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Preparation and Characterization of Naringin Loaded Chitosan Nanoparticles

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A B S T R A C T

The present study involves the formulation, optimisation and evaluation of naringin loaded chitosan nanoparticles by ionotropic gelation method using sodium tripolyphosphate as ionic cross-linker. Five nanoparticulate formulations of nanoparticles were prepared and evaluated for various parameters like particle size, zeta potential, % yield,% drug content,% entrapment efficiency, surface morphology, drug-polymer compatibility studies include (FTIR,DSC) and in vitro dissolution studies. The particle size and zeta potential of optimised formulation was found to be 352.4 nm, -31.5 mV respectively. SEM analysis revealed that the particles were of spherical in shape having smooth surface. The drug-polymer compatibility studies depicted that there was no interaction of between drug and polymer. The % entrapment efficiency was in the range of 47.4% to 69.7 %. In vitro dissolution studies showed highest % release for optimised formulation (92.0%) in 24 hrs. Kinetic modelling revealed that the drug release followed first order kinetics. The results obtained from this study proved that chitosan nanoparticles are potential candidates for drug delivery of naringin with enhanced bioavailability, safety and efficacy.

Keywords: Naringin, Chitosan, Sodium tripolyphosphate, Nanoparticles, Ionic gelation method.

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1. Introduction

Nanotechnology based oral drug delivery system provide an alternative strategy to administer drugs with improved bioavailability and therapeutic effect. Nanotechnology is a promising strategy in the development of drug delivery systems especially for those potent drugs whose clinical development failed due to their poor solubility, low permeability, inadequate bioavailability and other poor biopharmaceutical properties [1,2]. The use of nanoparticles for the development of new drug delivery system seems to be very promising. Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. By highlighting the chemical, physical and

biological processes associated with nanoparticles ranging from molecular size to 100 nm which exhibit improved properties and have novel applications due to their size [3]. Nanoparticles can enhance drug absorption by optimising their interaction with the absorption site in the GI tract walls or directly transporting these drugs through the intestinal mucosa to systemic circulation4. Nanoparticles are absorbed by different mechanisms but endocytosis is the most significant contributor to the cell entry.

2. Materials and methods

Materials

Naringin was purchased from Himedia. Enteric capsules were provided as gift sample from Nature Capsules Limited Bengaluru. Sodium tripolyphophate was purchased from Sigma Aldrich, chitosan was purchased from Himedia. All the other chemicals were of analytical grade procured from Himedia and Merck. Double distilled water was used throughout the study.

Methods

Preparation of drug loaded nanoparticles:

The chitosan nanoparticles (CH-NPs) were prepared according to the technique first reported by (Calvo et al., 1997) with suitable modification, based on the ionotropic gelation of chitosan (CH) with sodium tripolyphosphate (TPP) . Briefly, TPP aqueous solution was added to CH solution (in 1.5% v/v acetic acid) kept in a beaker. The solution was stirred for 30 min at 500 rpm with the help of magnetic stirrer (Remi Instruments, India) at room temperature. The CH to TPP ratio was varied in order to optimize the particle size and drug entrapment efficiency. The drug solution was added in to the CH solution in order to get drug loaded CH-NPs. Free unentrapped drug was removed by ultracentrifugation (Hitachi, Japan) at 12,000 rpm for 30 min. Supernatant of dispersion was discarded and NPs were washed with distilled water at least 3 times for complete removal of free drug. The naringin loaded CH-NPs were then dispersed in to distilled water, lyophilized and preserved till further use.

Characterization of nanoparticles

Particle size distribution:

The particle size of the nanoparticles was measured by Horiba scientific instruments(particle size analyser). Samples were prepared by diluting nanoparticles with sufficient amount of water in concentration of 0.0001% to 0.1%.The average particle size was determined by Laser Diffractometer .

Zeta potential:

The zeta potential was measured using Laser Doppler Micro electrophoresis (Horiba Scientific).

Scanning electron microscopy: (SEM)

The morphology and surface of nanoparticles were observed using SEM(S-3700).The samples of freeze dried nanoparticles were dispersed on a glass slide, and kept under a vaccum.The samples were coated with a thin gold/palladium layer using a sputter coat unit.

Percentage yield:

The percentage yield can be determined by calculating initial weight of raw materials and final weight of nanoparticles.

Percentage yield = Practical yield/Theoretical yield X 100.

In case of ACT1 to ACT5 ,

Theoretical yield =Total solids weight = Weight of naringin (drug)+ Weight of chitosan (polymer) +Weight of STPP (cross-linker)

Drug content:

The drug content in each formulation was determined by weighing nanoparticles equivalent to 40 mg of naringin and dissolving in 100 ml of phosphate buffer followed by stirring .The solution was filtered through 0.45µ membrane filter diluted suitably and absorbance of resultant solution was measured spectrophotometric ally at 292 nm using pH 6.8 phosphate buffer as blank. The drug content of prepared NPs was determined by the formula.

Drug content% = Weight of drug in nanoparticles/ Weight of nanoparticles X 100

Drug entrapment efficiency:

Also known as association efficiency. The drug loaded NPs were centrifuged at a high speed of 15000 rpm at -4ºC for about 30 min and supernatant was assayed for non bound drug concentration by UV spectrophotometer

Entrapment efficiency% = Bound drug- unbound drug/ Bound drug X 100

Drug-polymer compatibility studies

i) Differential scanning calorimetry (DSC):

The differential scanning calorimetry thermograms were recorded using differencial scanning calorimeter (DSC; Mettler Toledo,Japan).Indium standard were used to calibrate the DSC temperature and enthalpy scale.Approximately 2-5 mg of each sample was heated in a pierced aluminium pan from 30ºC to 300ºC at a heating rate of 10ºC/min under a stream of nitrogen at a flow rate of 50 ml/min. Thermal data analyses of the DSC thermogram were conducted using STARe software(version 5.21).

ii) Fourier transforms infrared spectroscopy (FT-IR):

The FT-IR spectra of naringin, Chitosan, Sodium tripolyphosphate and optimised formulation were recorded using Fourier transform infrared spectrophotometer (Bruker Alpha-T, Switzerland) to investigate any interaction between naringin and polymers in formulated nanoparticles.The samples were grounded with KBr and pressed into a disk shape for measurement. The prepared pellets were scanned over a frequency range of 4000-400 cm-1

In vitro drug release studies

The release of drug from the capsule filled with optimized nanoparticles was studied by using USP dissolution apparatus I (Basket type). Nanoparticles (equivalent to 40 mg of naringin) taken in a enteric capsule and the test was performed using 900 ml of 0.1 N HCl at 37± 0.5°C and 100 RPM for first 2 h. then replaced with 6.8 pH phosphate buffer and continued for 24 h. Aliquot volume of 5 ml was withdrawn at regular intervals and replaced with fresh buffer diluted.The samples were replaced with fresh dissolution medium. After filtration, the amount of drug release was determined from the standard calibration curve of pure drug.

At specified time intervals (0,0.5, 1, 2, 3, 4, 6, 8, 10, 12, 18 and 24 hrs) 5 ml aliquot of each sample was withdrawn and replaced by an equal volume of the release medium.

Samples were filtered and amounts of drug released were determined spectrophotometric ally at wavelength of 292 nm. The data were presented as mean ± SD of at least triplicates.

Kinetic modelling of drug release profiles

The drug release data were subjected to zero order, first order, Higuchi model (Higuchi et al., 1963), Korsmeyer model and Peppas model for analyzing the mechanism of drug release and release kinetics from the dosage form using MS Excel 2007. The model with the highest correlation coefficient was considered to be the best fitting one (Dorozynski et al., 2004).

Zero-order release kinetics:

Zero-order release kinetics, cumulative amount of drug released vs time and the release rate data are fitted to the following equation:

$$C = K0 .t$$

First–order release kinetics:

First-order release kinetics, log cumulative percentage of drug remaining Vs time and the release rate data are fitted to the following equation:

$$C = 100\times(1 - e\text{-}Kt)$$

Higuchi release model:

The Higuchi release, cumulative percentage of drug released vs square root of time and the release rate data are fitted to the following equation: $Q = Kt1/2$ Where, K is the constant reflecting the design variables of the system and t is the time in hours. Hence, drug release rate is proportional to the reciprocal of the square root of time (Hixson et al., 1931).

Mechanism of drug release:

The drug release were plotted in Korsmeyer et al's equation, as log cumulative percentage of drug released vs. log time, and the exponent n was calculated through the slope of the straight line.

$$Mt / M\infty = Ktn$$

Where $Mt/M\infty$ is the fractional solute release, t is the release time, K is a kinetic constant characteristic of the drug/polymer system, and n is an exponent that characterizes the mechanism of release of tracers (Korsmeyer et al., 1983). If the exponent $n = 0.45$, then the drug release mechanism is Fickian diffusion, and if $0.45 < n < 0.89$, then it is non-Fickian or anomalous diffusion. An exponent value of 0.89 is indicative of Case-II Transport or typical zero-order release (Siepmann et al., 2001).

3. Results and discussion

Preparation of nanoparticles and optimization:

Different formulations of naringin-loaded chitosan nanoparticles (NCT1–NCT5) were prepared by modified ionic gelation technique. The formation of CHNPs is governed by the controlled gelation of the CH/TPP crosslinking and evaluated to select the nanoparticles with the optimal composition. The evaluation criteria were the particle size, zeta potential, %yield, % EE, drug loading, morphological characters and the drug release profiles.

Optimization of CH to TPP ratio:

Optimization was done by preparing five different weight ratios of CH: TPP (1:1, 2:1, 3:1, 4:1 and 5:1) on the basis of size, zeta potential, and entrapment efficiency,% yield and drug loading (Table). Results showed that formulation NCT4 (CH: TPP ratio 4:1) is the optimised one. This formulation showed particle size of about 352.4±7.1 nm (Fig),zeta potential of -31.5mv (Fig), and acceptable entrapment efficiency (69.7±4.3 %). During optimization of CH:TPP ratio, it was noted that a high concentration of TPP was unfavourable as formulations NCT1, NCT2 and NCT3 showed larger particle size,which may be due to low zeta potential value and resulted in aggregation of NPs leading to increase in particle size as compared to formulation NCT4 .

Particle shape and morphology:

The shape and morphology was examined using Scanning electron microscopy (SEM) (Zeiss EVOMa 15). The SEM images of the optimized formulation NCT4 are shown in fig 3. The view of optimised nanoparticles of NCT4 formulation showed a spherical shape and smooth surface. From the results formulation NCT4 (4:1 ratio chitosan: sodiumtripolyphosphate) was selected as optimised one.

FTIR studies:

The characteristic bands of naringin, chitosan and sodium-tripolyphosphate (Table) were observed on the spectrum of the naringin-loaded chitosan nanoparticles (Fig 6) almost at the same frequencies, and thus indicating the absence of chemical interactions between the drug and polymer.

DSC studies:

DSC thermogram of pure naringin showed sharp peak at 164°C corresponding to its melting point. The thermogram of Atorvastatin nanoparticles showed a similar endothermic peak at 165.4°C which confirms that there was no polymer drug interaction.

In-vitro drug release studies:

The in-vitro drug release profiles of Atorvastatin loaded nanoparticles showed extended release up to 12 hrs for NCT1, NCT2, NCT3 formulations. This may be due to chitosan:sodium tripolyphosphate concentration was insufficient to coat drug. Hence the release was found to be retarded. Further stability of these formulations was also poor. The drug release from NCT4 and NCT5 formulations were found to be, controlled and extended for a period of 24hrs which may be due to high concentration of chitosan and no release of drug in acidic medium. NCT4 formulation was selected as optimised formulation based on particle size, zeta potential, % yield, entrapment efficiency, drug loading, drug release profile which were found to be superior when compared to NCT5. In-vitro release data obtained was fit to different kinetic models like zero-order; first-order, Higuchi, Hixson-crowell, Korsmeyer peppas plot and results were shown in the table. The coefficient of correlation (R2) was considered to be main parameter for interpreting the release kinetics of drug from the matrix systems. In case of optimised formulation, the higher values R2 of the linear regression curves in the first order plot than zero order plot showed that the drug release followed first order kinetics. As the matrix forming polymers were used, Higuchi model was applied which showed good linearity with regression R2 value of 0.968 suggested that the release

mechanism was diffusion controlled. In order to know the release mechanism, data so obtained was fitted to Korsmeyer peppas law. The release exponent value n= 0.69, indicates the dominant mechanism of drug release of drug release from the drug matrix of optimised formulation was swelling and erosion which is always associated with diffusion mechanism. It can be anomalous transportation i.e., non- fickian kinetics (combination of pure diffusion controlled coupled with dissolution controlled drug release). **Stability studies:** Stability study revealed that there is no significant change in physiochemical properties, drug entrapment efficiency and in-vitro drug release.

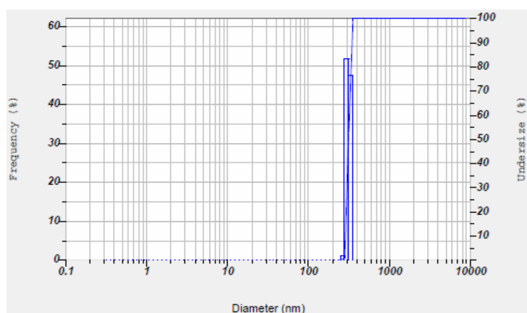

Fig 1: Particle size of NCT4- 352.4 nm

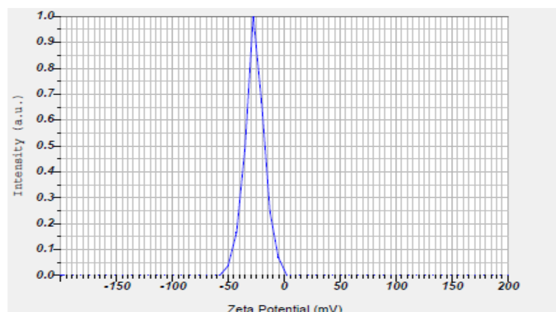

Fig 2: Zeta potential of NCT4- -31.5mv

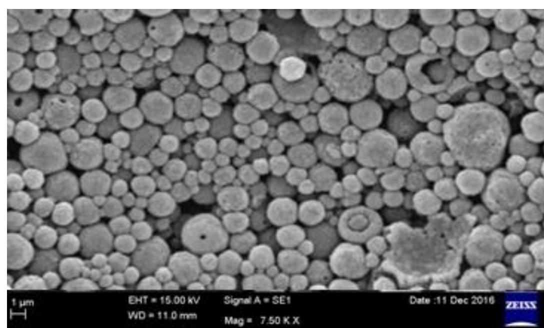

Fig 3:SEM image with Mag = 7.50 KX

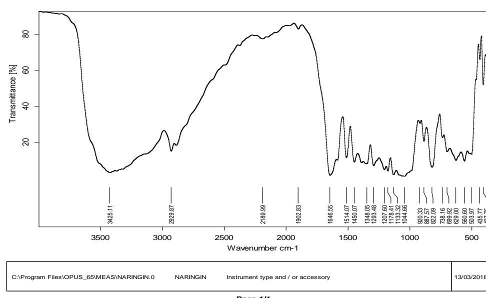

Fig 4: FTIR spectra of naringin

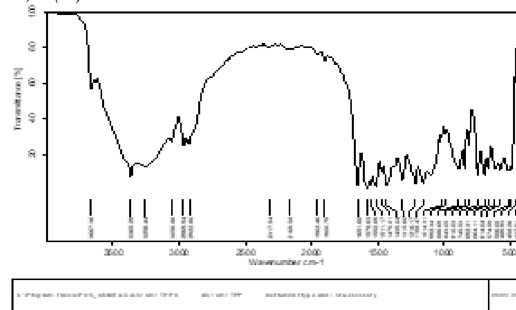

Fig 5:FTIR spectra of formulation

The FT-IR spectra of naringin and naringin loaded nanoparticles are shown in Fig 4 and 5 respectively. FTIR spectra of naringin demonstrated the characteristic absorption peaks at aromatic free O-H stretching at 3667 cm-1, 2927 cm-1 for aliphatic C-H stretching, at 1650 cm-1 for C=O stretching and 1514 cm-1 for C=C stretching .The absorption peaks with naringin loaded nanoparticles were almost similar to those obtained with the pure drug and formulation. This indicates the incorporation of naringin showed good compatibility of drug and polymer.

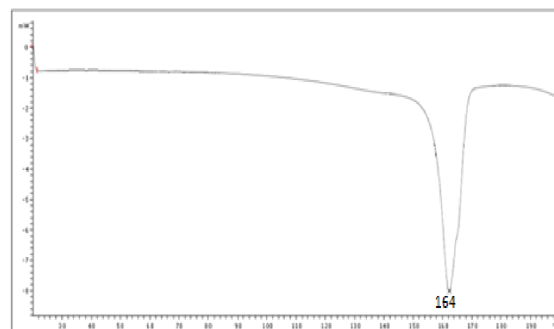

Fig 6:DSC thermogram of naringin

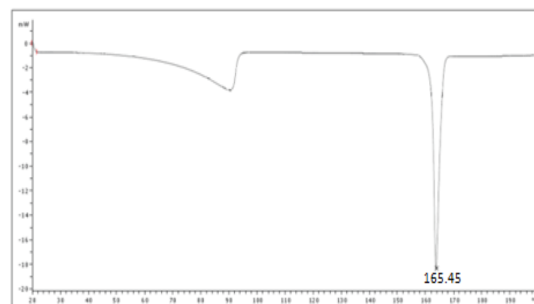

Fig 7:DSC thermogram of formulation

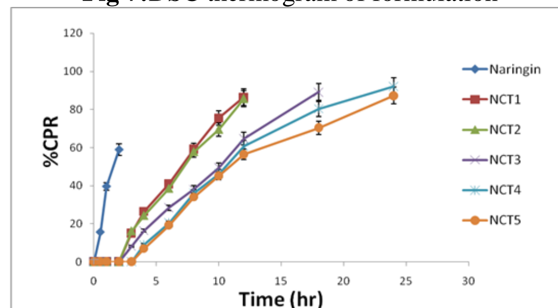

Fig 8: In vitro drug release profile of naringin and its formulations

4. Conclusion

In the present study naringin nanoparticles were prepared by ionic gelation method using chitosan as polymer and sodium tripolyphosphate as gelating agent. Various formulations were prepared and evaluated for particle size, zeta potential, yield, drug content, % entrapment efficiency and also for in vitro the optimized formulation was selected having desirable characteristics. Further the optimised formulation of naringin nanoparticles were incorporated into enteric capsule and were subjected to dissolution test. In vitro drug release studies of optimised formulation NCT4 have been performed in 0.1 N HCl for 2 hr and in pH 6.8 phosphate buffer for 22 hr. The capsules showed no release in acidic medium which is desirable and about 92±0.2% of drug was released at 24th hour. Decrease in particle size increases the surface to volume ratio and specific surface area. The use of particle size reduction to increase the surface area for dissolution and thereby increase in bioavailability of poorly-water soluble drugs has been widely investigated and accepted world-wide. Thus, the prepared nanoparticles proved to be the potential formulation for increase in aqueous solubility of drug and enhancement of bioavailability of naringin. % drug release studies. From the above data obtained

Table 1: Composition of chitosan nanoparticles

Ingredients	NCT1	NCT2	NCT3	NCT4	NCT5
Naringin (mg)	10	10	10	10	10
Chitosan :TPP	1:1	2:1	3:1	4:1	5:1
Acetic acid % w/v	1.5	1.5	1.5	1.5	1.5

Table 2:FT-IR data of naringin, chitosan, sodium-tripolyphosphate and naringin loaded nanoparticles

Group frequency (in cm^{-1})	Frequency of naringin (in cm^{-1})	Frequency naringin nanoparticles (in cm^{-1})	Frequency of chitosan (in cm^{-1})	Frequency of sodium tripoly Phosphate (in cm^{-1})
Aromatic Free O-H stretch	3667	3586	---	3367
C=O stretching	1650	1641	1664	1653
C=C stretching	1514	1493	1485	---
C-H stretching	2927	2904	2830	2926

5. References

[1] Lundquist P, Artursson P. Oral absorption of peptides and nanoparticles across the human intestine: opportunities, limitations and studies in human tissues. Adv Drug Deliv Rev. 2016;106: 256–276.

[2] Yadav SK, Kumari A, Yadav SC. Biodegradable polymeric nanoparticles based drug delivery systems. Colloids Surf B:Biointerfaces.2010;75:1-18.

[3] Majeti NV, Kumar R, Kumar N, Domb AJ, Arora M. Pharmaceutical polymeric controlled drug delivery systems.Adv.in polymer Sci.2012;160:47-108.

[4] Varma B V N, Hemant KSY, Ayaz A, Vasudha LS, Shiva KHG. Different techniques for preparation of polymeric nanoparticles- a review . Asian J Pharm Clin Res.2012; 5(3): 16-23.

[5] Mahapatro A, Singh DK. Biodegradable nanoparticles are excellent vehicle for site directed in vivo delivery of drugs and vaccines. J Nanobiotechnology .2011; 9:55.

[6] Wen F, Wei Y, Xu Z, Hong N. Formation mechanism of monodisperse, low molecular weight chitosan nanoparticles by ionic gelation technique. Colloids and Surf B: Biointerfaces. 2012;90: 21-27.

[7] Sailaja AK , Amareshwar P, Chakravarty P. Chitosan nanoparticles as a drug delivery system. Res J Pharm Biol Chem Sci.2010;1(3):474-484.

[8] Rashmi C, Nesalin AJ, Mani TT .Nanoparticulate drug delivery system - Review. International journal of research in pharmacy and chemistry. 2016; 6(3): 491-500.

[9] Choe SC, Kim HS, Jeong TS, Bok SH, Park YB. Naringin has an antiatherogenic effect with the inhibition of intercellular adhesion molecule-1 in hypercholesterolemic rabbits. J Cardiovasc Pharmacol. 2001;38:947–55.

[10] Martin MJ, Marhuenda E, Perez-Guerrero C, Franco JM. Antiulcer effect of naringin on gastric lesions induced by ethanol in rats. Pharmacology. 1994;49: 144–50.

[11] Benavente-Garcia O, Castillo J. Update on uses and properties of citrus flavonoids: new findings in anticancer, cardiovascular and anti-inflammatory activity. J Agric Food Chem. 2008;56 :6185–205.

[12] Mohammed MA, Jaweria TM , Wasan KM, Wasan EK.An overview of chitosan nanoparticles and its application in non-parenteral drug delivery. Pharmaceutics .2017; 9(5):1-26.

[13] Kalidoss S, Muthu KA.Formulation development and evaluation of colon targeted oral drug delivery system of atorvastatin . International Journal of Chemical and Pharmaceutical Sciences 2016; 7 (1):1-6.