Cutina based Nanoparticles of Clopidogrel

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ABSTRACT

The solubility and bioavailability of a drug is very important while preparing a formulation. BCS class-II drugs like clopidogrel have the problem of poor bioavailability because of less solubility. So many novel techniques were available to improve the solubility aspects of drug among which solid lipid nanoparticles is a promising approach. In the current study attempts were made to formulate and evaluate clopidogrel loaded solid lipid nanoparticles by employing cutina as lipid and lecithin soya and PEG-400 and TWEEN-80 were used as surfactant systems. Different formulations were prepared and analyzed for drug content, entrapment efficiency, drug release studies. The selected formulations were analyzed with stability studies at two different conditions which is, room temperature and refrigerated conditions.

Keywords: Clopidogrel, solid lipid nanoparticles, Cutina, PEG-400, TWEEN-80, lecithin soya, drug release studies.

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Received 20 September 2019, Accepted 28 September 2019
INTRODUCTION

Various novel techniques that are used to enhance the solubility are like solid lipid nanoparticles, Self emulsifying drug delivery systems, solid dispersions etc. Depending upon the problem associated with the drug the technique that is to be employed must be chosen\(^1\). Here we chose solid lipid nanoparticles for the study of the drug clopidogrel bisulphate is an antiplatelet drug used in the treatment of minor heart diseases. The drug clopidogrel bisulphate belongs to the BCS class II which has poor solubility and high permeability. The poor oral bioavailability is due to extensive first pass metabolism. The conventional preparations like solution, suspension or emulsion for drug delivery purpose has various boundaries like high dose and low availability, faster reach effect etc. The changes in plasma drug levels are also exhibited which do not provide sustained effect as well as reaching of drug to target site without any alteration in its physical and chemical properties\(^2\).

Therefore, there is a need for some novel carriers which could improve the above problems by reaching to its target site without making any adverse effects to body and can carry the drug easily and safely to its destination. Nanoparticles are such type of delivery carriers, which are colloidal drug delivery systems comprising particles with a size range from 10 to 1000 nm (diameter). The major advantages of nanoparticles are improved bioavailability by enhancing aqueous solubility, increasing residence time in the body (increasing half life for clearance/increasing specificity for its associated receptors and targeting drug to specific location in the body. This is why nanoparticle is increasingly used in variety of applications that includes drug carrier systems and to pass organ barriers such as the blood-brain barrier, cell membrane etc. They are based on biocompatible lipids that provide sustained effect by either diffusion or dissolution\(^3\).

Solid Lipid nanoparticles have ability to overcome the challenges associated with oral delivery of drugs that have low solubility, poor permeability, instability in the GIT and pre-systemic drug metabolism\(^4,5\). Thus to overcome the problems that are associated with drug clopidogrel bisulphate like low solubility and poor oral bioavailability, the clopidogrel bisulphate loaded solid lipid nanoparticles were prepared which are capable of improving above mentioned properties.

MATERIALS AND METHOD

Materials

Clopidogrel bisulphate was obtained as a gift sample from Aurobindo Labs, Cutina® HR (BASF), Tween 80, lecithin soya and PEG400, dialysis membrane (HiMedia, Mumbai) and other reagents used were of analytical grade.
Preparation of Clopidogrel bisulphate loaded solid lipid nanoparticle

Clopidogrel bisulphate loaded SLNs were prepared by hot homogenization method followed by sonication.

**Hot Homogenization Method**

Hot homogenization method is best suited method for the preparation of solid lipid nanoparticles as it can be performed at elevated temperatures to that of lipids melting point. The reduction in the particle size is due to cavitations and turbulences during homogenization. In hot homogenization technique the drug was dispersed in the lipid and soya lecithin (surfactant) by melting them above their melting point. This is considered as oil phase. The aqueous phase was prepared by adding other surfactant Tween 80 or PEG 400 in the distilled water and heated to the temperature of oil phase. The prepared oil phase was added to the aqueous phase drop by drop under continuous stirring of 3 hrs at 2700 rpm. The produced O/W emulsion is sonicated for half an hour and cooled to room temperature. At the room temperature the lipid recrystallizes and leads to formation of SLNs.

**Table 1 The various formulation prepared with cutina**

| Formulation code | Drug Clopidogrel (mg) | Polymer cutina (gm) | Lipid soya lecithin (gm) | Surfactant PEG 400 (ml) |
|------------------|-----------------------|--------------------|--------------------------|------------------------|
| C1               | 10                    | 1                  | 0.25                     | 0.25                   |
| C2               | 10                    | 1                  | 0.5                      | 0.5                    |
| C3               | 10                    | 1                  | 1                        | 1                      |
| C4               | 10                    | 1                  | 1.5                      | 1.5                    |
| C5               | 10                    | 1                  | 1                        | 2                      |
| C6               | 10                    | 1                  | 2                        | 1                      |

| Formulation code | Drug Clopidogrel (mg) | Polymer cutina (gm) | Lipid soya lecithin (gm) | Surfactant TWEEN 80 (ml) |
|------------------|-----------------------|--------------------|--------------------------|------------------------|
| D1               | 10                    | 1                  | 0.25                     | 0.25                   |
| D2               | 10                    | 1                  | 0.5                      | 0.5                    |
| D3               | 10                    | 1                  | 1                        | 1                      |

**Evaluation of Nanoparticles**

**Drug content**

5ml of nanoparticles suspension was taken, to this 10 ml of methanol was added. The dispersion was stirred thoroughly. Then the dispersion was filtered through whatman filter paper, the clear filtrate is further diluted and concentration of drug was measured U.V spectrophotometrically.

**Entrapment Efficiency**
Entrapment efficiency is an important parameter for characterizing nanoparticles. This parameter gives us an idea of the drug that was entrapped in nanoparticles by the carrier. In order to attain optimal entrapment efficiency, the varying concentrations of hydrophilic surfactant ratio to hydrophobic surfactant ratio were used. The entrapment efficiency of prepared nanoparticles was determined by the centrifugation method. Nanoparticles (containing equivalent to 10mg of drug) was centrifuged at 17000rpm for 40min in high speed research centrifuge to collect supernatant liquid. The collected liquid was filtered to measure amount of free drug concentration after suitable dilution with the fresh phosphate buffer of pH 6.8. The absorbance was measured at 220 nm in a UV spectrophotometer to calculate the entrapment efficiency using the formula:

\[ E.E = \frac{\text{Amount of total drug} - \text{Amount of drug in aqueous phase}}{\text{Amount of total drug}} \times 100 \]

In vitro Drug Release

The in vitro drug release of clopidogrel nanoparticles was determined by dissolution apparatus using USP II. An accurately weighed amount of clopidogrel nanoparticles containing the drug equivalent to 10mg was taken into the dialysis bag and sealed. This sealed dialysis bag was then suspended into the dissolution basket containing 900ml of phosphate buffer solution of pH 6.8 at the temperature of 37± 2°C, and stirred at a constant speed of 100rpm. Aliquotes were collected at each hour upto 24 hours and the same was replaced with the fresh buffer. The drug content was determined spectrophotometrically by measuring the absorbance at 220nm using the same buffer solution as the blank.

Stability Studies

Stability studies were carried out by storing the formulation at two different temperatures, in refrigerated condition and at room temperatures. The samples were analyzed for their physical appearance, drug content, entrapment efficiency and % drug release after a time period like at 0, 1, 2, and 3 months.

RESULTS AND DISCUSSION

Entrapment Efficiency

The entrapment efficiency of all the prepared nanoparticles formulations by hot homogenization is shown in Table 2. The entrapment efficiency of the prepared solid lipid nanoparticles by hot homogenization was found to be in the range of 54.2 to 72.3%. Among all the formulations D2 had shown better entrapment efficiency of 72.3%. Initially the entrapment was increased till D2 formulation and then decreased in further ratios. The high entrapment in D2 ratio was may be
because of the optimum concentration of hydrophobic surfactant ratio and hydrophilic surfactant ratio.

Table:2 Drug content and entrapment efficiency values

| Formulation code | Drug content (mg/ml) | Entrapment efficiency(%) |
|------------------|----------------------|--------------------------|
| C1               | 1.05                 | 64.2                     |
| C2               | 0.84                 | 42.6                     |
| C3               | 1.21                 | 51.3                     |
| C4               | 0.71                 | 63.1                     |
| C5               | 0.82                 | 38.7                     |
| C6               | 0.49                 | 54.2                     |
| D1               | 1.01                 | 67.8                     |
| D2               | 1.07                 | 72.3                     |
| D3               | 0.93                 | 46.2                     |

Figure: 1 Entrapment Efficiency Graph

**Drug Content**

The drug content values for all the prepared formulations were given in Table No: 2. The drug content values range from 0.84-1.75. Among all the prepared formulations, D2 had shown better drug content of 1.75 which is in accordance with the entrapment efficiency values.
Figure 2: Drug content graph

In Vitro Drug Release

The *in vitro* drug release profile of clopidogrel from various prepared nanoparticles formulation by hot homogenization were given in fig-2. The drug release over a period of 24 hrs from all the formulations was observed to be in the range of 30.4 % to 86.0 %. Among all the formulations D2 is showing 86.0 % of drug release in 24 hrs.

Figure 3: In-vitro drug release study graph

Stability Studies

The finalized formulation based on previous studies was kept for stability studies for 3 months at room temperature and refrigerated conditions shown in Table: 3
Table 3 Refrigerated Temperature

| Formulation D2 | before month | 1 month | 2 month | 3 month |
|----------------|--------------|---------|---------|---------|
| Drug content   | 79.2 %       | 79 %    | 75.8 %  | 73.9 %  |
| Entrapment efficiency | 72.3 %   | 72.1 %  | 70.9 %  | 70 %    |
| % drug release (24 hrs) | 86.3 % | 86.1 %  | 84.3 %  | 83.8 %  |

Room Temperature

| Formulation D2 | before month | 1 month | 2 month | 3 month |
|----------------|--------------|---------|---------|---------|
| Drug content   | 79.2 %       | 77.3 %  | 74.3 %  | 70.4 %  |
| Entrapment efficiency | 72.3 %   | 71.9 %  | 69.6 %  | 66.1 %  |
| % drug release (24 hrs) | 86.3 % | 86.1 %  | 83.5 %  | 80.0 %  |

Figure 4: In vitro drug release study graph before stability

Figure 5: In vitro drug release study graph after stability
FTIR Spectroscopy
A SHIMADZU P/N 206-73500-38 FTIR spectrometer was used for infrared analysis. Samples were prepared by KBr disc method (2 mg sample in 100 mg KBr) and examined in the transmission mode. A resolution of 4 cm⁻¹ was used and 64 scans were co-added for each spectrum over a frequency range of 4000-450 cm⁻¹. The software used for the data analysis was Perkin-Elmer spectra MA.

Figure 6 FTIR spectra of drug-clopidogrel

Figure 7 FTIR spectra for the optimized formulation -D2
FTIR spectroscopy showing no drug and excipients interactions. It showed C-S-C stretching bond on 2464, C=O stretching bond on 1752, C-O bond on 1188, C-Cl bond on 1221, pyridine ring on 1154 and chlorophyll ring on 1474.

ACKNOWLEDGEMENT

The authors wish to thank Dr. Sumakanth. This work was supported in part by a grant from R.B.V.R.R WOMENS COLLEGE OF PHARMACY.

CONCLUSION

In the present research, the different formulations were prepared by using Cutina HR, Tween-80, Lecithin soya and PEG400 by employing hot homogenization method. The entrapment efficiency and drug release profile were depended up on the concentration of hydrophilic surfactant and hydrophobic surfactant mixture employed. The results of in-vitro drug release studies demonstrated significantly controlled release of clopidogrel from prepared solid lipid nanoparticles. Among all the Preparations D2 formulation was best in terms of Entrapment efficiency of 72.3%, Drug release of 86.3% and in vitro drug release of 86.0 % in 24 hrs. Hot homogenization was found to be the best method with high entrapment efficiency. FTIR studies showed no drug and excipients reactions. This method was found to be simple, cost effective, easy and suitable to produce solid lipid nanoparticles. This method can be scaled up when compared with other preparations. Further it could be presumed that the obtained nanoparticles might increase oral bioavailability.

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