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Preparation of modified chitosan - Amino acid nanoparticles

Abdulhussien Mahdi Aljebory*, Tamadhr J. Alsalman

Dr. Abdulhussien Laboratory, College of Pharmacy, Babylon University, Iraq

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ABSTRACT

Chitosan nanoparticles have increased more consideration as medication transporters in view of their better solidness, basic arrangement and flexible courses of organization. Common and engineered degradable polymers are perfect bearer particles. The medication can be joined into the polymer where the discharge relies on upon either their progressive dissemination from the polymeric lattice, the disintegration of the network, or discharge from the surface of the grid. Chitosan is a remarkable characteristic polymer for the conveyance of helpful specialists since it is non-toxic, biocompatible, biodegradable, and has mucoadhesive properties. To be named "chitosan," the deacetylated chitin ought to contain no less than 60% of D-glucosamine deposits. By fusing drug particles in chitosan nanoparticles, the leeway can be diminished, and the flow half-existence of the medication augmented. The results of this study show that there is a good relationship between the weight of carrier (chitosan) and weight of drug (silymarin), it gives the best total releasing percent at the weight percent 10:1, while the best-encapsulating percent and loading capacity percent occurred at the weight percent of 10:2, and there is no effect on continuous increase in the weight of the carrier. With respect to the dialysis period, it seemed that there is no evidence of increasing the dialysis time more than 12 hours because of the stability of the total releasing percent and loading capacity percent.

*Corresponding Author

Name: Abdulhussien Mahdi Aljebory
Phone: +9647702782010
Email: abdulhussien@hotmail.com

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INTRODUCTION

Chitosan is a polymer with a starch spine structure like cellulose, which comprises of two sorts of rehashing units, N-acetyl-d-glucosamine and d-glucosamine, connected (Sahoo *et al.*, 2007; Chirra and Desai, 2012; Agarwal *et al.*, 2015; Dwivedi *et al.*, 2016), β-glycosidic linkage (Khalkhali *et al.*, 2015). It is a biopolyamino saccharide cationic polymer that is acquired from chitin by antacid deacetylation and portrayed by the nearness of extensive quantities of amino gatherings on its chain. Although chitosan is gotten from chitin, the utilizations of the last contrasted with chitosan are constrained because it is artificially dormant. A typical technique for chitosan blend is the deacetylation of chitin, normally got from the shells of shrimp and other ocean shellfish, utilizing an abundance of watery sodium hydroxide arrangement as a reagent. Chitosan is insoluble in water however solvent in weaken acidic arrangements of acidic, citrus, and tartaric yet not phosphoric or sulfuric at pH under 6.5 (Chirra and Desai, 2012). In weakening watery acidic arrangement, the free amino gatherings of chitosan glucosamine units that have an evident pKa of 6.5 experience protonation and change over into the ionizable dissolvable R-NH3+ form (Agarwal *et al.*, 2015).

Usually, weaken fluid acidic corrosive arrangement in fixations 1%–3% is utilized to make a dissolvable chitosan arrangement. Chitosan is accessible in low and high atomic weights, going somewhere around 3,800 and 20,000 Da, and with various evaluations of deacetylation degree (Figure 1). The sub-atomic weight and level of deacetylation unequivocally influence chitosan properties, especially amid the improvement of miniaturized scale and nanoparticles. Chitosan is hastened with polyanions and insoluble arrangements. In spite of the fact that chitosan has uncovered some restorative movement, for example, bringing down of cholesterol (Sahoo *et al.*, 2007), wound healing (Hou *et al.*, 2015), antiulcer (Prabaharan, 2008), and antimicrobial effects (Gainza *et al.*, 2015), it is generally utilized as a polymeric medication transporter attributable to its biocompatibility, biodegradability, and nontoxic characters.

Chitosan is described by mucoadhesive properties attributable to the electrostatic cooperation between the positive charge on ionizable R-NH^{3+} gather and the negative charge on the mucosal surfaces (Augst *et al.*, 2006). The connection of the protonated amine bunches with the cell film brings about a reversible auxiliary rearrangement in the protein-related tight intersections, which is trailed by opening of these tight intersections; (Khalkhali *et al.*, 2015) demonstrated that the sub-atomic weight, solid electrostatic collaboration, chitosan chain adaptability, probability of hydrogen security development because of accessibility of holding gatherings, for example, carboxylic and hydroxyl gatherings, and simplicity of spreading into the bodily fluid inferable from surface vitality properties are variables that credit to this character. Another favorable position that makes chitosan better than other polysaccharide polymers is the simplicity of synthetic alterations in the structure, particularly in the C-2 position, which gives subsidiaries distinctive attributes, with potential use in various applications (Hou *et al.*, 2015). Pharmaceutically, chitosan-based polymeric medication bearers have been effectively used in the conveyance of anticancer operators, proteins/peptides, development components, anti-infection agents, mitigating and different medications, and a technique in both antibody conveyance and quality therapy (Dwivedi *et al.*, 2016; Hellerbrand *et al.*, 2017).

Aims of the study

The aims of this study were,

1. To prepare the chitosan- amino acid nanoparticles at different pH.
2. Preparation of drugs coupled with the prepared nanoparticles.
3. Also, to study the effect of chitosan: drug percent on the loading capacity and encapsulating efficiency.

MATERIALS AND METHODS

This study was done in the University of Babylon; college of pharmacy, Babylon city; IRAQ

1. Chitosan (Mw of 100- 300 kDa, deacetylation grade of about 90.28%) perches from MP Biomedical was insoluble in water so, diluted acetic acidsolution was used to dissolve it, after dissolving the chitosan, the pH was adjusted to 5 using 1N- NaOH solution.
2. Hydrophobic amino acid (Leucine) is used in this study, a suitable weight of (EDC),1-ethyle-3-(3-dimethyl aminopropyl) carbo diimide hydrochloride and NHS (n-hydroxy succinimide) was added to the amino acid solution with continuous stirring for 24 at room temperature. After that solution was placed in a dialysis bag, cut- off 12.000 against bidistilled water for three days with continuous changing the water three times per day.
3. Tween 80 was added drop wise to 3ml of the above solution, with continuous stirring and heating. After that, the solution was placed in an ice bath.
4. Silymarin drug (2.5, 5.0 and 7.5 mg) was dissolved in ethanol; then was added drop wise with ultrasonic for about 20 minutes to the prepared solution of chitosan- amino acid nanoparticles prepared above. Then each of the above solutions was placed in a dialysis bag in 100 ml of phosphate buffer saline (PBS) buffer PH 7.4; after one-hour, 1ml of the buffer solution was taken and its absorbance was measured at a wavelength 288 nm represent the absorbance of unreacted drug with a nanoparticle.
5. After that the bag was placed in another container with 50 ml of the same buffer, 1ml solution from the buffer was taken and its absorbance was reading at the same wavelength to be used for calculation of releasing drug from nanoparticles, in the same time 1ml buffer was added to the container to maintain a stable volume. This prose was repeated each one hour for 12 times, and then the measurements were repeated at 12, 24, 48 and 72 hours.

These steps were done for each pH and each silymarin concentration.

6. To calculate the unbounding and to release drug, a standard curve for silymarin was done by serial dilution, five concentrations of silymarin (0.001, 0.002, 0.003, 0.004 and 0.005 molar) were used. The absorbance was measured using UV- vis spectrophotometer (Emclab – Germany) at a wavelength of 288 nm.

7. The loading capacity, encapsulating efficiency and releasing percent were calculated from the measured absorbance from the above steps according to (Hou *et al.*, 2015; Ahmed and Aljaeid, 2016).

RESULTS AND DISCUSSION

Chitosan NP prepared by ionotropic gelation technique was first reported by Calvo and has been widely examined and developed by Janes. The mechanism of chitosan NP formation is based on electrostatic interaction between the amine group of chitosan and negatively charge a group of poly-anion such as tripolyphosphate. This technique offers a simple and mild preparation method in the aqueous environment. First, chitosan can be dissolved in acetic acid in the absence or presence of the stabilizing agent, such as poloxamer, which can be added in the chitosan solution before or after the addition of poly-anion. Poly-anion or anionic polymers were then added, and nanoparticles were spontaneously formed under mechanical stirring at room temperature. The size and surface charge of particles can be modified by varying the ratio of chitosan and stabilizer (Dwivedi *et al.*, 2016).

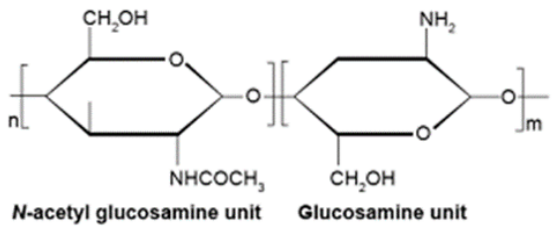

Figure 1: Chitosan unit

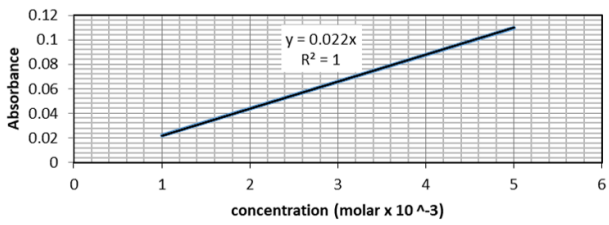

Figure 2: Standard curve for silymarin

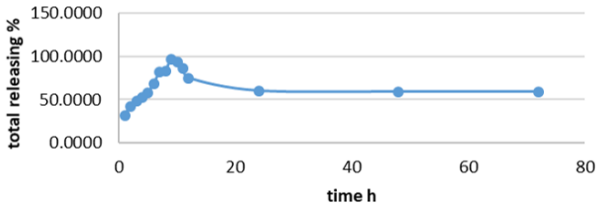

Figure 3: Chitosan + Leucine + Silymarin in PBS PH = 7.4 (0.01M); c=2.5mg silymarin. Chitosan: silymarin = 10 : 1 w\w

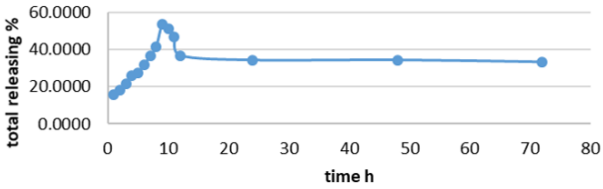

Figure 4: Chitosan + Leucine + Silymarin in PBS PH = 7.4 (0.01M); c=5mg Silymarin, Chitosan: Silymarin = 10 : 2

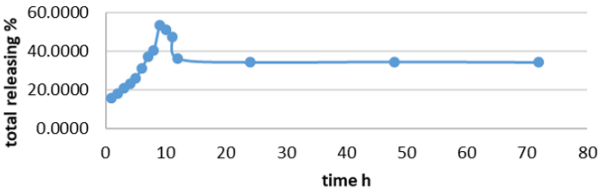

Figure 5: Chitosan + Leucine + Silymarin in PBS PH = 7.4 (0.01M); c=7.5mg Silymarin, Chitosan : Silymarin = 10 : 3

In order, to calculate the concentration of encapsulating and unreact drug in each sample, a standard curve of silymarin drug was don using five concentration as in Table 1 (the average of three repeating experiments), lambert – beer equation was used to calculate each concentration from this standard curve. Other calculations were performed using word excel 2016 according to the equation,

$$Y = aX + b$$

Where, a represents the slop and b represents the intercept of the standard curve (Figure 2).

It seemed that the loading capacity happened after 8-9 hours from the beginning time of dialysis it means that the drug was liberated from the nanoparticles nearly after this time in vivo, as shown in Figures 3, 4 and 5, there is an increase the absorbance until reach a maximum peak after 8-9 h from the zero time for the dialysis. This gives us the best results compared with the biological half-life of the drug (Hellerbrand *et al.*, 2017). The drug release behavior of nanoparticles was studied by (Ahmed and Aljaeid, 2016) at a physiological pH of 7.4 and acidic media with a pH of 5.3 in phosphate-buffered

Table 1: Data for a standard curve of silymarin drug

concentration (Molar) x 10^{-3}	Absorbance
1	0.022
2	0.044
3	0.066
4	0.088
5	0.11

Table 2: Calculation of EE % and loading capacity; wt. of Chitosan= 25mg

PH	Wt. of drug	A	Concentration	C *100	EE%	LC%
7.4	2.5 mg	0.091	4.136	413.63	83.45	7.58
	5.0 mg	0.086	3.909	390.91	92.18	15.36
	7.5	0.092	4.182	418.18	91.63	15.27

saline, PBS containing 0.5% (w/v) Tween 80. Typically, 10 mg of nanoparticles were placed into a dialysis bag (cut off 12 kDa) and introduced to PBS with the desired pH**,** at predetermined time intervals, to determine the drug concentration in dialysate and thereby time-dependent drug release profile and assayed by UV–Vis spectroscopy at a wavelength of 288 nm.

The releasing of the drug seemed to be stable after 24 hrs of dialysis; it may be due to equilibrium or to decrease the concentration of the drug-loaded on the nanoparticles.

Figure 3 shows that the total releasing % happened after 9 hours of dialysis. This means that the drug while is releasing in vivo nearly at the same time, so it is very efficient for treating the patients.

From Figure 4, we can have recognized that the maximum releasing percent occurred at the same time as in Figure 2, but the percentage of total releasing is low 53.5882 with respect to the first one 97.0855. These results indicate that the chitosan: silymarin ratio is very affected by the total releasing percent.

Figure 5 shows that the maximum total releasing percent happened after 9 hours, but the percent remains nearly constant with that of the Figure 4 53.5691. That's mean that the best chitosan: silymarin ratio to give maximum releasing percent of the drug (silymarin) from nanoparticles is 10:1.

With respect to the results show that the best concentration ratio for the carrier (chitosan) to the silymarin drug is 10: 2 weight/weight, give the best percent of loading capacity as shown in Table 2 which is represents the correlation between loading capacity (LC %), encapsulating efficiency (EE%) and chitosan concentration, at 50 mg of silymarin concentration give a LC percent 15.3636 from the remaining capsulations weight of the drug on the nanoparticles, while the best encapsulating happened at a weight percent of 1:1 between chitosan nanoparticle and silymarin drug.

CONCLUSION

The present study recommended that further work must be done using different pH and different types of amino acids to establish the suitable media to give a high loading capacity percent for the drug and the suitable amino acid to form the nanoparticles with chitosan. Also, we recommended that animal study must be done on these nanoparticles to show the behavior of the drug in vivo and the suitable target organ.

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