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ACYLATED CHITOSAN: A PROMISING CARRIER IN NOVEL DRUG DELIVERY OF ALBENDAZOLE

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ABSTRACT

Biodegradable and biocompatible polymers are suitable for human use and can be prepared into particles of various sizes. Chitosan is a positively charged natural biodegradable and biocompatible polymer. It is a linear polysaccharide consisting of h-1,4 linked monomers of glucosamine and N-acetylglucosamine. Chitosan possesses one reactive amino at the C-2 position of the glucosamine residue, and these amines confer important functional properties to chitosan which can be exploited for biofabrication to generate various chemically modified derivatives and explore their potential for pharmaceutical field..The present study describes the N-acylation of chitosan with succinic anhydried and fatty acids In present work, using modified chitosan for the formation of acylated chitosan nanoparticles by ionic crosslinking method by using sodium tripolyphosphate as cross linking agent and albendazole is selects as a drug molecule. The structure acylated chitosans was examined by Fourier-transform infrared (FT-IR), and X-ray diffraction analysis, and the data compared to those of native chitosan. Therefore, the major goal of the present study is to create a kind of new biodegradable nanoparticle as novel drug delivery system. The nanoparticles have been characterized in terms of mophology, drug loading efficiency. Also in vitro release was investigated to determine the efficacy of this system.

Key words: Nanopartical,chitosan, albendazole, acylation, crosslinking, in-vitro release

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INTRODUCTION

The colloidal drug delivery systems are capable of carrying high drug load and of controlling the drug release in a predictable manner and provide site specific and targeted drug delivery. Injectable, colloidal drug delivery systems especially the nanoparticles have gained much interest during

last few years as they improve the distribution of drugs in the body because of their enhanced efficiency against tumors and reduced toxicity. The submicron size of nanoparticles offers numerous advantages over microparticles. Nanoparticles have relatively higher intracellular uptake compared to microparticles. In the last decade, significant effort has been made to develop nanoparticles for drug delivery. Considerable research has been directed towards developing safe and efficient chitosan based particulate drug delivery systems such as nanoparticles. Nanoparticles are thought to enter cells via an endocytic pathway through either specific or nonspecific interaction with cell membrane.

Chitosan is derived from chitin and is prepared by deacetylation of chitin For preparation of chitosan, main source are the crustacean shell waste such as crab,shrimp, lobster and crawfish. The shells contain approximately 30-40% protein, 30-50% calcium carbonate, and 20-30% percent chitin on dry basis. The isolation of chitosan from these wastes involves four traditional steps, viz. demineralization (DM), deproteination (DP), decoloration (DC) and deacetylation (DA).There are numerous reports highlighting the low toxicity and biocompatibility of chitosan. physical and chemical properties of chitosan depend mainly on its molecular weight and degree of deacetylation.As a natural product, chitosan is a renewable pharmaceutic adjuvant with good biocompatibility. Chitosan and its derivatives have strong potential for application as drug carriers.

In the present research work chitosan derivatives were synthesized by N acylation with longer chain fatty acids (namely, Lauric, Palmitic acid and succinic anhydride) to prepare derivatives possessing increased hydrophobic/hydrophilic character (as assessed by their solubility studies). The synthesized derivatives were studied for their physicochemical characteristics and they were used as nanoparticle prepration as novel drug delivery system . The effect of acylation on chitosan leads to increased hydrophobic character which is reflected as prolongation in the release of drug to varying degrees as compared to parent. We report the formation of N-acylchitosan nanoparticles by ionic crosslinking. The structure of these derivatized chitosans was examined by Fourier-transform infrared (FT-IR), and X-ray diffrac-tion analysis, and the data compared to those of native chitosan. Therefore, the major goal of the present study is to create a kind of new biodegradable nanoparticle as novel drug delivery system. The nanoparticles have been characterized in terms of mophology, drug loading efficiency. Also in vitro release was investigated to determine the efficacy of this system.

MATERIAL AND METHOD

MATERIAL

Albendazole was received as a gift sample from alpa lab (indore).chitosan was procured from SDFCL (Mumbai).All other reagent like(Ethanol, acetone methanol NaOH, NaCL, Thinoyl chloride,palmitic and lauric acid, succinic anhydride ,DMF, Pyridine etc.) were of analytical grade and procured from MERCK(Mumbai) and SDFCL(Mumbai)

METHOD

Modification of chitosan

Synthesis of lauryl and palmitoyl chitosan

Synthesis of acyl chitosan is require 2 step

1. Preparation acyl Chloride As Intermediate For acyl Chitosan Synthesis
2. Synthesis Of acyl Chitosan By Reacting acyl Chloride With Chitosan

Succinyl chitosan

One gram chitosan was dissolved into 200 ml of 1% w/v acetic acid solution and then transferred into a flask. Succinic anhydride (0.2 g) was dissolved in acetone (20 ml), and added into the flask by drop-wise for 30 min at room temperature, and then the reaction was allowed for 4 h at 40 °C. The reaction mixture was cooled to room temperature. The mixture precipitated in an excess of acetone, filtered to remove the solvent and then washed with acetone, respectively. Finally, the product was dried at 40 °C under vacuum for 4 hr

Nanoparticle preparation

Chitosan solutions of 0.25% (w/v) were prepared in 1%acetic acid solution. Tripolyphosphate (TPP)

solution(1.0%) was added dropwise to 20 ml of chitosan solution with magnetic stirring, followed by sonication. The resulting chitosan particle suspension was subsequently centrifuged at 15,000 rpm for 10 min. The precipitated particles were resuspended in DI water with sonication, centrifuged again and then freeze dried. The freeze-dried chitosan nanoparticles were then resuspended in a saline solution.

Characterization Of Acylated Chitosan Nanoparticle

Drug Loading Efficiency

The amount of albendazole entrapment in the nanoparticles was calculated by the difference between the total amount of drug added to the nanoparticles and the amount of non entrapped drug remaining in the aqueous supernatant. The latter was determined following the separation of drug loaded nanoparticles from the aqueous medium by centrifugation at 5000 rpm for 30 min. The supernatant was collected and the particles were washed with water and then subjected to another cycle of centrifugation.1 The amount of free albendazole in the supernatant was determined by UV-Visible spectrophotometer

In-vitro drug release

After separation of the free drug, the nanoparticle preparation was transferred to a dialysis tube and subjected to dialysis with the dialysis tube immersed in a phosphate buffer saline pH 7.4 (100 ml). At different time intervals, samples were withdrawn from the receptor compartment and the drug content was determined spec-trophotometrically at 294 nm. An equal volume of phosphate buffer saline replaced the samples that were withdrawn.

Scanning Electron Microscopy

The surface morphology of chitosan was visualized by scanning electron microscopy (SEM).The sample for SEM was prepared by lightly sprinkling the powder of chitosan and derivatives on a double adhesive tape, which stuck on a metal stub. The powder was observed at an excitation voltage of 20kv on different magnifications using JEOL JSM 5600 Scanning electron microscop

RESULT

Chitosan Derivatives Characterization

Solubility studies

The final product of each derivative was checked for its solubility in different solvent like Water, Dimethylsulphoxide (DMSO), Dimethylformamide (DMF), Pyridine, Acetone, and Hydrochloric acid(0.1M HCl), Sodium Hydroxide(0.1M NaOH). The results observed from the solubility study are shown in the table below:

Table 1: Comparison Of Chitosan And Acyl Chitosan Solubility

Chitosan Derivative	Water	DMSO	DMF	Pyridine	Acetone	0.1M HCl	0.1M NaOH
Chitosan	--	--	--	--	--	+	--
Lauryl chitosan	__	+	__	+	p.s.	__	__
Palmitoyl chitosan	__	+	__	+	p.s.	__	__
Succinyl chitosan	+	__	__	__	__	__	__

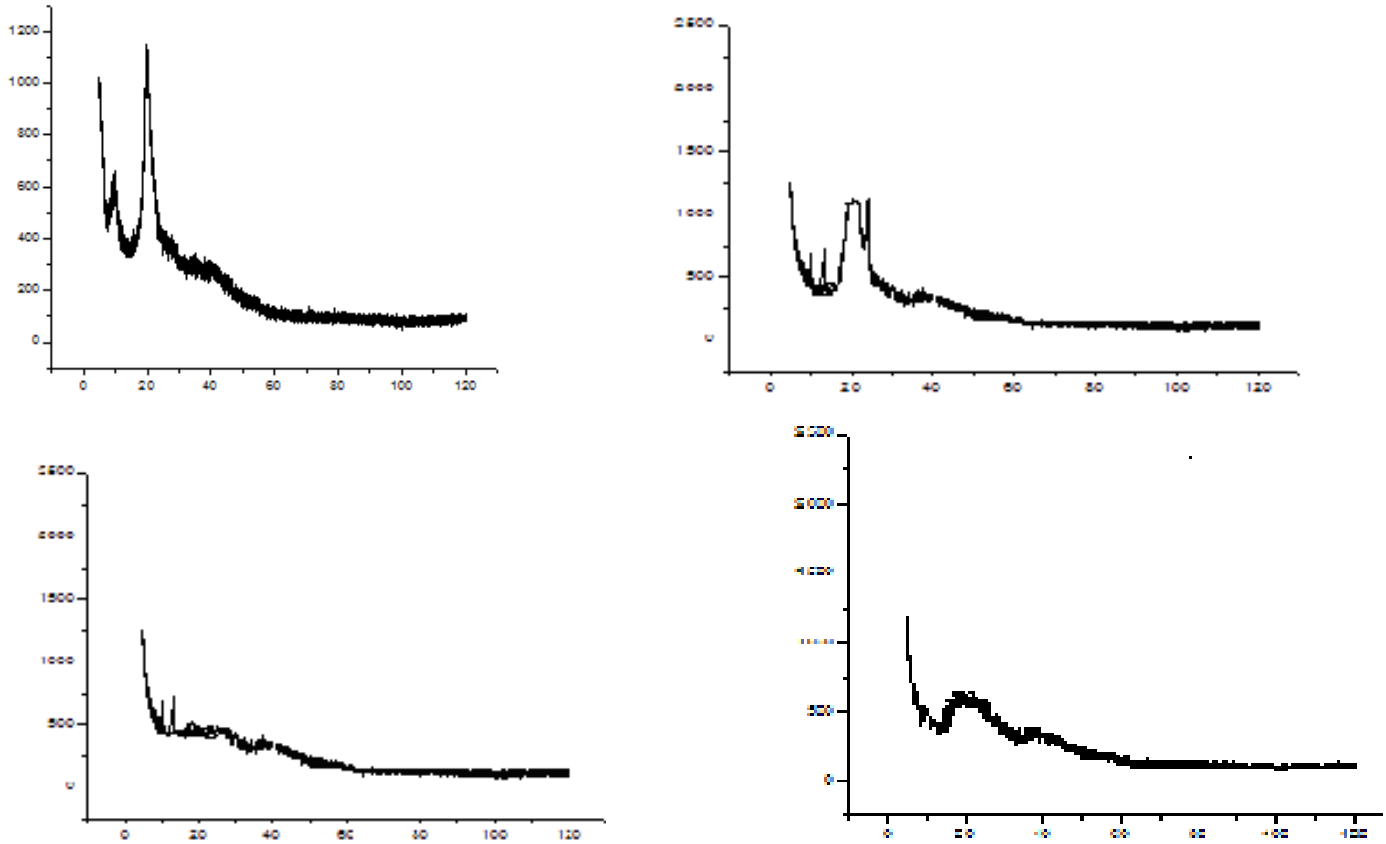
FTIR Analysis Of Chitosan Derivatives:

Structural changes of chitosan and its derivatives were confirmed by FTIR. The FTIRspectrum of chitosan and synthesized chitosan derivatives were recorded by Perkin Elmer 2400 Spectrophotometer

Table 2: Interpretation Of Chitosan And Acyl Chitosan Spectra

S. No.	Compounds	Functional group	peak
1	Chitosan	$-NH_{(bend)}$ -OH -CH2 -C-O	1560 cm^{-1} 3447 cm^{-1} 2877cm^{-1} 1027cm^{-1}
2	Lauryl Chitosan	-NH $-CH_2$ -C=0	-------- 2916 cm^{-1} 1633 cm^{-1}
3	Palmitoyl Chitosan	$-NH_{bend}$ $-CH_{2strch}$ -C=O	-------- 2930 cm^{-1}, 1638 cm^{-1}
4	Succinyl Chitosan	-NH CH_{2Strch} Amide I Amide II	------- 2917 cm^{-1} 1656 cm^{-1} 1380cm^{-1}

➢ X- Ray differaction analysis



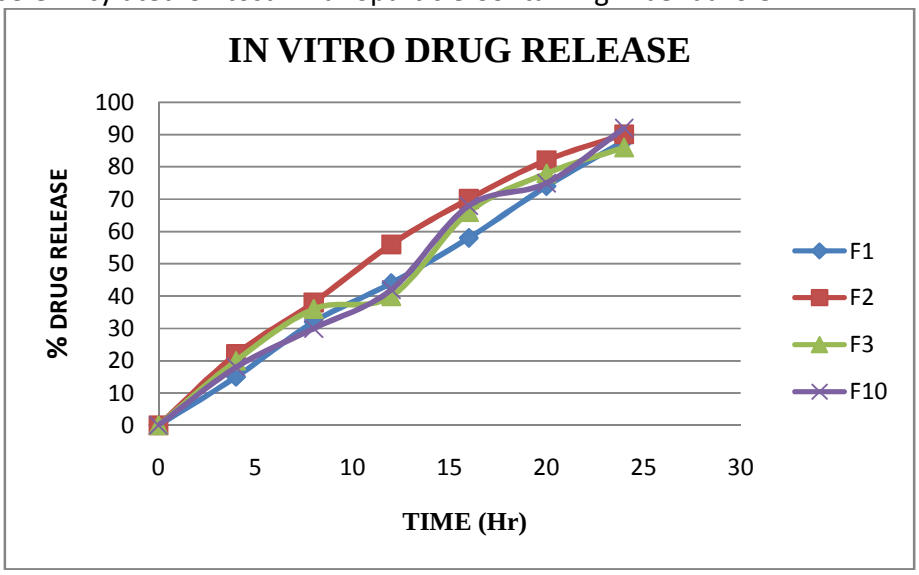
(Fig.1 Xrd graph of chitosan, lauryl chitosan, palmitoyl chitosan,and succinylchitosan)

Drug Loading Efficiency

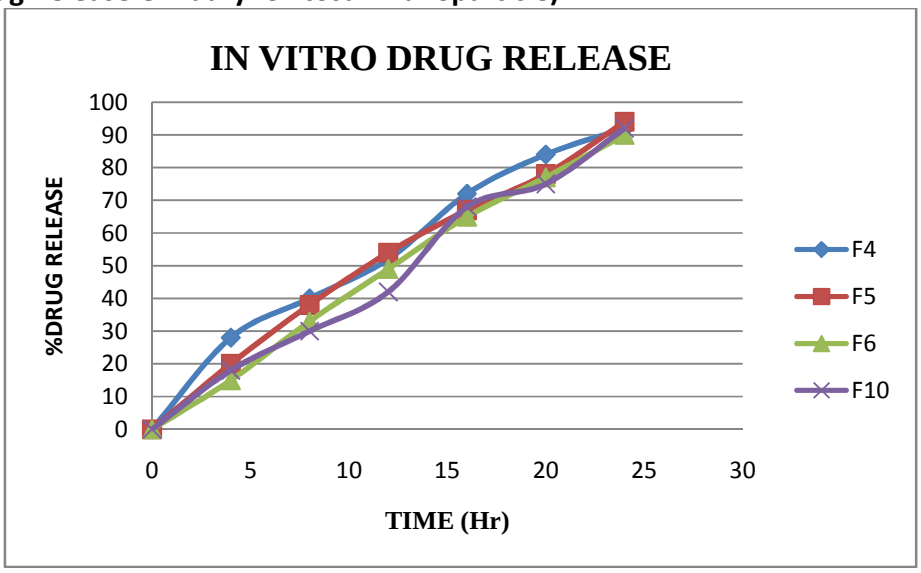
Table3: Drug Loading Efficiency

formulation	FN (LC)	FN2 (LC)	FN3 (LC)	FN4 (PC)	FN5 (PC)	FN6 (PC)	FN7 (SC)	FN8 (SC)	FN9 (SC)	FN10 (C)
% Drug loading	75	80	78	86	90	82	76	86	84	70

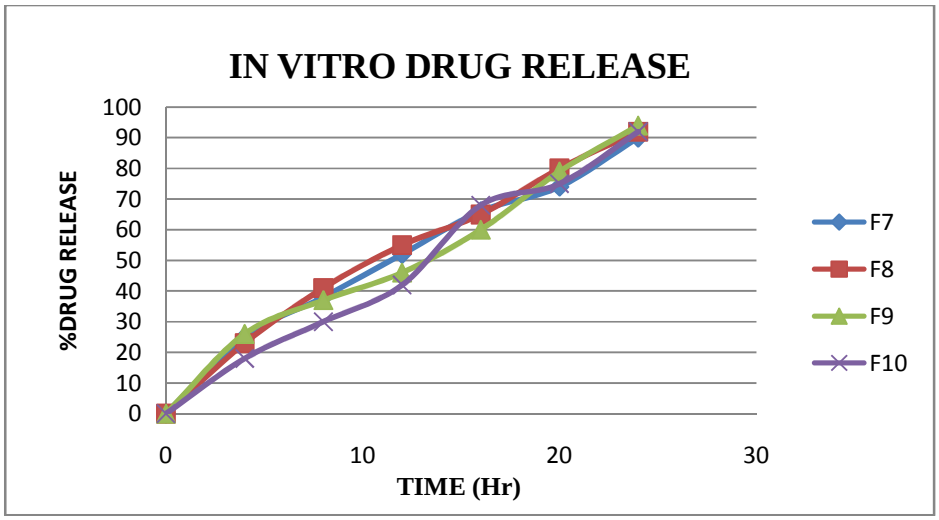
In Vitro Drug Release Of Acylated Chitosan Nanoparticle Containing Albendazole



(Fig 2. In Vitro Drug Release Of Lauryl Chitosan Nanoparticle)

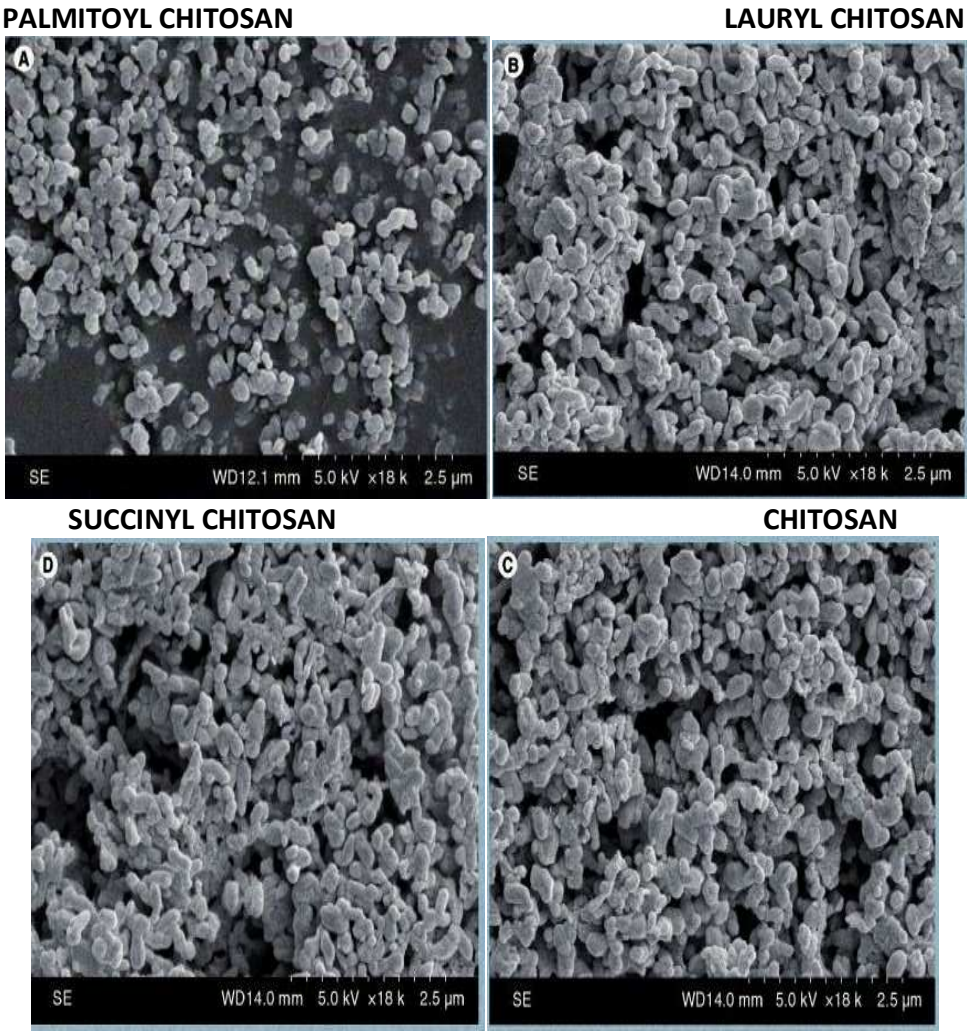


(Fig 3.In Vitro Drug Release Of palmitoyl Chitosan Nanoparticle)



(Fig4. In Vitro Drug Release Of Succinyl Chitosan Nanoparticle)

Scanning Electron Microscopy Of Chitosan And Acylated Chitosan Nanoparticle



(Fig 5. SEM Of Chitosan And Acylated Chitosan Nanoparticle)

DISCUSSION

Solubility study

Chitosan is soluble in hydrochloric acid and insoluble in water and organic solvent

Laury land palmitoyl chitosan is soluble in organic solvents but chitosan insoluble

Succinyl chitosan is water soluble it shows that after acylation chitosan solubility is changed

FTIR Analysis

In concerned with chitosan, peak at 2917 cm^{-1} appears due to streching of -CH_2- , the peak at 1586 cm^{-1} diappeared greatly .the peak at 1656 cm^{-1} Amide I, and 1380 cm^{-1} Amide III increases, these result confirmed that succinylation took place at N-position of chitosan

Peak at 1586 cm^{-1} disappeared, peak at 1633.16 cm^{-1} corresponds to C=0 stretching of amide, peak in area 2850 –2950 cm^{-1} (C-H for hydrocarbons) increased and observed at 2850 cm^{-1}, 2916.3 cm^{-1}, thus suggest the formation of lauryl chitosan

Peak at 1586 cm^{-1} disappeared, peak at 1638.52 cm^{-1} corresponds to C=0 stretching of amide, peak in area 2850 –2950 cm^{-1} (C-H for hydrocarbons) increased and observed at2850 cm^{-1}, 2920.03 cm^{-1}, thus suggest the formation of palmitoyl chitosan

X ray diffraction

X-ray diffraction pattern were recorded for chitosan and its derivatives using the same Instrumentation and procedure as used for chitosan. When compared with the diffraction pattern of acylated chitosan it was observed that, the chitosan shows two distinct peaks, which actually are two distinct crystal form I and II. As per Samuel these two are orthorhombic form of crystals .The strongest reflections falls at 2Θ =20.81°. These reflections are the typical crystalline peak of chitosan.

In case of succinyl chitosan one broader peak at 2Θ = 20° is diappear suggest that crysallinity of chitosan destroyed that's why succinyl chitosan is water soluble .

In case of lauryl and palmitoyl chitosan crystallinity structure was not destroyed and but more crystalline form is observed. However for acylated chitosan peak becomes sharper and it shows only a single peak at around 15.64° suggesting a more crystalline and stable structure which could be due to loss in hydrogen bonding .Palmitoyl chitosan shows more crystalline as they are more stabilized by hydrophobic interaction

Nanoparticle characterization

Drug loading efficiency

Loading efficiency of all 10 formulations is done result show that Loading efficiency of drug increases with increasing drug concentration after saturation point loading efficiency decreases.

In-vitro drug release

In-vitro release of drug from chitosan and acyl chitosan nanoparticle show that f2 ,f5 and f8 is best formulation and show linear drug release as compared to native chitosan nanopaticle

Scanning electron anaysis

Electron microscopy analysis confirmed the presence of nanoparticles and provided morphological information of the typical albendazole loaded CS/LC,PC,SC nanoparticles. With the scanning electron microscope, particles were seen to be spherical, aggregates and regular.palmitoyl chitosan nanoparticle seen less aggregated due to hydrophobic interaction as compared to native chitosan

CONCLUSION:

Chitosan is natural biodegradable , non toxic and biocompatible polymer but due to its limited physiochemical properties it require derivatization.

Acylation of chitosan modified its physio chemical properties which increases its utility.

Acylated chitosan is more stable derivative and require simple experimental condition so acylated chitosan is prepare easily.

Acylated chitosan is play important role in novel drug delivery due to its biocompatibility and biodegradability.

The modified chitosan is characterize by SEM analysis ,FTIR ,XRD and solubility gives valuable result

After characterization the acylated chitosan is use to prepare nanoparticle as a novel carrier.

Acylated chitosan Nanopaticle is prepared by cross linking method by simply addition of sodium tri-polyphosphate as cross linking agent.

Nanoparticle of acylated chitosan more stable than native chitosan and also is blood compatible and biodegradable

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