Original Article

Xylometazoline Loaded Chitosan Nanoparticles: Fabrication, Optimization and Evaluation for Nasal Congestion

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ABSTRACT

Aim: The present study aims at exploring the development of xylometazoline hydrochloride loaded chitosan nanoparticles (XYL-NP) cross linked with calcium chloride for the treatment of nasal congestion. **Materials and Methods:** XYL-NP were prepared using different polymer like chitosan, cross linking agent calcium chloride and glacial acetic acid as a solvent enhancer using ionotropic gelation method, which was further optimized and validated by Box-Behnken Design. Further, these particles were characterised for size, morphology and evaluated for its release and permeability studies. **Results:** XYL-NP showed a particle size of 172 + 0.4 nm and a PDI of 0.27. The obtained charged nanoparticles showed encapsulation efficiency of 90.5%. Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) studies showed nanoparticles have small size and smooth morphology and an *in-vitro* drug release of 81.2 %, following Peppas-korsemeyer release model.96.3% of the drug was permeated through the nasal mucosa of the goat in a time period of 8 hr. **Conclusion:** Release and permeation results shows the capability of the nanoparticles to retain large amount of XYL-NP inside the mucosa. Hence XYL-NP can be used in treating nasal congestion as the fabricated formulation has good bioadhesive and mucoadhesive property.

Keywords: Xylometazoline hydrochloride, Nasal Congestion, Chitosan, Box-Behken Design, Calcium Chloride, Ionic gelation, Peppas-korsemeyer.

INTRODUCTION

Airway disorders are the obstructions in the tubes that transfer oxygen and other gases in and out of the lungs. These airway pathways are frequently narrowed or blocked which leads to several disorders such as nasal congestion, asthma, sinus, cystic fibrosis and rhinitis.[1] Amongst the above airway disorders, nasal congestion is a prevalent and chronic condition affecting nearly one-quarter of the US population.[2] Nasal congestion or obstruction is a common symptom of upper respiratory tract disorders having otitis media and asthma which leads to obstructive sleep apnea.[3] To overcome the problem of nasal congestion various nasal decongestants are available in market like L-deoxyephedrine (Vicks vapo Inhaler), Phenylephrine (Nose Drops), Oxymetazoline nasal spray, Xylometazoline nasal spray. Xylometazoline hydrochloride (XYL) is a nasal decongestant which causes systemic vasoconstriction directly affecting the adrenergic alpha-agonist that reduces nasal congestion.[4]

The availability of XYL nasal spray causes several side effects such as temporary burning, stinging, dryness in the nose and hence, repeated dose is required to overcome these side-effects.[5] Since XYL nasal spray has a repeated frequency of drug administration which causes patient incompliance. It also causes rapid drug clearance (mucociliary clearance) from the nasal cavity due to low residence time.[6]

Nanoparticle technology has emerged as a feasible drug delivery technique, allowing for regulated release, active component protection from enzymatic or

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environmental deterioration, and confined retention.[7] Nanoparticle production technique are scalable as well as used to make a wide variety of medications. Polymeric nanoparticles have risen to prominence among nanoparticle drug delivery approaches because they are biodegradable, biocompatible, and have more broadly available formulation methods; the scope of utilization has been enlarged to incorporate a range of chemical drug types and dosage forms.[8] This can lead to fewer side effects and a more comfortable administration method, resulting in better patient compliance and therapeutic outcome.[9] They are made up of chitosan, a biocompatible and biodegradable polymer that has furthermore GRAS approved (Generally Recognized as Safe by the United States Food and Drug Administration [US FDA]).[10] Chitosan nanoparticles have efficient drug delivery agents that also improve medication therapeutic efficacy.[11] In this research, fabrication of XYL loaded chitosan nanoparticles for the treatment of Nasal congestion were prepared in order to have sustained release having patient compliance as well. Since, marketed formulation of XYL require repeated dose that causes rebound and atrophy in nose, these problems were circumvented with the fabrication of chitosan nanoparticles showing a novel approach in prolonging the residence time of the formulation into the nasal cavity.[12]

MATERIALS AND METHODS

Materials

Xylometazoline hydrochloride was provided as a gift sample by Scott Edil Pharmacia Ltd. (Baddi, India). Chitosan was purchased from Kemphasol (Mumbai, India). Calcium chloride, Glacial Acetic acid (1% v/v) and sodium dihydrogen orthophosphate were purchased from MolyChem (Mumbai, India). Potassium dihydrogen phosphate was purchased from Central Drug House (P) Ltd. (CDH) (New Delhi, India). Milli-Q (Millipore, Billerica, MA) water was used during the experimental work.

Formulation of Xylometazoline-hydrochloride chitosan nanoparticles (XYL-NP)

XYL-NP were prepared by ionic gelation method using calcium chloride as a cross-linking agent. 0.55% w/v of chitosan was dissolved in 80 ml water with the help of 1 ml of glacial acetic acid and kept overnight for proper dissolution. 100 mg Xylometazoline Hydrochloride was dissolved in distilled water and 3% w/v calcium chloride was added to it. Finally, 20 ml of calcium chloride and drug solution were added drop wise to 80 ml solution of chitosan with stirring at 800 rpm for 2 hr. After completion of stirring, the solution was sonicated to get proper nano sized of formulation.[13]

Optimization of the formulation using Box-Behnken design (BBD)

For the formulation optimization of XYL-NP, the independent variables such as chitosan (0.01-0.1% w/v),[14] calcium chloride (1-5% w/v)[15] and sonication time (1-5 min) were used. The nanoparticles prepared were then evaluated with respect to the dependent variables such as particle size, polydispersity index (PDI) and entrapment efficiency as shown in Table 1 using Box –Behnken Design with Design of Expert Software. (Version 8.0.7.1; Stat-Ease Inc., Minneapolis, MN). Seventeen runs were given by design expert and the experiment was performed in triplicates. For better interpretation of the responses between independent and dependent variables, response surface and contour plots were plotted. Optimization of XYL-NP were carried out using Box-Behnken design Software.

Particle Size and Polydispersity Index (PDI)

Particle size and PDI were analyse during dynamic light scattering (DLS) method with the help of Zetasizer Nano ZS (Nano ZS; Malvern Instruments, Malvern, UK) and examine by using "DTS nano" Software. Particle size and Polydispersity Index were determined by utilizing a 200-fold dilution of the formulation in aqueous phase, vigorous shaking to acquire 100-250 kilo counts per second.[16,17]

Transmission Electron microscopy (TEM)

In order to determine the morphological studies of the XYL-NP, TEM was employed to evaluate the optimized

Table 1: Independent and dependent variables range lower to higher.

Factors	Coded Levels		
Independent	**Variable high(+1)**	**Low (-1)**	**Medium (0)**
X1= Chitosan (%)	0.55	0.1	1
X2= Calcium Chloride(%)	1	3	5
X3=Sonication time (min)	1	3	5
Dependent variables	**Constraints**		
Y1= Particle Size (nm)	(200-500)		
Y2= Polydispersity Index (PDI)	Minimum		
Y3= Entrapment Efficiency	100%		

formulation.[17] This was done using a Tecnai T20 (FEI, USA). A drop of the XYL-NP was placed on carbon-coated grid with 2% phosphor-tungstic acid (PTA) then viewed on a TEM operating at 60-80 kV TEM.

Scanning Electron Microscopy (SEM)

SEM (Carl Zeiss, Supra 55, Germany) was used to examine the optimised formulation's shape and surface morphology, using previously published methodologies.[18]

Drug-Excipient Compatibility

FT-IR spectra were used to determine the surface chemistry, physical interaction and functional groups between the polymers and the Drug. The samples for this spectroscopy (Cary630, Agilent, USA) were combined with KBr and compressed into transparent tablets prior to the analysis over the 4000-400cm^{-1} range.

Encapsulation Efficiency

Nanoparticles were separated from the aqueous phase by ultracentrifugation (instrument name) at 15000 rpm at 4°C for 20 min. The supernatants were collected and evaluated for xylometazoline hydrochloride residue by UV spectroscopy.[19]

$$EE\% = \frac{100 \times (\text{amount of total drug} - \text{amount of free drug})}{\text{Amount of total drug}}$$

In-vitro release studies

Xylometazoline hydrochloride was released from XYL-NP was evaluated using dialysis membrane method. The formulation was incubated at 37°C in Phosphate-buffered saline (PBS) pH 7 for 24 hr. Individual samples were removed and centrifuged at 30,000g for 30 min at 4°C at intervals of 0.25,0.5,1,2,4,6,8,12,24 h with the supernatant being replaced with fresh PBS.[17] Release of XYL was determined by UV at λ_{max}=218nm. Different kinetic models were used to fit the *in-vitro* release data such as Zero order, First order, Higuchi model, Hixon Crowell model and Peppas-koresmeyer model. In order to obtain the best-fit model, the regression coefficient was calculated in each model. The Peppas-korsemeyer models were applied to describe the mechanism of release, as well as the component of release, time, and shape parameter were determined.

Ex-vivo Permeation studies

The fresh mucosa of the nose was extracted from a goat's nasal cavity which was procured from a nearby slaughterhouse. To prevent bacterial growth, the mucosa was preserved in normal saline with a few drops of gentamycin sulphate injection. The mucosal membrane is ready to utilise after blood and bone cartilage have been removed. The Franz Diffusion Cell was used to investigate the drug's *ex-vivo* drug permeation profile.[19] The acceptor chamber was filled with 11 mL of Phosphate Buffer, pH 5.5, at 37°C. By circulating hot water, the temperature within the chamber was kept at 37°C. After a 20-min pre-incubation period, 3 ml of XYL-NP formulation was placed in the donor chamber. After each sampling, 0.5ml of sample was taken from the acceptor compartment and refilled with Phosphate buffer pH 5.5 at predefined time intervals. Phosphate Buffer was used to dilute the samples to 10ml, filter them, and use them for analysis. The amount of permeated drug was calculated by using UV-visible spectrophotometer. The sampling was carried out in triplicates.

RESULTS AND DISCUSSION

Development and Optimization of xylometazoline hydrochloride encapsulated chitosan nanoparticles using BBD.

XYL-NP were prepared using ionic gelation methodology and optimized through BBD. The effect of several independent variables, including chitosan loading concentration (A) (0.55-1%), calcium chloride concentration (B) (1-5%) and sonication time (C) (1-5 min), on the evaluative standard characteristics of XYL-NP was analysed. The best values for the various process variables were found as 0.55% of chitosan, 3% the concentration of calcium chloride and the sonication time of 3 min and the resultobtained has a particle size of 172 nm, PDI of 0.27 and an EE of 90.5%. Figure 1 shows the response graphs for particle size, PDI and Entrapment Efficiency. Effect on particle size can be seen in Figure 1a).

First Equation in terms of coded factors for Particle Size

$(Y1) = +174.6-23.19A+0.30B+7.59C+1.97AB-6.50AC-3.48BC+22.77A^2+10.95B^2-10.12C^2$

The effect on PDI exhibits similar effect as shown on particle size as shown in Figure 1b).

First equation in terms of coded factors for PDI

$Y2=(+0.21)-0.0316A-0.00105B+0.0314C-0.0030AB-0.0053AC+0.0390BC+0.0469A^2+0.0951B^2+0.0159C^2$

Effect on EE can be seen in Figure 1c)

First equation in terms of coded factors for EE

Particle Size (Y3) = $+91.00-5.62A-4.00B-4.63C+6.50AB-4.75AC+5.00BC-18.13A^2-8.88B^24.62C^2$

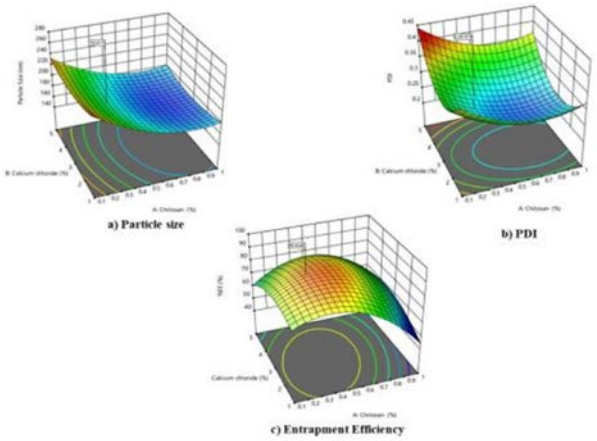

Figure 1: Box-Behken design for optimization of chitosan nanoparticle with dependent variables a) Particle Size b) PDI c) Entrapment Efficiency.

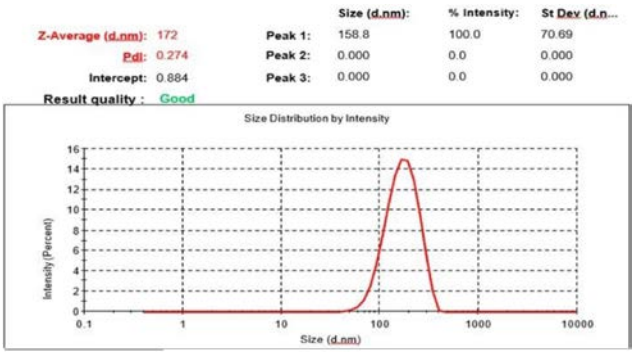

Figure 2: Particle size of the optimized formulation.

The Box-Behnken design (BBD) was utilized to investigate and verify the impact of changing process (independent) variables on nanoparticle quality characteristics (dependent variables). To acquire the qualitative features of xylometazoline hydrochloride, it was clear that optimization of all independent parameters was required.

Particle Size and PDI

The particle size and Polydispersity Index (PDI) of the XYL-NP optimized formulation were 172± 0.3 nm and 0.274, respectively as shown in Figure 2.

TEM, SEM & EE

Optimised XYL-NP were determined to be in the 150-200nm size range, from TEM and SEM which was in-consistent with results of the zetasizer study (Figure 3a and Figure 3b). The xylometazoline hydrochloride showed an entrapment efficiency of 90.5%.

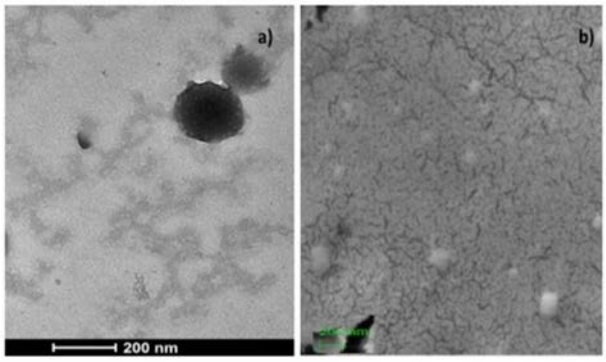

Figure 3: a) Transmission electron microscopy (TEM) b) Scanning electron microscopy (SEM) of the optimized formulation.

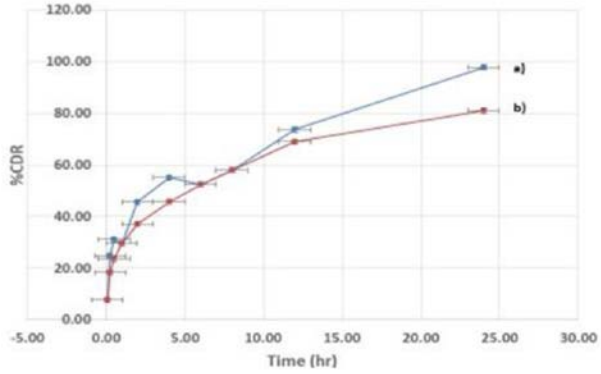

Figure 4 i): a) Percentage cumulative drug release of xylometazoline solution, b) Percentage cumulative drug release of XYL from XYL-NP.

In –vitro release of Xylometazoline-hydrochloride loaded chitosan nanoparticles

In-vitro release investigation of XYL-NP revealed continuous, slow release for 24hr. Figure 4i) and Figure 4 ii) shows the proportion of cumulative drug release, which was determined to be 81.02% and the fitting parameters were established after the *in vitro* data was fitted into various kinetic models respectively. XYL-NP showed high regression coefficient in Peppas-Korsmeyer model out of all the different kinetic models.

Ex-vivo release of Xylometazoline hydrochloride loaded chitosan nanoparticles

Ex vivo permeation studies were performed for XYL-NP to show has better permeation when compared with xylometazoline solution under the same experimental conditions given in 2.5.6. Permeation flux (F) was determined from the slope of the graph by plotting the amount of drug permeated (lg) v/s time (min) whereas Figure 5 shows the *ex-vivo* release study for XYL-NP

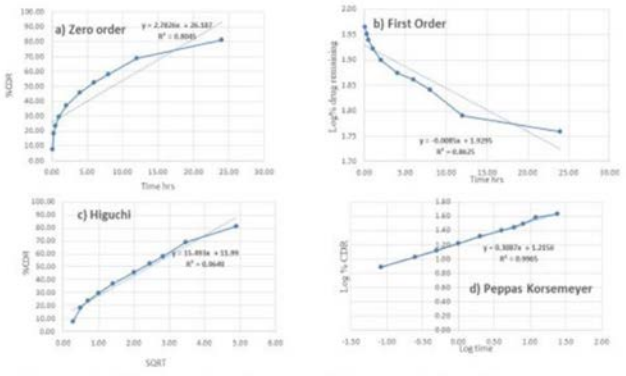

Figure 4 ii): Kinetic modelling of *in-vitro* release of XYL a) Zero order b) First order c) Higuchi model d) Peppas Korsemeyer.

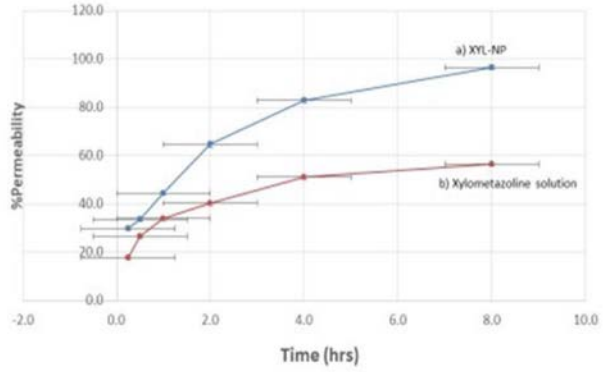

Figure 5: *Ex-vivo* Permeation release of a) XYL b) Xylometazoline solution through goat nasal mucosa.

and XYL solution. In a time period of 8 hr 96.53% of the drug was permeated through the nasal mucosa in the Franz Diffusion cell apparatus. Thus, it is clearly demonstrated that permeation of XYL is much more enhanced as compared to the drug solution.

DISCUSSION

Nasal Congestion has been reported to be an airway disorder which requires sustained release of the medicament and longer residence time in the nasal cavity. In order to resolve the above purpose, XYL-NP were prepared using ionic gelation method as it is shows nanoparticle variability according to our convenience, non-toxicity and absence of any side reaction.[20]

The formulation was optimized using BBD software which showed the effect of different independent variables on the dependent variables. Effect on particle size elucidates that when the calcium chloride concentration increases and chitosan concentration decreases the particle size will be decreased. In our research it is shown that the effect of $CaCl_2$ solution with concentration 3% gives suitable swelling %.[15] Same effect can be seen for PDI as in case of the particle size. Increase in sonication time and increase in Calcium chloride concentration as a result, monodisperse small particles form, which are more effective at encapsulating the drug than polydisperse large particles.[19] The results for particle size and PDI shows that the particles are distributed evenly throughout the formulation. The optimized XYL-NP formulation was discovered to be lower than 200 nm in size, that is the best size of nanoparticles for avoiding identification from the reticulo-endothelial system (RES), and thus particles of this size have a longer half-life.[17] SEM and TEM images revealed that the nanoparticles have a small size, spherical and smooth surface morphology. The dialysis membrane bag computed the *in-vitro* release samples showing continual slow release over time would help in maintaining the optimal therapeutic concentration for a longer period of time while also reducing administration frequency. The release study showed is Peppas-Korsmeyer model suggesting the drug release by diffusion and swelling.[21] *Ex-vivo* permeation studies showed most of the drug gets permeated through the nasal mucosa when compared with the drug solution. This is due to the presence of chitosan in the formulation delivers percutaneous penetration enhancement and bio adhesive property.[22]

CONCLUSION

In conclusion, XYL-NP were prepared using ionic gelation technique. The optimized formulation showed a particle size of 172 nm, uniform particle size distribution and high entrapment efficiency. The optimized formulation showed an *in-vitro* release of 81.03% for XYL-NP which a controlled drug release following Peppas -Korsemeyer drug kinetic model. In order to determine the permeation of the XYL-NP *ex-vivo* permeation studies were carried out and a release of 96% was observed for XYL-NP. Hence, XYL-NP can be subjected in treating nasal congestion as formulation fabricated has good bioadhesive and mucoadhesive property.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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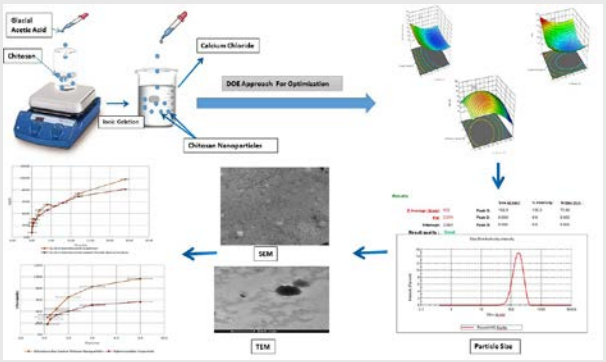
PICTORIAL ABSTRACT



SUMMARY

The fabrication of XYL-loaded chitosan nanoparticles for the treatment of nasal congestion was done in this study to ensure long-term release and patient compliance. Because current XYL formulations require repeated doses, causing rebound and atrophy in the nose, these issues were avoided by fabricating them into chitosan nanoparticles, which demonstrated a novel strategy in extending the formulation's residence time in the nasal cavity. The ionic gelation process was used to prepared XYL-NP. The improved formulation had a 172 nm particle size, a homogeneous particle size distribution, and a good entrapment efficiency. According to the Peppas-Korsemeyer drug kinetic model, the improved formulation showed an in-vitro release of 81.03 % for XYL-NP, indicating a regulated drug release. Ex-vivo permeation experiments were carried out to determine the permeation of the XYL-NP, and a release of 96 % was detected. As a result, XYL-NP can be used to treat nasal congestion because the formulation is bioadhesive and mucoadhesive.

About Authors

Kavita Attri, is a PhD scholar at Department of Pharmaceutics, SGT College of Pharmacy, SGT University, Gurugram. She has a teaching and research experience of 7 years. She has worked novel drug delivery system and formulation development.

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Dr.Vijay Bhalla, presently working as Professor & Principal, SGT College of Pharmacy, SGT University, Gurgaon, Haryana. Prof. Bhalla passed Diploma in Pharmacy (1980), Bachelor of Pharmacy (1983), Master of Pharmacy (1985) from University of Delhi, and also secured Doctorate of Philosophy in Pharmacy. Prof Bhalla is a registered Pharmacist since 1980 with Delhi State Pharmacy Council. Prof Bhalla has exposure of working for NATIONAL AIDS CONTROL ORGANIZATION (NACO) in Delhi and Uttar Pradesh Projects as Capacity Building Manager & Manager Operations. Prof Bhalla has rich experience of working for ESI project SANKALP as Capacity Building Manager in Uttar Pradesh to train work force of Industries for Prevention and control of HIV AIDS, Life Style Disorders like Hypertension, Diabetes Mellitus (NIDDMS), Obesity and Vector Borne Diseases like Malaria, Dengue, Chickangunia and Japanese Encephalitis. Prof Bhalla has served with Pharmaceutical Multinational Organization in various capacities for 18 years with Upjohn, Unichem, Ciba Geigy, Sandoz & Novartis.

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