Oral delivery of chitosan microspheres for peyer's patch targeting against diseases associated with small instestine

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INTRODUCTION

Chitosan microspheres as drug delivery system have attained importance and attracted the attention of researchers recently. Microspheres have been explored extensively for their use in the field of drug delivery and various polymers have been utilized for the formulation of the microspheres which in turn have been assessed for different purposes (Woo B.H. 2001). Recently, dosage forms that can precisely control the release rates and target drugs to a definite body site or organ have played a pivotal role and provided a major thrust in the development of the novel drug delivery field. Chitosan which is deacetylated derivative of (-4)2- acetaamido-2-deoxy b-d- glucose or chitin, has been extensively explored for its various biomedical and pharmaceutical applications. As a drug carrier chitosan has been investigated for the sustained delivery of many oral formulations and parentral formulations. In the present project the chitosan microspheres were developed and characterized for the effective delivery of drug to small intestine via oral route. The developed systems can be used to deliver the bioactives for the selective uptake by the peyers patches or release drug in the small intestine for diseases associated with this specific part of Gastrointestinal tract.

MATERIAL AND METHODS

Chitosan (purified, viscosity grade 50) obtained from Central Institute of Fisheries and Technology, Cochin India was used without further purification. Span 85 and Glutaraldehyde (biological grade, 25% v/v aqueous solution) and Eudragit S-100 were from Sigma chemical, USA. Light liquid paraffin, Petroleum ether, Acetone, Acetic acid and other solvents were from Loba Chemie Pvt Ltd. Mumbai, India and were utilized as received. Deionised Millipore water was used throughout the study. Ciprofloxacin-HCl was a kind gift from Solisto Pharma, Sagar (M.P), India.

Preparation of Microspheres

Microspheres were prepared by slight modification of emulsification method (Gohel M.C. 1998). Glutaraldehyde was used as cross linking agent. Microspheres were prepared by taking 1:1 mixture of Petroleum Ether and Light liquid paraffin. Ciprofloxacin-HCl was taken as model drug. Briefly, the drug was already dissolved in 4% acetic acid solution and then chitosan was dissolved in the drug solution to produce 2% w/v concentration of the polymer. The drug to polymer ratio was kept 1:2. This aqueous phase (5 ml) was added drop wise to the beaker containing 50 ml of oil phase and 0.5% w/v Span 85 as emulsifying agent (previously mixed through stirring) under constant stirring. Stirring was carried out at 3000 rpm utilizing a three blade mechanical stirrer (Remi Instruments, Mumbai). 750 µL glutaraldehyde was added after 20 minutes. The stirring was continued for 3 hours. The oil phase containing microspheres was centrifuged at 1000 g for one minute. The supernatant oil was decanted and the microspheres at the bottom were then washed three times with petroleum ether by centrifugation to remove the oil.

They were then washed with water to remove traces of glutaraldehyde and then they were washed with acetone and then they were freeze dried in a freeze drier (Heto Hilton, Germany).

Electron Microscopy

Shape and surface morphology of the different microspheres formed was investigated through Scanning Electron Microscope (Leo 435 VP, Oxford Instruments, England). Briefly, microspheres were sprinkled on double sided tape, sputter coated with gold and were examined in the microscope.

Particle Size

Microspheres were sized using a Malvern mastersizer (model Nano ZF-90, Malvern Instruments Private Ltd. UK). The volume mean diameter and distribution of particle size were measured. Average mean size of different microspheres prepared was found to be 3.15±0.04 µm.

Zeta Potential

Zeta potentials were measured by electrophoresis performed on a Malvern Zetasizer (model Nano ZF-90, Malvern Instruments Private Ltd. UK). Phosphate buffer pH 7.4 was used as the medium to suspend the microspheres for the measurement of the zeta potential. The zeta potential was found to be 5.03±0.06 mv.

Drug content and Entrapment Efficiency

Microspheres were crushed in a glass mortar and pestle. The powered microspheres were suspended in 10 ml of phosphate buffer (pH 7.4). After 24 hours, the solution was filtered and the filtrate was analysed for the drug content through UV-spectrophotometer (Shimadzu-1601, Kyoto, Japan) at 241.5 nm. The drug entrapment efficiency was calculated using the following formula: Practical drug content/ Theoretical drug content x 100.

In vitro Drug release

In vitro drug release studies were performed by suspending 50 mg of microspheres in PBS buffer (pH 7.4). This suspension was then filled in a dialysis bag and this was placed in release media (PBS buffer pH 7.4). Aliquots were withdrawn at predetermined intervals of time and volume was made up with an equal amount of phosphate saline buffer pH 7.4. The aliquots were analyzed for the drug content at 241.5 nm using UV spectrophotometer (Shimadzu-1601, Kyoto, Japan).

Coating of Microspheres

The microspheres were coated with Eudragit S-100 as reported by Lorenzo-Lamosa *et.al.,* with slight modifications (Lorenzo-Lamosa M.L. 1998). Briefly the prepared chitosan microspheres were dispersed in 5 ml of an organic solvent in which Eudragit S-100 was previously to give 10:1 coat /core ratio. This organic phase was poured into 70 ml of liquid paraffin containing Span 85. The system was maintened under agitation at 1500 rpm at room temperature.The solvent was evaporated and the coated microspheres were collected and rinsed with n-hexane and freeze dried.

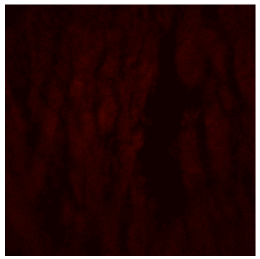
Confocal Microscopy study

The CLSM study was performed using Rh-6G loaded chitosan microspheres which were administered to the Balbc mice orally. Plain dye was administered to the other group of mice and was treated as control. The studies were performed on Zeiss LSM 510 meta microscope .

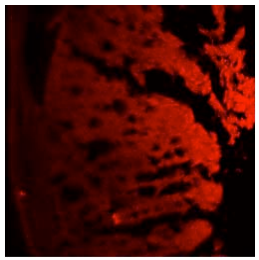
RESULTS AND DISCUSSION

Uniform and small microspheres of 3.15±0.04 µm and 68.87±1.03 percent drug entrapment were obtained. Zeta potential of the microspheres was found to be 5.03±0.06 mv. A higher entrapment value in this case is due to the hydrophllic nature of the drug which got entrapped in the hydrophllic matrix of the microspheres. Lower zeta potential values are due to the crosslinking of the amine groups of the chitosan by glutaraldehyde.

Since the amine groups are positively charged and contribute to the potential of the carrier system and hence the crosslinking reduces the free amine groups and hence the effect. Microspheres were coated with Eudragit S-100 to prevent the drug release in acidic environment of stomach. Eudragit S-100 is a pH responsive polymer and degrades only at the pH similar to that of small intestine. Therefore it was used so as to release the contents specifically at small intestine. In vitro drug release in phosphate buffer pH 7.4 was calculated for the microspheres which showed 70±2.46% release of drug after an interval of 7 hrs. Confocal microscopy studies were performed in Balbc mice where Rhodamine 6G loaded coated microspheres as well plain dye was administered orally to the animals in different groups. The confocal laser scanning microscopy study revealed that Rh-6G loaded chitosan microspheres were present in peyers patch which confirm the effective uptake of the microspheres to peyer's patches whereas the plain dye did not show any uptake into the peyers patches (Figure 1a & 1b).



1a: Plain dye (Rh6G)



1b: Rh6G loaded microspheres

Figure 1: CLSM photomicrograph showing uptake of the plain dye and chitosan microsphers microspheres to peyer's patches

This could be due the fact that Eudragit coated microspheres reached the small intestine and did not release their content in the stomach. Moreover these microparticles are effectively taken up by the peyers patch tissue where they are trapped depending upon their size. On the contrary the dye may have been washed off by the gastrointestinal fluid and hence no uptake resulted as a result.

CONCLUSION

Results revealed that microspheres effectively localize in the peyer's patch of small intestine. Further this also can be utilized for the development of the vaccine by delivering the antigen

through these systems which would elicit the immune response. This work was a part of our ongoing thrust and project to develop microparticulate drug delivery system for oral delivery.

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