Formulation and Evaluation of Paliperidone Palmitate Loaded Nanoparticles Using Biodegradable Polymers

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***Abstract*:-Paliperidone palmitate also known as 9-hydroxyrisperidone, is a dopamine antagonist and 5-HT_2A antagonist of the typical antipsychotic class of medications. The present study intends to carry out the formulation and evaluation of Paliperidone palmitate loaded biodegradable nanoparticles. The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties, and release of pharmacologically active agents so as to achieve the site specific action of the drug at the rational rate and dose. Nanoparticles are synthesized by the nano-precipitation method. Nanoparticles of paliperidone palmitate were obtained with high encapsulation efficiency 60-80%. Nanoparticles are generally characterized by their size, morphology, surface charge using the advanced microscopic techniques such as Scanning electron microscopy (SEM). Drug entrapment efficiency was determined by centrifugation. These studies suggest the feasibility of formulating paliperidone palmitate loaded nanoparticles using a biodegradable polymer (Gelatin) for the treatment of psychotic disorders.**

***Keywords*:-Paliperidone palmitate, biodegradable polymers, gelatin, nanoprecipitation.**

I. INTRODUCTION [2],[7],[9],[16]

The most emerging branch in pharmaceutical sciences known as "Pharmaceutical nanotechnology*"*presents new tools, opportunities and scope, which are expected to have significant applications in disease diagnostics and therapeutics. Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. Recently nano-pharamceuticals reveal enormous potential in drug delivery. Drugs that are transformed in to nano-range offer some unique features which can lead to prolonged circulation, improved drug localization, enhanced drug efficacy etc. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen.

The main aim of this work is to formulate and evaluate paliperidone palmitate loaded nanoparticles using biodegradable polymers. Paliperidone palmitate, also known as 9-hydroxyrisperidone, is dopamine antagonist and 5-HT_2A antagonist of the typical anti- psychotic class of medications. While its specific mechanism of action is unknown, it is believed paliperidone and risperidone act via similar, if not identical, pathways. Paliperidone has antagonist effect at α1 and $\alpha_2$adrenergic receptors and at H_1 histamine receptors. It does notbind to muscarinic acetylcholine receptors. In addition, it blocks dopamine and serotonin receptors. Paliperidone has less affinity for D4 receptors than risperidone.

Many opportunities exist for the application of synthetic biodegradable polymers in the biomedical area particularly in the fields of tissue engineering and controlled drug delivery. Degradation is most important in biomedicine for many reasons. Gelatin is extensively used in food and medical products and is attractive for use in controlled release due to its nontoxic, biodegradable, bioactive and inexpensive properties. It is a polyampholyte having both cationic and anionic groups along with hydrophilic group.

The selection of appropriate method for the preparation of nanoparticles depends on the physicochemical character of the polymer and the drug to be loaded. Nanoparticles can be prepared from a variety of materials such as proteins, polysaccharides and synthetic polymers.

Characterization of nanoparticles is based on the size, morphology and surface charge, using such advanced microscopic techniques as atomic force microscopy (AFM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Properties such as the size distribution, average particle diameter, charge affect the physical stability and the *in vivo* distribution of the

nanoparticles. Properties like surface morphology, size and overall shape are determined by electron microscopy techniques. Features like physical stability and redispersibility of the polymer dispersion as well as their *in vivo* performance are affected by the surface charge of the nanoparticles.

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II. MATERIALS

Paliperidone palmitate is gift sample from Leiutis pharmaceuticals, gelatin, dimethyl sulfoxide, acetone from Nice chemicals. Remi cooling centrifuge C-24L, Rotec magnetic stirrer, Shimadzu UV-1800 spectrophotometer, Superfit rotary flash evaporator.

III. METHODOLOGY

A. Pre- Formulation Studies.[8], [12]

Pre optimization was done by FTIR spectroscopy and UV-Visible spectroscopy.

1. Fourier Transform Infrared Spectroscopy:

FTIR spectra of the drug Paliperidone Palmitate were recorded. This spectra is compared with the nanoparticle of the same drug prepared using the polymer Gelatin.

2 UV-Visible Spectrophotometric Determination of Paliperidone Palmitate:

Preparation of Sample Solution:

10 mg of Paliperidone was weighed accurately and transferred into a 100 ml standard volumetric flask. The contents were dissolved in methanol and sonicated for 30 Minutes. This entire solution was filtered through 0.45 micron Whatmann filter paper (No. 41) and the final solution was made with methanol to get the solution of 1000µg/ ml. This solution was further diluted with methanol as per the requirement. The drug solution was scanned (200-400 nm) against reagent blank i.e. methanol and the absorption spectrum was recorded.

B. Preparation of Paliperidone Palmitate Loaded Nanoparticles Using Gelatin As Polymer.[3], [5], [13]

Nanoparticles are prepared by slight modification in nanoprecipitation method. 200 mg of gelatin was dissolved in 25ml of acetone, to this polymer solution add 100mg of the drug Paliperidone palmitate previously dissolved in 2ml of Dimethyl sulfoxide was added. Stir well for 10 minutes. Then 50ml of distilled water is added and stirred on a magnetic stirrer for 30 minutes. The acetone was removed by reduced pressure using Rotary flash evaporator and the volume was adjusted to 10ml. This solution was centrifuged at 15000rpm at 4^0 C for 30min. The supernatant was discarded and the precipitated nanoparticles were filtered and washed with distilled water for 3 times. The nanoparticles were dried at 60^0 C in a hot air oven and stored in a dessicator. By following the above mentioned procedure three batches of nanoparticles ratio of 1:1, 1:2 and 1:3 were prepared and named F1, F2 and F3 respectively.

TABLE I: WORKING FORMULA FOR NANOPARTICLES

Batch code	Amount of drug(mg)	Amount of gelatin(mg)	Drug:carrier ratio
F1	500mg	500mg	1:1
F2	500mg	1000mg	1:2
F3	500mg	1500mg	1:3

C. Evaluation of Nanoparticles.[3][6],[8],[14],[15]

1. Percentage Yield:

Percentage practical yield is calculated to know about the efficiency of any method, thus it helps in selection of appropriate method of preparation. Practical yield was calculated as the weight of nanoparticles recovered from each batch in relation to the sum of starting material. The percentage yield of prepared nanoparticles was determined by using the formula.

$$\text{Percentage yield} = \frac{\text{Practical yield X 100}}{\text{Theoretical yield}}$$

2. Preparation of Calibration Curve of Paliperidone Palmitate[4]

The standard solution of paliperidone palmitate was prepared by dissolving accurately 100mg of drug with small quantity of methanol in 100ml standard flask and sonicated for 20 minutes. The solution was further diluted to get 2, 4, 6, 8, 10µg/ml. Then measure the absorbance at 278nm in UV Visible spectrophotometer. Calibration curve was plotted by taking the concentration of the drug solution on X axis and absorbance at 278nm on Y axis.

3. Drug Entrapment Efficiency.[10],[11]

The entrapment efficiencies of prepared systems were determined by measuring the concentration of free drug in the dispersion medium. The obtained suspension was centrifuged for 60 min at 10,000 rpm. The supernatant was separated and the absorbance was measured at 278 nm. The amount of free

drug was detected and entrapment efficiency was calculated using the following equation.

$$\text{Entrapment Efficiency} = \frac{\text{weight of initial drug} - \text{weight of freedrug}}{\text{weight of initial drug}} x\ 100 \ldots$$

4. Particle Size, Shape And Surface Morphology:[6],[8]

Particle size, shape and surface morphology of nanoparticles was done by Scanning Electron Microscopy. SEM has been used to determine surface topography, texture and to examine the morphology of fractured surface. Small volume of nanoparticulate suspension was placed on an electron microscope brass stub. The stubs were placed briefly in a drier and then coated with gold in an ion sputter. Pictures of nanoparticles were taken by random scanning of the stub. The shape and surface morphology of the nanoparticles was determined from the photomicrographs of each batch.

5. In-Vitro Drug Release Studies:[3]

The *in vitro* drug release of the formulation was studied by using membrane diffusion. The dissolution medium was freshly prepared phosphate buffer of pH 7.4. Egg membrane previously soaked overnight in the dissolution medium and was tied to one end of the diffusion tube. Amount of nanoparticle equivalent to 100mg of paliperidone palmitate was placed in the tube. The tube containing formulation was placed in beaker containing 250 ml of phosphate buffer pH 7.4, maintained at $37^0C \pm 2^0C$. The dissolution medium was stirred at low speed using a magnetic stirrer. Aliquots, each of 5 ml volume were withdrawn at various intervals of time over a period of 6hrs. The aliquots were suitably diluted with dissolution medium and analyzed by UV Spectrophotometer at 278 nm.

D. Interpretation of Data.[5]

1. Determination of Order of Release of Drug from Nanoparticle by Graphical Method:

To determine the order of release of drug by graphical method using the dissolution data, a graph was plotted with % drug release vs time. Zero order release can be confirmed if a straight line or linearity is obtained. To find out the release rate constant, the slope of the curve was found out and multiplied with 2.303. Regression coefficient of the curve was determined to confirm the correlation between X and Y.

In the second stage, using the same dissolution data, a graph was plotted with log % remaining to release vs time. If a straight line or linearity is observed, it can be confirmed that the drug release follows first order kinetics. The slope of the graph was found out and multiplied with 2.303 to obtain the first order rate constant k_1.Regression co-efficient of the graph was found out to confirm the correlation between X and Y.

2. Mechanism of Drug Release Study:

In order to predict and correlate the release behavior of drug from the hydrophilic matrix, it is necessary to fit the *in vitro* release data in to a suitable model. Hence the dissolution data were fitted according to the well-known exponential equation, which is often used to describe the drug release behavior from a polymeric system. The equation which is used to describe drug release mechanism is:

$$M_t/m_\alpha = kt^n$$

Where, M_t/m_α is the fraction release of drug,'t' is the release time, 'k' is the constant, which indicates the properties of the macromolecular polymeric system, and 'n' is the release exponent indicative of the mechanism of release. The 'n' value was used for the analysis of drug release mechanism from drug loaded nanoparticles. The 'n' value was determined for all batches of drug loaded nanoparticles by graphical method, which is explained below.

In the first stage, a graph plotted with log % remaining to release vs log time. If a straight line is obtained, then the regression co-efficient was found out to confirm the linearity between X and Y. The slope (n) of the line was found out and if

$n \leq 0.5$: The release is by Fickian diffusion
$n > 0.5$ and <1: The release mechanism is swelling
$n=1$: Release is by case II transport release mechanism
$n>1$: Release is by Super case II transport

In the second stage, a graph was plotted with % release Vs time. If linearity is observed, the release mechanism is by Higuchi's diffusion.

IV. RESULTS AND DISCUSSION

A. Pre-Formulation Studies.

1 Ftir Spectroscopy:

Figure 1: FTIR spectra of Paliperidone palmitate

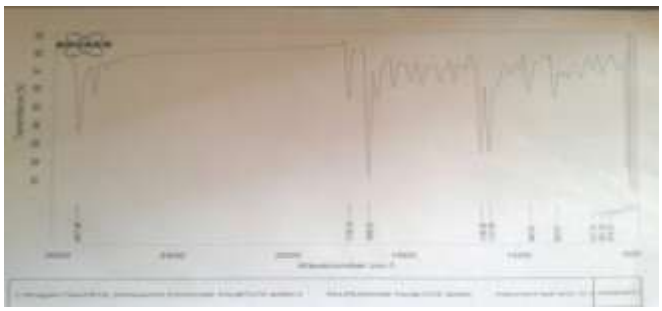

Figure 2: FTIR spectra of Paliperidone palmitate nanoparticles

The presence of intense peak at around 1650-1750 cm-1 indicate the presence of C=O stretching. The peak at 1647.24 indicate C=C stretching. The presence of weak absorption band at 2850-2950cm-1 indicate C-H stretching. The peaks in the spectra of pure drug Paliperidone palmitate are similar to that of the peaks of Paliperidone-Gelatin nanoparticles. It means the polymer do not have an incompatibility with the drug and it is suitable for preparing nanoparticles.

2 UV-Visible Spectrophotometric Determination of Paliperidone Palmitate:

The drug solution was scanned (200-400 nm) against reagent blank i.e. methanol and the absorption spectrum was recorded. The absorption maximum (λ max) was observed at 278nm which confirms the drug is paliperidone palmitate.

B. Preparation of Nanoparticles.

The nanoparticles of three different ratios (1:1, 1:2, 1:3) were prepared using the drug Paliperidone palmitate and Gelatin as polymer by Nanoprecipitation method.

Figure 3: Prepared nanoparticles

C. Evaluation of Nanoparticles.

1 Percentage Yield:

The percentage yield of the three different drug –polymer ratio (1:1, 1:2, 1:3) are found to be:

TABLE II: PERCENTAGE YIELD OF NANOPARTICLE FORMULATIONS.

Formulation	Practical Yield (gm)	Theoretical Yield (gm)	Percentage yield
F1(1:1)	0.20	1.0	20%
F2(1:2)	0.52	1.5	34%
F3(1:3)	0.96	2.0	48%

The Formulation with drug Paliperidone palmitate and the polymer gelatin which are in the ratio 1:3 is having high percentage yield.

2 Calibration Curve of Paliperidone Palmitate:

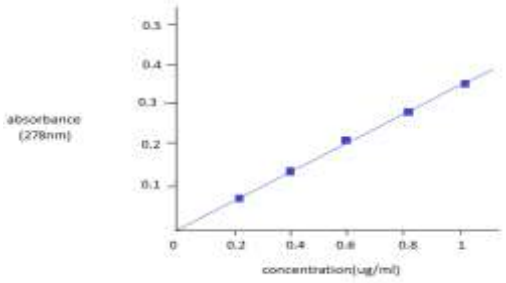

Figure 4: calibration curve of paliperidone palmitate

3. Drug Entrapment Efficiency:

The drug entrapment efficiency of the formulations was determined by centrifugation. From the absorbance values the amount of free drug was calculated. The drug entrapment efficiency for three different drug: polymer ratios are:

TABLE III: DRUG ENTRAPMENT EFFICIENCY OF NANOPARTICLE FORMULATIONS

Formulation	Drug entrapment efficiency (%)
F1 (1:1)	56
F2 (1:2)	62
F3 (1:3)	78

The drug entrapment efficiency of nanoparticles F3 prepared in the ratio1:3 (drug: polymer) having the high drug entrapment efficiency on comparing with other ratio.

4 Particle Size, Shape And Morphology:

From percentage yield and entrapment efficiency determination the formulation F3 shows best entrapment efficiency as well as yield. Hence formulation F3 is selected for further evaluations.

The scanning electron microscopy was performed to determine the particle size, shape and morphology of F3 nanoparticles showed that each particle unit exhibited a nanostructure.

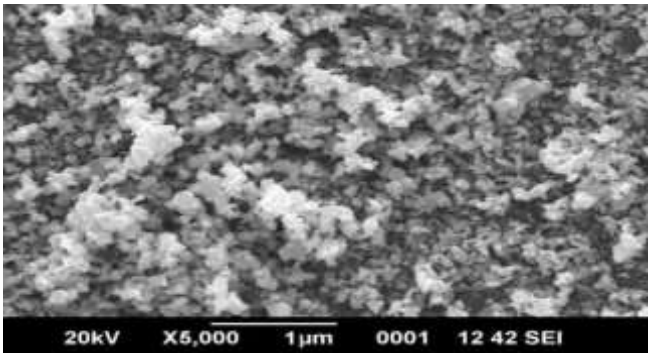

Figure 4: SEM Image of prepared nanoparticle F3

5 In-Vitro Drug Release Studies:

The cumulative percentage drug release of the drug polymer ratio 1:3 (F3) was determined.

TABLE IV:CUMULATIVE PERCENTAGE DRUG RELEASE OF F3

Time (hrs)	Absorbance 278nm	Concentration (ug/ml)	Amount of drug released (mg)	Cumulative amount of drug release (mg)	cumulative Percentage drug release
0	0	0	0	0	0
1	0.3657	1.042	26.071	26.071	33.42
2	0.3918	1.119	27.985	28.506	36.54
3	0.5214	1.489	37.242	37.801	48.46
4	0.6207	1.773	44.335	45.07	57.79
6	0.6413	1.832	45.807	46.693	59.86

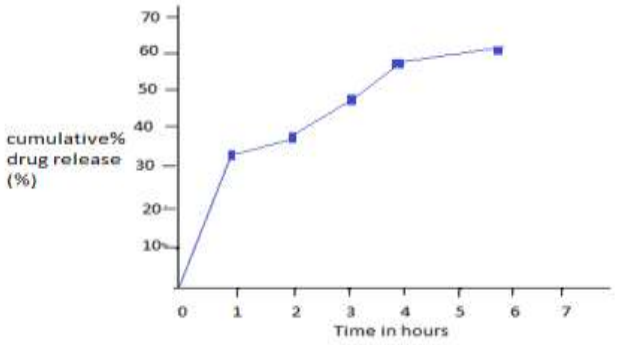

Figure 5: Drug release profile for the nanoparticle formulation F3

D. Interpretation of Data.

1 Determination of The Order of Release of Drug from Nanoparticle Using Graphical Method:

The zero order and first order plots for F3 nanoparticles was determined

Zero order kinetics

The graph was plotted with percentage drug release on y axis and time on x axis. The zero order rate constants for the formulation F3 was found to be 13.2. The regression coefficient was also determined to find the correlation between X and Y values.

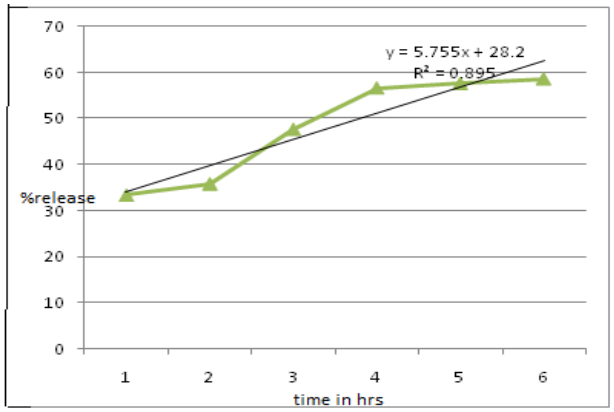

Figure 6: Percentage drug release vs time for F3

First order kinetics

The graph was plotted with Log percentage drug remaining to release on X axis and time on Y axis. The First order rate constant for the formulation was found to be 0.105. The regression coefficient was also determined to find the correlation between X and Y values.

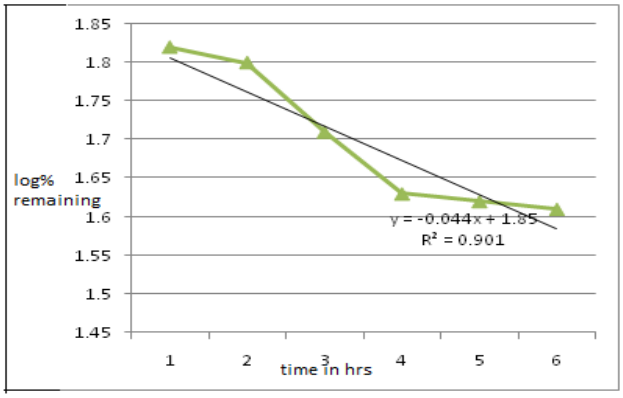

Figure 7: Log percentage drug remaining to release vs Time

The R^2 values for the formulation shows that maximum values are in first order kinetics. Hence it follows a first order kinetics.

2 Mechanism of Drug Release Study:

In the first stage the graphs were plotted with log percentage drug release on Y axis and log time on X axis.

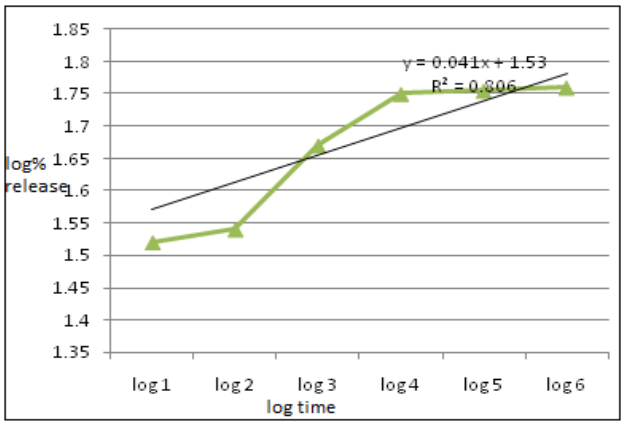

Figure 8: Log percentage drug release vs log time

The slope (n) of F3 was found to be 0.041 which indicate that n values are less than 0.5 which indicate that the mechanism of drug release is Fickian Diffusion.

V. CONCLUSION

Paliperidone palmitate loaded gelatin nanoparticles were prepared and evaluated. The prepared nanoparticles by nanoprecipitation method by using gelatin have a good entrapment efficiancy. Among the prepared nanoparticles drug-polymer ratio in 1:3 shows highest entrapment efficiency, percentage yield and cumulative drug release of

59.86 within six hours. These studies suggest the feasibiliy of formulating paliperidone palmitate loaded nanoparticles using a biodegradable polymer gelatin for the treatment of psychotic disorders.

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