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Formulation and Optimisation of k-Carragenan based oral anticancer nanoparticles by *In Vitro* Bioavailability Enhancement technique

S.Rajarajan, Beny Baby, K.Ramesh and Rashmi Mathews
Department of Pharmaceutics, Karnataka College of Pharmacy, Bangalore -560064

ABSTRACT
The current research works to formulate the Paclitaxel (PTX) k-carragenan(kC) based nanoparticles along with chitosan (CS). Formulation of paclitaxel loaded Chitosan, k-carragenan nanoparticle was prepared by ionic gelation method using cross linking agent tripolyphosphate (TPP). Evaluation of nanoparticles were subjected to particle size, entrapment efficiency, drug release and drug content. The optimization design was performed by using response surface method and Box-Behnken design model with concentration of chitosan, k-carragenan and tri polyphosphate as variables and particle size, entrapment efficiency, drug release as responses. The characterization study of optimized formulations were subjected for particle size, DSC and SEM. The polymeric nanoparticle formulation was demonstrated enhanced invitro bioavailability and uniform size of particle with size range of 263 nm with highly loaded drug entrapment efficiency were observed in addition other evaluation and optimization of paclitaxel loaded nanoparticles were identical in their properties. The results suggest that chitosan, k-carragenan with paclitaxel polymeric nanoparticle is a promising formulation for effective oral cytotoxic agent.

Keywords: Paclitaxel, Chitosan, k-carragenan, Tripolyphhosphate, oral cyotoxic agent.

INTRODUCTION

Over the past twenty years various studies are according on seaweeds-derived polysaccharides for medicine and biological applications (tissue engineering, drug delivery, wound healing, and biosensor). Alginate, carrageenan, fucoidan, and ulvan are wide used marine derived polysaccharides for biological and medicine applications because of their biocompatibility and availableness[1].

The exploration of seaweed polysaccharides for drug delivery applications remains in its infancy. These natural polymers are often regenerate into nanoparticles (NPs) by differing kinds of strategies, like ionic gelation, emulsion, and polyelectrolyte complexing. Ionic gelation and polyelectrolyte complexing are usually used by adding cationic molecules to those anionic polymers to provide NPs of a desired form, size, and charge[2].

Historically, most of cytotoxic agents medication were delivered by the blood vessel route that is that the most direct one resulting in immediate and complete bioavailability of the medication. However, this administration route may end in many facet effects and needs a clinic or hospitalization visit, nursing and palliative treatment[3].

Many cytotoxic agents medication ar meagrely water soluble and that they show a low permeability at the viscus level. moreover, their oral bioavailability is impaired as a result of they're substrates of the haemoprotein P450 and of the effluence pumps. The prodrug strategy, represents one among the various approaches to overcome these obstacles[4].

In the past few years, the expansion of nanometric size drug delivery systems (DDS) has burst into challenging innovations enabling real progresses to attain oral delivery of metastatic tumor medication. DDS like compound nanoparticles, micelles, dendrimers and lipid-based formulations modify physico-chemical properties of cytotoxic agents to be improved and oral bioavailability to be increased[5].

Based on the above literature survey there were very few report were available on seaweed polysaccharide application on drug delivery. Recently by researchers around globally insisting importance and potential of using seaweed polysaccharide application on nanopaticles application in drug delivery system has increased. So keep all this information in mind, the current study has been designed to develop by the interest of utilisation and application of marine seaweeds polysaccharide based oral anticancer nanoparticle to use for oral dosage form application. Since this area of research may have potential to use for patient safety.

MATERIALS AND METHODS

Materials

Paclitaxel was purchased from labon, Bangalore, chitosan and Tri-polyphosphate was purchased from Yarrow chem pvt ltd, Mumbai India, k-Carrageenan was purchased from sigma aldrich chemicals pvt ltd, All the chemical ingredients and solvents used were of Analytical grade.

Methods

Preparation of Nanoparticles

Chitosan and k-carragenan nanoparticle was prepared by calvo et al.1997 method ionic gelation. Appropriate concentrations with chitosan and k-carragenan solution were prepared by dissolving chitosan and k-carragenan of various concentrations in 1% acetic acid solution. 1mg/ml concentration of paclitaxel was added to each chitosan and k-carragenan solution into the TPP solution. Drop wise TPP solution was incorporated to chitosan and k-carragenan solution in the ratio 1:5 with constant magnetic stirring for 1h. The obtained dispersion was was centrifuged for 15 min at 9000 RPM. Final product was dried by vacuum dryer.

The dried product were collected for characterization and in vitro release study. It is shown in table 1.[6]

OPTIMIZATION

The runs or formulation, which are designed based on Response surface method, are evaluated for the response. The tool used here is Design Expert trial version with Box-Behnken model. Variables used here are chitosan, k-carragenan, and TPP and responses are particle size, entrapment efficiency, drug release. The responses values are subjected to Quadratic model to find out the relationship between the variables used the response values obtained.

Particle size analysis

Particle size of all the formulations of drug loaded nanoparticles was distributed in deionized water, centrifuged for five minutes at 5000 revolutions per minute and filtered with 0.2 membrane filter. Particle size were determined by utilisation Malvern Zetasizer Nano S90 at a temperature of 25°C at a measure angle of 90° to the incident beam.[7]

Entrapment efficiency

Loading of PTX in chitosan nanoparticles was determined by extracting 5 mg nanoparticles with 1 mL methanol for 8 h. From this solution, 0.2 ml was diluted with phosphate buffer pH 6.8 and analysed by ultraviolet (UV) spectrophotometer (Shimadzu UV-1800, Japan) at 230 nm against appropriate blank.[7]

In Vitro Release Studies

Drug release of formulation was performed using a Franz diffusion cell with a receptor compartment capacity of 150 ml. The dialysis membrane was fixed between the receptor and donor portion of the diffusion cell. The prepared nanoparticle was placed on dialysis membrane and covered with aluminium foil.

The acceptor compartment of the diffusion cell was filled up with phosphate buffer pH 1.2, 6.8, 7.2 respectively. The whole assembly was fixed on a hot plate magnetic stirrer, and solution in the receptor compartment was continuously and constantly stirred by use of magnetic beads and the temperature was mentioned at 37 ± 0.5°C. The samples remained obtained at different time intervals and analysed the drug content in U.V. spectrophotometer. The receptor phase was replenished with an equal volume of phosphate buffer (pH 7.2) at each sample withdrawal.[8]

Determination of Drug Content

About 10 mg of paclitaxel equivalent suspension nanoparticles was dissolved in 10 mL of methanol and sonicated for 45 minutes. Then 10 mL of the above solution was diluted to 100 mL with methanol. 1 mL of from the above solution was taken and diluted to 10 mL with methanol and measured at 230 nm under UV spectrophotometer. The percentage drug content in the nanoparticle was calculated.[9]

Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) experiments were carried out in order to characterize the physical state of the drugs. Samples of polymeric nanoparticle formulation were placed in aluminium pans and thematically sealed. The heating rate was 10°C per minute exploitation nitrogen because the purge gas. The DSC instrument was graduated for temperature with indium. Additionally, for H standardisation indium was sealed in aluminium pans with sealed empty pan as a reference.[10]

Scanning Electron Microscopy

The prepared loaded nanoparticles were subjected for Scanning Electron Microscopy (SEM) analysis on the access their surface morphological characteristics of prepared polymeric nanoparticles using magnifications as shown in Fig**. 5.**

RESULTS AND DISCUSSION

Particle size analysis for nanoparticle formulation was carried by using optical microscope at 40X and Malvern particle size analyzer. It showed that the average diameter of the nanoparticles was found to be 262.5 and the single peak obtained from analysis indicates uniformity of the particle size which is the result of the use of conventional technique. High micro mix potency increased the mass transfer and therefore the rate of diffusion between the polyphase, that induced solid super saturation in brief time and therefore fast nucleation to supply smaller drug particles. Hence higher homogenization is favored for the formation of the smaller and more uniform drug particles.

The entrapment efficiency was in the range 75 to 87%. It showed that entrapment efficiency decreases with increase in drug to polymer ratio. Amount of drug entrapment was observed with increased concentration upto 1:1 ratio of chitosan with k-carragenan shown entrapment efficiency.

The in-vitro release of Paclitaxel from the prepared nanoparticles formulation was studied in buffer 0f H 1.2, 6.8, 7.4 for 8 hours. The results are shown as the concentration of polymer increased, the drug release also increased proportionally upto 1:1 ration of chitosan with drug further more concentration of chitosan were shown increased particle size and decreased drug release pattern with preliminary study for this development of formulation. This may be because chitosan retards release to more extent. The release of the drug at 8th ranged from 77.58% to 83.53 %. The result has shown that Paclitaxel released the drug improved the concentration and ratio of k-carragenan with chitosan formulation and release of drug as sustained drug releases for longer period of time for 8 hr. Chitosan permits a protection for therapeutic agent from the hostile conditions of the higher channel and unleash the entrapped agent specifically within the stomach through degradation of the glycosidic chitosan. K-carragenan is observed with enhanced drug release with chitosan, by this

observation it can be a synergistic effect of k-carragenan and chitosan nanoparticle to improve the drug release of Paclitaxel and shown Controlled drug delivery systems. Here it is followed either purely diffusion or erosion controlled.

The optimization of Paclitaxel (PTX) k-carragenan (kC) based nanoparticles was done by response surface method and Box-Behnken design model. Chitosan, k-carragenan and tri polyphosphate as variables and particle size, entrapment efficiency, drug release as responses were selected. The constraints were opted in the range to obtain the optimised formulation prediction as mentioned in table 3. The analysis of DOE was performed by quadratic model was utilised ANOVA results as observed in table 2. The optimized formula shows a particular percent combination of different variables % Chitosan, k-carragenan and TPP for formulation design F15. The quadratic model is selected for these responses with model F-ratio which were indicated that the model is significant.

ANOVA were shown significant in all the responses. 3D surface graph shows the effect on Particle size, Entrapment efficiency and drug release were observed enhanced which were shown Fig 1-3, RAMP model graph of optimised formulation with constraints selection values and overlay plot of optimised formulation by graphical optimisation model were shown in Fig 4, 5. Eeffect on k-carragenan was observed when increase in the ratio or concentration at particular proportion with chitosan for the Paclitaxel k-carragenan based nanoparticle formulation for the oral drug delivery. The optimised formulations with predicted and actual results presented in table 4.

In DSC Studies melting peak appeared at 222.1^0C for Paclitaxel. There was no change in melting point of binary mixture of Paclitaxel, chitosan, k-carragenan and tripolyphosphate Nanoparticles which indicate that there is no interaction between drug and polymers. The peak observed.

SEM was performed for optimised formulations F15P to assess their surface which was shown in Fig 6. The polymer surface of the Nanoparticles appeared irregular with rough texture surface distinct nature, and distinct particle size and morphology with a rough surface. The morphology particles were measured. The particle size strongly depends preparation conditions. The average particle size of the optimised formulation measured particles was as 263.5 nm.

Table 1: Formulation and Evaluation Results Table

Formulation Code	Drug	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
	Paclitaxel	A:Chitosan	B:K Carragenan	C:TPP	Particle Size	Entrapment Efficiency	Drug Release
	mg	mg	mg	%	nm	%	%
F1	10	8.75	7.5	0.675	261.8	72.8	81.9
F2	10	5	7.5	0.5	254.4	68.8	78.5
F3	10	5	7.5	0.85	254.1	69.2	78.6
F4	10	12.5	5	0.675	269.1	73.9	82.5
F5	10	8.75	5	0.5	261.2	72.5	80.1
F6	10	8.75	7.5	0.675	261.6	73.1	82.2
F7	10	12.5	7.5	0.85	269.8	74.3	83.2
F8	10	12.5	10	0.675	272.5	74.4	83.5
F9	10	8.75	5	0.85	269.2	74	80.3
F10	10	8.75	10	0.85	262.2	73.6	82.5
F11	10	12.5	7.5	0.5	270.1	74.2	83.3
F12	10	5	5	0.675	252.5	68.5	78.2
F13	10	5	10	0.675	255.2	69.6	79.2
F14	10	8.75	10	0.5	262	73.6	82.5

Table: 2 ANOVA Results by Quadratic model

Response	p-value	Model
Particle Size	0.0271	Significant
Entrapment Efficiency	0.0002	Significant
Drug Release	0.0046	Significant

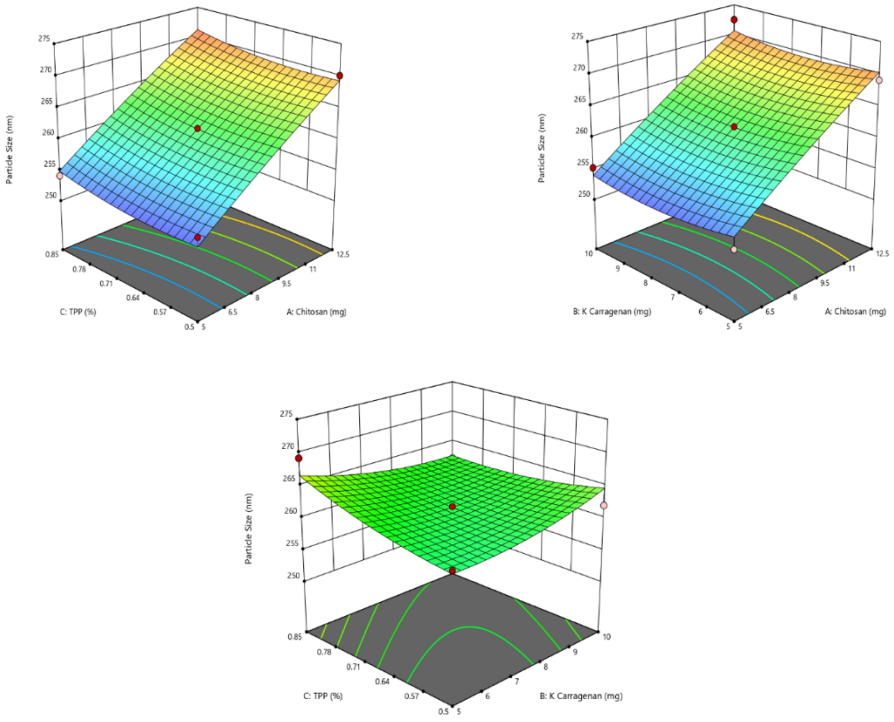

Figure 1: Three-dimensional (3D) response surface diagrams represent the effect of Particle Size. (A) The influence of particle size on TPP and chitosan. (B) The influence of particle size on k-carragenan and chitosan. (C) The influence of particle size on k-carragenan and TPP.

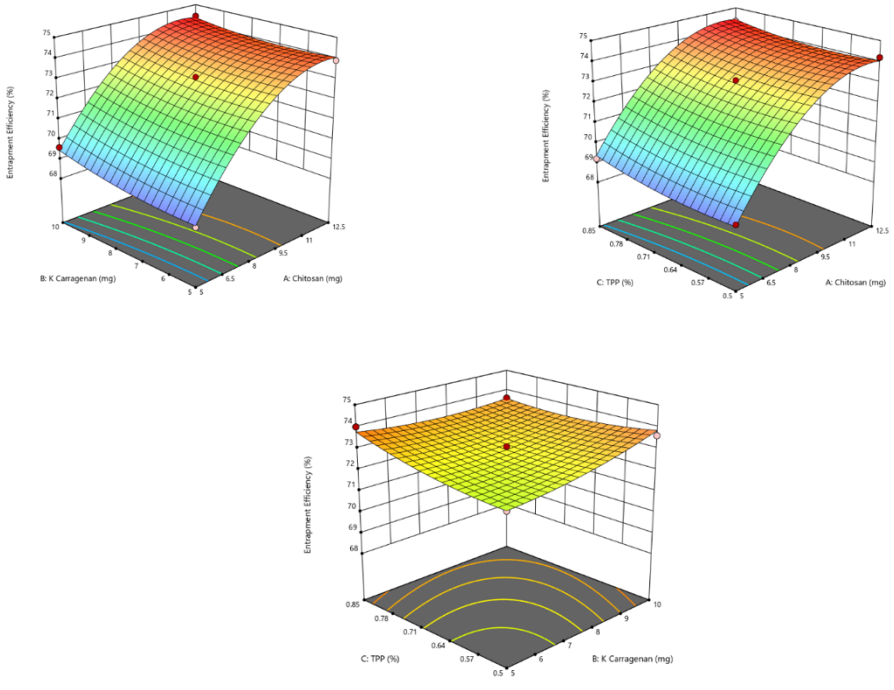

Figure 2: Three dimensional (3D) response surface diagrams represent the effect of Entrapment Efficiency. (A) The influence of entrapment efficiency on k-Carragenan and chitosan. (B) The influence of entrapment efficiency on TPP and chitosan. (C) The influence of entrapment efficiency on k-Carragenan and TPP.

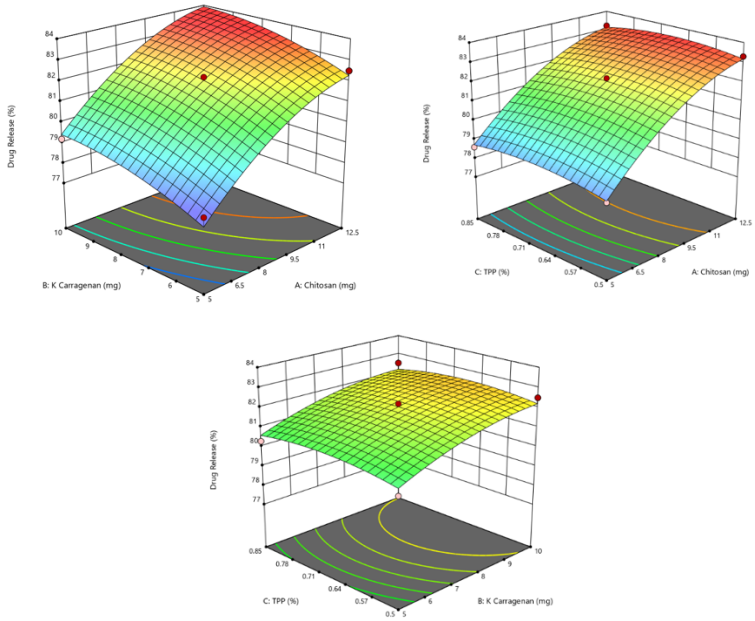

Figure 3: Three-dimensional (3D) response surface diagrams represent the effect of Drug Release. (A) The influence of drug release on k-Carragenan and chitosan. (B) The influence of drug release on TPP and chitosan. (C) The influence of drug release on k-Carragenan and TPP.

Table 3: Constraints for the selection of Optimised formulation in DOE

Name	Goal	Lower Limit	Upper Limit
A:Chitosan	is in range	5	12.5
B:K Carragenan	is in range	5	10
C:TPP	is in range	0.5	0.85
Particle Size	is in range	250	275
Entrapment Efficiency	is in range	70	75
Drug Release	is in range	80	85

Table 4: Optimised formulations with predicted and actual results

Formulation Code	Paclitaxel (mg)	Chitosan (mg)	K-Carragenan (mg)	TPP (%)	Particle Size (nm)	Entrapment Efficiency (%)	Drug Release (%)
F15 (Predicted)	10	9.057	5.888	0.731	263.6	73.25	81.53
F15 (Predicted)	10	9.057	5.888	0.731	263.5	73.27	81.60

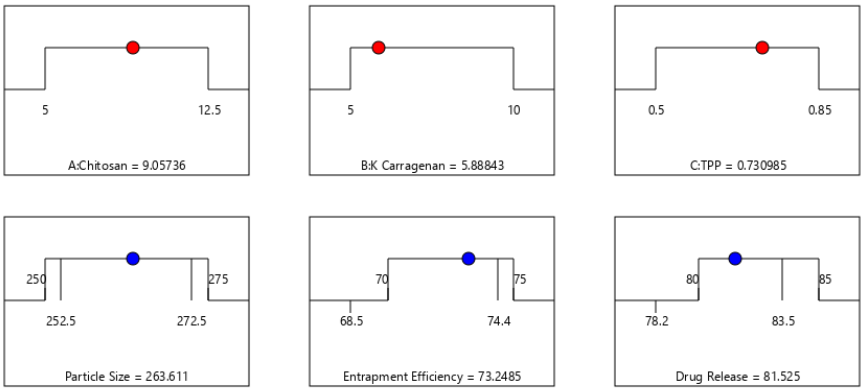

Figure 4: RAMP Model graph of optimised formulation with constraints selection values

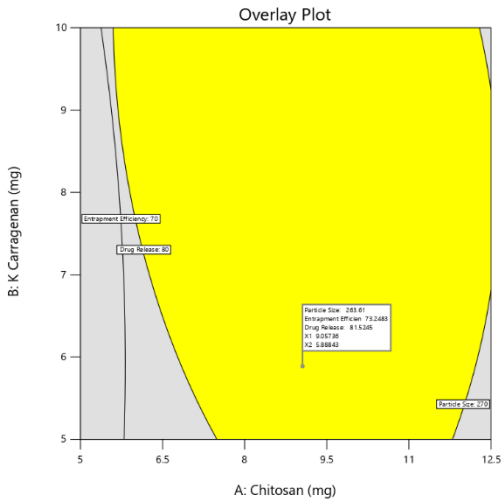

Figure 5: Overlay Plot of Optimised formulation by Graphical Optimisation Model

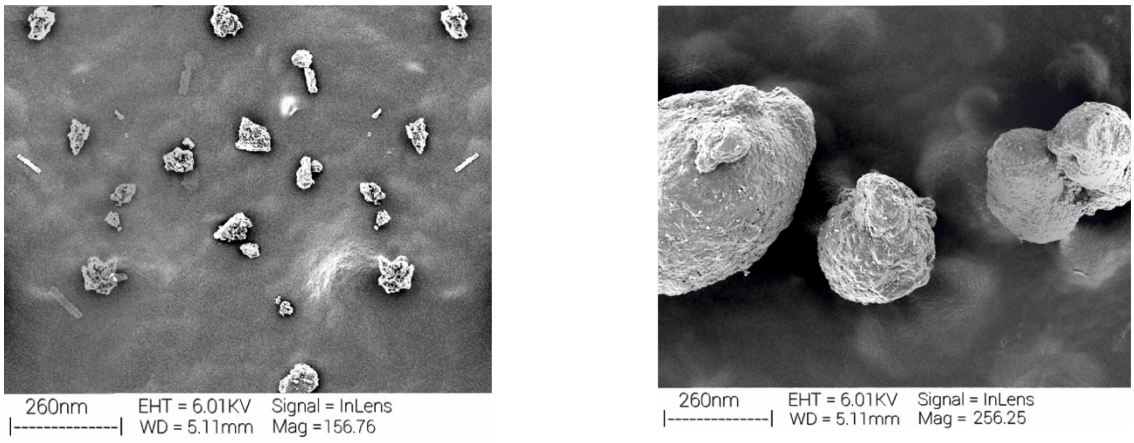

Figure 6: SEM Photograph of k-carragenan based Paclitaxel Nanoparticles

CONCLUSION

Formulated nanoparticles using PTX using kC and chitosan by inotropic gelation method was successful. The prompt physicochemical properties of prepared nanoparticle were observed. PTX nanoparticles showed increased solubility in presence of k-carragenan. PTX nanoparticles formulations can be further more to investigate bioequivalent study to understand the clinical efficiency. Therefore, it is finally concluded that inotropic gelation nanoparticle technology can be successfully applied for achieving improved drug release for paclitaxel using chitosan, k-carragenan and Tripolyphosphate using response surface design statistical model application.

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