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PREPARATION AND OPTIMIZATION OF PROCESS VARIABLES OF NANOPARTICLES CONTANING ANTI CANCER DRUG

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ABSTRACT

The objective of the present study is to formulate nanoparticles that contain 5-Flurouracil, an anticancer drug and optimization for chemical properties, drug concentration, polymer concentration, cross-linking agent and stirring speed. Nanoparticles of 5-Flurouracil were formulated using chitosan polymer and pregelated Sodium alginate by Ionotropic pregelation method. Calcium chloride was also included in the formulation for pregelation of sodium alginate. Prepared 1% acetic acid solution of chitosan and pregelated calcium alginate suspension further cross linked with Gluteraldehyde. Different formulations of nanoparticles were prepared using different concentrations of chitosan, stirring speed, time of rotation and polymer to drug ratio in the nanoparticles. The average particle size ranged between 246 nm to 620 nm. Drug entrapment ranged between 71.97%-89.90%. The result indicated that the drug loaded nanoparticles of 5-Flurouracil showed optimum particle size and maximum drug entrapment with drug polymer ratio 05:75, cross-linking agents 02 ml, stirring rate 800 rpm and stirring time 90 min.

KEY WORDS

Ionotropic pregelation, Drug entrapment, Gluteraldehyde, Average particle size

INTRODUCTION

The colloidal carriers based on biodegradable and bio-compatible polymeric systems have largely influenced the controlled and targeted drug delivery concept. Nanoparticles are sub-nanosized colloidal structures composed of synthetic or semi-synthetic polymers[1].

Colloidal drug delivery systems offer a number of advantages over conventional dosage forms. Due to their small particle size, colloidal preparations lend themselves to parenteral administration and may be useful as sustained release injections for the delivery to a specific organ or target site. Targeting the drug to the desired site of action would not only improve the therapeutic efficiency but also enable a reduction of the amount of drug which must be administered to achieve a therapeutic response, thus minimizing unwanted toxic effects[2].

Nanoparticles useful for sustained drug release can be also obtained by electrostatic interaction between alginate and chitosan[3]. Both alginate and chitosan have been widely used in drug delivery[4]. Chitosan is a natural cationic polysaccharide derived by deacetylation of chitin, a copolymer consisting of combined units of glucosamine and N-acetyl glucosamine[5, 6]. In the pharmaceutical field chitosan's advantageous biological properties have prompted its extensive study as a carrier of both drugs[7, 8] and proteins[9, 10]. Drug loaded nanoparticles made of polyelectrolytes complexation have shown its potentiality for use as drug delivery systems[11, 12]. Polyelectrolyte complexes are formed by interactions between macromolecules that carry oppositely charged ionizable groups[13]. A more selective drug delivery was achieved using water soluble drug–polymer conjugates[14].

Gemcitabine, a nucleoside analog related to cytarabine, is one of the most effective cytotoxic agents for non small cell lung cancer (NSCLC). It is a pyrimidine antimetabolite that is anabolized sequentially to the nucleoside monophosphate, diphosphate, and triphosphate intracellularly. This drug may be a cell cycle-specific agent inhibiting DNA synthesis, and it also induces apoptosis[15].

MATERIALS AND METHODS

Chitosan (deacetylation degree 85%) low MW (50 kDa) was obtained as a gift sample from Central Institute of Fishery Technology (Trivandrum, Kerala). Sodium alginate (low viscosity), Calcium chloride and Gluteraldehyde of analytical grade were purchased from Loba chemicals (Pune). Gemcitabine pharmaceutical grade (as per USP) was obtained as a gift sample from Shilpa Medicare Limited, (Raichur), Karnataka, India. Glacial acetic acid of analytical grade was procured from Qualigens Fine Chemicals. A549 human non small cell lung cancer cell line was purchased from NCCL (Pune). All other chemicals were of analytical grade and used as received. Double distilled water was used throughout the study. Magnetic stirrer was used of Rami.

PREPARATION OF DRUG-LOADED ALGINATE NANOPARTICLES

Alginate/chitosan particles were prepared in a two-step procedure based on the ionotropic pre-gelation of polyanion with calcium chloride followed by polycationic crosslinking through an adapted protocol initially described[13], but modified according to ideal pre-gelation stoichiometric ratio and time of drug association[16]. 7.5 ml of 18 mM calcium chloride solution was added drop wise for 60 min under gentle stirring (800 rpm) into a beaker containing 117.5 ml of a 0.063% alginate solution to provide an alginate pre-gel. Then, 25 ml of different

concentration (0.05–0.09%) chitosan solution was added drop wise into the pre-gel over 90 min. The pH of alginate and chitosan solutions was initially set to 4.9 and 4.6, respectively. A colloidal dispersion at pH 4.7 formed upon polycationic chitosan addition, (visible as the Tyndall effect). Nanoparticles were stirred for 30 min to improve curing and subsequently collected by centrifugation (20,000g/45 min) at 4˚C. For Gemcitabine-loaded nanoparticles, 5 mg of insulin was mixed with the alginate solution before calcium chloride addition.

Drug-loaded nanoparticles were recovered by centrifugation at 19,000 rpm for 30–45 min and washed thrice with distilled water to obtain the final pellet.

Glutaraldehyde (GLA) cross-linking nanoparticles were prepared as follows: a known mass of 0.25% (w/w) GLA solution was dropped in CS–ALG suspension or drug loaded CS–ALG suspension under magnetic stirring. This mixture was further stirred for three hours under room temperature (G-CS–ALG)[17].

OPTIMIZATION

Optimization of formulation variables

Various formulation variables were optimized to prepare nanoparticle viz. polymer concentration and cross-linking agents concentrations. The effect of these variables on the particle size, shape, size distribution entrapment efficiency was studied.

Optimization of process variables

Process various variables that could affect the preparation and properties of final preparations were optimized i.e. stirring speed (500, 600, 700, 800, 1000 rpm.) and stirring time (hrs.). Effect of these variables on particle size, shape, size distribution and entrapment efficiency was studied.

Estimation of entrapped drug (5-FU) in nanoparticles:

The 50 mg of nanoparticles was dispersed in 50 ml of PBS pH 7.4 for 24 hrs. and the homogenate was centrifuged at 300rpm for 5 min., and the supernatant was assayed for 5-FU, spectrophotometrically. The percentage drug entrapped was calculated and reported in table (1, 2, 3, 4 and 5).

RESULT AND DISCUSSION

Using Ionotropic pregelation method, average particle size and entrapment with high drug contents could be produced (Fig. 1,2,3,4 & 5).

In the study of drug: polymer (Sodium alginate) ratio, Sodium alginate concentration was effected the particle size from 230-575 and entrapment of drug and 92.89%-69.00%.

The drug concentration also affects the particle size and drug entrapment with respect to the concentration of chitosan from 229-480 and 69.97%-91.98% respectively. The same parameters were affected with using the different concentration of cross-linking agents 232-589 and 83.65%-90.88%.

Table no. 4. indicates that the effect of stirring rate on the particle size and drug entrapment. With an increase in stirring speed, the particle size of nanoparticles was reduced from 627-236 and entrapment of drug content was increased from 78.00%-90.00%. Stirring time (at 800 rpm) also affected the nanoparticle size and drug entrapment from 557-236 and 78.00%-90.90%.

Estimation of entrapped drug in nanoparticle

The 50 mg of nanoparticles were dispersed in 50 ml of pH 7.4 was added and kept for 24 hrs. The digested homogenate was centrifuged at 3000 rpm for 5 min., and the supernatant was assayed for 5-FU Spectrophotometrically.

Table 1

Effect of drug: polymer ratio on particle size and size distribution of nanoparticles

S.No.	Drug:Polymer(sodium ginate) Ratio (mg)	Average particle size (nm)	Drug Entrapment (%)
1	05:40	252	84.00
2	05:50	262	87.00
3	**05:75**	**256**	**88.84**
4	05:100	288	80.22
5	05:125	289	79.37
6	05:135	310	76.19
7	05:150	567	70.32
8	05:170	654	72.85

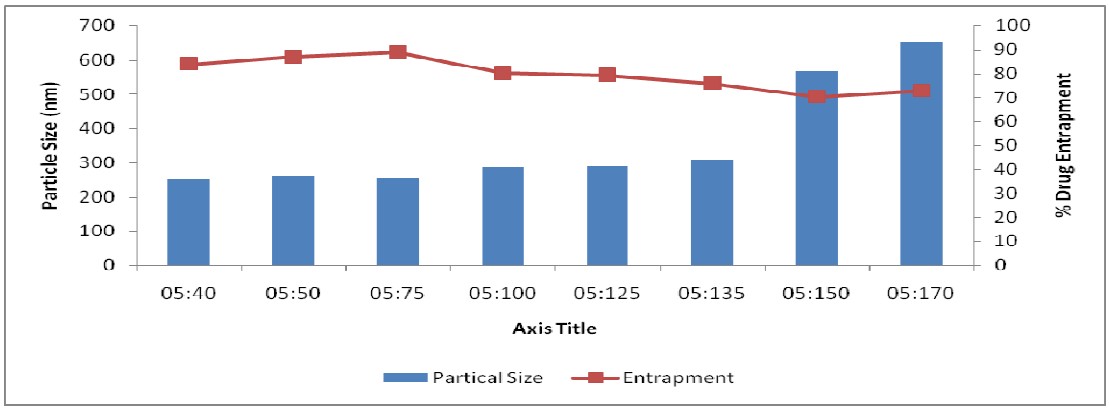

Figure . 1

Effect of drug: polymer ratio on particle size and size distribution of nanoparticles

Table 2

Effect of drug: polymer (chitosan) ratio on the particle size and size distribution of nanoparticle

S.No.	Drug:Polymer Ratio (mg)	Average Partical Size (nm)	Drug Entrapment (%)
1	0.5:50	261	65.73
2	01:50	297	68.00
3	02:50	281	71.97
4	03:50	267	81.00
5	**05:50**	**266**	**89.12**
6	06:50	379	90.12
7	07:50	600	86.00
8	08:50	674	85.83

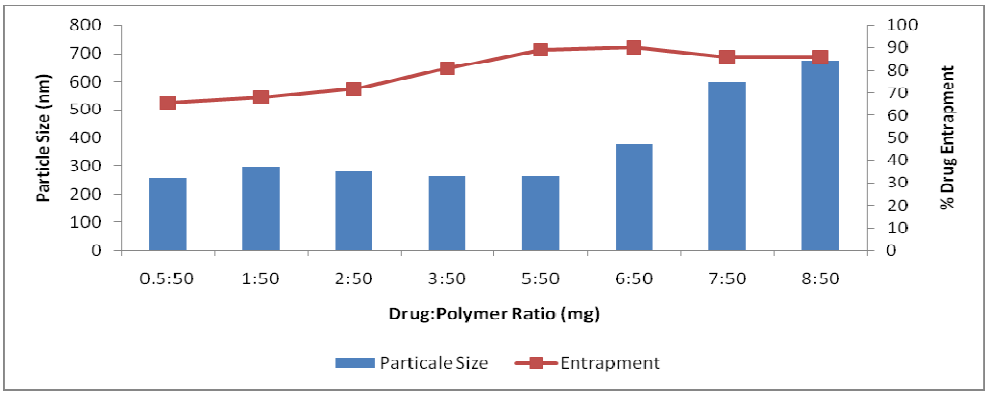

Figure . 2
Effect of drug: polymer (chitosan) ratio on the particle size and size distribution of nanoparticle

Table 3
Effect of cross-linking agent concentration on particle size and size distribution of nanoparticles

S.No.	Volume of cross-linking agents (ml.)	Average Particle Size (nm)	Drug Entrapment (%)
1	0.25	244	81.51
2	0.5	257	85.13
3	01	232	87.20
4	1.5	239	88.11
5	**02**	**251**	**88.87**
6	2.5	340	85.08
7	03	566	83.65
8	04	622	86.76

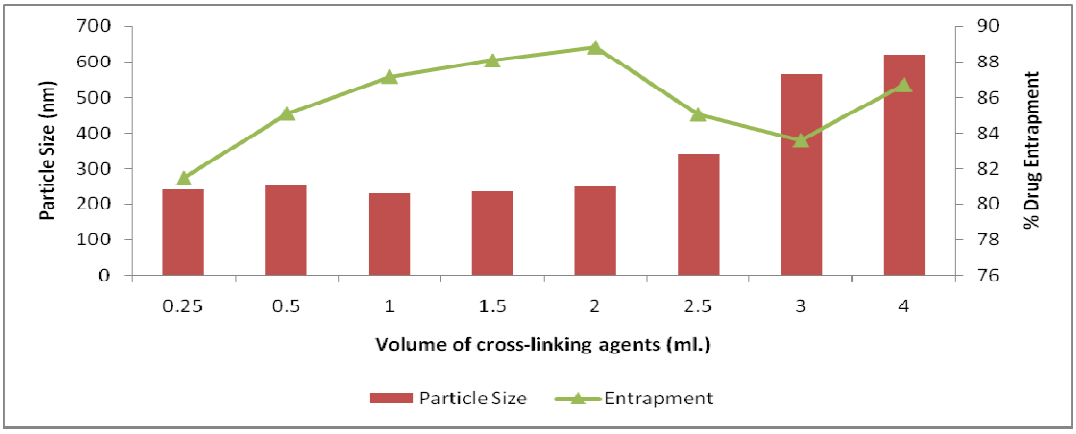

Figure . 3
Effect of cross-linking agent concentration on particle size and size distribution of nanoparticles

Table 4
Effect of stirring rate on the particle size and size distribution of nanoparticle

S.No.	Stirring speed (rpm)	Average Particle Size (nm)	Drug Entrapment (%)
1	400	611	71.94
2	500	620	72.36
3	600	565	80.64
4	700	347	85.10
5	**800**	**262**	**87.99**
6	1000	259	85.90
7	1100	256	81.87
8	1200	242	79.00

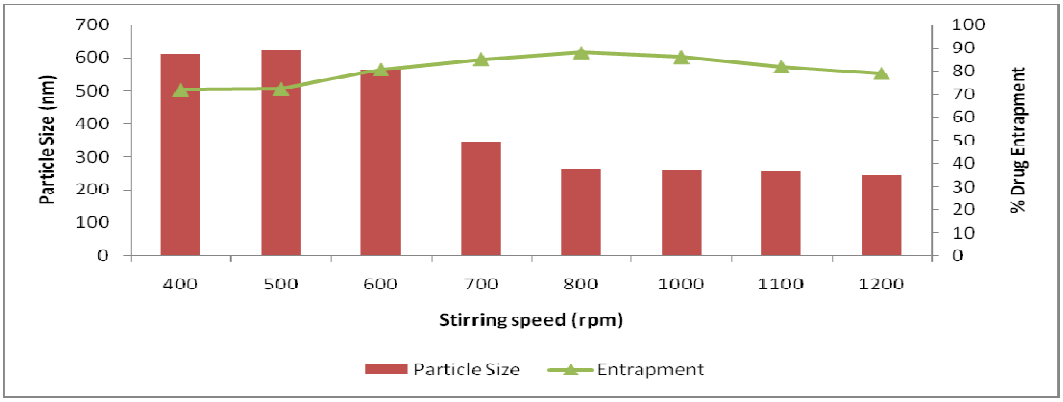

Figure . 4
Effect of stirring rate on the particle size and size distribution of nanoparticle

Table 5
Effect of stirring time at 800 rpm on the particle size and size distribution of nanoparticle

S.No.	Stirring time (min.)	Average Particle Size (nm)	Drug Entrapment (%)
1	30	623	73.65
2	45	557	74.00
3	60	345	79.67
4	**90**	**246**	**89.90**
5	120	295	87.00
6	180	385	84.94
7	200	390	83.98
8	220	400	81.00

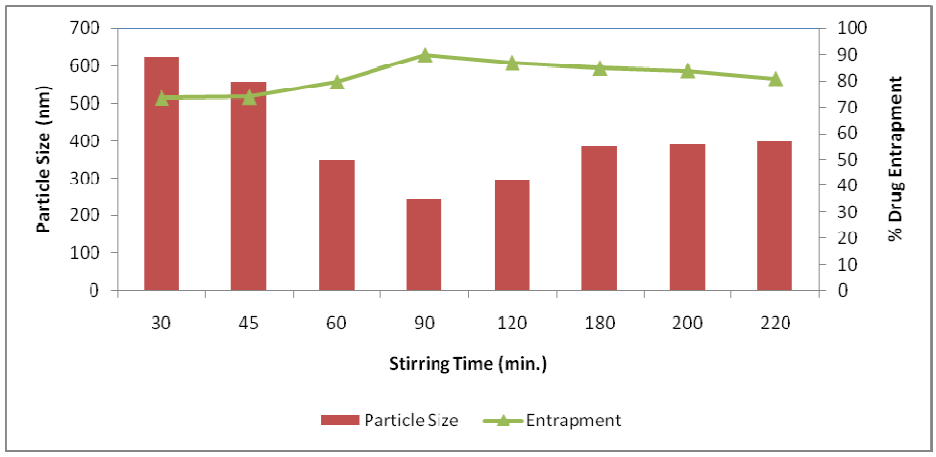

Figure . 5
Effect of stirring time at 800 rpm on the particle size and size distribution of nanoparticle

CONCLUSION

In conclusion, Ionotropic pregelation method can produce chitosan-alginate nanoparticles with optimum particle size and maximum entrapment of drug contents. The physical properties/parameters of nanoparticles can be varied by changing a number of process variables.

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