

FORMULATION AND EVALUATION OF BIOPOLYMER BASED MICROSPHERES FOR NASAL DRUG DELIVERY

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ABSTRACT: Nasal drug delivery has been recognized as a very promising route for delivery of therapeutic compounds. In the present work microspheres are designed by emulsification technique to provide the absorption of high polar drug through nasal mucosa. Gentamicin Sulphate is used as model drug, by varying the Drug/Polymer ratio with or without absorption enhancer. Chitosan and HPMC were used as mucoadhesive polymers. The prepared microspheres of all the formulations were evaluated for particle size, encapsulation efficiency, shape and surface properties, drug-polymer interaction, mucoadhesive property, stability and in vitro drug release. The present study concludes that, the prepared microspheres can be used to achieve nasal drug delivery system and improved bioavailability.

Key words: Chitosan, HPMC, Bioadhesive Microspheres, Gentamicin Sulphate.

INTRODUCTION

The concept of the advanced drug delivery systems especially those offering a sustained and controlled action of drug to desired area of effect, attained greater appeal. The microparticulate delivery systems are considered and accepted as a reliable means to deliver the drugs to target site with specificity, if modified, and to maintain the desired concentration at the site of interest without untoward effect(s). The microspheres are characteristically free flowing powders, which are biodegradable in nature, and ideally having the particle size less than 200 μm . Solid biodegradable microspheres containing the drug dispersed or dissolved through the polymer matrix have the potential for the controlled release of drug. The nasal route of drug delivery is convenient for administering active pharmaceutical agents. Mucosa has a protective function against foreign material penetration [1]. The systemic therapy has greater advantages for certain drugs that get metabolized in gastrointestinal tract, or by first-pass metabolism. The rate of absorption from the nasal cavity is quite rapid and offers improved bioavailability. The absorption of drug from nasal

mucosa is also influenced by the contact time between drug and epithelial tissue. This contact time is dependent upon the clearance of drug formulation from the nasal cavity. The mean clearance is about 25 min. The short half-life is caused by rapid clearance from the nose to the throat by ciliary movement [2]. The nasal mucoadhesive drug delivery systems have several biological and pharmaceutical considerations, applications in delivery of small organic molecules, antibiotics, proteins, vaccines. The bioadhesive microparticles of Gentamicin using chitosan hydroglutamate, hyaluronic acid and of its combination by solvent evaporation method. The particles were administered via insufflators to rabbits. The Gentamicin was also administered as a solution and powder, intravenously, and intramuscularly. The in vivo studies showed that the bioavailability of Gentamicin was poor when administered as a nasal solution (1.1%) and dry powder (2.1%) when compared to intravenous. However the microparticles showed less bioavailability compared to intravenous, there was enhancement in bioavailability when

compared to solution and dry powder [3,4]. The influence of absorption enhancers like sodium deoxycholate, hydroxypropyl β cyclodextrin, sodium caprate, sodium tauroglycholate on nasal absorption of Acyclovir. They found the absorption increased with use of enhancer. Hydroxypropyl β Cyclodextrin was more effective than other [5]. Microspheres containing either Amoxicillin or Clarithromycin via interpolymer complexation of poly (acrylic acid) with poly (vinyl pyrrolidone), the loading efficiency of clarithromycin in microspheres was higher than the Amoxicillin due to the stronger interaction of Clarithromycin with the poly (acrylic acid) [6]. The mucoadhesive drug delivery system intended for intravesical application. The microspheres were prepared using different polymers like chitosan hydrochloride, sodium carboxy methyl cellulose, polycarbophil [7]. Mucoadhesive micro capsules of Glipizide, using various polymers carried out in vitro and in vivo evaluation. The microcapsules were made to achieve controlled release and drug targeting which prolonged the residence time of dosage form at the site of action [8]. The mucoadhesive microspheres of Gentamicin for nasal administration [9]. Biodegradable microspheres of Metoprolol tartrate using chitosan by phase separation emulsification technique. Drug and carrier ratio was varied [10]. To increase the gastric residence time using inter polymer complexation of poly acrylic acid) with poly vinyl pyrrolidone [11]. The starch microspheres in aqueous drug solution followed by solvent evaporation or lyophilization, the microspheres provided the best platform as the material adheres to the nasal mucosa [12]. The effect of starch microspheres on absorption enhancing efficiency by various enhancer systems in formulation with insulin. the bioadhesive starch microspheres and absorption enhancers act synergistically [13]. The microspheres of hydrophilic polymers by emulsification solvent evaporation technique for nasal administration through insufflation [14]. The soluble polymers in microparticulate for improved, vaccine response in mucosal delivery [15]. The mucoadhesive microspheres containing Beclomethasone for prolonging the drug action or reducing drug dosage forms. Bioadhesive excipients were used to prolong the residence time of formulation. The formulation was subjected to physico-chemical characterization and also for stability. The stability studies were carried out for 6 months at different temperature and relative humidity [16]. The effect of polyvinyl pyrrolidone, on physical characteristics of Ketoprofen-loaded polystyrene microparticles. The mean diameter of particles remained narrow and there was increase in diameter of particles with increase in drug loading [17]. Gentamicin sulphate is an important member of the amino glycoside class of antibiotics and is widely used in treatment of serious gram -ve aerobic bacterial infections, such as Urinary tract infection, Respiratory

tract infection, Gastro-intestinal infection, skin, soft tissues, bone infection, extreme burns. In the present work microspheres are prepared by emulsification technique, where in, the aqueous drug-polymeric solution is added to oil phase containing surfactant while stirring continuous under mechanical stirrer. The microspheres formed were collected, washed and subjected to physico chemical studies. Melatonin was released from the microspheres in a sustained manner in vitro. Nasal clearance of 99mTC labeled starch microspheres was investigated using gamma scintigraphy. It was revealed that >80% of the starch microspheres could be detected in the nasal tissue 2h after administration, compared to 30% for a solution [19]. Addition of formulation aid could improve microspheres to have the appropriate size, morphology, and flowability for aerosolization for appropriate nasal deposition. Mannitol and propylene glycol have been investigated as particle filler and shaper [20].

EXPERIMENTAL

MATERIALS

Gentamicin Sulphate was obtained from M/s Micro labs Pvt. Ltd, Hosur, Bangalore, India as a gift sample. It is buff colored, Odorless, Hygroscopic powder, moderately soluble in ethanol, methanol and acetone insoluble in benzene and halogenated hydrocarbons. It is having a broad spectrum bactericidal antibiotic of the aminoglycoside group. It is readily absorbed and reaches maximum serum concentrations within 30-90 minutes after intramuscular administration. Effective concentrations in the blood persist for 6 - 8 hours. About 90 % of gentamicin is excreted unchanged in urine by glomerular filtration. Chitosan was procured from Indian fisheries, Cochin, Kerala, India. HPMC **K100** from Zydus Recon, Bangalore. β -Cyclodextrin, Petroleum Ether 40-60, Potassium dihydrogen orthophosphate were procured from S.D.Fine Chemicals Ltd, Mumbai, India, and all other chemicals were of analytical grade.

Preparation of HPMC Microspheres:

Three different formulations of HPMC with drug named H₁, H₂ and H₃ with different drug polymer ratio 1:1, 1:1, and 1:2 were prepared by adding aqueous drug polymeric solution containing β -Cyclodextrin of 1% w/w of total amount of drug and polymer (H₁ formulation do not contain β -Cyclodextrin) into a 150ml of preheated (60°C) liquid paraffin (containing 1% v/v span 80) with the syringe with continuous stirring using mechanical stirrer for 8 hrs. The temperature was maintained through out the process, which helps in evaporation of dispersed aqueous phase. The resultant solid microparticles were separated by vacuum filtration and further washed with petroleum ether and dried at 40° C in hot air oven.

Preparation of Chitosan Microspheres:

Three different formulations of chitosan with drug named CH₁, CH₂ and CH₃ with different drug polymer ratio 1:1, 1:1, and 1:2 were prepared by adding aqueous acidic solution of drug and polymer containing β -Cyclodextrin of 1% w/w of total amount of drug and polymer (CH₁ formulation do not contain β -Cyclodextrin) into a 150ml of preheated (60°C) liquid paraffin (containing 1% v/v span 80) with the syringe with continuous stirring using mechanical stirrer for 8 hrs. The temperature was maintained through out the process, which helps in evaporation of dispersed aqueous acidic phase. The resultant solid microparticles were separated by vacuum filtration and further washed with petroleum ether and dried at 40° C in hot air oven.

Measurements**Characterization of Microspheres****Production yield**

The yield was calculated by dividing the weight of the collected microspheres by the weight of all the non-volatile components used for preparing the microspheres and expressed in percentage.

Particle Size Analysis:

The microspheres were suspended in n-Hexane, by sonicating for 5 min, and then the samples were analyzed by a Malvern Mastersizer 2000S laser diffraction spectrometer.

FTIR spectrophotometry

In order to evaluate the integrity and compatibility of the drug/polymer formulations, IR spectra of the drug and its formulations were obtained by FTIR spectrophotometer using potassium bromide pellet method, Jasco-4100, Japan.

Scanning electron Microscopy:

SEM (Jeol JSM-840A and 5600LV) was used to examine the shape and surface morphology of the microspheres of all the batches. Samples were analyzed after they had been gold sputtered (25nm gold film thickness), with 1×10^{-9} probe current. The Samples were imaged using 20KV electron beam.

Drug content:

The Drug content of all the formulations of Starch, Carbopol Microspheres was determined spectroscopically at 251nm using Shimadzu (UV-1601) after digestion of known amount of microspheres (theoretically containing 50mg of drug) in phosphate buffer pH 7.4 for one hour at room temperature using rotary shaker.

Drug Encapsulation Efficiency:

The encapsulation efficiency was calculated from the ratio of actual to theoretical drug content and expressed in percentage.

$$\frac{\text{Actual Drug content}}{\text{Theoretical Drug content}} \times 100$$

In- vitro release studies:

The Dialysis membrane was washed in running water for 3 hrs in order to remove glycerin. Then the membrane was soaked in 90 % alcohol for 24 hours. For removal of sulphur from the membrane, it was treated with 0.3% w/v sodium sulphide at 80°C for 2 min. Then the membrane was washed with warm water at 60°C for 2 min followed by acidification with 0.2% v/v solution of sulphuric acid and rinsed with hot water to remove the acid. Finally the membrane was rinsed in receptor media (phosphate buffer pH 7.4) for 12 hours. The *in-vitro* drug release studies of microspheres were carried out using Franz diffusion cell. A treated dialysis membrane was used to keep the microspheres on the donor side and free diffusion of Gentamicin Sulphate was allowed to the receptor compartment containing 50ml of phosphate buffer solution (pH 7.4). The temperature was maintained at $37 \pm 2^{\circ}$ C. The content of receptor compartment was continuously stirred with a magnetic stirrer. Samples of 1ml were withdrawn from receptor compartment at hourly intervals and replaced with the same amount of fresh buffer solution. The withdrawn samples were measured for the drug content at 251nm.

Preparation of Porcine Nasal Mucosa

Porcine nasal mucosa was used as the model substrate for the study. The porcine nose was collected from local abattoir and placed in isotonic phosphate buffer (pH 7.4) to prevent dehydration during transportation. Mucosa was isolated and made used for studies.

Bioadhesion Testing

The mucosa was attached to the plastic support, with a nylon line. About 60mg of microspheres were compressed to the flat-faced disc. The disc was attached to the surface of the weight, which was then attached to a top pan balance with a nylon line in such a way that the disc of sample comes in contact with mucosa. After 2 min of contact the weights were added gradually on other side of the panel till the disc gets separated from the mucosal surface. The force required to separate was expressed in terms of gms/cm².

Thin layer chromatography

The TLC plates were prepared using Silica gel G. The solvent system used was a mixture of Chloroform:

Methanol: Ammonium hydroxide: Water (1: 4: 2: 1). The Gentamicin Sulphate and the drug loaded microspheres were dissolved and using the capillary tube the samples were spotted on the plate, air dried and kept for development. The plates were dried and sprayed with ninhydrin for visualization of spot. R_f of samples was compared with R_f of pure drug.

Stability Studies:

The microspheres were placed in screw capped glass container and stored at ambient humidity conditions, at various temperatures like $25 \pm 2^\circ\text{C}$ ($60 \pm 5\text{RH}$), $30 \pm 2^\circ\text{C}$ ($65 \pm 5\text{RH}$), $40 \pm 2^\circ\text{C}$ ($75 \pm 5\text{RH}$) for a period of 60 days. The samples were analyzed for physical appearance and for the drug content at regular interval of 15 days.

RESULTS AND DISCUSSION

FTIR spectrophotometry

The FTIR spectra of Gentamicin Sulphate and its formulations with polymer blends are shown. OH , NH_3^+ , NH_2^+ stretch at (3421.42) , NH_3^+ , NH_2^+ symmetric bend at (1637.19) , NH_3^+ , NH_2^+ symmetric bend at (1533.71) , C-O, HSO_4^- stretch at (1123.10) and SO_2 bend at 617.59 . From the studies indicates that there is no chemical interaction occurred between the drug and the polymers used.

Mechanical properties

The measured mechanical properties such as bioadhesion strength, % of yield, Mean particle size, Drug content and Drug encapsulation for the formulations are given in Table 1.

Scanning electron Microscopy

The particles of all formulations were discrete, spherical, and smooth in surface.

In vitro release studies

The percentage drug release for all the formulations are given in table 2. The studies shows that the β -Cyclodextrin enhances the release of the drug by more

than 2 folds and the release was than decreased with increase in polymer ratio which may be due to increase in path length for the drug to penetrate through the polymer layer. With the compilation of the results we found that the carbopol formulations are better than formulations of starch as they have better bioadhesion with almost same amount of the drug release. The slight enhancement in release of drug from carbopol may be due to its better swelling property. Overall the CH_3 formulations were considered the best among the rest for nasal delivery of gentamicin as it showed good bioadhesion strength and the better release.

Stability studies

Stability studies of the formulations were carried out to determine the effect of contents on the stability of the drug at $25^\circ\text{C}/60\% \text{RH}$, $30^\circ\text{C}/65\% \text{RH}$ and $40^\circ\text{C}/75\% \text{RH}$ for 60 days. There was no significant change in the drug content.

CONCLUSION

All the formulations were analyzed for drug content and encapsulation efficiency. The encapsulation efficiency ranged between 58-80%. The mean particle size of microspheres ranged from 11.891 – 17.031μ . The chitosan formulations showed more uniformity in size around 11μ . The scanning electron microscopy reveals smooth and discrete in surface, the *in-vitro* release and the bioadhesion studies shows CH_3 formulation was the best which released 99.33% drug at the end of 6th hour. The release studies of all the formulations was 40 – 50 % enhancement in absorption of drug using enhancer and increase in polymer ratio decreases the percentage drug release. The bioadhesiveness also increases with increase in Polymer concentration. The FTIR and TLC studies showed that there is no drug-polymer interaction. Stability studies reveals formulations were stable till 60 days.

Table 1. Mechanical parameters of all the formulations

Formulation	Tensile strength (gms/cm ²)	% of yield	Mean particle size (μm)	Drug content (mg)	Drug encapsulation (%)
H ₁	12.85	77.50	11.983	33.33	66.66
H ₂	13.	74.00	11.891	33.25	66.50
H ₃	16.44	73.75	14.973	29.00	58.00
CH ₁	16.11	68.5	14.895	33.58	67.00
CH ₂	16.00	66.62	13.997	34.00	68.00
CH ₃	18.73	67.50	17.031	36.97	73.94

* Indicates average of three readings \pm SE.

Table 2: In vitro release studies of all formulations

Time (hrs)	H ₁	H ₂	H ₃	CH ₁	CH ₂	CH ₃
01	18.45	35.18	31.59	18.22	40.07	38.75
02	31.46	55.12	56.01	24.20	56.22	62.42
03	37.24	63.75	65.95	29.00	68.20	75.80
04	41.76	69.13	75.83	33.63	76.87	88.98
05	43.91	74.32	80.90	36.96	85.16	96.94
06	45.69	78.78	87.13	39.10	89.02	99.33

- Indicates average of three readings \pm SE.

Table 3: Thin layer chromatography of all formulations

	R _f Values		
	C _{1a}	C ₂	C ₁
Pure Drug	0.69	0.76	0.71
H ₁	0.69	0.76	0.71
H ₂	0.69	0.76	0.72
H ₃	0.69	0.76	0.71
CH ₁	0.69	0.77	0.71
CH ₂	0.69	0.76	0.72
CH ₃	0.69	0.76	0.71

In Vitro Release Profile of H₁- H₃ and CH₁-CH₃ formulations

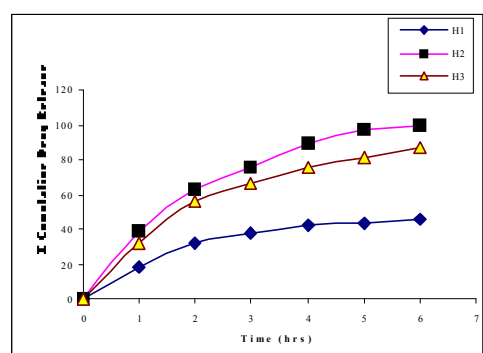


Fig:1

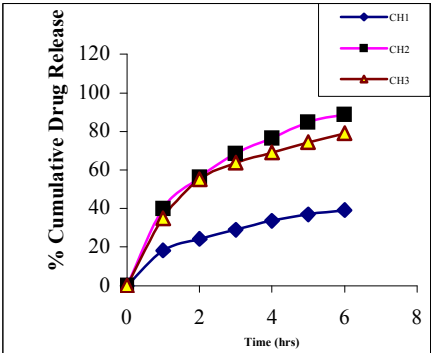


Fig: 2

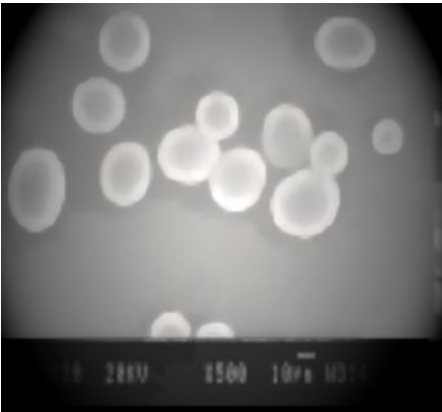


Fig: 3 SEM of Chitosan Microspheres

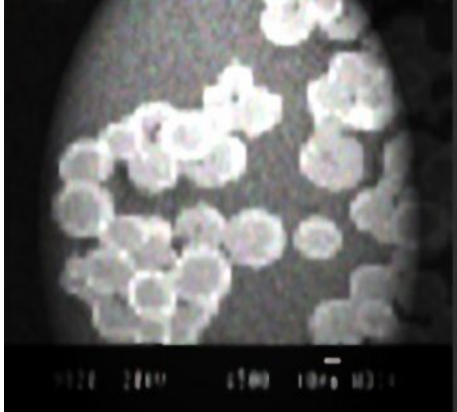


Fig: 4 SEM of HPMC Microspheres

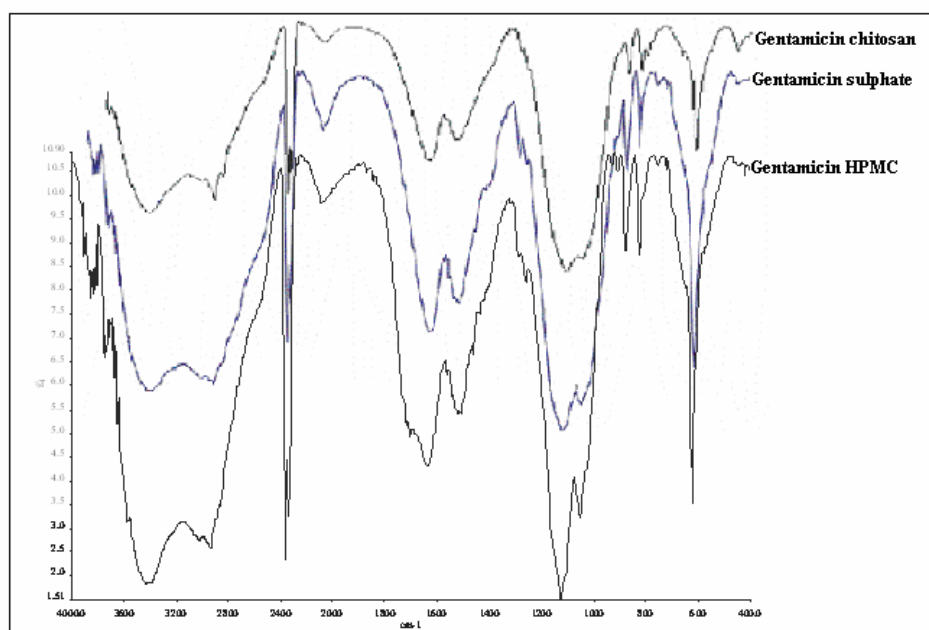


Fig:5 FTIR Spectra of Pure Drug and its polymer blend

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