FORMULATION AND EVALUATION OF IRBESARTAN LIPOSPHERES

Dr. C. Aparna*, Rithika Chilukuri

Sri Venkateshwara college of pharmacy, 86, Hitech City Rd, Vittal Rao Nagar, Madhapur, Hyderabad, Telangana 500081, India.

ABSTRACT:

The objective of the present study was to formulate and evaluate irbesartan-loaded lipospheres to enhance its solubility and achieve sustained drug release. Irbesartan, a BCS Class II drug with poor aqueous solubility, was incorporated into lipid-based carriers using both the melt dispersion and solvent evaporation techniques. The solvent evaporation technique produced uniform, stable lipospheres with better structural integrity.

Preliminary solubility screening identified Caproyl 90, glyceryl monostearate (GMS), and Compritol 888 ATO as suitable lipid excipients. FTIR analysis confirmed the absence of drug–excipient interactions, indicating compatibility of the formulation components. The prepared formulations were evaluated for solubility enhancement, drug content, morphological characteristics, and *in vitro* release behavior. Formulation LS9 containing GMS and Compritol 888 ATO exhibited the highest solubility enhancement compared to the pure drug. SEM and optical microscopy images revealed spherical, well-dispersed particles with porous surfaces. *In vitro* release results of lipospheres demonstrated sustained release of irbesartan over 24 hours, with the GMS and Compritol 888 ATO formulation LS9 achieving the highest cumulative drug release of 96%. Overall, the study confirms that liposphere-based systems can effectively enhance the solubility and provide sustained release of irbesartan.

Keywords: Lipospheres, Irbesartan, Solubility, Sustain release

INTRODUCTION:

Irbesartan is a highly potent, long-acting angiotensin II receptor blocker (ARB) that acts non-competitively and exhibits selective affinity for the AT_1 receptor subtype[1]. It is widely used for the treatment of hypertension and diabetic nephropathy. It is classified as a BCS Class II drug, exhibiting low aqueous solubility and high permeability, which makes it a suitable candidate for lipid-based delivery systems to enhance its dissolution and absorption.

Lipid-based drug delivery systems (LBDDS) have emerged as a promising approach for enhancing the solubility, bioavailability, and therapeutic efficacy of poorly water-soluble drugs. In general, lipid-based drug delivery systems are preferred as carriers because they offer good stability, can hold a high amount of drug, allow incorporation of both lipophilic and hydrophilic compounds, and support multiple routes of administration, such as oral, topical, parenteral, and pulmonary. Lipid-based formulations improve the solubility of poorly water-soluble drugs by several mechanisms. These systems utilize physiological lipids, such as triglycerides, fatty acids, phospholipids, and cholesterol, to encapsulate the drug and promote its absorption through the lymphatic route. Lipid-based drug delivery systems are generally divided into emulsion-based systems, Vesicular systems, and lipid particulate systems.[2]

Eldem and co-workers were the first to introduce the concept of using lipid microparticles. Later, Domb and Maniar described lipospheres as highly scattering, spherical, lipid-based particles that are dispersible in water. These solid particles, typically ranging from 0.01 to 100 μm in diameter, contain a hydrophobic lipid core in which the drug is either dissolved or uniformly dispersed within a solid fat matrix. Lipospheres function as a single-unit system, providing uniform drug distribution and consistent absorption throughout the gastrointestinal tract.[3] In addition, lipospheres offer formulation-related benefits, including simple preparation methods, easy scalability, and the use of cost-effective excipients. They demonstrate strong physical stability, can accommodate a high load of hydrophobic drugs, and disperse readily in water even when carrying lipophilic compounds. In addition, the lipid matrix restricts internal drug movement, supports sustained and extended release, and provides excellent biocompatibility, making them highly suitable for pharmaceutical use[4]. Lipospheres can be prepared by the melt dispersion technique, the Solvent evaporation technique, the Water-in-oil-in-water double emulsion (w/o/w) method, the Multiple microemulsion technique, the Sonication method, the Microfluidizer method, and the Solvent extraction method[5,6].

The research work aims to formulate and evaluate the irbesartan lipospheres to enhance solubility and sustain the release of the drug.

LITERATURE REVIEW:

Charan Singh, et al (2015) developed rifampicin loaded phospholipid lipospheres containing sulfphobutyl ether β-cyclodextrin and Vitamin C for inhalation to test their potential for deep lung delivery. The findings of the solid-state characterization revealed the amorphous nature of the lipospheres. These exhibited a better flowability, an aerodynamic diameter in the range of 1.76 to 3.99 μm. Moreover, the fine particle fraction and emitted dose was found in the range of 68.84–83.73% and 80–93%, respectively. Moreover, lipospheres exhibited enhanced/equivalent efficacy in vitro in H_{37}Rv strain. Hence, the results show the potential of lipospheres for pulmonary delivery of rifampicin.

Mumuni Audu Momoh*, Charles Okechukwu Esimone (2012) formulated gentamicin liposphere by solvent-melting method using lipids and polyethylene glycol 4000 (PEG-4000) for oral administration. Gentamicin lipospheres were prepared by melt-emulsification using 30% w/w Phospholipon® 90H in Beeswax as the lipid matrix containing PEG-4 000. These lipospheres were characterized by evaluating encapsulation efficiency, loading capacity, change in pH, and the release profile. Antimicrobial activities were evaluated against Escherichia coli, Pseudomonas aeruginosa, Salmonella paratyphi, and Staphylococcus aureus using the agar diffusion method. Photomicrographs revealed spherical particles within a micrometer range with minimal growth after 1 month. The release of gentamicin in vitro varied widely with the PEG-4,000 contents. Moreover, a significant ($P>0.05$) amount of gentamicin was released in vivo from the formulation. The encapsulation and loading capacity were all high, indicating the ability of the lipids to take up the drug. The antimicrobial activities were very high especially against Pseudomonas, compared to other test organisms. This strongly suggested that the formulation retain its bioactive characteristics. This study strongly suggests that the issue of gentamicin stability and poor absorption in oral formulation could be adequately addressed by tactical engineering of lipid drug delivery systems such as lipospheres.

Siriporn Toongsuwan, et al (2004) prepared Bupivacaine lipospheres as a parenteral sustained-release system for post-operative pain management. Bupivacaine free base was incorporated into micron-sized triglyceride solid particles coated with phospholipids, which were formed via a hot emulsification and cold resolidification process. The bupivacaine liposphere dispersions were characterized with respect to drug loading, particle-size

distribution, and morphology. Gelation of the fluid liposphere dispersions was observed at different time intervals upon storage. The type of phospholipids used in the formulation was found to have a major impact on the gelation of the dispersion. The use of synthetic phospholipids instead of the natural phospholipids in the formulation yielded bupivacaine liposphere dispersions exhibiting prolonged gelation time. The addition of a hydrophilic cellulosic polymer can further improve the physical stability of the dispersion.

CONCLUSION FROM LITERATURE REVIEW:

- Lipospheres are a drug delivery system designed to improve the therapeutic performance of poorly water-soluble drugs.
- Various studies have successfully employed liposphere-based formulations to enhance the solubility, bioavailability, and sustain the release of drugs.
- Irbesartan is a BCS Class II drug, which has low solubility and high permeability.
- The objective of the study was to formulate irbesartan lipospheres to enhance solubility and sustain the release of the drug.

MATERIALS AND METHODS:

Materials:

Irbesartan was obtained as a gift sample from CTX Lifesciences Pvt. Ltd., Gujarat. Compritol 888 ATO and Caproyl 90 was obtained as a gift sample from Gattefossé India Pvt. Ltd., Mumbai. Glyceryl Monostearate was purchased from Virat Laboratories, Hyderabad. Stearic acid, Cetyl alcohol, and polyvinyl alcohol were procured from SD Fine Chem Limited, Hyderabad. Tween 80 was purchased from the Sisco Research Laboratories Pvt. Ltd, Maharashtra.

Methodology:

Preformulation studies:

Melting point:

The melting point of irbesartan was determined by the Capillary tube method[7], and the observed value was compared to the value reported in the literature.

Solubility study of irbesartan:

The solubility of irbesartan was tested in various lipids, including glyceryl monostearate, Capryol 90, Compritol 888 ATO, beeswax, oleic acid, Cetyl alcohol, stearic acid, Gelucire 44/14, and Labrafac WL 1349. The small amount of each lipid is weighed and then melted. A certain amount of Irbesartan was then added to the molten lipid, and the solubility was determined by visually checking whether any undissolved drug remained[8].

Fourier-transform infrared (FTIR) spectroscopy:

FTIR analysis of Irbesartan and its lipospheres was carried out using an Agilent Cary 630 FTIR spectrophotometer with an ATR accessory. The pure drug and a small amount of the liposphere formulation were placed on the ATR crystal, and spectra were recorded from 4000–650 cm^{-1} to check for characteristic peaks and possible drug–excipient interactions. The crystal was cleaned after each run[9].

Analytical method development of irbesartan:

Determination of λmax in methanol and 0.1 N HCl:

10 µg/ml solution of irbesartan was prepared in methanol and 0.1N HCl. The absorbance of this solution was measured using a UV spectrophotometer in the range of 200- 400nm.

The blank solution of methanol and 0.1 N HCl was also scanned between 200 and 400 nm. The peaks obtained from the blank and drug solution were compared simultaneously for absorbance value to check for any analytical interference.

Calibration curve construction in methanol and 0.1N HCl:

Dilutions of irbesartan were prepared in methanol and 0.1 N hydrochloric acid. The absorbance of these solutions was measured using UV spectrophotometer at wavelengths of 217 nm and 207 nm respectively. A standard calibration curve was constructed by plotting absorbance values on the Y-axis and concentrations on the X-axis.

METHOD OF PREPARATION:

Melt dispersion method:

At 70–72°C, the lipids were melted on a water bath, and the drug, in finely powdered form, was incorporated into the molten lipid. Separately, the aqueous phase containing water, surfactant, and stabilizer was heated slightly above73^{o}C. The molten lipid mixture was

gradually added to the hot aqueous phase to create an o/w (oil-in-water) emulsion, and the mixture was continuously sonicated to achieve uniform emulsification. The resulting milky dispersion was rapidly cooled to 20°C in an ice bath with constant stirring, producing a homogeneous liposphere dispersion. The lipospheres were subsequently washed with water and collected by filtration[10].

Table 1: Formulation of Lipospheres by Melt Dispersion Method

Ingredients	LS1	LS2	LS3	LS4	LS5	LS6
Drug(mg)	150	150	150	150	150	150
Glyceryl monostearate(g)	-	-	1.2	1.5	1.5	2
Cetyl alcohol(g)	0.3	1.2	-	-	-	-
Stearic acid(g)	0.2	-	-	-	-	-
Poly vinyl alcohol(g)	0.2	0.2	0.2	0.4	0.4	0.4
Caproyl 90(ml)	-	3	3	3	3	4
Tween 80 (ml)	0.4	0.4	1	1	1.5	1
Water(ml)	20	20	20	40	40	40

Solvent Evaporation Method:

Irbesartan (150 mg) was dissolved in dichloromethane with glyceryl monostearate and Compritol 888 ATO to create the organic phase. The water phase, containing polyvinyl alcohol and Tween 80, was heated to 75 °C to get a clear solution. The aqueous phase was continuously stirred while the organic phase was added dropwise using a syringe to create an emulsion of oil and water (O/W), which was sonicated for 45 minutes. The emulsion was poured into cold water to solidify the lipids, and the resulting lipospheres were recrystallized, filtered, washed, and dried for further evaluation[11].

Table 2: Formulation of Lipospheres by the Solvent Evaporation Method

Ingredients	LS1	LS2	LS3	LS4	LS5	LS6	LS7	LS8
Irbesartan(mg)	150	150	150	150	150	150	150	150
Glyceryl monostearate(g)	1	1	1.5	1.5	1.5	1.5	2	2
PVA(g)	-	0.5	1	1.5	1	1	1	1.5
Tween 80(ml)	1	1	1	1	1	1	1	1
Caproyl 90(ml)	-	-	-	-	2.5	3	3	3
Dichloromethane(ml)	15	15	15	15	10	12	15	15
Water(ml)	50	50	50	50	50	50	50	50

Ingredients	LS9	LS10	LS11	LS12	LS13	LS14
Irbesartan(mg)	150	150	150	150	150	150
Glyceryl monostearate(g)	2	1	1.5	0.5	-	-
Compritol 888 ATO (g)	0.5	1	0.5	1.5	1.5	2
PVA(g)	1	1	1	1	1	1
Tween 80(ml)	1	1	1	1	1	1
Caproyl 90(ml)	3	3.5	3.5	3.5	3	3.5
Dichloromethane(ml)	15	10	10	10	15	15
Water(ml)	50	50	50	50	50	50

EVALUATION OF LIPOSPHERES:

Solubility study of lipospheres in 0.1 N HCl:

Solubility was determined using the shake flask method.

Weighed amounts of pure drug and each formulation were dissolved in 10 mL of 0.1 N HCl. Samples were kept on a rotary shaker for 24 hours to reach equilibrium. After 24 hrs. samples were filtered, diluted, and analyzed using a UV spectrophotometer at 207 nm.

Drug content:

Accurately weighed 10 mg of lipospheres were dissolved in 10 ml of methanol. The dispersion

was sonicated for 10–15 minutes to ensure complete dissolution and then filtered through a 0.45 µm filter. The solution was measured at 217 nm using a UV spectrophotometer [12].

Drug Content (%) = Practical content / Theoretical content ×100

***In Vitro* Drug Release Study of Lipospheres:**

The drug release studies of irbesartan lipospheres were performed using USP type II Paddle apparatus. The lipospheres were filled in the capsule and placed in 900 ml of 0.1 N HCl medium maintained at 37 ± 0.5 ºC. The apparatus was rotated at a constant speed of 100 rpm. At regular intervals, 5 ml of the sample is withdrawn, and fresh buffer was added to maintain sink conditions. The collected samples were analyzed by a UV Spectrophotometer at 207 nm after calibration with the respective blank[13].

CHARACTERIZATION OF IRBESARTAN LIPOSPHERES:

Optical Microscopy:

The prepared lipospheres were observed under an optical microscope to study their shape. A small amount of sample was placed on a glass slide, covered with a cover slip, and examined under 10x magnification. The images were captured using a digital camera [14].

Morphology of lipospheres:

Morphological analysis of lipospheres was performed using a Hitachi S-3800N® Scanning Electron Microscope (SEM). A small quantity of the liposphere dispersion was placed on a glass-covered stub and allowed to air dry. After drying, a thin layer of gold was applied to the sample using sodium aurothiomalate, then examined under SEM at 10,000× magnification.

RESULTS AND DISCUSSION:

Preformulation studies:

Melting point:

The melting point was determined by the capillary tube method to confirm the identity and purity of the drug sample. The observed melting point of the sample is close to the reported value, confirming the drug’s purity and identity.

Table 3: Melting point of irbesartan

Melting point	
Reference value	**Experimental value**
180 - 181°C	180°C

Solubility study of irbesartan:

The solubility was seen visually in various lipids. It was more soluble in Capryol 90 compared to Glyceryl monostearate and Compritol 888 ATO.

Fourier-transform infrared (FTIR) spectroscopy:

The FTIR study was conducted to evaluate the interaction between the drug and excipients. FTIR was performed for both the drug as well as the formulation. Table 4 indicates the peaks of the drug and formulation.

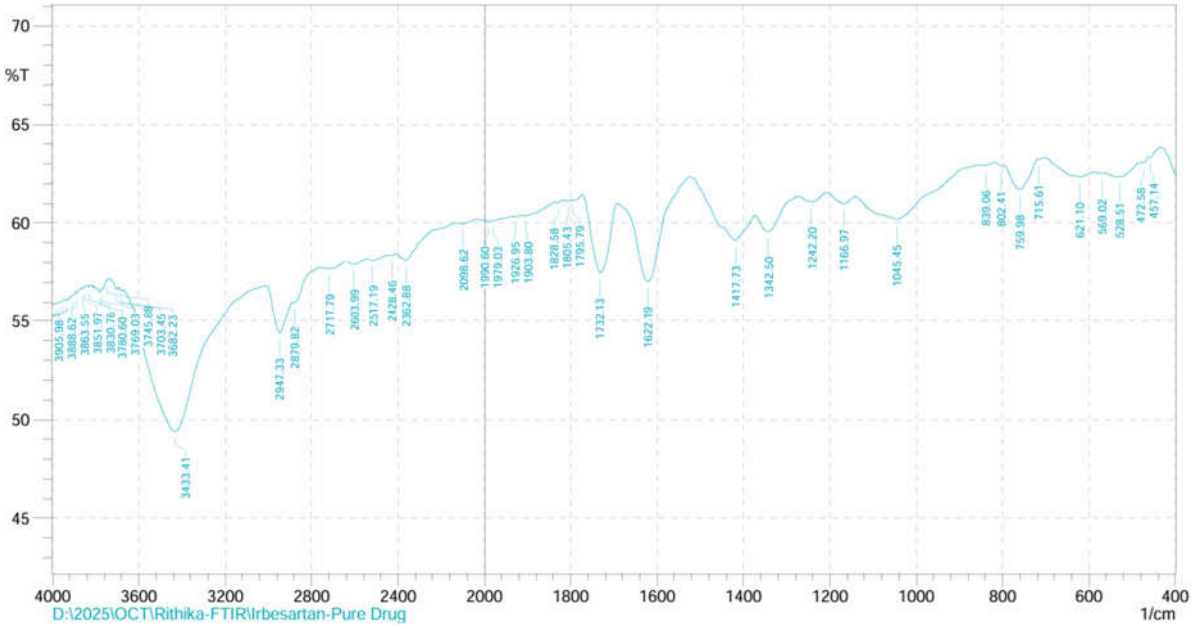

Fig 1: FTIR Spectrum of Irbesartan

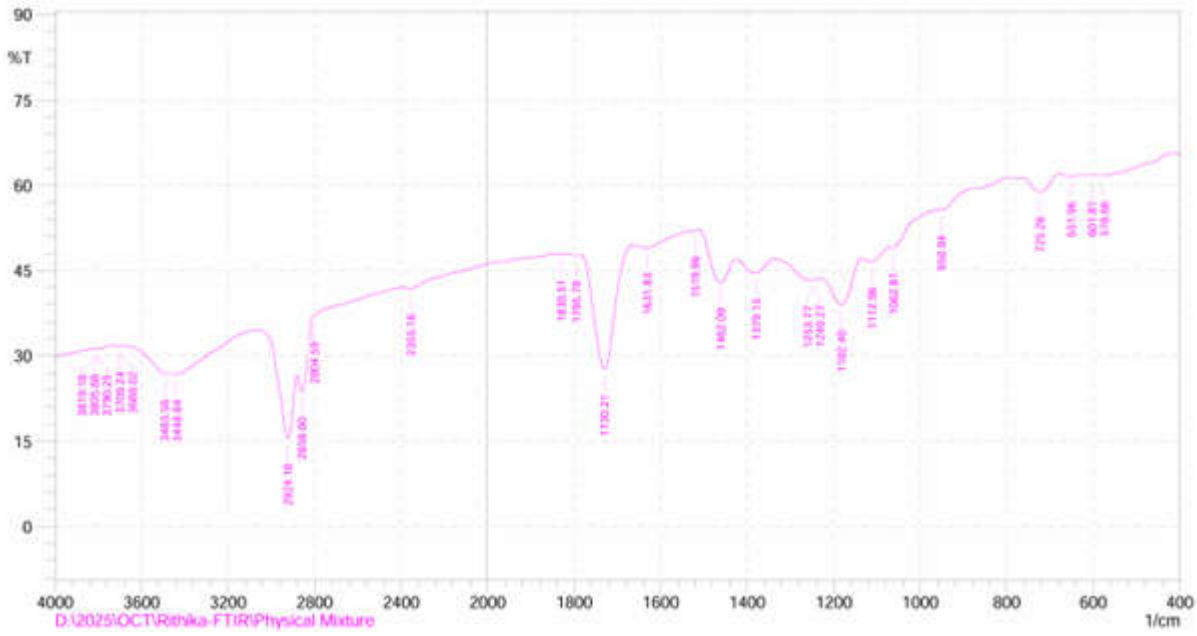

Fig 2: FTIR Spectrum of Irbesartan Lipospheres

Table 4: Wave number measurements of drug and lipospheres

Range	Functional groups	Wavenumber (cm^{-1}) values of Irbesartan drug	Wavenumber (cm^{-1}) values of Lipospheres
3000 – 2880 cm^{-1}	N-H	2947.33	2924.18
1740 – 1720 cm^{-1}	C=O	1732.13	1730.21
1420 – 1330 cm^{-1}	O-H Bending	1342.50	1379.15
2870 – 3000 cm^{-1}	Symmetric C-H	2947.33	2924.18
1600 – 1680 cm^{-1}	C=C	1622.19	1631.83
1600 – 1400 cm^{-1}	Aliphatic C=C	1417.23	1462.09

As shown in Table 4, there is no change in the position of the peaks, indicating the absence of intermolecular interaction. There is no appearance of new peaks or disappearance of the present peaks.

Analytical method development of irbesartan:

The absorption maximum λ_{max} of Irbesartan was determined to identify the wavelength with the highest absorbance for precise and reliable analysis.

Determination of λmax in methanol:

10 µg/ml solution of irbesartan was prepared and scanned in the 200–400 nm range using a UV spectrophotometer. Irbesartan showed maximum absorbance at 217 nm. To check for any analytical interference, the blank was also scanned and compared with the irbesartan solution.

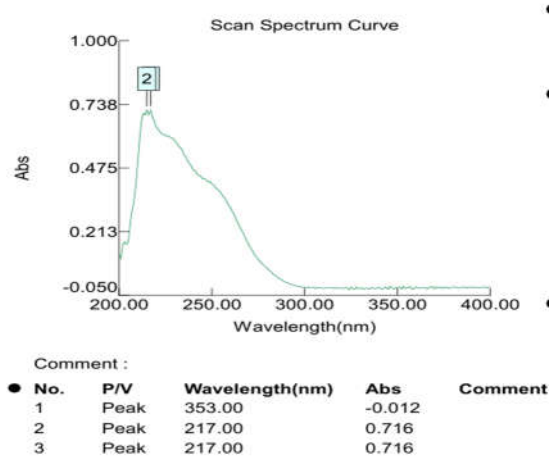

Fig 3(a): λmax of irbesartan in methanol

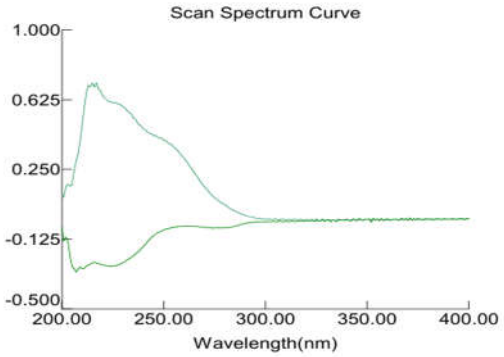

Fig 3(b): Peak comparison between drug & blank

Determination of λmax in 0.1 N HCl:

10 µg/ml solution of irbesartan was prepared and scanned in the 200–400 nm range using a UV spectrophotometer. Irbesartan showed maximum absorbance at 217 nm. To check for any analytical interference, the blank was also scanned and compared with the irbesartan solution.

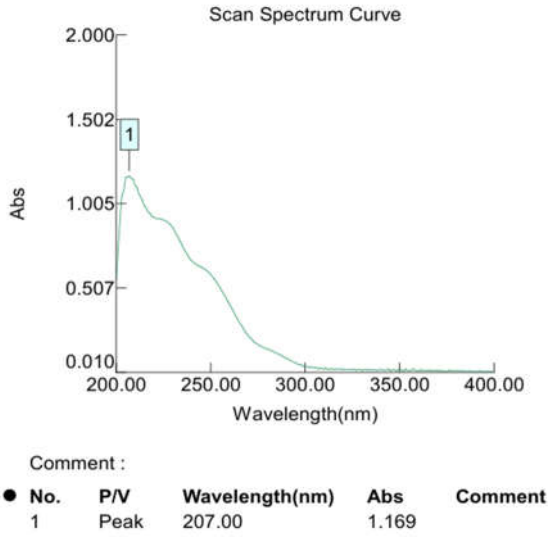

Fig 4(a): λmax of irbesartan in 0.1 N HCl

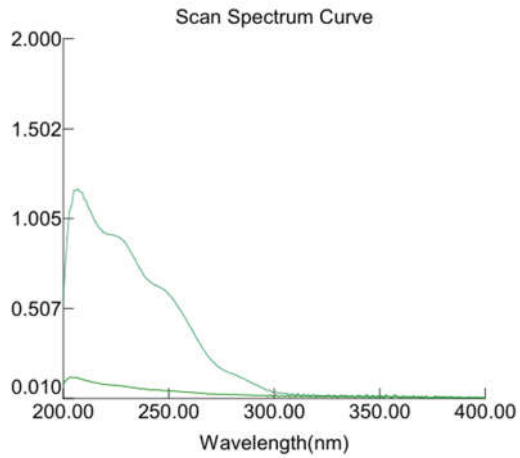

Fig 4(b): Peak comparison between drug and blank

Calibration curve construction in methanol and 0.1N HCl:

Standard stock solutions of irbesartan were prepared in methanol (2–16 µg/ml) and in 0.1 N HCl (2–10 µg/ml). The absorbance values were recorded using a UV–visible spectrophotometer at 217 nm and 207 nm. The corresponding calibration curve is presented in the figures.

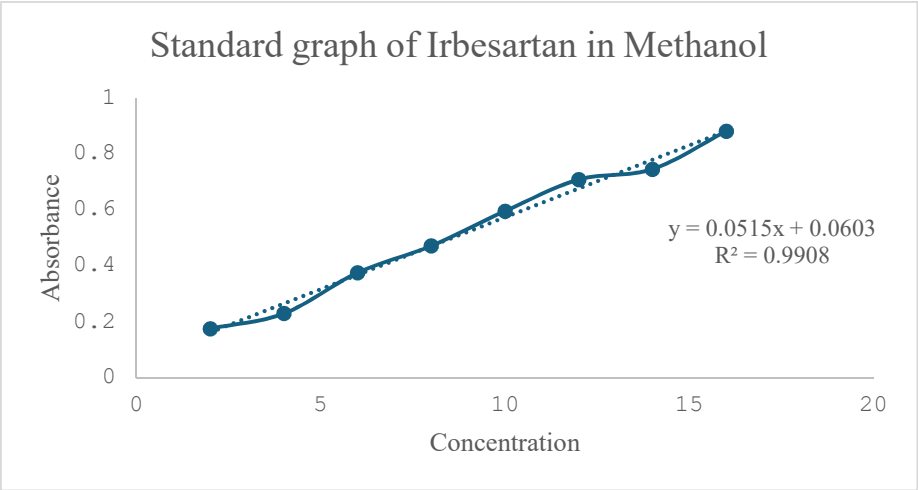

Fig 5: Calibration curve of irbesartan in methanol

The equation of the graph was found to be y = 0.0515x + 0.0603 with an R^2 value of 0.9908. Hence, the graph is linear in the range of 2–16 µg/ml.

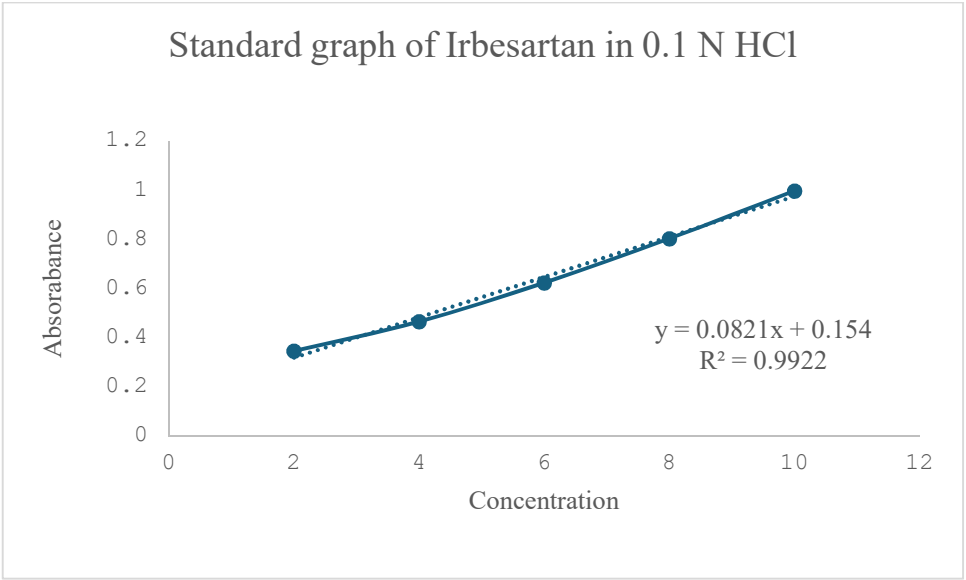

Fig 6: Calibration curve of irbesartan in 0.1 N HCl

The equation of the graph was found to be y = 0.0821x + 0.154, with an R^2 value of 0.9922. Hence, the graph is linear in the range of 2-10 µg/ml.

Preparation of Lipospheres:

Irbesartan lipospheres were prepared by the melt dispersion technique and solvent evaporation method. The solvent evaporation method was chosen over the melt dispersion method for the preparation of lipospheres. Lipospheres didn't form using the melt method; this may be due to improper solidification. Additionally, the melt dispersion method has drawbacks such as reduced drug encapsulation efficiency and susceptibility to thermal and oxidative degradation. To overcome these limitations, the solvent evaporation method was chosen. The solvent evaporation method provides better control over particle formation and produces a more uniform and stable dispersion.

EVALUATION OF LIPOSPHERES:

Solubility study of lipospheres in 0.1 N HCl:

Table 5: Solubility study of lipospheres

Sample	Observed Solubility (mg/ml)
Pure drug	0.504
LS6	4.021
LS9	7.17
LS12	6.93
LS14	4.91

The liposphere formulations exhibited markedly greater solubility in 0.1 N HCl compared to the pure drug. This enhancement is likely due to the ability of GMS and Compritol carriers to improve the wetting characteristics of irbesartan and facilitate its uniform dispersion within the lipid matrix, thereby promoting increased solubility.

Drug content:

The total drug content of the best selected formulation (LS9) was found to be 69.15%, indicating adequate incorporation of irbesartan into the liposphere matrix.

***In Vitro* Drug Release study of lipospheres:**

The *in vitro* drug release study was carried out using a USP Type II (paddle) apparatus in 0.1 N HCl for a period of 24 hours, and the cumulative percentage of drug released was calculated for each formulation. The GMS-based formulation LS6 demonstrated a sustained-release profile, showing 87.6% cumulative drug release in 24 hours. The best selected formulation F9 exhibited the highest release, of 96% in 24 hours.

The mixed lipid formulations LS9 and LS12 (containing both GMS and Compritol) also showed sustained-release behavior, with LS12 releasing 83.76% of the drug over 24 hours. The Compritol based formulation LS14 exhibited a similarly sustained release pattern, with a cumulative release of 86.92% in 24 hours. Although these values were lower than those of the best selected formulation, they clearly demonstrated sustained release compared to the remaining formulations.

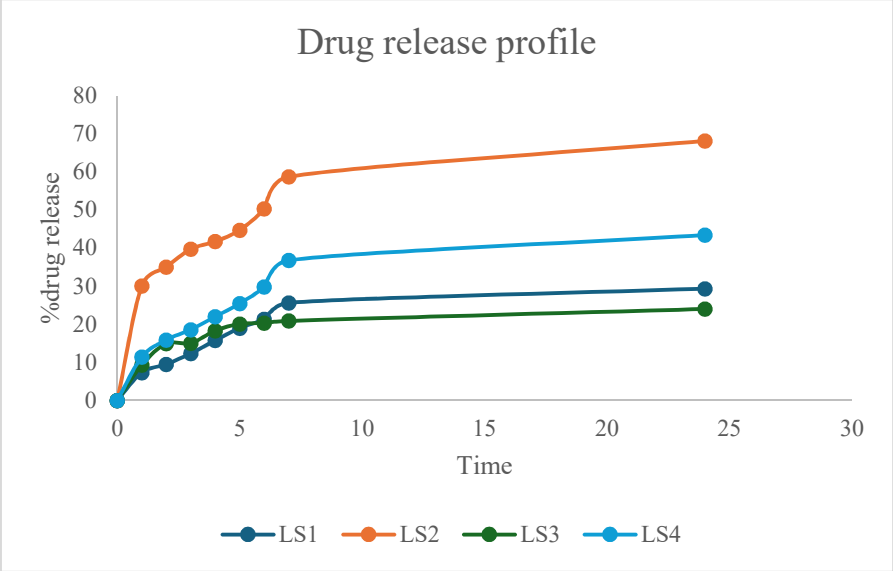

Fig 7(a): Drug release profile of formulations LS1-LS4

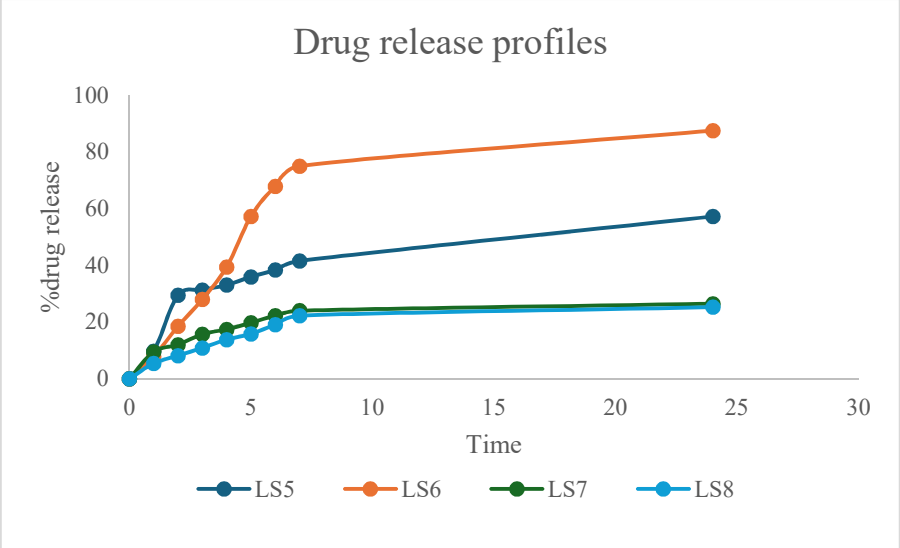

Fig 7(b): Drug release profile of formulations LS5-LS8

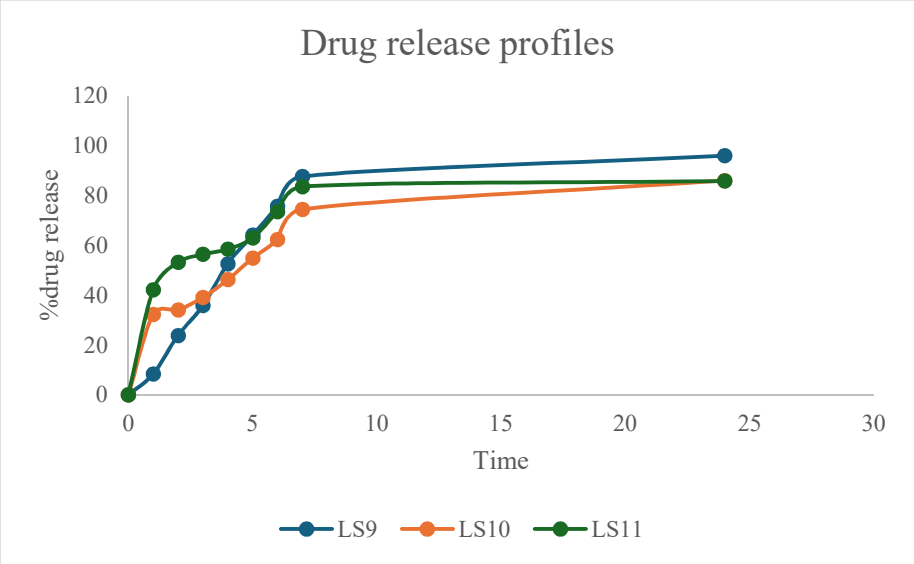

Fig 7(c): Drug release profile of formulations LS9-LS11

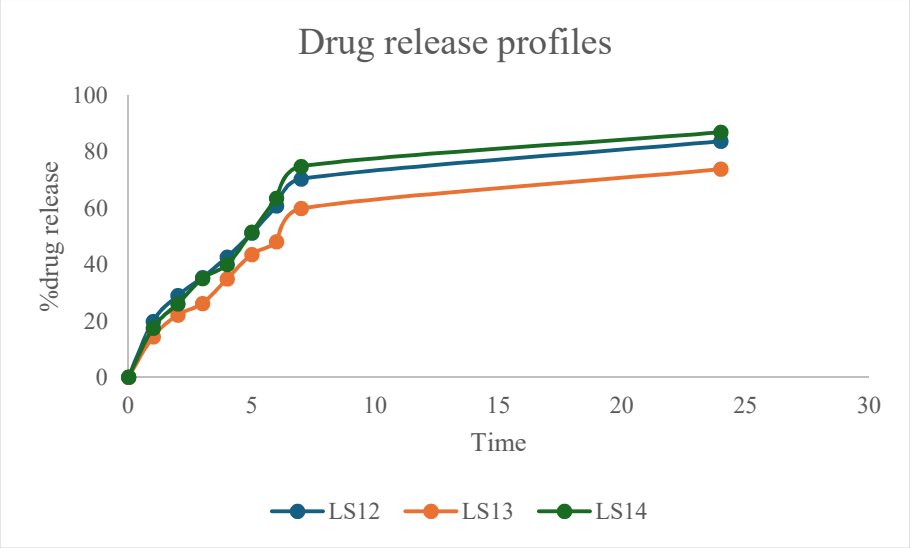

Fig 7(d): Drug release profile of formulations LS12-LS14

CHARACTERIZATION OF IRBESARTAN LIPOSPHERES:

Optical Microscopy:

The figure shows the photomicrographs of the lipospheres of the formulations LS6, LS9, LS12, and LS14.

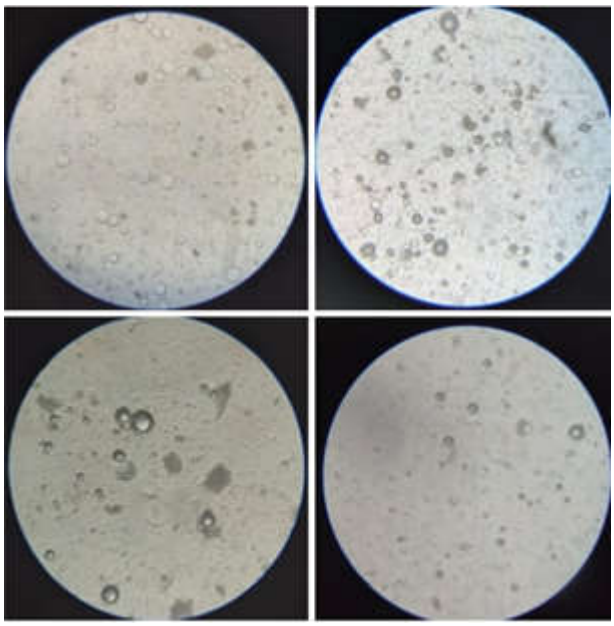

Fig 8: Photomicrographs of formulation LS6, LS9, LS12, and LS14

The microscopic images revealed that the prepared lipospheres were predominantly spherical with good dispersion and uniform morphology, confirming successful liposphere formation.

Morphology of the Surface (SEM):

The surface morphology of the prepared lipospheres was examined by Scanning Electron Microscopy. It was observed that the lipospheres were spherical and porous in structure.

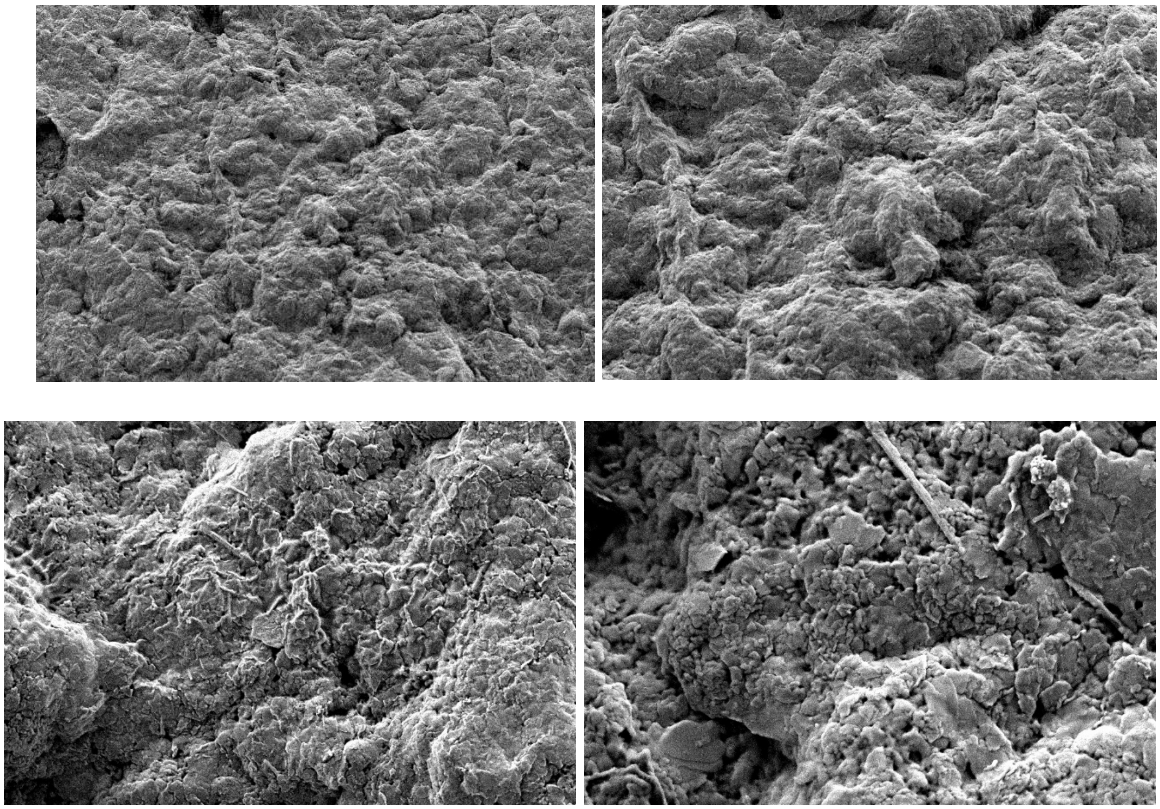

Fig 9: SEM images of irbesartan lipospheres

CONCLUSION:

The irbesartan lipospheres were developed to improve solubility and provide sustained release. Among the formulations, LS9 showed the best performance, with improved solubility in 0.1 N HCl, 69.15% drug content, and 96% drug release over 24 hours with spherical and uniform particles. Overall, lipospheres proved effective for improving the oral delivery of irbesartan.

ACKNOWLEDGEMENTS:

The authors are thankful to the management of Sri Venkateshwara College of Pharmacy, Madhapur, Hyderabad for providing the necessary facilities to carry out the research work.

REFERENCES:

1. Pouleur HG. Clinical overview of irbesartan: a new angiotensin II receptor antagonist. American journal of hypertension. 1997 Dec 1;10(S9):318S-24S.
2. Chime SA, Onyishi IV. Lipid-based drug delivery systems (LDDS): Recent advances and applications of lipids in drug delivery. Afr. J. Pharm. Pharmacol. 2013 Dec 29;7(48):3034-59.
3. Katta. Manogna S. Vanitha Sagar. A Detailed Review on Lipid-Based Carrier Drug Delivery System: Lipospheres. International Journal of Scientific Research, 2019; Vol. 8 (7).
4. Ramakrishna G, Kakarparthy R. A Review on Lipospheres. Journal of Clinical and Pharmaceutical Research. 2023 Jan 26:8-11.
5. Misbah Nikhath, Vazir Ashfaq Ahmed, Anees Fathima, and Afroj Ansari. Liposphere-Lipid-Based Drug Delivery System: A Review. World Journal of Pharmacy and Pharmaceutical Sciences, 2019; 9(1): 599-611.
6. Cortesi R, Esposito E, Luca G, Nastruzzi C. Production of lipospheres as carriers for bioactive compounds. Biomaterials. 2002 Jun 1;23(11):2283-94.
7. Organic laboratory techniques 4, 26 March 2020, University of Calgary, www.chem.ucalgary.ca.
8. N.S. Kurup, J. More. Nanostructured Lipid Carrier System for Dissolution Rate Enhancement of Irbesartan. International Journal of Science and Research Methodology, 2016; vol. 4(2).
9. Maddula VR, Dachuru RS. Physicochemical Characterization of an Irbesartan Inclusion Complexes with Modified β-Cyclodextrins Prepared by Optimizing Process Variables and Employing Microwave Irradiation Technique. Biointerface Research in Applied Chemistry, 2023; Vol. 13(6): 585.
10. Patel S, Manigauha A, Singhai N. Formulation and Characterization of Lipospheres of Telmisartan. Cahiers Magellanes-NS. 2024; 6(2).
11. Hanif M, Khan HU, Afzal S, Majeed A, Iqbal N, Afzal K, Andleeb M, Rauf A, Farooq A. Formulation, characterization and optimization of nebivolol-loaded sustained release lipospheres. Tropical Journal of Pharmaceutical Research. 2019 Mar 12;18(2):223-31.

12. Galgatte UC, Bhosale UM, Chaudhari PD. Formulation development, optimization and in-vitro evaluation of Glimepiride lipospheres. International Journal of Pharmaceutical Sciences Review and Research. 2015;34(2):157-62.
13. Saini S, Mittal A. Formulation and in-vitro evaluation of Glipizide (Anti diabetic drug) Liposphere. Journal of Drug Delivery & Therapeutics. 2018 Nov 2;8.
14. Nasr M, Mansour S, Mortada ND, El Shamy AA. Lipospheres as carriers for topical delivery of aceclofenac: preparation, characterization and in vivo evaluation. Aaps Pharmscitech. 2008 Mar;9(1):154-62.