Abstract

Systemic drug delivery through the nasal route has gained great interest as it provides several advantages over other routes of drug administration. These include rapid absorption, avoidance of the hepatic first-pass effect and high potential for drug transfer to the cerebrospinal fluid. Unfortunately, the mucociliary clearance, which reduces the residence time of the nasally applied drugs and the poor nasal permeability made it difficult for many drugs to be delivered through this route. Alternative approaches have been adopted to overcome these problems. These include the use of mucoadhesive formulations or chemical penetration enhancers. Colloidal drug carrier systems provide promising alternative for enhanced and controlled nasal drug delivery.

Carvedilol is a nonselective β-adrenergic antagonist used in the treatment of hypertension and angina pectoris. It is rapidly absorbed after oral administration from the gastrointestinal tract (80%) with low bioavailability (~25%) due to significant first-pass hepatic metabolism by cytochrome P450 and short plasma half-life of 6 hr.

The objective of work is to administer carvedilol by nasal route loaded into different nanocarriers to be incorporated in a mucoadhesive thermosensitive *in situ* gel, in order to improve its bioavailability and therapeutic efficacy. Thus the work in this thesis is divided into five chapters:

Chapter I: Formulation and Evaluation of Carvedilol-Loaded Transfersomes

Chapter II: Formulation and Evaluation of Carvedilol-Loaded Solid Lipid Nanoparticles

Chapter III: Formulation and Evaluation of Carvedilol-Loaded Chitosan-Sodium Alginate Nanoparticles

Chapter IV: Formulation and Evaluation of Carvedilol Mucoadhesive Thermosensitive *In Situ* Gel Formulations

Chapter V: Bioavailability Study of Carvedilol from Some Selected Formulations

Chapter I

Formulation and Evaluation of Carvedilol-Loaded Transfersomes

The aim of work in this chapter was to develop phospholipid based vesicular systems encapsulating carvedilol for its nasal delivery after incorporating different edge activators (EAs) in the lipid bilayer. An anionic as well as non-ionic surfactants were utilized in the preparation of carvedilol transfersomes. The effect of different EAs with different ratios with respect to phosphatidylcholine (lecithin) was investigated.

Preformulation studies were carried out to detect interaction between carvedilol and components of transfersomes using differential scanning calorimetry (DSC) and Fourier-transform infra red spectroscopy (FT-IR).

Different carvedilol transfersomal vesicles were prepared according to the thin film hydration technique. Transfersomal formulations were prepared using lecithin and different EAs including Span 20, Span 60, Tween 20, Tween 80 and sodium deoxycholate (SDC) and each EA was used in three different ratios with respect to lecithin including 95:5%, 85:15% and 75:25% (w/w) (lecithin:EA). An amount of 10 mg carvedilol was used in each formulation.

The prepared formulations were characterized with respect to entrapment efficiency (EE%), particle size measurement by dynamic light scattering (DLS), transmission electron microscopy (TEM), *in vitro* release, *ex vivo* permeation, confocal laser scanning microscopy (CLSM) and stability studies.

In vitro release studies were performed using vertical diffusion Franz cells with an effective diffusion area of 5 cm^2. A 50 ml of simulated nasal electrolyte solution (SNES) pH 5.5 containing 20% propylene glycol was used as receptor medium. The receptor compartment was maintained at 37 ± 0.5°C and stirred by a magnetic bar at 100 rpm for 8 hr. The donor compartment was separated from the receptor compartment by cellulose dialyzing membrane with molecular

weight cut off of 12000 Da. The linear regression analysis was applied to all *in vitro* release results. The correlation coefficient [r] was determined in each case and accordingly, the orders of release were determined.

The excised superior camel nasal membrane mounted on Franz diffusion cell was used for *ex vivo* permeation studies. Franz diffusion cell used for *ex vivo* diffusion studies had a surface area 5 cm^2 and mucosa thickness 0.2 ± 0.1 mm. The temperature of the receiver chamber containing 50 ml of diffusion media (phosphate buffer saline (PBS) pH 6.4 containing 20% propylene glycol) was controlled at 37 ± 0.5°C under continuous stirring with magnetic bar at 100 rpm for 24 hr.

Stability testing was carried out on formulation T14 (containing SDC with ratio of 85:15% w/w lecithin:EA) by measuring vesicle size, EE% and zeta potential monthly for a period of three months.

The results revealed the following:

- The results of DSC and FT-IR obtained showed no interaction between carvedilol and components of transfersomes.
- Carvedilol was successfully embedded in transferosomal shell and the EE% for all vesicles was in the range of 43.68–88.72%, where T4 (containing Span 60 with ratio of 95:5% (w/w) lecithin:EA) gave the highest values.
- Mean diameter of the prepared carvedilol vesicles was in the range of 295–443 nm with polydispersity indices ranging from 0.08 to 0.2. T14 gave the smallest particle size while T7 (containing Tween 20 with ratio of 95:5% (w/w) lecithin:EA) gave the largest particle size.
- The release rate of carvedilol from all transferosomes was lower than the corresponding control solution. The ratio (85:15%, w/w) showed optimum release of carvedilol from all transferosomal formulations containing different EAs. Formulation T14 showed the highest release percentage, while formulation T4 showed the smallest release percentage.

- Linear regression analysis of the release data revealed that carvedilol was released from the majority of transfersomes by a diffusion-controlled mechanism except formulations T7, T11, T13 and T15 which followed zero-order kinetics while carvedilol solution followed first-order kinetics.
- The developed transfersomes exhibited a significant higher ($p < 0.05$) nasal mucosa permeation compared to the control solution containing equivalent amount of carvedilol. Formulation T14 gave the best permeation parameters, while the lowest permeation parameters were obtained in formulation T4.
- CLSM study results revealed that transfersomal formulation T14 was highly penetrated throughout different layers of nasal mucosa with a high fluorescence intensity and homogeneous distribution as compared to solution of rhodamine.
- Transmission electron micrograph of formulation T14 showed the outline and core of the well-identified ellipsoidal vesicles. They were smooth and showed bilayers structure of their membrane.
- Formulation T14 was found to be stable on storage for a period of three months, so it was selected to be incorporated into three different thermosensitive *in situ* gel matrices for further study.

Chapter II

Formulation and Evaluation of Carvedilol-Loaded Solid Lipid Nanoparticles

This chapter included preparation of carvedilol-loaded SLNs by emulsion/solvent evaporation method. Furthermore, the influence of some formulation variables on the characteristics of the developed SLNs was also investigated.

Preformulation studies were carried out to detect interaction between carvedilol and components of SLNs using DSC and FT-IR.

Eight formulations were proposed adopting a factorial design (2^3), in which three factors were tested. They were namely, lipid type (Compritol or Precirol), surfactant concentration (1 and 2% (w/v) poloxamer 188) and co-surfactant concentration (0.25 and 0.5% (w/v) lecithin). SLNs were prepared using a modified emulsion/solvent evaporation method. An amount of 10 mg carvedilol was used in each formulation.

The prepared formulations were characterized with respect to EE%, particle size measurement by DLS, TEM, *in vitro* release, *ex vivo* permeation, CLSM and stability studies.

The used procedure for *in vitro* release and *ex vivo* permeation studies was similar to that performed in chapter I.

Stability testing was carried out on formulation S8 (containing precirol as a solid lipid material, 0.5% (w/v) lecithin and 2% (w/v) poloxamer 188) by measuring particle size, EE% and zeta potential monthly for a period of three months.

The results revealed that:

- The results of DSC and FT-IR obtained showed no interaction between carvedilol and components of SLNs.
- Carvedilol was successfully embedded in SLNs' core with high EE% reached 87.62%. SLN formulations containing compritol (S1–S4) showed a significant higher EE% ($p < 0.05$) compared to those containing precirol (S5–S8). Also, increasing the concentration of surfactant from 1 to 2% (w/v) resulted in a significant decrease in the EE% of the produced SLNs ($p < 0.05$). On the other hand, increasing lecithin concentration resulted in a consequent increase in EE% ($p < 0.05$). The combination of Compritol with 1% (w/v) poloxamer 188 and 0.5% (w/v) lecithin, S3, gave the highest EE% while the combination of Precirol with 2% (w/v) poloxamer 188 and 0.25% (w/v) lecithin, S6, gave the lowest EE%.

- All the prepared SLNs exhibited small particle size with narrow particle size distribution ranging from 66 to 352 nm. Formulations containing Compritol showed larger particle sizes than those containing Precirol and a gradual decrease in particle size was observed with increasing surfactant concentration ($p < 0.05$). Also, the particle size was found to decrease with increasing lecithin concentration ($p < 0.05$). The combination of Precirol with 2% (w/v) poloxamer 188 and 0.5% (w/v) lecithin, S8, gave the smallest particle size while the combination of compritol with 1% (w/v) poloxamer 188 and 0.25% (w/v) lecithin, S1, gave the largest particle size.
- Carvedilol–loaded SLN formulations were able to retard its release and the percentage of carvedilol released up to 8 hr ranged from 35.04% to 55.51%. Higher release was achieved with Precirol (S5–S8) compared to Compritol (S1–S4). Increasing the poloxamer 188 concentration led to corresponding increase in the percentage of carvedilol released. Also, increasing lecithin concentration resulted in an increase in the percentage of carvedilol released. Formulation S8 exhibited the highest percentage of carvedilol release.
- Most of carvedilol–loaded SLNs had a release order of Higuchi–type release except formulations S4 and S6 that exhibited zero–order release model. Carvedilol solution followed first–order kinetics.
- The produced carvedilol–loaded SLNs showed better diffusion compared to the control solution as confirmed by CLSM study. Formulation S8 gave the best permeation parameters.
- TEM revealed that carvedilol–loaded SLNs had a spherical shape with thin layer surrounding the particles which postulated a drug–enriched core model.

- Formulation S8 was found to be stable on storage for a period of three months, so it was chosen to be incorporated into three different thermosensitive *in situ* gel matrices for further study.

Chapter III

Formulation and Evaluation of Carvedilol-Loaded Chitosan-Sodium alginate nanoparticles

This chapter included with Preparation of carvedilol–loaded chitosan–sodium alginate nanoparticles based on the formation of a polyionic complex between the two biopolymers.

Preformulation studies were carried out to detect interaction between carvedilol and components of chitosan–sodium alginate nanoparticles using DSC and FT-IR.

Nine formulations of carvedilol–loaded chitosan–sodium alginate nanoparticles were prepared on the basis of a 3^2 factorial design. The independent variables were chitosan concentration (X_1) and sodium alginate concentration (X_2). Chitosan–sodium alginate nanoparticles were prepared by ionic gelation technique. Different chitosan–sodium alginate nanoparticulate formulations of carvedilol were prepared using the following composition: chitosan (0.1, 0.2 and 0.3% w/v) and sodium alginate (0.2, 0.4 and 0.6% w/v). An amount of 10 mg carvedilol was used in each formulation.

The prepared formulations were characterized with respect to EE%, particle size measurement by DLS, TEM, *in vitro* release, *ex vivo* permeation, CLSM and stability studies.

The used procedure for *in vitro* release and *ex vivo* permeation studies was similar to that performed in chapter I.

Stability testing was carried out on formulation I5 (containing 0.2% (w/v) chitosan and 0.4% (w/v) sodium alginate) by measuring particle size, EE% and zeta potential monthly for a period of three months.

The results revealed that:

- The DSC and FT-IR studies showed that there was no interaction between carvedilol and both chitosan and sodium alginate.
- Carvedilol was successfully incorporated into chitosan–sodium alginate nanoparticles and the EE% of the nanoparticles was found to vary between 33.85% and 67.75%. It was also observed that the entrapment of carvedilol into chitosan–sodium alginate nanoparticles was highest when the chitosan and sodium alginate were used at intermediate concentrations. The combination of chitosan and sodium alginate at the intermediate levels, I5, gave the highest EE% while the combination of chitosan at the higher level and sodium alginate at the lower level, I7, gave the smallest EE%.
- The particle size of all formulations ranged from 122 to 416 nm with polydispersity index values ranging from 0.1 to 0.22. The combination of chitosan and sodium alginate at the lower levels, I1, gave the smallest particle size while the combination of chitosan and sodium alginate at the higher levels, I9, gave the largest particle size.
- The release rate of carvedilol from all chitosan–sodium alginate nanoparticles was lower than the corresponding control solution. Formulations containing lower level of the polymers showed higher release than other formulations.
- Most of carvedilol–loaded chitosan–sodium alginate nanoparticles had a release order of Higuchi–type release except formulations I6 and I7 that exhibited zero–order release model.
- The prepared carvedilol–loaded chitosan–sodium alginate nanoparticles exhibited enhanced diffusion compared to the control solution. Formulation I5 containing the intermediate concentrations of both chitosan and sodium alginate gave the best permeation parameters.

- CLSM study revealed higher nasal mucosa permeation compared to the control solution
- TEM revealed that carvedilol–loaded chitosan–sodium alginate nanoparticles had a spherical shape with particle size somewhat smaller than that obtained by DLS.
- Formulation I5 was found to be stable on storage for a period of three months, so it was chosen for further study.

Chapter IV

Formulation and Evaluation of Carvedilol Thermosensitive *In Situ* Gel Formulations

In this chapter, formulation of carvedilol (either plain or being loaded in transfersomes, SLNs and chitosan–sodium alginate nanoparticles) mucoadhesive thermosensitive *in situ* gel using a combination of poloxamer 407 and poloxamer 188 was carried out. Three mucoadhesives (Carbopol 971P, HPMC K15M or chitosan) were adjuncted with poloxamers.

Preformulation studies were carried out to detect interaction between carvedilol and *in situ* gel components using DSC and FT-IR.

A combination of 20% (w/w) poloxamer 407 and 10% (w/w) poloxamer 188 with mucoadhesives was used for the preparation of *in situ* gel formulations of carvedilol. The mucoadhesives used were the neutral polymer HPMC K15M (1% w/w), the cationic polymer chitosan (0.75% w/w) as well as the anionic polymer Carbopol 971P (0.5% w/w). The selected formulations of carvedilol–loaded transfersomes (T14), SLNs (S8) and chitosan–sodium alginate nanoparticles (I5) as well as plain carvedilol were incorporated into the different *in situ* gel bases adopting the cold method. Mixture of 0.1% methylparaben and 0.01% propylparaben was used as preservatives. For carvedilol–loaded chitosan–sodim alginate nanoparticles, only poloxamer 407 and poloxamer 188 were added as they already contain the mucoadhesive polymers chitosan and

sodium alginate. The final concentration of carvedilol was 12.5 mg/ml in all preparations.

The prepared *in situ* gel formulations were characterized by visual inspection, measuring homogeneity percentage, pH and spreadability. Also, evaluation of the prepared formulations for their sol–gel transition temperature, mucoadhesion strength, rheological behavior, *in vitro* drug release, *ex vivo* permeation and histopathological studies were carried out.

The results revealed that:

- The DSC and FT-IR studies revealed that carvedilol was found to be compatible with *in situ* gel components.
- All *in situ* gel formulations were found to be homogenous either transparent in case of plain carvedilol or opaque in case of carvedilol–loaded transfersomes, SLNs and chitosan–sodium alginate nanoparticles, free from lumps, had high spreadability and the pH was within acceptable range.
- All the prepared carvedilol *in situ* gel formulations were found to gel at temperature ranged from 28.5 to 31.9°C, which are considered to be suitable for nasal application.
- All *in situ* gel formulations showed high mucoadhesive strength ranging from 3655 to 8437 (Dyne/cm^2), where formulations prepared using HPMC K15M exhibited the highest values followed by formulations containing Carbopol 971P then those containing chitosan.
- The rheological behaviors of the *in situ* gel formulations revealed that they exhibited a shear rate thinning behavior with thixotropy.
- Plain carvedilol had better release from chitosan (F6) than Carbopol 971P (F4) followed by HPMC K15M (F5) *in situ* gel matrix while for *in situ* gel of carvedilol–loaded transfersomes and SLNs, the highest release percentages were observed in Carbopol 971P (F7 and F10) followed by

HPMC K15M (F8 and F11) then chitosan (F9 and F12). All *in situ* gel formulations followed Higuchi diffusion model.

- The best permeation parameters were obtained in F7 (carvedilol–loaded transfersomes in Carbopol 971P polymer) followed by F10 (carvedilol–loaded SLNs in Carbopol 971P polymer) then F13 (carvedilol–loaded chitosan-sodium alginate *in situ* gel).
- Histological examination of nasal mucosa revealed that *in situ* gel formulations (F7, F10 and F13) had good nasal tolerability.

Chapter V

Bioavailability Study of Carvedilol from Some Selected Formulations

In this chapter, the pharmacokinetic parameters of carvedilol from the selected intranasal *in situ* gel formulations F7 and F10, oral carvedilol suspension and intravenous solution were studied in rabbits. Also, the absolute bioavailability of carvedilol from the selected formulations was determined.

On the basis of the previous studies, four formulations were selected for the bioavailability study:

• F7 (equivalent to 12.5 mg/ml carvedilol), composed of carvedilol–loaded transfersomes containing SDC as an EA with 85:15% (w/w) (lecithin:EA) ratio incorporated into a thermosensitive *in situ* gel base composed of poloxamer 407/poloxamer 188 (20/10 w/w) and 0.5% Carbopol 971P polymer.

• F10 (equivalent to 12.5 mg/ml carvedilol), composed of carvedilol–loaded SLNs containing Precirol (100 mg), 2% (w/v) poloxamer 188 and 0.5% (w/v) lecithin incorporated into a thermosensitive *in situ* gel base composed of poloxamer 407/poloxamer 188 (20/10 w/w) and 0.5% Carbopol 971P polymer.

• Carvedilol coarse suspension (1 mg/ml) in purified water.

• Carvedilol solution (1 mg/ml) prepared in sterile water for injection containing 1% (w/v) Tween 80 and 2% (w/v) ethanol.

A cross over design was carried out using six rabbits. Plasma samples were analyzed using a modified LC–MS/MS method.

The results revealed the following:

- The LC–MS/MS assay was validated and had a good linearity from 5–300 ng/ml with acceptable within and between day reproducibility. The lower limit of carvedilol quantification in plasma was 5 ng/ml.
- Results showed that the mean values for the peak plasma concentration (C_{max}) of carvedilol were 57.02 ± 7.62 ng/ml, 157.47 ± 21.68 ng/ml and 122.49 ± 16.17 ng/ml following the administration of oral carvedilol suspension, intranasal *in situ* gel formulations F7 and F10, respectively. There was a significant difference between peak plasma concentration of oral carvedilol suspension and the selected intranasal formulations F7 and F10 ($p < 0.001$). It was also clear that there was significant difference between C_{max} of the two tested *in situ* gel formulations ($p < 0.05$).
- Concerning the area under plasma concentration time curve, $AUC_{(0-\infty)}$ for carvedilol, the mean values were 601.21 ± 147.88 ng.hr/ml, 1582.97 ± 310.88 ng.hr/ml, 1263.83 ± 373.39 ng.hr/ml and 2496.86 ± 391.21 ng.hr/ml following the administration of oral carvedilol suspension, intranasal *in situ* gel formulations F7, F10 and intravenous solution, respectively. Statistical analysis revealed that there was a significant difference between the oral suspension and the intranasal *in situ* gel formulations.
- The percentage absolute bioavailability was calculated and found to be 24.08%, 63.4% and 50.62% for oral carvedilol suspension, intranasal *in situ* gel formulations F7 and F10, respectively.
- The intranasal *in situ* gel formulations F7 and F10 exhibited 2.63 and 2.1 folds increase in the bioavailability, respectively compared to oral carvedilol suspension.

- The developed transfersomes and SLNs could be considered as suitable drug carrier systems for the noninvasive nasal delivery of drugs exhibiting poor oral bioavailability.