Archives of Pharmacy Practice

Research Article

ISSN 2045-080X Vol. 3 , Issue 2 , 2012

# Mucoadhesive Nasal Delivery of Raloxifene: Effect of cyclodextrin Complexation and Chitosan Grades on Extent of Drug Loading and Release

**Pramod Bhise[1], Aliasgar Shahiwala[1], Manju Misra[1]**

[1] Department of Pharmaceutics, NIPER, Ahmedabad, C/O B.V Patel PERD center, S.G. Highway, Thaltej, Ahmedabad 380054, India

**Citation:** Pramod Bhise, Aliasgar Shahiwala, Manju Misra. **Mucoadhesive Nasal Delivery of Raloxifene: Effect of cyclodextrin Complexation and Chitosan Grades on Extent of Drug Loading and Release.** Archives of Pharmacy Practice. 2012; 3(2) pp 136-143.

## Abstract

**Objectives:** Raloxifene (RLX) is a BCS class II drug, with low aqueous solubility, extensive first pass metabolism and only 2 % bioavailability .Oral administration of RLX tablet exhibits side effects like hot flushes, musculoskeletal pain, venous thromboembolic events, nausea, vomiting and diarrhea. To overcome these limitations a mucoadhesive micro particulate system of RLX is developed, with the objective of overcoming hepatic first and improving oral bioavailability.**Material and methods:** Complexation of cyclodextrin (CD) with chitosan was evaluated using DSC. Nanoparticles of RLX with chitosan and glycol chitosan were developed using ionic gelation method and were evaluated for particle size, zeta potential, entrapment efficiency , and in-vitro and ex-vivo drug release.**Results:** The results revealed improved aqueous solubility of RLX due to formation of inclusion complex with Carboxymethyl-β-CD (CM-β-CD) and chitosan. The chemical nature of chitosan and anionic CD significantly affected the size, shape, zeta potential and entrapment efficiency of formed nanoparticles. The release of drug from the matrix of nanoparticles followed higuchi kinetics. **Conclusion:** Cyclodextrin-chitosan complexed RLX nanoparticles exhibited improved solubility of RLX with 27 % entrapment efficiency.

**Key words**

*Raloxifene, chitosan, Carboxymethyl-β-CD, ionic gelation, inclusion complex*

**Manuscript History**

*Article Received on: 15th Oct, 2011*
*Revised on: 23rd Jan, 2012*
*Approved for Publication: 10th Feb, 2012*

**Corresponding Author**

***Dr. Manju Misra,*** *Asst. professor, Department of Pharmaceutics, NIPER, Ahmedabad, C/O B.V Patel PERD centre, S.G. Highway, Thaltej, Ahmedabad 380054, India. Phone: 91-79-27439375, 27416409, Mob Number: 91-*9099069333

***Email:*** *mtbitat@gmail.com*

## Introduction

Raloxifene (RLX) is a selective estrogen receptor modulator (SERM) and is shown to be effective in the prevention of osteoporosis with potential utility as a substitute for long term female hormone replacement therapy (1,2). RLX is a BCS Class II drug, exhibiting very less aqueous solubility and undergoes extensive first pass metabolism with only 2% absolute bioavailability and erratic absorption of up to 60%. It is metabolized mainly by glucuronide conjugation & shows enterohepatic recirculation. The existing formulation of RLX is a 60 mg tablet which is associated with side effects like hot flushes, musculoskeletal pain, venous thromboembolic events, nausea, vomiting and diarrhea(3). Taking into account these limitations of delivering RLX orally, nasal administration could be considered an alternative route to overcome hepatic first pass and improve drug bioavailability.

Nasal route is one of the most permeable and highly vascularized site for drug administration ensuring rapid absorption and onset of therapeutic action besides being a good alternative route for drugs that are susceptible to enzymatic or acidic degradation or first pass hepatic metabolism (4,5). The major limitation though in nasal drug delivery remains the rapid mucocilliary clearance which is the cause of a limited contact period allowed for drug absorption through nasal mucosa. This limitation however can be overcome using mucoadhesive nano and microparticulate system. Particulate drug carrier systems administered through nasal mucosa may protect the drug from enzymatic degradation, increase the drug dissolution rate, intensify the contact of the formulation with the mucosa, enhance the uptake by the epithelium, and act as a controlled release system resulting in increased drug absorption through nasal cavity(6-10). Crosslinked chitosan nanocarrier system has been extensively investigated for nasal drug and vaccine delivery during the past decade (3). Chitosan has been shown to have mucoadhesive properties because of its viscosity and interaction of the positively charged amino groups with the negatively charged sites on mucosa surface. The nasal delivery of Chitosan was demonstrated to greatly enhance the absorption of insulin across the nasal mucosa of rats

and sheep. This was due to mucoadhesive property of polymer, leading to reduced clearance rate of drug from the nasal cavity, thereby prolonging the contact time with nasal epithelium. In addition it has been also shown that Chitosan caused opening of tight junctions and allowed large hydrophilic compounds to be transported across the epithelium (11, 12).

In case of RLX which is poorly water soluble, solubility is another parameter that will affect nasal bioavailability. It is therefore necessary to increase drug solubility to allow the delivery of therapeutically relevant dose. One of the possible approaches is the formation of drug cyclodextrin inclusion complexes. The inclusion complex of RLX with Hydroxybutenyl-β- cyclodextrin showed significant improvements in oral bioavailability when compared with RLX formulated with microcrystalline cellulose alone (13). Cyclodextrins may be used to improve nasal bioavailability of lipophilic drug, because the formation of dynamic noncovalent inclusion complexes can enhance apparent water solubility of the drug with no influence on their permeability across biological membranes. Also cyclodextrins may interact with the lipophilic components of biological membranes changing their permeability. Clinical trials have shown that nasal application of cyclodextrins to humans was well tolerated with only minor adverse effects (14). Hydroxypropyl- β-cyclodextrin(HP-β-CD) showed superior tolerability compared with other cyclodextrin derivatives because long term nasal application of 20% HP-β-CD dose had no influence on the integrity of the mucosa in rats(15) . Also studies involving human cell cultures have shown that HP-β-CD had no significant cilio-inihibitory effect(16).

With this background the aim of the current work was to develop and evaluate a new mucoadhesive nanocarrier based nasal delivery system of RLX. The work involves characterization of Raloxifene: and Carboxy-Methyl- β-cyclodextrin (CM-β-CD) complex using DSC followed by development of drug loaded nanoparticles using Chitosan, Glycol Chitosan, and CM-β-CD. The effect of grade of Chitosan on extent of drug entrapment in presence CM-β-CD which is anionic in nature and its subsequent effect on drug release are also evaluated. The final objective was to design a delivery system that involves the cyclodextrin inclusion complex for improved solubilization of raloxifene and use of mucoadhesive polymer like Chitosan to enhance the uptake of RLX via mucoadhesion and reduce nasal clearance.

## Materials And Methods

Raloxifene HCl was kindly donated by Aurobindo Pharma Ltd. (Hyderabad, India). Chitosan was received from Mahatani Chitosan Pvt Ltd, Veraval Gujarat. Glycol Chitosan, CM-β-CD and Pluronic F-68 were purchased from Sigma Aldrich chemicals Ltd., Mumbai. Remaining all chemicals used were of HPLC or analytical grade.

### Analytical Method Development

A calibration curve in methanol for RLX was obtained by measuring the absorbance of diluted solution (2-20µg/ml) at 287nm by UV against methanol as a blank. To determine the amount of drug released from nanoparticles during in-vitro release studies and for analysing amount of drug permeated through Sheep nasal mucosa during ex-vivo permeation studies, calibration curves were also constructed in Phosphate Buffer pH 6.4 and Phosphate Buffer pH 7.4 respectively. All the readings were recorded in triplicate.

### Screening of Raloxifene: BCD Solid Powder Complexes Using DSC

RLX-complex with CM-β-CD was formed by co-grinding RLX and CM-β-CD in 1:1 ratio using mortar pestle. The thermal behavior of RLX, CM-β-CD alone and grounded mixture of RLX and CM-β-CD in 1:1 ratio was analyzed using DSC. Analysis was performed using DSC-7 (Perkin Elmer-UK) under nitrogen flow (60 Kg/cm2) at a scanning rate of 10°C/min from 50°C to 300°C in hermetically sealed aluminum pans. Empty aluminum pan was used as a reference.

### Preparation of drug loaded Chitosan and Glycol Chitosan Nano particles

Nanoparticles (NPs) were prepared using the ionotropic gelation method as described by Calvo et.al. (17). 0.2% chitosan solution (2mg/ml) was prepared in acetic acid (0.2%) and pH of the solution was adjusted till 5 using 1M NaOH. RLX was added to the Chitosan solution in the concentration of 2 mg/ml. This mixture was kept to equilibrate for 24 hrs. for effective complexation. After complexation Pluronic F- 68 (which acts as a surfactant) was added to this solution. The cross-linking agent TPP (1mg/ml) was added drop wise to the above solution, which was stirred continuously, leading to the controlled gelation of Chitosan to form the NPs. Different batches containing varying ratio of Chitosan: TPP (w/w) were prepared in the ratios of 2:1, 2.5:1 and 3:1.

Glycol Chitosan Nanoparticles were also prepared using similar method. Glycol chitosan was dissolved in water and pH was adjusted to 5 by using 1M HCl. Similar to chitosan Nanoparticles, different batches containing varying ratio of Glycol Chitosan: TPP (w/w) were prepared in the ratios of 4:1, 5:1, 5.5:1 and 5.7:1.

The NPs were isolated by centrifugation on a glycerol bed at 16,000g for 30 min and resuspended in MiliQ water. The supernatant remained after centrifugation was analyzed by UV spectrophotometer at 287nm to calculate the amount of unentrapped drug. Finally the NPs were lyophilized using 5% mannitol as a cryoprotectant to get solid product.

Only those ratios which exhibited minimum particle size, more positive zeta potential and maximum RLX entrapment were selected for complexation with CM-β-CD.

### Preparation of CM-β-CD Complexed-RLX loaded nanoparticles

For the preparation of carboxymethyl-β-cyclodextrin (CM-β-cd) complex with drug loaded nanoparticles, initially CM-β-CD solution of fixed concentration was prepared in MiliQ water. RLX was added to this solution and kept for 24 hrs stirring on magnetic stirrer to facilitate

complexation. In next step RLX- CM-β-CD complex solution was added drop wise to 0.2% CS or GCS solution (pH 5) containing 0.1% Pluronic F-68 to form pregel. Complete gelation was achieved by dropwise addition of TPP (1mg/ml) to the pregel leading to formation of nanoparticles. The amount of TPP required for gelation was optimized based on particle size, zeta potential and entrapment efficiency. The nanoparticles were also prepared without using TPP, to verify the ability of anionic cyclodextrin derivative i.e. CM-β-CD, to form complex with cationic chitosan. The ratio of Chitosan/Glycol Chitosan: CM-β-CD was optimized to produce the nanoparticles.

The NPs were isolated by centrifugation on a glycerol bed at 16,000g for 30 min and resuspended in MiliQ water. The supernatant remained after centrifugation was analyzed by UV spectrophotometer at 287nm to calculate the amount of unentrapped drug. Finally the NPs were lyophilized using 5% mannitol as a cryoprotectant to get solid product .

**Physicochemical Characterization of Nanoparticles**

**Measurement of Particle size and zeta potential**

Particle size and zeta potential of the nanoparticles was determined by light scattering based on laser diffraction using Zetasizer Nano ZS90 (Malvern Instruments Inc., U.K.). The apparatus consisted of a He-Ne laser and a sample holding cell made up of polystyrene. The instrument settings used were as follows: temperature 25° C, viscosity 0.8872 centipoise, dispersant refractive index 1.33, material refractive index 1.58 and run time 10 sec for each run.

**Determination of entrapment efficiency of nanoparticles**

Entrapment of RLX in nanoparticle matrix was determined by indirect method. After addition of TPP the nanoparticle suspension was centrifuged at 16000 g and supernatant was analyzed for unentrapped drug after suitable dilution by UV spectrophotometer at 287nm.

The entrapment efficiency was calculated using following equation

$$\text{Entapment efficiency (\%)} = \frac{\text{Total amount of RLX added} - \text{Amount remained in supernatant}}{\text{Total amount of RLX added}} \times 100$$

**In-Vitro Release Studies**

These studies were performed to determine rate and kinetics of release of RLX from NP's matrix. For in-vitro release study, lyophilized NP's (containing 2mg entrapped RLX) were resuspended in 50ml phosphate buffer (pH 6.4) and maintained under agitation, at 37°C on magnetic stirrer. Aliquots of the release medium were withdrawn at predetermined time intervals and replaced with same volume of fresh buffer to maintain sink condition. The samples were further centrifuged at 16,000g for 30 min. The drug released from the NP's, present in the supernatant, was determined by UV spectrophotometer at 287 nm.

**Ex-Vivo Permeation through Sheep Nasal Mucosa**:

Flux And Effective Permeability Coefficients Measurement (19)

Fresh nasal mucosa was carefully removed from the nasal cavity of sheep obtained from the local slaughterhouse. Freshly excised Sheep nasal mucosa was dipped immediately in phosphate buffer (pH 6.4). Cartilages were removed properly and the mucosal membrane was isolated and washed with phosphate buffer. Mucosal preparation was mounted on receptor compartment of Franz diffusion cell displaying a permeation area of 5.84 cm2. 20 mL of phosphate buffer pH 7.4 was added to the receptor compartment. Mucosal preparation was mounted on the diffusion cell with the mucosal and serosal sides facing the donor and receiver phases, respectively (20).A small Teflon coated magnetic bead was placed in receptor compartment for stirring the media. The donor and receptor compartments were securely closed with stainless steel clip. The tissue sample was pre-incubated for 20 mins. maintaining temperature at 37°C. After a pre-incubation time of 20 min, the lyophilized drug loaded nanoparticles (equivalent amount of 2.0 mg of RLX) were reconstituted with 1 ml phosphate buffer (pH 6.4) which was then added into the donor compartment on the outer surface of the nasal mucosa (20).Permeation study was carried out for seven hours. At predetermined time intervals, the aliquot were withdrawn and diluted appropriately with phosphate buffer and UV absorbance was measured at 287nm. Percentage drug permeation was calculated from the calibration curve of RLX prepared in phosphate buffer (pH 7.4).

## Results and Discussion

**Calibration curve of Raloxifene Hydrochloride in methanol, phosphate buffer pH 6.4 and pH 7.4**

Absorption maxima (λmax) of RLX was found to be 287nm which was determined by scanning 10 µg/ml solution against methanol as a blank on UV-Visible Spectrophotometer between 200 –400 nm. The developed analytical method was found to be linear 2-20 µgm /ml with r2 value of 0.999

**Screening of RLX CM-β-CD solid powder complexes using DSC**

Differential scanning calorimetry is one of the most widely used techniques for characterization of complexation. In the results of DSC analysis represented in Fig. 1, the DSC plot of pure RLX drug powder shows a sharp endothermic peak at 254.4°C, which is attributed to RLX melting. In the DSC thermogram of CM-β-CD no thermal features were seen in the range analyzed. The absence of peak in case of cyclodextrins is attributed to their amorphous nature. The grounded mixture of RLX and cyclodextrins and CM-β-CD in 1:1 ratio showed the absence of endothermic peak for RLX. The absence of endothermic peak in case of grounded mixture can be explained by the fact that, cyclodextrins forms inclusion complex with the drug. Due to the complex formation the endothermic peak of RLX disappears in case of grounded mixture.

These results were in agreement with work of Mura et.al.

(21,22) wherein they have reported disappearance of endothermic peak of drug as an indication of amorphization due to strong drug-carrier interactions and/or drug inclusion complexation of metformin HCl with triacetyl-cyclodextrin (23).

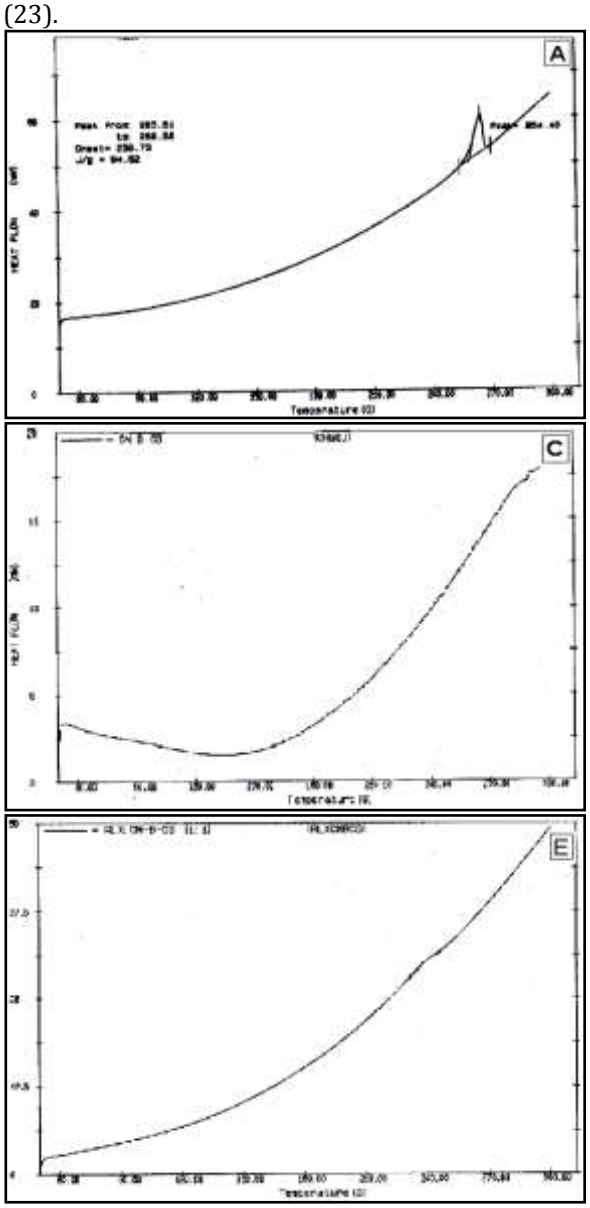


**Fig 1 :** DSC thermograms of (A) Raloxifene HCl, (C) CM-β-CD, and (E) Grounded mixture(1:1) of RLX: CM-β-CD at temperature gradient 10ºC/min

## Preparation and Characterization of Drug-Loaded Nanoparticles

### Chitosan Nanoparticles

Different ratios of Chitosan: TPP were selected for formation of Nanoparticles (NP's) (2:1, 2.5:1 and 3:1). Among the different ratios studied, 2.5:1 Chitosan: TPP ratio gives the NP's with minimum particle size and maximum entrapment efficiency. Hence this ratio was further selected for in-vitro release and ex-vivo permeation studies. The evaluated parameters for the finally formulated NP's are shown in Table 1.

Table: 1- Physicochemical properties of RLX loaded NP's with different CS: TPP (w/w) ratio. (Mean ± S.D., n = 3).

| CS:TPP | Size (nm) | PDI | Zeta potential (mV) | Entrapment Efficiency (%) |
|---|---|---|---|---|
| 2:1 | 247.1±10.8 | 0.229±0.012 | 27.3±1.75 | 17.89±1.42 |
| 2.5:1 | 160.9±5.8 | 0.236±0.023 | 33.0±1.92 | 25.07±1.68 |
| 3:1 | 184.4±13.0 | 0.268±0.029 | 33.6±1.86 | 17.84±1.25 |

### Glycol Chitosan Nanoparticles

Similar to Chitosan NP's, for the preparation of Glycol chitosan NP's initially different ratios of Glycol chitosan: TPP were screened in presence of 10 mM/ml CM-β-CD. Glycol chitosan: TPP w/w ratios of 4:1, 5:1, 5.5:1 and 5.7:1, leads to formation of NP's indicated by formation of opalescent solution. Below this range NP's were not formed and above this range aggregates were formed. In the ratio of 5.7:1 Glycol chitosan: TPP, the NP's with minimum particle size and maximum entrapment efficiency was formed. Hence this ratio was further selected for in-vitro release and ex-vivo permeation studies. Table 2 lists the physicochemical properties of RLX loaded NP's with different Glycol chitosan: TPP.

From the results it appears that particle size of Glycol chitosan NP's is very sensitive to Glycol chitosan: TPP ratio than chitosan NP's. Particle size was found to be ≥1000nm for Glycol chitosan: TPP ratios below 5.5:1.

### Nanoparticles Containing Drug Complexed with CM-β-CD

The ratios of Glycol chitosan: CM-β-CD: TPP w/w for preparation of NP's was optimized on the basis of particle size and entrapment efficiency of RLX. The results are shown in Table: 3. It was observed that when anionic cyclodextrin (CM-β-CD) was used for complexation of RLX, the amount of TPP required to form NP's was reduced. Amongst these the 2:1:0.7 and 2:1:0.2 ratios of Chitosan: CM-β-CD: TPP and Glycol chitosan: CM-β-CD: TPP respectively were selected for further in vitro and ex vivo studies.

Due to its anionic nature CM-β-CD has tendency to form complex with cationic polymer. Hence the tendency of CM-β-CD to form NP's was also evaluated in absence of polyanion TPP. Glycol chitosan: CM-β-CD w/w ratios were optimized for formation of NP's. The NP's formed in this case were larger in size than the NP's which were prepared by combination of CM-β-CD and TPP. The larger size of NP's was probably due to incorporation of more amount of CM-β-CD in NP's which has high molecular weight than TPP. For both Glycol chitosan and chitosan 1:2 ratio of Glycol chitosan: CM-β-CD was evaluated for further in vitro and ex vivo studies.

Table: 2 Physicochemical properties of RLX loaded NP's with different GCS: TPP (w/w) ratio. (Mean ± S.D., n = 3).

| GCS: TPP | Size (nm) | PDI | Zeta potential (mV) | Entrapment Efficiency (%) |
|---|---|---|---|---|
| 4:1 | 1023.7±25.7 | 0.22±0.03 | +20.4±2.73 | 12.33±1.12 |
| 5:1 | 1261.3±40.6 | 0.24±0.15 | +24.4±4.5 | 24.48±2.01 |
| 5.5:1 | 947.83±45.7 | 0.16±0.05 | +31.0±1.61 | 25.59±0.78 |
| 5.7:1 | 267.0±23.8 | 0.06±0.02 | +38.3±1.96 | 27.45±1.65 |

Table: 3- Physicochemical properties of RLX loaded NP's with different Glycol chitosan: CM-β-CD (w/w) ratio.

| Batch | G/CS: CM-β-CD | Size (nm) | PDI | Zeta potential (mV) | Entrapment Efficiency (%) |
|---|---|---|---|---|---|
| CS-III | 1:2 | 352.8 | 0.185 | 34.4 | 23.34 |
| GCS-III | 1:2 | 342.5 | 0.167 | 36.8 | 27.40 |
| GCS-IV | 1:2.25 | 1711.1 | 0.216 | 32.5 | 26.51 |

**In-Vitro Release Studies**

The results of the drug release experiments from the NPs are shown in Fig. 2. All the formulations tested showed similar release profiles in phosphate buffer, pH 6.4. These profiles were characterized by an initial fast release phase followed by a delayed release. These results suggest that a certain amount of drug-Cyclodextrin complexes, which are present near to the surface of NP's, can be easily released by simple diffusion through the polymer network. However, there is an additional amount of complex that cannot be released from the particles unless the polymer matrix is degraded.

Release of drug from NP's containing Glycol chitosan was more rapid than that of NP's containing chitosan, as seen in figure 2. This release behaviour can be explained as; Glycol chitosan is soluble in neutral pH, release is faster than NP's containing Chitosan due to faster solubilization. In case of chitosan NP's the release is slower as drug is released only by diffusion through polymer matrix.

From the release profile it appears that in NP's which contains anionic cyclodextrin derivative i.e. CM-β-CD forms more rigid complex with cationic polymer chitosan resulting in slower release of drug from CM-β-CD containing NP's.

Also the NP's which were prepared by using TPP only, drug releases readily than NP's which were formed either by TPP in combination with CM-β-CD or by CM-β-CD only. This nature of release from NP's containing anionic CD derivative i.e.CM-β-CD, in all probability can be explained by the fact that, anionic CD derivative has tendency to extensively crosslink with cationic polymer than TPP.

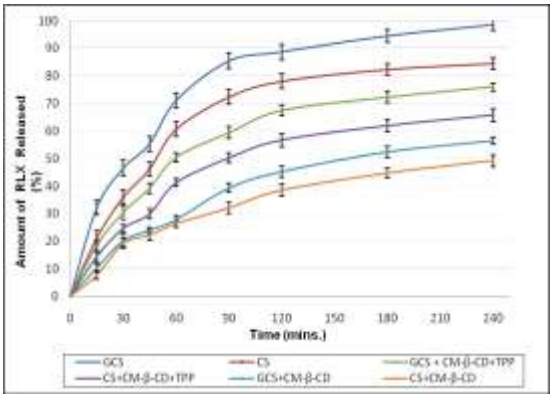


Fig.2: In-vitro release profile of RLX from nanoparticles in phosphate buffer pH 6.4

Due to this release of drug from NP's containing TPP in combination with CM-β-CD or CM-β-CD only is slower than the NP's prepared with the help of TPP as a cross linker.

**Ex-Vivo Permeation through Sheep Nasal Mucosa**

Results of the ex-vivo permeation of nanoparticles across the nasal mucosa are given in Fig.3. Results indicate that the permeation of RLX depends on the amount of drug released from NP matrix. Permeation profiles indicate that initially more amount of drug is permeated through mucosa, i.e. for Glycol chitosan NP's more than 50% of drug permeated within first 2hrs. after that drug permeates at slower rate. This indicates that the permeation of drug was concentration dependant.

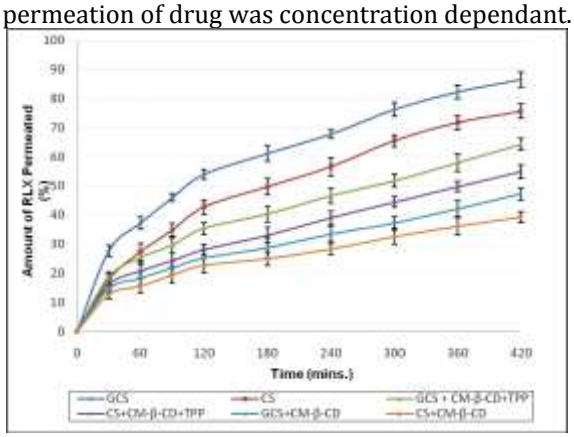


Fig. 3: Ex-vivo permeation profiles of RLX through Sheep nasal mucosal membrane

As the concentration of drug in donor compartment depletes, the permeability of drug also decreases. This fact is also proven by calculating the flux i.e. amount of drug permeated per cm2 per hour. Glycol Chitosan NP's shows more permeation of drug than Chitosan NP's, this is due to the fact that Glycol Chitosan NP's releases drug more readily at the mucosal surface due to its soluble nature than chitosan NP's. In case of NP's containing anionic CM-β-CD, permeation of drug is slow due to late dissociation of anionic CD complex from NP matrix.

NP's prepared either by TPP in combination with CM-β-CD or by CM-β-CD only shows lesser permeability of drug

than the NP's prepared by using TPP alone, most probably due to extensive crosslinking by anionic CD derivative.

**Data Analysis**

**Determination of Flux Per Hour Through Mucosal Membrane**

Nature of flux per hour across Sheep nasal mucosa is shown in Fig.4. From the profile of flux vs. time it was observed that, initially flux across the mucosal membrane was high and it decreases as time proceeds. This was due to presence of drug on the surface of NP's which resulted in burst release. Due to initial burst release more amount of drug is available for permeation through mucosa and hence higher initial flux across the membrane. As time proceeds there is depletion of drug in NP matrix and less drug is available for permeation across the membrane. Due to this the flux decreases gradually with time finally remaining nearly constant after 3 hrs. due to complete depletion of drug from NP's matrix after 3hrs.

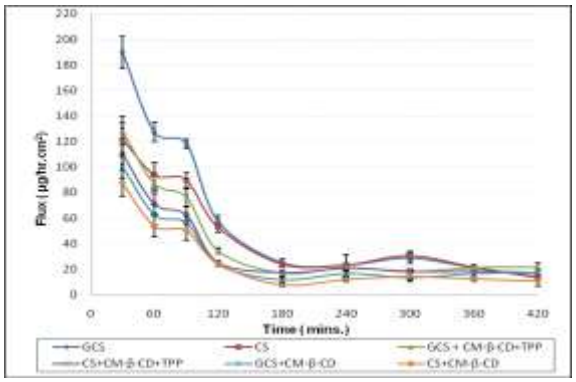


Fig. 4: Graph representing change in flux per hour across Sheep nasal mucosal membrane

**Drug Release Kinetics**

The ex-vivo permeation data was analyzed according to zero- and first-order kinetics as well as diffusion- controlled mechanism (Higuchi), Korsmeyer-Peppas model and Hixson-Crowell model using linear regression analysis. The results, as shown in Table: 4 revealed that the drug release from the nanoparticles followed Higuchi diffusion model with a correlation coefficient ranging from 0.992 to 0.997, which means an excellent model fit. This finding indicates that the rate-controlling stage in the permeation process was diffusion of the dissolved drug through the matrix of NP's to the external medium.

**Determination of Effective Permeability Coefficients (Papp)**

Effective permeability coefficients (Papp) were calculated for all batches and their values were given in Table: 5. Results of Papp indicate that the permeability of RLX across the Sheep nasal mucosal membrane depends on the release of RLX from matrix of NP's. Permeability coefficients are higher for Glycol chitosan than Chitosan due to soluble nature of Glycol chitosan

Table 4: Kinetic treatment of the permeation data of Raloxifene HCl from different nanoparticles formulations

| Batch | r2 | | | | |
|---|---|---|---|---|---|
| | Zero Order | First order | Higuchi | Korsmeyer -Peppas | Hixson - Crowell |
| GCS | 0.875 | 0.874 | 0.993 | 0.995 | 0.960 |
| CS | 0.907 | 0.854 | 0.997 | 0.942 | 0.960 |
| GCS+CM-β-CD+TPP | 0.900 | 0.916 | 0.995 | 0.979 | 0.983 |
| CS+CM-β-CD +TPP | 0.926 | 0.947 | 0.995 | 0.994 | 0.995 |
| GCS+CM-β-CD | 0.912 | 0.941 | 0.992 | 0.989 | 0.991 |
| CS+CM-β-CD | 0.899 | 0.921 | 0.992 | 0.979 | 0.982 |

Table 5: Effective Permeability Coefficients (Papp) of Raloxifene HCl from different nanoparticles formulations *n=3; S.D. =Standard Deviation.

| Batch | Papp (*10-5 cm/sec) ±S.D.* |
|---|---|
| GCS | 8.2049 (±0.51726) |
| CS | 7.4583 (±0.060292) |
| GCS + CM-β-CD+TPP | 6.2719 (±0.095524) |
| CS+CM-β-CD+TPP | 5.5242 (±0.033323) |
| GCS+CM-β-CD | 4.5301 (±0.029705) |
| CS+CM-β-CD | (±0.031433) |

.

Permeability coefficients for NP's containing CM-β-CD were less due to more intimate contact between anionic cyclodextrin with chitosan or Glycol chitosan; thereby resulting in association of complex with polymer for longer duration than that of neutral cyclodextrin derivative. This leads to decrease Papp in case of anionic cyclodextrin derivative i.e. CM-β-CD. Also since TPP is weak crosslinker than CM-β-CD, Papp for NP's prepared using TPP was less than NP's prepared using CM-β-CD along with TPP or CM-β-CD alone.

## Conclusion

Aqueous solubility of RLX was significantly improved due to formation of inclusion complex with CM-β-CD and chitosan does not hinder the formation of such inclusion complex. During the formation of RLX-cyclodextrin it was found that nanoparticle preparation method is very sensitive to the ratio between polymer and crosslinker, as was evident from either nanoparticles being not formed or large aggregates of polymer being formed depending upon the ratio used . Also the chemical nature of chitosan

and anionic cyclodextrin significantly affected the size shape, zeta potential and entrapment efficiency of formed nanoparticles. The inherent tendency of anionic cyclodextrin derivative to crosslink with cationic polymer like Chitosan, was further substantiated by the lesser amount of tripolyphosphate required for complexation during preparation of nanoparticles. The release of drug from the matrix of nanoparticles showed initial burst release followed by delayed release. It is also observed that effect of both Chitosan and cyclodextrin was synergistic and resulted in improvement in solubility of drug and enhancement of mucoadhesion. It is believed that utilization of nanoparticulate system for delivery of RLX through nasal route would increase its bioavailability than oral route, thereby decreasing the dose of drug which is required to treat osteoporosis and side effects associated with high oral dose.

## Acknowledgement

The authors would like to thank Aurobindo Pharma Ltd Hyderabad, India for the gift sample of Raloxifene HCl.

## Conflict of Interest

The authors declare no conflict of interest.

## Funding Disclosure

Pramod Bhise would like to thank Department of Pharmaceuticals, Ministry of chemicals and fertilizers, Govt. of India, for providing financial assistance during project.

## References

1. Delmas PD, Bjarson NH, Mitlak B, Ravoux AC, shah A, Huster W, Draper M, Christiansen C. Effect of Raloxifene on bone mineral density , serum cholesterol concentrations and uterine endometrium in post menopausal women . N Engl J Med 1997; 337:1641-1647.
2. Mitlak BH, Cohen FJ. In search of optimal long-term female hormone replacement: the potential of selective estrogen receptor modulators. Horm Res 1997; 48: 155-163.
3. Raloxifene Patient information leaflet http://www.accessdata.fda.gov/Scripts/cder/DrugsatFDA/index.cfm?fuseaction=Search.Label_ApprovalHistory (Cited 2011 June 27).
4. Illum L. Nasal drug delivery-possibilities, problems and solutions. J Control Rel. 2003; 87: 187-198.
5. Illum L. Is nose-to-brain transport of drugs in man a reality? J Pharm Pharmacol. 2004; 56: 3-17.
6. Pontiroli AE. Peptide hormones: review of current and emerging uses by nasal delivery. Adv Drug Deliv Rev 1998; 29: 81-87.
7. Song Y, Wang Y, Thakur R, Meidan VM, Michniak B. Mucosal drug delivery: membranes, methodologies, and applications. Crit Rev Ther Drug Carrier Sys 2004; 21:195-256.
8. Fernández-Urrusuno R, Calvo P, Remuñán-López C, Vila- Jato JL, Alonso MJ. Enhancement of nasal absorption of insulin using chitosan nanoparticles. Pharm Res 1999; 16: 1576-81.
9. Van der Lubben I M, Kersten G, Fretz M M, Beuvery C, Coos Verhoef J, Junginger HE. Chitosan microparticles for mucosal vaccination against diphtheria: Oral and nasal efficacy studies in mice. Vaccine 2003; 21: 400-8.
10. Shaoyun Yu, Ying Zaho, Fenglan Wu, Xuan Zhang, Wanliang Lu, Hua Zhang, Qiang Zhang. Nasal insulin delivery in the Chitosan solution: invite and invivo studies. Int J pharm 2004; 281: 11-23.
11. Artursson P, Lindmark T, Daris S S, Illum L. Effect of chitosan on the permeability of monolayers of intestinal epithelial cells ( Caco -2 ). Pharm Res 1994; 11: 1358-1361 .
12. Schipper N G M, Hermens, W A J J, Romeijn S G, Verhoef J, Merkus, F W H M. Nasal absorption of 17 β estradiol and progesterone from a dimethyl β-cyclodextrin inclusion formulation in rats. Int J pharm 1990; 64: 61-66.
13. Wempe M F, Wacher V J, Ruble K M, Ramsey M G, Edgar K J, Buchanan N L, Buchanan C M. Pharmacokinetics of Raloxifene in male Wistar-Hannover rats: Influence of complexation with hydroxybutenyl- beta- cyclodextrin. Int J Pharm 2008; 346: 25-37.
14. Merkus F W H M, Verhoef J C, Marttin E, Romeijn S G, van der Kuy P H M, Hermens, W A J J, Schipper N G M. Cyclodextrins in nasal drug delivery. Adv Drug Deliv Rev 1999; 36:41-57.
15. Asai K, Morishita M, Katsuta H, Hosoda S, Shinomiya K, Noro M, Nagai T and Takayama K. The effect of water soluble cyclodextrin on the histological integrity of the rats nasal mucosa. Int J Pharm 2002; 264:25-35.
16. Agu R U, Jorissen M, Willems T, den Mooter G V, Kinget R, Verbeke N , Augustijns P . Safety assessment of selected cyclodextrin-effect of cilliary activity using a human cell suspension culture model exhibiting in-vitro ciliogenesis. Int J Pharm 2000; 193:219-226.
17. Calvo P, Remunan-Lopez C, Vila-Jato JL, Alonso MJ. Chitosan and chitosan/ethylene oxide-propylene oxide block copolymer nanoparticles as novel carriers for proteins and vaccines. Pharm Res 1997a; 14 (10): 1431–1436.

18. Fernández-Urrusuno R, Calvo P, Remuñán-López C, Vila- Jato J L, Alonso M J. Enhancement of nasal absorption of insulin using chitosan nanoparticles. Pharm Res 1999 ; 16 : 1576–1581.
19. Janes K, Alonso M J. Depolymerized chitosan nanoparticles for protein delivery: preparation and characterization. J Appl Polym Sci 2003;12: 2769–2776.
20. Gungor S, Erturk -Toker A O S, Baktir G, Ozsoy Y. Ondansetron -loaded chitosan microspheres for nasal antiemetic drug delivery: an alternative approach to oral and parenteral routes. Drug Dev Ind Pharm 2010; 36(7): 806-813.
21. Mora P C, Cirri M, Mura P. Differential scanning calorimetry as a screening technique in compatibility studies of DHEA extended release formulations. J Pharm Biomed Anal 2006; 42: 3-10.
22. Mura P, Furlanetto S, Cirri M, Maestrelli F, Corti G, Pinzauti S. Interaction of naproxen with ionic cyclodextrins in aqueous solution and in the solid state, J Pharm Biomed Anal 2005;37: 987-994.
23. Corti G, Capasso G, Maestrelli F, Cirri M, Mura P. Physical-chemical characterization of binary systems of Metformin hydrochloride with triacetyl-cyclodextrin. J Pharm Biomed Anal 2007; 45: 480-486.