International Journal of Drug Development & Research
| April-June 2012 | Vol. 4 | Issue 2 | ISSN 0975-9344 |
Available online http://www.ijddr.in
Covered in Official Product of Elsevier, The Netherlands
SJR Impact Value 0.03 & H index 2

FULL Length Research Paper
Covered in Index Copernicus with IC Value 4.68 for 2010

Effect of different molecular weights of chitosan on preparation and characterization of insulin loaded nanoparticles by ion gelation method

Maryam Kouchak[1], Mohammadreza Avadi[1,2], Mohammadreza Abbaspour[1*], Alireza Jahangiri[1], Sara Kargar Boldaji[1]

[1] Nanotechnology Research Center and School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

[2] Faculty of Pharmacy, Azad University of Medical Sciences, Tehran, Iran

Abstract

Nanoparticulate drug delivery systems have several opportunities to overcome bioavailability or stability problems of peptide and protein drugs. In this study a 2^3 full factorial design was used for preparation of insulin containing nanoparticles using different concentrations of low, medium and high molecular weight chitosan (CS) and tripolyphosphate (TPP) by ion gelation method. Encapsulation efficiencies (EE) of each formulation were determined by HPLC method. Regression analysis and surface plots were used in order to evaluating the effect of variables on EE and choosing the optimum formulations. The morphology of selected nanoparticles was obtained by transmission electron microscopy (TEM). Particle size, poly dispersity index (PDI) and zeta potential were also measured. Freeze-dried nanoparticles were used for drug release studies in phosphate buffer (pH=6.8). Resulted nanoparticles had mean size of 112-419 nm with PDI< 0.5 and positive zeta potential. Insulin concentration and molecular weight of chitosan had pronounced effect on EE, but chitosan concentration had no considerable effect on EE. The maximum EE of CS nanoparticles with low, medium and high molecular weights were 61.88 %, 70.89 % and 53.73 %, respectively. The in vitro drug release profiles from the low molecular weight chitosan nanoparticles showed an initial burst release followed by a slow release within 3 hours.

*Corresponding author, Mailing address:
Mohammadreza Abbaspour
E-mail: abbaspourmr@ajums.ac.ir

Key words:
Chitosan, Molecular weight, Insulin, Nanoparticle, Ion gelation

***How to Cite this Paper*:**
Maryam Kouchak, Mohammadreza Avadi, Mohammadreza Abbaspour*, Alireza Jahangiri, Sara Kargar Boldaji "Effect of different molecular weights of chitosan on preparation and Characterization of Insulin loaded Nanoparticles by Ion Gelation Method", Int. J. Drug Dev. & Res., April-June 2012, 4(2): 271-277

Article History:----------
Date of Submission: 26-04-2012
Date of Acceptance: 01-05-2012
Conflict of Interest: NIL
Source of Support: NONE

Introduction

Chitosan [a (1→ 4) 2-amino-2-deoxy-β-D-glucan] is a cationic polysaccharide obtained by the alkaline deacetylation of chitin [1]. Chitosan (CS) has been widely used in pharmaceutical and medical areas because of its favorable biological properties such as biodegradability, biocompatibility, low toxicity, haemostatic, bacteriostatic, fungistatic, anticancerogen and anticholestrolemic properties [2-4]. Furthermore the word chitosan refers to a large number of polymers, which differ in their degree of N-deacetylation (40 – 98%) and molecular weight (50 – 2,000 kDa). These two characteristics are very important to the physico-chemical properties of chitosan and hence, they have a major effect on their biological properties [5]. The primary amino groups of chitosan led to special properties useful for pharmaceutical applications, such as pH and charged dependant solubility, ability to interact with incorporated drugs, proteins and peptides. Thease amino groups also contribute to the biological membrane permeability of chitosan through paracellular pathway, leading to greater bioavailability of drug or protein [6]. Chitosan nanoparticles have been prepared by several approaches, including physical crosslinking by ionic gelation by specific negatively charged macromolecules such as TPP [5], arabic gum [7], and EDTA [8]. In particular, CS–TPP nanoparticles have been used as a drug delivery system for a wide range of active ingredients. Some advantages of this nanoparticulate drug delivery system are the capacity to protect hydrophilic macromolecules against degradation and ability to overcome mucosal barriers. Thus its application has been mainly focused in non invasive routes of administration via ocular, nasal and oral mucosa [9-11].

The aim of this study was to develop insulin nanoparticulate system as a model drug based on chitosan and study the effect of various molecular weight of chitosan on characteristics of resulted nanoparticles. The effect of different formulation parameters such as concentration of chitosan and TPP and concentration of insulin solution were also investigated on drug encapsulation efficiency, particle size and zeta potential.

Experimentals

1- Materials

Low molecular weight of chitosan (CSL, degree of deacetylation = 98%, viscosity of 1% solution in 1% acetic acid = 22 cP), medium molecular weight of chitosan (CSM, degree of deacetylation = 92%, viscosity of 1% solution in 1% acetic acid = 715 cP) and high molecular weight of chitosan (CSH, degree of deacetylation = 96%, viscosity of 1% solution in 1% acetic acid = 1234 cP) were purchased from Primex (Siglufjordur, Iceland). Crystalline recombinant human insulin (Novo Nordisk, Denmark) provided by Exir pharmaceutical company (Iran) and TPP was purchased from Sigma-Aldrich (UK). The other materials were of pharmaceutical and analytical grades and were used as received.

2- Factorial design

Insulin nanoparticles were formulated based on 2^3 full factorial designs. Independent variables were CS concentration (X_1), TPP concentration (X_2) and insulin concentration in TPP solution (X_3). The factors and their levels are shown in table 1. Based on preliminary studies, the optimum range of CS concentration to produce nanoparticles was different for low molecular weight, so CS levels in this case were chosen different from two other grades. The dependent variable or response was insulin encapsulation efficiency of the nanoparticles (Y). Analysis of the effect of variables on response was carried out by multiple linear regression and obtained mathematical polynomial models (SPSS 16).

3- Insulin determination

Insulin determination was carried out using high performance liquid chromatography (HPLC, Waters 1525, USA). The methods has been developed on a Waters Breeze system equipped with Waters 2487 dual wavelength absorbance detector, Waters 1525 binary pump and Eurospher 100 C8 column (250 mm x 4.6 mm, 5 µm) using a mixture of triflouroacetic acid/acetonitrile/water solution (0.1:65:35 v/v) adjusted to pH 2.3 as mobile phase at the flow rate of 2.0 mL/min. UV detection was performed at 210 nm. All samples were measured in triplicate. The method was validated in terms of linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ).

4- Preparation of insulin nanoparticles

According to the factorial design (table 1, 2), known amounts of low, medium and high molecular weight CS were dissolved in 1% v/v of acetic acid aqueous solution to obtain concentrations of 0.5 mg/mL, 1 mg/mL and 3 mg/mL under magnetic stirring at room temperature. Subsequently, TPP was dissolved in distilled water to obtain concentrations of 1mg/mL and 3mg/mL. Known amount of insulin were added to TPP solution under slowly stirring at room temperature to obtain concentrations of 0.5 and 1 mg/mL. Nanoparticles were prepared by adding premixed solutions of TPP-insulin drop-wise to 10 mL of each molecular weight CS solution at room temperature, until a stable colloidal suspension were formed spontaneously under gentle magnetic stirring.

5- Determination of insulin encapsulation efficiency

Nanoparticles were separated from aqueous phase by ultracentrifugation (Sigma 3k30, Germany) at 15000 rpm and 4°C for 20 minutes. The supernatants were collected and evaluated for insulin residue by HPLC. The encapsulation efficiency (EE) was determined indirectly by measurement of the amount of free insulin in the supernatant after ultracentrifugation and was calculated according to the following equation:

EE% = 100 (amount of the total drug - amount of the free drug) / amount of the total drug

6- Physical characterization of nanoparticles

The formulations which showed higher encapsulation efficiencies (for each CS molecular weight) were selected for physical characterization such as particle size, poly dispersity index (PDI), surface charge (zeta potential) and morphology assessment. The sizes of the nanoparticles were determined by dynamic laser light scattering (DLS) (Malvern zetasizer, Malvern Instruments, UK). The measurements were performed at a scattering angle of 90° at 25°C. The particle size distribution is reported as PDI. The PDI defined as dispersion homogeneity, has the range of 0-1. Values close to 1 indicate heterogeneity and those less than 0.5 showed more homogeneity. The zeta potential of the nanoparticles was also obtained by zetasizer (Malvern ZS, UK).

The morphology of the nanoparticles was observed by transmission electron microscopy on a drop of nanoparticles' suspension by uranyl acetate staining (TEM, Zeiss, EM 902 A, Germany).

7- Release Studies

The release profile for selected chitosan nanoparticles is carried out in modified USP II dissolution apparatus (medium =30mL phosphate buffer pH 7.4, at 37 °C). Samplings were done in definite times and released drug determined by HPLC. The test was done in triplicate.

Results and discussion

In this study, chitosan with different molecular weights and TPP were successfully used to prepare the insulin loaded chitosan nanoparticles based on ion gelation method. The effect of formulation

variables on the encapsulation efficiency (EE) as the main response was studied by linear regression (table 3). The resulted polynomial mathematical models were obtained as following and showed as 3D surface plots (figures 1-3):

Y_L=69.325-15.085 X_3-0.673 X_2X_2

Y_M=51.802-23.791 X_1X_1-12.441 X_2X_2-49.746 X_3X_3+29.236 X_1X_2+56.477 X_2X_3-17.639 $X_1X_2X_3$

Y_H=52.763+5.121 X_1X_2-8.687 $X_1X_2X_3$

Where Y_L, Y_M and Y_H are the EE of low, medium and high molecular weight chitosan nanoparticles, respectively. As demonstrated in figures 1-3 the EE of nanoparticles decreased with increasing insulin concentration in the case of low and high molecular weight chitosan, while EE of medium molecular weight chitosan nanoparticles increased with increasing amount of drug. It has been shown that for nanoparticles containing bovine serum albumin (BSA), low amount of protein and chitosan may result in increasing encapsulation efficiency [12]. Regarding the highest EE of medium M_W nanoparticles (71%) it can be concluded that the loading capacity of medium M_W CS is higher than two other types of CS, so increasing amount of drug can cause the higher EE. While in the case of low and high M_W CS, the drug loading is limited and increasing drug concentration led to higher amount of free drug and lower EE. As shown in table 4 the maximum EE of CS nanoparticles with low, medium and high molecular weights were 61.88 %, 70.89 % and 53.73 %, respectively. It has been reported that using chitosan quaternized derivatives, encapsulation efficiency could be increased up to 90.3% [13]. The procedure of nanoparticle formation in this study was the ionic interactions between positive charges of CS and negatively charge TPP, thus the higher viscosity of high M_W chitosan solution containing the longer fragments made its free amino groups harder to protonate and restricted the ionic interaction with TPP. This can explain the low EE of high M_W CS nanoparticles. The same findings were reported for chitosan nanoparticle containing 5FU [14]. As mentioned in preparation of nanoparticles the optimum concentration range of CS was different M_W CS, i.e it was 1-3 mg/mL for low M_W CS and 0.5-1 mg/mL for medium and high M_W CS. figure 1 displays that chitosan concentration for each molecular weight had no effect on EE, figures 2 and 3 also indicated that concentration of CS had no pronounced effect on EE, while the chitosan molecular weight had greater impact on EE of nanoparticles.

Based on higher EE, two formulation of each CS molecular weight were selected for characterization of nanoparticles. The results of EE, size, poly dispersity index and zeta potential of these selected formulations are reported. As shown in table 4, drug loaded nanoparticles had mean size of 112-419 nm with a relatively narrow particle size distribution as indicated by a relatively low poly dispersity index values (PDI<0.5). Also nanoparticles had a positive zeta potential which is higher in the case of formulations with higher CS concentration. The TEM photographs of three formulations (F1.L, F7.M, and F1.H) are represented in Figure 4. In vitro drug release study on selected formulation (F1.L) exhibits a burst release of insulin in the first 5 minutes with a maximum drug release of about 70% after 3 hours. This release pattern could be representative of shell-enriched drug loading model in studied nanoparticles.

Conclusion

This study demonstrated that ionic gelation method can be used to prepare insulin loaded chitosan nanoparticles with different molecular weights of chitosan. Chitosan molecular weight has clear effect on encapsulation efficiency of nanoparticles. EE could be reached to 70% choosing optimum concentration of TPP, insulin and medium M_W chitosan. The results of in-vitro drug release revealed

that insulin incorporated mainly on to the surface of nanoparticles.

Acknowledgment

This work was Pharm.D thesis of Sara K. Boldaji and was supported by a grant (U 86138) from vice chancellor for research, Ahvaz Jundishapur University of Medical Sciences. We would like to extend our thanks to Dr. Behzad Sharif Makhmalzadeh for his supporting ideas.

Table 1: Independent variables and their levels; (CS_L; low molecular weight chitosan, CS_M; medium molecular weight chitosan, CS_H; high molecular weight chitosan)

Independent Variables (factors)		Level	
		-1	+1
X_1: Chitosan concentration ($mg.ml^{-1}$)	CS_L	1	3
	CS_M, CS_H	0.5	1
X_2: TPP concentration ($mg.ml^{-1}$)		1	3
X_3: Insulin concentration ($mg.ml^{-1}$)		0.5	1

Table 2: Design of experiments

Formulations	Factors		
	X_1	X_2	X_3
F1	1-	1-	1-
F2	1+	1-	1-
F3	1-	1+	1-
F4	1-	1-	1+
F5	1+	1+	1-
F6	1+	1-	1+
F7	1-	1+	1+
F8	1+	1+	1+

Table 3: Results of multiple linear regression analysis of encapsulation efficiencies of nanoparticle formulations based on chitosan with different molecular weight

Types of Mw	Model	Coefficients		Sig
		B	Std. Error	
Low	Constant	69.325	2.977	0.000
	X_3	-15.085	3.502	0.000
	X_2X_2	-0.673	0.219	0.006
Medium	Constant	51.802	3.572	0.000
	X_1X_1	-23.791	4.814	0.000
	X_2X_2	-12.441	1.197	0.000
	X_3X_3	-49.746	4.814	0.000
	X_1X_2	29.236	5.404	0.000
	X_2X_3	56.477	5.404	0.000
	$X_1X_2X_3$	-17.639	5.777	0.007
High	Constant	52.763	1.020	0.000
	X_1X_2	5.121	1.083	0.000
	$X_1X_2X_3$	-8.687	1.222	0.000

Table 4: Encapsulation efficiency, particle size, polydispersity index (PDI) and zeta potential of selected formulations.

Molecular Weight of Chitosan	Formulation	Encapsulation efficiency (Mean±SD)	Size (nm)	Polydispersity (PDI)	Zeta potential (mV)
Low	F1.L	61.61±4.52	261	0.40	+27.2
	F2.L	61.88±5.59	419	0.45	+48.4
Medium	F7.M	70.89±3.32	132	0.28	+25.1
	F8.M	70.59±1.70	343	0.49	+39.3
High	F1.H	53.50±2.61	112	0.27	+27.5
	F2.H	53.73±2.29	160	0.28	+29.0

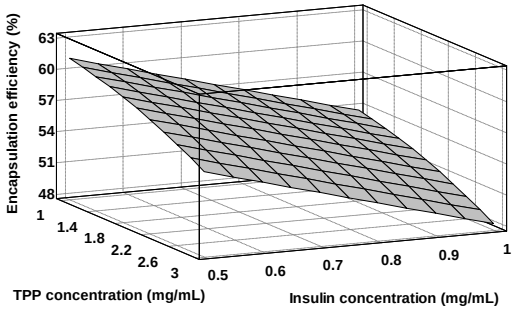
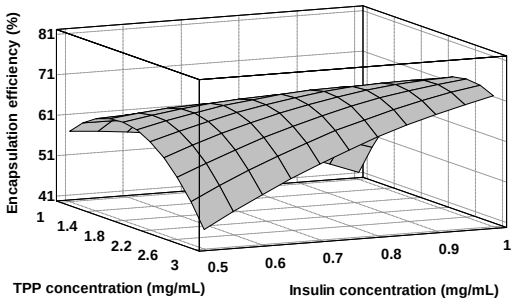
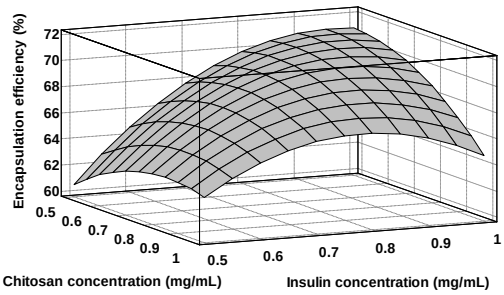


Figure 1: Surface plot of the effect of different variables on encapsulation efficiency of nanoparticles prepared with low molecular weight of chitosan





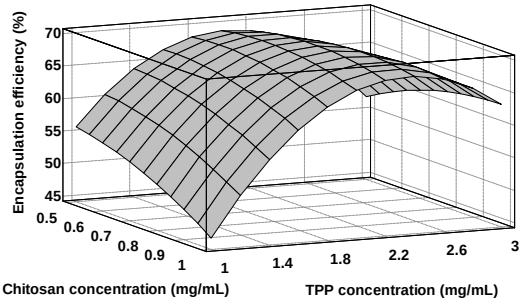
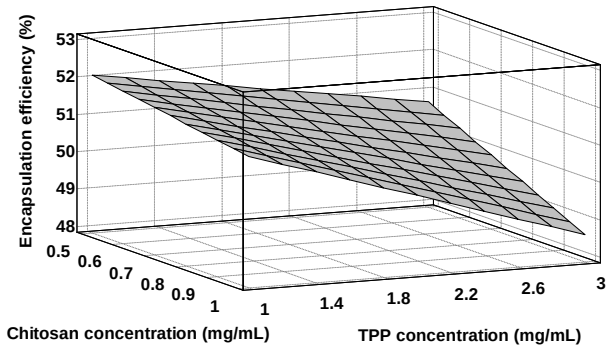
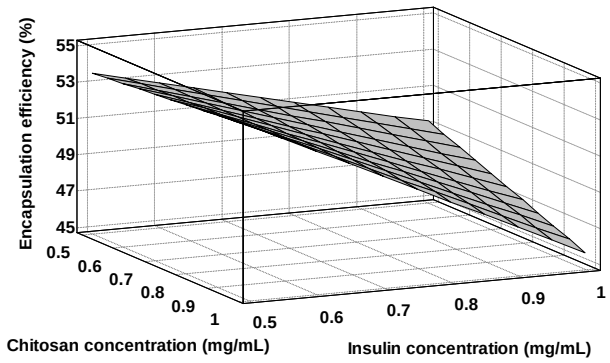


Figure 2: Surface plot of the effect of different variables on encapsulation efficiency of nanoparticles prepared with medium molecular weight of chitosan





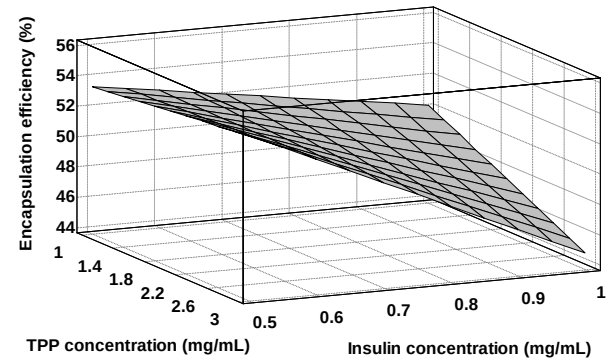


Figure 3: Surface plot of the effect of different variables on encapsulation efficiency of nanoparticles prepared with high molecular weight of chitosan

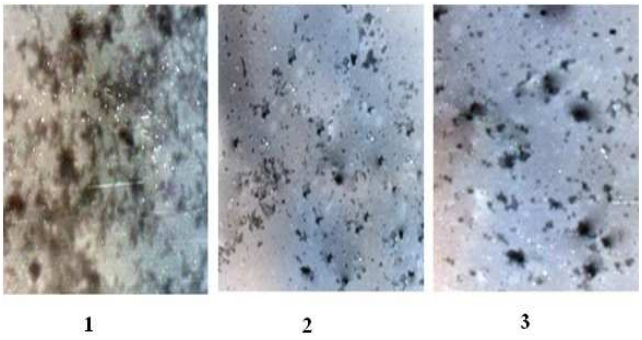


Figure 4: TEM photographs of chitosan nanoparticles with different molecular weights (1=Low, 2=Medium, 3=High)

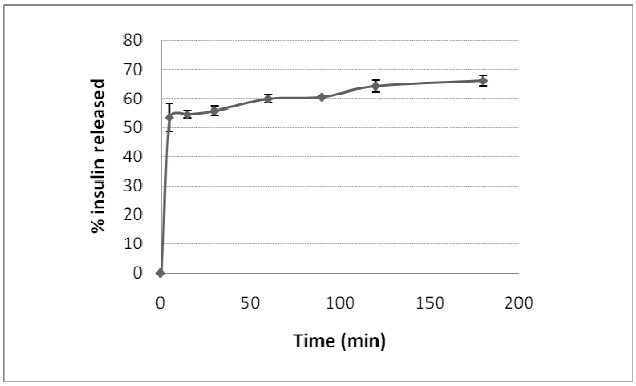


Figure 5: Insulin Release Study in Formulation F1-L

References:

1) Felt O, Buri P, Gurni R. Chitosan: a unique polysaccharide for drug delivery, Drug. Dev. Ind. Pharm. 1998; 24: 979-93.

2) Kobayashi T, Otsuka S, Yugari Y. Effect of chitosan on serum and liver cholesterol levels in cholesterol-fed rats, Nutr. Rep. Int. 1979; 19: 327–334.

3) Malette WG, Quigley HJ, Gaines RD, Johnson ND, Rainer WG. Chitosan: a new hemostatic, Ann. Thorac. Surg. 1983; 36: 55-8.

4) Thanou M, Verhoef JC, Junginger HE. Chitosan and its derivatives as intestinal absorption enhancers, Adv. Drug. Deliver. Rev.2001; 50: S91–101.

5) Calvo P, Remuñán–López C, Vila–Jato JL, Alonso MJ. Novel hydrophilic chitosan–polyethylene oxide nanoparticles as protein carriers, J. Appl. Polym. Sci. 1997; 63: 125–32.

6) Ma Z, Yeoh HH, Lim LY. Formulation pH modulates the interaction of insulin with chitosan nanoparticles, J. Pharm. Sci. 2002; 91: 1396-404.

7) Avadi MR, Sadeghi AM, Mohammadpour N, Abedin S, Atyabi F, Dinarvand R, Rafiee-Tehrani M.

Preparation and characterization of insulin nanoparticles using chitosan and Arabic gum with ionic gelation method,. Nanomed.: Nanotech. Biol. Med. 2010; 6: 58-63.

8) Loretz B, Bernkop–Schnürch A. In vitro evaluation of chitosan–EDTA conjugate polyplexes as a nanoparticulate gene delivery system, AAPS J. 2006; 8: E756-764
9) Csaba N, Garcia–Fuentes M, Alonso MJ. The performance of nanocarriers for transmucosal drug delivery, Expert. Opin. Drug Deliv. 2006; 3: 463–78
10) Alonso MJ, Sanchez A. The potential of chitosan in ocular drug delivery, J. Pharm. Pharmacol. 2003; 55: 1451–63.
11) Prego C, Torres D, Alonso MJ. The potential of chitosan for the oral administration of peptides, Expert. Opin. Drug Del. 2005; 2: 843–54.
12) Xu Y, Du Y. Effect of molecular structure of chitosan on protein delivary properties of chitosan nanoparticles, Int. J. Pharm. 2003; 250: 215-26.
13) Bayat A, Larijani B, Ahmadian S, Junginger HE, Rafiee-Tehrani M. Preparation and characterization of insulin nanoparticles using chitosan and its quaternized derivatives, *Nanomed.*: Nanotech. Biol. Med. 2008; 4: 115-20.
14) Yang HC, Hon MH. The effect of the molecular weight of chitosan nanoparticles and its application on drug delivery, Microchem. J. 2009; 92: 87-91.