DESIGN, OPTIMIZATION AND CHARACTERIZATION OF PH SENSITIVE NANOPARTICLES OF DULOXETINE HYDROCHLORIDE FOR ENHANCEMENT OF ORAL BIOAVAILABILITY

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ABSTRACT

The aim of the study was to develop pH sensitive nanoparticles of duloxetine hydrochloride for increasing the oral bioavailability. Depression is a mental disorder that affects millions of people worldwide. The conventional drugs available for its treatment have less bioavailability hence such a system is needed which protects the drug in adverse conditions, enhances the oral bioavailability and also provide sustained drug release to reduce frequent dosing. Duloxetine hydrochloride was the drug of choice and was incorporated into nanoparticles by controlled precipitation method. To deliver drugs at predetermined rate for fixed time period, pH sensitive drug delivery system was prepared to deliver the drug orally. The prepared nanoparticulate formulation was made pH sensitive by using Eudragit L100 to increases the stability of drugs in gastric fluids and to release the drug in the intestinal pH by the dissolution of the polymer in a pH and time controlled manner. The formulation was characterized for particle size, shape, entrapment efficiency, in vitro drug release. Prepared nanoparticle formulation showed enhanced bioavailability and pH sensitive drug release in the intestine.

KEYWORDS: Duloxetine HCl, pH Sensitive, Nanoparticles, Eudragit L100.

1. INTRODUCTION

Depression is a widely prevalent and a recurrent chronic disabling syndrome that affects nearly 340 million people worldwide. This disease exhibits symptoms such as intense feeling
of sadness, hopelessness and guilt, sleep disorders, impaired concentration, decreased energy and suicidal thoughts.\textsuperscript{[1, 2]} Depression can be of different types: 1) A major depressive disorder is characterized by a combination of symptoms that interfere with a person's ability to work, sleep, study, eat, and enjoy any pleasurable activity,\textsuperscript{[3]} 2) dysthymic disorder is characterized by long term (two years or longer) but less severe symptoms that may not disable a person but can prevent one from functioning normally or feeling well,\textsuperscript{[4]} 3) psychotic depression occurs when a severe depressive illness is accompanied by some form of psychosis, such as a break with reality, hallucinations, delusions,\textsuperscript{[5]} 4) postpartum depression diagnosed in new mothers within one month of delivery,\textsuperscript{[6]} 5) seasonal affective disorder is characterized by the onset of a depressive illness during the winter months, when there is little natural sunlight available.\textsuperscript{[7]} Depression may also accompany diseases like generalized anxiety disorder,\textsuperscript{[8]} stress-induced urinary incontinence,\textsuperscript{[9]} fibromyalgia,\textsuperscript{[10, 11]} or diabetic peripheral neuropathic pain.\textsuperscript{[12, 13]} However, the exact reasons of depression are currently unknown.\textsuperscript{[1, 2]} Different antidepressant drugs are available for the treatment of depression involving reversible MAO-A inhibitors, tricyclic antidepressants, selective serotonin reuptake inhibitors, or serotonin norepinephrine reuptake inhibitors. Currently, an acid labile, dual serotonin and noradrenaline reuptake inhibitor called duloxetine hydrochloride is prescribed for depression. The antidepressant efficacy of duloxetine is comparable to tricyclic antidepressant.\textsuperscript{[14]}

The oral route has certain advantages like it is safe, economical and easy administration of drug but oral formulations face several common problems, as most of the drugs are acid labile and hence prone to degradation in the gastric environment, less amount of drug reaches to the absorption site which results in low bioavailability, frequent dosing is to be done which is not suitable for patients who are already in depressive state. Hence such a system is needed which protects the drug from unfavorable conditions, enhances the oral bioavailability and sustained the release profile of the drug which results in the less dosing to the depressed patients.\textsuperscript{[15]}

Different carriers can be used to deliver the drug and to overcome the limitations of oral route. Polymeric nanoparticles are colloidal spherical, branched or shell structures solid particles which can penetrate capillaries and are taken up by cells because of their small size and hence increases the accumulation of drugs at target sites. For targeting, the drug can be conjugated to cell specific ligand and can also be coupled to macromolecules that reach the
target organs.\textsuperscript{[16,17]} Liposomes are small spherical vesicles developed from phospholipids.\textsuperscript{[18-20]} The liposomes possess certain limitations like low encapsulation efficiency, rapid leakage of water-soluble drug in the presence of blood components and poor storage stability.\textsuperscript{[21,22]} The oral uptake of dendrimers which are highly branched nanostructures with inner core and are produced from macromolecules such as polyamidoamine, polypropyleneimine and polyarylam.\textsuperscript{[23, 24]} is not preferred due to their flocculation and aggregation properties in vivo.\textsuperscript{[25]} Solid lipid nanoparticles (SLNs) are nanostructures made from solid lipids such as glyceryl behenate, stearic triglyceride and cetyl palmitate.\textsuperscript{[26, 27]} The SLNs possess poor drug loading, drug expulsion during storage due to polymeric transition and high water content of the dispersions.\textsuperscript{[28]} Polymeric micelles formed from amphiphilic block copolymers, such as poly(ethylene oxide)-poly (benzyl-Laspartate)\textsuperscript{[29]} have limited targeting ability due to low drug loading and low drug incorporation stability which cause the loaded drug to be released before getting to the site of action.\textsuperscript{[30]} Nanocapsules are spherical hollow structures in which the drug is confined in the cavity and is surrounded by a polymer membrane.\textsuperscript{[31]} But the drug loading capacity of nanocapsules makes them slightly less favourable for oral delivery of drugs.\textsuperscript{[32]} The main disadvantage of nanoemulsions which are the are emulsions with droplet size below 1\textmu m but usually between 20-200nm\textsuperscript{[33]} is the requirement of special production technique and thus high production cost.\textsuperscript{[34]} Hence among different carriers available for drug delivery like liposomes, SLNs, polymeric micelles, nanocapsules and polymeric nanoparticles; polymeric nanoparticles are most effective as they encapsulate and protect the drugs and release them in a controlled manner and also show more target specificity.

A pH-sensitive delivery system is defined as a system in which the drug release rate is dependent on the environmental pH value.\textsuperscript{[35, 36]} To make the formulation pH sensitive the eudragit is used. Some of the most important reasons for the application of pH sensitive nanoparticulate dosage form are to protect the acid-labile drugs like duloxetine from the acidic pH of gastric fluid, to prevent gastric distress or nausea due to irritation caused by some drugs, to deliver drugs for the local action in intestine, to deliver the drugs to their site of action in a concentrated form and to bypass systemic absorption in the stomach.\textsuperscript{[37, 38]} Eudragit polymers are copolymers derived from esters of acrylic and methacrylic acid, whose physicochemical properties are determined by functional groups. Eudragit polymers are available in a wide range of different physical forms (aqueous dispersion, organic solution
granules and powders). Eudragit L100 is available in powder form and gets dissolved above pH 6.0.\textsuperscript{[39]}

2. MATERIALS AND METHODS

2.1. Materials

Duloxetine hydrochloride was a gift from Hetro Drugs, Hyderabad. Eudragit L100 was purchased from Yarrow Chem, India. All remaining chemicals and solvents were of analytical grade and purchased from local suppliers.

2.2. Preparation of pH Sensitive Nanoparticles Formulation

The eudragit nanoparticles were prepared by controlled precipitation method.\textsuperscript{[40]} Eudragit L100 and Duloxetine hydrochloride were dissolved in 20 ml of 0.1 N NaOH and volume was made up to 100 ml with distilled water. Glacial acetic acid (0.1\% v/v) was added slowly using syringe fitted with a 24 gauge needle to adjust pH of the system to 3.5. The formed nanoparticles were stirred at 2000 rpm initially for 4 hours. The formed nanosuspension was centrifuged at 20,000 rpm for 1 hr. The supernatant was separated and analyzed for free drug content. The formed nanoparticles were isolated, washed three times with distilled water and freeze dried.

2.3. Characterization and Evaluation of pH Sensitive Eudragit Nanoparticles

The nanoparticle formulations were characterized for morphology, particle size, entrapment efficiency and cumulative \% drug release.

2.3.1. Morphology

The surface view of the nanoparticles was observed via Scanning Electron Microscopy (SEM). The internal view of the particles was seen by Transmission Electron Microscopy (TEM).

2.3.2. Particle size analysis

The particle size of the nanoparticles formulations was characterized by using digital microscope (BA-310, Motic, USA) under the 40X magnification. The size of Nanoparticles was also analyzed using Zeta sizer.
2.3.3. Entrapment efficiency
Accurately weighed powdered nanoparticles were suspended in methanol. After 12 hours the solution was filtered and the filtrate was analyzed for the drug entrapment efficiency using UV-Visible spectrophotometer at 290 nm.\textsuperscript{[26]}

2.3.4. \textit{In Vitro} Drug Release
The \textit{in vitro} release studies of Duloxetine hydrochloride nanoparticles were carried out using dialysis bag method.\textsuperscript{[41]} 5 ml of nanoparticles suspension was transferred to dialysis bag (m.w. cutoff 12,000 Da, Himedia, India) and immersed in 500 ml of 0.1N HCl for 2 hrs and then transferred to 500 ml phosphate buffer pH 6.8 maintained at 37\textdegree{}C. The medium was stirred at 50 rpm. An aliquot of 5 ml was removed at predetermined time points \textit{viz.} 0.5, 1, 2, 6, 8, 12, 18 and 24 hrs and medium was replaced with equal quantity of fresh buffer. The samples were filtered and then analyzed using UV-visible spectrophotometer at the wavelength of 290 nm.

Statistical Data Analysis and Formulation optimization
The multiple regression analysis was done using DESIGN EXPERT 8.0.7.1 demo version software. Analysis of data was carried out using ANOVA and the individual parameter was evaluated with F-test. Using the regression coefficient of factor, the polynomial equation for the each response is generated \textit{i.e.}
\[
Y = b_0 + b_1A + b_2B + b_{12}AB + b_{11}A^2 + b_{22}B^2
\]

Where \(Y\) is the dependent variable, \(b_0\) is the arithmetic mean response of the nine runs and \(b_1\) is estimated coefficient for the factor A. The main effects (A and B) represent the average result of changing one factor at a time from its low to high value. The interaction terms (AB) show how the response changes when two factors are simultaneously changed. The polynomial term (\(A^2\) and \(B^2\)) are included to investigate nonlinearity. A numerical optimization technique using the desirability approach was employed to develop an optimized formulation with the desired responses.

2.4. \textit{In Vivo} Study
\textit{In vivo} study was carried out for eudragit nanoparticles on albino rats and compared with the drug (Duloxetine HCl) suspension. Weighed amount of nanoparticles equivalent to dose 45mg/kg were suspended in 1.0 ml saline and administered orally using a rubber canula under non-anaesthetic condition. Blood samples were collected at 1, 2, 4, 6, 8, 12, 18, 24 hrs
time intervals, from retro-orbital region in ependorff tubes and centrifuged at 3000rpm for 20 min. The blood sample volume withdrawn was immediately replaced with an equal volume of physiological saline. The serum was separated by placing the tubes in a centrifuge 20 min at 300 rpm and then drug analysis was carried out using HPLC.\textsuperscript{[42]}

**Estimation of drug by HPLC**

The Duloxetine HCl concentration in rat plasma samples was analyzed by HPLC. To 200μl of each plasma sample, 600μl acetonitrile was added and vortex-mixed in a microcentrifuge tube. The mixture was permitted to stand for about 10 mins and centrifuged for 5 mins at 14,000 rpm. The clear supernatant was evaporated to dry and then redisssolved with 400μl acetonitrile. After ultrasonic extraction for 3 mins and centrifuging for 5 mins at 14,000 rpm, the clear supernatant was removed and evaporated, and the dried residue was dissolved with 50μl mobile phase and filtered through 0.45 μ syringe filter and injected directly into HPLC system. The mobile phase consisting of acetonitrile: potassium dihydrogen phosphate buffer (pH 5.4 adjusted with orthophosphoric acid) (80:20) at a flow rate 1.0 ml/min. and the detection was carried out at 229 nm.\textsuperscript{[43]}

**Data analysis**

The standard pharmacokinetic parameters from plasma concentration vs. time profiles of Duloxetine HCl $C_{\text{max}}$, $t_{\text{max}}$, AUC, $t_{1/2}$, apparent plasma clearance and mean residence time (MRT) were calculated by noncompartmental method using the Win Nonlin computer program.

2.5. Stability Studies

2.5.1. Effect of storage on particle size and visual appearance

The effect of storage on particle size of the blank and drug loaded pH sensitive nanoparticles formulations were determined by zeta sizer and digital microscope. The particle size and morphological characteristics of the blank and drug loaded nanoparticles formulations were observed after 15, 30, 45, 60, 75 and 90 days at 4°C ± 1°C and 25 ± 2°C, 60 ± 5% RH.

2.5.2. Effect of storage on % drug remaining

Drug remaining (%) of eudragit containing pH sensitive nanoparticles was determined by measuring the free drug concentration. 10 mg of drug-loaded nanoparticles from each batch was placed in 100 ml conical flask containing 50 ml of methanol. The microspheres were magnetically stirred. The solution was filtered through a 0.45μm membrane filter. Then the
drug was quantified at 290nm spectrophotometrically after appropriate dilution with methanol.

3. RESULTS AND DISCUSSION

3.1. Preparation, Optimization and Evaluation of pH Sensitive Eudragit Nanoparticles

The pH sensitive eudragit nanoparticles of Duloxetine hydrochloride were prepared by controlled precipitation method and optimized by using 3-level, 2-factor full factorial design. Ratio of drug: polymer and stirring time were selected as independent variables while particle size, entrapment efficiency and cumulative % drug release were selected as dependent variables.

Optimization and Model validation

The responses were recorded and analysed using ANOVA by Design Expert 8.0.7.1 demo version. The individual parameters were evaluated using F-test. Polynomial equation for each response was generated.

The polynomial Equations for Particle size, entrapment efficiency and % cumulative drug release were obtained.

Particle size = +243.01+0.50* A-30.62* B-1.13* A * B-6.76 * A²+17.92* B²  \[1\]

EE =  + 61.53 - 5.25* A + 16.95 * B \[2\]

% CDR = +84.03-1.55 * A+6.12 * B \[3\]

The Model F-value of 76.66 for particle size implied that the model is significant. The results of the polynomial equation 1 indicated that positive value of A and negative value of B i.e. as stirring time was increased, the particle size was decreased. Reduction in particle size with increase in stirring time may be due to increase in kinetic energy of molecules within emulsion which may lead to more collision among the particles. There was slight increase in particle size with increase in drug: polymer ratio. This was due to the corresponding increase in viscosity of drug-polymer dispersion comprising the internal phase of the emulsion. The increased viscosity within the internal phase resulted in the generation of a coarser emulsion with larger droplets, leading to the formation of larger nanoparticles as represented graphically in Figure 1.
The model F-value for entrapment efficiency 58.18 showed that the model is significant. Results of the polynomial equation 2 indicated that B is positive and more significant than A i.e. with increase in drug: polymer ratio there was a decrease in entrapment efficiency while it was increased with increase in stirring time. Increase in the amount of polymer in drug: polymer ratio leads to increase in cross link density which reduces the free volume spaces within the polymer matrix and hence a reduction in the entrapment efficiency. Whereas with increase in stirring speed particle size was reduced due to which surface area was increased and hence increase in entrapment efficiency which has been represented graphically in Figure 2.

Model F-value of 44.50 implied that the model is significant for % cumulative drug release. The polynomial equation 3 showed that factor B has positive effect on cumulative drug release whereas factor A shows negative effect. But effect of B is more significant than effect of A (drug: polymer ratio). Increase in % CDR with increase in stirring time was due to the reduction in particle size. It leads to large surface area which in turn is responsible for more dissolution and hence more CDR. This relation has been graphically represented in Figure 3. Decrease in cumulative drug release with increase in polymer ratio may be due to decrease in entrapment efficiency.

Design Expert software selected the Batch F7 as optimized batch, having maximum desirability i.e 0.936 as shown in Figure 4.

3.2. Characterization of the Optimized Batch

The characterization of the pH sensitive eudragit nanoparticles was done using SEM, TEM showed that the nanoparticles were spherical in shape and this may attribute to uniform drug distribution within polymeric matrix. The Photomicrographs 1 & 2 obtained via SEM and TEM respectively showed the morphology of nanoparticle formulation. Zeta seizer showed that the average size of nanoparticles found to be 221.9±11 nm. The polydispersity index as shown in Figure 5 was found to be 0.62 which was found to be within range.

3.3. In Vitro Drug Release Study

The in vitro release studies of Duloxetine hydrochloride nanoparticles was carried out using dialysis bag method for 24 hrs. The cumulative drug release of different formulations found to be 80.6± 4.09, 76.8± 3.26, 77.3± 2.19, 84.9± 2.69, 86.4± 1.96, 82.6± 2.8, 93.7± 3.06, 89.9± 2.6 and 87.8±3.16 after 24hrs have been shown in Figure 6. The $R^2$ value for Duloxetine HCl
were found to be 0.986, 0.922, 0.972 and 0.982 in zero order, first order, Higuchi model and Korsmeyer-peppas model, respectively. The $R^2$ value 0.986 indicated that the drug release follows the zero order release kinetic model. This model mainly describes the mechanism of constant release of drug from the nanoparticles formulation without depending on the concentration.

3.4. **In Vivo Study**

The data obtained was subjected to pharmacokinetic study. A shift in $t_{\text{max}}$ value towards higher side indicates the sustained release behavior of pH sensitive nanoparticles. The $C_{\text{max}}$ of Duloxetine HCl was found to be 574.06 µg/ml while in case of drug loaded nanoparticles was 842.24 µg/ml. It was clear from the pattern of peak and valley in **Figure 7** that the drug solution showed fluctuation in plasma concentration whereas in case of eudragit nanoparticles steady state plasma concentration was maintained. The mean AUC$_{0-24}$ values after the treatment with Duloxetine HCl solution was 2103.95 h*µg/ml and drug loaded nanoparticles 8224.72 h*µg/ml. There was a significant difference between the AUC values for drug loaded nanoparticles and standard drug solution ($P < 0.05$). The difference in AUC value indicates that higher amount of drug was available in case of drug loaded nanoparticles than drug solution. The drug loaded nanoparticles formulation was found to enhance the bioavailability of Duloxetine HCl by 3.9 times with reference to drug solution. The increased bioavailability may be due to the eudragit that releases the drug at the target site, this resulted in protection of the drug from acidic environment and hence more drug available at absorption site. The drug loaded nanoparticles showed the ability to maintain the Duloxetine HCl plasma concentration up to 24 hrs as compared to the drug solution that could maintain this level of drug only for 3 hrs. These results confirmed the sustained release potential of drug loaded nanoparticles of Duloxetine HCl prepared from eudragit L 100.

The MRT of the drug loaded nanoparticles was found to be 9.75 hrs i.e. higher than 3.05 hrs of the drug solution. The plasma clearance of the drug solution, 5614.22 ml/hr higher than 1216.76ml/hr of drug loaded nanoparticles which indicated the higher relative bioavailability of drug loaded nanoparticles. The high plasma clearance and low $t_{1/2}$ for drug solution due to the extensive elimination of drug from the body but in the case of drug loaded nanoparticles formulation the plasma clearance was low and $t_{1/2}$ was high, this indicated that nanoparticles formulation enhanced the shelf life and delayed the clearance of drug from the body. Thus, the drug loaded pH sensitive eudragit nanoparticles formulation was found to provide
prolonged steady-state concentration of Duloxetine HCl with minimal fluctuations and improved bioavailability.

3.5. Stability Studies

The formulations were kept at different temperatures (4±1°C and 25±2°C with 60±5% relative humidity) for three months and formulations were further examined for % drug remaining, particle size and visual appearance. From the obtained data, it was observed that formulation when stored at 4±1°C, after three months (or 90 days), % drug remaining decreased to 91.78±0.11% in the formulation and the particle size was increased from 221.9±11nm to 261.3±10.3 nm whereas when the formulation stored at 25±2°C, the % drug remaining reduced to 87.24±0.82 % and the particle size was found to be increased from 221.9±9 nm to 279.5±10.4 nm. The graphs of particle size and % drug remaining are represented graphically in Figures 8, 9 respectively. From the results, it was observed that more increase in particle size in case of higher temperature (25±2°C) may be due to aggregation of particles and degradation of the polymer at higher temperature. The decrease in % residual drug content more in case of storage at 25±2°C was due to increased particle size which increases the pore size leading to the drug leakage from the pores of the nanoparticles. The statistical analysis was carried using analysis of variance (ANOVA) and Dunnett post test by using software PRISM (Graph Pad). From the ANOVA analysis, the particle size and % drug remaining P values were found to be 0.262 and <0.0004 respectively. The ANOVA and Dunnett post test concluded that no significant change in particle size and drug remaining (%) of eudragit containing nanoparticles was found when these were stored at 4±1°C compared to 25±2°C. Hence, eudragit containing pH sensitive nanoparticles formulation of Duloxetine HCl having sufficient stability at 4±1°C and was further evaluated for in vivo efficiency.

Figure1: RSM plot to study the effect of drug: polymer and stirring time on particle size.
Figure 2: RSM plot to study the effect of drug: polymer and stirring time on Entrapment Efficiency.

Figure 3: RSM plot to study the effect of drug.

Figure 4: RSM plot of desirability polymer and stirring time on % CDR.
Photomicrograph 1: SEM of Nanoparticles.

Photomicrograph 2: TEM of Nanoparticles.

Figure 5: Zeta sizer of the eudragit nanoparticles.
Figure 6: *In vitro* release of Duloxetine HCl from different batches of pH sensitive Nanoparticles.

Figure 7: Plasma–drug profile curve.

Figure 8: Effect on particle size and appearance in drug loaded Nanoparticles at 4 ± 1 °C and 25 ± 2 °C, 60 ± 5% RH.
4. CONCLUSION
The purpose of this study was to design the pH sensitive eudragit nanoparticulate formulation of the antidepressant duloxetine hydrochloride in order to protect the drug from the gastric acid, enhance the bioavailability and sustained the release profile of DLX. Duloxetine HCl was successfully incorporated in eudragit containing nanoparticles formulations using controlled coprecipitation method. The formulation was found to be stable at 4±1°C. The in vitro drug release study concluded that the pH sensitive eudragit formulation delayed the drug release for two hours in stomach and sustained the drug release upto 24 hrs in intestine. It can be concluded from the results obtained that the eudragit nanoparticles formulation developed for oral delivery of Duloxetine HCl possessed high pH sensitivity, better stability and higher entrapment efficiency and increase in relative oral bioavailability of Duloxetine HCl. Thus the problems associated with the oral bioavailability of Duloxetine HCl could be overcome by incorporating it into a novel oral drug delivery system, pH sensitive eudragit nanoparticles.

5. ACKNOWLEDGEMENT
I would like to acknowledge the support of Hetro Drugs, Hyderabad for providing the gift sample of duloxetine hydrochloride.

6. REFERENCES


