Formulation and Evaluation of Lacidipine Nanosphers

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Abstract

Lacidipine (LCDP) is a calcium-channel blocker with low aqueous solubility and bioavailability. Nanosphere is one of the popular methods that has been used to solve the solubility problems of many drugs. To develop the best formulations of lacidipine nanoparticulate. screening of polymers for the production of nanospheres, such as ethanol and eudragits.Examining surfactants such Tween 80, sodium lauryl sulphate, and ploxamer for nanospheres to evaluate the nanoparticle compositions' technological qualities and in-vitro drug release. Due to the positive results of the in vitro drug release experiments, which could increase patient compliance, the product should probably be used in future in vivo research. According to the findings, formulation F9, which makes use of a number of polymers and incorporates lacidipine nanospheres, is the most successful formulation and releases more than 98.9% of the drug in 20 hours. There are no drug-excipient interactions in the altered formulation, according to IR spectroscopic measurements. The improved formula F9 may be able to keep the release going. Lacidipine nanospheres, a type of drug delivery technology, offer virtually zero order drug release over the course of a 24- hour period.

KEY WORDS: Nanosphers, Lacidipine, Evaluation, Formulation, Drug, Excipients, IR Spectroscopy, Technology, Drug Delivery.

Introduction:

Oral delivery of drugs is regarded as the optimal route to achieve therapeutic and prophylactic effects against various diseases, especially chronic conditions. It may have poor bioavailability as a hurdle, leading to challenges for pharmaceutical manufacturers to design delivery system that can provide improved pharmacokinetic profiles and therapeutic responses.Lacidipine (LCDP) is a dihydropyridine calcium- channel blocker developed for oral administration .It used in the treatment of hypertension and atherosclerosis and possessed an antioxidant effect . Chemical name of LCDP is (E)-4-[2-[3-(1,1- Dimethylethoxy) -3-oxo-1-propenyl] phenyl]- 1,4- dihydro -2,6-dimethyl -3,5-pyridine dicarboxylic acid diethyl ester, The molecular weight (455.5) and pka 2.5, log P (octanol/water) is 5.51,the powder is a white to paleyellow powder, melt at 178°C

LCDP poorly absorbed from the gastrointestinal tract after oral doses and undergoes extensive first-pass metabolism the bioavailability has been reported to be 2 to 10%. The rate limiting Step for drug absorption in this class is dissolution. This study aims to prepare LCDP as a nanosphere to improve its dissolution rate.[1]

NANOSPHERES

Nano drug delivery system is one of the field of science, the explosive growth of nanotechnology has brought rapid development in drug delivery is called Nano drug delivery system (NDDS). [2,3] It’s the study to individual molecules, atoms or compounds into structures to produces materials having special properties. It’s the study of small structures whose dimensions in the nanometers scale length (1-100). [4,5] Nano DDS can cause the drug to remain in blood circulation for a long time, thereby leading to lesser fluctuations in plasma levels and therefore, minimal side effects.

In these Nano DDS includes large range of Nano carriers such as nanoparticles, micelles,

nanogels, Dendrimer, solid lipid nanoparticles, polymersomes, liposomes, carbon nanotubes, nanocrystals, silica nanoparticles, nanocapsules and nanospheres (show in figure1) [2,6,7]

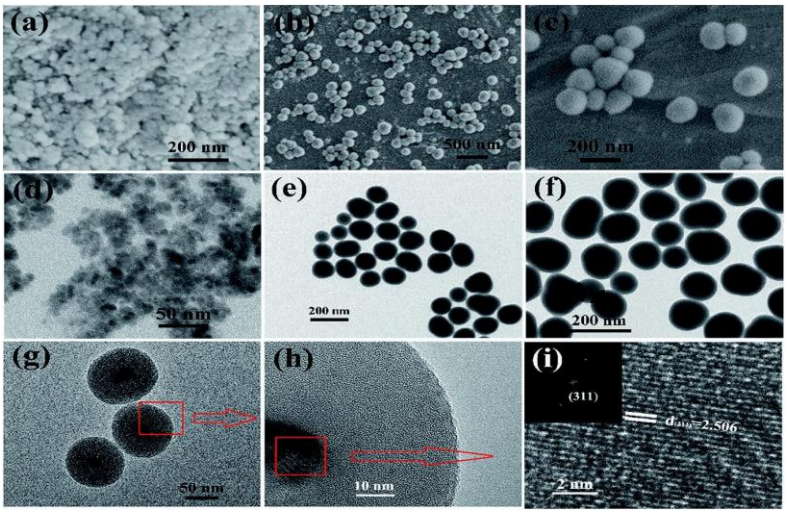


Fig.1. Different sizes of nanospheres

Advantages of Nanospheres

- Nanospheres can easily pass through the smallest capillary vessels.
- It can be used target the organs like liver, spleen, lungs, spinal cord. [8]
- Nanospheres reduce the toxicity and reduction in the frequency of the dosages.
- Nanospheres can be administered via various routes (oral, nasal, parenteralroute).
- Rapid clearance & Sitespecific targeting [9]

Disadvantages of Nanospheres

- Nanospheres are difficult to handle in liquid and dry form
- Requires skills to manufacture.
- They are prone to particle aggregation due to size and larger surface area. [10]

METHOD OF PREPARATION OF NANOSPHERES

Various types of methods are used for the preparation of nanospheres. Different methods should include

- Polymerization method
- Solvent evaporation
- Solvent displacement technique
- Double emulsion method
- Controlled gellification method
- Desolvation technique
- Ionic gelation method
- Salting out method. [11]

Materials and Methods:

Chemicals:

All Chemicals are obtained from Active Pharma labs, Hyderabad.

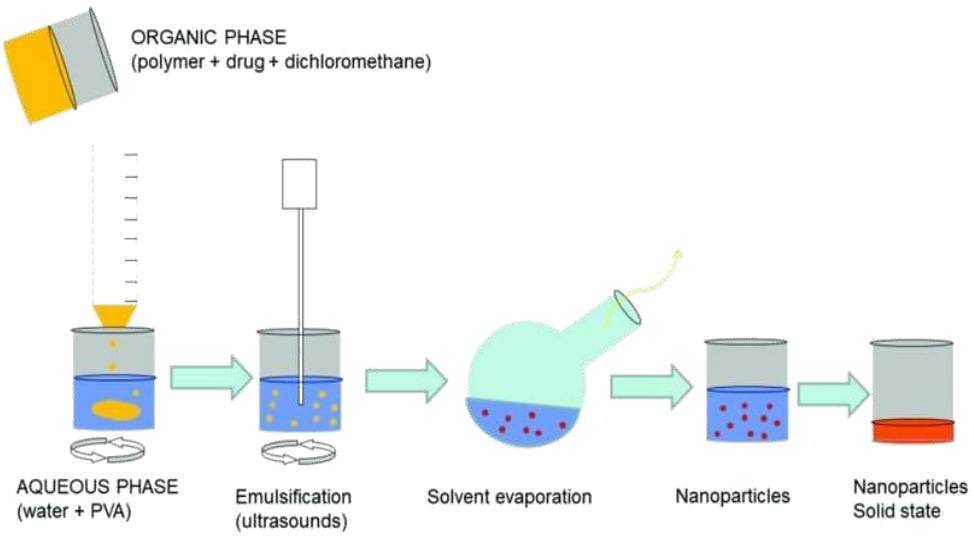
METHOD OF PREPARATION

Lacidipine drug nanospheres were prepared by Emulsion followed by Solventevaporation method and different types of polymers were used

Solvent evaporation method

In this method polymer dissolved in a suitable organic solvent, thus mixture sonicated for 2min, and then the drug dispersed into the previous solution and again sonicated for 2min. this above mixture is then emulsified using the suitable emulsifying agent (e.g. poly vinyl alcohol, gelatin) to form O/W emulsion. To formed emulsion is subjected to solvent evaporation via continuous mixing or increasing temperature or by reducing pressure. After then process completed nanosphere are formed.[12]

Fig-2: Solvent evaporation



Preparation of Polymer and drug Solution:

- Weighed the required amount of polymer and placed in a dry beaker.
- Required quantity of solvent (methanol) was taken in a measuringcylinder.
- Now, methanol was added to the beaker containing polymer slowly.
- Then, it was stirred with glass rod continuously to form polymer solution.
- add accurately weighed amount of Lacidipine 300mg and mixthoroughly.

Preparation of aqueous solution:

Weighed the required amount of SLS 1g in 1000mL of water and mix then kept a side for removing air bubbles

As required, one gramme of SLS was added to one thousand millilitres of water, and the mixture was then allowed to sit for a short period of time to remove air bubbles.

SIMPLE MIXING:

Lacidipine Nanospheres were prepared by using Emulsion followed by solvent evaporation technique as an effective technology in preparation of nanodrugs. Polymers dissolved in chloroform then 10mg of drug of Lacidipine was completely dispersed in polymer solution and 1 % SLS solution add to this under stirring at 400-
500 rpm up to 20min then beaker placed into probe sonicatoe for 15min after sonication kept for continuous stirring by magnetic stirrer and temperature was maintained at 10˚c by using ice bath. nanospheres occurred immediately upon mixing.

Table.-1: Formulation

Ingredients	**Formulation code**								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Lacidipine (mg)	3	3	3	3	3	3	3	3	3
HPMC K4M (mg)	75	150	225	-	-	-	75	150	225
HPMC K 100 (mg)	-	-	-	75	150	225	75	150	225
Ethyl cellulose(mg)	75	150	225	75	150	225	-	-	-
Dichloromethane(ml)	10	10	10	10	10	10	10	10	10
Methanol (ml)	10	10	10	10	10	10	10	10	10
2% SLS (ml)	50	50	50	50	50	50	50	50	50

Evaluation of Nanospheres:

1. Assay

It's crucial to weigh the 3 mg of nanocrystalline lactidipine manufactured accurately. Afterwards, it needs to be dissolved in 40 mL of methanol and titrated with 0.1 mol/L sodium hydroxide VS (potentiometric titration, Endpoint Detection Method in Titrimetry). Each milliliter of the solution contains 35.419 milligrams of $C_{16}H_{13}Cl_2NO_4$ at 0.1 mol/L VS sodium hydroxide. When dried, lactidipine has a concentration of99.0% to 101.0%.

2. Modified Dissolution Test:

The 25mL nanoparticle solution solution and a beaker containing 100mL of a 1% sodium lauryl sulphate (SLS) solution in distilled water were used for the in vitro dissolving investigations. The studies lasted the entire day. A thermostatically regulated water bath with a temperature of 37 0.05 °C was used to maintain the dissolving medium. The basket's spinning speed was set to 50 rpm. 3 ml samples were taken at regular intervals, and the drug release was calculated spectrophotometrically at 275 nm. To keep the sink condition, 3 ml of new matching media were added each time a sample was removed from the dissolution flask.[13]

3. FT-IR Analysis:

To discover any potential interactions between medications and the polymers or excipients, IR spectral matching investigations are used. In the present, FT-IR was used to assess the medicine Lacidipine's compatibility with various polymers (PERKIN ELMER FT-I Insf. USA). The samples were scanned using an FT-IR spectrophotometer with a range of 4000 to 400 cm-1. In a similar manner, the IR spectra of each distinct drug and produced nanocrystal were recorded. To look for any potential physical and chemical interactions, the materials' exterior features and the existence or lack of peaks in the spectra were examined.[13]

SEM, or Scanning Electron Microscopy

The particle morphology of both drug nanospheres that had not yet been treated and those that had was investigated using scanning electron microscopy. Each drug powder sample was divided into little bits and attached to a piece of double-sided carbon conductive tape. A Pt-Pd alloy coating with a thickness of 5 nm was then applied to cover the whole surface of the tape. A Zeiss DSM 982 Field Emission Gun Scanning Electron Microscope was used to take the micrographs. (Carl Zeiss AG, Germany).[13,14]

5. Particle size distribution(Size distribution of the particles):

Immediately following precipitation, the size of drug nanospheres was measured using dynamic laser light scattering (Nanoparticle size analyzer, Malvern). The drug solution was diluted with purified water to a concentration of 0.2 mg/ml before analysis. The findings of the particle size study were interpreted using the graphic mean size (Mz) and computed surface area.[13,15,16]

6. Measurement with Differential Scanning Calorimetry (DSC):

Using DSC-41 equipment, the thermal characteristics of the lyophilized powder samples were examined (Shimadzu, Japan). Each lyophilized powder sample had its scanning temperature adjusted between 25 and 200 ℃ at a heating rate of 10 ℃/min.
In an open aluminum pan, 10 mg of each sample were examined, with magnesiumacting as the control. Thermal analysis was done on Lacidipine and the excipients toassess the internal structure alterations brought on by the nanosizing process.[17] **Zeta potential**

A zeta sizer was used to assess the nanospheres' size, size distribution, and zeta potential (ZS 90 malvrn). The lyophilized substances were diluted with PBS to a pH of 6.0 on mg/ml and 67 mm prior to testing. These samples were stored in a separate, clean cubet throughout the size analysis method in order to obtain several peaks and then compute its average zeta size. Surface charge potential (also known as zeta potential) samples were kept in the zeta sizer analysis chamber while it was operating in order to collect zeta potential data. Usually, the monodisperse feature of this data is taken into account rather than the polydisperse feature.[13,14]

8.8 Drug entrapment efficiency (EE%) and Drug loading (DL%) :

Nanospheres are centrifugation, washing, re-centrifugation and subsequent filtration, an aliquot from the supernatant is taken and diluted. The free drug can be estimated from the filtrate using UV-Visible spectrophotometer. Amount of entrapped drug was calculated by subtracting the amount free drug from the total amount of drug added in the formulation. [18]
Calculating the formula:-

$$\text{Drug entrapment efficiency (W/W\%)} = \frac{\text{Amount of entrapped drug} \times 100}{\text{Total amount of the drug added}}$$

$$\text{Drug loading (W/W)\%} = \frac{\text{Amount of entrapped drug} \times 100}{\text{Amount polymer} - \text{entrapped drug}}$$

RESULTS AND DISCUSSION:

Preformulation studies

Characterization of Pharmaceutical Active Ingredients was carried out.

API characterization (appearance, identification test by FTIR, and assay) was carried out as part of preformulation research, and it was discovered that all of the results are within pharmacopoeial parameters.

Table 2: defining the active medicinal component

Description	Specifications	Observations
Appearance	White Crystalline powder	White
Identification	FTIR	Complies
Assay	Not less than 99.0% w/w and not more than 101.0% w/w of Carvedilol	99.97%w/w

Standard curve for lacedipine in 0.1% SLS solution

The standard graph of lacidipine was produced with 0.1% SLS. Concentrations between 2 and 10 g/ml were created. After calibrating with a blank sample, the resulting concentrations' absorbance was measured at 301 nm. In order to describe the data, a graph showing the concentration and absorbance was created along with the best fit line, regression value, and equation.

Concentration (µg/ml)	Absorbance
0	0
4	0.2
8	0.39
12	0.55
16	0.72
20	0.89

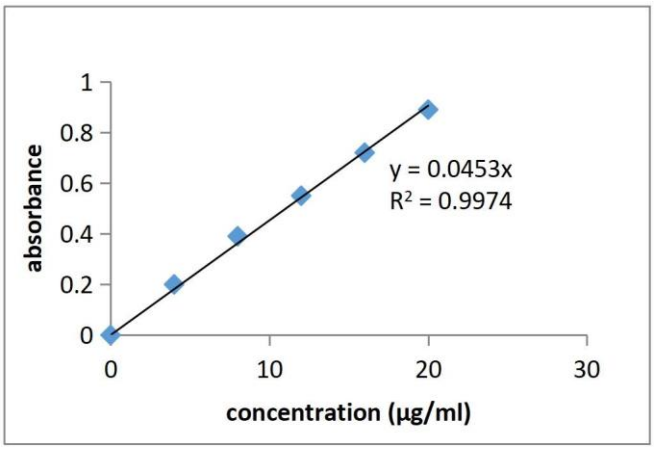


Fig-3: Caliberation curve of lacidapine

Table 4 : Evaluation Particle size of nanospheres determine by Malver sizer

Formulation	Particle size	Percentage yeild	Entrapment efficacy	Drug content
F1	200.5	98.5	77.8	298.5
F2	210.2	80.7	87.5	297.8
F3	246.7	79.5	97.6	298.2
F4	198.2	96.2	75.2	298.0
F5	205.3	87.5	80.2	298.2
F6	226.7	79.8	91.8	297.4
F7	197.2	98.8	77.4	298.4
F8	220.2	84.2	83.4	296.3
F9	245.3	75.8	95.2	295.5

Fig.4: particle size Zeta sizer is used to determine zeta potential

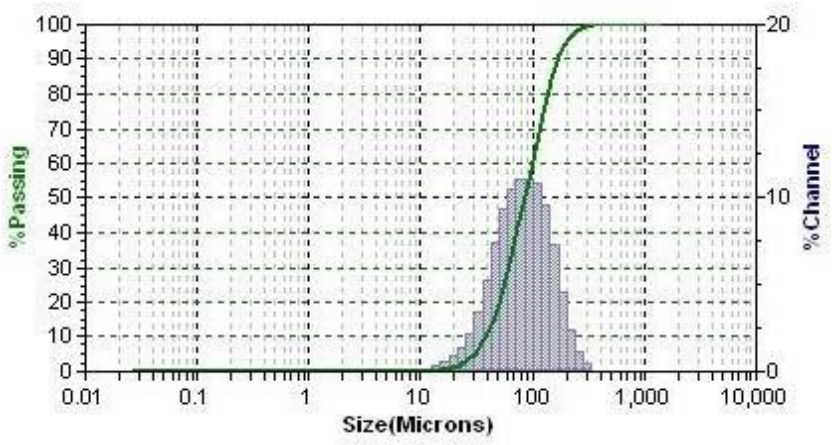
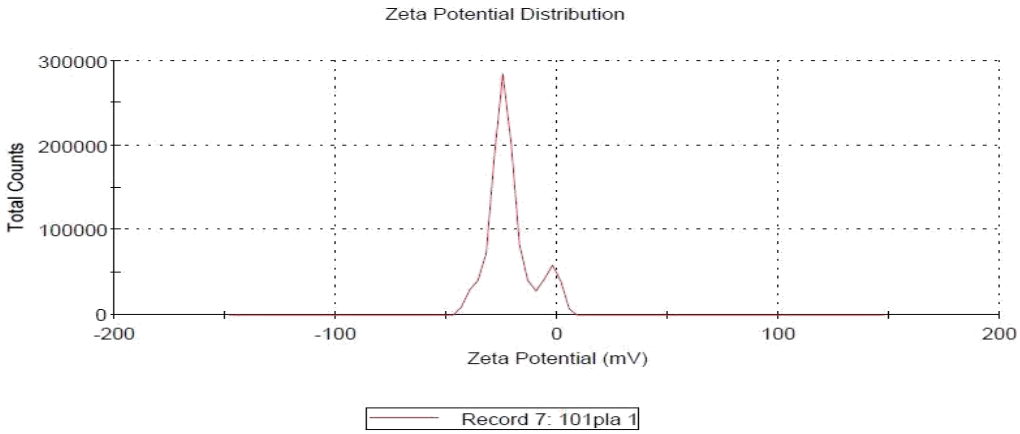


Fig-5: zeta potential



Percentage yield

Calculate the percentage yield using formula

$$\textbf{Percentage yield} = \frac{\text{Actual yield}}{\text{Theoretical yield}} \times 100$$

Drug entrapment efficiency (EE%) and Drug loading (DL%) : Nanospheres are centrifugation, washing, re-centrifugation and subsequent filtration,an aliquot from the supernatant is taken and diluted. The free drug can be estimatedfrom the filtrate using UV-Visible spectrophotometer. Amount of entrapped drug wascalculated by subtracting the amount free drug from the total amount of drug added inthe formulation.[34]

Calculating the formula:-

$$\text{Drug entrapment efficiency (W/W\%)} = \frac{\text{Amount of entrapped drug} \times 100}{\text{Total amount of the drug added}}$$

$$\text{Drug content} \quad \text{Drug loading (W/W)\%} = \frac{\text{Amount of entrapped drug} \times 100}{\text{Amount polymer} - \text{entrapped drug}}$$

In vitro dissolution:

Using a modified dissolving method apparatus and a solvent solution containing 0.1% SLS, in vitro dissolve tests are carried out on prepared nanospheres. It was demonstrated that the dissolving rate increased linearly with polymer concentration. The best formulations were (F9), which over the course of 24 hours recorded 98.9%of the medication

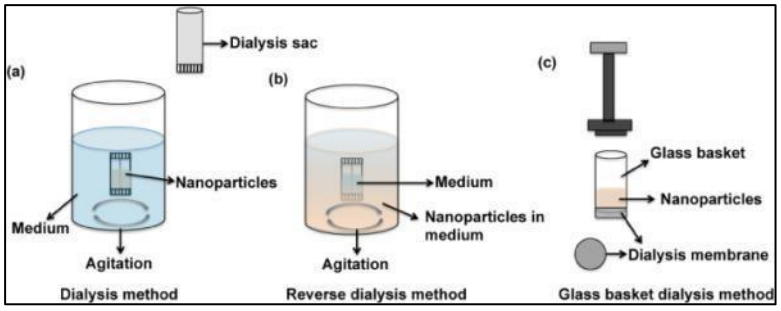


Table 5 : In-Vitro drug release:

Time (hr)	% drug release								
	Formulation code								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	15.2	12.5	10.8	20.8	17.8	15.2	25.4	22.8	9.5
2	38.9	25.9	21.6	28.9	25.9	21.8	38.9	30.2	17.8
4	46.4	34.5	30.8	35.4	31.8	29.6	46.2	42.7	28.3
6	54.8	46.4	42.7	48.9	43.6	40.9	54.8	51.8	39.4
8	68.9	58.5	55.8	56.1	50.7	49.4	61.7	59.7	47.5
10	82.5	67.5	63.7	69.8	59.8	56.8	76.8	71.5	54.5
12	96.7	85.4	81.6	84.7	76.8	71.2	89.5	85.3	67.6
14	-	94.8	90.8	96.8	87.8	83.5	97.6	94.5	79.7
16	-	-	96.2	-	95.5	93.7	-	97.8	87.2
20	-	-	-	-	-	-	-	-	98.9

Fig-6: PercentaFig-6: of Drug Release

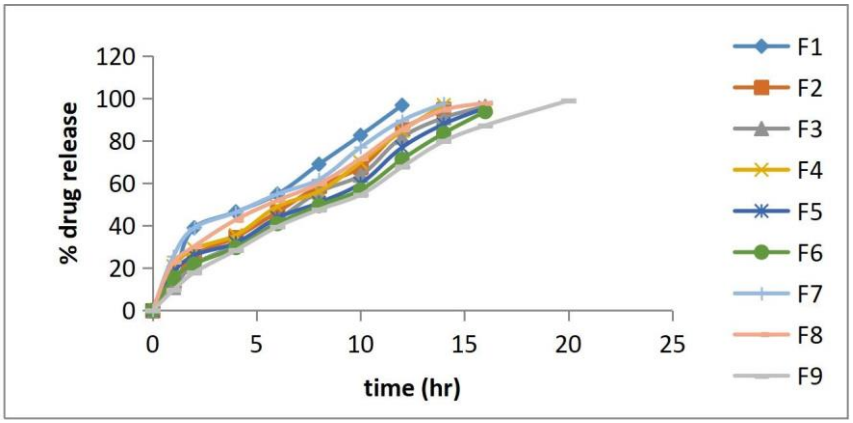


Fig-7: FT IR spectra of Lacidipine

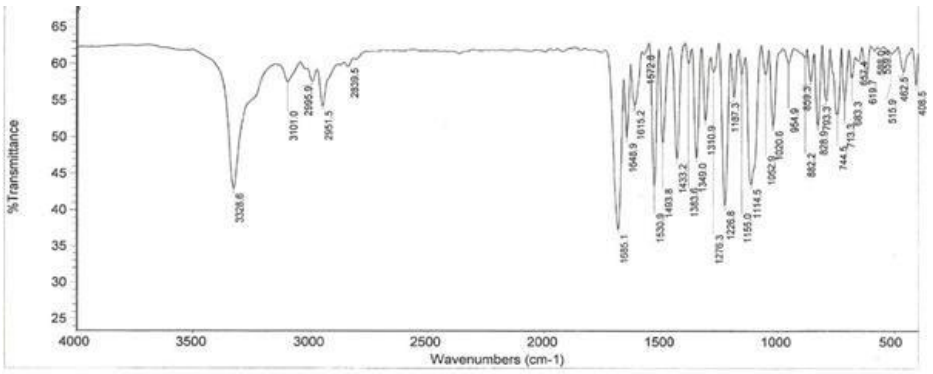


Fig-8: FTIR Spectra For Lacidipine and Excipients:

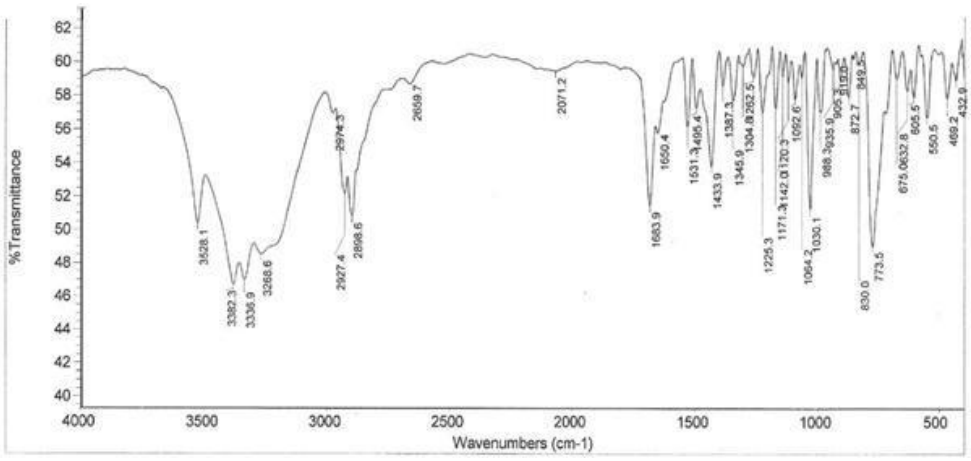
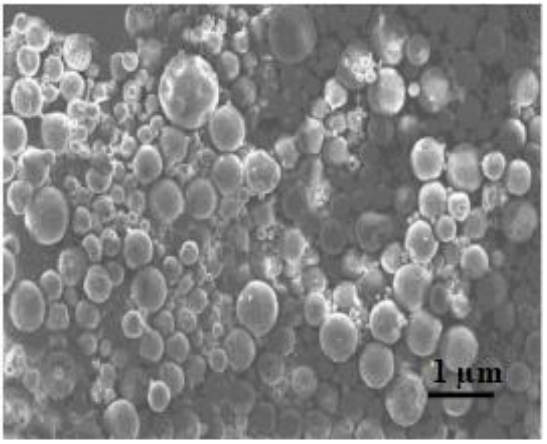


Table-6: lacidipine excipients

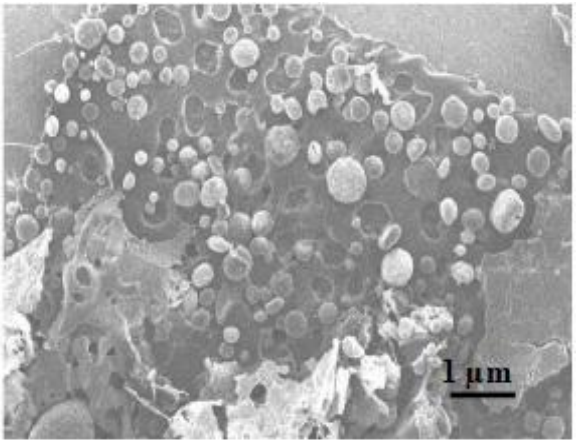
Transition	IR Range (cm-1)	Absorption wave number of pure drug	Best formulation Absorption wave number
Carboxylic acid	3400-2400 cm^{-1}	2939.04 cm^{-1}	2953.35 cm^{-1}
Alcoxy (O-C)	1300-1000 cm^{-1}	1274.60 cm^{-1}	1275.36 cm^{-1}
Benzene	1500-1400 cm^{-1}	1474.71 cm^{-1}	1473.76 cm^{-1}
Amine (C-NH)	1640-1560 cm^{-1}	1613.18 cm^{-1}	1619.65 cm^{-1}

Morphology

Using a scanning electron microscope, these Lacidipine nano particles were formed into spheres (SEM). The particles have rounded and irregular surfaces. According to reports, as the ratio of the polymer grew, so did the tendency for the relative widths of the pores to grow. (Nayak et al., 2009).



Surface structure of Lacidipinewith GMS(80mg)



Surface structure of Lacidipinewith GMS(100mg)

KINETIC ANALYSIS OF DATA FROM DISSOLUTION:

The in-vitro release data was fitted into a variety of release equations and kinetic models, including the zero order, first order, Higuchi, and Korsmeyer Peppasmodels,to analyze the drug release mechanism. The release kinetics of the improved formulation are displayed in table.

Table-7:kinetic analysis of data from dissolution

Formulation code	Zero order	First order	Higuchi	Peppas	
	R2	R2	R2	R2	n
F9	0.99	0.8	0.96	0.99	0.8

Fig-7: First Order

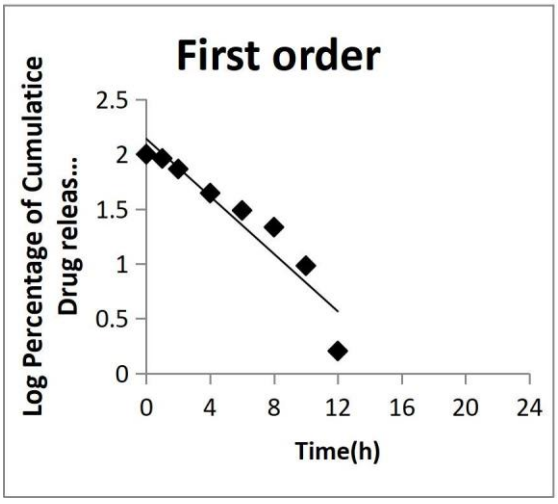


Fig-8: Zero order

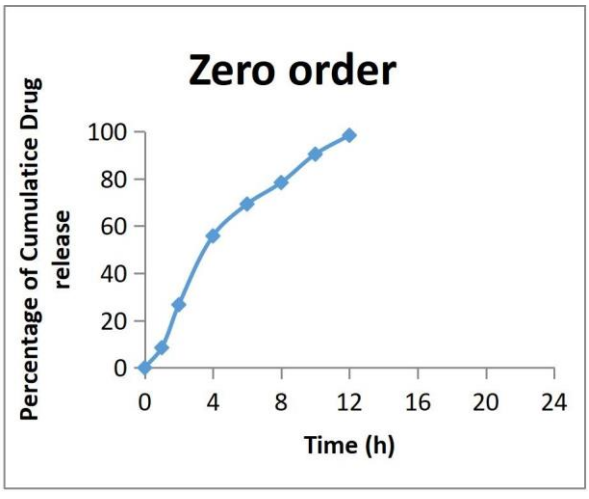
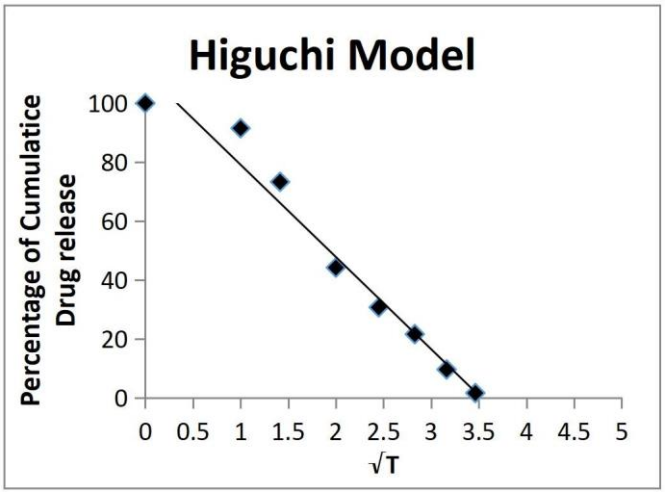


Fig-8: Higuchi Model



Stability Studies

The physical or chemical features of the formulation F-9 pill were unchanged after three months. The parameters that were measured at various intervals were shown.

Table 8 Findings from stability tests on the improved formulation F-9

S.NO	Parameters	Initial	1 month	2 month	3 month	Limits as per specification
1	40^0C/75% RH % Release	98.9	98.52	97.79	96.56	Not less than 85 %
2	40^0C/75% RH Assay Value	98.9	97.96	96.22	96.00	Not less than90 % Not more than 110 %

Discussion:

The objective of the current effort was to fabricate lipid nanospheres that were lacidipine-solid.

Solid lipid nanospheres:

Different polymers were used to create additives like GMS, Chitosan, PEG6000 SLN, and others. The solvent evaporation method was used to prepare the nanospheres. In total, nine formulations were developed and evaluated.

Particle Size Analysis:

The presence of the stabiliser affects particle size, according to the particle size inquiry for the lacidipine fabricated nanospheres utilizing different polymers. The results of the particle size inquiry were analyzed using the graphic mean (Mz) and approximated surface area (Cs). Graphic Mean delivers a different and perhaps better control value because it considers both tiny and large particles, even though it includes the median value. Therefore, the mean diameter of the volume distribution is less coarse-particle weighted than the mean particle size. When GMS (F3) was applied at 10%, smaller graphic mean (Mz) values suggesting smaller particles were discovered. The maximum Mz value of the formulation F7, which was determined to be 275 nm, indicates larger particles. It has been demonstrated that polymer concentration affects particle size. The particle size was reduced when the concentration of the majority of the examined polymers was raised from 6 to 10%.

In vitro dissolution:

Using a modified dissolving method apparatus and a solvent solution containing 0.1% SLS, in vitro dissolve tests are carried out on prepared nanospheres. It was demonstrated that the dissolving rate increased linearly with polymer concentration. The best formulations were (F9), which over the course of 24 hours recorded 98.9%of the medication.

Drug Release Kinetics:

In order to determine the mechanism of drug release, in vitro drug release data from all Sustained formulations were submitted to a goodness of fit test by linear regression analysis in accordance with zero order and first order kinetic equations, Higuchi's, and Korsmeyer-models. Peppa's The data above ('r' values between 0.900 and 0.965) demonstrate that all of the formulations had first order release kinetics. According to Higuchi and Peppas' research, the medication is delivered using a non- fickian diffusion technique (n0.5). The kinetic data of the factorial formulations demonstrate zero order kinetic drug release in the F9 formulation. The r values for Higuchi's formulation equation. This information reveals that the Higuchi model of non-Fickian diffusion controls drug release.

Conclusion:

Lacidipine is a antihypertensive drug having low solubility because of highly lipophilic nature.Hence lacidipine nanosphere preparation by using different polymers and surfactants. Finally, I am succeeded in increasing the solubility and bioavailability of the drug by the preparation of the lacidipine nanospheres.

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