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Evaluation of the effect of the chitosan/carrageenan ratio on lovastatin release from chitosan/carrageenan based biomaterials

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Evaluation of the effect of the chitosan/carrageenan ratio on lovastatin release from chitosan/carrageenan based biomaterials

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Abstract

The chitosan/carrageenan/lovastatin biomaterials were prepared using a solution technique with 5 % lovastatin and a variable chitosan/carrageenan mass ratio. Fourier transform infrared spectroscopy (FT-IR) and field emission scanning electron microscopy (FESEM) images revealed that the complex's components were well dispersed into each other without changing the chemical structure, forming dipole interactions and hydrogen bonds among them. The abilities to release Lovastatin (Lov) in pH 2 and pH 7.4 solutions, corresponding to the environments in stomach and intestinal fluids, respectively, are both affected by the ratio of polymer components. The best results were obtained with the chitosan/carrageenan/lovastatin (w/w/%w = 10/90/5%) (CsCL195) biomaterial sample in which the particles are evenly dispersed. While the drug release process was poor in the pH 2 solution (gastric juice), a good release observed in the pH 7.4 solution (intestinal fluid) allows facilitating drug absorption through the intestinal mucosa cells into the blood, from which the effects of hypercholesterolemia treatment can be obtained.

Keywords. Chitosan, carrageenan, lovastatin, biomaterials, drug delivery.

1. INTRODUCTION

Lovastatin (Lov) is a commercial statin compound commonly used to treat high blood cholesterol. It is the preferred treatment for coronary artery disease and atherosclerosis. This drug inhibits the enzyme hydroxyl methylglutaryl CoA (HMG-CoA reductase) to limit cholesterol production.[1] Recent research has found that Lov demonstrated the ability to kill tumor cells by suppressing the genes involved in cell division, increasing the activity of cell cycle inhibitors,[2,3] to inhibit the growth of human acute myeloid leukemia (AML) cells[4] and the metabolism of breast cancer cells.[5]

However, the Lov which is mainly absorbed in the gastrointestinal tract is poorly soluble in water (4.10^{-3} mg/mL)[6] leading to its low bioavailability.[7] A number of works have been conducted to improve Lov's water solubility and thus drug bioavailability such as the fabrication of Lov-carrying nanoparticles [8], Lov-containing lipid bilayer with a core-shell structure,[9] or poly(lactic acid) microspheres containing Lov.[10] Research has shown that the solubility and bioavailability of Lov were significantly improved. However, the use of organic solvents, and additives is harmful to the environment and increases production costs.

Besides these works, bio-based nanocomposite fabrication has emerged as a promising way to produce effective, biocompatible, and environmentally friendly drug carriers.[11-14] The drug release control has been reported to be affected by the nature and composition of composite biomaterials as well as the pH of the drug release medium. Some of the composite biomaterials that have been studied can be mentioned such as poly(lactic acid)/chitosan/nifedipine,[11] carrageenan/allopurinol,[12] carrageenan/collagen/ allopurinol (CCA) membranes,[13,14] etc. Previous studies also showed the influence of combinations of chitosan/alginate,[15,16] chitosan/collagen,[17] carrageenan/gellan,…[18] with different ratios on the ability to control Lov release in fluid simulation solution and human body. Among studied biomaterials, the combination of carrageenan and chitosan to fabricate composites carrying lovastatin has not been given much attention.

Therefore, this work aims to evaluate the influence of carrageenan and chitosan ratios on the characterization, properties, and release capacity of lovastatin in solutions simulating human body fluids.

2. EXPERIMENTAL

2.1. Materials

Carrageenan was provided by Aladdin - China (Potassium: 0-11 %, Sodium 0-2 %, pH: 7-10; Water by Karl Fischer: 0-12 %; Calcium: 0-3.5 %; White to Yellow or Light Brown Crystals or Powder Gel Test Consistent with Past Lots); Chitosan (medium viscosity, 200-400 mPa.S) provided by Aladdin - China; Lovastatin powder (≥ 98 %) manufactured by Rshawn - China. Some other chemicals such as CH_3COOH, C_2H_5OH, HCl, KCl, KH_2PO_4, Na_2HPO_4, CH_3COONa, etc. are commercial products of Vietnam and China.

2.2. Fabrication of carrageenan/chitosan/ lovastatin biomaterials

The solution method was used to prepare the carrageenan/chitosan/lovastatin biomaterial samples, with the ratio of carrageenan and chitosan being varied. The synthesis procedure of the carrageenan/chitosan/lovastatin biomaterials was as follows: carrageenan was dissolved in distilled water on a magnetic stirrer (carrageenan/water ratio = 1/200 g/mL). The solution was heated to 80 ^oC for 30 minutes before being allowed to cool (solution A). On a magnetic stirrer, chitosan was dissolved in a 1 % acetic acid solution at room temperature with a chitosan/acetic acid solution ratio of 1/1050 (g/mL) (solution B). Lov was dissolved in ethanol on a magnetic stirrer (solution C). Then, the solution C was poured into the glass burette and slowly added to the solution A while stirring with a homogenizer (speed of 20000 rpm) to obtain solution D. This solution was stirred on a stirrer for 1 hour. Next, the chitosan solution (solution B) was added to solution D and homogenized with a homogenizer (speed 20000 rpm), the resulting mixture was continuously stirred for 60 minutes on a magnetic stirrer, followed by centrifugation to collect the solid and freeze-drying to obtain a white powder product. The composition of the produced carrageenan/chitosan/ lovastatin (CsCL) samples is presented in table 1.

Table 1: Sample codes and the composition of carrageenan/chitosan/lovastatin samples

Number	Sample codes	Chitosan (g)	Carrageenan (g)	Lovastatin (g)
1	CsCL915	0.09	0.01	0.005
2	CsCL735	0.07	0.03	0.005
3	CsCL555	0.05	0.05	0.005
4	CsCL375	0.03	0.07	0.005
5	CsCL195	0.01	0.09	0.005

2.3. Characterization of carrageenan/chitosan/ lovastatin biomaterials

Fourier transform infrared (FT-IR) spectroscopy was performed to determine the functional groups of the material structure. A Thermo Scientific Nicolet iS10 spectrometer and FTIR/NIR software were used to record FT-IR spectra of CsCL bioactive substances. Powdered samples were pressed into pellets with KBr and scanned in the range of 400 cm^{-1} to 4000 cm^{-1}, with a resolution of 8 cm^{-1} and a scanning number of 32 rounds at room temperature.

Field emission scanning electron microscopy (FESEM) images of the samples were taken on a FESEM S-4800 instrument (Hitachi, Japan).

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The thermal properties of CsCL biomaterials were analyzed on a DSC-60 thermal analyzer (Shimadzu) under a nitrogen atmosphere from room temperature to 600 °C at a heating rate of 10 °C/min.

Ultraviolet (UV-Vis) spectroscopy was used to determine the content of Lov released from the CsCL biomaterials in different pH buffers. This test was performed on a UV- Vis spectrometer (S60 Libra, Biochrom, UK) in the 200-400 nm range using 1-cm quartz cuvettes.

2.4. Investigation of the drug release

Drug release studies were conducted with CsCL samples in a buffer of pH 2 (simulating gastric juice) and a buffer of pH 7.4 (simulating intestinal fluid) at 37 ±0.1 °C. Samples of definite mass were added to a 300 mL beaker containing 200 mL of pH 2 or pH 7.4 solution. The solution was stirred continuously during the test. At each interval, 5 mL of solution was withdrawn and 5 mL of new buffer was added to keep the constant study solution volume. UV-Vis spectra of the withdrawn drug carrier solutions were recorded on a UV-Vis spectrophotometer to measure their absorbance.

Each experiment was performed in triplicate. The content of lovastatin released from CsCL samples in the studied buffers was calculated using the standard curve equation established for lovastatin in the pH 2 buffer ($y = 2598x - 0.1468$, $R^2 = 0.9972$) and in the pH 7.4 buffer ($y = 3978x - 0.1975$, $R^2 = 0.9985$, where x is the concentration of lovastatin in solution and y the absorbance)

3. RESULTS AND DISCUSSION

3.1. FT-IR spectra of CsCL biomaterials

Figure 1 presents the FT-IR spectra of chitosan (Cs), carrageenan (C), and lovastatin (Lov). It can be seen on the FT-IR spectrum of chitosan that in addition to the typical peaks of the –CH and C–O groups at 2883 cm^{-1} and 1083 cm^{-1}, chitosan also has an obtuse peak at 3448 cm^{-1}, which is typical for –OH groups and $–NH_2$ groups; the peak at 1598 cm^{-1} is typical for the $–NH_2$ group;[19] the peak at 895 cm^{-1} is typical for the saccharide ring structure and the peak at 1651 cm^{-1} is due to the valence oscillation of the C=O group in chitosan.

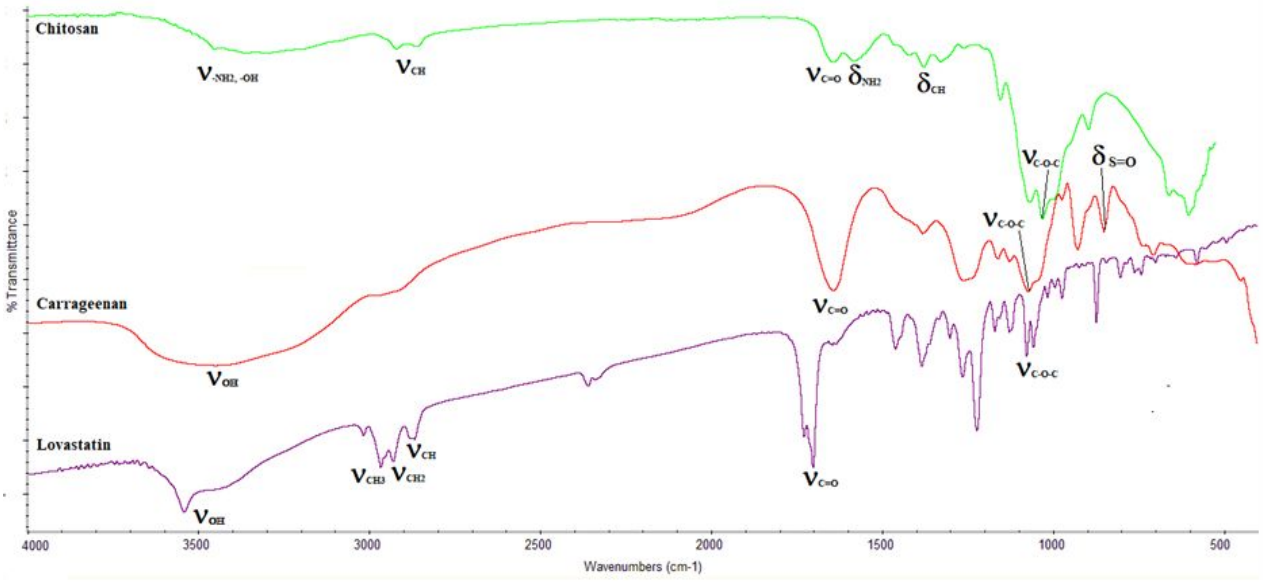

Figure 1: IR spectra of chitosan, carrageenan, and lovastatin

The FT-IR spectrum of Lov shows valence fluctuations of the –OH group at 3644 cm^{-1}. The three peaks at 2966, 2925, and 2868 cm^{-1} characterize the vibrations of the $–CH_3$, $–CH_2$, and –CH saturated groups, respectively.[20] The sharp peak at 1696 cm^{-1} is characteristic of the C=C bond oscillation while the peaks at 1714, and 1071 cm^{-1} are due to the C=O and –C–O–C group vibration.

FT-IR spectra of CsCL biomaterials containing 5% Lov and different chitosan/carrageenan ratio are shown in Figure 2, revealing a good correlation between peaks of the biomaterial with those of the characteristic groups in Cs, C, and Lov. However, there is a small shift in the peaks corresponding to the characteristic groups in Cs, C, and Lov of the CsCL biomaterial. This signal shift can be explained

by strong contacts existing in the biomaterial composites, such as dipole interactions between the HSO_3^- groups in carrageenan and the $–NH_3^+$ groups of chitosan protonated by acetic acid, and hydrogen bonds between the –OH and $–NH_2$ groups. These signals did not considerably change when the ratio of polymers was varied, indicating that the polymers interacted well with each other and with medications. Table 2 lists the distinctive signals of the material samples.

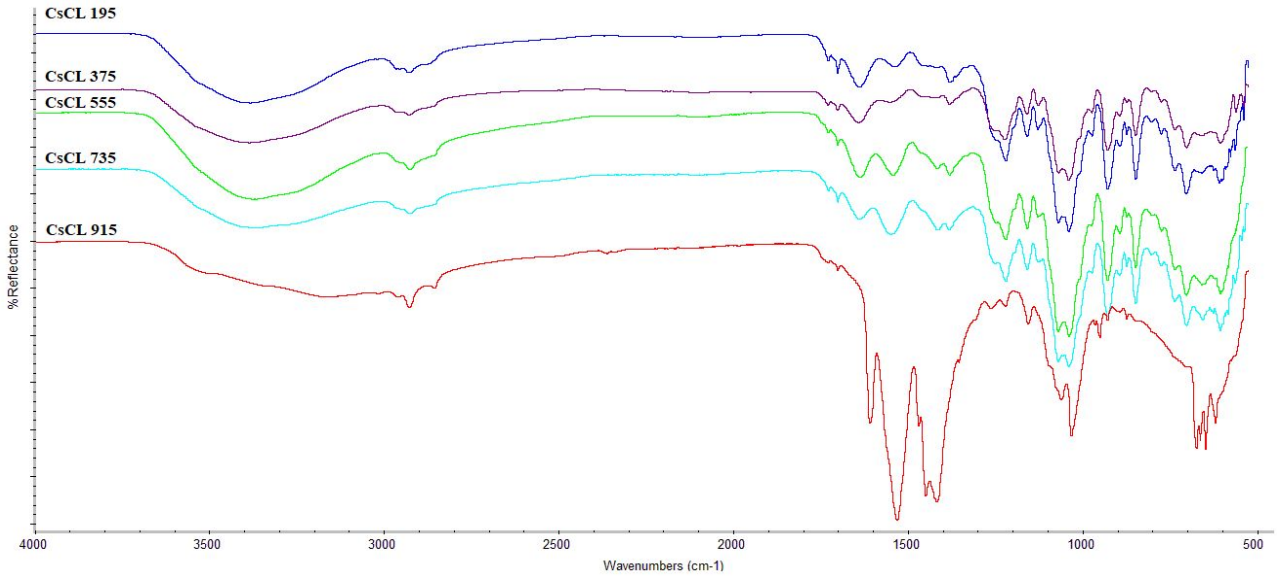

Figure 2: FT-IR spectra of CsCL samples with different chitosan/carrageenan ratios

Table 2: Characteristic wave numbers for functional groups of Cs, C, Lov and composites CsCL

	Cs	C	Lov	CsCL195	CsCL375	CsCL555	CsCL735	CsCL915
$\nu_{OH, NH2}$	3360	3431	3644	3384	3391	3371	3391	3534
$\nu_{CH\ sat.}$	2920	2966	2966	2929	2929	2936	2936	2929
$\nu_{C=O}$	-	1639	1696	1638	1637	1637	1644	1609
δ_{NH}	1598	-	-	1538	1550	1537	1550	1524
δ_{CH}	1376	1380	-	1223	1229	1216	1229	1269
ν_{C-O-C}	1028	1077	1071	1042	1035	1042	1042	1029
$\nu_{S=O}$	-	847	-	861	848	855	848	875

3.2. Morphology of CsCL biomaterials

The FESEM images of Lov and CsCL biomaterial samples are displayed in figure 3. Lov is visible as a rod-shaped crystal with large size of 5-10 µm on the FESEM images. Lov particles were dissolved in ethanol when disseminated in the CsCL materials, so when spread into the polymer matrix, Lov particles converted into spheres. The particles have varying sizes and tend to cluster together as well as return to their original bar (rod) form depending on the ability to scatter Lov into the polymer matrix.[20]

Lov particles have a spherical form of roughly 50-100 nm in size and are optimally disseminated into the polymer matrix, as seen in the sample CsCL195. The particles in the remaining samples tended to clump together and return to bars, and they were poorly disseminated in the polymer matrix. This can be explained by the fact that *(i)* the sulfate group of C and the protonated amine group of Cs produce dipole interactions; *(ii)* hydrogen bonding forms the steric structure; and *(iii)* the Lov particles enter the structure. It occupies this area and creates hydrogen bonds with the matrix polymers, allowing it to be disseminated throughout the matrix polymer system. Because the amount of dipole interactions is reduced at low rates, the ability to form hydrogen bonds between Lov molecules and the matrix polymer is improved, as well as the ability to disperse into the matrix. Increasing the Cs ratio will result in more dipole interactions, reducing the number of hydrogen bonds between Lov molecules and the matrix polymer, and therefore reducing the capacity to distribute Lov particles into the matrix polymer, causing Lov particle aggregation.[2

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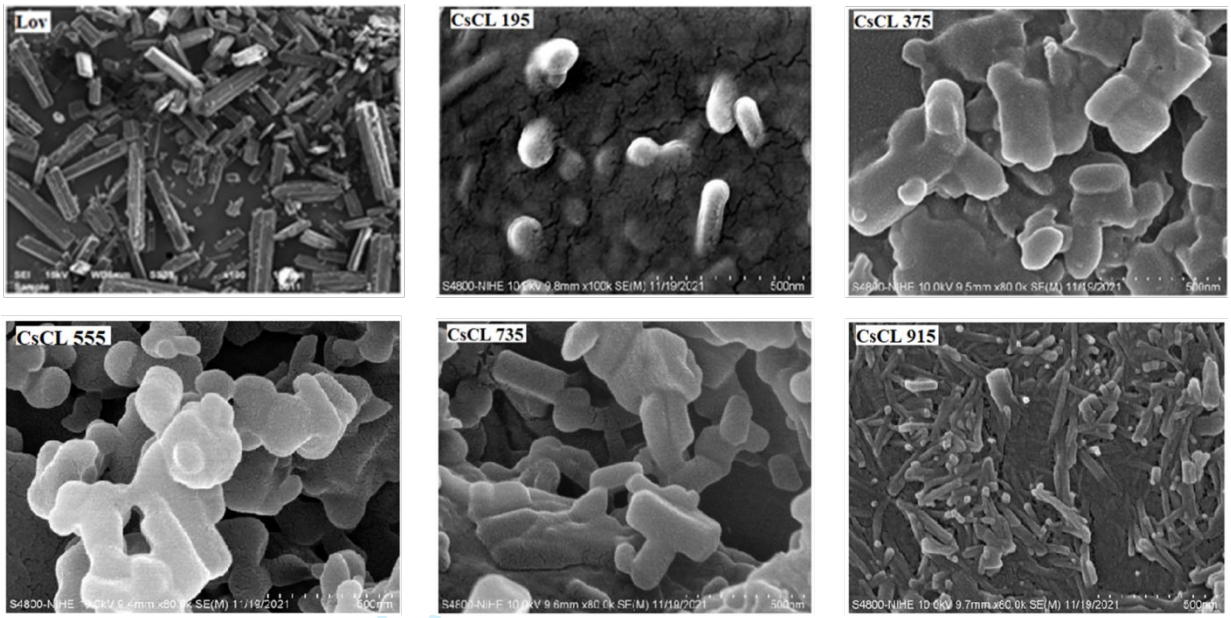

Figure 3: FESEM image of Lov and biomaterial CsCL

3.3. Thermal properties of CsCL biomaterials

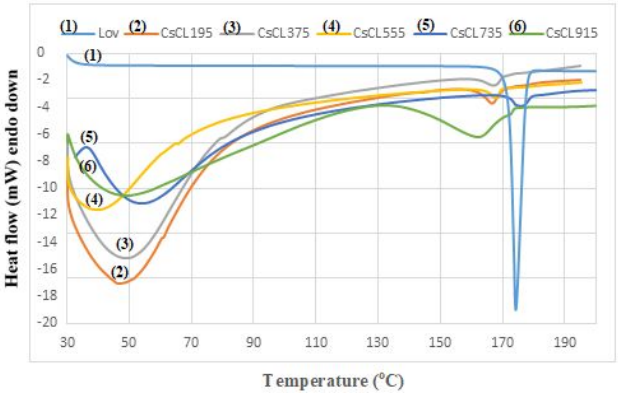

Figure 4: DSC diagrams of Lov and CsCL biomaterials

The DSC diagrams and statistics of DSC parameters of Lov and CsCL composites with different carrageenan/chitosan ratios are indicated in figure 4 and table 3.

The DSC plot of Lov shows that there is only one endothermic peak, which corresponds to the melting of Lov at 174.3 °C. The melting process begins at 172 °C and ends at 177 °C, indicating that Lov has high purity, which is consistent with previous studies.[21, 22] Meanwhile, the DSC plot of the biomaterial samples shows two endothermic peaks, which correspond to the dehydration of chitosan's hydrophilic groups and the melting of Lov. When the chitosan-carrageenan ratio is decreased, the dehydration temperature of the complex increases. The possible reason is that when chitosan content is reduced, the content of hydrophilic groups in chitosan decreases, causing the dehydration temperature of the sample to rise.[20]

Table 3: DSC parameters of Lov and CsCL biomaterials

Sample	Heat collector peak 1 (°C)	Enthalpy 1 (J/g)	Heat collector peak 2 (°C)	Enthalpy 2 (J/g)
Lov	-	-	174.3	-88.523
CsCL915	47.8	-71.091	161.7	-27.046
CsCL735	47.9	-67.908	171.7	-3.604
CsCL555	44.7	-68.992	172.6	-2.214
CsCL375	54.5	-204.409	172.0	-2.292
CsCL195	52.3	-141.722	171.7	-3.120

When comparing the second endothermic peak with the melting point of Lov, it can be seen that the melting point of Lov is slightly reduced as the chitosan/carrageenan combination is added. At the same time, the melting point of the complex is significantly lower than that of Lov. The decrease in melting temperature can be attributed to the sample's crystallinity.

3.4. Investigation of drug release

Figure 5 depicts Lov drug release from the composites at pH 2 and pH 7.4. The solution pH and chitosan/carrageenan ratio had a significant impact on the Lov release from the composites. In pH 2 solution, Lov tends to release more quickly. This could be due to the fact that at pH 2, proton H^+ attacks chitosan and carrageenan molecules, loosening the composite structure and making it easier to dissolve.[20] The ratio of chitosan to carrageenan changes, which affects the unconventional release of Lov. It can be explained by the differences in the interactions between drug molecules and polymer molecules. Based on the drug release content, a chitosan/carrageenan ratio of 1/9 can be chosen as appropriate because Lov release is better in the pH 7.4 buffer and slower in the pH 2 solution.

Thus, the drug is protected in the gastric medium and released in the intestine, where the drug is better absorbed through the cell wall.

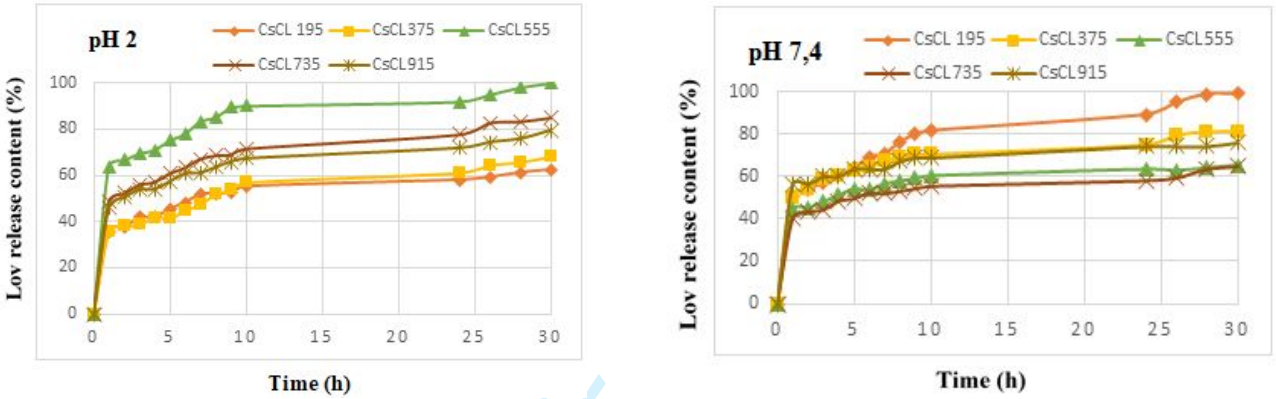

Figure 5: Graph of Lov drug release from CsCL biomaterials in pH 2 and pH 7.4 solutions

Table 4: Regression value R^2 of models release Lov from CsCL biomaterials in pH 2 and pH 7.4 solutions

		Zero-order	First-order	Higuchi	Crowell	Korsmeyer-Peppas	n
pH = 2							
CsCL195	SR	0.9689	0.8781	0.9727	0.9689	0.9439	0.3385
	FR	0.9884	0.8000	0.9879	0.9884	0.9875	0.1952
CsCL375	SR	0.9680	0.9319	0.9070	0.9735	0.8425	0.3308
	FR	0.9735	0.8492	0.9764	0.968	0.9761	0.1414
CsCL555	SR	0.9895	0.9521	0.9599	0.9895	0.9033	0.6021
	FR	0.9896	0.8902	0.9924	0.9896	0.9932	0.2728
CsCL735	SR	0.9788	0.8831	0.8537	0.9788	0.9641	0.4687
	FR	0.8441	0.2696	0.9879	0.8441	0.8568	0.2404
CsCL915	SR	0.9783	0.8616	0.987	0.9783	0.9674	0.4497
	FR	0.9742	0.8557	0.9709	0.9742	0.9713	0.1812
pH = 7.4							
CsCL195	SR	0.9933	0.9763	0.982	0.9933	0.9652	0.4276
	FR	0.8698	0.5203	0.8806	0.8698	0.8865	0.1972
CsCL375	SR	0.9521	0.6942	0.99	0.9521	0.9897	0.4922
	FR	0.7990	0.1680	0.8121	0.799	0.8021	0.2454
CsCL555	SR	0.9681	0.7736	0.9799	0.9681	0.9499	0.4220
	FR	0.7128	0.6000	0.6988	0.7128	0.6827	0.3994
CsCL735	SR	0.9554	0.6000	0.9394	0.9554	0.8846	0.5440
	FR	0.9532	0.6000	0.5852	0.6000	0.5704	0.5502
CsCL915	SR	0.9306	0.4149	0.9630	0.9306	0.9702	0.3837
	FR	0.9431	0.8000	0.9424	0.9431	0.9418	0.1072

SR: Slow release; FR: Fast release

The results obtained when studying the drug release kinetics according to the zero, first-order, Higuchi, Hixson-Crowell, Korsmeyer-Peppas models[20] are presented in table 4, revealing that the

drug release from CsCL biomaterials in the fast phase has a rebound coefficient higher linearity than the drug release from the biomaterials at the slow stage. In general, the carrageenan/chitosan ratio has little influence on the drug release mechanism from the CsCL biomaterial particles. The drug release from these CsCL particles almost follows the zero-order kinetics with the release rate independent of the initial drug concentration.

4. CONCLUSION

The research has successfully produced carrageenan/chitosan biomaterials carrying 5 % (by weight) of the drug lovastatin (Lov) and determined the structural characteristics of the materials. The IR spectral signals show that the two polymers have been combined with each other and the Lov has been well dispersed into the blend of carrageenan/chitosan without modifying the polymer structure, at the same time maintaining the drug Lov structure. In other words, carrying the drug on the biomaterial does not change its properties. The release of Lov medication from the Cs/C composites in the pH buffer that matches the pH of the stomach and intestinal juices was investigated. All samples were released according to two kinetic models of zero-order ($R^2 \approx 0.99$) and Korsmeyer-Peppas; with $n < 0.5$, the Lov drug release mechanism from CsCL biomaterials obeys the Fick diffusion law.

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