Chapter 6

SUMMARY AND CONCLUSION
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6.1 Introduction
Epilepsy is a common and complex pathology characterized by anomalous neuronal discharges (Chang et al., 2003). Chronic treatment with antiepileptic drugs aims to minimize the recurrence of convulsions by reducing the neuronal activity and excitability. At the same time, their inherent mechanism of action can eventually affect different neurobiological systems related to the cognitive process. From this perspective, both the disease and its treatment trigger cognitive impairment that could be especially critical in the earlier stages of the learning process (Browne and Holmes, 2000). Scientists proclaimed that Gabapentin (GBP), a well-known add-on antiepileptic drug used under chronic management, had minimal effects on cognitive functions and improved memory and attention (Kalviainen et al., 1997).

GBP [1-(aminomethyl)cyclohexane acetic acid] is a structural analog of γ-aminobutyric acid (GABA), with an incorporated cyclohexyl ring. GBP is freely soluble in water and across a wide range of pH and is characterized by a marked bitter taste (Thomas et al.; EP1628656 B1, 2008).

GBP is currently marketed as an adjunctive therapy for partial seizures in adults with epilepsy and for the management of postherpetic neuralgia (Bryans and Wustrow, 1999). In clinically recommended doses, it is rapidly absorbed following oral, single-dose administration to healthy volunteers (Vollmer et al., 1989; Hooper et al., 1991). The site of action of GBP is, the alpha-2—delta (α2—δ) protein, an auxiliary subunit of voltage-gated calcium channels. It subtly reduces the synaptic release of several neurotransmitters, apparently by binding to α2—δ subunits, and possibly accounting for its actions in vivo to reduce neuronal excitability and seizures.

Currently in market tablets, capsules and oral Neurontin solution is available. Although all these dosage forms allow the satisfactory concentration of GBP in the plasma, they are, however, unsuitable for paediatric patients because of bitter taste. In addition to bitter taste this drug has another issue of stability. It is moisture sensitive and is to undergo intramolecular cyclization to form lactam impurity (Zong Z, Desai S et al., 2011) which should be below 0.4%. Therefore, in present research work an attempt was made to develop taste masked stable formulations of GBP for conventional delivery. Many taste masked oral solid dosage forms like tablets,
powders and multiparticulate preparations were investigated, as they are more stable in comparison to liquid dosage forms.

GBP has a relatively short half-life (i.e., 5-7 hours). Therefore, frequent dosing is necessary to maintain reasonably stable plasma concentrations. The effective dose of GBP is 900 to 1800 mg/day, which is given in divided doses, leads to significant noncompliance in epilepsy patients (Richter et al., 2003).

It has been determined that GBP is typically absorbed from the upper intestine, i.e., it has a narrow absorption window and is absorbed by active transport through a large neutral amino acid (LNAA) transporter (Stewart et al., 1993; Uchino et al., 2002). This transporter is located in the upper small intestine, has limited transport capacity, and becomes saturated at high drug concentrations. Consequently, the plasma levels of GBP are not dose proportional and, therefore, higher doses do not give proportionately higher plasma levels. Since the LNAA transporter responsible for GBP absorption is present only in the upper region of the intestine, the dosage form used to provide GBP should be designed to release GBP in the stomach at a rate such that the maximum amount of the drug is available in the intestinal segment.

Prolonged, stable exposure to GBP may provide other clinical benefits, including greater efficacy, prolonged duration of action, and a reduced incidence of adverse effects related to peak drug levels.

Therefore, attempts were made for designing Controlled release mucoadhesive drug delivery systems of gabapentin that will release drug over an extended period of time, and majorly in upper gastrointestinal tract to provide therapeutically effective plasma levels.

Hence the project was taken up with an objective to initially develop taste masked and conventional formulations and further to attempt controlled release matrix formulations using various parameters and to investigate pharmacodynamic and pharmacokinetic efficacy of the gabapentin.

6.2 Preformulation studies

6.2.1 Standardization of drug

Gabapentin was purchased from Sashun chemicals Ltd. The drug was screened and standardized as per monographic specifications and Certificate of Analysis. Table 2.1.1 illustrate various tests, observations and specifications for GBP. It passed the tests for identity, purity and the results were found to comply with all the standards
and hence was used for further incorporation in the taste masked formulations and mucoadhesive matrix delivery systems.

6.2.2 Standardization of excipients

All the excipients used for taste masking viz. sucralose, mannitol, microcrystalline cellulose, glyceryl monostearate, cetyl alcohol, glycerine, propylene glycol ion exchange resins Indion 234, Indion 294, Indion 414 and Indion 464, Pearlitol DC, Pharmaspheres 200 µ, beta-cyclodextrin, Eudragit EPO and the ones which were selected for incorporating in mucoadhesive matrix formulation viz. HPMC K100M, Carbopol 971 P, Klucel HXF, Natrosol 250HHX, Polyox 301 and 303, Sodium Alginate, Blanose 7HF, Avicel PH 101, Aerosil R972, Compritol 888 were standardized and complied as per the tests given in the respective monographs and Certificates of Analyses provided by the manufacturers. All these excipients were used in formulation development of proposed taste masked trials and oral controlled release mucoadhesive drug delivery systems.

6.2.3 Drug excipients compatibility studies

To investigate the stability of GBP in developed formulation and its interaction with polymers and other excipients, DSC studies were carried out. Endotherm of drug was obtained at 174°C. Endotherms of other excipients did not overlap with that of the drug indicating compatibility. Drug contents of excipient admixtures stored at 55°C for 1 month were determined, and there was no significant difference in drug content after storage indicating stability and absence of lactam formation.

6.2.4 Analytical Method Development and Validation

UV spectrophotometric, colorimetric, HPTLC and HPLC methods were developed for quantification of Gabapentin in various media as well as in plasma for in vivo studies.

i. UV Spectroscopy for GBP

GBP when scanned by UV spectroscopy it, showed absorption maxima of 215nm. Initially an UV visible spectrophotometric method for determination of GBP was explored. The calibration curve was obtained in methanol and in 0.1N HCl. The calibration curves exhibited linearity in both the media. GBP was found to be sensitive to UV spectroscopy in concentrations above 500µg/ml. Linear calibration curve in methanol, distilled water and in 0.1N HCl was obtained within a
concentration range of 500-3000 µg/ml. LOQ was found to be 500 µg/ml. Hence, UV spectrophotometric method developed was not found to be very sensitive to determine the low drug contents of dissolution aliquots in an initial (1-4) hours and drug content of those taste masked formulations where more amount of solvent for extraction of GBP was needed yielding concentrations below 500 ppm. Hence to enhance sensitivity and in turn detectability, a more sensitive colorimetric method was developed.

ii. Colorimetric method development for GBP
Colorimetric method was developed for GBP by derivatising it with ninhydrin reagent in N, N¹-DMF. The method was found to be sensitive in the concentration range of 40-240 µg/ml with wavelength maxima at 572 nm. The linearity was obtained with $r^2= 0.9909$. The developed method was validated for linearity, precision, accuracy, LOD, LOQ and robustness. This method was found to be more sensitive compared to the UV method i.e. in the range of 40-240 ppm. But since the gabapentin react with ninhydrin at the neutral pH only, it was difficult to estimate the drug content in acidic and basic vehicles. In dissolution studies of the Gabapentin tablets the dissolution media for mucoadhesive tablets was 0.1N HCl. Also analysis and quantitation of lactam impurity was not practically possible with the said method.

iv. HPTLC Method development for GBP
More sensitive HPTLC method for the quantitation of Gabapentin in oral dosage form was also developed for routine analysis. In this method the mobile phase of n-butanol: water: Glacial acetic acid (2.4:1.2:0.6) was used. The plate was then sprayed with 0.2% alcoholic ninhydrin solution and dried at 105°C for 10 min. Detection and quantification of gabapentin was performed by densitometry at $\lambda$, 490nm. Method was found to be sensitive in the concentration range of 200ng/ml to 1200ng/ml and linearity was obtained with $r^2= 0.9916$ for GBP. This procedure was too lengthy and tedious for day-to-day routine analysis of the dissolution samples so this method was discontinued. In addition, the quantitation of lactam was difficult with HPTLC. Therefore, HPLC method was further investigated for analysis of both gabapentin and lactam in dissolution samples.

iii. HPLC Method development:
HPLC method was developed for GBP and to determine lactam impurity content using Perfectsil C18 column (46mm x 250 mm, 2.5 µm) using Tosoh HPLC, Japan
system. For HPLC analysis of drug, the mobile phase consisted of Monobasic Ammonium Phosphate Buffer pH 1.8: Acetonitrile (76:24). The retention time for GBP was 6.8 ± 0.5 min. and that of lactam was 26.2 ± 0.5 min. Method was found to be sensitive in the concentration range of 50-500 µg/ml and 5-50 µg/ml. The linearity was obtained with $r^2 = 0.9991$ for GBP and $r^2 = 0.9998$ for lactam. The developed method was validated for linearity, precision, accuracy, LOD, LOQ and robustness.

**v. HPLC method with Spectrofluorimetric detection for estimation of GABA in the brain tissue**

All the chromatographic measurements were carried out by using a HPLC Merck Hitachi Model comprising of La Chrom L-7100 pump, Fluorimetric detector and L-7200 auto sampler by using C18, 150 m X 4.6 mm column and Buffer: Acetonitrile (77:23) as mobile phase. The retention time for GABA was found to be 18.2 ± 0.5 mins and the method was linear in range of 10-100 ng/ml. The method was validated for all the validation parameters like accuracy, linearity, precision, etc.

**vi. HPLC method with Spectrofluorimetric detection for estimation of Gabapentin in plasma**

For pharmacokinetic study of GBP, active drug content need to be analysed in plasma from blood samples collected from retro-orbital region of Wistar rat after administration of GBP. HPLC method with UV detector is not sensitive enough to detect the concentration of drug in plasma so more sensitive HPLC method employing spectrofluorimetric detection was developed for estimation of Gabapentin in plasma. Gabapentin content in plasma was calculated by using a HPLC Merck Hitachi Model comprising of La Chrom L-7100 pump, Fluorimetric detector and L-7200 auto sampler by using C18, 150 m X 4.6 mm with 0.33M acetate buffer (Containing 100 mg/ml EDTA): methanol: Acetonitrile (40:30:30, v/v) as mobile phase. Developed method was found to be sensitive in the range of 1 to 10 µg/ml and linearity was obtained with $r^2 = 0.9997$ for GBP in plasma.

6.3 Taste masked formulations of gabapentin

Several strategies for taste masking of gabapentin were explored to get a stable taste masked gabapentin formulation. In the present research project work, initially efforts
were carried out to attain taste masking by the traditional techniques like wet granulation, melt granulation, extrusion spherisation etc. followed by novel techniques such as spray drying, beta-cyclodextrin complex formation, adsorption of an ion exchange resins and many more as described in the following section. The method was validated for all the validation parameters like accuracy, linearity, precision, etc.

1) Wet granulation

i) Wet Granulation with Fillers: Wet granulation was tried initially using drug and varying ratios of various excipients like HPMC K15, microcrystalline cellulose, mannitol and PVP K30 as binders. For developed formulations taste assessment in human volunteers was carried out with the objective of evaluation of degree of taste masking of the developed formulations. Panel of six volunteers between the age group of 23 and 26 years was selected and explained the purpose of the study. Study protocol followed principles in the Declaration of Helsinki. Each volunteer tasted 1, 5 and 10 mg/ml solution of drug and taste-masked formulation with in-between washings of water. Volunteers were asked to give scores on a scale of 1–10. Score of 1 was assigned to very bad tasting formulations and a score of 9 was assigned to very good tasting formulations. Each score was converted to the corresponding percentage. Score of 9 was equivalent to 90% acceptance, whereas a score of 5 indicated 50% acceptance. Score for water was taken as 10 which is equivalent to 100% acceptance by human volunteers. Taste masking of the drug was not accomplished in absence of sweeteners. Therefore different concentrations of high intensity sweeteners like sucralose and flavors were tried out. Developed formulations were having very bad taste. (Taste score 1)

ii) Wet granulation with concentrated solutions of polymers without fillers: The drug was granulated with concentrated solutions of polymers like gelatin, guar gum, PVA and with supersaturated solution of sucrose and evaluated for taste masking. Taste making was not achieved in any of these formulations. (Taste score 1)

iii) Wet granulation by dispersion method: The drug was dispersed in 10% PVA solution to which starch was added as diluent under stirring with overhead stirrer. As the solid excipients were added, the dry agglomerates were formed and they were
2) Melt granulation: Here taste masking potential of melt granulation technology employing lipophilic carriers was checked.

I) Simple Melt Granulation: Initially waxes like cetyl alcohol and glyceryl monostearate were melted and the premix of drug and wax was added while stirring under overhead stirrer. The agglomerate was allowed to cool and the mass was passed through the 40# sieve. (Taste score 2)

II) Modified Melt granulation: In this method different ratios of Glyceryl monostearate and Triethyl citrate were tried. Glyceryl monostearate (GMS) was warmed till it melted completely and triethyl citrate was incorporated and stirred to form a homogenous solution. To this solution, gabapentin was added and the granular mass was mixed and allowed to cool to RT. This granular mass was first passed through # 16 and then through # 40 followed by extragranular mixing of premix of aerosol, sucralose and strawberry flavor. (Taste score 3)

3) Polymeric granules prepared by dispersion method: In this method initially premix of drug, aerosil and sucralose was prepared and this premix was then added to the polyox solution. This powder mix was homogeneously dispersed in polyox solution by means of stirring. This suspension was then poured in the petriplate and dried for 24 hrs. The dried mass was then passed through #16 to get granules. (Taste score 3).

4) Spray drying: Taste masking of GBP was further attempted by employing polymeric carriers like gelatin, Polyox WSR N10, Carbopol 971 P and lipid carriers like glyceryl monostearate and cetyl alcohol. Spray drying process parameters for trials with different polymers were optimized separately. In some of the trials high intensity sweeteners like sucralose and trehalose were also added. But irrespective of sweeteners appreciable taste-masking was not achieved in all the trials (taste score 2) except the trials with lipid carriers (taste score 4).

5) Taste masking by dissolution and adsorption of gabapentin on diluents: Initially the solubility of gabapentin in various non-aqueous solvents was quantified. Amongst the various solvents screened for assessing gabapentin solvation potential,
propylene glycol was selected as it exhibited maximum solvation ability i.e. 90mg/ml. Gabapentin was solubilized in propylene glycol and resulting solution was adsorbed on inert diluent microcrystalline cellulose to yield granules. Several strategies were adopted under this approach, viz. by altering the excipients, by addition of lipid carriers, sequence of addition and its effect on taste masking were also evaluated. Average taste score obtained by this approach was found to be 4 while some formulation trials containing lipid carriers exhibited taste score 9 reflecting complete taste masking. But when analyzed for lactam content it was above permissible limit.

6) **Pellets of gabapentin:** Spherical pellets were prepared using microcrystalline cellulose (MCC). Diluents like Avicel PH 101 and dicalcium phosphate were incorporated in spheronisation. Formulations with different binders like PVP K30 and polyox WSR N10 with their varying concentrations were prepared. Rotation speed of spheroniser and spheronising time was optimized to get pellets in particle size in the range of 800-1000µ with minimum fines. Ratio of water: IPA for dissolution of Polyox was also optimized during preparation of binder solution to decrease contact time of drug with water and minimize time for drying. When solution of PVP K30 in water was used as binder, formulations were slightly bitter. While on binding of pellets with alcoholic solution of Polyox, resulting formulations were moderately bitter. Optimized formulations with desired particle size range were subjected to coating with Opadry AMB (Colorcon Asia Pvt. Ltd) and with Instashield (Ideal cures). Coating solutions were prepared as per guidelines mentioned in the information sheet provided by respective manufacturers. Taste score value of 7 was assigned to the coated pellets. Pellets were evaluated for drug release behavior at salivary pH, %drug content and monitored for lactam impurity formation. Gabapentin pellets when subjected to stability studies were found to be unstable at all storage conditions with gradual decrease in percent drug content and increase in lactam impurity.

7) **Rapidly dissolving oral films (RDFs) or wafers:** Complexes of drug with Carbopol 971 P, Viscarin GP 109, Eudragit L100-55 and Blanose 7 LF were prepared and poured in petriplates followed by drying in vacuum oven at 55°C. Wafer like film was obtained in case of Viscarin GP 109 and glassy transparent films were obtained in case of Blanose 7LF while elastic films were obtained with Eudragit L100-55. Before subjecting to drying, sucralose was incorporated in low concentrations to all the
complexes to attain taste masking. Complete taste masking was observed in developed films (Taste score 9). But very low concentrations of drug could be incorporated in the films as only 5 ml of sample was used for casting films containing around 100mg of drug per film.

8) **Loading on non-pareil seeds:** Taste masking of gabapentin was also attempted by loading onto the non-pareil seeds. Non-pareil seeds are specially designed spherical particles of lactose that have uniform diameter and are available in different grades. Non-pareil seeds were rotated in Erweka multipurpose coating pan unit at 30 rpm and were wetted by spraying aqueous solution of 5% Polyox followed by addition of drug on this wetted bed. The pan was allowed to rotate for 5 minutes and then dried with a dryer. These loaded seeds were then coated with polymers for attaining taste masking. Two coating solutions i.e.4% Polyox + 0.6% triacetin in IPA: DCM (90:10) and 5% Cetyl Alcohol+1% Medium chain triglyceride in IPA were used. (Taste score 1)

9) **Ion exchange resin complex:** Taste masking of gabapentin was further attempted by loading on ion-exchange resin complexation. Formulations containing varying ratios of various cation exchange resins, viz Indion 214, 234, 204, 414, 294 and anion exchange resins viz. Indion 454 and controlled release cation exchange resins viz, Indion 244 were explored as discussed in chapter 3. Parameters like drug: resin ratio and time of stirring were optimized to attain taste masking and to enhance drug loading in the resin. (Taste score 9)

10) **Taste masking by inclusion complex formation with beta-cyclodextrin (BCD):** Dispersion method was used for preparing beta-cyclodextrin (BCD) inclusion complexes with gabapentin. Several drug: betacyclodextrin ratios were attempted and evaluated for taste masking potential. Complete taste masking was attained at 1:10 ratio of drug: BCD. This formulation when subjected to stability studies and analyzed for impurity formation, it was found to contain 1.5% lactam impurity. Further attempts are needed to minimize lactam formation.

11) **Preparation of Microspheres with Eudragit EPO:** Microspheres of Eudragit EPO with GBP were prepared by emulsion solvent evaporation and evaluated for taste masking ability. Process parameters, viz. drug: polymer ratio, composition of internal phase and external phase were optimised to get taste masked free flowing powder formulations. Here composition with 1:10 ratio of GBP: Eudragit EPO, produced
tasteless formulation (Taste score 9), but when the formulation was subjected to stability studies, lactam impurity was to the extent of 1.2% was observed in the said formulation. This may be due to process parameters viz. composition of internal phase and external phase, prolonged exposure to solvent during stirring period during microsphere formation. Hence further studies were discontinued.

12) Preparation of Chewable tablets: Scope of this approach in taste making was also evaluated by incorporating excipients, viz. mannitol, menthol, sucralose and xylitol. Powder blends for tablet formulations were assessed for bulk density, tapped density, angle of repose and tablets were assessed for hardness and friability. All the preliminary compositions of tablets were when subjected to taste analysis, they were extremely bitter. (Taste score 2)

13) Chemical approach: Chemical modification with cinnamic acid was attempted. Salt formed was characterised by I.R, NMR and Mass spectroscopy. The compound prepared exhibited identification peaks of both the starting materials reflecting salt formation. But after analysis of these salt, impurity was observed to be 1% which was above recommended limits. Taste score by this method was found to be 9 which is not further investigated.

For developing taste masking methodology for gabapentin taste masking massive experimentation was carried out. Several approaches for taste masking were explored. Some of the techniques apparently masked the bitter taste of gabapentin to larger extent while other approaches were not successful in masking the bitter taste.

- Complete taste masking was achieved with spray drying with lipids, by dissolving drug in PEG and then adsorbing it on inert substrate, by formulating rapidly dissolving films, by forming ion exchange resins complexes, by Inclusion complex formation with beta-cyclodextrin, by preparing microspheres with Eudragit EPO and by using chemical approach.

- Trials where complete taste masking was observed i.e. taste score of 9 was obtained, were subjected to stability studies and monitored for stability in terms of impurity formation. As gabapentin is highly unstable and a tricky molecule, it was very difficult to get any formulation without impurity. When these formulations were analyzed by HPLC using developed analytical method though impurity was
found to be within limits initially, after certain storage period lactam formation as impurity above the recommended limits i.e. 0.4% was detected.

6.4 Controlled release mucoadhesive matrix tablets of GBP

Efforts have been made to develop oral controlled release mucoadhesive controlled drug delivery systems of GBP by direct compression using hydrocolloid polymers. Oral dose of 900mg of GBP was decided for incorporating into once a day controlled release mucoadhesive tablets. Based on precise dose calculation desired drug release profile is found to be 25 to 30% in first 2hrs, 58 to 63% in next 6hrs and 83 to 88% in next 10 hrs. The formulations were optimized to achieve calculated desired release profile.

6.4.1 Preliminary trials

For developing this formulation, various hydrocolloid mucoadhesive polymers, viz. Sodium alginate, Klucel HXF, Polyox N303, Blanose 7 HF and Natrosol different grades were screened in preliminary trials. Factors affecting the drug release such as polymer viscosity, polymer ratios, combination of hydrophilic polymer with inert diluent, and mechanical strength of the polymer were also investigated. Precompression blend for the trial formulations were assessed for angle of repose, bulk density, tapped density, car’s Index, Hausner’s ratio and blend uniformity and compressed tablets were assessed for hardness, friability, weight variation, thickness and drug content uniformity.

In vitro dissolution study on the formulations containing HPMC K100M, HPMC 100LV, Klucel LF Sodium Alginate, Polyox WSR N80 and Blanose 7HF showed faster drug release so further study on these batches was discontinued. Formulation R9 and R10 containing 23% and 15.3% of Klucel HXF, respectively and in Formulation R12 and R13 containing 23 % Polyox 301 and Polyox 303 drug release was retarded for more than 12 hrs. Formulations R9, R10, R12, R13 had showed scope for yielding desired release profile at the lower concentrations as they retarded the drug release for more than 12 hrs. Klucel HXF and Polyox 301 and Polyox 303 polymers showed potential for carrying out the further trials and investigated in detail to attain desired release profile.
Two prototype mucoadhesive matrix formulations were optimized by taking nonionic polymers Klucel HXF and Polyox 301 polymers, respectively for comparative study as they exhibited the desired release profile with minimum tablet weight and more stability as compared to anionic polymer like Carbopol.

### 6.4.2 Trials with Klucel HXF

From the preliminary experience of various polymers and their different viscosity grades on the *in vitro* drug release profile of GBP, we attempted to formulate Controlled release mucoadhesive matrix tablets of GBP using putative hydrophilic matrix material Hydroxyl Propyl Cellulose (Klucel HXF) in combination with inert diluent microcrystalline cellulose and to study the *in vitro* release characteristics and release rate kinetics of the prepared formulations. A Central composite design was employed to get an optimum formulation suitable for once a day administration. The amounts of Klucel HXF (A) and Avicel PH113 (B) were selected as the factors, studied at three levels each. An account of 9 experimental runs studied, their factor combinations, and the translation of the coded levels to the experimental units employed during the study is summarized in Table 4.1.6. The % of drug released in 2hrs (Q2), % of drug released in 6 hrs (Q6), % of drug released in 10hrs (Q10) and mucoadhesive force were taken as the response variables.

Physicochemical evaluation of the pre-compression blend and tablets for factorial batches was carried out for various parameters and results are shown in the section 4B. Physicochemical parameters were within limits corresponding to good flow property of the precompression blend. Optimised formulation R15 gave desired drug release profile with optimum mucoadhesive strength and optimum swelling properties. Optimised batch R15 was selected kinetic modelling for drug release characteristics and was placed on stability at 25° C/60% RH for 12 months and 40° C/75% RH for 6 months. The *in vitro* release profiles of drug from R15 formulation could be best expressed by Higuchi model, as the plots showed high linearity ($R^2 = 0.998$). It was found to be stable up to a period of 12 months under accelerated storage conditions.

### 6.4.3 Trials with Polyox 301 and Polyox 303

In preliminary trials it was found that Polyox grades 301 and 303 were found to exhibit potential for yielding tablets with desired release profiles. From literature it
was evidenced that Polyox Water-Soluble Resins NF offer a number of important properties for mucoadhesion – water solubility, hydrophilicity, high molecular weight, hydrogen bonding functionality, and good biocompatibility. The flowability, compactibility and lubricity provide Polyox™ with desirable properties to be utilized in direct compression applications.

6.4.3A Trials with Polyox 301

Different formulation trials were conducted using varying concentrations of Polyox 301. Physicochemical evaluation of the precompression blend and tablets was carried out for various parameters and results are shown in the section 4B. Physicochemical parameters were within limits corresponding to good flow properties of the precompression blend. All the factorial formulations were also assessed for mucoadhesive force and their swelling properties. Formulation R27 gave desired release profile with optimum force of mucoadhesion i.e. 0.674N. In vitro drug release data of the optimized formulation R27 was applied to various drug release kinetic models. The in vitro release profiles of drug from the R27 formulation could be best expressed by Korsemeyer–Peppas equation as the plots showed high linearity ($R^2 = 0.9966$)

But it was observed that formulations made with 301 intended to lose their integrity after around 7-8 hours. It may be due to erosion of polymeric chains or solubilisation of polymer. Therefore, formulation trials were also taken with Polyox 303 with higher viscosity compared to Polyox 301.

6.4.3B Formulation trials with Polyox 303

Different formulation trials were conducted with varying concentrations of Polyox 303. Physicochemical evaluation of the precompression blend and tablets for formulation trials with Polyox 303 was carried out for various parameters and results are shown in the section 4D. Physicochemical parameters were within limits corresponding to good flow property of the precompression blend. All the trial formulations were assessed for mucoadhesive force and their swelling properties. Formulation R31 with the desired drug release profile and optimum force of adhesion of 0.968 N was selected for accelerated stability study and kinetic modelling. The in vitro release profiles of drug from the R31 formulation could be best expressed by Korsemeyer–Peppas, as the plots showed high linearity ($R^2 = 0.9592$). Release exponent was found to be 0.58 which lies between 0.5 and 1.0 that indicates non-
Fickian release; which indicates a coupling of diffusion and erosion mechanism. Formulation R 31 was stable in terms of the physicochemical characteristics at storage conditions of 25°C/60 % RH for 12 months and 40°C/ 75% RH for 6 months.

6.5. Preclinical studies

6.5.1 Pharmacokinetic Studies

Pharmacokinetics of the developed oral mucoadhesive formulations of GBP was initiated in Wistar rats to investigate the time course of systemic absorption of GBP from developed controlled release mucoadhesive formulations in comparison to conventional GBP formulation using rat as animal model. For estimation of GBP in plasma more sensitive bioanalytical method was developed encompassing HPLC with fluorometric detector. Mucoadhesive tablets, specially designed for administering to rats were administered orally with the help of a catheter, blood samples were withdrawn at specific time intervals and analysed for drug content. Various pharmacokinetic parameters like $C_{\text{max}}$, $T_{\text{max}}$, AUC, bioavailability and $t_{1/2}$ was determined. The $t_{1/2}$ of the developed formulations was found to be above 4 hrs as compared to conventional GBP tablet that exhibited a $t_{1/2}$ of 2.35 hrs. The results are discussed in chapter 5. The mean area under plasma time curve AUC$_{0-12}$ from controlled release tablets was 35-40 µg h/ml, while the mean AUC$_{0-8}$ of oral reference tablet was 16µg h/ml. Thus, the overall absorption of gabapentin from the controlled release tablets was around two times higher than its reference tablets, which shows enhancement of bioavailability of test formulation with respect to reference product at the same dose.

6.5.2 Development of epilepsy induced model

The study was designed to investigate the anticonvulsant potential of GBP which acts on the alpha$_2$—delta (α$_2$—δ) protein, an auxiliary subunit of voltage-gated calcium channels. Effect of GBP on pentylenetetrazole (60 mg/kg) induced seizure activity model in wistar rats was assessed. Seizures were assessed in terms of Latency to onset of seizure, seizure duration, seizure score and percentage mortality. These parameters were assessed after administration of for conventional tablets as well as developed mucoadhesive formulations. Conventional and Mucoadhesive tablets based experimental data was compared with respect to latency to onset of seizure, seizure duration and seizure score. Results are discussed in chapter 5.
Gabapentin in dose 98.5mg/kg attenuated pentylenetetrazole induced seizure activity in mice, as reflected by a significant increase in Latency to onset of seizure, decrease in seizure duration, decrease in seizure score and decrease in percentage mortality. Efficacy of mucoadhesive controlled release formulation was proved by reversal pentylenetetrazole induced epilepsy model.

6.5.3 GABA content in brain tissue
For estimating GABA content brain tissues, the epilepsy induced experimental rat’s brain tissue were isolated and GABA was extracted and analysed using developed HPLC method. GABA content in brain tissue was decreased to 53 ng/gm of tissue after PTZ administration whereas control group showed GABA levels upto 83 ng/gm of tissue. After oral administration of developed mucoadhesive tablets formulation I and II as well as conventional tablet formulations, GABA content was significantly increased to 78.66 ng/gm, 77 ng/gm and 68.16 ng/gm of tissue, respectively. The data was statistically analysed using ANOVA which showed significant difference in GABA content at P> 0.05.

6.5.4 Toxicity Studies
The acute potential toxicity of the developed formulation was assessed at three dose levels. Mucoadhesive matrix tablets with dose equivalent to 92.5 mg, 185 mg and 277.5 mg of gabapentin per kg were orally administered to rats. The rats were observed for 14 days and at the end of 14th day hematological profiles and serum biochemistry was determined. The histopathological examination of vital organs was also performed. No mortality was observed at 277.5 mg/kg of dose and no significant change in any of the biochemical and histological parameters were observed. The results of the acute toxicity studies are given in table. The results of histopathological studies showed that the developed formulations were safe for administration. The results are presented in chapter 5 in detail. Developed formulations were found to be stable for oral administration.

6.6 Conclusion:
Oral mucoadhesive matrix once a day formulations of GBP with the desired drug release profile were developed using Klucel HXF and Polyox 303. The results of pharmacokinetic studies indicated that developed mucoadhesive tablets maintained drug concentrations in plasma for 12 hrs as compared to conventional tablets in which 100% drug is released within 2hrs. Such systems based on mucoadhesive controlled
delivery with comparatively higher bioavailability will provide patient compliance by reducing the dosing frequency. Since once a day formulations of gabapentin are much needed for treatment and monitoring of epilepsy the developed formulations have a great market potential in India.

6.7. Future Scope of the Research Work:
Although the developed formulations were found to be promising based on in vitro drug release profiles, pharmacokinetics and pharmacodynamic efficacy studies, they need to be further investigated by in vivo bioavailability studies in higher animal models like beagle dogs and clinical studies in human volunteers to assess their in vivo performance.