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Research Article

NANOPARTICLES FORMULATION USING COUNTER-ION INDUCED GELIFICATION TECHNIQUE: *IN-VITRO* CHLORAMPHENICOL RELEASE

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ABSTRACT

In the past decades polymeric nanoparticles have received increasing scientific and industrial interests because of their potential site-specific drug delivery to optimize drug therapy as well as it resolves solubility problems of poorly soluble drugs. The present study was designed to formulate Chloramphenicol (CP) nanoparticles encapsulating with a natural polymer (Sodium alginate stabilized with Chitosan) using counter-ion induced gelification technique to enhance *in-vitro* drug release so that the *in-vivo* bioavailability would be improved. Physicochemical properties of the formulated nanoparticles such as Particle size and Poly dispersity index using Photon Correlation Spectroscopy (PCS), Particle size, Shape and Morphology using Transmission Electron Microscope (TEM), Zeta potential using Zetasizer Nano ZS, Drug entrapment efficiency, and Fourier Transform Infrared were performed. *In vitro* cumulative drug release in both simulated gastric fluid (SGF, pH 1.2) and Borate buffer solution (BBS, pH 7.4) were conducted for 24 hr. CP loaded sodium alginate nanoparticles showed better *in-vitro* release in BBS at pH 7.4 with in 24 hr in comparison to the plain CP. There were no significant changes in physical and chemical stability of CP loaded sodium alginate nanoparticles observed when stored at 2-8°C ± 1°C and 25°C ± 1°C. The results emphasized the power of nanotechnology to make the concept of enhancement of *in-vitro* release of CP comes to reality.

Keywords: Nanoparticles, Chloramphenicol, Sodium alginate, Counter-ion induced gelification, Cumulative drug release.

INTRODUCTION

Nanoparticles are the sub-nanosized colloidal structures composed of natural and synthetic polymers and size range from 10nm to 1000nm. The merits of nano-encapsulation includes the enhanced stability of labile drugs, controlled drug release and an enhanced drug bioavailability owing to the fact that particles in the nano-size range are efficient in crossing through the permeable barriers. Even though, the flexibility of being administered through various routes is offered by polymeric nanoparticles. Many techniques have been used to enhance the bioavailability of poor aqueous soluble drugs including amphiphilic macromolecule cross-linking, polymerization based methods, and polymer precipitation methods[1]. The CP is used in the treatment of typhoid fever and ear infection but an oral administration of the drug often results in a low systemic bioavailability which is mainly attributable to the premature degradation and/or poor solubility of the drug in the gastrointestinal tract. Grey baby syndrome and bone marrow depression are the dose related side effects of the drug[2, 3]. Nanotechnology has been found to be a possible approach which alleviates many problems that affecting a delivery system such as poor solubility, bitter taste and poor bioavailability of drugs. The ideal goal of the present investigation was to formulate sodium alginate nanoparticles of CP for possible use in improving *in-vitro* bioavailability of the drug.

MATERIALS AND METHODS

Chloramphenicol (CP) was procured from Pharma Synth, Haridwar as a gift sample, Sodium alginate (Medium viscosity, a 2%w/v solution give 3500 cps range) was purchased from Chemical Drug House, Mumbai, Chitosan (minimum 85% deacetylated) was purchased from Sigma Aldric, Buch, Calcium chloride was purchased from Chemical Drug House, Mumbai, and all other chemicals used in the study were of analytical grade.

Methods optimization and formulation of drug-loaded sodium alginate nanoparticles

Nanoparticles of alginate were obtained by counter-ion induced gelification method[4,5,6]. Calcium chloride (0.5ml, 18mM), a cross linking agent, was added to 9.5 ml of sodium alginate solution (0.06%w/v) containing CP under stirring condition. The initial ratio of polymer:drug was 1:7.5. Chitosan solution (2ml, 0.05% w/v) was added followed by sonication at 25W for 7min and the mixture was kept at room temperature overnight. Drug-loaded nanoparticles were recovered by centrifuging at 19,000 rpm for 30-45 min and washed thrice with distilled water to obtain the final nanoparticles. Optimization during the sodium alginate nanoparticles formulation[5] is done and the influence of the order of addition of calcium chloride and chitosan on the size of the alginate nanoparticles is shown (Figure 1A and 1B). The method of nanoparticles formulation is shown (Figure 2).

Characterization of drug-loaded alginate nanoparticles

Size determination and morphological characterstics

The sample was prepared by dispersing nanoparticles with water which showed the viscosity of 0.8872 cP as a dispersant. The system temperature and count rare were maintained during the process at 25° C and 104.9 kcps respectively. The sample was placed in the disposable sizing cuvette and the particle size and poly dispersity index (PI) were carried out on a Zetasizer Nano ZS (Malvern Instrument, UK) based on Photon Correlation Spectroscopy (PCS).

Surface charge (Zeta potential) measurement

The sample was prepared by dispersing nanoparticles with water which showed the viscosity of 0.8872 cP and the dielectric constant of 78.5 as a dispersant and the sample was placed in the disposable zeta cell and the surface charge of the nanoparticles was determined using Zetasizer Nano ZS (Malvern Instrument, UK). The Zeta potential distribution was measured between Zeta potential (mV) versus intensity (kcps) and the measurements were performed at 25° C with the count rate of 2272.3 kcps.

Drug encapsulation efficiency

The amount of CP entrapped in the nanoparticles was determined by extracting the drug from the formulation and assaying the same. The efficiency was calculated by using the formula:

Drug encapsulation efficiency = 100 - [amount of drug extracted (mg) x 100/amount of drug taken (mg)

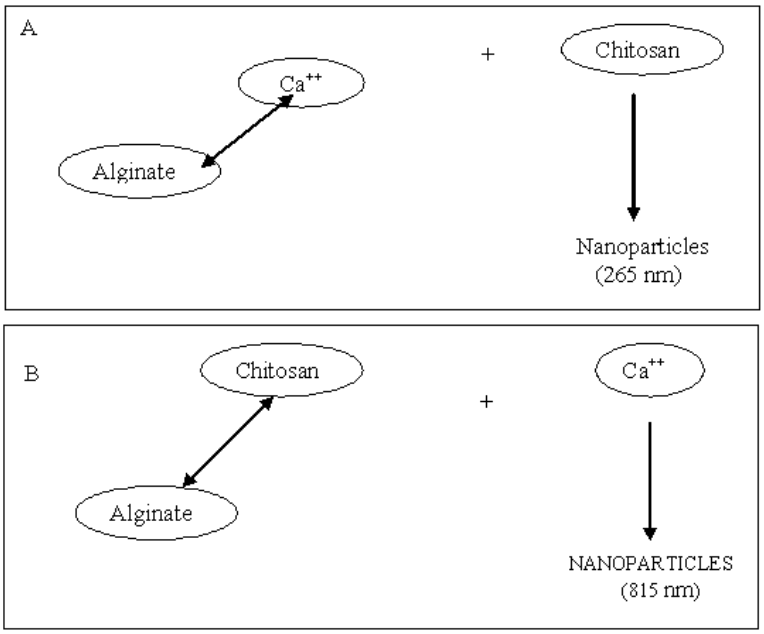

Fig. 1: Influence of the order of addition of calcium chloride and chitosan on the size of the alginate nanoparticles: (A) addition of calcium chloride to the sodium alginate solution; (B) addition of chitosan to the sodium alginate solution.

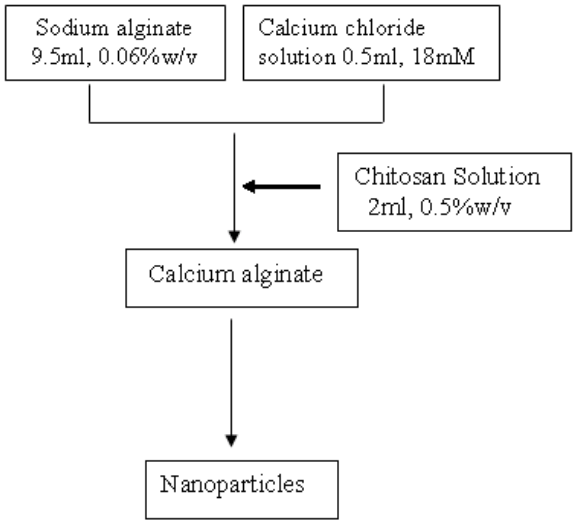

Fig. 2: Method for the formulation of drug-loaded sodium alginate nanoparticles

FTIR analysis

The FTIR spectra using Perkin Elmer 2000 spectrophotometer (Norwalk, CT, USA) of pure drug, polymer alone and drug loaded sodium alginate nanoparticles was recorded from 4000 – 400 as scanning range between wave number (cm^{-1}) and % Transmittance. Samples were prepared in KBr discs (2mg sample in 200mg KBr) with a hydrostatic press at a force of 5ι cm^{-2} for 5min and the resolution was 4 cm^{-1}. The data is tabulated (Table 1). The experiments were duplicated to check the reproducibility.

***In vitro* drug release studies**

In vitro drug release studies were carried out in simulated gastric fluid (SGF, pH 1.2) prepared according to the United State Pharmacopoeia (USP 26/NF 21, 2003) and Borate buffer solution (BBS, pH 7.4) at 37°C ± 1°C. Aliquots were drawn at different time intervals for 24 hr and replaced with equal volume of buffer.

The dissolution studies were carried out in triplicate and the mean values are plotted as percentage cumulative release versus time.

Stability studies

The nanoparticles were subjected to stability studies for 2 months at 5-7˚±1˚C and 25˚±1˚C. The samples were collected at 15 days interval for 60 days and evaluated their characteristics like drug loading and % cumulative release of drug from the nanoparticles.

Table 1: FTIR data of Chloramphenicol (raw material), sodium alginate, and drug loaded sodium alginate nanoparticles formulated by the addition of calcium chloride before the addition of chotosan (265 nm)

S.No	Group	Raw material Chloramphenicol bands cm^{-1}	Drug-loaded sodium alginate nanoparticles bands cm^{-1}
1.	Amide portion of 2,2 dichloracetamide moiety (NH bending vibration) Amide I **(1° amide)** Amide II **(2° amide)**	 1689 1568	 1687.0 1608.9
2.	Nitro group (nitro phenyl) (NO2-Ph)	1530	1522.8
3.	Hydroxyl group **(Bending 2°-OH)**	1069	1095.1
4.	Bonded hydroxyl group **(-OH stretching vibration)**	3340	3421
5.	Sodium alginate polymer (-COOH)	1725	
6.	Ester (-OCOR)	1740	
7.	Saturated acyclic, ester stretching vibration (bonding between **-OH stretching vibration of chloramphenicol and –COOH of sodium alginate polymer)**		Found at 1746.5 (normal range of ester group –OCOR 1735-1750)

RESULTS AND DISCUSSION

The Chloramphenicol nanoparticles were formulated using sodium alginate and chitosan as rate controlling agent with calcium chloride as crosslinking agent by counter - ion induced gelification technique.

The effect of addition of calcium chloride and chitosan were performed during the formulation of nanoparticles and the addition of calcium chloride with the sodium alginate solution before addition of chitosan produced stable nanoparticles with the size of 265 nm.

The size distribution by intensity diagram is shown (Figure 3 and 4). The particle size, shape and morphology were examined using a Transmission Electron Microscope (TEM) and the TEM image is shown (Figure 5).

Surface charge of the nanoparticles were performed with Zetasizer Nano ZS (Malvern Instrument, UK) and the nanoparticles were showed the negative surface charge of –2 mV. The Zeta potential distribution diagram is shown (Figure 6).

The maximum drug entrapment efficiency of drug loaded sodium alginate nanoparticles was achieved with the drug-polymer ratio of 1:7.5 of 97.4%.

There was no significant difference in the spectra of drug raw material, polymer alone and the drug loaded nanoparticles ensured there is no any interaction i.e. chemical and functional group changes during the processing of gelification.

Drug-loaded sodium alginate nanoparticles released maximum of 87.80% in Borate buffer solution (BBS, pH 7.4) at 37°C±1°C within 24 hrs. The cumulative drug release was calculated and expressed as a percent of the theoretical drug content and its shown (Figure 7).

The mathematical model of release and Higuchi mathematical model were applied to the dissolution profile of drug-loaded alginate nanoparticles. The release of CP followed first order kinetics with non-fickien diffusion (n value <0.9). The formulation contained 7.5 : 1 ratio of drug : sodium alginate showed linearity and controlled manner of their drug release in term of its 'r' value and the release was significant in term of its 'P' value (<0.005).

There were no significant changes in chemical stability when subjected to stability studies for two months at 5-7°±1°C and 25°±1°C with 60%RH. Drug loading and % cumulative release of drug from the nanoparticles during the sability study is shown (Table 2 and 3).

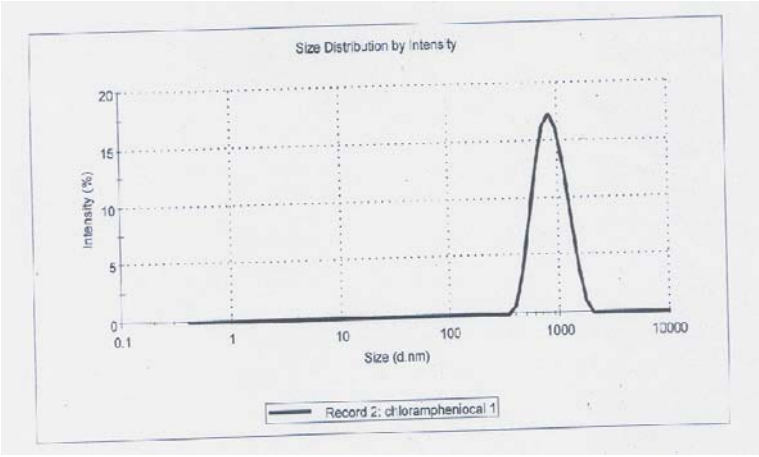

Fig. 3: Size distribution of drug loaded sodium alginate nanoprticles (815nm).

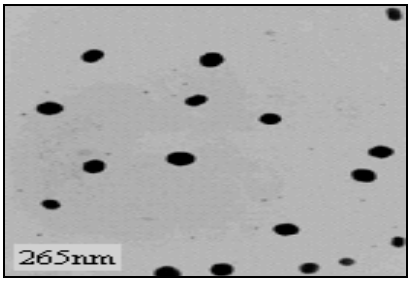

Fig. 5: TEM image of drug loaded sodium alginate nanoparticles.

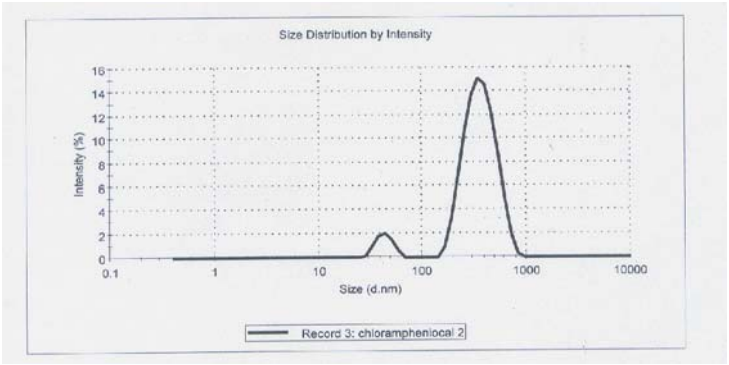

Fig. 4: Size distribution of drug loaded sodium alginate nanoprticles (265 nm).

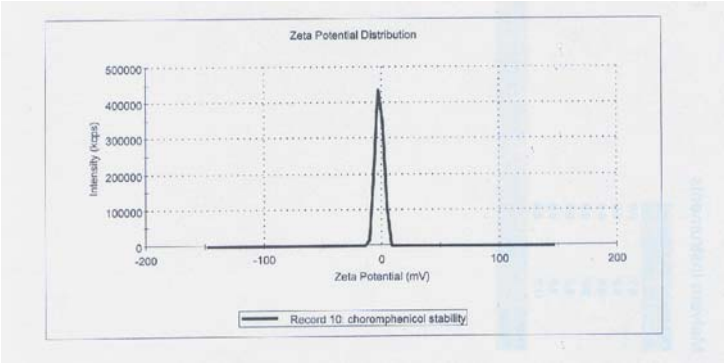

Fig. 6: Zeta potential distribution of drug loaded sodium alginate nanoparticles (–2mV).

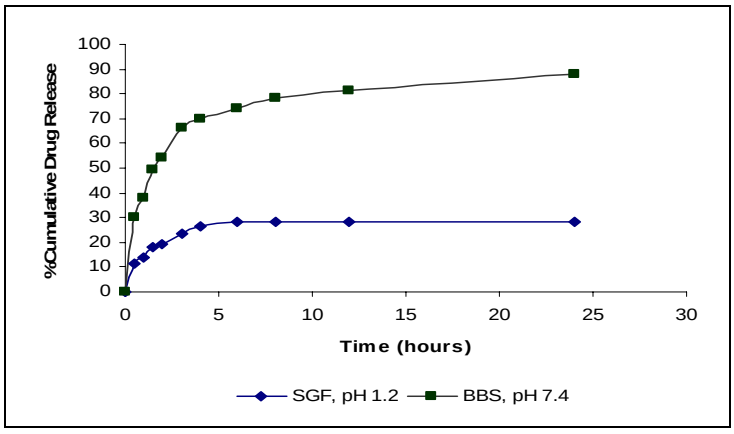

Fig. 7: In-vitro drug release profile of drug loaded sodium alginate nanoparticles formulated by the addition of calcium chloride before the addition of chitosan (265 nm) in SGF (pH 1.2) and BBS (pH 7.4).

Table 2: Drug loading and % cumulative release of Chloramphenicol loaded sodium alginate Nanoparticles formulated by the addition of calcium chloride before the addition of chotosan (265 nm) at 5-7° ±1°C

Time (Days)	Drug loading*	% Cumulative drug release*
15	97.42±0.4	87.80±0.3
30	96.11±0.3	86.09±0.2
45	95.91±0.4	85.61±0.4
60	95.00±0.2	85.24±0.4

* Each observation is the mean (±SD) of three determinations (n=3)

Table 3: Drug loading and % cumulative release of Chloramphenicol loaded sodium alginate Nanoparticles formulated by the addition of calcium chloride before the addition of chotosan (265 nm) at 25°± 1°C

Time (Days)	Drug Loading*	% Cumulative drug release*
15	96.72 ±0.2	86.12 ±0.4
30	96.12 ±0.4	85.61 ±0.2
45	95.51 ±0.1	85.40 ±0.2
60	94.93 ±0.2	84.87 ±0.1

* Each observation is the mean (±SD) of three determinations (n=3).

CONCLUSION

Nanoparticles with highly improved solubility, masked the bitter taste and acceptable dissolution profile were successfully formulated by counter-ion induced gelification method using sodium alginate, confirming that the concept of producing controlled release nanoparticles. Optimized formulation concentrations and addition of first compound were confirmed in subsequent experiments. The results suggest that alginate is a potentially useful polymer for making controlled release nanoparticles by the counter-ion induced gelification method. The preparation of CP aqueous formulations is rather difficult thus a sustained release nanoparticles system would be an interesting and suitable way for its administration by promising to extend the release and period of action of this drug. The system could further benefit from the use of natural biodegradable polymers in order to prevent chronic toxicity encountered after parenteral administration of non-biodegradable polymers.

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