SYNOPSIS

OF THE THESIS ENTITLED

DESIGN AND DEVELOPMENT OF TASTE MASKED FORMULATIONS OF GABAPENTIN FOR ORAL CONTROLLED DRUG DELIVERY

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INTRODUCTION

Taste has an important role in development of oral pharmaceuticals, with respect to patient acceptability and compliance and is one of the prime factors determining the market penetration and commercial success of oral formulations, especially in pediatric medicine. Hence, pharmaceutical industries invest time, money and resources into developing palatable and pleasant tasting products and adopt various taste-masking techniques to develop an appropriate formulation.

Taste, smell, texture and after taste are important factors in the development of dosage forms. Enhanced taste, flavour and texture are found to significantly affect the sales of the product and its preference. Current taste masking technologies offer a great scope for invention and patents.

The simplest method of taste masking involves use of flavor enhancers. Where these methods fail more complex technologies are adopted. Various techniques have been identified for taste masking viz. polymer coating, inclusion complex formation with cyclodextrin, use of ion exchange resins incorporation of anesthetic agents, solubility limiting methods or entrapment of drugs in liposomes, nanoparticles or multiple emulsions. Several approaches along with novel methods of taste assessment like psychopharmacological evaluation and animal preference tests have been tried out in order to achieve taste masked product.

Gabapentin, also known as 1-aminomethylcyclohexaneacetic acid, is a γ-aminobutyric acid (GABA) analogue having anti-convulsant properties. The site of action of gabapentin is, the alpha2—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. From the point of view of pharmacokinetics, it is essential for the concentration of gabapentin in the plasma to reach its peak in 2 to 3 hours. This is one of the reasons why gabapentin is currently sold in France under the trade name NEURONTIN.RTM. by Parke-Davis in the form of gelatin capsules. Although gel-capsules allow the satisfactory concentration of gabapentin in the plasma to be obtained, they are, however, unsuitable for pediatric use. In addition to bitter taste this drug has another issue of stability. It is moisture sensitive and
tends to form lactam impurity which should be below 0.1%. Gabapentin degrades via intramolecular cyclization to form a $\gamma$-lactam: 3, 3-pentamethylene-4-butyrolactam (2-azaspiro [4,5]decan-3-one). Therefore an attempt was made to develop taste masked stable formulations of gabapentin for conventional delivery.

Gabapentin is thought to be absorbed from the intestine of humans and animals by a low capacity solute transporter localized in the upper small intestine. Saturation of this transporter at doses used clinically leads to dose-dependent pharmacokinetics and high inter-patient variability, potentially resulting in suboptimal drug exposure in some patients.

Frequent dosing is necessary to maintain reasonably stable plasma concentrations. The effective dose of gabapentin is 900 to 1800 mg/day, which is given in divided doses. Gabapentin conventional dosage forms like tablets or capsules are administered three times a day.

The controlled release drug delivery systems that can be retained in the stomach for extended periods may also be designed as gastroretentive drug delivery systems (GRDDS). They help in optimizing the oral controlled delivery of drugs with absorption window in the stomach or small intestine thus continuously releasing the drug in the region of the absorption and ensure optimal bioavailability.

Design of controlled release gastroretentive bioadhesive drug delivery systems for drugs like gabapentin will be undertaken to modulate drug release over an extended period of time, and throughout the gastrointestinal (GI) tract to provide therapeutically effective plasma levels.

**OBJECTIVES**

A. Development of taste masked formulations of Gabapentin for conventional oral drug delivery:

i) To explore various strategies for attaining taste masking of gabapentin viz. wet granulation, melt granulation, spray drying, incorporating in ion exchange resins or beta-cyclodextrins and loading on non-pariel seeds, etc.

i) Characterization and Evaluation of the developed taste masked formulations.

ii) *In-vitro* and *in-vivo* taste assessment of the developed formulations.
B. Development of oral controlled release dosage forms of gabapentin by direct compression using hydrocolloid polymers like Klucel HXF, Polyox N303, Natrosol and Carbopol.

- Evaluation of the developed formulations for *in-vitro* release profiles, mucoadhesive strength using porcine gastric and physicochemical properties.
- Scale up and reproducibility studies.
- Stability studies as per ICH guidelines.

C. *In-Vivo Studies*

- Comparative *in-vivo* evaluation to investigate the efficacy of the developed formulations and to assess their pharmacokinetic parameters and bioavailability.
- Safety and toxicity studies on the developed formulations.

**Gabapentin**

![Gabapentin structure]

**Molecular formula:** $C_9H_{17}NO_2$  
**Molecular Weight:** 171.24

**PREFORMULATION STUDIES**

1. **Standardisation of drug and polymers:**
Gabapentin was procured from Shasun Chemicals and Drugs Ltd. The drug was standardized as per the certificate of analysis. Various grades of HPC and HEC were obtained as gift samples from Signet Chemicals. Various grades of HPMC, Polyox and compritol ATO888 were procured as gift samples from Colorcon Asia Pvt. Ltd. All the excipients were standardized and were found to be within the limits as per specifications.

2. **Drug-Exciipient compatibility studies**
The stability of gabapentin and its interaction with polymers and other excipients viz. Polyox 301, Klucel HXF was investigated by DSC studies. Endotherms of excipients did not overlap with the drug, indicating compatibility.
3. Analytical Method development
With reference to the method available in USP the analytical method for routine estimation of the drug was developed.

1) Colorimetric method for estimation of Gabapentin
This method is based on the reaction of the primary amino group of gabapentin with ninhydrin reagent in N, N1-dimethylformamide (DMF) medium producing a coloured product which absorbs maximally at 572 nm. This method was found to be sensitive in the range of (40-240 ppm)

2) HPTLC method for estimation of the gabapentin
A Camag, Linomat sampler applicator was used for spotting of plates. In this method n-butanol: water: glacial acetic acid (2.4:1.2:0.6) was used as mobile phase. 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 λ, 490nm. This method was found to be sensitive in the range of (200-1000ng)

3) HPLC method for estimation of Gabapentin:
HPLC method was developed using Hypersil C18 gold column (250 x 4.6 mm, particle size 5 micron) using buffer pH 3.1, acetonitrile and methanol (70:30:10 v/v) as mobile phase. This method was found to be sensitive in the range of 100-600µg. Tosho, Japan with UV-Visible detector and data management HPLC system was utilized for the analysis.

4) HPLC method with spectoflurimetric detection for gabapentin estimation in the plasma and brain:
A rapid and simple method for determination of the antiepileptic compound gabapentin in plasma and brain was developed. Blank human plasma and brain homogenate were spiked with gabapentin (1.0-10/µg/ml). Individual samples were treated with 2 M perchloric acid, centrifuged and then derivatised with o-phthalaldehyde-3-mercaptoethanol. Separation was achieved on a Beckman Ultrasphere 5 /µm reversed-phase column with mobile phase consisting of 0.33 M acetate buffer (pH 3.7; containing 100 mg/l EDTA)-methanol-acetonitrile (40:30:30 v/v). Eluents were monitored by fluorescence spectroscopy with excitation and emission wavelengths of 330 and 440 nm, respectively.

5) HPLC method with spectoflurimetric detection for GABA estimation in the brain:
A rapid and simple method for determination of the neurotransmitter in plasma and brain was developed. Blank human brain homogenate was prepared and samples were treated with 2 M perchloric acid, centrifuged and then derivatised with o-phthalaldehyde-3-mercaptopethanol. Separation was achieved on a Beckman Ultrasphere 5 µm reversed-phase column with mobile phase consisting buffer pH 5.4: Acetonitrile (77:23 v/v). Eluents were monitored by fluorescence spectroscopy with excitation and emission wavelengths of 330 and 440 nm, respectively.

4. Formulation trials for achieving taste masked products of Gabapentin

As gabapentin forms lactam impurity in contact with aqueous media several strategies were tried out for taste masking of gabapentin.

I) STRATEGIES FOR TASTE MASKING OF GABAPENTIN

1) Wet granulation

i) Wet Granulation with Fillers:
Wet granulation method was used by incorporating drug with varying ratios of various excipients like HPMC K15, microcrystalline cellulose, mannitol and PVP K30 as a binder. Different concentrations of high intensity sweetener, sucralose and flavors were also tried out. Although partial taste masking was achieved, very high concentrations of excipients were required for the same.

ii) Wet granulation with concentrated solutions of polymers without fillers:
Here drug was granulated with concentrated solutions of polymers like gelatin, guar gum PVA and with supersaturated solution of sucrose and evaluated for taste masking. This method was unable to mask the taste of the gabapentin.

iii) Wet granulation by dispersion method
The drug was dispersed in 10% PVA to which diluent like starch was added while stirring with overhead stirrer. As the solid excipients were added, the dry agglomerates were formed. The agglomerates were sieved and dried to get granules. Partial taste masking was attained.

2) Melt granulation:
Waxes like cetyl alcohol and glyceryl monostearate were melted and the premix of drug and polymers was added while stirring under overhead stirrer, allowed to cool and the mass was
passed through the 40# sieve. Even this method was unsuccessful in attaining the complete taste masking of the gabapentin and hence it was modified in further investigations.

3) **Modified melt granulation:**
   
   a) **Mix melt granulation:** In this method different ratios of glycercyl monostearate and triethyl citrate were tried. Glycercyl monostearate (GMS) was warmed till it melted completely and stirred to form a homogenous solution. Gabapentin was added, mixed and allowed to cool at RT.

   b) **Organic solution granulation using polymers:** Polyox was dissolved in an organic solvent such as IPA. 5% solution of polymer was made and Gabapentin was granulated with this solution. These granules were subjected to air drying to remove traces of organic solvent followed by incorporation of flavor and sweetener.

   c) **Combination approach:** Initially dry mix of Gabapentin + Mannitol (1:1) was prepared. This premix was binded with 5% PVP K30 solution. Then premix of aerosil, sweetener and flavour was incorporated extragranularly. Granules were prepared as mentioned above and subjected to the melt granulation using GMS and TEC. Melt granulation technique was found to mask the taste to greater extent as compared to the other techniques. When these formulations were subjected to stability studies and after a month evaluated for impurity formation by HPLC. The content of lactam impurity was found to be 0.5%.

4) **Dispersion method:** In this method initially premix of drug, aerosil and sucralose was prepared. This powder mix was homogeneously dispersed in polyox solution by means of stirring with glass rod. This suspension was then poured in the petriplate and allowed to dry for 24 hrs. The dried mass was subjected to sieving to get granules. When these formulations were evaluated for taste, they were found to be moderately bitter so other methods were investigated to achieve complete taste masking.

5) **Adsorption of drug solution on diluents:** Drug was dissolved in mixture of glycerine and glycercyl monostearate and was adsorbed onto the premix of microcrystalline cellulose and sucralose. Here bulk of the formulation was increased and also larger amount of propylene glycol was added. When the taste masked formulation was evaluated for impurity formation after 2 weeks, 0.2% of impurity was found to be formed in the granules.

6) **Pellets of gabapentin:** Spherical pellets were prepared using microcrystalline cellulose (MCC). Trials with diluents like Avicel PH 101 and dicalcium phosphate were taken.
Formulations with different binders like PVP K30 and polyox WSR N10 in varying concentrations were prepared. Optimized formulation was coated with two different moistureshield coating solutions procured from Colorcon and Ideal cures. These pellets were evaluated for taste masking potential by panel of volunteers and *In vitro* drug release in first 10 minutes was also noted. These pellets were also subjected to the complete drug release and stability studies. Gabapentin pellets during stability studies were found to be unstable.

7) **Loading of non-pariel seeds**

Both original gabapentin as such (available in supplied form) and micronised gabapentin were used for loading on non-pariel seeds. This method was also unable to achieve complete taste masking to a greater extent so other methods were explored.

8) **Use of ion exchange resins:**

Taste masking of gabapentin was attempted by ion-exchange resin complexation using a number of resins. Formulations containing varying ratios of cation exchange resins namely Indion 214, 234, 204, 414, 294 and anion exchange resin namely Indion 454 and controlled release cation exchange resin namely Indion 244 were tried out. With the help of ion exchange resins complete taste masking was successfully achieved. But the drug loading was in the range of 10 to 20% only. Therefore around 3 gms of the drug-resin complex equivalent to 300mg dose would be required to incorporate the desired dose in the formulation. It is reported that such a high dose of resins can cause electrolyte imbalance in the body. So this approach was discontinued and other methods were investigated.

9) **Use of beta-cyclodextrin:** Different ratios of drug: beta-cyclodextrin were tried out to obtain taste masked powder. At 1:10 ratio of drug: BCD taste masking was accomplished. This powder was further subjected to the stability studies by filling into alu-alu pouches. After one month 1.5% impurity was formed indicating instability of gabapentin. Therefore other methods were explored to attain complete taste masking with minimal impurity formation.

10) **Preparation of chewable tablets:** Flavors like chocolate, mango and lemon along with salt (NaCl) and lactose were used for preparation of conventional chewable tablets. The taste was not successfully masked in all the tablet formulations so this approach was also discontinued.

11) **Preparation of gabapentin microspheres with Eudragit EPO:** Drug and Eudragit were dissolved in suitable mixture of optimized organic solvents depending on solubility and
emulsified in liquid paraffin. Varying ratios of drug: Eudragit EPO were tried to obtain taste masked microspheres. A drug: polymer ratio of 1: 10 produced tasteless microspheres with the highest entrapment efficiency of 95.8 %. This batch was subjected to the stability studies. Initially the microspheres were showing 0.1 % of impurity. After one month of storage at room temperature around 1.2% of lactam impurity was formed.

12) Spray drying:
Attempts were made to entrap gabapentin in natural polymers like gelatin, semisynthetic polymer like carbopol 971 P, synthetic polymer, polyox WSR N10 and waxes like glycercyl monostearate and cetyl alcohol. Various trials were taken by altering ratios of drug and entrapping agent and by using excipients like aerosil, sucrose and lemon flavour. When 1:2 ratio of drug: wax was used, formulations obtained were slightly bitter (Taste score 2) and some degree of taste masking was achieved. With carbopol 971 P powder generated was slightly bitter and hygroscopic rather fibrous with very less yield.

13) Preparation of taste masked films 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. This approach was discontinued since it was difficult to incorporate such a large dose i.e. 300mg of gabapentin in film.

14) By forming salts of aromatic acids: In situ reactions of drug with different aromatic acids were carried out and the salt formed was characterized by IR, NMR, and Mass spectroscopy. Taste score of 0 could be assigned to the compound which was formed due to the reaction of cinnamic acid and gabapentin. From the spectral analysis it can be concluded that the derivative cinnamate ester of the gabapentin was formed. After one month of storage at room temperature around 1.0% of lactam impurity was formed. This salt formation and characterization needs to be further explored.

Evaluation of developed taste masked formulation:

Monitoring for lactam impurity: The developed formulations were monitored for presence of lactam impurity by performing HPLC analysis of the samples.

Human panel studies: Sensory analysis of tastants in trained healthy human volunteers was carried out and for taste assessment subjective or hedonic scales were used. Sample equivalent
to a normal dose was held in mouth for 10 sec. Bitterness level was recorded and scored from 0-5.

**In vitro drug release studies:** Pharmacopoeial release tests modified by altering the chemical composition of the dissolution media (e.g. artificial saliva) and reducing the size of the basket screen or using Flow-through cell was used to assess extent of drug released during early exposure to dissolution media. Release of drug substance in the early time points from 0 to 5 min was monitored and the amount of the drug release in these time points was estimated by HPLC to indicate any possibilities of drug dissolving in salivary fluid and produce taste sensation.

**Conclusion:** The study conclusively demonstrated that complete taste masking of gabapentin was difficult due to lactam impurity formation. However two of the above approaches i.e. adsorption of drug solution on diluents and microspheres of Eudragit EPO showed great potential for further investigations.

**II) Development of Controlled release Tablets of gabapentin:**
A controlled release dosage form of gabapentin was formulated and adapted to release the drug over an extended period of time (10-12 hrs). Different stomach specific mucoadhesive tablets were attempted.

**A. Hydrocolloid based gastro retentive controlled release systems of gabapentin**
Different hydrocolloid based polymers like Carbopol 971 P, Natrosol 250 HHX, Klucel HXF, Viscarin GP 109, Blanose 7 HF and Polyox WSR 303, HPMC K 100M and Polyox WSR 301 were screened to obtain optimized formulation with desired bioadhesive strength, swelling property and release profile. Depending on these characteristics two tablet formulations were developed for comparative study.

**i) Mucoadhesive tablets of gabapentin developed using Klucel HXF as Polymer**
Tablets were prepared by direct compression and evaluated for physicochemical properties and *in vitro* dissolution parameters. A central composite design of 2 factors at 3 levels each was employed to systematically optimize drug release profile and bioadhesive strength. Concentrations of Klucel HXF and microcrystalline cellulose were taken as the independent variables. Response surface plots and contour plots were drawn and optimum formulations were selected by feasibility and grid searches.
ii) Mucoadhesive tablets of gabapentin developed using Polyox 301 as Polymer

Based on the early findings more concentrations i.e. higher and lower levels of polymer were attempted out to get desired drug release profile and bioadhesive strength.

**Evaluation of the developed tablet formulation:**

Physicochemical parameters like appearance, uniformity of weight, drug content and friability were determined.

**In-vitro release kinetics:** *In-vitro* drug release were studied in dissolution media comprising 0.06 M HCl at two agitation rates 50 and 100 for both Klucel based and Polyox based formulations. The rate of drug release from the developed formulations was found to be slightly dependent on the agitation rate following zero order kinetics.

**Bioadhesive strength:** The developed oral mucoadhesive tablets were assessed for their mucoadhesive strength using modified analytical balance technique. Mucoadhesive efficiency was investigated using porcine gastric mucosa. The bioadhesive force required to detach the applied formulations from porcine gastric mucosa was expressed in dyne/ cm².

**Swelling index:** The swelling properties of Klucel HXF and Polyox WSR 301 matrices containing drug were determined by placing tablet matrices in dissolution test apparatus, containing 900ml dissolution media at 37°C. The tablets were removed periodically excess surface water was removed carefully using filter paper and weight gain was measured.

**Conclusion:** Hydrophilic matrix of HPC and Polyox 301 could control the gabapentin release effectively for 12 hours.

**Scale up and Reproducibility Studies:** The optimized taste masked formulations and controlled release mucoadhesive tablets of gabapentin were scaled upto 10 times of the original batch size. The reproducibility of manufacturing process was validated by preparing same batch in triplicate. The developed formulations were found to be reproducible without any significant changes in their physicochemical parameters and percent of lactam impurity.

**Stability Studies:** The selected batches of developed formulations were subjected to stability studies as per ICH guidelines. The mucoadhesive tablets and optimized taste masked formulations were packed in Alu-Alu strip pack and stored at 25°C/ 60 % R.H. and 40°C/ 75 % R.H for a period of 12 months. Mucoadhesive drug delivery systems were regularly
assessed for physicochemical parameters like appearance, hardness, moisture content, drug content, impurity formation and in-vitro drug release profile. No significant changes were observed up to the period of 12 months of storage.

**In-Vivo studies:** In vivo studies are necessary to determine the release profile of the drug from the dosage form that will provide enhanced bioavailability.

i. **Development of Epilepsy induced animal model:**

The study was carried out based on the protocol approved by Animal Ethical Committee of C. U. Shah College of pharmacy. Epilepsy induced animal model was developed in Wistar strain rats. The disease was induced by administering pentylenetetrazole 60 mg/ kg subcutaneously once. Induction of the disease and efficacy of the developed formulations was confirmed by determining changes in locomotor activity of the epilepsy induced animals and by Estimation of GABA after oral administration of mucoadhesive tablet formulation.

ii. **Striatal GABA content:** The rats were euthanized by cervical dislocation and the brains were rapidly removed and stored at -70°C until analyzed. The brain tissue was homogenized in 0.1M, 250 µl perchloric acid, and 2 ml ethanol for 2 minutes and the resulting homogenate was centrifuged at 4000 rpm at 4°C for 20 minutes. The supernatant solution was filtered through 0.2µ filter and analyzed by the developed HPLC method with fluorimetric detection. Striatal gabapentin content was decreased to 50.9 ng/gm of tissue after PTZ administration whereas control group showed GABA levels up to 83 ng/gm of tissue. After oral administration of stomach specific mucoadhesive tablet in Epilepsy induced rat the GABA content was significantly increased to 76.2 ng/gm of tissue.

iii. **Pharmacokinetic studies:** Pharmacokinetics of developed optimized mucoadhesive tablet formulations was studied in Wistar Rats. The tablets were administered orally with the help of catheter. At different time intervals the blood was collected and and concentration of the drug in blood was estimated by HPLC with fluorimetric detection. Various pharmacokinetic parameters like $C_{\text{max}}$, $T_{\text{max}}$, AUC, bioavailability and $t_{1/2}$ was determined. The developed stomach specific mucoadhesive tablets maintained concentration of gabapentin in plasma over a period of 12 hrs. The relative bioavailability was found to be 63%.
iv. Toxicity studies:

To prove the safety of developed tablet formulations the toxicity studies were carried out as per OECD guidelines. The tablets were subjected for acute toxicity studies in Wistar rats.

**Acute toxicity:** The acute toxicity of the developed mucoadhesive tablets was assessed at three dose levels. The rats were observed for 14 days and then their hematological profile and serum biochemistry were determined. The histopathological examination of the vital organs was performed. No significant changes in any of the biochemical and histological parameters were observed. The results obtained from histopathological studies proved that the developed formulations were safe for oral administration.

**Conclusion:**

Several techniques were explored to achieve optimum taste masking of gabapentin. The study conclusively demonstrated that complete taste masking of gabapentin was obtained with two of the above mentioned approaches i.e. by dissolving drug and absorbing it on diluents and microspheres of Eudragit EPO which has shown great potential for further investigation. Developed taste masked formulations of gabapentin were assessed with Human panel studies and *in-vitro* solubility studies. Developed bioadhesive drug delivery systems of gabapentin were prepared and evaluated. These bioadhesive delivery systems exhibited the desired controlled release profile over a period of 12 hrs. Also the taste masking of tablets formulated in this investigation may possibly help in administration of gabapentin in a more palatable form. The results of pharmacokinetic studies on tablets indicated that, the developed Gastroretentive system maintained gabapentin concentration in plasma for 12 hrs. Formulations developed as mucoadhesive tablets would be cost effective with ease of manufacture.

**References:**


Research guide

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