IBUPROFEN/KETOPROFEN ENTRAPMENT IN CHITOSAN BASED VESICLE CARRIER

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Abstract. Vesicles are relatively small and hollow spherical structures appear as a result of the self-assemblation in aqueous solution of amphiphilic molecules. They are important to be studied for understanding biomembranes and for developing new vehicles for drug delivery. Ibuprofen and ketoprofen are nonsteroidal anti-inflammatory drugs, reducing inflammation, fever and pain in the body. Due to the allergic reactions induced in organism there is necessary to use encapsulated systems. In this paper studies regarding obtaining and stability of these systems are presented. The dimension measurements determined by the laser diffraction method reveal that the dimension of vesicles varies from tens to hundreds of nanometers depending on the concentrations and the physical parameters of precursors; by the addition of associating chitosan biopolymer the size and morphology are changed. Spectral UV-VIS concentration measurements show that these vesicles are capable to entrap drug water solution (50 μg/mL) with a large efficiency.

Key words: lecithin/surfactant vesicles, amphiphilic molecules, ibuprofen/ketoprofen, chitosan.

INTRODUCTION

In this paper we are investigating a class of self-assembling vesicles formed by an equilibrated mixture of single-tailed cationic and anionic surfactants, named "equilibrium" vesicles. These vesicles offer some advantages over conventional phospholipid vesicles, since they are easier to product and present long-term stability.

These studies are significant because they provide a fundamental perception of self-assembly processes and represent a basis for controlled release applications which can be valuable for the biomedical (bandages for wound healing, tissue reconstruction), pharmaceutical (drugs with high bioactivity encapsulated in vesicles), cosmetic (skin treatment, topical ointments, creams for anti-aging), agrochemical and food industries.

Vesicles are formed by the self-assembly of amphiphilic molecules in aqueous solution. During this process an important role is played by the geometry of molecules, knowing that only amphiphiles with a cylinder-like shape tend to

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form bilayers. These include lipids (two-tailed biological amphiphiles) as well as mixtures of oppositely-charged single-tailed surfactants [1, 5].

For controlled release and drug delivery, chemicals are entrapped inside the vesicle which will deliver and release them to the desired area (Fig. 1). In this manner it is possible to transport hydrophilic as well as hydrophobic molecules. Depending on the environmental variables (pH, temperature, ionic strength, chemicals) vesicles can get easily disrupted and, thereby, they will release the containing molecules.

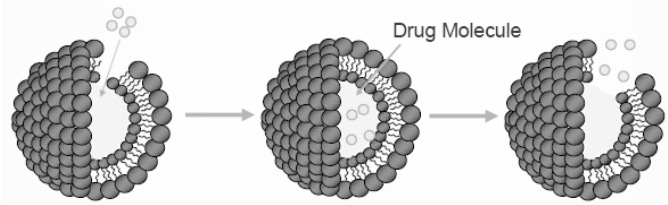

Fig. 1. Model for use of vesicles in drug entrapment and delivery.

When a polymer is added to a solution of vesicles the following processes can take place: vesicles stabilization by adsorption, vesicles destabilization, changing the shape of vesicles or linking them into a network. The interactions between polymer and vesicles are essential to the behavior of cell membranes, knowing that every biological membrane consists of lipids and polymers (proteins or polysaccharides) combination [6].

In the presence of high polymer concentration, some of the unilamellar vesicles transform into bilamellar structures. Similar co-existence of unilamellar and bilamellar vesicles is observed in all eukaryotic cells [3].

As polymer, we used chitosan that acts like an additional transport barrier which enables a slower and more extended rate of release for molecules encapsulated in the vesicles. Moreover, encapsulation within vesicles may also help in maintaining the bioactivity of drugs and proteins [8].

MATERIALS AND METHODS

The cationic surfactant, cetyl trimethylammonium bromide (CTAB), was purchased from Chemapol and the anionic surfactant, sodium lauryl sulfate (SLS), from Sigma.

The lipid used, Egg Yolk L-α-phosphatidylcholine (L-α-lecithin), approximately 99% (TLC) pure, was obtained from Sigma.

The ibuprofen (with ≥ 98% (GC) purity and molecular weight of 228.26) and ketoprofen (with ≥ 98% (TLC) purity and molecular weight of 254.28) were acquired from Sigma.

The chitosan was given by Sherbrooke University (Quebec, Canada). The N-deacetylation degree was 88%, average molecular weight number was M_n = 150000, average molecular weight was M_w = 350000, the polydispersity index being 2.33. Chitosan was dissolved in a 1% (wt/wt) acetic acid solution. The 0.5 (wt/wt) chitosan homogeneous solutions were prepared by stirring at room temperature for 24 hours, followed by centrifugation at 1500 rpm/30 minutes.

All solutions were made using distilled water.

SURFACTANT VESICLES

Surfactant vesicles were prepared by mixing the cationic surfactant, CTAB, and the anionic surfactant, sodium lauryl sulfate (SLS) [4]. The surfactant solution is a 1% mixture of CTAB/SLS at a 70/30 weight ratio in distilled water.

The ternary system CTAB/SLS/Water is highlighted in the literature; in Figure 2 is shown the water-rich corner of the CTAB/SLS/Water ternary phase diagram [7]. All concentrations are expressed in weight %. From this diagram it can be seen that wormlike micelles are formed in the CTAB-rich corner, the spherical micelles are formed in the SLS-rich corner, and unilamellar vesicles (V) are formed in the two lobes extending from the water corner. The focus of this study is on the cationic vesicle phase, with the composition of choice being a 70/30 CTAB/SLS weight ratio mixture (dashed arrow).

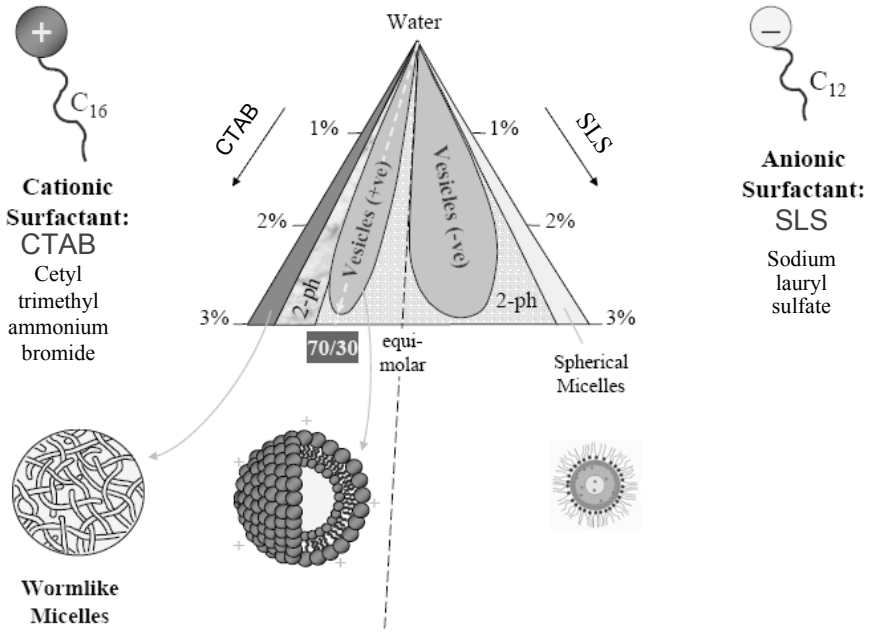

Fig. 2. Water-rich corner of the CTAB/SLS/Water ternary phase diagram.

LIPID VESICLES

First, the lipid (lecithin) was dissolved in an organic solvent (chloroform). Thereafter, the solvent was removed by evaporation and a dry lipid film was obtained. The film was then hydrated by adding distilled water.

The solution was gently stirred during this process, the result being the formation of large multi-lamellar vesicles (MLVs) in solution. To convert the MLVs to unilamellar vesicles (ULVs), the lipid solution was sonicated [9].

DRUG ENCAPSULATION IN VESICLES

The ibuprofen/ketoprofen (dissolved in ethanol) was added to the aqueous solution used in preparing either lipid or surfactant vesicles [10].

CHITOSAN-VESICLES INTERACTION

Vesicles with or without encapsulated drug were combined with a 0.5% chitosan solution, and the mixture was mildly heated at 50°C for two hours, followed by centrifugation to remove bubbles [2].

MEASUREMENTS

The dimension of the vesicles was determined by the laser diffraction method with Shimadzu-SALD-7001 Laser Diffraction Particle Size Analyzer.

The vesicles obtained were visualized using a Nikon E600 optical microscope. Images were recorded with a Coolpix 950 camera.

The transmission spectra were recorded in the 190 – 500 nm range using an UV-VIS M-40 (Carl Zeiss) spectrophotometer.

RESULTS AND DISCUSSIONS

In Figure 3 is presented the Dynamic Light Scattering (DLS) particle distribution for a 0.1% surfactant solution.

In this case, it was obtained a vesicles median diameter value of 28 nm. But, from the optical microscopy image it was observed that they are much larger. This means that at low concentrations, vesicles are transparent, so that the light instead of being scattered on vesicles passes through them.

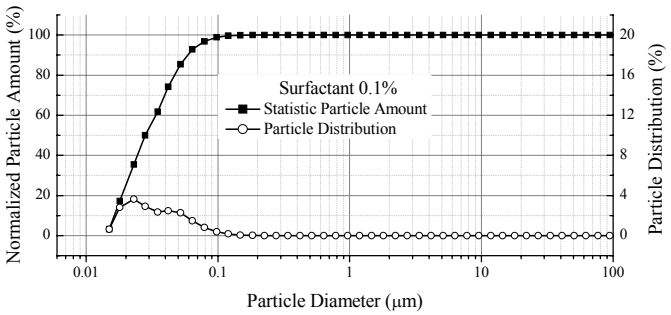

Fig. 3. DLS diagram for 0.1% surfactant solution. Median diameter value of vesicles is 28 nm.

After adding chitosan in surfactant solution, vesicles become spherical, very stable, more confined and the solution is clear. In this case, they are better seen on DLS, the median diameter value of vesicles being of 585 nm (Fig. 4).

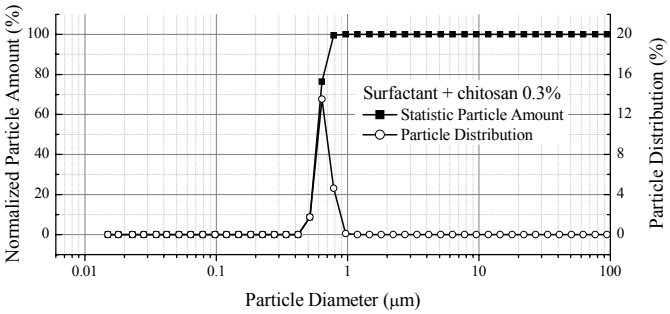

Fig. 4. DLS diagram for 0.3% surfactant (3 ml) + chitosan (1.8 ml) solution.
Median diameter value of vesicles is 585 nm.

For a solution obtained from mixing surfactant with ibuprofen it can be seen a disruption process (Fig. 5). Vesicles are no longer spherical. Considering de fact that the concentration was very low, we notice the same sub dimensioning case due to their transparency.

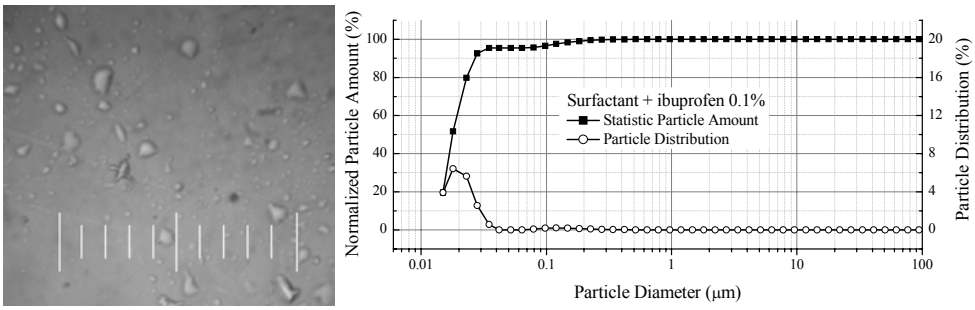

Fig. 5. Optical microscopy image and DLS diagram (median diameter value of vesicles is 18 nm) for surfactant + ibuprofen (50 μg/ml) solution. The dimensioning scale represents 10 μm.

Similar results were obtained in the case of ketoprofen as active drug. It is noticed that in this case the vesicle dimension is of micrometer order.

By adding chitosan to previous solutions, we observe spherical vesicles with a different containing, this being the encapsulated drug. Also, they are well dispersed in solution and very stable (Fig. 6).

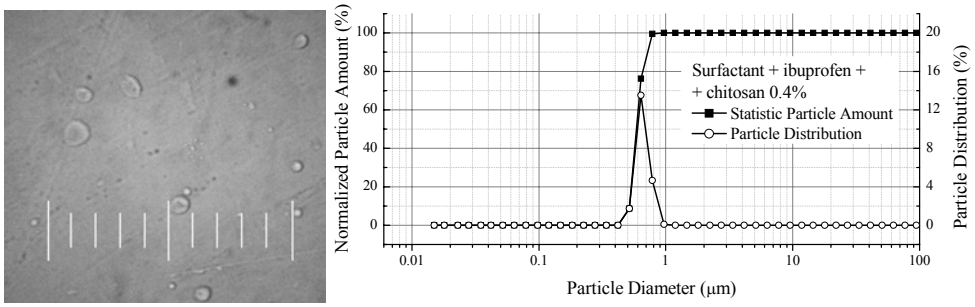

Fig. 6. Optical microscopy image and DLS diagram (median diameter value of vesicles is 585 nm) for (surfactant + ibuprofen) (3 ml) + chitosan (1.8 ml) solution. The dimensioning scale represents 10 μm.

In the case of lipid vesicles, these appear to be smaller than surfactant vesicles both from optical microscopy image and DLS diagram (Fig. 7).

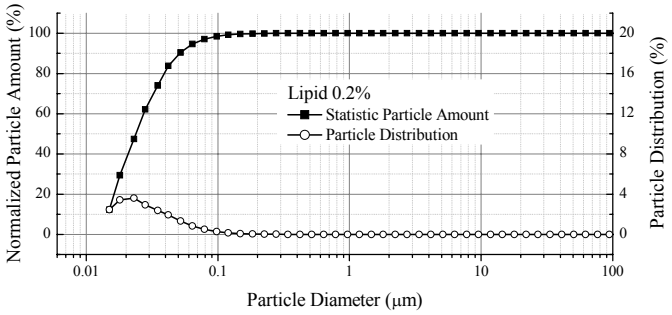

Fig. 7. DLS diagram for 0.2% lipid vesicles solution. Median diameter value of vesicles is 24 nm.

Vesicles formed from lipid and chitosan are, also, spherical, stable and well dispersed, but they have a diameter of 25 nm only (Fig. 8).

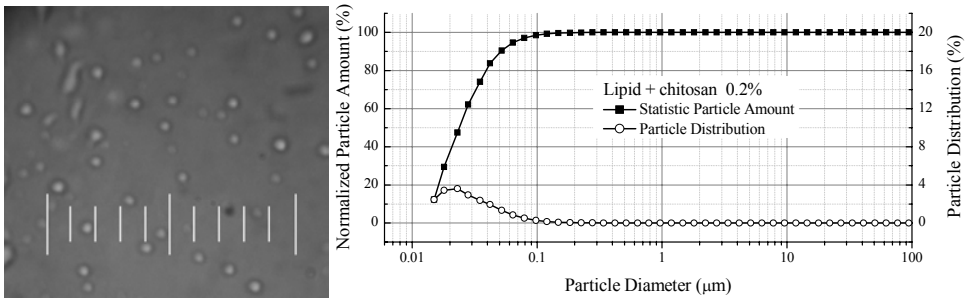

Fig. 8. Optical microscopy image and DLS diagram (median diameter value of vesicles is 25 nm) for lipid (3 ml) + chitosan (1.8 ml) solution. The dimensioning scale represents 10 μm.

For a lipid + drug solution we notice, again, that introducing drug in system leads to a dynamic process between vesicles (Fig. 9).

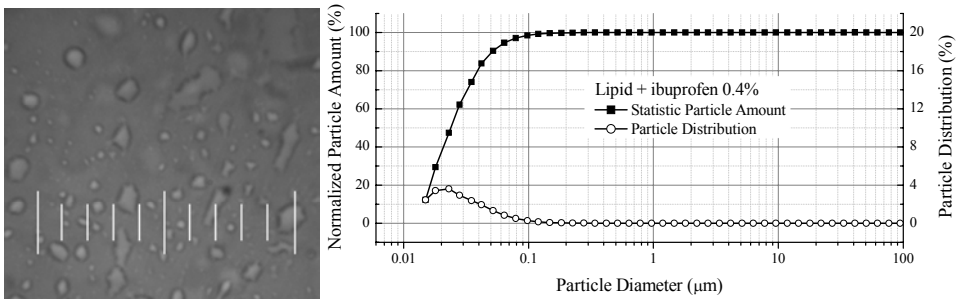

Fig. 9. Optical microscopy image and DLS diagram (median diameter value of vesicles is 22 nm) for lipid + ibuprofen (50 μg/ml) solution. The dimensioning scale represents 10 μm.

In a lipid-drug-chitosan solution it is very clear the influence of chitosan, because the vesicles become smaller, but more defined and stable (Fig. 10 – 11).

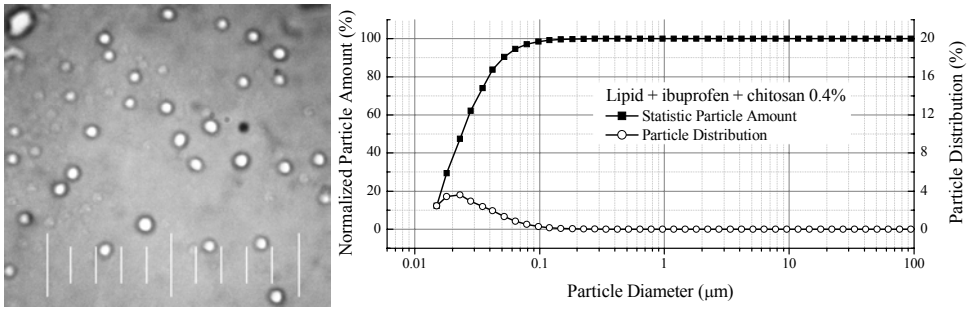

Fig. 10. Optical microscopy image and DLS diagram (median diameter value of vesicles is 18 nm) for (lipid + ibuprofen) (3 ml) + chitosan (1.8 ml) solution. The dimensioning scale represents 10 μm.

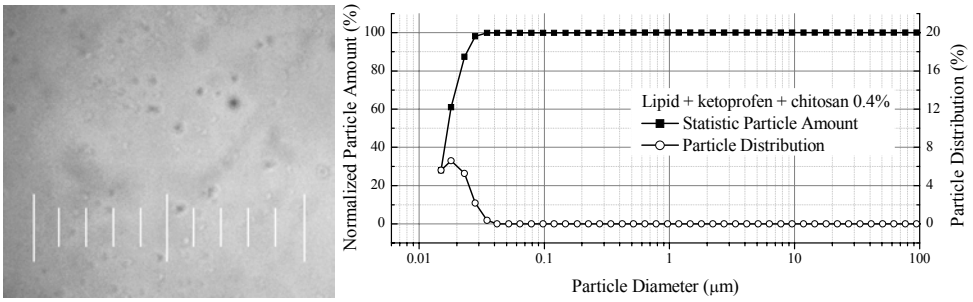

Fig. 11. Optical microscopy image and DLS diagram (median diameter value of vesicles is 17 nm) for (lipid + ketoprofen) (3 ml) + chitosan (1.8 ml) solution. The dimensioning scale represents 10 μm.

In order to see the degree of drugs encapsulation in vesicles, there were recorded UV-VIS transmission spectra of drugs (Fig. 12), for building the etalonation curve of drugs and to determine the drug free in vesicle solutions (Fig. 13).

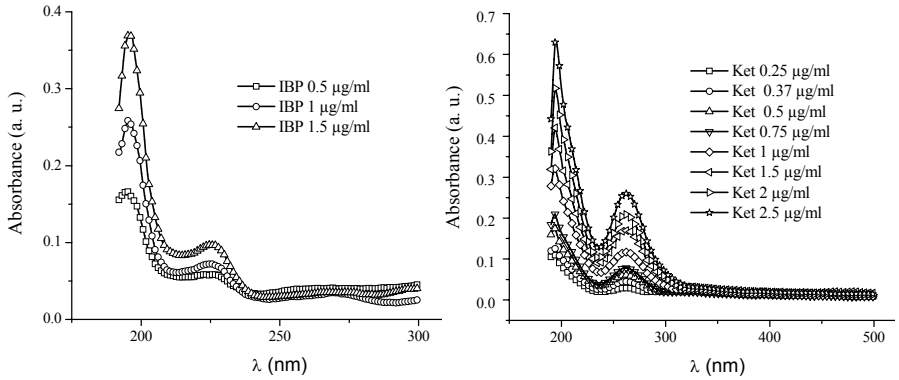

Fig. 12. UV-VIS Spectrum for different concentrations of ibuprofen/ketoprofen solution.

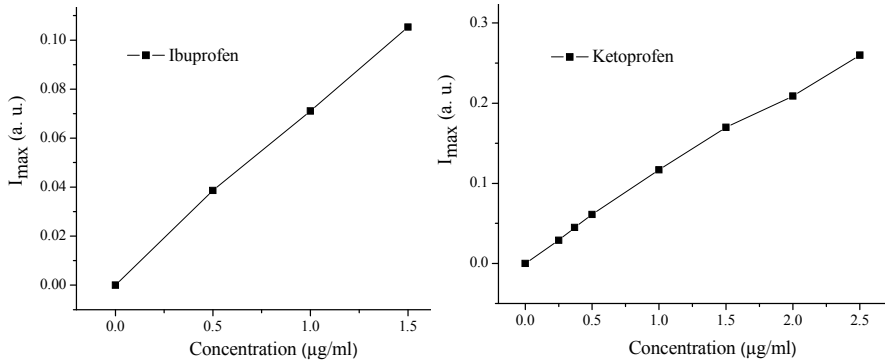

Fig. 13. Etalonation curves for ibuprofen/ketoprofen solutions.

The UV – VIS spectra of surfactants, lipid, surfactants with chitosan and lipid with chitosan (Figs. 14 and 15) were performed for missing bands visualization at 226 nm (maximum for ibuprofen band) and 262 nm (maximum for ketoprofen band).

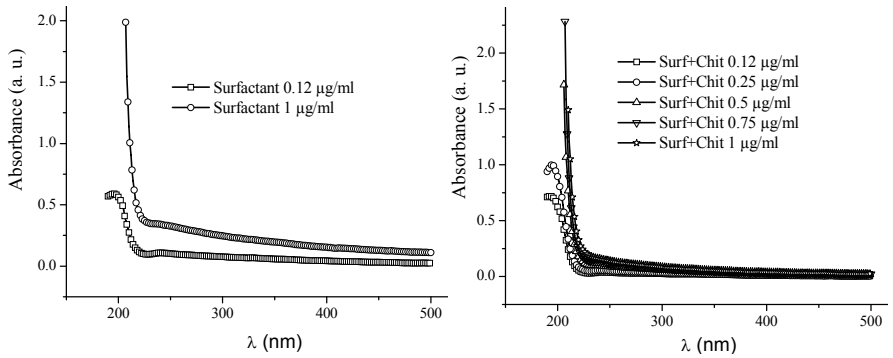

Fig. 14. UV-VIS Spectra for surfactant and surfactant + chitosan solutions.

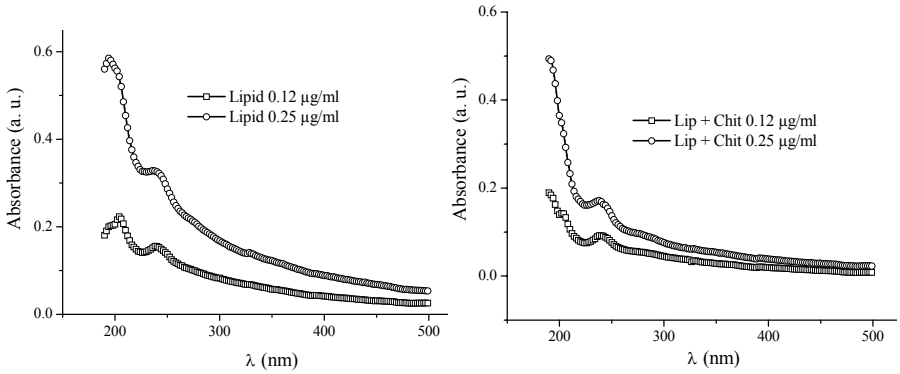

Fig. 15. UV-VIS Spectra for lipid and lipid + chitosan solutions.

In Figures 16–19 the UV-VIS spectra of surfactant/lipid vesicle with ibuprofen/ketoprofen and chitosan associated polymer were presented.

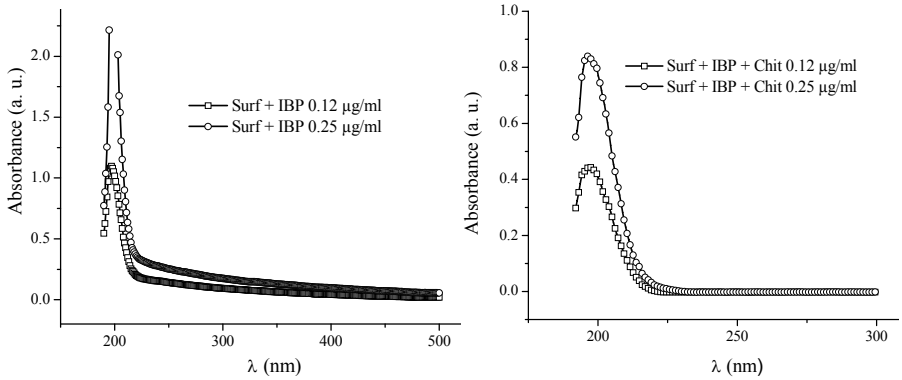

Fig. 16. UV-VIS Spectra for surfactant + ibuprofen and surfactant + ibuprofen + chitosan solutions.

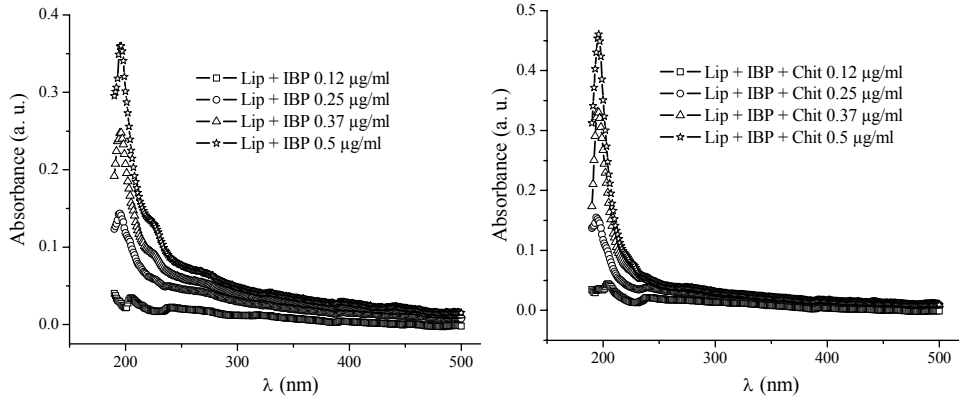

Fig. 17. UV-VIS Spectra for lipid + ibuprofen and lipid + ibuprofen + chitosan solutions.

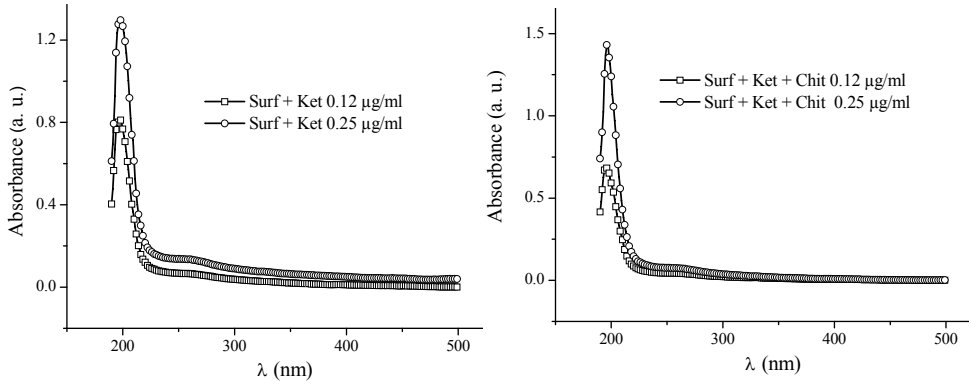

Fig. 18. UV-VIS Spectra for surfactant + ketoprofen and surfactant + ketoprofen + chitosan solutions.

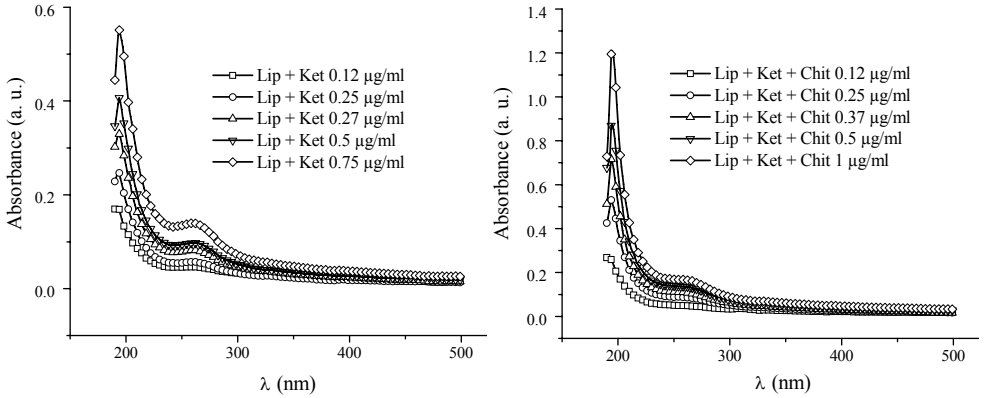

Fig. 19. UV-VIS Spectra for lipid + ketoprofen and lipid + ketoprofen + chitosan solutions.

By corroborating with etalonation drug curves results an efficiency of 50 μg/mL encapsulation drug with a linkage of about 1 μg/mL.

CONCLUSIONS

The vesicle container in aqueous media was obtained from 70/30 ratio of CTAB and SLS respectively, and from lecithin.

The dimension of vesicles varies from tens of nanometers to hundreds depending on the concentrations and the physical parameters of precursors.

It was demonstrated that these vesicles are capable to entrap ibuprofen and ketoprofen water solution (50 μg/mL) with a large efficiency.

By the addition of associating chitosan biopolymer changes in the size and morphology of unilamellar vesicles (ULVs) were produced. The chitosan binds to vesicles, causing the bilayer to rigidify and in turn driving a decrease in the size of ULVs.

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