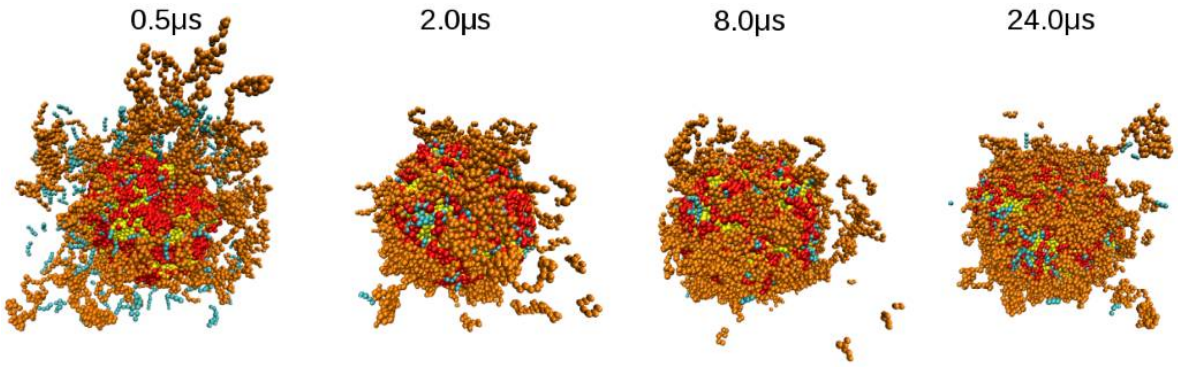

Figure 8. Snapshots of adsorption of CS on the nanolipsome at various times during simulation. In these pictures the conformation of the nanocapsule along different times can be observed. The color code in this Figure is the same as the one in Figure 2. The solvent molecules are not shown for clarity purposes. These Figures were prepared with the VMD package [47].

4. Conclusions

We performed DPD simulations to analyze the stability of nanocapsules formed by nanoliposomes with a polyelectrolyte shell (CS). Results obtained from density maps showed that the nanocapsule is stable with size but comparable to those of nanocapsules experimentally obtained. The information provided by the potentials of mean force showed that the interaction between capsaicin and CS is very weak compared to that with lecithin. An association between capsaicin and CS, in presence of lecithin, is not likely to occur. Under experimental conditions, the solvent may harbor other, molecular compounds that can reduce the absorption of capsaicin by the nanoliposome.

Supplementary Materials: The following are available online at www.mdpi.com/link.

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Author Contributions: AGG, RLR and KATM conceived and designed the project; KATM and EMB performed the simulations. JH, IHC, CV, ZD and AGG analyzed the data; JH, IHC, FMGV, WAM, RLR and AGG contributed to the discussion of results. All authors contributed to the reading, writing and approval of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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