



Synthesis of Insulin Loaded Nanoparticles and Their Antidiabetic Studies

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ABSTRACT

The goal of this present work turned into synthesis of polymeric nanoparticles from natural polymeric material. Natural polymeric nanoparticles have some unique physicochemical properties like stability, biocompatibility, biodegradability, and manage the drug release, so it's used for drug transport. On this look natural polymeric material casein and chitosan are used for the synthesis of nanoparticles. Insulin drug was introduced to these polymeric nanoparticles, which are used for the drug delivery. These drug loaded polymeric nanoparticles are synthesised by nano precipitation technique. The particles size of drug loaded polymeric nanoparticles based totally the rate of stirring and time of nanoparticles synthesised. Insulin was used as a drug and Glutaraldehyde was used as a linking agent. This insulin drug becomes adsorbed on the surface of the nanoparticles. Those drug loaded nanoparticles are characterized by FT-IR spectrum and physical status is analysis by means of XRD pattern. The morphology of the drug loaded nanoparticles is studied by using Scanning Electron Microscopy (SEM). From those characterisation studies insulin drug was efficaciously loaded with the nanoparticles. It has high inhibitory property towards kind II diabetics.

Keyword: Casein, Chitosan, Insulin, Nanoparticles, Anti-diabetic.

INTRODUCTION

Diabetes is the continual disease. Now a day's diabetes mellitus is the widespread metabolic disease. Around 463 million humans dwelling with diabetics. In 2045 the number of suffers will increase to 700 million. People with kind II diabetic affected person's wide variety accelerated in maximum of the countries¹. Insulin is the important key to glucose of physical body. However kind I diabetes didn't produce the insulin and type II didn't responds to the insulin.

So it leads to increase the blood sugar level².

Natural polymeric nanoparticles derived from dwelling organism. And it has a few special residences like non poisonous, low immunogenicity, biodegradability, biocompatibility and so on. So this biopolymer like casein chitosan becomes used as a natural drug delivery vehicle³. Casein was a protein, which was found in milk. Because of the biological function, nutrients transfer from the mother to her youngsters⁴. It is a non toxic and high stable milk



protein. This casein is proline-wealthy and has lacks of disulfide bonds and additionally poor secondary and tertiary structures⁵. Casein has four fundamental phosphate-rich sub-units, that are amphiphilic and assembled them self into a bilayer structure in the size 50-300 nm. But for hydrophobic interactions, the bilayers are also held collectively with the aid of calcium phosphate nanoclusters performing as bridges connecting those subunits⁴.

Chitosan is a linear polysaccharide; it's acquired through partial deacetylation of chitin. It is received from the exoskeletons of marine arthropod including crabs, shrimps, lobsters, prawns and fungi⁶. Chitosan consisting 2-amino-2-deoxy-D-glucose units joined with glycosidic linkages^{7,8}. Like casein, chitosan additionally have some special characters like non toxic, high drug maintaining ability, low immunogenicity, biodegradability, biocompatibility etc⁹. Chitosan is used in bandages, haemostatic vehicle, and wound recuperation belongings and also used as a anti microbial agent¹⁰⁻¹². It is insoluble in water, but soluble in acids.

MATERIAL AND METHODS

Low molecular weight chitosan (LMW), Casein powder was purchased from Merck. Novolin R human insulin was purchased from Novo Nordisk. PBS, Glacial acetic acid and Glutaraldehyde was purchased from Sigma Aldrich. α -amylase, α -glucosidase enzymes was purchased from SRL Pvt. Ltd., Mumbai. Calcium Chloride and NaOH also purchased from Merck.

EXPERIMENTAL

Synthese of nanoparticles

2 g of Casein was dissolved in 10mM calcium chloride salt solution with stirring then heating to 40°C for 15 minutes. 2.5 g of chitosan was dissolved in 1% acetic acid. This chitosan solution was added to casein solution as drop wise, with continuous stirring. 0.1 mL of glutaraldehyde was added to this mixture and stirrer for 3 hours. Now cross linking Casein chitosan nanoparticles are formed.

Synthesis of Insulin loaded nanoparticles

0.1 mL of insulin delivered to that casein chitosan nanoparticles combination and nonstop stirred. Ultimately insulin drug loaded casein chitosan polymeric nanoparticles are synthesised. This aggregate become centrifuged at 7000 rpm for 30 min and washed with water. Store that product as powder form for future research.

Characterization

Fourier Transform-Infrared (FT-IR) Spectroscopy

FT-IR spectrum become received from a PerkinElmer 1000 paragon FT-IR spectrometer. FT-IR spectrum was used to study the interplay between the drug and the polymeric nanoparticles. Surface morphology and physical state analysis.

Scanning electron microscopy becomes used to look at the morphology of synthesised drug loaded polymeric nanoparticles. The physical state of insulin loaded casein chitosan nanoparticles become studied the usage of XRD analysis.

In vitro anti-diabetic assay

Inhibition of α -amylase assay

Different concentration of (100, 200, 400, 600, 800, 1000 μ L/mL) drug loaded nanoparticles and standard drug was added to 500 μ L of 0.20 mM phosphate buffer (pH6.9), which contains α - amylase solution and it had been incubated at 25°C for 10 minute. 500 μ L of starch solution in phosphate buffer become added to the mixture again incubated at 25°C for 10 minute. The reaction was stopped with the aid of the addition of 1 mL of 3,5 dinitrosalicylic acid colour reagent. Again it had been incubated in boiling water and allows cooling at temperature. The reaction combination turned into diluted with water and absorbance was measure at 520 nm against positive control.

Inhibition of α - glucosidase assay

To determine the inhibition of α - glucosidase activity incubating 1 mL of starch solution with various concentration (100, 200, 400, 600, 800, 1000 μ L/mL) of drug loaded nanoparticles for 5 min at 37°C. Add 200 μ L of α -glucosidase (0.1U/mL) to initiate the response and incubated for 5 min at 37°C. To forstall the response the reaction mixture turned into heated with boiling water both for 2 minute. Using glucose oxidase peroxidase approach the amount of liberated glucose become measured.

Calculation of IC₅₀ value

$$\text{Inhibition \%} = (A_a - A_b) / A_a \times 100$$

Whereas,

A_a = Absorbance of control reaction

A_b = Absorbance in the presence of sample of drug loaded nanoparticles

Using this formula IC₅₀ value for both inhibition of α -amylase and Inhibition of α -glucosidase was calculated.

RESULT AND DISCUSSION

FT-IR spectroscopy

Figure 1. The peak at 1598 cm^{-1} is determined in the chitosan spectrum; it is the amide II group of bending vibration. In casein spectrum, the peak at 1650 cm^{-1} is absorbed it is denoted the carboxyl group of casein.

Figure 2. The spectrum of insulin loaded chitosan casein nanoparticles. In this spectrum, the peak at 1598 cm^{-1} of chitosan was shifted to 1524 cm^{-1} . The peak at 1650 cm^{-1} of casein was disappearing. The cause of the change of peak position is the interaction between the opposite groups of the nanoparticles. Those peaks display the formation of the casein chitosan nanoparticles. The peak at 1660 cm^{-1} shows the interaction between the drug insulin and the nanoparticles. Consequently this FTIR spectrum confirmed the formation of casein chitosan nanoparticles due to the interaction between the protonated amino group of chitosan and carboxyl group of casein¹³.

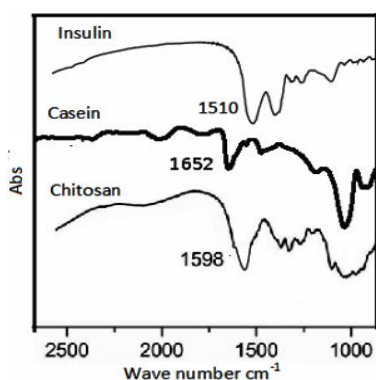


Fig. 1. FT-IR spectrum of Insulin Casein, Chitosan

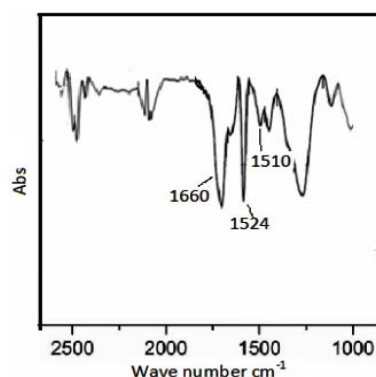


Fig. 2. FT-IR spectrum of Insulin loaded casein chitosan nanoparticles

X-ray diffraction studie

Figure 3. Shows XRD diffraction studies of drug loaded chitosan casein nanoparticles. The XRD pattern of drug loaded nanoparticles showed the broad diffractions peaks at 2θ values is 19.45° . The lower intensity shows via the drug loaded nanoparticles, that they're amorphous in nature. Due to the absence of another diffraction peaks means, no impurities found in the drug loaded nanoparticles¹⁴.

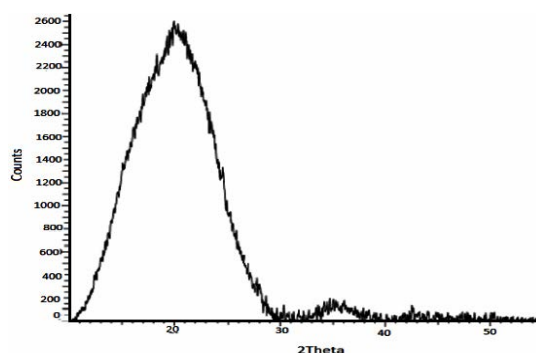


Fig. 3. XRD pattern of Insulin loaded casein chitosan nanoparticles

Scanning electron microscopy

Scanning electron microscopy is used to study the morphologies of the nanoparticles. Fig.4. shows the insulin loaded polymeric nanoparticles are spherical in shapes and uniform distributions.

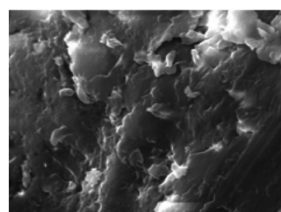


Fig. 4. SEM image of Insulin loaded casein chitosan nanoparticles

In vitro anti-diabetic assay

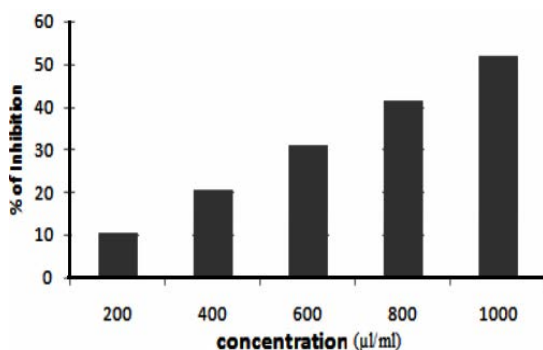
In vitro anti-diabetic activity was carried out in insulin loaded casein chitosan polymeric nanoparticles using α -amylase and α -glucosidase enzymes.

Inhibition of α -amylase activity

Gra 1. Shows Inhibition of α -amylase activity of Insulin loaded polymeric nanoparticles, it has more effect on α -amylase enzyme. Various concentrations (100, 200, 400, 600, 800, 1000 $\mu\text{L}/\text{mL}$) of drug loaded nanoparticles are subjected to α -amylase inhibitory assay. The maximum inhibition value 51.70 was obtained in 1000 $\mu\text{L}/\text{mL}$. The IC_{50} value of α -amylase inhibition assay is 36.193.

Inhibition of α -glucosidase activity

Gra 2. Shows Inhibition of α -glucosidase activity Insulin loaded polymeric nanoparticles, it has more effect on α -glucosidase enzyme. Various concentrations (100, 200, 400, 600, 800,

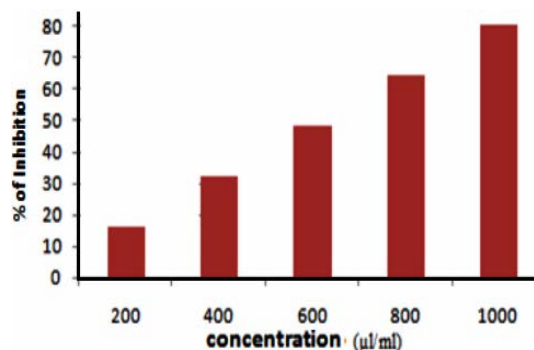


Graph 1: % of inhibition of α -amylase against various concentration of Insulin loaded casein chitosan nanoparticles

CONCLUSION

The Insulin loaded casein chitosan nanoparticles was efficaciously synthesised by nanoprecipitation technique. The functional group of drug loaded polymeric nanoparticles was confirmed with the aid of FT-IR spectrum. The synthesised nanoparticles are amorphous state, which was confirmed by XRD evaluation. SEM morphologies research showed that nanoparticles are spherical in shape. The synthesised insulin loaded casein chitosan

1000 $\mu\text{L/mL}$) of drug loaded nanoparticles are subjected to α -glucosidase inhibitory assay. The maximum inhibition value 80.40 was obtained in 1000 $\mu\text{L/mL}$. The IC_{50} value of α -glucosidase inhibition assay is 40.20.



Graph 2: % of inhibition of α -glucosidase against various concentration of Insulin loaded casein chitosan nanoparticles

nanoparticles have more effective on α -glucosidase assay whilst examine with α -amylase assay.

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Conflict of interest

The authors declare no conflict of interest.

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