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RESEARCH ARTICLE

Enhancement of Oral Bioavailability of Irbesartan by Niosomal Formulation

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Abstract: The purpose of this research was to prepare the Irbesartan niosome in a trial to improve its oral bioavaibility. Niosome are vesicles mainly consisting of nonionic surfactant. These NSVs were prepared by the conventional film hydration method. The mixture consisted of cholesterol, span-80 & chloroform in the molar ratio 65:60:5 respectively. The entrapment ~10% of Irbesartan used in the hydration, for hydration phosphate buffer pH 7.5 solution was used, the vesicles have an average size of 0.95m, the most probable size of 0.8m and average size range 0.4 to 2.2 m, most of the noisome have unilamellar or spherical shape. The niosomal formulation significantly retard release compared with free drug. The *in-vivo* study revealed that niosomal dispersion significantly improved oral bioavaibility of Irbesartan in rabbit, after a single oral dose of 40mg/kg. The average relation bioavaibility of the drug from the niosomal dispersion in relation to the free solution was 2.55 indicating more than two folds increase in drug bioavaibility. In conclusion, the niosomal formulation would be an improving delivery system for Irbesartan, with improved bioavaibility and prolonged drug release profiles.

KeyWords: Niosome, Oral bioavaibility, Irbesartan.

Introduction:

Irbesartan is an nonpeptide tetrazole derivative and angiotensin II receptor (AT1 subtype) antagonist used to treat hypertension, diabetic neuropathy, and in the reduction of renal disease progression in patients with Type 2 diabetes[1, 2]. This drug is a specific competitive antagonist of AT1 receptor with a much greater affinity (more than 8500-fold) for the AT1 receptor than for the AT2 receptor. According to Biopharmaceutical Classification System (BCS) classification Irbesartan belongs to BCS class II, having poor solubility in water and biological fluids with high permeability which ultimately results into its poor bioavailability (26%) after oral administration[3,4]. Aqueous solubility of a drug can be a critical limitation to its oral absorption. Solubility and dissolution are the main parameters for the therapeutic outcome of a drug and to attain desired concentration of drug in systemic circulation for pharmacological response[5-7]. For the enhancement of oral bioavailability of poorly soluble drugs remnants one of the most challenging aspects of drug development. Although salt formation, solubilisation and particle size reduction have commonly been used to enhance dissolution rate and so oral absorption and bioavailability of such drugs, there are some practical restrictions of these techniques[8,9].

Niosomes are the surfactant vesicles prepared from different nonionic surfactants. These are spherical lipid bilayers capable of entrapping water soluble molecules within an aqueous domain or alternatively lipid molecules within lipid bilayers. They may be unilamellar or multilamellar depending upon the approach used for their preparation. In recent years, niosomes have been broadly studied for the prospective to serve as carriers for delivery of drugs, antigens, hormones, and other bioactive agents. Niosomes are nonionic surfactant vesicles that are well recognized as drug delivery vehicles. Niosomes can carry hydrophilic drugs by encapsulation are quite stable, and require no special conditions for production or storage. Preliminary studies indicate that niosomes may increase the absorption of certain drugs from the gastrointestinal tract following oral ingestion[10, 11].

In the present study, Irbesartan loaded niosomes were formulated and evaluated for their in vitro as well as in vivo characteristics in an attempt to improve the oral bioavailability of the drug. The in vivo evaluation of Irbesartan niosomes in comparison with free drug solution was conducted in rats after a single oral dose.

2. MATERIALS AND METHODS

2.1. Materials

Materials used for the preparation of Irbesartan niosome were Irbesartan, recived as gift sample from Nicholas Piramal India Ltd. Mumbai, Cholesterol, Span 80, Chloroform and PBS, Trichloroacetic acid, Perchloric acid, Acetonitrile, Triton X-100 All other chemicals except pure drug Irbesartan were of analytical grade and purchased from Himedia laboratories pvt.ltd Mumbai and High purity laboratory chemical Mumbai.India.

2.2. Preparation of Irbesartan Niosomes[12]

The nonionic surfactant vesicles were prepared by the conventional thin film hydration method. Cholesterol, span 80 and chloroform (49.5 mg CHOL, 150 mg span 80 and 10 ml chloroform) in a molar ratio of 65:60:5. Both of this content dissolved in chloroform (10 ml). The lipid mixture was added to a 100ml round bottom flask, and the solvent was evaporated under reduced pressure at a temperature of 40-45ºC by a rotary evaporator until a thin lipid film was deposited on the wall of the flask. The excess organic solvent was removed by leaving the flask in a desiccator under vacuum overnight. The lipid film was hydrated with 5ml of the aqueous phase containing 20mg Irbesartan. The hydration was continued for 1 hour, while the flask was kept rotating at 40-45^{0}C. It was essential to prepare the vesicles at a temperature above the gel-liquid transition temperature. The niosomal suspension was further hydrated at room temperature for 2 hours in order to complete the swelling process. The hydrated niosomes were sonicated for 20 minutes in a bath type sonicator. This niosomal dispersion containing both free and entrapped drug was used for in vivo study. Niosomes were separated from un-entrapped drug by gel permeation chromatography. The niosomal fraction was diluted with the eluent to obtain a total lipid concentration of 5 mg/ml. This purified niosomal dispersion was used for in vitro study.

2.3. Particle Size Determination

The freshly purified niosomal dispersion was scanned and imaged using an optical microscope attached to video camera (Panasonic, Japan) with a magnification power of X40.

2.4. Determination of Entrapment Efficiency[13]

An aliquot of the freshly purified niosomal dispersion (5 mg/ml) was diluted with 10% Triton X-100 in a ratio of 1:99 v/v. The detergent dissolved the niosomes and yielded a clear solution. The

resultant solution was analyzed for Irbesartan concentration using the described high performance liquid chromatography (HPLC) method to calculate the amount of entrapped Irbesartan. The percentage of entrapped Irbesartan was calculated by applying the following equation:

$$\% \boldsymbol{Entrapment} = \frac{(\boldsymbol{AE} - \mathbf{100})}{(\boldsymbol{AI})}$$

Where,

AE is the amount of entrapped drug, and

AI is the initial amount of drug in the aqueous phase

2.5. *In-Vitro* Release Study[14]

The release of Irbesartan from niosomes was studied by employing the dialysis method. The dialysis sacks (cellulose tubing, 35/100 mm flat width/length and left to soak in normal saline for 24 hours before use. A 3ml sample, either of the freshly purified niosomal dispersion or of free Irbesartan solution in normal saline, was transferred to the dialysis sacks. The concentration of Irbesartan in each of the 2 samples was ~80µg/ml (determined according to the calculated entrapment efficiency of the niosomal dispersion). The sack was placed in 200ml magnetically stirred normal saline at 37^0C. Two milliliter samples were withdrawn at specified time intervals of 0.5, 1, 2, 3, 4, 5, and 6 hours and replaced by fresh medium, and drug content was determined according to the described HPLC method.

2.6. *In-Vivo* Study

2.6.1. Experimental Design[15]

New Zealand White Rabbits (2.0-2.5 kg) of either sex were selected for study. *In-vivo* animal studies were conducted in accordance with the protocol approved by the Institutional Animal Ethical Committee of Truba Institute of Pharmacy, Bhopal, (M.P.). Animals were housed under standard conditions with room temperature of 21±2°C, Relative humidity of 65% and 12:12 hour light dark cycle and starved for 18 hours before the experiment with free access to water. The animals were divided into three groups, each group containing three animals. The first group was treated as control. Second and third groups were treated with a single oral dose of 40 mg/kg of

free Irbesartan solution and the freshly prepared unpurified niosomal dispersion containing both the free (89%) and the entrapped drug (11%) by oral route. Blood samples (1 ml) were collected directly from marginal ear vein of each animal with a 24-G, 1-in. needle and collected directly in fresh Eppendorf tubes containing small quantity of EDTA at 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0, 10.0, and 12.0 hours after administration of free drug solution and at 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 4.0, 6.0, 9.0, 12.0, 15.0, and 24.0 hours after administration of the niosomal Irbesartan dispersion. Eppendorf tubes containing blood with EDTA were placed in centrifuge apparatus and centrifuged for 15 minutes at 5000rpm. After 15 minutes Eppendorf tubes were removed from centrifuge apparatus and supernatant was collected in fresh Eppendorf tubes then equal quantity of acetonitrile was mixed in the supernatant and again centrifuged for 15 minutes at 5000rpm. After 15 minutes Eppendorf tubes were removed from centrifuge apparatus and the supernatant (Plasma) was collected in fresh eppendorf tubes leaving coagulated proteins. This collected plasma was stored at 2-8ºC for further *in-vivo* study.

2.6.2. HPLC Analysis of Irbesartan

The concentrations of Irbesartan were measured in plasma samples using the HPLC technique described by Peh and Yuen[16] with the slight modification. An aliquot (250 µL) of plasma was mixed with 100 µL of 30% trichloroacetic acid and the mixture was vortexes for 30 seconds and centrifuged for 25 minutes. A 50µL sample of the clear supernatant was injected onto the HPLC system. A waters HPLC system was used consisting of 600-controller, fluorescence detector in combination with a data module integrator chromatographic separation accomplished using a C-18 column, made up of stainless steel with a guard per column of same packing material. The column effluent was monitored at an excitation wavelength of 250 nm and an emission wavelength of 370 nm, and the eluent flow rate was 1.2 ml min^{-1}. The mobile phase consisted of acetonitrile in 0.02M disodium hydrogen orthophosphate buffer adjusted to pH 2.5 with perchloric acid in the ratio of 60:40v/v. Calibration curves were constructed in rabbit plasma by spiking the blank samples with the standard amounts of drug Irbestarn. Peak areas were used in the determination of drug concentrations in the analyzed samples. The data were acquired and processed. The obtained chromatograms showed no interfering peaks, and the retention time of Irbesartan was 4.185 minutes. The calibration curves were linear over the range of 100 to 6000 mg/ml. The sensitivity of the assay under these conditions was 50 mg/ml in rabbit plasma.

Interlay precision was determined by assaying 5 samples; the coefficients of variation were 19.5% and 5.1% at concentrations of 100 mg/ml and 600 mg/ml, respectively.

2.6.3. Pharmacokinetic Analysis

Pharmacokinetic parameters were calculated from the individual plasma concentration-time curves for Irbesartan after the oral administration of its free solution or niosomal dispersion. The values of peak height (Cmax) and peak time (Tmax) were obtained directly from individual plasma drug concentration time curves. The areas under the plasma concentration time curves (AUC_0) were estimated by the linear trapezoidal rule. The ratio of individual AUC_0 values of niosomal Irbeatarn suspension to those of from Irbesartan solution (relative bioavailability) was calculated to assess the extent of absorption from each formulation. The terminal elimination rate constants (K) were calculated by applying linear regression on the log concentration vs. time curve (3-4 points).

3. RESULTS AND DISCUSSION

3.1. Size Distribution

Niosomes appeared as large unilamellar vesicles with spherical shape. Sonication may be responsible for the breakdown of the multilamellar vesicles to form unilamellar ones. Particle size analysis of the freshly prepared niosomes shows that the average size is ~0.95µm and the most probable size is 0.8µm (Figure 2). The use of high cholesterol content in the formulation of Irbesartan niosomes may lead to large vesicle size. McIntosh *et al*[17] found that cholesterol increases the width of lipid bilayers and consequently increases the vesicle size. Yoshioka *et al*[18] found that the mean size of the niosomes showed a regular increase with increasing the hydrophilic lipophilic balance (HLB) of the surfactant because surface free energy decreases with increasing hydrophobicity.

This result was in good agreement with that obtained in the present study, where the average size of Irbesartan niosomes is 0.95µm. The particle size distribution of the prepared niosomes reflects a wide size range of 0.4 to 2.2µm as shown in Figure 2. This finding may be owing to the influence of certain preparation conditions such as the hydration time and the degree of shaking.

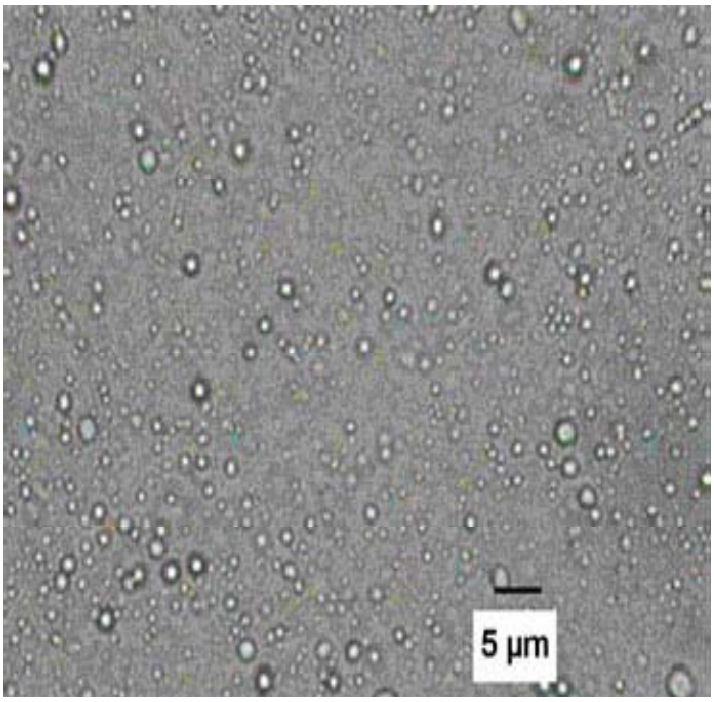

Figure 1: Photomicrograph of niosomes after sonication (original magnification 40X)

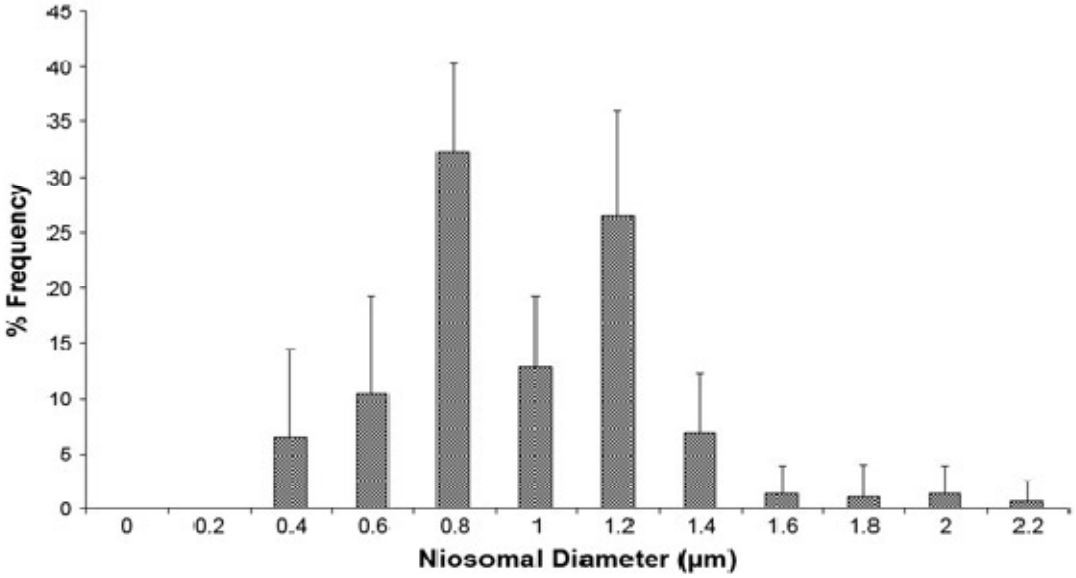

Figure 2: Particle size distribution of Irbesartan niosomes (±SD)

3.2. Entrapment Efficiency

Entrapment efficiency was expressed as a percentage of the total amount of Irbesartan used initially. The calculated average percentage entrapment efficiency of the niosomes was 9.003%±0.1% (n=2). This means that ~14.5µg of Irbesartan was entrapped per 1.0mg of the lipid

phase. This result may be explained by the high cholesterol content (~50% of the total lipids). Yoshioka *et al*. reported that entrapment efficiency was increased with increasing cholesterol content when niosomes were prepared by changing the molar ratio of nonionic surfactant to cholesterol. The authors found that vesicles prepared with span 80 (HLB-4.7) showed the most efficient entrapment compared with those prepared with other spans because of its highest phase transition temperature of ~50^0C. The large size of the prepared unilamellar niosomes may also be responsible for the high drug entrapment.

3.3. *In-Vitro* Release

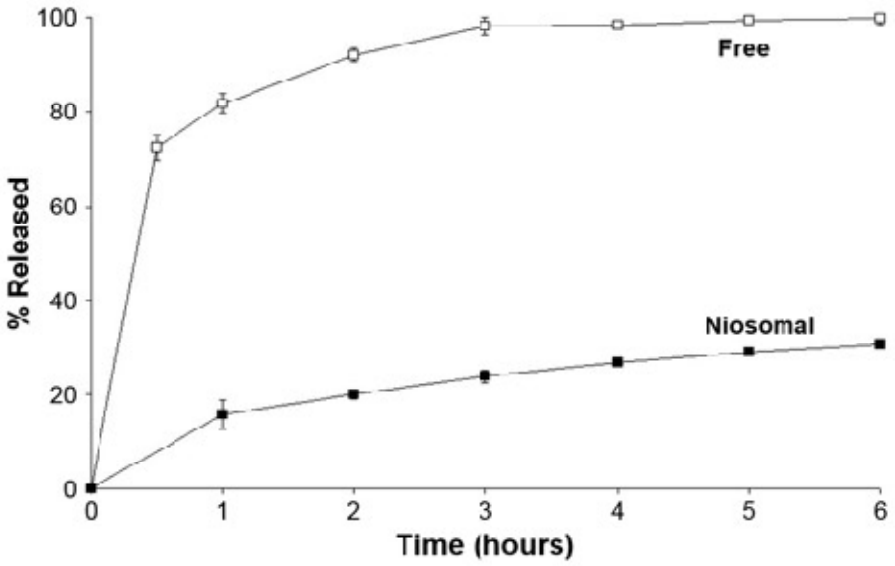

Figure 3: Illustrates the drug release profiles from its free solution and niosomal dispersion

The release data were analyzed mathematically according to zero-order, first-order, and Higuchi's equations. The data were best fitted to Higuchi's equation for both free and niosomal drug with average R2 values of 0.9864 and 0.9869, respectively. The release rate constants (K) and the half-life values ($T_{1/2}$) were calculated from the corresponding release profiles beyond 0.5 hour and 1.0 hour from the beginning of the release tests for free and niosomal drug, respectively. As a result, K values do not reflect the initial amounts of drug released that differ significantly (P G .05) according to the tested form. Free drug solution gave a high initial percentage drug release of ~72% after 0.5 hour, whereas the niosomal drug dispersion showed only 16% drug release after 1.0 hour. The release rate of Irbesartan drug from the niosomal dispersion (K = 0.0293 mg/hour) was significantly lower (PG .001) than that from the free

solution (K = 0.0654 mg/hour). The drug release from the free solution began to plateau after 3 hours, whereas, the release from the niosomal dispersion was continued for 6 hours without reaching plateau. These results pointed to sustained release characteristics with a Higuchi pattern of drug release, where niosomes act as reservoir system for continuous delivery of drug. This slow release pattern of entrapped drug may indicate the high stability of the niosomal formulation. Presence of cholesterol in a high percentage and the use of span 80 in the niosomal formulation may explain this high stability of the niosomal membrane.

3.4. Bioavailability and Pharmacokinetics

The average plasma drug concentration time curves in new zealand white rabbits after a single oral dose of Irbesartan (40 mg/kg) as free solution and niosomal dispersion (containing both free and entrapped drug) are shown in Figure 4.

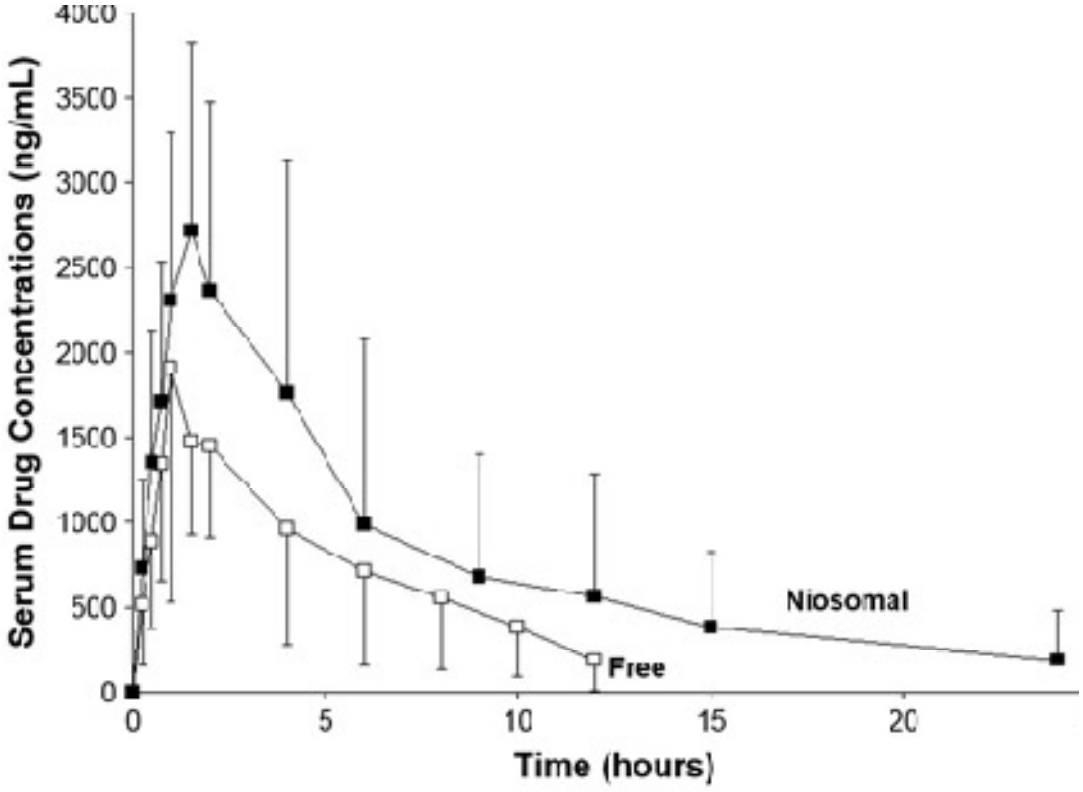

Figure 4: Mean plasma concentration time profiles (± SD) in rabbits after oral administration of free solutions (□) and niosomal dispersions (■) as a single dose of 40 mg/kg (n = 12)

The sustained release effect was also investigated by the in vitro release study. A possible explanation for this sustained release effect is that niosomes act as a carrier and a slow release vehicle. The drug is carried by the niosomes through the epithelium into deeper layers of the mucosa, where the encapsulated drug is slowly released.

This sustained release effect can improve the bioavailability of drugs with slow and limited absorption and narrow absorption windows. The significant increase of C_{max} values may be owing to enhanced absorption of the free drug included in the tested unpurified niosomal formulation (containing both the free and niosomal drug). The individual $AUC_{0\rightarrow\infty}$ values for the niosomal dispersions were compared with those for the free drug solutions to determine the relative bioavailability and the mean ratio was found to be 2.55 (±1.82). This result indicated that more than 2 fold increase in the oral bioavailability of Irbesartan was achieved by the niosomal formulation. The oral bioavailability of Irbesartan is low (15% to 30%), highly variable in humans, and is species dependent. Studies of the mechanisms of oral absorption of Irbesartan have produced conflicting results. The existence of a saturable process in the oral absorption of Irbesartan by mice,and dogs has been proposed based on a decline in the fraction of dose absorbed with rising dosage levels. The same suggestion has been made for humans based on a decline in percentage Irbesartan urinary recovery with increasing dosage. In the present study, the niosomal dispersion enhanced the bioavailability up to 2.5-fold despite the low content of entrapped Irbesartan (10%) in comparison with free Irbesartan (89%). This effect may be explicable in view of the poor oral bioavailability of Irbesartan in its conventional forms (15%-30%). The improved oral bioavailability may be owing to the lipophilic nature of the niosomal formulation and the effect of the nonionic surface-active agent on the permeability of the gastrointestinal membrane. Improved portioning of the lipophilic system to the mucosa, a direct effect of the surface active agent (span 80) on the barrier function of the mucosa, and prolonged localization of the drug-loaded niosomes at the site of absorption may be possible reasons for the improved bioavailability. The present in vivo results support previously published in vitro findings indicating that passive diffusion is the main mechanism underpinning the intestinal absorption Irbesartan.

4. CONCLUSION

The prepared Irbesartan niosomes have unilamellar spherical shape with an average size of 0.95 μm and percentage drug entrapment of 10%. The niosomal formulation showed sustained release characteristics with Higuchi pattern of drug release. In vivo study in rabbits revealed that more than 2-fold increase in the oral bioavailability and MRT was achieved by the niosomal formulation. So, the prepared niosomes could be promising delivery systems Irbesartan with sustained drug release profiles.

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