DEVELOPMENT AND OPTIMISATION OF HYDROCHLOROTHIAZIDE -LOADED SOLID LIPID NANOPARTICLES

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ABSTRACT : ***The hydrochlorothiazide (HCT) has low solubility and permeability which give rise to limited and variable bioavailability; its low stability makes it difficult to develop stable aqueous liquid formulations, the aim of this study was to investigate the effectiveness of a strategy based on the development of solid lipid Nanoparticles as an innovative formulation of HCT with improved therapeutic efficacy. The hydrochlorothiazide solid lipid nanoparticles were prepared by emulsification solvent evaporation technique by applying ultrasonic energy through Sonicator, The different formulations with various ratios of drug-lipid and surfactant were evaluated and optimised. The method used for the formulation of Hydrochlorothiazide containing soya lecithin solid lipid nanoparticles was solvent evaporation method followed by sonication to reduce the particle size. The prepared nanosuspensions were characterised for particle size, surface morphology by SEM, drug excipient compatibility by FTIR and in-vitro drug release studies. Formulation (F-4) showed the highest encapsulation efficiency. In this research, a drug encapsulation efficiency as high as 81% has been achieved. It was found that as the concentration of soya lecithin increased, the % of encapsulation efficiency was also increased. The present study revealed that solvent evaporation technique followed by sonication can be used as an effective tool for preparation of Hydrochlorothiazide solid lipid nanoparticles.***

Key words: Hydrochlorothiazide drug, solid lipid Nano Particles, Solvent Evaporation, lipid,FTIR, invitro drug release.

I.INTRODUCTION

Colloidal particles ranging in size between 10 and 1000 nm are known as nanoparticles. They are manufactured from synthetic/natural polymers and ideally suited to optimize drug delivery and reduce toxicity.[1] Over the years, they have emerged as a variable substitute to liposomes as drug carriers. The successful implementation of nanoparticles for drug delivery depends on their ability to penetrate through several anatomical barriers, sustained release of their contents and their stability in the nanometre size.[2] However, the scarcity of safe polymers with regulatory approval and their high cost have limited the wide spread application of nanoparticles to clinical medicine. To overcome these limitations of polymeric nanoparticles, lipids have been put forward as an alternative carrier, particularly for lipophilic pharmaceuticals.[3] These lipid nanoparticles are known as solid lipid nanoparticles (SLNs), which are attracting wide attention of formulators world-wide. SLNs are colloidal carriers developed in the last decade as an alternative system to the existing traditional carriers (emulsions, liposomes and polymeric nanoparticles).[4] They are a new generation of submicron-sized lipid emulsions where the liquid lipid (oil) has been substituted by a solid lipid. SLN offer unique properties such as small size, large surface area, high drug loading and the interaction of phases at the interfaces, and are attractive for their potential to improve performance of pharmaceuticals, neutraceuticals and other materials.[5] SLNs are attracting major attention as novel colloidal drug carrier for intravenous applications. The SLNs are sub-micron colloidal carrier which is composed of physiological lipid, dispersed in water or in an aqueous surfactant solution.[6] The aim was to improve oral bioavailability either by increasing GI absorption or bypassing the first-pass metabolism Several drugs have been incorporated in to SLN formulations for oral administration and enhancement of oral bioavailability of Hydrochlorothiazide. Preparation of solid lipid nanoparticles by two methods solvent emulsification followed by evaporation method followed by ultrasonication method and optimization of the sonication time for the desired particle size.[7] Hydrochlorothiazide is used to treat edema (fluid retention; excess fluid held in body tissues) caused by various medical problems.[8]

II. MATERIALS AND METHOD

2.1 MATERIALS

Hydrochlorothiazide was collected as a gift sample from Hetero labs, HYD, lipids and other excipients were purchased from AR Chemicals, Hyd.

2.2 METHODODOLOGY

Compatibility studies:

The drug-polymer compatibility was ascertained by subjecting the drug and homogenates of drug and polymer to Infrared spectrophotometric study.

Fourier Transform Infrared Spectroscopy (FTIR)[9,10]

Assessment of possible incompatibilities between an active drug substance and different excipients forms an important part of the preformulation stage during the development of a dosage form. The use of FTIR technique allows pointing out the implication of the different functional groups of drug and excipients by analysing the significant changes in the shape and position of the absorbance bands. In this method individual samples as well as the mixture of drug and excipients were ground mixed thoroughly with potassium bromide (1:100) for 3-5 mins in a mortar and compressed into disc by applying pressure of 5 tons for 5 mins in hydraulic press. The pellet was kept in the sample holder and scanned from 4000 to 400 cm-1 in FTIR spectrophotometer. Then the characteristics peaks were obtained of all sample as well as mixtures.

Method of preparation of Hydrochlorothiazide loaded nanoparticles:

HCL loaded SLN were prepared by solvent emulsification/evaporation method. The composition of all the formulations 20 mg of drug was dissolved in 10 mL methanol, and Phosphatidylcholine was dissolved in 20 mL chloroform separately; drug and lipid solutions were mixed together. The organic solvent mixture was completely evaporated at 70°C using rotary evaporator to remove the of organic solvent. Drug embedded lipid layer was then poured into 100 mL of aqueous solution containing poloxomer 407 surfactant and the mixture was Sonicated for 15 minutes by using Sonicator followed by homogenized for 15 minutes at different homogenization speed using high speed homogenizer. The suspension was then allowed to cool at room temperature. The suspension was filtered through membrane filter. The filtrate was centrifuged (1000 rpm for 10 minutes) and nano particles was collected [11]

Table -: 1 composition of Hydrochlorothiazide for preparation of solid lipid nanoparticles

Ingredients	**F1**	**F2**	**F3**	**F4**
Hydrochlorothiazide	25	25	25	25
Phosphatidylcholine	25	50	75	100
Poloxamer 407	10	20	30	40
Solvent(Methanol)	10	10	10	10
Chloroform	20	20	20	20

Evaluation of Hydrochlorothiazide loaded nanoparticles:

Particlesize:

All the prepared batches of nanoparticles were viewed under microscope to study their size. Size of Nanoparticles from each batch was measured at different location on slide by taking a small drop of nanoparticle dispersion on it and average size of nanoparticles were determined[12].

SEM analysis

The morphology of NPs was studied by a scanning electron microscope. For this purpose, the sample was

lyophilized and placed on aluminum stubs and the surface was coated with a layer of gold particles using a sputter coater. The shape of the NPs was determined by scanning electron microscopy (SEM) (XL30, Philips, the Netherlands) at 15 kV and 750 mA.[13]

Drug encapsulation efficiency:

Lyophilized nanoparticles 50mg were dissolved in 100ml of phosphate buffer and the drug amount was determined by UV analysis. The encapsulation efficiency was determined as the mass ratio of entrapped Hydrochlorothiazide in nanoparticles to the theoretical amount of the drug used in the preparation. The entrapment of the Hydrochlorothiazide nanoparticles was expressed as loading capacity.[14]

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Amount entrapped}}{\text{Total drug loaded}} \times 100$$

In-vitro drug release studies:

The release studies were carried out by franz diffusion cell. It containing 10 ml Phosphate buffer. Phosphate buffer pH 7.4 (100 ml) was placed in a 10 ml of beaker. The beaker was assembled on a magnetic stirrer and the medium was equilibrated at 37±5^{0}C. Dialysis membrane was taken and one end of the membrane was sealed. After separation of non entrapped Hydrochlorothiazide dispersion was filled in the dialysis membrane and other end was closed. The dialysis membrane containing the sample was suspended in the medium. 1ml of aliquots were withdrawn at specific intervals, filtered after withdrawal and the apparatus was immediately replenished with same quantity of fresh buffer medium.[15]

Percentage of drug release was determined using the following formula.

$$\text{Perentage drug release} = \frac{Da}{Dt} \times 100$$

Where, Dt = Total amount of the drug

Da = The amount of drug released

Stability studies[16]:

Selected Formulation was subjected to stability studies as per ICH guidelines.

Following conditions were used for Stability Testing.

1. 25^{0}C/60% RH analysed every month for period of three months.
2. 30^{0}C/75% RH analysed every month for period of three months.
3. 40^{0}C/75% RH analysed every month for period of three months.

III. RESULTS AND DISCUSSION

Drug - excipient compatibility studies (FT-IR)

The compatibility between the drug and the selected lipid and other excipients was evaluated using FTIR peak matching method. There was no appearance or disappearance of peaks in the drug-lipid mixture, which confirmed the absence of any chemical interaction between the drug, lipid and other chemicals.

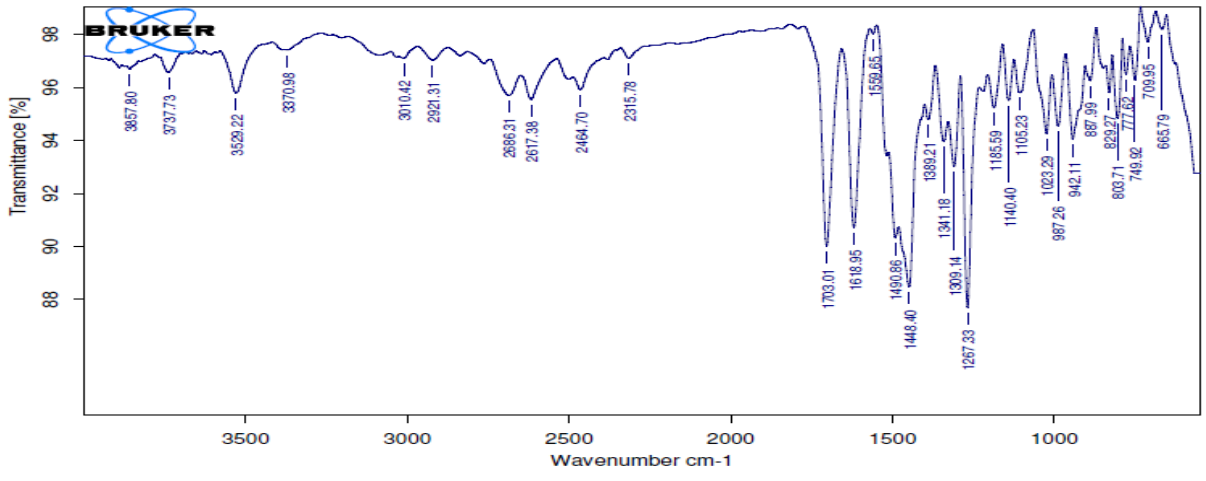

Fig.1. FT-IR Sample for Hydrochlorothiazide

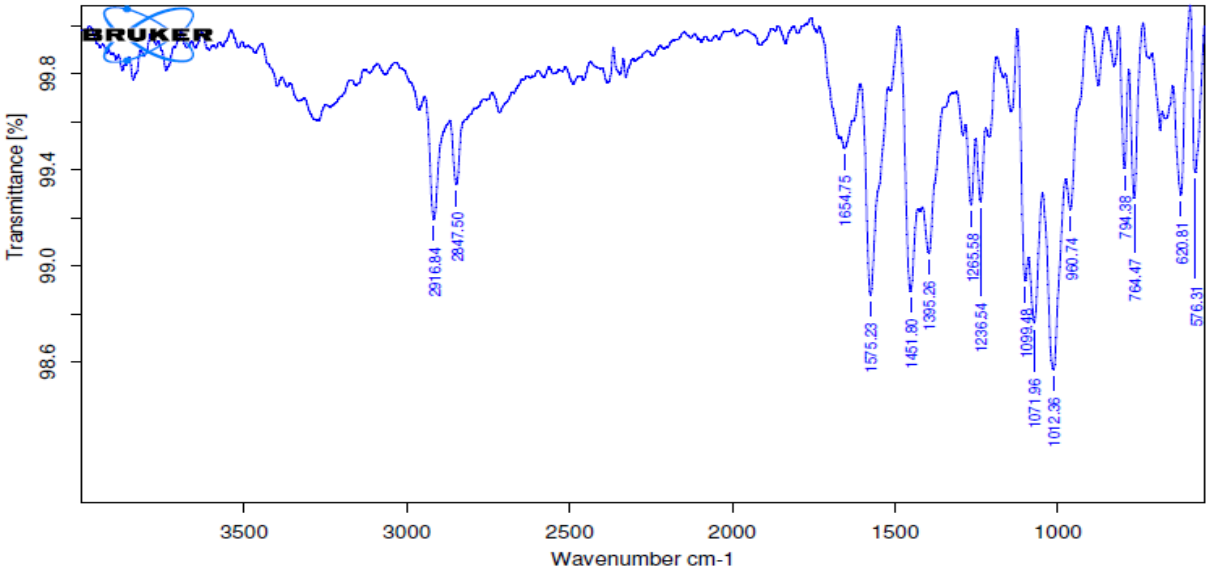

Fig.2. FT-IR Sample for Optimized Formulation

EVALUATION PARAMETERS

The solid lipid nanoparticles prepared were evaluated as per the following parameters-

Particle size:

The particle size increased with increasing of lipid concentration. Based on particle size distribution and entrapment efficiency.

Surface morphology:

Scanning electron microscopy (SEM) SEM revealed that the MTX solid lipid nanoparticles were smooth and spherical without any aggregation.

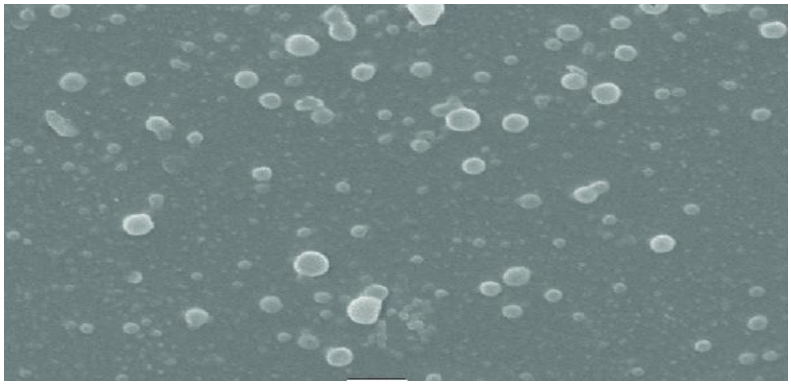

Fig.3. SEM analysis of Optimized Solid lipid solid lipid nanoparticle

Drug entrapment efficiency:

The first part of the plan of work was to optimize the concentration of Lipid to be used in the formulation of solid lipid nanoparticles. The optimization of lipid concentration was done on the basis of particle size and entrapment efficiency of solid lipid nanoparticles obtained.

Table: 2 Evaluation Studies of Prepared solid lipid nanoparticles: Entrapment Efficiency and Particle size

Batch No	Particle size (nm)	Entrapment Efficiency (%)
F1	332	81
F2	362	80
F3	375	79
F4	395	90

In vitro drug release studies

Results indicate that the formulation showed initial burst release followed by sustained release of the drug for a prolonged period of time. The rapid initial release may be attributed to the fraction of Hydrochlorothiazide on the surface of solid lipid nanoparticles. The in vitro drug release results revealed that the prepared Hydrochlorothiazide solid lipid nanoparticles would be able to control drug release for extended period of time.

Table-: 3 Drug release study profiles for all formulations

Time (hrs)	F_1	F_2	F_3	F_4
0	0	0	0	0
1	28.55	26.45	26.55	29.55
2	35.25	34.26	31.6	38.5
3	43.82	34.7	42.55	48.12
4	52.65	53.54	55.55	55.65
5	61.28	62.85	65.58	65.55
6	69.25	72.8	72.9	77.2
7	78.85	81.63	83.52	83.85
8	88.56	90.55	91.29	95.55

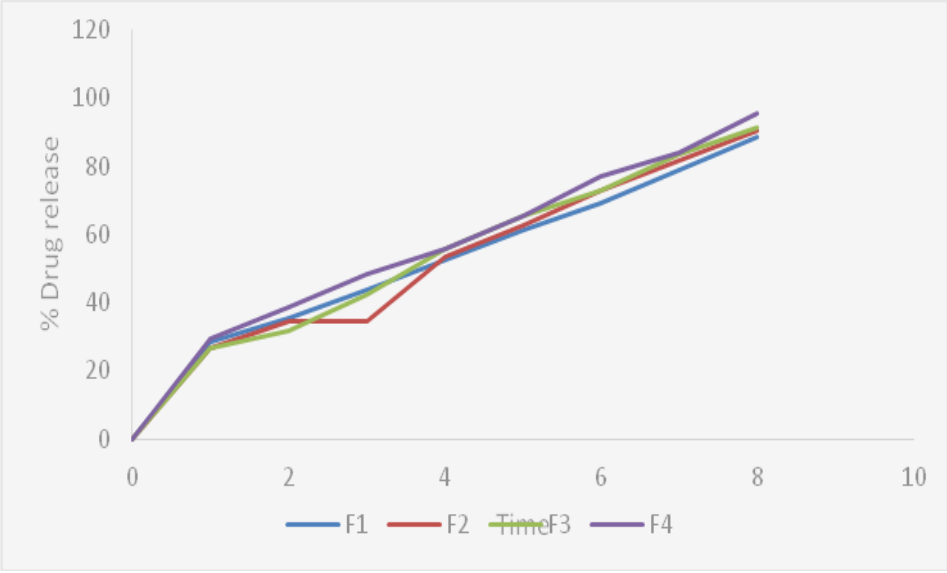

Fig.4. In vitro drug release studies for all formulations

The in vitro diffusion studies were performed in pH 7.4 buffer using Dialysis membrane for 8 hours. Initially the release of drug from all the three batches was found to be about 25-30% in 8 hours. This was due to the release of adsorbed drug from the surface of Solid lipid solid lipid nanoparticles. Later on a constant and slow drug release was observed for 8hrs. F4 formulation which had lipid and surfactant ratio was decided to be the optimized formulation.

Stability studies:

There was no significant change in physical and chemical properties of the nanoparticles of formulation F-4 after

3 months. Parameters quantified at various time intervals were shown

Table- : 4 Results of stability studies of optimized formulation F-4

Formulation Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-4	25^0C/60%RH % Release	95.55	95.41	95.38	95.34	Not less than 85 %
F-4	30^0C/75% RH % Release	95.55	95.45	95.37	95.32	Not less than 85 %
F-4	40^0C/75% RH % Release	95.55	95.50	95.35	95.30	Not less than 85 %

IV.CONCLUSION

The present research proposed a novel formulation Hydrochlorothiazide solid lipid nanoparticles for controlled release. Investigation of the preparation, characterization and in-vitro release of the solid lipid nanoparticles was carried out. The different formulations of with various ratios of drug-lipid and surfactant were evaluated and optimised. In this research, a drug encapsulation efficiency as high as 81% has been achieved. The method used for the formulation of Hydrochlorothiazide containing soya lecithin solid lipid nanoparticles was solvent evaporation method followed by sonication to reduce the particle size.

solid lipid nanoparticles formulations showed good results in terms of the assayed drug content and encapsulation efficiency. This indicates that the method used for the formulation produced good yield and it was suitable and reproducible in nature. Formulation (F-4) showed the highest encapsulation efficiency. It was found that as the concentration of soya lecithin increased, the % of encapsulation efficiency was also increased. Permeation studies with dialysis membrane were carried out as per the method reported. The formulations showed good drug release from the lipid, the *in vitro* drug release profiles of all the formulations showed an initial burst effect, and followed by a slow drug release. The burst release of drug is associated with those drug molecules dispersing close to the solid lipid nanoparticle surface, which easily diffuse in the initial incubation time. The Hydrochlorothiazide release was faster for those solid lipid nanoparticles with higher drug content.

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