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Comparative Study of Clopidogrel Using PVA and Cutina

Madiha Fatima*, Tripura Sundari
R.B.V.R.R Womens College of Pharmacy, Barkatpura, Telangana, Hyderabad-500020, India

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

*Corresponding Author Email: madihamkp@gmail.com
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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]. Clopidogrel bisulphate belongs to 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[2]. 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[3,4].

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[5].In the present work, attempts were made to prepare SLNs of clopidogrel. Clopidogrel is a BCS class –II drug which has low solubility and permeability. 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), PVA , 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 cavitation 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 in the distilled water and heated above the 80^0C 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. The formulations prepared by hot homogenization.

The comparative formulations were prepared using PVA and Cutina HR as polymer and lipid ,soya lecithin,Tween 80 as lipophilic and hydrophilic surfactants and by keeping the lipid and polymer constant i.e,1 gm and drug as 10mg.All the formulations are shown in the table ,1.

Table 1 Prepared formulations with PVA and Cutina

Formulation Code (PVA)	Soya Lecithin mg (Surfactant)	Tween 80 (ml) (Surfactant)
F1	0.25	0.25
F2	0.5	0.5
F3	0.5	1
F4	1	1
Formulation Code (PVA)	**Soya Lecithin mg (Surfactant)**	**Tween 80 (ml) (Surfactant)**
C1	0.25	0.25
C2	0.5	0.5
C3	0.5	1
C4	1	1

Evaluation of Nanoparticles

Drug content

5 ml 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 by using UV 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= Amount of total drug - Amount of drug in aqueous phase X100 /Amount of total drug.

In vitro Drug Release

The in vitro drug release of clopidogrel nanoparticles was determined by dissolution apparatus using USP II with the help of dialysis membrane. 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 6.8at the temperature of 37± 2°C, and stirred at a constant speed of 100rpm. Aliquotes were collected at the time up to 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, to calculate the amount of drug released from the nanoparticles.

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 fortheir physical appearance, drug content, entrapment efficiency and % drug release after a time period like at 0, 1, 2, and 3 months.

RESUTS AND DISCUSSION

Drug Content

The drug content values for all the prepared formulations were given in table 3. The drug content values ranges from 1.01 – 2.59 mg/ml. Among all the prepared formulations, comparatively the drug content were high for the formulations prepared with PVA.

Table 2 Comparative studies of various formulations

Formulation Code	Drug content	Formulation code	Drug content
F1	2.59	C1	1.01
F2	2.13	C2	1.75
F3	2.35	C3	1.13
F4	2.21	C4	1.04

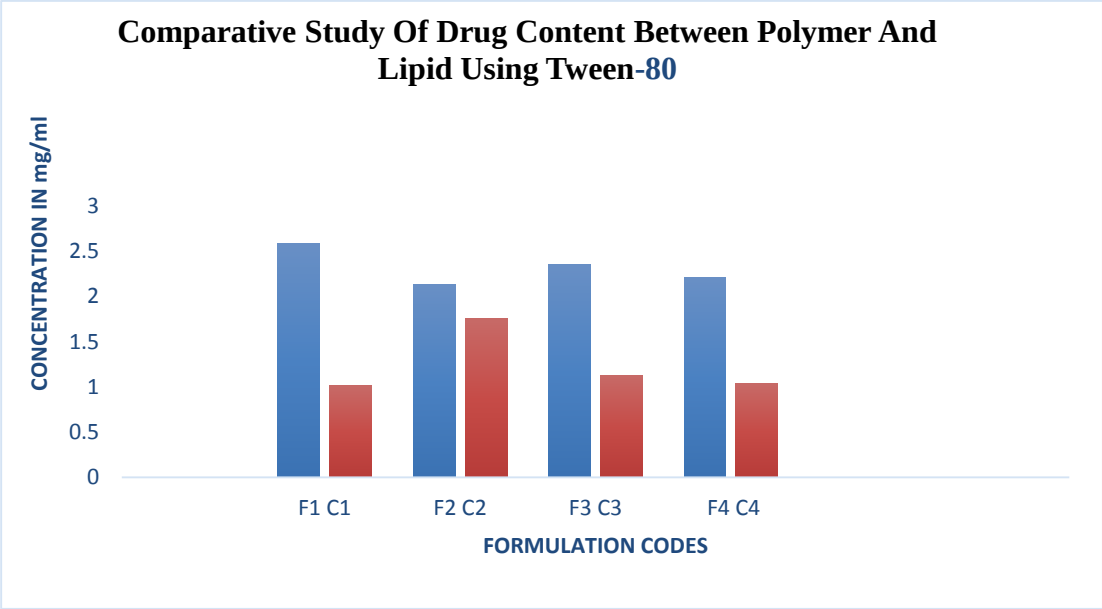

Figure 1: Comparative study of drug content of various formulation

Entrapment Efficiency (E.E)

The entrapment efficiency of all the prepared nanoparticles formulations by hot homogenization method is shown in Table 2. It was in the range of 53% - 72 %.

Initially the comparative studies of entrapment efficiency was studied. Comparatively % entrapment efficiency were high for PVA formulations, may be because of the optimum concentration of hydrophobic surfactant ratio and hydrophilic surfactant ratio.

Table 3 Entrapment efficiencies of the prepared formulations

Formulation Code	% E.E	Formulation Code	% E.E
F1	72 %	C1	55 %
F2	45 %	C2	61 %
F3	59 %	C3	46 %
F4	53 %	C4	59 %

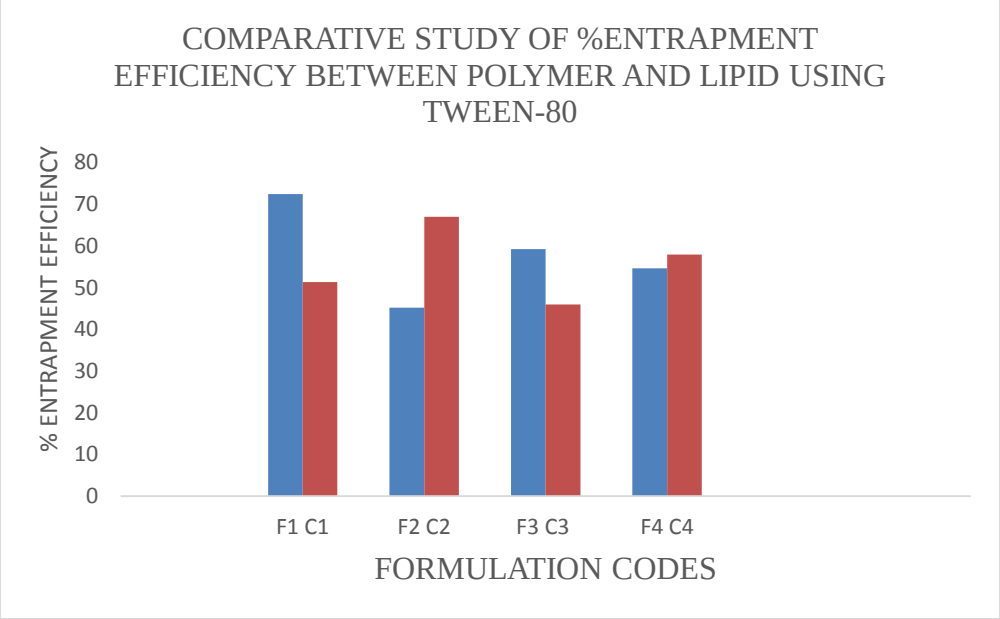

Figure 2 Comparative study of % EE of various formulation

In Vitro Drug Release

The *in vitro comparative* drug release profile of clopidogrel from various nanoparticles formulation by solvent evaporation. The *in vitro* release of clopidogrel , Among all the formulations, (F1) formulation was found to be 86.3% at the end of 24 hours shown Figure: 3. Comparatively the %drug release studies with polymer (PVA) were showing the best results when compared with lipid(cutina) using Tween-80.

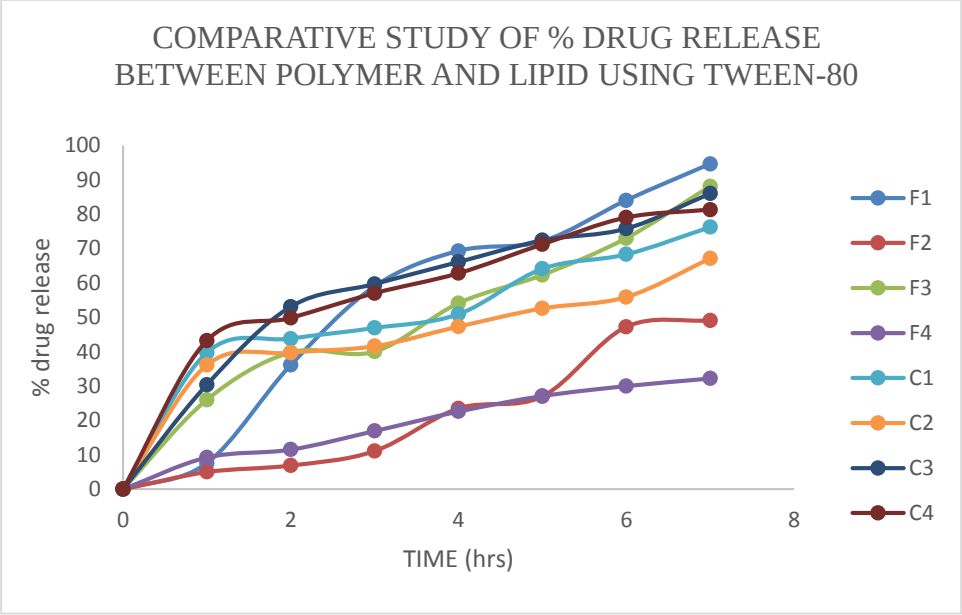

Figure 3: Comparative % Drug Release Studies Between Polymer and Lipid By using Tween 80

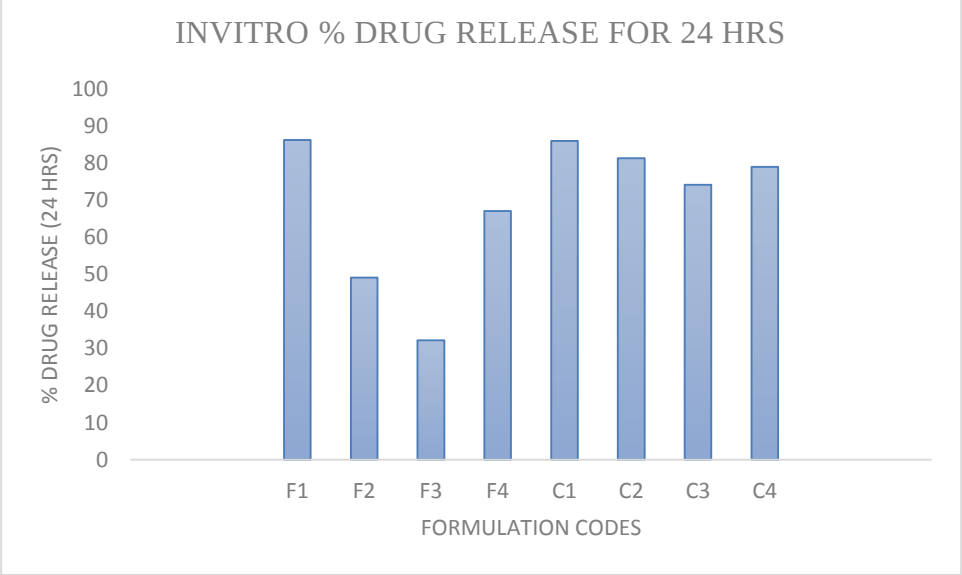

Figure 4 In-vitro % drug release for 24 hrs

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.The stability of the formulation ranges from 86.3 % - 69.1 % within 3 months.

Table 4 Refrigerated Temperature

Formulation	0 month	1 month	2 month	3 month
% Drug content	79.2 %	77.5 %	73.6 %	71.2 %
% Entrapment efficiency	72.3 %	72 %	71.5 %	69.2 %
% drug release(24hrs)	86.3 %	86.1 %	85.9 %	84.3 %

Room Temperature

Formulation	0 month	1 month	2 month	3 month
% Drug content	75.1 %	72.3 %	70.3 %	69.4 %
% Entrapment efficiency	72.3 %	72 %	71.6 %	69.1 %
% drug release(24hrs)	86.3 %	82.6 %	78.5 %	72.3 %

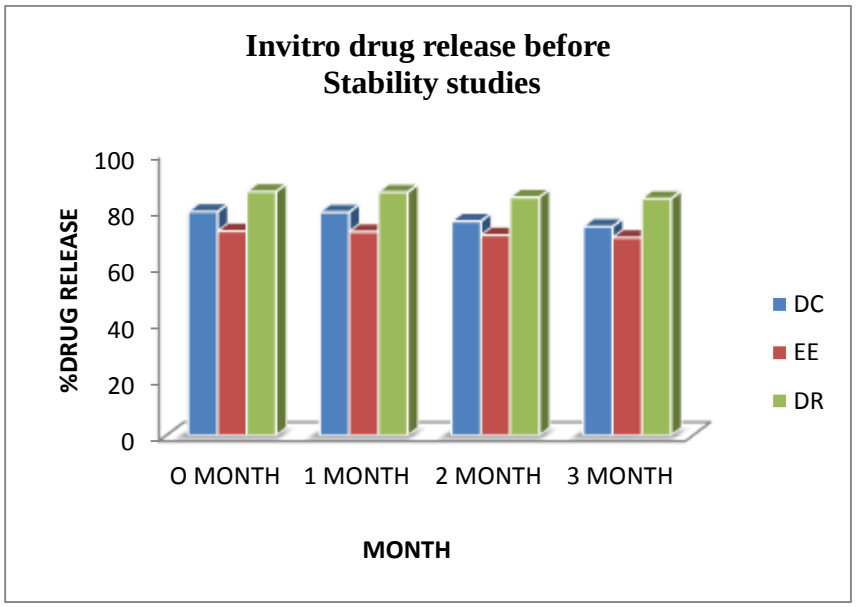

Figure 5 In-vitro drug release studies before stability studies

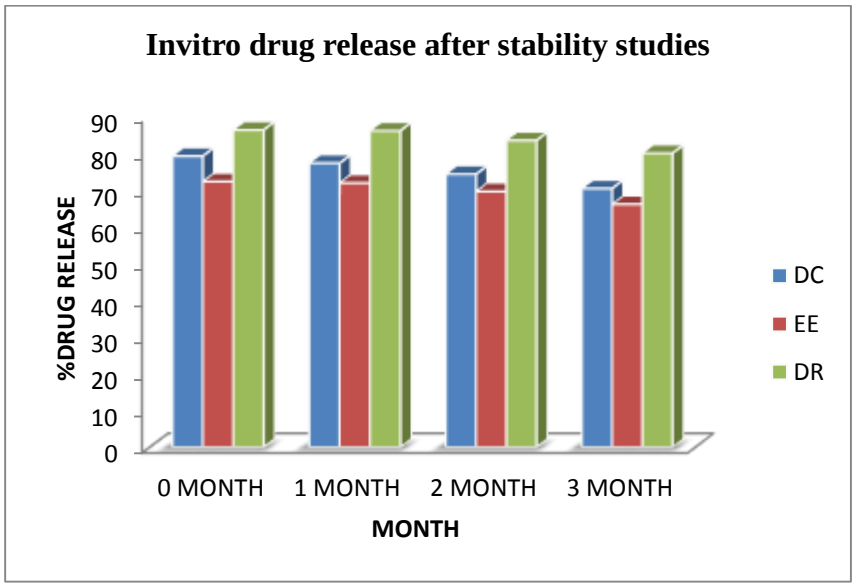

Figure 6 In-vitro drug release after stability studies

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.

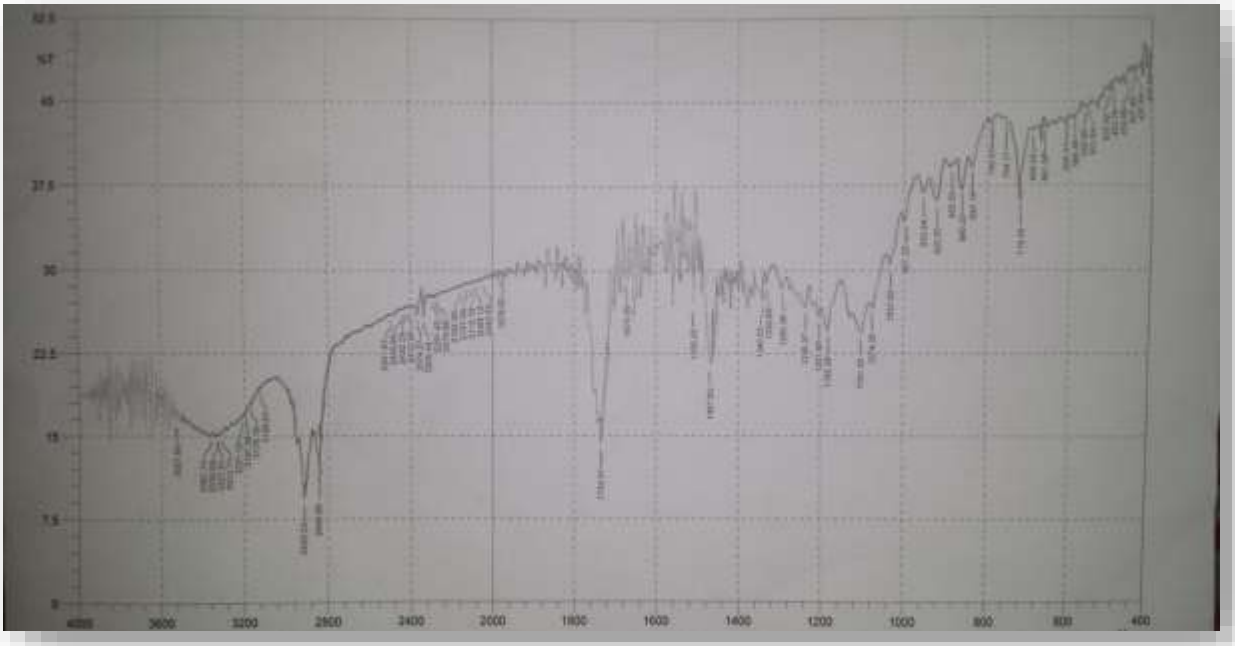

Figure 7 FTIR spctra of drug-clopidogrel

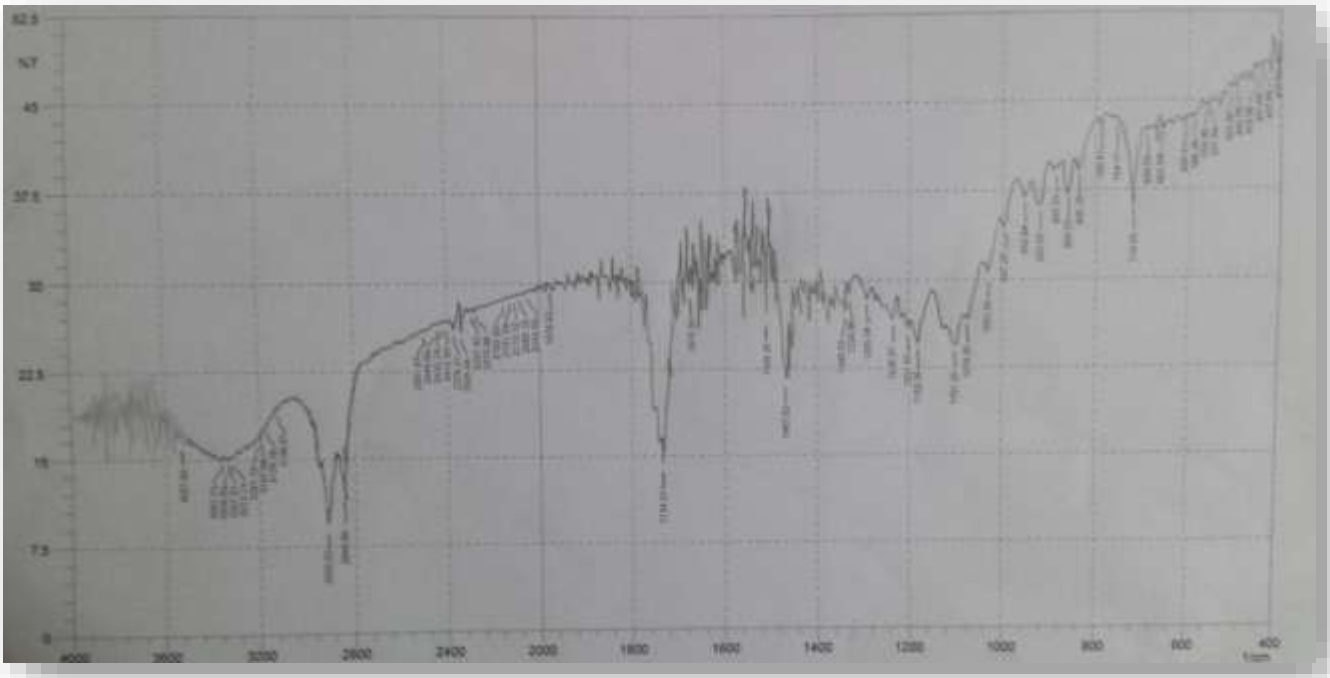

Figure 8 FTIR spectra for the optimized formulation

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.

CONCLUSION

In the present research, the different formulations were prepared and compared by using polymer and lipid (polyvinyl alcohol, cutina), Tween-80, and Lecithin soya by employing hot homogenization method. The better results were shown with polymer (PVA) when compared with lipid (cutina) using Tween-80. The better results with polymer showing % drug content(79.2 %), % entrapment efficiency(72.5 %) and % drug release(86.3 %). The comparative results of in-vitro drug release studies demonstrated significantly controlled release of clopidogrel from prepared nanoparticles. Hot homogenization was found to be the best method with high entrapment efficiency. This method was found to be simple, cost effective, easy and suitable to produce 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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