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Development of Zein-Pectin Nanoparticle as Drug carrier

Dhanya AT[1], Haridas KR[1], Divia N[1], Sudheesh S[1]*

*Corresponding author:

Sudheesh S

[1]School of Chemical Sciences, Kannur University, Payyanur Campus, Kerala, India

Abstract

Recent years have witnessed tremendous growth of nanotechnology based drug delivery system which reduces drug toxicity and side effects and increases the therapeutic index of the drug. Aim of the study is to develop a biodegradable, non-toxic nanoparticle, solely from natural polymers.
Zein – pectin nanoparticle comprising of a hydrophobic zein core and a hydrophilic pectin shell was developed by ultrasonication method. SEM images confirm the nanosize of the nanoparticle. UV-Visible and FT-IR spectroscopic results confirm the incorporation of zein, pectin and the encapsulation of the model drug quercetin in the nanoparticle.
Zein is a prolamine class of protein found in wheat, maize etc and pectin is a polymer of galacturonic acid units found in plant cell wall
Keywords: Zein, Pectin; Nanoparticle; cytotoxicity; quercetin; ultrasonication

Introduction

Improving delivery techniques that minimize toxicity of drug has a significant effect on its efficacy [1]. Drug delivery with the aid of nanoparticles enhances delivery or uptake by target cells and reduces the toxicity of the free drug to non-target organs and thereby results in an increase of therapeutic index [2]. The cytotoxicity of nanoparticles or their biodegradation products remains a major problem, and improvements in biocompatibility obviously are a main concern of future research [1]. The employment of natural polymers enables the achievement of materials with many interesting features such as low toxicity, biocompatibility and biodegradability and thereby allows their use as carriers. Several works have been published relating to the use of natural polymers such as zein, chitosan, alginate, pectin etc in drug carrier development [3-8].
Zein, a major prolamine class protein from corn is considered and generally recognized as safe (GRAS) and food grade ingredient by the Food and Drug Administration (FDA). It contains three quarter of lipophilic and one quarter of hydrophilic amino acid residues. Although zein is a water- insoluble protein, it remains soluble in aqueous solutions containing at least 70% alcohol. Because of its high hydrophobicity, zein has been successfully applied as a promising carrier for encapsulation and controlled release of fat soluble compounds [3, 8].
Pectin is a natural, non-toxic and amorphous polysaccharide present in cell walls of plant tissues with considerable potential for uses in pharmaceutical formulations, chemistry, natural science and so forth [9, 10]. It has high molecular weight heteropolymers containing at least 65% (by weight) of D- galacturonic acid units which are joined to one another by means of α(1⟶4) glycosidic linkages [4]. Pectin macromolecules are able to bind with some organic or inorganic substances via molecular interactions. So, pectin can be used to construct matrices to absorb desired materials and deliver them in a controlled manner [9]. Incorporating low methoxy pectin in the polymer matrix is expected to increase the efficiency of encapsulation [4]. Moreover we have reported the hypolipidemic and hypoglycaemic activity of pectin earlier [11, 12].
Hence our study aims at developing a biodegradable, nontoxic nanoparticle by using a low methoxy pectin and zein with an average size most appropriate for drug delivery. The developed nanoparticle was encapsulated with a model drug quercetin. It was subjected to different characterization studies and confirmed the incorporation of all the components.

Materials & Methods

Zein was purchased from Sigma Aldrich Co, U.S.A. Pectin was isolated from locally available fruits of *Coccinia indica* (Ivy Gourd) by the modified method Mohamadzadeh *et.al* [13]. All other chemicals required for the study was purchased from Merck India Ltd.

Estimation of Degree of esterification

Degree of esterification of isolated pectin was calculated by estimating total uronic acid content and by HPLC method [14].

Preparation of nanozein

The nanozein was prepared by the modified method of Nicholas *et. a/* [5]

Preparation of drug encapsulated core-shell nanopectin

The drug encapsulated core-shell nanopectin was prepared by the modified method of Nicholas *et. a/* and Vinicius *et.a/* [3,5]. To 0.01% silicone oil under sonication, Zein and Quercetin (model drug) solution in 80% ethanol was added and sonicated until a single phase was formed. Pectin solution (1.04mg/ml) was added drop wise during sonication at a flow rate of 2.5ml/ min and continued sonication for 5 minutes. The solution was then freezed, lyophilized and stored.

Characterization of nanozein and drug encapsulated core-shell nanopectin

Morphology of nanozein and drug loaded core-shell nanopectin was investigated by Hitachi SU 6600 variable Pressure field Emission Scanning Microscope. The nanoparticle surfaces were vacuum sputter coated with gold for allowing the SEM visualization. The diameters of nanoparticles were determined.

UV- Visible absorption spectra of the lyophilized samples of drug encapsulated zein nanoparticle, drug encapsulated zein-pectin nanoparticle, pure zein, pectin and quercetin (model drug) were recorded in the wavelength in the range of 200-500 nm with Shimadzu spectrophotometer by using water as reference standard and 80% ethanol and water as solvents. The chemical structure of zein, pectin, quercetin, quercetin/zein nanoparticle, quercetin/zein/pectin nanoparticle was observed by FT-IR spectroscopy (Shimadzu).

Results and Discussion

Degree of esterification of pectin from *Coccinia indica* shows 1.9% and 0 .062% of degree of methylation and degree of acetylation respectively and confirms that it is low methoxy pectin. It has been reported earlier that low methoxy pectin enhances the drug encapsulation. Hence pectin from *Coccinia indica* can be used for drug encapsulation.

Morphological observation

Morphological observation of zein nanoparticles and drug encapsulated zein- pectin nanoparticle by Scanning Electron Microscope are shown in figure 1 & 2. Zein nanoparticles formed smooth surfaced nanospheres with an average diameter of approximately100nm. However the size ranged from 54nm to 300nm. More homogenous and smaller nanospheres than zein nanospheres were formed when pectin solution was added. The approximate size was 93nm and the size ranged from 56nm to178nm. The reduced size of nanopectin may be due to the electrostatic interactions existing between quercetin/zein and pectin particles.

The zein nanoparticle and drug encapsulated zein –pectin nanoparticle was characterized by means of UV- Visible and Fourier Transform Infra Red spectroscopic analysis. The analysis shows that the drug has been incorporated in the zein-pectin nanoparticle.

Overlay spectrum (Fig.3) shows UV-Vis spectra of the zein, quercetin, nanozein, drug loaded zein-pectin nanoparticle (80% ehanol as solvent), pectin and drug loaded zein-pectin nanoparticle (water as solvent). From the UV- Visible spectrum it can be observed that λ_{max} of pure pectin matches with the λ_{max} of drug loaded core shell – nanopectin (water as solvent for both) which confirms the presence of pectin in the drug loaded zein-pectin nanoparticle. λ_{max}'s of all other components were not visible since those are insoluble in water. When 80% ethanol was taken as solvent, λ_{max}'s of pure zein and quercetin was also seen in drug loaded nanozein in which zein and quercetin are incorporated and in drug loaded zein-pectin nanoparticle in which pectin, zein and quercetin was incorporated. From these data it can be assumed that core- shell nanopectin is formed and drug has been incorporated in the nanoparticle.

FT-IR spectrum of drug loaded zein-pectin nanoparticle (Fig.7) shows very prominent peaks at 3300, 1650, 1540 and 1450 cm^{-1}. The peak at 3300 cm^{-1} which is assigned to – OH is found to be shifted from 3400 cm^{-1} range in pectin, zein and quercetin, this may be due to interactions of –OH groups among them. The peak at 1650 cm^{-1} may be due to the C-C multiple bonds stretching in tetra substituted alkene shifted towards a higher wave number. The peaks at 1540 and 1510 cm^{-1} may be assigned to – NH bending vibrations which is seen in pectin and zein as well. Another peak at 1450 cm^{-1} can be due to C-C multiple bonds stretching of aromatic compounds which show the presence of quercetin in zein-pectin nanoparticle. A new band at 1748 cm-1 assigned to C=O of –COOH confirms the presence of pectin in the nanoparticle developed. Another band observed at 1300 cm-1 which can be assigned to OH bending and C-O stretching vibrations of phenols confirms the presence of the model drug quercetin.

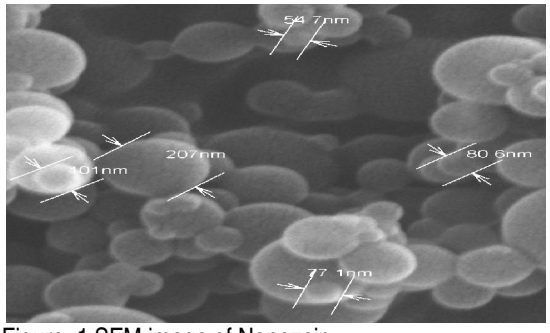

Figure: 1 SEM image of Nanozein

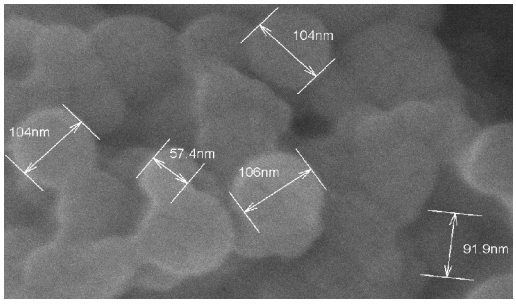

Figure: 2 SEM image of drug encapsulated zein – pectin nanoparticle

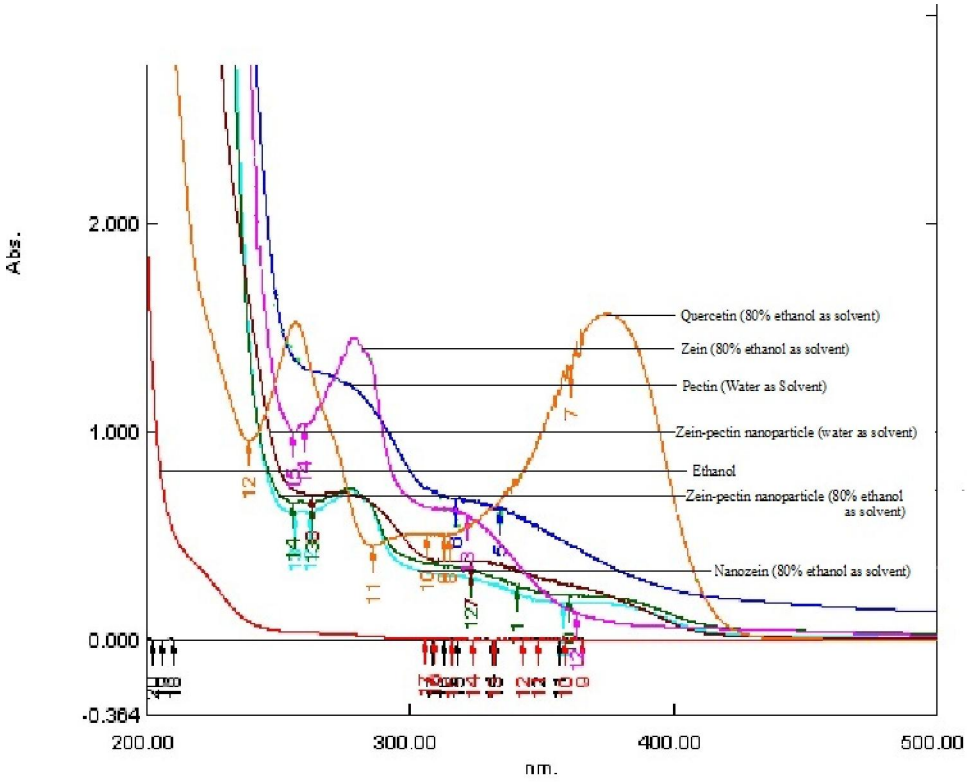

Figure: 3 Overlay spectrum of zein, Quercetin, pectin, nanozein and zein-pectin nanoparticle

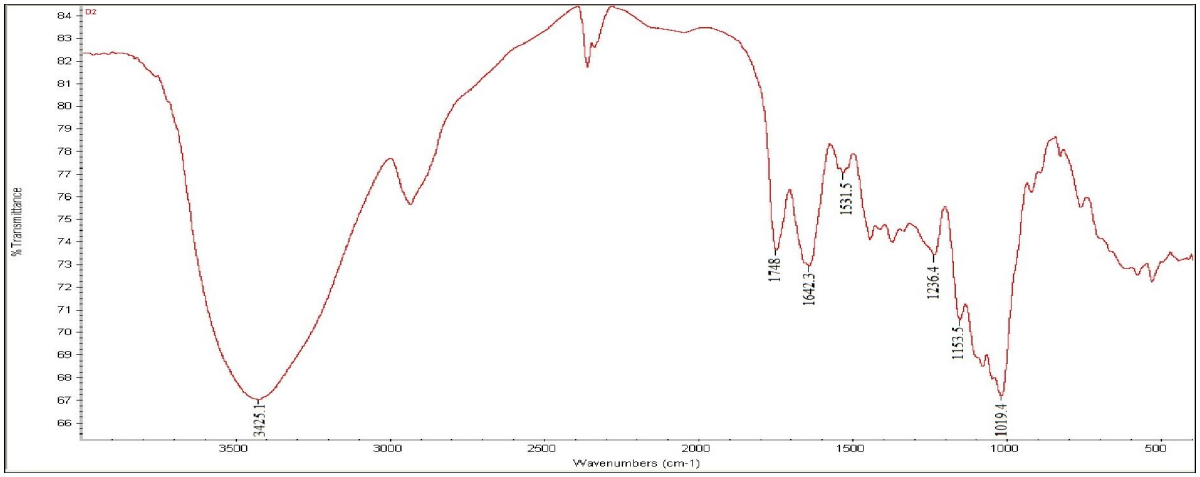

Figure: 4 FT- IR Spectrum of Pectin from *Coccinia indica*

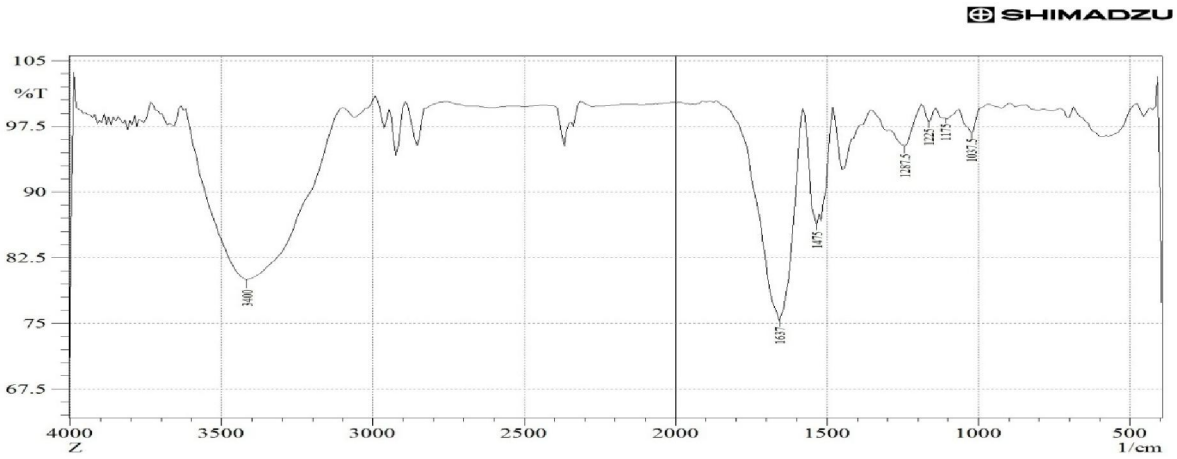

Figure: 5 FT-IR spectrum of Zein

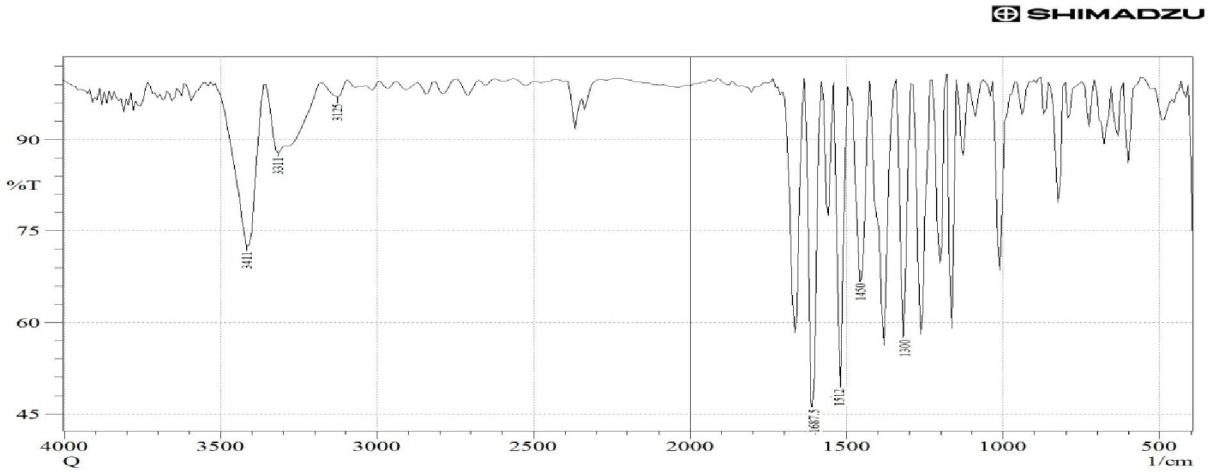

Figure: 6 FT-IR spectrum of Quercetin

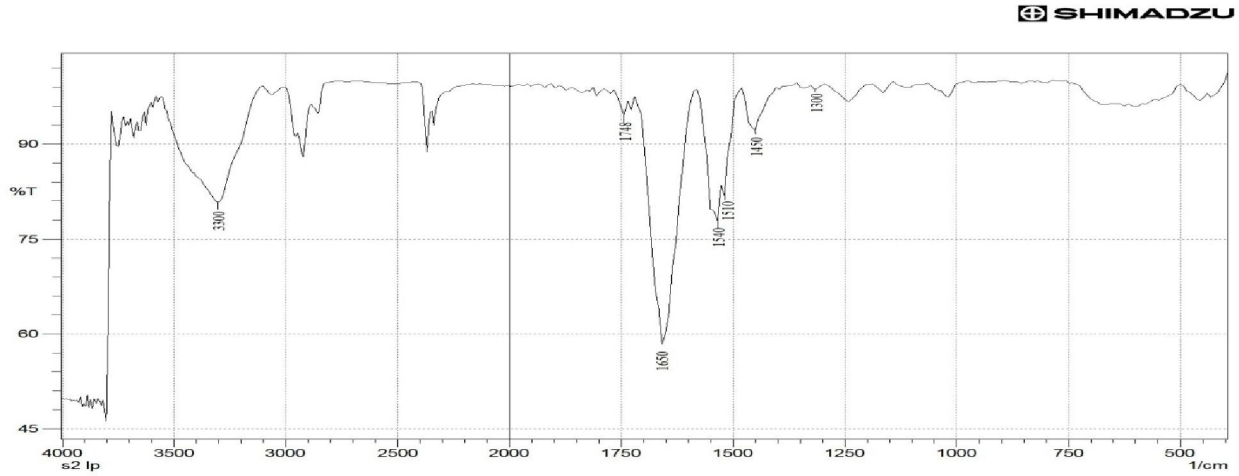

Figure: 7 FT-IR spectrum of Drug loaded zein – pectin nanoparticle

FT-IR spectrum of drug loaded zein-pectin nanoparticle (Fig.7) shows very prominent peaks at 3300, 1650, 1540 and 1450 cm^{-1}. The peak at 3300 cm^{-1} which is assigned to – OH is found to be shifted from 3400 cm^{-1} range in pectin, zein and quercetin, this may be due to interactions of –OH groups among them. The peak at 1650 cm^{-1} may be due to the C-C multiple bonds stretching in tetra substituted alkene shifted towards a higher wave number. The peaks at 1540 and 1510 cm^{-1} may be assigned to – NH bending vibrations which is seen in pectin and zein as well. Another peak at 1450 cm^{-1} can be due to C-C multiple bonds stretching of aromatic compounds which show the presence of quercetin in zein-pectin nanoparticle. A new band at 1748 cm^{-1} assigned to C=O of –COOH confirms the presence of pectin in the nanoparticle developed. Another band observed at 1300 cm^{-1} which can be assigned to OH bending and C-O stretching vibrations of phenols confirms the presence of the model drug quercetin was taken as solvent, λ_{max}'s of pure zein and quercetin was also seen in drug loaded nanozein in which zein and quercetin are incorporated and in drug loaded zein-pectin nanoparticle in which pectin, zein and quercetin was incorporated. From these data it can be assumed that core- shell nanopectin is formed and drug has been incorporated in the nanoparticle.

FT–IR Spectroscopy

Figures 4-7 shows the FT-IR spectra of pectin from *Coccinia indica*, zein, quercetin (model drug), and drug loaded zein – pectin nanoparticle respectively.

FT – IR spectrum of pectin (Fig. 4) exhibits a broad intense peak at 3425.1 cm^{-1} which may be assigned to O-H vibrational stretching. An intense peak at 1748 cm^{-1} is due to C=O of –COOH and the peak at 1642 cm^{-1} may be due to C-C multiple bond stretching. Another peak at 1531.5 cm^{-1} is due to N-H bending vibrations. The peaks 1236.4 cm^{-1} and 1153.5 cm^{-1} are assigned to O-H bending and C-O stretching of 1^0 and 2^0 alcohols respectively. The peak seen at 1019.4 cm^{-1} may be due to the aliphatic C- N vibration.

FT – IR spectrum of zein (Fig. 5) exhibits an -OH peak at 3400 cm^{-1} and another peak at 1637 cm^{-1} may be due to C-C multiple bond stretching. The peak at 1475 cm^{-1} is due to N-H bending vibrations of amine salts. Less intense peaks for -OH bending and stretching vibrations of 1^0, 2^0 and 3^0 alcohol are observed at 1287.5 cm^{-1} to1175 cm^{-1}. Two bands at 1225 cm^{-1} and 1037.5 cm^{-1} may be assigned to C- N vibrations of aliphatic and aromatic moieties.

FT-IR spectrum of quercetin (Fig. 6) shows intense peaks at 3411 cm^{-1} and 3311 cm^{-1} which may be due to the presence of –OH group. Another peak observed at 3125 cm^{-1} may be assigned to -OH stretching of the chelate compounds due to the polymeric association. A peak observed at 1687.5 cm^{-1} may be due to the ketone stretching. The peaks observed at 1512 cm^{-1} and 1450 cm^{-1} are due to the presence of aromatic C-C multiple bond stretching. The peak observed at 1300 cm^{-1} may be assigned to OH bending and C-O stretching vibrations of phenols.

Overlay spectrum (Fig.3) shows UV-Vis spectra of the zein, quercetin, nanozein, drug loaded zein-pectin nanoparticle (80% ehanol as solvent), pectin and drug loaded zein-pectin nanoparticle (water as solvent). From the UV- Visible spectrum it can be observed that λ_{max} of pure pectin matches with the λ_{max} of drug loaded core shell – nanopectin (water as solvent for both) which confirms the presence of pectin in the drug loaded zein-pectin nanoparticle. λ_{max}'s of all other components were not visible since those are insoluble in water. When 80% ethanol

Conclusion

A natural core-shell nanoparticle comprising of zein and pectin was successfully developed and the model drug quercetin was encapsulated within.

From overall results of morphological and physical characterization, it can be concluded that the zein-pectin nanoparticle developed can be used as a drug carrier. A further study on temperature stability, drug release and drug targeting is under progress.

Author's Contributions

DAT carried out extraction of pectin from *Coccinia indica*, estimation of degree of esterification, preparation of nanozein and zein – pectin nanoparticle and preparations for SEM, UV-Visible, FT-IR studies etc.SS guided the study by designing and coordinating the work and helped in drafting the manuscript.

HKR participated in interpreting the FT – IR spectral data.

DN performed UV- Visible analysis of the samples and interpreted the overlay spectrum

Conflict of Interest

There is no conflict of interest

Acknowledgment

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