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Encapsulation of Silymarin via Chitosan-PLGA Nanoparticles for Drug Delivery

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Abstract

The herbal extracts of milk thistle, known as Silymarin (SM), are used to treat liver diseases. Chitosan is a natural polymer with high biodegradability, which is used as a coating for many drugs to improve drug delivery. Polylactic-co-Glycolic Acid (PLGA) is nontoxic and one of the most successful polymers used in drug delivery. In this study, in addition to SM loading in each of the coatings, a combination of polymeric coatings of chitosan and PLGA was used as carrier agents. The second stage is the rate of release and swelling in phosphate buffer solution (pH=7.4), as well as the morphology of the coated drugs using Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). SEM images indicate that the particles obtained are spherical. In addition, by adding PLGA nanoparticles to the combination of chitosan nanoparticles and the drug, the density and integrity of the nanoparticles increase, and their size will be diminished due to the interaction between the molecules. By examining the percentages of encapsulation, chitosan showed the best results at lower concentrations, which is equal to 84%. Also, with the addition of PLGA polymer, it yields a higher efficiency, reaching 92 %. The results showed that PLGA nanoparticles delay the release of the drug and increase the drug's residence time inside the body. Generally, the presence of chitosan-PLGA increases the Encapsulation Efficiency (EE). As a result, the coating of nanoparticles of chitosan-PLGA can be a suitable polymeric carrier for releasing control, as well as delivering SM-based herbal drugs.

Keywords: Silymarin, Chitosan, Polylactic-co-glycolic acid nanoparticles, Drug delivery, Encapsulation.

1 | Introduction

Since ancient times, natural drugs have been used as essential ingredients in pharmaceutical systems. To date, natural products continue to be a significant source of medication. For example, roughly 60% of anti-cancer compounds and 75% of medications are infectious diseases, natural products, or natural product derivatives. However, the evolution of the natural formulation of the drug was slow over the years, because the quality and quantity of data on the safety and efficacy of natural medicines were not enough to meet the criteria for

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supporting its use worldwide. Although the effectiveness of natural medicines has not yet been clarified, the recent improvement in drug delivery systems for synthetic drugs stimulates the usage of modern drug delivery techniques for natural drugs. In recent decades, many new drug delivery systems or pharmaceutical forms using natural medicines, including solid dispersion, incorporated compounds, self-emulsifying delivery systems, release systems, nanoparticles, and microspheres, have been developed. The use of new drug delivery technologies seems to be somewhat delayed. This problem is related to the complexity of the components of natural drugs and requires maintaining the ratio of the mass of active molecules. A herbal drug is defined as a set of practical molecular components in a specific mass ratio.

The pharmacologic effect heavily depends on these components, which are significant changes in their impact on the drug. Therefore, it is desirable to maintain the ratio of the mass of multiple components to dosage forms and to be performed simultaneously in both in vitro and in vivo conditions [1]. Milk thistle is a widely consumed botanical used for an array of purported health benefits. The primary extract of milk thistle is termed SM, a complex mixture that contains a number of structurally related flavonolignans, the flavonoid taxifolin, and a number of other constituents. The major flavonolignans present in most extracts are silybin A, silybin B, isosilybin A and isosilybin B, silydianin, silychristin, and isosilychristin [2], and its name is derived from two features of its leaves. It has white thistly leaves and also has a milky extract. For 2000 years, botanists have used the seeds of this plant to maintain and care for the liver against toxins and treat chronic diseases.

In addition, silybinin has multiple anti-cancer effects in a variety of tumor cells, such as prostate, colon, and bladder. Its multiple effects against tumor cell types are without any harm [3]. Chitosan, the second most abundant next to cellulose, is a naturally occurring amino polysaccharide derived from a deacetylated form of chitin. Its nontoxic, biocompatible, antibacterial, and biodegradable properties have led to significant research towards biomedical and pharmaceutical applications, such as drug delivery, tissue engineering, wound-healing dressing, etc. [4], [5]. Choosing the proper carrier is very important in the delivery of oral medication. A wide range of materials, such as natural and synthetic polymers, cyclodextrins, and dendrimers, have been selected as carriers to improve the bioavailability of drugs. Poly (lactic-co-glycolic acid) or Polylactic-co-Glycolic Acid (PLGA) is a synthetic copolymer that is biodegradable and biocompatible. PLGA can be used as a protein carrier in the delivery system [6], [7].

2 | Materials and Methods

2.1 | Materials

PLGA: Sigma-Aldrich; lactic-glycolic acid ratio: 50:50, Mw = 30,000-60,000, Sigma-Aldrich, Polyvinyl Alcohol (PVA): Sigma-Aldrich, Mw ~ 25,000, 88% hydrolysed, Sigma-Aldrich, chitosan (Low molecular weight (LMwt), Sigma-Aldrich), Silymarin (SM): Zardband Company, Ethanol (98%), Penta-Sodium Tripolyphosphate (TPP): Sigma-Aldrich, Dichloromethane (DCM): Sigma-Aldrich, acetic acid.

2.2 | Preparation of Polymeric Coating of Chitosan Containing Silymarin

In this method, at first, chitosan with ratios of 0.24 % w/v, 0.32 % w/v, 0.4 % w/v, and 0.48 % w/v dissolved in acetic acid 2% and also SM with a concentration of 1 mg/ml dissolved in ethanol [8]. Chitosan solution was placed on a magnetic stirrer for 30 minutes, and the solution of SM and ethanol was added dropwise to the chitosan solution. In the next step, the chitosan solutions were adjusted to pH 5 with 1 molar NaOH [9]. On the other hand, a solution of TPP with a concentration of 0.133% w/v was prepared with distilled water as solvent [8], and its pH was adjusted to 5 using 0.1 M hydrogen chloride solution [9]. Then, the TPP solvent with a ratio of 1 to 4 was added to the chitosan-drug solution as a droplet [8]. Then, it was placed on a magnetic stirrer for 1 hour at a speed of 800 rpm to make the solution uniform. The nanoparticles were obtained at a speed of 11,000 rpm for 45 minutes at 10 °C in a refrigerated centrifuge apparatus. The centrifuge sludge was separated from the suspension and mixed with a certain amount of distilled water. Then it was placed in the ultrasonic bath for 30 minutes. Finally, the various concentration solutions were put in to

the -80 °C freezer and after one day that they were frozen, they were entered into the Freezer Dryer (FD -10V, Iran) until they were completely dried and became nanoparticle packs [9].

2.3 | Preparation of Polymeric Coatings of Polylactic-Co-Glycolic Acid Carrier Silymarin

In this section, the emulsion evaporation method is used. In this method, 50 mg of PLGA and 5 mg of SM were dissolved in 3 ml of DCM and 2 ml of acetone, respectively. Then, 0.5% w / v of PVA was mixed with 10 ml of distilled water and placed in a hot water bath to be well dissolved in distilled water. The PLGA solution was then added to a solution of PVA and distilled water at a temperature of 4 °C. The solution was placed in an ultrasonic bath for 5 minutes until the solution was uniform, and then placed at a temperature of 4 °C for one day on a magnetic stirrer at 100 rpm for complete solvent Evaporation. Finally, the solution was placed in a refrigerator centrifuge at 20 °C for 20 minutes at 4 °C at a speed of 15,000 rpm to separate sediment from Suspension. The precipitate was frozen at -80 °C, and they were completely dried by a Freezer Dryer (FD-10V, Iran) [10].

2.4 | Preparation of Chitosan Nanoparticles - Polylactic-co-Glycolic Acid Nanoparticles Loaded with Silymarin

In order to make the combined coating of chitosan-PLGA nanoparticles loaded with SM, according to the previous step, after centrifuging the solution, the precipitate was extracted again with 10 ml of a solution of 0.5% w / v of PVA with water. The operation was achieved at 4 °C. On the other hand, in order to make a chitosan solution, the best concentration with the highest value of encapsulation was used. The 0.24% chitosan was added to 2% acetic acid, and the pH was adjusted to 5. To prepare a PLGA-chitosan nanoparticle solution, PLGA nanoparticles were passed through a 0.45 μm membrane filter and added to chitosan with ratios of 5: 5, 7: 3 and3: 7. The solutions were placed on a magnetic stirrer for 4 hours at 4 °C and then centrifuged for 20 minutes at 4 °C at a speed of 15,000 rpm to separate the nanoparticles fromsuspension. The prepared nanoparticles were frozen at-80 °C and dried by a Freezer Dryer (FD-10V, Iran) to obtain nanoparticles [10].

2.5 | Determination of Encapsulation Efficiency

After centrifuging, the clear prepared sediment was separated from suspension and diluted with distilled water to define the value of SM by using a UV-visible spectrophotometer at λ_{max} = 287 nm. Encapsulation Efficiency (EE) of nanoparticles was calculated by using the following equation [9]:

$$\mathrm{EE}(\%) = \left(\frac{\text{total amount of drug added} - \text{amount of free drug}}{\text{total amount of drug added}}\right) \times 100.$$

2.6 | Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) defined the shape and surface characteristics of freeze-dried nanoparticles. The beads were sputter-covered with Au using a vacuum evaporator and examined using a scanning electron microscope [11].

2.7 | Fourier Transforms Infrared Spectroscopy

The FTIR transmission spectrum of three samples (chitosan nanoparticles with SM, PLGA nanoparticles with SM, PLGA–chitosan nanoparticles with SM) was determined. Each of the samples was powdered and mixed with dry Potassium Bromide (KBr), and then prepared under low pressure in a hydraulic pressure. Each pill is placed in a special chamber and then by the Raylei'gh model WQF 510 FTIR device, and each of the spectra was determined [12].

2.8 | Swelling Characteristics

In order to determine the values of nanoparticle swelling, each sample was weighed after drying and then placed within 40 ml of phosphate-buffered solution, with a pH of 7.4, for 6 hours. Subsequently, the swollen samples were weighed after removing surface water. The percentage of nanoparticle inflation was obtained using the following equation:

$$S(\%) = \frac{Wt - Wo}{Wo} \times 100,$$

Where W_o is the initial weight and W_t is the final weight of the swollen nanoparticles at time t [13], [14].

2.9 | Drug Release Study

In order to determine the amount of drug release from the synthesized nanoparticles, after drying and weighing the samples, they were put inside 40 ml of phosphate buffer with a pH of 7.4 on a magnetic stirrer. The ambient temperature of release was 37 ° C, and the speed of the stirrer was 100 rpm. At an interval of one hour, 2 ml of the solution was taken by the sampler, and the absorbance was read by the UV-visible spectrophotometer (UV1800-DP200A, Taiwan) in λ_{max} = 287 nm. Each time, 2 mg phosphate buffer was replaced. The operation took place within 6 hours. The data were placed in the following equation, and the release rate was obtained for each sample [15]–[17].

$$\text{Drug release percentage} = \frac{Mt}{Mn} \times 100,$$

where M_t is the amount of drug released at time t, and M_n is the amount of drug loaded with nanoparticles.

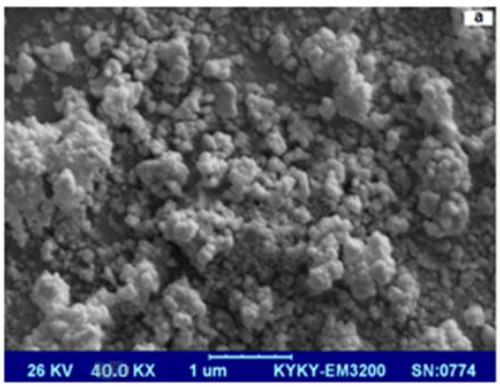
3 | Result and Discussion

3.1 | Entrapment Efficiency of Nanoparticles

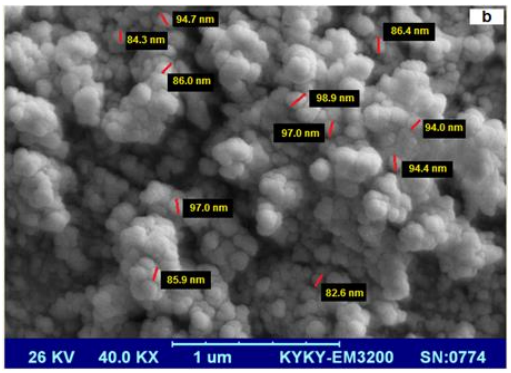
The previous studies showed that the best ratio for chitosan: TPP was considered about 4:1, which was used in this study, too [8]. Also, the highest entrapment efficiency with a value of 81.52 % was related to the lowest concentration [9]. In the current study, entrapment efficiencies at concentrations of 0.24, 0.32, 0.4, and 0.48 % were 84, 79, 57, and 51 %, respectively, and show that the lowest concentration has the highest yield. On the other hand, using the best ratio for SM loaded PLGA nanoparticles (1: 20) from recent reports [1] gave us the value of 84 %. Moreover, the best efficiency was related to the 50:50 ratio of SM loaded chitosan–PLGA nanoparticles (92 %).

3.2 | Microstructure and Surface Morphology

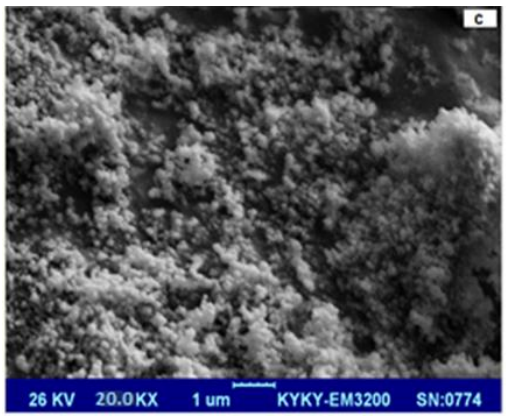
The morphology of the particles obtained by SEM shows the porosity of the particles, as well as their spherical size and the particle diameter, as shown in *Fig. 1*.



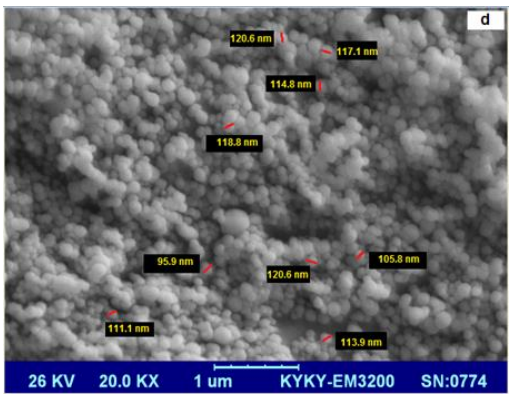
a.


b
94.7 nm
84.3 nm
86.4 nm
86.0 nm
98.9 nm
97.0 nm
94.0 nm
94.4 nm
97.0 nm
85.9 nm
82.6 nm
26 KV 40.0 KX 1 um KYKY-EM3200 SN:0774

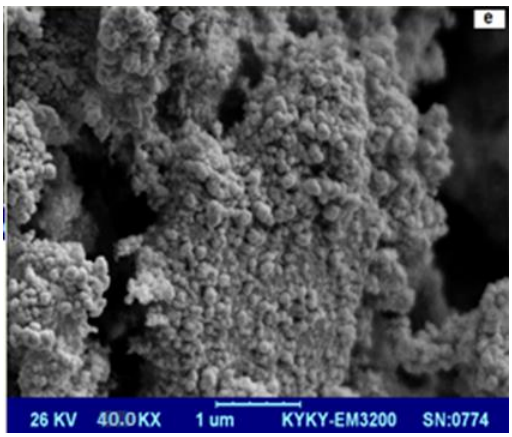
b.


c
26 KV 20.0KX 1 um KYKY-EM3200 SN:0774

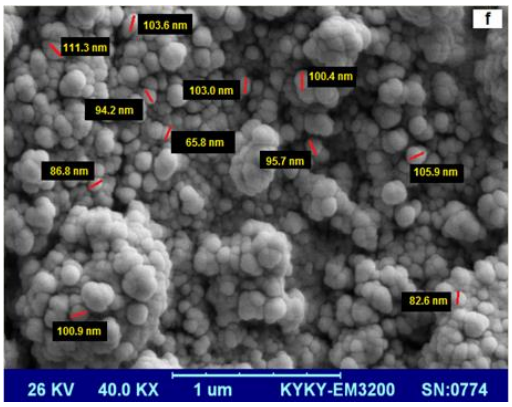
c.


d
120.6 nm
117.1 nm
114.8 nm
118.8 nm
95.9 nm
120.6 nm
105.8 nm
111.1 nm
113.9 nm
26 KV 20.0 KX 1 um KYKY-EM3200 SN:0774

d.


e
26 KV 40.0KX 1 um KYKY-EM3200 SN:0774

e.



f.

Fig. 1. Scanning electron microscopy images; a. Silymarin loaded with chitosan nanoparticles at a concentration of 0.24% by the magnification of KX 40.0, b. Determination of particle size in Silymarin loaded with chitosan nanoparticles at a concentration of 0.24%, c. Silymarin loaded in polylactic-co-glycolic acid nanoparticles with a magnification of KX 20.0, d. Determination of particle size in Silymarin loaded in chitosan nanoparticles, e. Silymarin loaded in a combination of chitosan nanoparticles with a concentration of 0.24% - polylactic-co-glycolic acid with a ratio of 5 to 5 by the magnification of KX 40.0, and F. Determination of the particle size of the Silymarin loaded in the combination of chitosan nanoparticles with a concentration of 0.24% - polylactic-co-glycolic acid with a ratio of 5 to 5.

According to the images obtained from chitosan nanoparticles with SM, it is possible to see how the drug is placed in chitosan particles. It can also be seen that its particle diameter is less than 100 nm *(Figs. 1.a* and *1.b),* which had a similar result to the mean particle size of SM loaded chitosan nanoparticles in previous studies [9]. Also, it is cited from recent studies that the particle shapes are spherical and the mean particle size of SM loaded PLGA nanoparticles is about 80 nm [1]. Also, present images that were obtained from PLGA nanoparticles containing SM show the porosity and very high dispersion between particles. Due to the presence of PLGA nanoparticles, the particle diameter varies between 90 and 120 nanometers *(Figs. 1.c* and *1.d).* The images obtained from the synthesized nanoparticles with SM show that the bond between the amino acid group of chitosan and the PLGA's polyester group is well formed, resulting in smaller cavities and less porosity. Also, the size of the particles in the combined coatings of chitosan and PLGA nanoparticles with SM is also between 90 and 120 nanometers (*Figs. 1.e* and *1.f*). The results showed that by increasing the chitosan concentration, the particle size also increased. The increase of particles is due to the presence of intermolecular bonding (due to the presence of –OH groups) and electrostatic intermolecular explosion (according to the +NH groups) between the chitosan [18]. As chitosan concentrations increase, more molecules of chitosan interfere with each other and bind to TPP to form a larger particle [19].

3.3|The Fourier Transform Infrared Spectroscopy Transmission Spectrum of Nanoparticles

The data of this study and previous studies were compared, and the following results were obtained. *Fig. 2* shows the FTIR spectrum of pure SM, pure chitosan, and chitosan nanoparticles with SM. Index peaks for pure SM show 3437 cm^{-1} for OH stretching [20], 1700 cm^{-1} for carbonyl ester stretching, 1636 cm^{-1} for the reactive flavonolignan ketone, 1560 cm^{-1} for the aromatic ring stretching vibrations, and 1033 cm^{-1} for the benzopyran ring *(Fig. 2.a)* [21], [22]. According to the peaks in the pure chitosan spectrum, it can be found that adsorption in region 3419 cm^{-1} is related to the N-H groups and in zone 2922 cm^{-1}, the tensile vibrations of the C-H stretch are shown. Absorption of region 1653 cm^{-1} of the vibrational groups -NH2 (amide i link), the region between 1458 and 1325 cm^{-1} related to the CH bend, and 1260 cm^{-1} belongs to the C-O group. In area 667 cm^{-1}, the group is C-H *(Fig. 2.a)* [9, 17, 23]. The FTIR transmission spectrum of chitosan- SM nanoparticles shows 2923.6 cm^{-1} for O-H stretching, 1727.9 cm^{-1} for C=O and $–NH_2$ stretching, 1122.4 cm^{-1} for C-O stretching, and 671.1 cm^{-1} for C-H stretching *(Fig. 2.c)* [9].

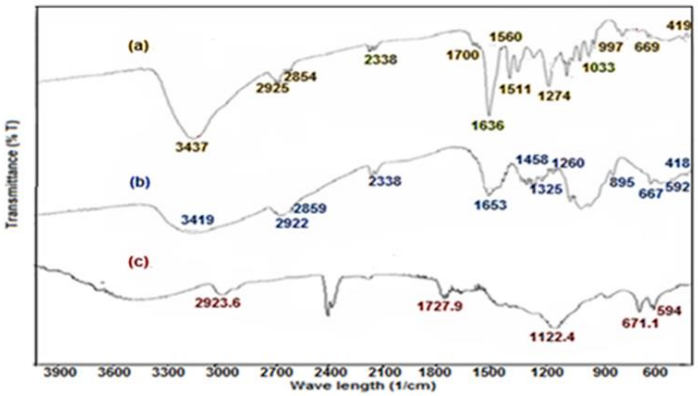


Fig. 2. Fourier transform infrared spectroscopy spectrum for (a) pure Silymarin, (b) pure chitosan, (c) chitosan nanoparticles / Silymarin.

Fig. 3 shows the FTIR spectrum of pure SM, pure PLGA, and PLGA nanoparticles with SM The FTIR transmission spectrum of pure PLGA shows the C=O absorption band at 1651 cm^{-1} [6]. FTIR spectrum of PLGA nanoparticle-SM shows 2942 cm^{-1} for O-H stretching, 1739.5 $cm^{-1}$for C=O stretching, and 1157.1 cm^{-1} for C-O stretching *(Fig. 3.c)*.

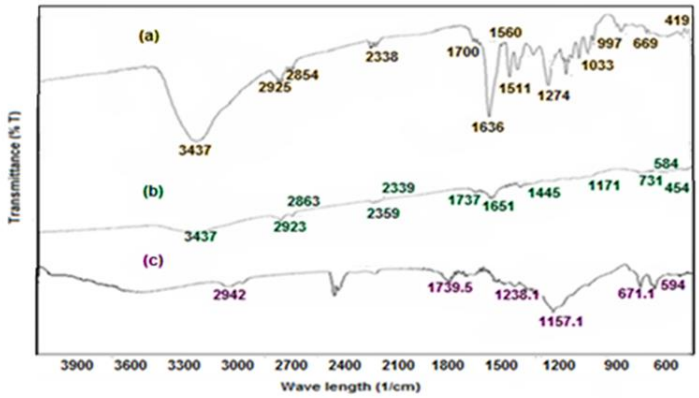


Fig. 3. Fourier transform infrared spectroscopy spectrum for (a) pure Silymarin, (b) pure polylactic-co-glycolic acid, (c) polylactic-co-glycolic acid nanoparticles/silymarin.

Fig. 4 shows the FTIR spectrum of pure SM, pure chitosan, pure PLGA, and chitosan-PLGA nanoparticles with SM. The FTIR transmission spectrum of PLGA-chitosan nanoparticle with SM demonstrates 2942.8 cm^{-1} for C-H stretching, and this spectrum and 1724 cm^{-1} indicate that the chitosan is a very suitable coating onto the PLGA nanoparticle surface in physical and chemical aspects. The chitosan peak at 1730-1660 cm^{-1} is shown in the PLGA-Chitosan nanoparticles, which means that the chitosan has been successfully coated onto the PLGA nanoparticle. The amine group ($-NH_2$) attached to the chitosan on the PLGA nanoparticle surface enables drug delivery carriers that are based on PLGA *(Fig. 4.d)* [17].

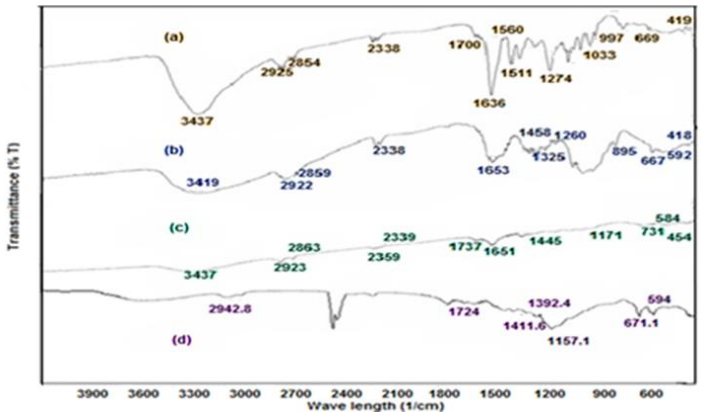


Fig. 4. Fourier transform infrared spectroscopy spectrum for (a) pure Silymarin, (b) pure chitosan, (c) pure polylactic-co-glycolic acid, (d) PLGA-chitosan nanoparticles/silymarin.

3.4 | Swelling Results for Nanoparticles

Due to the amount of swelling of each of the samples tested during the same period of time and the calculation of the inflation percentage of each of the samples, as presented in *Fig. 5*, it is seen that in different concentrations of chitosan, the highest inflation is related to the highest concentration for every sample. In addition, it is clear that by adding a certain concentration of chitosan to PLGA, the percentage of swelling of the samples has increased that by adding a particular concentration of chitosan to PLGA, the percentage of swelling of the samples has increased. Previous studies showed that increasing chitosan concentration causes a concentration gradient in the solution, which means that the driving force of chitosan increases with the content of the solution and raises the rate of the reaction and molecular diffusion. Therefore, it enhances the swelling values [24], [25].

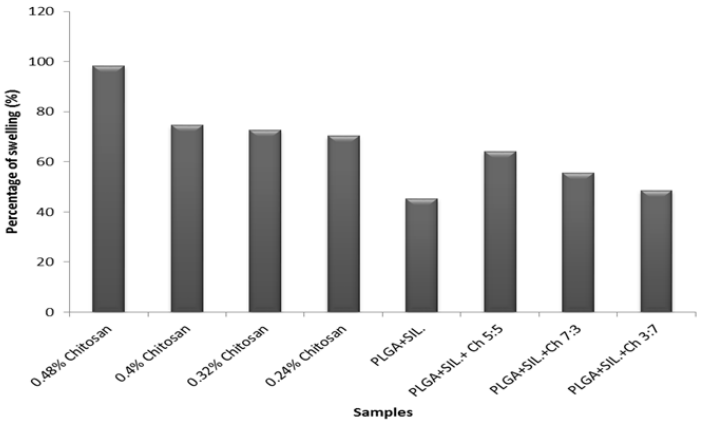


Fig. 5. Swelling percentages.

3.5 | Drug Release Results

Fig. 6 shows the release behavior of various concentrations of chitosan nanoparticles with SM. Due to increased time, drug release increases. Also, the drug release rate has a direct correlation with chitosan concentrations in the particles.

According to previous studies, the formulations of the PLGA and chitosan–PLGA nanoparticles showed that the release was fast during the initial period and then slowed down over time [12]. *Fig. 7* shows the release behavior of PLGA nanoparticles with SM, which is much lower than that of chitosan release with SM, due to the presence of PLGA polymer in the mixture, because this polymer increases the amount of residence

time and half-life in the body, and drug release happens over a longer period of time. However, because of the lack of sufficient facilities in the laboratory, a 6-hour release for each sample was counted.

Fig. 8 shows the release behavior of the various ratios of chitosan nanoparticles to PLGA-SM, as in the previous figures, due to the presence of PLGA polymer, its release rate was significantly lower than that of the chitosan nanoparticle containing SM.

Chitosan is more hydrophilic than PLGA, which helps to penetrate easily into the networks of nanoparticles in PBS solution (pH = 7.4), and more drug will be released over time [17]. Therefore, in *Fig. 6*, the drug release is much higher than the other cases. Moreover, the drug release in the curve that is related to the ratio of 30:70 in PLGA to chitosan nanoparticles is greatest, which is shown in *Fig. 8*.

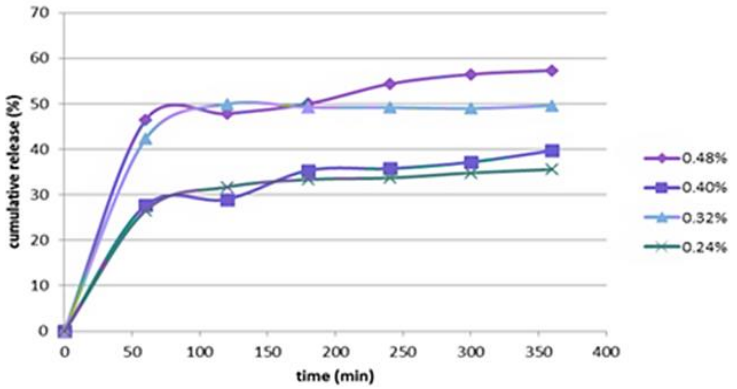


Fig. 6. Drug release of chitosan nanoparticle with Silymarin.

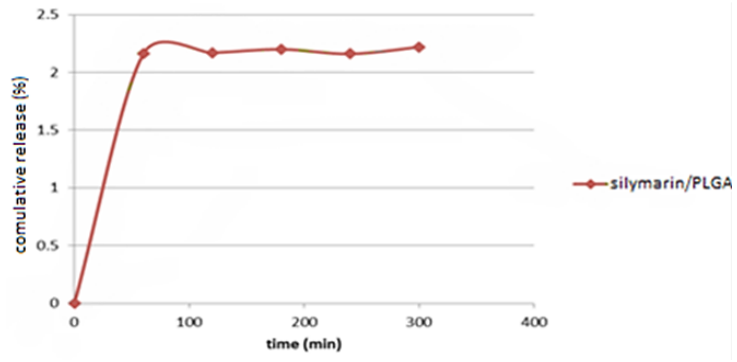


Fig. 7. Drug release of polylactic-co-glycolic acid nanoparticle with Silymarin.

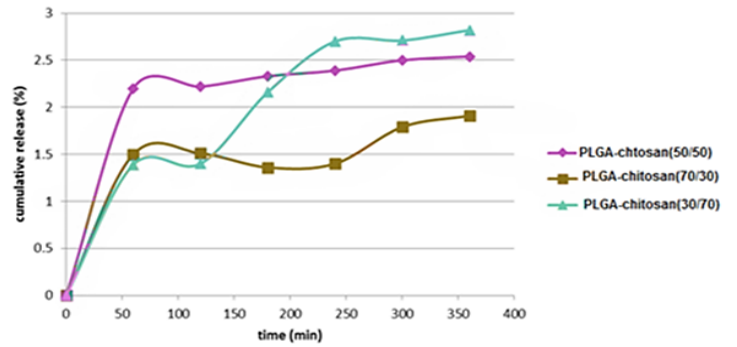


Fig. 8. Drug release of chitosan / polylactic-co-glycolic acid nanoparticle with Silymarin.

4 | Conclusion

This study described a new combination of polymeric coatings to improve SM's properties. By adding a TPP solution to a chitosan solution, as well as a PVA solution to a solution of PLGA and SM, spherical beads

were formed. By increasing the concentration of chitosan in chitosan-containing SM nanoparticles, the amount of encapsulation decreased, whereas its drug release increased. Due to the rising half-life and the residence time of drugs loaded with PLGA nanoparticles, adding these nanoparticles to other particles will reduce their release and also extend it over a longer period of time. Inflammation levels in various concentrations of chitosan nanoparticles containing SM were higher than in other concentrations. Also, increasing the amount of PVA and PLGA, along with chitosan, increased the amount of inflammation as well as encapsulation. The results obtained from SEM images showed that by increasing the chitosan concentration, particle size also increased. Also, with the addition of PLGA nanoparticles, the density and integrity of the particles increase, so it can be seen that the combination of chitosan and PLGA nanoparticles loaded by SM gives the best particles, and due to the increase in intermolecular interactions, the particle size is reduced.

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