ISSN: 0975-766X
CODEN: IJPTFI

Available Online through ***Research Article***
www.ijptonline.com

SYNTHESIS, CHARACTERIZATION AND *IN VITRO* RELEASE PROFILE OF GENTAMICIN LOADED CHITOSAN NANOPARTICLE

Kushagri Singh[1], Amit Singh[2] and Abha Mishra[1]*
1. School of Biochemical Engineering, Indian Institute of Technology (BHU), India.
2. Department of Pharmacology, Institute of Medical Sciences, Banaras Hindu University, India.
Email: abham.bce@gmail.com

Received on: 02-03-2017 *Accepted on: 05-04-2017*

Abstract

Chitosan has prompted the continuous movement for the development of safe and effective drug delivery systems because of its unique biological and physiochemical characteristics. Gentamicin loaded chitosan nanoparticles were prepared for the treatment of microbial infections. Nanoformulation of gentamicin was prepared using 85% deacetylated chitosan as a biodegradable polymer and tripolyphosphate as a crosslinking agent by ionotropic gelation method. It was further evaluated and characterized on the basis of morphology, drug loading efficiency, zetapotential value, FTIR study and also *In-Vitro* release behavior of CS1 formulation of gentamicin. The FTIR spectral studies indicated that there was no interaction between the drug and chitosan. The formulation CS1 was found stable with good drug entrapement efficiency, fair zeta potential value and its size ranged between 30-60nm. The in-vitro study showed sustained release with steady rise in cumulative drug release (> 90%) upto about 4h and thereafter no significant release observed. The characterized formulations were also used to study antimicrobial activity.

Key words: Chitosan, Nanoparticles, Gentamicin, Ionotropic Gelation, Cumulative drug release.

Introduction

Drug delivery and development involve highly challenging, laborious, and expensive processes. Many drugs in clinical phase, fails to achieve favorable clinical outcomes because they do not have the ability to reach the target site of action. A significant amount of the drug administered is distributed over the normal tissues or organs which are not involved in the pathological processes, often leading to severe side effects. Therefore, an effective approach to overcome this critical issue is the development of targeted drug delivery systems that releases the drug at the desired site.(1-4).

The targeted dug delivery system is comprised of three components: A therapeutic agent, a targeting moiety and a carrier system. The drug can be either incorporated passively via absorption or through chemical conjugation into the carrier system. Range of materials such as natural or synthetic polymers, lipids, surfactants and dendrimers, has been employed as drug carrier. (5-8). Among these, polysaccharides have received increasing attention because of their outsanding physical and biological properties. Chitosan is a cationic linear copolymer polysaccharide made up of random distribution of β(1→4) linked 2-amino-2-deoxy-D-glucose(D-glucosamine) and 2-acetamido-2-deoxy-D-glucose(N-acetyl-D-glucosamine) units. Presence of large percentage of nitrogen (6.89%), chitosan shows much commercial interest than synthetically substituted cellulose (1.2%). This is responsible for the chelating properties of chitosan. Chitosan, a linear aminopolysaccharide obtained by the deacetylation of chitin, a natural polysaccharide found in the exoskeleton of crustaceans such as crabs and shrimps. (9)

Chitosan in drug delivery:

The drug delivery systems offer many advantages in therapy which include:

a) Reduces toxicity

b) Increases therapeutic index of drug

c) Prevents frequent, expensive and unpleasant dosing.

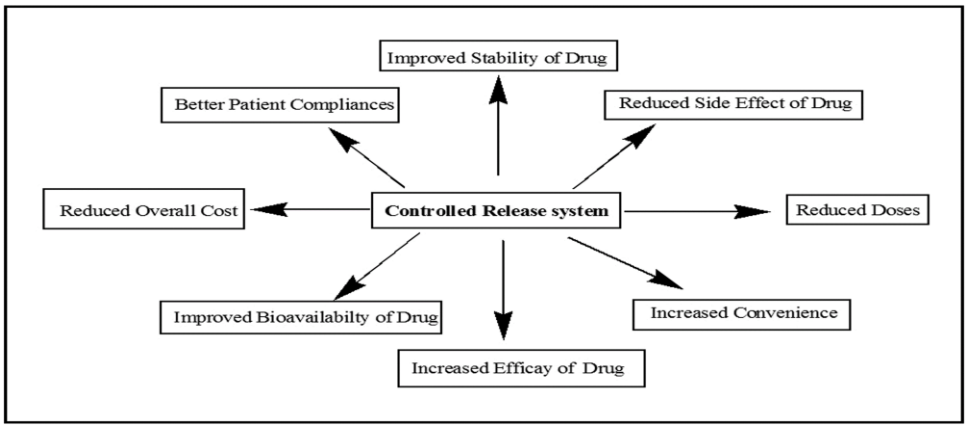


Fig 1.Advantages of controlled drug delivery systems (10).

Polymer based drug delivery systems can improve the pharmacokinetic of a drug, improves their therapeutic index, decreases their side effects and hence increase the efficiency of the whole system. Biodegradable and biocompatible polymers are best suggested for this application because of the need of appropriate release of the drug as well as easy removal of the carrier after drug administration. (11-14)

The polycationic behavior of chitosan enables it to interact with the negatively charged mucous membrane and thereby increases the adhesion to the mucosa and as a result enhances the time of contact for penetration of the drug molecules through it. (15). Nanoparticles of chitosan with tripolyphosphate have excellent capacity for association with hydrophobic drugs such as Gentamicin loaded with the chitosan nanoparticles displayed high stability with rapid release kinetics. These make chitosan nanoparticles an interesting system for drug delivery of poorly water soluble drugs.

Gentamicin

Gentamicin, sold under brand names **Garamycin** among others, is anantibiotic used to treat many types of bacterial infections. This may includebone infections, endocarditis, pelvic inflammatory disease, meningitis, pneumonia, urinary tract infections, and sepsis among others. Even though the therapeutic efficacy of numerous antimicrobial drugs has been well established, inefficient drug delivery can result in the inadequate therapeutic index (16). Gentamicin sulphate is a water-soluble antibiotic of the aminoglycoside group, derived from Micromonospora purpurea, an actinomycete (17). Inhabitation of protein synthesis and induction of a significant increase in misreading of messenger RNA is the mechanism of gentamicin action. Although the gentamicin is the first choice aminoglycoside for the treatment of serious infections but because of its important side effects, mostly related to nephrotoxicity and ototoxicity, its usage is restricted in the recent years (18,19). The structure of gentamicin was shown in figure 2.

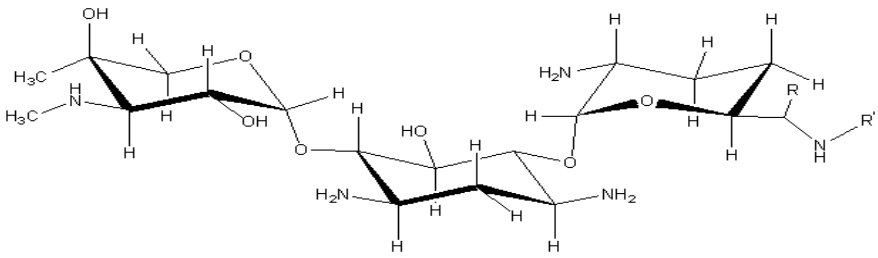


Fig 2. Structure of Gentamicin.

Improvement in gentamicin pharmacokinetic profile with the aim to reduce the harmful effects and to increase the effectiveness of the drug via achievement and maintenance of a safe and efficacious drug level have been the ideal goals in all the therapeutic strategies concerning gentamicin. The aim of the present study was to prepare biocompatible & biodegradable chitosan nanoparticles loaded with gentamicin and to evaluate their potential as drug delivery vehicle. A set of CS nanoparticles were prepared using different concentrations of polymer via ionotropic gelation method and then analyzed/characterized on the basis of size, surface morphology, zetapotentiality, FTIR plot study, antimicrobial activity and in vitro release behavior.

Materials & Methods

Chitosan with molecular weight of 200kD & TPP of Sodium salt purchased from Sigma Aldrich, Mumbai, India. The minimum degree of deacetylation of chitosan was 85 %(the data provided by the company). Gentamicin was obtained as a gift from Hi-Media laboratory, Mumbai, India. All other reagents used were of analytical grade.

Chitosan nanoparticle preparation

Chitosan nanoparticle was prepared using ionotropic gelation method by Calvo et al, 1997(20-21). 1% acetic acid solution was prepared in distilled water. Different concentrations of chitosan solution were prepared by dissolving chitosan in concentrations such as 1mg/ml, 2mg/ml & 3mg/ml in 1% acetic acid solution. 1mg/ml concentration of gentamicin added to each chitosan solution. 100ml of TPP solution was prepared by dissolving 0.85gms of TPP in 100ml distilled water. TPP solution was added drop-wise using syringe to chitosan solution in the ratio 1:2 under constant magnetic stirring for 1 hour. The resulting suspension was subsequently centrifuged at 15,000RPM for 20mins. The pellets obtained were freeze dried using lyophilizer. The dried pellets were collected for characterization and *in-vitro* release study.

Measurment of particle size & morphology study

The gentamicin loaded nanoparticle size & morphology was determined by Scanning electron microscope study (Department of Metallurgical Engineering, IIT(BHU),Varanasi) under low vaccum condition.

Determination of Zetapotential

The zetapotential of Gentamicin – loaded chitosan nanoparticles was measured on a Zetasizer (Malvern Instruments) by determining the electrophoretic mobility in microelectrophoresis flow cell. All the samples were measured in water at 25°C in triplicates.

Determination of Entrapment efficiency

The encapsulation efficiency of nanoparticles was determined by first separating the nanoparticles formed from the aqueous medium by ultracentrifugation at 15,000RPM for 30mins.

The amount of free gentamicin in the supernatant was measured by UV spectrophotometer at 257nms. The gentamicin entrapped in the nanoparticles was calculated as:

$$Entrapment\ Efficiency\ (\%) = (T_p\text{-}T_f)\ 100/T_p$$

Where T_p is the total gentamicin used to prepare the nanoparticles and T_f is the free gentamicin in the supernatant.

Practical yield calculation & In-Vitro release profile

Freeze dried nanoparticles were collected and weighed to determine practical yield (PY) from following equation:

$$PY\% = \text{Nanoparticle weight} / \text{Theoretical mass (polymer+drug+TPP)} \times 100$$

For the FTIR study a specified quantity of potassium bromide and samples were blended uniformly. The resultant blend was then compressed to prepare the pellet as desired. The pellet was subjected for the analysis.

Assessment of antimicrobial activity

Nutrient agar media was prepared and autoclaved. The strain selected for the study, was *Pseudomonas aeroginosa.* The bacterial strain was cultured on the Nutrient agar. Nutrient agar plates were prepared by pouring 20ml of the media into a sterile petridish. Bacterial culture was spread over the media and incubated at 37°C for 2 h for stabilization. Further 50µl of free drug and gentamicin loaded chitosan nanoparticles were dropped over the plate on the marked points. Plates were then kept for incubation at 37°C for 48h. Zone of inhibition were determined for both samples after 24 and 48hrs to evaluate extent of microbial inhibition by the preparation.

Results & Discussion

The most satisfactory nanoparticles of chitosan were obtained at a chitosan concentration of 1 mg/ml in 1% acetic acid and TPP of 0.85%(w/v) in d/w.

Measurement of particle size & morphology study

It is evident from the SEM study of nanoparticles sample that size ranges between 30-60nm for the sample with 1mg/ml chitosan concentration and with the increase in chitosan concentration size of nanoparticles also increases. Further clear, spherical and uniform morphology was observed (Fig 3).

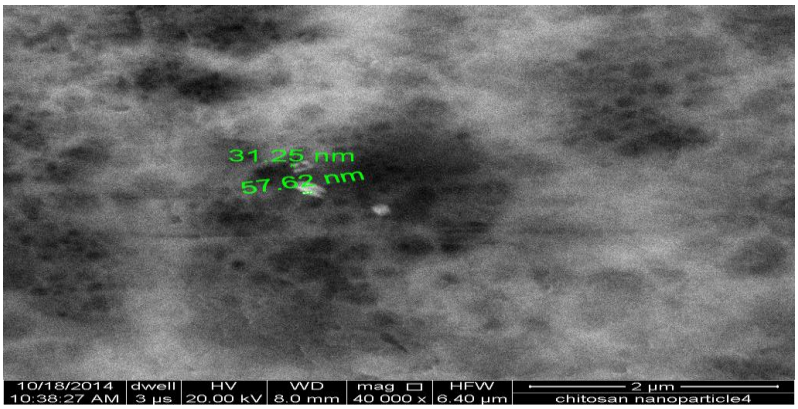


Fig 3. SEM images of gentamicin loaded chitosan nanoparticles.

Entrapment Efficiency

The results of entrapment efficiency (Fig 4) revealed that drug entrapment efficiency is dependent on the polymer concentration (Table 1). The entrapment efficiency found to be maximum for the CS I formulation and it decreases with increasing polymer concentration at physiological pH and temperature.

Table 1. Entrapment efficiency of different concentrations of polymer and drug.

Serial No.	Chitosan concentration (mg/ml)	Drug concentration(µls/ml)	Entrapment Efficiency (%)
1. CS I	1	2.0	87.1
2. CS II	2	2.0	66.9
3. CS III	3	2.0	49.2

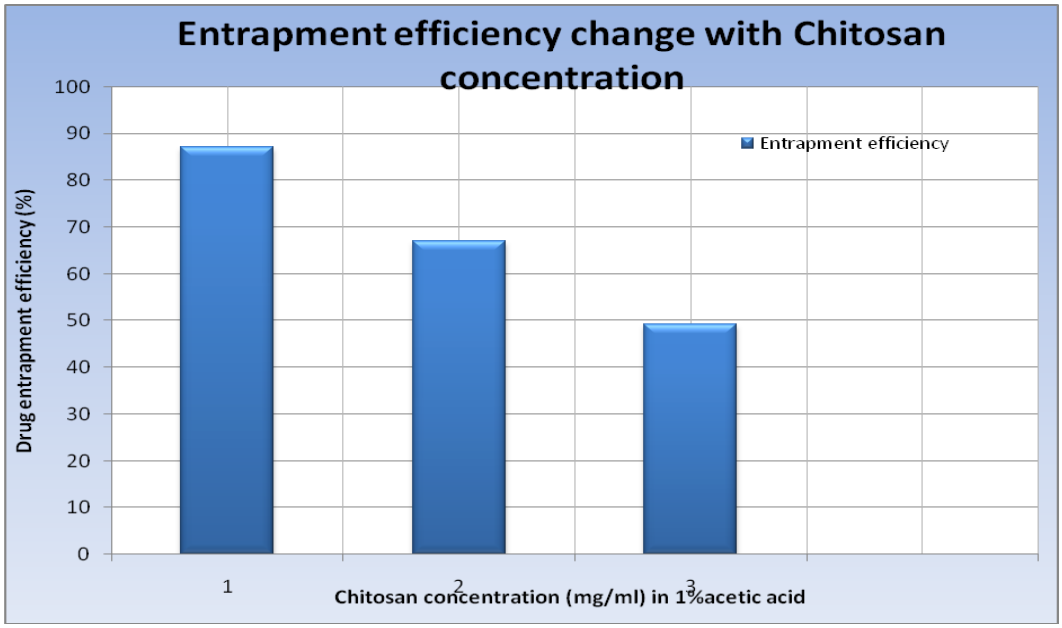


Fig 4. Bar diagram showing effect of chitosan concentration on efficiency of drug entrapment by nanoformulation at physiological pH and temperature.

Practical yield and in-vitro release profile

Practical yield for gentamicin loaded CS1 nanoparticle was found to be 39.07%. The *in-vitro* release profile of gentamicin loaded chitosan nanoparticles formulation indicates that the CS1 formulation exhibit sustained release with a steady rise in cumulative drug release (>90%) upto 4 hours. Thereafter, no significant release observed (Fig 2).

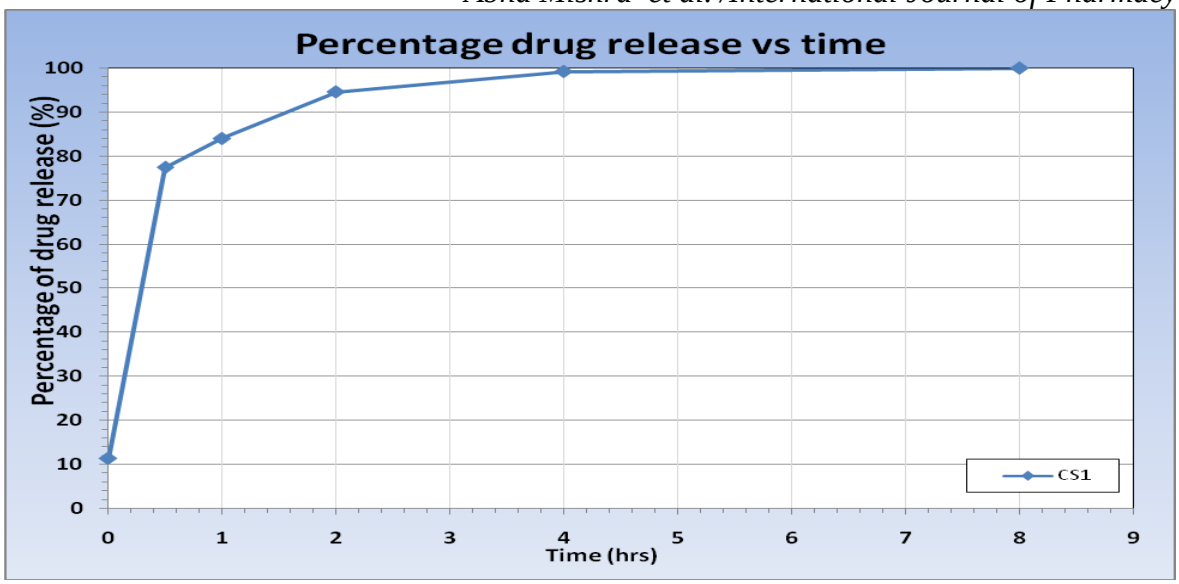
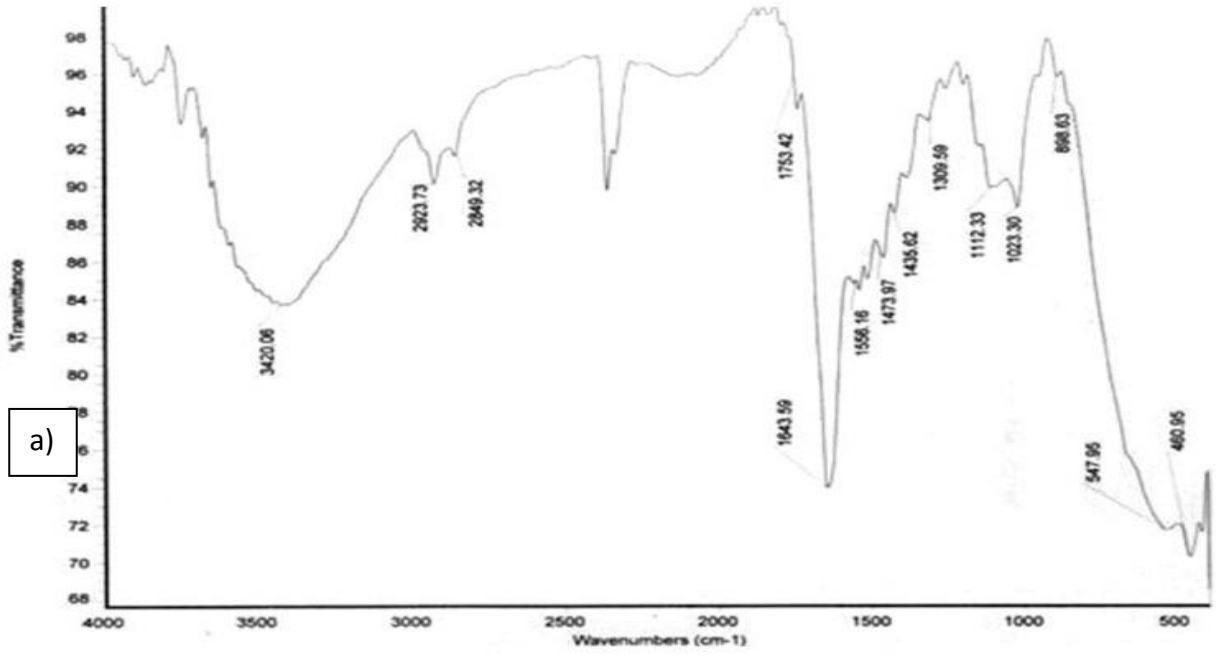


Fig 5. Cumulative in-vitro Gentamicin release profile from CS1 nanoparticle formulation upto 8h.

Fourier transform infrared study

The ability of the ionotropic gelation process to gentamicin-loaded chitosan nanoparticles was assessed by employing FTIR to determine gentamicin-chitosan interactions. The FTIR spectra of chitosan matrix (a)(22), and gentamicin loaded chitosan nanoparticles(b) are shown in Fig 6.It may be suggested that the tripolyphosphoric groups of TPP are linked with ammonium groups of chitosan in spectra (b). The inter- and intra- molecular interactions are enhanced in chitosan nanoparticles but there was not much difference in the peaks found in drug loaded nanoparticles. Therefore, It can be predicted that gentamicin entrapped in chitosan nanoparticles without any interaction.



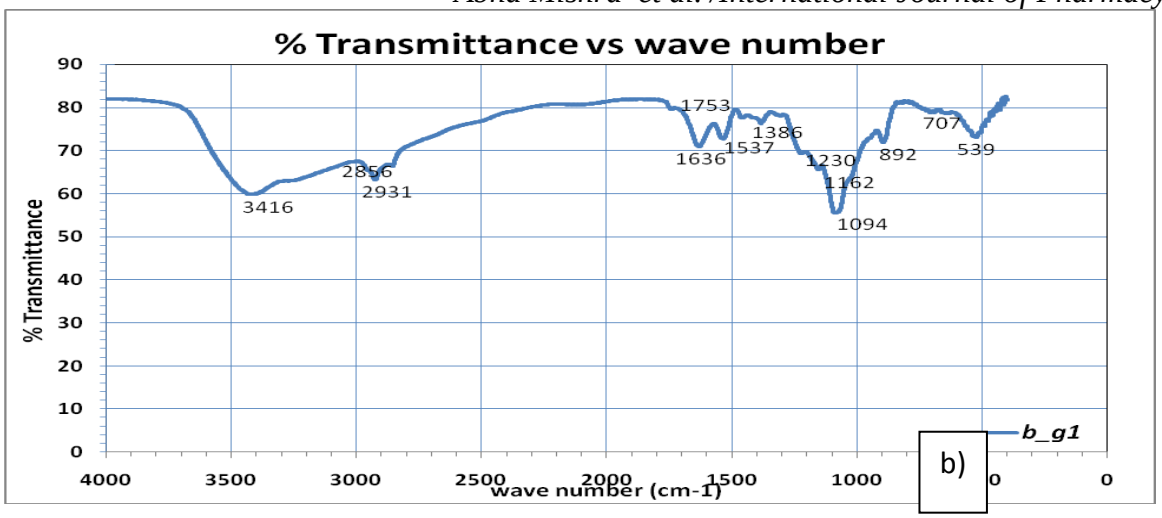


Fig 6. ***FTIR Spectra of chitosan matrix (a), & gentamicin loaded chitosan nanoparticle(b).***

Zetapotential determination

Zetapotential value for the chitosan nanopaticle was found to be +3.14mV. Zetapotential value provides an important criterion for the stability of colloidal system. This is the electric potential that exists at the shear plane of a particle, which is related to both the surface charge and local environment of the nanoparticle. Value of zetapotential between +10mv to -10mv indicates that the particles are neutral and stable in nature.

Antimicrobial acitivity

Fig(7) indicates that all the test preparations containing the gentamicin loaded chitosan nanoparticles, including the free drug gentamicin demonstrated that equal zone of clearance was seen in both the cases for upto 48 hours of incubation. Therefore the antimicrobial activity of both the drug as well as drug loaded nanoparticles is nearly same.

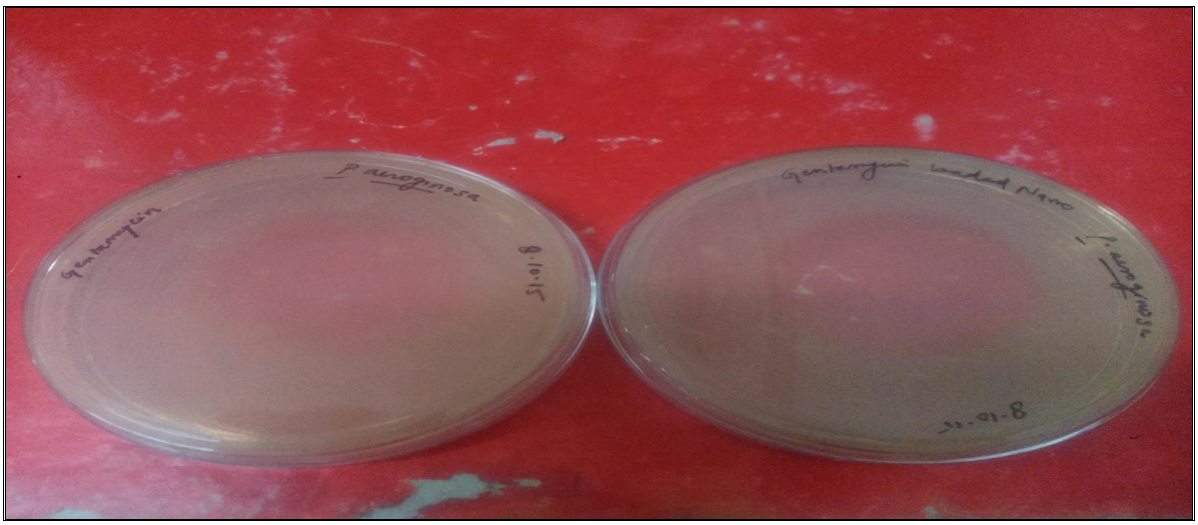


Fig 7. ***Zone of clearance in the lawn of Pseudomonas sp. by free drug and drug loaded chitosan nanoparticle after 48h of incubation.***

Conclusion

This work confirms that the stable gentamicin loaded chitosan nanoparticles formulation is useful and efficient enough to treat antomicrobial infections. They are prepared to maintain a controlled and effective release of drug and thereby reduces toxicity and patient compliance. With this work, three different formulations of chitosan nanoparticles were developed using different concentrations of polymer and they showed different entrapement efficiencies. In- vitro drug release behavior of CS1 was also observed and >90% of the drug released in 4 hours. Three formulations were evaluated where CS1 was found to be most suitable for the delivery of gentamicin because of its small particle size with more surface area, fair zetapotential value and good entrapment efficiency. Antimicrobial activity of both the drug and gentamicin loaded nanoparticles was found to be same.

Acknowledgements

Author would like to acknowledge for the financial support from the Department of Science & Technology-INSPIRE fellowship, New Delhi, India. Prof. R.K. Mandal (Department of Metallurgical Engineering, IIT,BHU,Varanasi) for providing SEM facility & Dr.S.K.Singh (Department of Pharmaceutics, IIT,BHU,Varanasi) for providing Zetapotential and FTIR facility.

References

1. J.A. DiMasi, R.W. Hansen, H.G. Grabowski, The price of innovation: new estimates of drug development costs, J. Health Econ. 22 (2003) 151–185.
2. R. Langer, Drug delivery and targeting, Nature 392 (1998) 5–10.
3. G. Poste, R. Kirsh, Site-specific (targeted) drug delivery in cancer therapy, Biotechnology 1 (1983) 869–878.
4. G. Gregoriadis, Targeting of drugs, Nature 265(1977) 407–411.
5. K. Strebhardt, A. Ullrich, Paul Ehrlich's magic bullet concept: 100 years of progress, Nat. Rev. Cancer 8(2008) 473–480.
6. J.K. Vasir, M.K. Reddy, V.D. Labhasetwar, Nanosystems in drug targeting: opportunities and challenges, Curr. Nanosci. 1 (2005) 47–64.
7. R. Duncan, The dawning era of polymer therapeutics, Nat. Rev. Drug Discov. 2 (2003) 347– 360.
8. R. Duncan, Polymer conjugates for drug targeting. Frominspired to inspiration! J.Drug Target 14 (2006) 333–335.

9. M.N. Kumar, R.A. Muzzarelli, C. Muzzarelli, H. Sashiwa, A.J. Domb, Chitosan chemistry and pharmaceutical perspectives, Chem. Rev. 104 (2004) 6017–6084.
10. Saikia C, Gogoi P, Maji TK (2015) Chitosan: A Promising Biopolymer in Drug Delivery Applications. J Mol Genet Med S4:006. doi:10.4172/1747-0862.S4-006.
11. Smolen VF (1978) Bioavailability and pharmacokinetic analysis of drug responding systems. Annu Rev Pharmacol Toxicol 18: 495-522.
12. Farokhzad OC, Langer R (2009) Impact of nanotechnology on drug delivery. ACS Nano 3: 16-20.
13. Liechty WB, Kryscio DR, Slaughter BV, Peppas NA (2010) Polymers for drug delivery systems. Annu Rev Chem Biomol Eng 1: 149-173.
14. Hanou M, Verhoef JC, Junginger HE (2001) Chitosan and its derivatives as intestinal absorption enhancers. Adv Drug Deliv Rev 50 Suppl 1: S91-101.
15. Funkhouser JD, Aronson NN Jr (2007) Chitinase family GH18: evolutionary insights from the genomic history of a diverse protein family. BMC Evol Biol 7: 96.
16. Ahangari A, Salouti M, et al, 2013. Development of gentamicin-gold nanospheres for antimicrobial drug delivery to Staphylococcal infected foci. Drug Deliv, 20(1): 34–39.
17. Goitein, K., J. Michel, and T. Sacks, 1975. Penetration parenterally administered gentamicin into the c erebrospifluid in experimental meningitis. Chemotherapy, 21: 181- 188.
18. Tulkens, P. M, 1986.Experimental studies on nephrotoxicity of aminoglycosides at low doses. Mechanisms and perspectives. American Journal of Medicine, 80: 105–14.
19. Royal Pharmaceutical Society, 1997. British Medical Association, London and The Pharmaceutical Press, Wallin-gford, Oxon, p. 230.
20. P. Calvo, C. Remuan –Lopez, J.Vila-Jato, M.Alonso, 1997. J Appl polym sci 63: 125132.
21. P.Calvo, C.Remuan-Lopez, J.Vila-Jato, 1997. Pharm Res 14: 1431-1436.