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Research Article

Formulation and *Invitro* Characterization of Neomycin Loaded Chitosan Nanoparticles

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Abstract

The main objective of this work is to formulate and evaluate of neomycin loaded chitosan nanoparticles and to extend the drug release time by the preparation of Nanoparticles using ionic gelation method. In present work different formulations were prepared by using different ratios of polymer, and sodium tri polyphosphate (cross linking agent). Prepared Nanoparticle was evaluated for its Particle Size, zeta potential, scanning electron microscopy, Percentage practical yield, Drug Entrapment Efficiency, anti-microbial studies and *In-Vitro* drug release studies. The optimized CSNPs was found percentage practical yield was 87.7%. Entrapment efficiency (%EE) of 65.5%, scanning electron microscopy round shape and smooth surface. The *in-vitro* release profile was found to be 96.65% sustained up to 330 minutes. Thus, incorporation of Neomycin into CSNPs results in enhanced the drug release when compared to pure drug.

Keywords: Neomycin Sulphate; Sodium Tripolyphosphate; Chitosan; Ionic Gelation Method; Anti-Microbial Studies

Abbreviations

Cs: Chitosan; Stpp: Sodium Tripolyphosphate; NP: Nanoparticles

Introduction

Definition

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 1 - 1000 nm [1].

Chitosan Nanoparticles

Chitosan is a polysaccharide comprising copolymers of glucosamine and N-acetyl glucosamine and can be derived by the partial deacetylation of chitin. It is a biodegradable, biocompatible and hydrophilic polymer of low toxicity. It is a material found in abundance in shells of crustacean such as lobsters, prawns and crabs. It is insoluble under alkaline and neutral conditions, but can react with inorganic and organic acids such as hydrochloric acid, lactic acid, acetic acid and glutamic acid under acidic conditions. It has OH and NH2 groups that give rise to hydrogen bonding and these groups could act as nucleophilic agent to initiate the polymerization of methylmethacrylate leading to an irreversible attachment between chitosan and methylmethacrylate through different multipoint linkages [2]. The cationic polyelectrolytic nature of chitosan could interact with a negatively charged mucosal surface. It was also confirmed that coating liposomes with chitosan improved their adsorption to mucosal surface. Chitosan has been used as a nanoparticle material owing to its versatile biodegradability, biocompatibility, and natural origin. Its hydrophilicity and solubility permit the design of nanoparticles capable of protecting the loaded drug and controlling its release [3].

1. Nanoparticles are defined as particles sized below 1 mm and can consist out of different biodegradable materials like natural or synthetic polymer, lipid or phospholipids. The drug is dissolved, entrapped, encapsulate or attached to a nano particle matrix. Submicron particles possess very high surface volume ratios.
2. Polymer nanoparticles offer some specific advantages over liposome for instance; they increase the stability of drug and possess useful controlled release properties.
3. Nanoparticulate systems for improved drug delivery are the recent advances in nano medicine. Nanoparticulate systems show their promise as a potential ideal drug delivery system for poorly soluble, poorly absorbed and labile substances [4].

Furthermore, it possesses positively charge and exhibits absorption enhancing effect. These properties render chitosan a very attractive material as a drug delivery carrier. Controlled drug delivery systems offer numerous advantages over conventional dosages forms, including improved efficacy, reduce toxicity, and improved patient compliance, and can be utilized in the form of nanocarriers in drug delivery. They consist of macromolecular materials in which the active ingredient is dissolved, entrapped, encapsulated, adsorbed or chemically attached. They may be prepared from a variety of materials such as proteins, polysaccharides and synthetic polymers. The selection of materials is dependent on many factors including, Size of nanoparticles required, inherent properties of the drug, e.g. solubility and stability, surface characteristics such as charge and permeability, degree of biodegradability, biocompatibility and toxicity; and drug release profile desired. Nanotechnologies are attractive candidates for the delivery of, amongst others, antibiotics in infections caused by bacteria [5].

Among water-soluble polymers available, chitosan is one of the most extensively studied. This is because chitosan possesses some ideal properties of a polymeric carrier for nanoparticles such as biocompatibility, biodegradability, non-toxicity, and low cost. It possesses a positive charge and exhibits an absorption enhancing effect. This characteristic can be employed to prepare cross-linked chitosan nanoparticles. The mechanism of Chitosan nanoparticles formation is usually based on electrostatic interaction between the amine group of chitosan and a negatively charged group or polyanion such as tripolyphosphate.

Furthermore, it possesses antimicrobial property and adsorbs toxic metals such as mercury, cadmium, lead, etc [6].

Why we go for developing nanoparticles

The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents so as to achieve the site specific action of the drug at the rationale rate and dose. Two Polymeric nanoparticles offer some specific advantages over liposomes. For instance, they help to increase the stability of drugs/proteins and possess useful controlled release properties [7].

Properties of chitosan nanoparticles

CS has been explored as a material of choice to form nanoparticles for the last decade. The properties of chitosan have been enhanced by making their nanoparticles. The unique character of NP for their small size and quantum size effect could make CSNP exhibit superior activities They are simple and inexpensive to manufacture and scale-up and have unique size and large surface-to-volume ratio. They are mucoadhesive and hydrophilic in nature due to which they provide good protection to encapsulated drug, increase its clearance time and stability in the body. Thus they are applicable to a broad category of drugs, small molecules, proteins and polynucleotides. The benefits of encapsulating active agents in a polymer matrix include their protection from the surrounding medium or processing conditions and their controlled release [2].

Advantages chitosan nanoparticles

- Easy to synthesize and characterize
- Inexpensive
- Biocompatible
- Biodegradable
- Non-immunogenic
- Non-toxic
- Water soluble

Nanoparticle delivery systems

- Simple and inexpensive to manufacture and scale-up
- No heat, high shear forces or organic solvents involved in their preparation process
- Reproducible and stable
- Applicable to a broad category of drugs; small molecules, proteins and polynucleotides
- Ability to lyophilize
- Stable after administration
- Non-toxic [8]

Limitations

In spite of these advantages nanoparticles do have limitations like,

- Altered physical properties which lead to particle – particle aggregation, making physical handling of nanoparticles difficult in liquid and dry forms due to smaller size and larger surface area.
- Smaller the particles size greater the surface area and this property makes nanoparticles very reactive in the cellular environment.
- Small particles size results in limited drug loading and burst release.
- These practical problems have to be sorted out before nanoparticles can be used clinically or made commercially available [9].

Mechanism of drug release from particulate system

Drug release from chitosan nanoparticulate systems depends upon the extent of cross-linking, morphology, size and density of the particulate system, physicochemical properties of the drug as well as the presence of adjuvant. *In vitro* release also depends upon pH, polarity and presence of enzymes in the dissolution media. CSNPs releases the drug either by swelling of nanoparticles due to hydration followed by release of drug through diffusion or by an enzymatic reaction resulting in rupture or cleavage or degradation of polymer site of delivery or simply by dissociation of drug from the polymer and its de-adsorption release from swelled nanoparticles. In majority of cases, drug release follows more than one type of mechanism. More the drug loading greater the burst and faster the release rate [10]. To develop a successful nanoparticulate system, both drug release and polymer biodegradation are important consideration factors. In general, drug release rate depends on solubility of drug, desorption of the surface bound/adsorbed drug, drug diffusion through the nanoparticles matrix, nanoparticles matrix erosion/degradation and combination of erosion/diffusion process. Thus solubility, diffusion and biodegradation of the matrix materials govern the release process. Furthermore release rate can also be affected by ionic interaction between the drug and addition of auxiliary ingredients. When the drug forms a less water soluble complex by the interaction with the auxiliary ingredient, then the drug release can be very slow with almost no burst release effect [11].

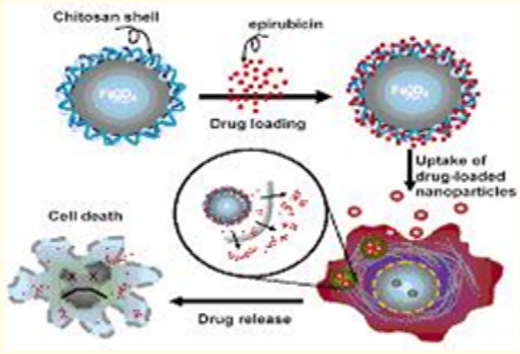


Figure 1: Mechanism of drug release.

Materials and Methods

Method: Ionic Gelation Method

Chitosan nanoparticles were prepared by ionic cross linking of chitosan solution with TPP anions. Chitosan was dissolved in aqueous solution of acetic acid (3 ml) in 100 ml of distilled water. Under magnetic stirring at room temperature, prepared 10 ml of (w/v) S.TPP aqueous solution was added dropwise using syringe needle into 100 ml chitosan solution containing 100 mg of neomycin. The stirring was continued for about 2.30 minutes. The resultant nanoparticles suspensions were centrifuged at 12000 × g for 15 minutes using C24 centrifuge. Samples were washed with methanol and dried. The formation of the particles was a result of the interaction between the negative groups of the TPP and the positively charged amino groups of chitosan (ionic gelation) [12,13].

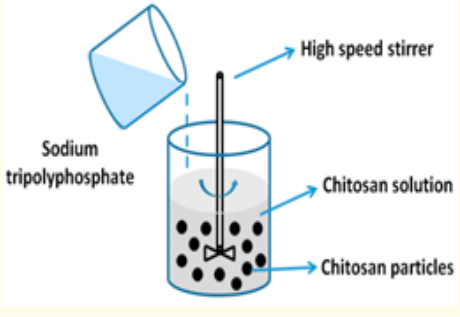


Figure 2: Method of preparation.

Method: Ionic Gelation Method

S. No.	Ingredients	Formulations				
		F1	F2	F3	F4	F5
1.	Neomycin	100 mg	100 mg	100 mg	100 mg	100 mg
2.	Chitosan	100 mg	200 mg	300 mg	400 mg	500 mg
3.	Acetic acid	3 ml	3 ml	3 ml	3 ml	3 ml
4.	Sodium tripolyphosphate	1%	1%	1%	1%	1%
5.	Distilled water	100 ml	100 ml	100 ml	100 ml	100 ml
6.	Methanol	q.s	q.s	q.s	q.s	q.s

Table 1: Formulation of neomycin loaded chitosan nanoparticles.

Physico – Chemical Characterization of Nanoparticles

Solubility studies

The solubility of the neomycin was tested in various solvents including water, ethanol, acetone, ether, chloroform, Glacial acetic acid etc.

Fourier transform infra-red spectroscopy (FT-IR) analysis

The FT-IR spectra of pure neomycin, chitosan and their mixture were recorded using Shimadzu IR spectrophotometer, Model 840, Japan, to check drug polymer interaction and stability of drug [14].

Surface morphology study

Scanning electron microscopy (SEM) of the chitosan Nanoparticle was performed to examine the surface morphology. The nanoparticles were mounted on metal stubs and the stub was then coated with conductive gold with sputter coater attached to the instrument. The photographs were taken using a Jeol scanning electron microscope under magnification of 7500 - 20000×.

Zeta potential

The Zeta-potential of drug loaded nanoparticles was measured by Zeta sizer (Malvern Zetasizer 3000HS, UK). To determine the zeta potential, nanoparticles samples were diluted with water (0.1 ml) and placed in electrophoretic cell where an electrical field of 15.2 V/cm was applied. Each sample was analyzed in triplicate [15,16].

Percentage Practical yield

Dried nanoparticles were collected and weighed to determine practical yield (PY) from the following equation 1.

$$PY(\%) = \frac{nanoparticle\ weight}{theoretical\ mass\ (polymer + drug + s.tpp)} \times 100 \quad \text{------ (1)}$$

Drug entrapment efficiency

Drug content was determined by centrifugation method. The redispersed nanoparticles suspension was centrifuged at 12000 rpm for 15 minutes at 25°C to separate the free drug in the supernatant. Concentration of neomycin in the supernatant was determined by using UV-visible spectrophotometer at 277 nm after suitable dilution. The drug entrapment efficiency (% EE) was determined using the relationship in equation 2 [17,18].

$$EE(\%) = \frac{experimental\ drug\ content}{theoretical\ drug\ content} \times 100 \quad \text{------ (2)}$$

Evaluation of *in vitro* drug release

The neomycin loaded chitosan nanoparticles, after separation by ultracentrifugation, were re-dispersed in 2 ml, 0.1N HCL buffer solution, placed in a dialysis membrane bag, tied and immersed in 100 ml of 0.1N HCL solution in a 250 ml beaker. The entire system was stirred continuously at 37°C with a magnetic stirrer. At pre-determined time intervals, 5 ml of the release medium was removed and replaced with 5 ml of fresh 0.1N HCL solution. The amount of neomycin in the release medium was evaluated by UV Spectrophotometry at 277nm [19-21].

Assessment of antimicrobial activity

The method employed for assessment of antibacterial activity of neomycin nanoparticles is cup plate method The strain selected for the study, *Staphylococcus aureus* (G+ve), and *E. coli* (G-ve) was cultured on the nutrient agar medium. Nutrient agar plates were prepared by pouring 150 ml of autoclaved nutrient agar into sterile Petri dishes. Four cups each of 8 mm diameter were prepared by scooping out medium with a sterilized cork borer. Prior to this, the agar was seeded with the test organisms. The solution of each formulation i.e. 0.06 ml of drug-loaded nanoparticles of different formulations of F1, F2, F3, F4, F5 and commercial formulation of Neomycin sulphate ointment, and drops (0.04 mg/ml)) - were added separately to the cups and the Petri dishes were incubated for 48 h at 37 ± 2°C. Zone of inhibition was determined after 12, 24 and 48 h to evaluate the extent of bacterial inhibition by the preparations. All the readings were performed in triplicate [22-24].

Results and Discussion

Solubility of drug

Neomycin is soluble in water, insoluble in acetic acid.

Compatibility Studies: Drug – Excipients Interaction-FTIR studies

FTIR spectra of the neomycin, chitosan and physical mixture performed using FTIR spectrometer are as follow

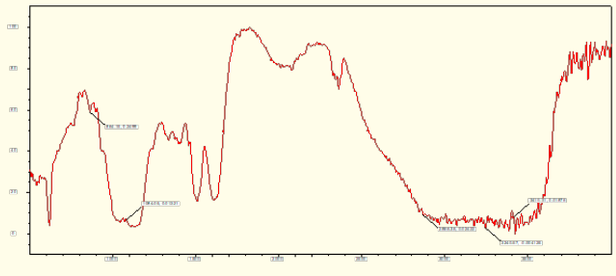


Figure 3: FTIR spectra of Neomycin sulfate.

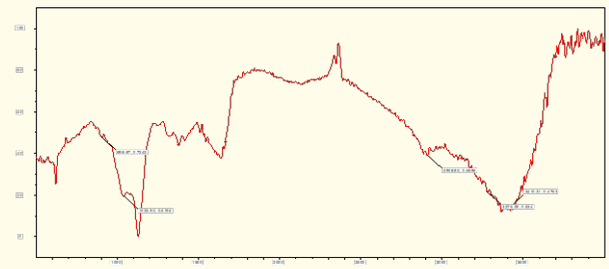


Figure 4: FTIR spectra of Chitosan.

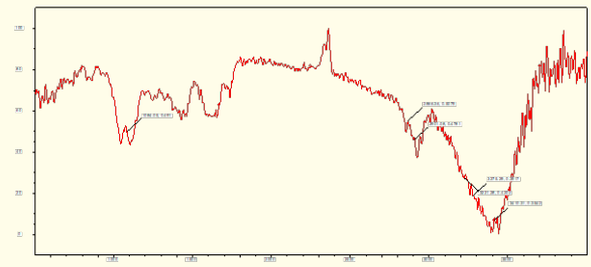


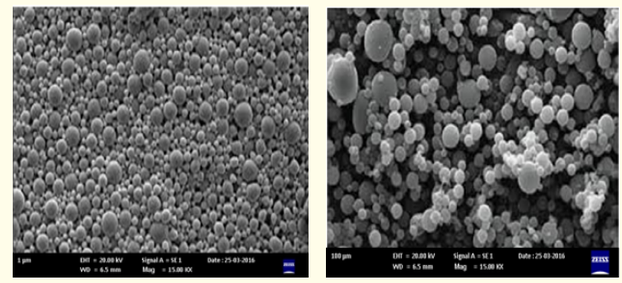
Figure 5: FTIR spectra of Neomycin sulphate and chitosan.

S. No.	Observed range cm^{-1}			Characteristic peak	Functional group
	Neomycin	Chitosan	Physical mixture		
1.	3410.31	3410.31	3410.31	3300 – 3500	N – H
2.	3240.57	3275.29	3275.29	3550 – 3200	O – H
3.	2866.36	2908.80	2866.36	2950 – 2850	C – H
4	1084.05	1033.90	1084.05	1260 – 1000	C – O

Table 2: Drug and excipients interaction study FT IR.

From the above figures and data we conclude that there is no possible interactions between Neomycin and other ingredients within the formulation and the presence of functional groups are within the range. So this may not affect the formulation stability during its shelf life.

Morphology: SEM Analysis



(a) (b)

Figure 6: SEM images of the nanoparticles a) plain naoparticles b) drug loaded nanoparticles

Elucidates the morphology of chitosan nanoparticles. The nanoparticles were spherical non-aggregated with uniform size and smooth surface.

Percentage Yield

S. No	Formulation	Percentage yield
1	F_1	63.6%
2	F_2	75.6%
3	F_3	87.7%
4	F_4	67.2%
5	F_5	80.1%

Table 3: Percentage yield.

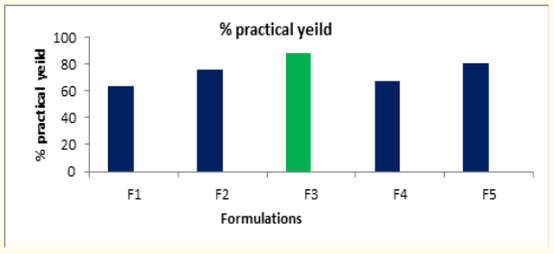


Figure 7: % percentage yield.

When different formulations were prepared with a varying ratio of drug and polymer, there was no much difference in the percentage yield of the product. All the five formulations showed good percetnage yields between 63.6 - 87.7%. Where the formulation F3 showed a maximum % practical yeild of 87.7%.

Drug Entrapment Efficiency

S. No	Formulation	% DEE
1	F_1	54.3%
2	F_2	56.1%
3	F_3	65.5%
4	F_4	52.9%
5	F_5	61.6%

Table 4: Drug entrapment efficiency.

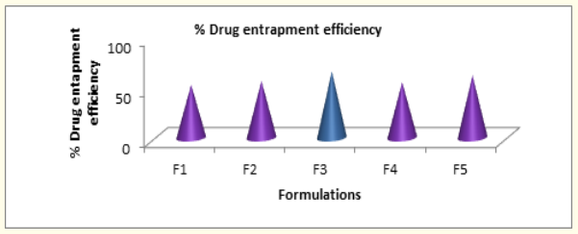


Figure 8: Entrapment efficiency.

The average percent drug entrapment efficiency of the the Five formulations ranges from to 52.3 -6 5.5% where the formulation F_3 showed a maximum drug entrapment of 65.5%.

Drug Release Studies

Calibration Curve of Neomycin Sulphate

100 mg of the drug was dissolved in the 100 ml of the distilled water the concentration of the above solution was 1000 µg/ml and it is noted as stock 1. From the above stock 1 sample 1 ml of the sample was taken and makeup to the 10 ml in the volumetric flask and the concentration of the above solution was find to be 100 µg/ml and it is noted as the stock 2 from this series of the sample was taken such a 1 ml, 2 ml 3 ml, 4 ml, 5 ml and make it up to the 10 ml and the concentration of the above sample was found to be 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml and 50 µg/ml. And the absorbance of the samples was check through the UV-spectroscopy in triplicate.

S. No.	Concentration (µg/ml)	Absorbance
1	10	0.066
2	20	0.131
3	30	0.195
4	40	0.251
5	50	0.31

Table 5: Calibration curve data of Neomycin sulphate.

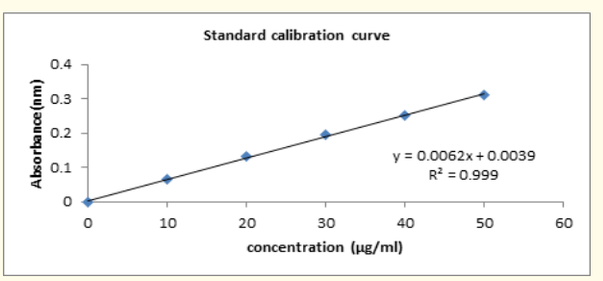


Figure 9: Standard calibration curve of Neomycin sulphate.

Drug release profile of neomycin sulphate loaded chitosan nanoparticles

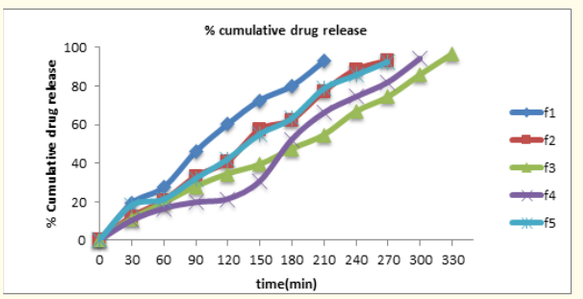


Figure 10: Cumulative drug release.

Time (min)	Cumulative Drug Release				
	F1	F2	F3	F4	F5
30	19.05	12.18	11.12	10.13	18.02
60	27.35	20.41	19.22	16.42	21.09
90	45.95	32.98	27.87	19.62	31.99
120	60.20	40.83	34.42	21.32	42.04
150	72.54	57.31	39.31	30.54	55.01
180	80.06	62.43	47.43	52.24	63.24
210	92.97	77.10	54.62	66.14	78.62
240		88.42	66.72	74.42	85.72
270		93.00	74.22	82.06	92.63
300			85.93	94.32	
330			96.65	.	

Table 6: Cumulative drug release.

When five formulations were carried for the *in-vitro* drug release studies, all the five formulations showed good release F_3 formulation released the drug for an extended peroid of time and were considered as optimised formulations.

Anti-Microbial Studies

Anti-bacterial studies of Staphylococcus aureus (Gram positive)

Anti-bacterial studies of Escherichia coli (Gram negative)

Figure 11: Antibacterial studies of nanoparticles and marketed formulations

S. No.	Formulations	Zone of inhibition (diameter in mm)	
		Staphylococcus aureus (Gram positive)	*Escherichia coli* (Gram negative)
1	F1	3.3	3.5
2	F2	2.8	3.5
3	F3	3.0	3.1
4	F4	3.2	3.5
5	F5	3.0	3.3
6	Marketed formulation (Ointment)	3.5	3.0
7	Marketed formulation (Eye drops)	2.5	3.5

Table 7: Zone of inhibition of nanoparticles and marketed formulations.

Elucidates the zone of inhibitions of prepared nanoparticles and marketed formulations. Stating that nanoparticles possessed antimicrobial property among the five formulation F3 showed the zone of inhibition similar to that of marketed formulations i.e. neomycin ointment and eye drops.

Conclusion

The present study has been a satisfactorily attempt to formulate Nanoparticles of Neomycin sulphate with chitosan by Ionic gelation method. Chitosan can be used to formulate an efficient nanoparticulate system with acceptable results like good entrapment efficiency of 52.3 - 65.5% and practical Percentage yield of 63.6 - 87.7%. Pertaining to *in-vitro* drug release studies all the five formulations showed good release. Among all the formulations the F3 formulation showed good release of 96.65% within 5 - 6 hrs in a controlled manner. All the formulations were studied the F3 formulation is optimized. Morphology of chitosan nanoparticles were spherical, non-aggregated, uniform size and smooth surface area. antibacterial property F3 formulation showed the zone of inhibition similar to marketed formulations. Hence the present study was a successful attempt to formulate and extend the drug release of Neomycin sulphate by nanoparticulate system with a view of conventional delivery of a drug.

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