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Research Article

FORMULATION AND EVALUATION OF NANOSUSPENSION BY EMULSIFICATION METHOD

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ABSTRACT

Olmesartan medoxomil (OLM), an anti-hypertensive agent administered orally has absolute bioavailability of only 26% due to the poor aqueous solubility. The present investigation aimed at enhancing the oral bioavailability of OLM by improving its solubility and dissolution rate by preparing nanosuspensions. The nanosuspensions of OLM were prepared using emulsification solvent evaporation method. Various formulation as well as process parameters were optimized in order to achieve desirable size and saturation solubility. Characterization of the prepared nanosuspension was done with respect to particle size, zeta potential, saturation solubility, dissolution rate, morphology study (SEM), in-vitro dissolution study. The results indicated that Increase in the stabilizer concentration of PVP K90 shows 98.65% of drug release, so the formulations prepared by using PVP K90 releases more drug release at the end of 30mins than the other stabilizers and follows first order kinetics. Nanosuspension seems to be a promising approach for bioavailability enhancement because of the simple method of its preparation and its universal applicability.

Key Words: Olmesartan medoxomil, PVP K90, Poloxamer 184, SEM.

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INTRODUCTION

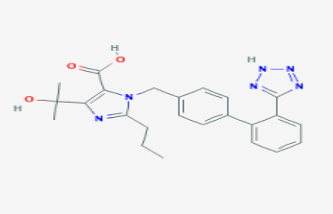
The design of oral controlled DDS should be primarily aimed to achieve more predictable and increased bioavailability. Now-a-days most of the pharmaceutical scientists are involved in developing the ideal DDS (Chaudhary A, 2012). This ideal system should have advantage of single dose for the whole duration of treatment and it should deliver the active drug directly at the specific site. Controlled release implies the predictability and reproducibility to control the drug release, drug concentration in target tissue and optimization of the therapeutic effect of a drug by controlling its release in the body with lower and less frequent dose (Deoli Mukesh *et al*., 2012). However, this approach is be filled with several physiological difficulties such as inability to restrain and locate the controlled drug delivery system within the desired region of the GIT due to variable gastric emptying and motility (Keck CM *et al*., 2006). Furthermore, the relatively brief GET in humans which normally average 2-3 hrs through the major absorption zone, i.e., stomach and upper part of the intestine can result in incomplete drug release from the drug delivery system leading to reduced efficacy of the administered dose.

Therefore, control of placement of a DDS in a specific region of the GI tract offers advantages for a variety of important drugs characterized by a narrow absorption window in the GIT or drugs with a stability problem (Sharma D, 2009).

DRUG PROFILE

Olmesartan

Structure



Synonyms

4-(1-hydroxy-1-methylethyl)-2-propyl-1-{[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl}-1H-imidazole-5-carboxylic acid

4-(hydroxy-1-methylethyl)-2-propyl-1-{[2'-(1H-tetrazol-5-yl)-1,1'-biphenyl-4-yl]methyl}-1H-imidazole-5-carboxylic acid

Categories: Agents Acting on the Renin-Angiotensin System; Agents causing hyperkalemia; Antihypertensive Agents; BSEP/ABCB11 Substrates; Cardiovascular Agents

CAS number: 144689-24-7
Weight: Average: 446.5016
Monoisotopic: 446.206638728
Chemical Formula: $C_{24}H_{26}N_6O_3$
IUPAC Name: 4-(2-hydroxypropan-2-yl)-2-propyl-1-({4-[2-(1H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)-1H-imidazole-5-carboxylic acid
Pharmacodynamics: Overall, olmesartan's physiologic effects lead to reduced blood pressure, lower aldosterone levels, reduced cardiac activity, and increased excretion of sodium.

Mechanism of action

Olmesartan belongs to the angiotensin II receptor blocker (ARB) family of drugs, which also includes telmisartan, candesartan, losartan, valsartan, and irbesartan. ARBs selectively bind to angiotensin receptor 1 (AT1) and prevent the protein angiotensin II from binding and exerting its hypertensive effects (Shayana Gora *et al*., 2016).

Volume of distribution: The reported volume of distribution of olmesartan is of 17 L.

Protein binding

Olmesartan is highly bound to plasma proteins hence, even 99% of the administered dose is found in a bound state with no penetration in red blood cells.

Half life

The mean plasma olmesartan half-life is reported to be from 10-15 hours after multiple oral administration.

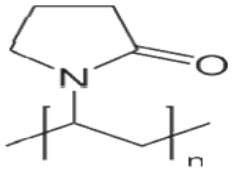
Clearance

Total plasma clearance of olmesartan is reported to be of 1.3 L/h and the renal clearance is 0.6 L/h.

Excipients Profile

Polyvinyl Pyrrolidone

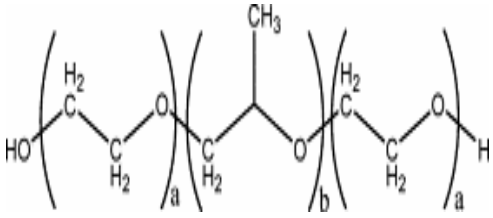
Polyvinyl pyrrolidone (PVP) is a water-soluble polymer made from the monomer N-vinyl pyrrolidone. Molecular formula is (C6H9NO)n, Molar mass is 2.500 - 2.5000.000 g/mol and melting point is 110 to 180°C (glass temperature).



Structure of polyvinyl pyrrolidone

Polyvinyl pyrrolidone (PVP) is cross-linkable to a water insoluble, swellable material either in the course of vinyl pyrrolidone polymerization, by addition of an appropriate multifunctional co-monomer, or by post-reaction; typically through hydrogen abstraction chemistry.

Poloxamer



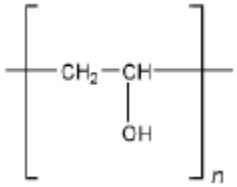
Poloxamers are non-ionic poly (ethylene oxide) (PEO)– poly (propylene oxide) (PPO) copolymers. They are used in pharmaceutical formulations as surfactants, emulsifying agents, solubilizing agent, dispersing agents, and in vivo absorbance enhancer Poloxamers are often considered as "functional excipients" because they are essential components, and play an important role in the formulation.

Poloxamers as Pharmaceutical excipients

Poloxamers possesses properties which appear to make it suitable for use in the formulation of topical dosage forms. Poloxamer 407 had been used in vehicles for fluorinated dentifrices, eye applications and

contraceptive gels. A poloxamer based dental gel product has been in use several years for treating patients with sensitive gums and teeth. Moreover, P-407 gel has been shown to possess many favorable characteristics for use as a burn dressing.

Polyvinyl alcohol
Structural formula



Empirical formula and Molecular weight
(C2H4O)n & 20000–200000
Melting point
228°C for fully hydrolyzed grades
180–190oC for partially hydrolyzed grades
Density 1.19–1.31 for solid at 25oC;
1.02 for 10% w/v aqueous solution at 25oC.
Viscosity (dynamic)
High viscosity 40.0–65.0
Medium viscosity 21.0–33.0
Low viscosity 4.0–7.0
Refractive index: 1.49–1.53

Methanol

Methanol also known as methyl alcohol among others, is a chemical with the formula CH_3OH (often abbreviated MeOH). Methanol acquired the name "wood alcohol" because it was once produced chiefly as a byproduct of the destructive distillation of wood.

MATERIALS AND METHODS

Pre-formulation studies

Prior to the development of dosage form, it is essential that certain fundamental physical and chemical properties of the drug molecule alone and when combined with excipients are determined. This first learning phase is known as pre-formulation. The overall objective of the pre-formulation is to generate information useful to the formulator in developing stable and bioavailable dosage forms which can be mass produced. The goals of pre-formulation studies are:

- To evaluate the drug substance analytically and determine its necessary characteristics
- To establish its compatibility with different excipients.

Spectroscopic study
Identification of pure drug
Melting Point

The temperature at which the first particle of the substance completely melts is regarded as melting point of the substance. The temperature at which the first particle starts to melt and last particle completely melts is regarded as the range of melting point.

Solubility studies

Solubility of Olmesartan was determined in Methanol, Ethanol, pH 1.2, pH 6.8 and pH 7.4 phosphate buffers. Solubility studies were performed by taking excess amount of Olmesartan in different beakers containing different solvents. The mixtures were shaken for 48 hrs in rotary shaker. The solutions were centrifuged for 10mins at 1000 rpm and supernatant were analyzed at 246nm by using UV Spectrophotometry.

Drug-Excipient Interactions Studies

There is always possibility of drug- excipient interaction in any formulation due to their intimate contact. The technique employed in this study is IR spectroscopy. IR spectroscopy is one of the most powerful analytical technique, which offers possibility of chemical identification. The IR spectra was obtained by KBr pellet method. (Perkin-Elmer series 1615 FTIR Spectrometer).

Preparation of Calibration Curve of Olmesartan
Procedure for standard curve in pH 6.8

10 mg of Olmesartan was dissolved in 10 ml of pH 6.8 by slight shaking (1000 µg/ml). 1 ml of this solution was taken and made up to 10 ml with pH 6.8, which gives 100 µg/ml concentration (stock solution). From the stock solution, concentrations of 5, 10, 15, 20, 25& 30 µg/ml in pH 6.8 were prepared. The absorbance of diluted solutions was measured at 246nm and a standard plot was drawn using the data obtained.

Method of Preparation of Nanosuspension
Preparation of Olmesartan Nanosuspension by Emulsification solvent evaporation method

Nanosuspension was prepared by the Emulsification solvent evaporation technique. Olmesartan was dissolved in methanol at room temperature (organic phase). This solution is followed by its emulsification into water containing different stabilizers of PVP K90, PVA, Poloxamer 184, and TWEEN 80 maintained at room temperature. Addition of organic solvents by means of a syringe positioned with the needle directly into stabilizer containing water, and subsequently stirred on magnetic stirrer to allow the volatile solvent to evaporate. Evaporation leads to precipitation of the drug.

Evaluation parameters of Nanosuspension Olmesartan

The Nanosuspension was evaluated for various

parameters:-

1. Entrapment efficiency
2. Particles size analysis
3. Zeta potential
4. In-vitro drug release studies
5. Scanning electron microscopy

Entrapment efficacy

The freshly prepared nanosuspension was centrifuged at 20,000 rpm for 20 min at 5°C temperature using cool ultracentrifuge. The amount of un incorporated drug was measured by taking the absorbance of the appropriately diluted 5 ml of supernatant solution at 246 nm using UV spectrophotometer against blank/control nanosuspensions. DEE was calculated by subtracting the amount of free drug in the supernatant from the initial amount of drug taken.

The entrapment efficiency (EE %) could be achieved by the following equation

%Entrapment efficiency= Drug content *100/Drug added in each formulation

Scanning electron microscopy

The morphological features of Olmesartan nanosuspension are observed by scanning electron microscopy at different magnifications.

Particle size and shape

Average particle size and shape of the formulated nanosuspensions was determined by using Malvern Zetasizer ZS using water as dispersions medium. The sample was scanned 100 times for determination of particle size.

***In vitro* drug release study**

In vitro dissolution study was performed by USP dissolution apparatus-type II using 900 ml of 6.8pH buffer as a dissolution medium maintained at 37 ± 0.5°C and stirring speed (50 rpm). The freshly prepared nanosuspensions were added to the dissolution medium, five-milliliter samples were withdrawn at specific intervals of time, then filtered through a 0.45 µm filter paper and analyzed for their drug concentrations by measuring at 246nm wavelength. The results of in vitro release profiles obtained for the NDDS formulations were fitted into Two models of data treatment as follows:

1. Cumulative percent drug released versus time (zero order kinetic model).
2. Log cumulative percent drug remaining versus time (first- order kinetic model).

1. **Zero Order Kinetics**: A zero-order release would be predicted by the following equation.

$$\mathbf{A_t = A_0 - K_0 t}$$

Where:

A_t = Drug release at time 't'

A_0 = Initial drug concentration.

K_0 = Zero-order rate constant (hr^{-1}).

When the data is plotted as cumulative percent drug release versus time, if the plot is linear then the data obeys zero-order release kinetics, with a slope equal to K_0 (Thorat YS, 2011).

2. **First Order Kinetics:** A first-order release would be predicted by the following equation

$$\mathbf{Log\ C = Log\ C0 - \frac{Kt}{2.303}}$$

Where:

C = Amount of drug remained at time't'

C_0 = Initial amount of drug

K = First-order rate constant (hr^{-1}).

When the data is plotted as log cumulative percent drug remaining versus time yields a straight line, indicating that the release follows First-order kinetics. The constant 'K' can be obtained by multiplying 2.303 with slope values.

Zeta potential

There are three ways by which a solid particle (colloid) dispersed in a liquid media can acquire a surface charge. First, by the adsorption of ions present in the solution. Second, by the ionization of functional groups on the particle's surface. Third, due to the difference in dielectric constant between the particle and the medium. Attention should be paid to the formation of electric double layer at the solid-liquid interface. The zeta Potential is defined as the difference in potential between the surface of the tightly bound layer (shear plane) and the electro-neutral region of the solution. The potential gradually decreases as the distance from the surface increases (Wongmekiat A *et al*., 2002).

As the concentration of electrolyte increases in the medium, the zeta potential falls off rapidly due to the screening effect of the counter ions. The zeta potential cannot be measured directly; however, it can be calculated using theoretical models and from experimentally determined electrophoretic mobility data. The theory is based on electrophoresis and can be expressed as:

$$\mu = \zeta\varepsilon/\eta$$

Where (μ) is the electrophoretic mobility, (ε) is the electric permittivity of the liquid, (η)

Is the viscosity and (ζ) us the zeta potential.

Table 1. Composition of Nanosuspension of Olmesartan

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Olmesartan (mg)	160	160	160	160	160	160	160	160	160
Poloxamer 184 (%)	0.25	0.5	0.75	--	--	--	--	--	--
PVP K-90(%)	--	--	--	0.25	0.5	0.75	--	--	--
PVA (%)	--	--	--	--	--	--	0.25	0.5	0.75
Tween 80 (%w/v)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Methanol (ml)	10	10	10	10	10	10	10	10	10
Water (ml)	40	40	40	40	40	40	40	40	40

Table 2. Drug release kinetics

Kinetic Model	Relation	Systems Following the Model
First order	$\ln Q_t = \ln Q_o + K_t$ release is proportional to amount of drug remaining	Water-soluble drugs in porous matrix
Zero order	$f_t = K_o t$ (independent of drug concentration)	Transdermal systems Osmotic systems

RESULTS AND DISCUSSION

Table 3. Solubility data

Solvent	Solubility(mg/ml)
Ethanol	21.46
Methanol	26.58
0.1N HCL	12.53
pH 6.8 phosphate buffer	19.85
pH 7.4 phosphate buffer	16.45

Table 4. Standard graph of Olmesartanin pH 6.8 (λmax 246 nm)

Concentration (µg/ml)	Absorbance
0	0
5	0.125
10	0.249
15	0.373
20	0.509
25	0.635
30	0.755

Table 5. Entrapment efficiency of formulated Nanosuspensions

Formulation code	Mean % entrapment efficiency
F1	83.23±0.26
F2	85.15±1.1
F3	87.45±0.56
F4	89.63±0.14
F5	88.23±0.56
F6	97.14±0.47
F7	93.26±0.59
F8	95.15±0.47
F9	97.26±0.63

Table 6. *In-vitro* drug release data of formulation F1to F9

Time (min)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
5	12.65	26.53	32.45	39.78	46.08	53.64	32.56	36.49	49.84
10	29.63	34.18	39.85	46.07	61.63	69.53	39.86	46.18	56.48
15	39.86	39.86	52.68	53.94	69.04	77.61	46.75	57.49	68.49
20	48.16	51.63	63.49	68.19	76.48	86.49	53.94	62.89	76.52
30	55.08	60.35	79.62	73.65	80.65	98.65	69.85	73.49	89.34
45	62.65	72.58	82.68	79.45	97.05		76.18	86.04	96.53
60	76.48	85.39	92.65	89.36			83.69	98.61	

Table 7. Stability studies of optimized formulation (F6)

Time (min)	F6 Initial	F6 1M	F6 2M	F6 3M
0	0	0	0	0
5	53.64	55.29	51.94	53.07
10	69.53	72.52	70.42	69.34
15	77.61	79.05	75.19	76.98
20	86.49	87.05	84.06	86.19
30	98.65	99.63	97.34	98.96

Table 8. Kinetic data of the formulation F6

Order of Kinetics	Zero Order	First Order
Regression	0.781	0.937

Fig. 1. Solubility studies of Olmesartan

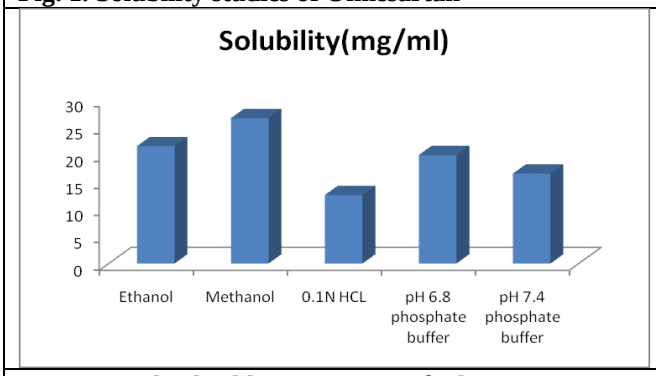


Fig. 2. UV spectrum of Olmesartan

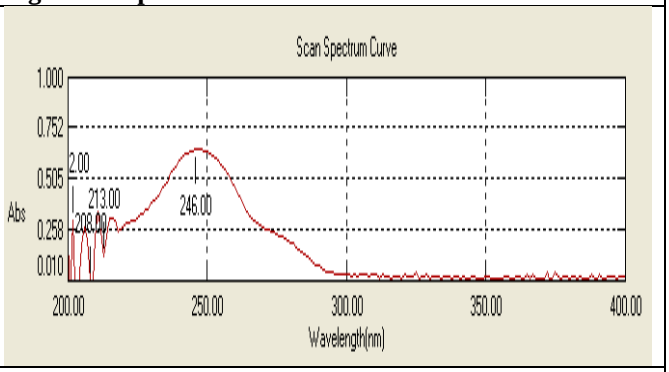


Fig. 3. Standard calibration curve of Olmesartanin pH 6.8

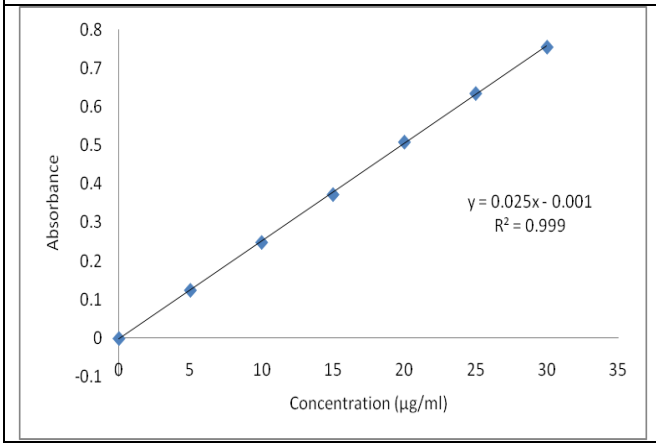


Fig. 4. Scanning Electron Microscopy Of Optimized Formulation

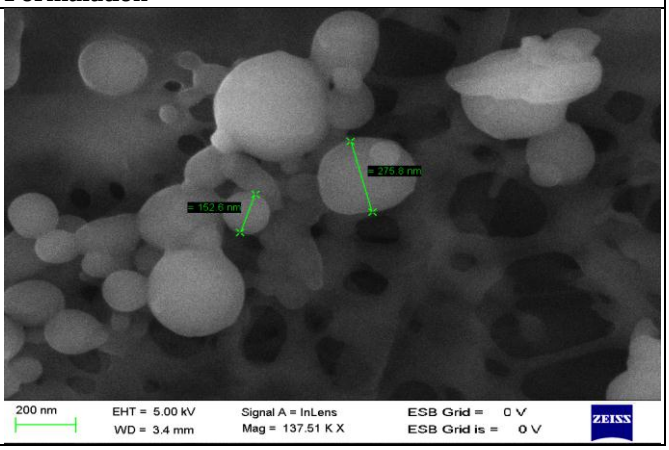


Fig. 5. Zeta potential value for the optimized formulation (F6)

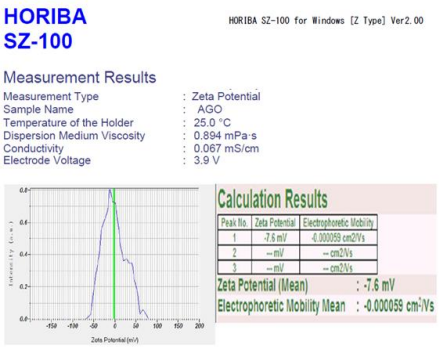


Fig. 6. Particle Size Analysis Of Optimized Formulation

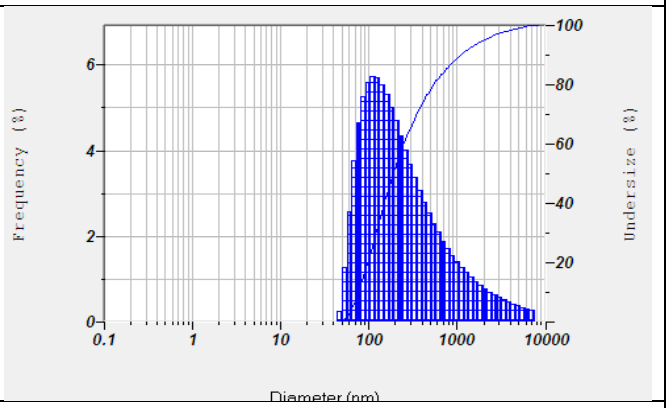


Fig. 7. Zero order release profile of formulation F6

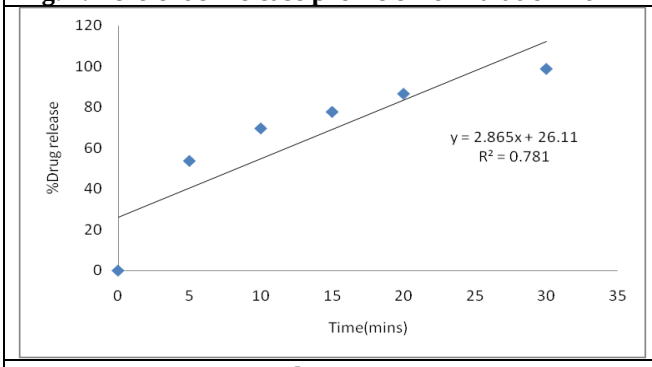


Fig. 8. First order release profile of formulation F6

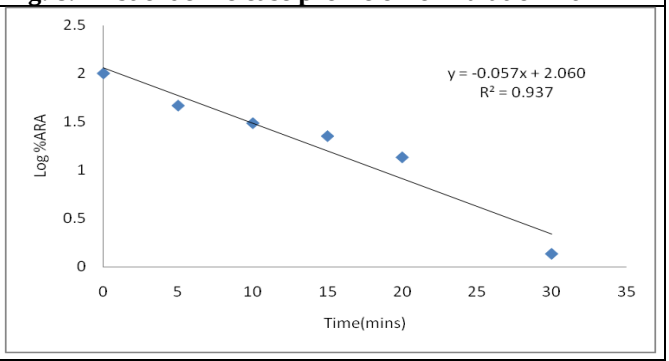


Fig. 9. IR spectrum of Olmesartan

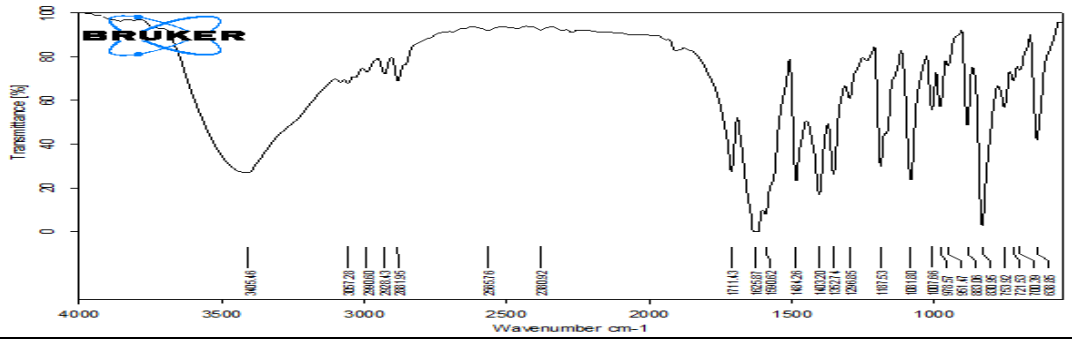
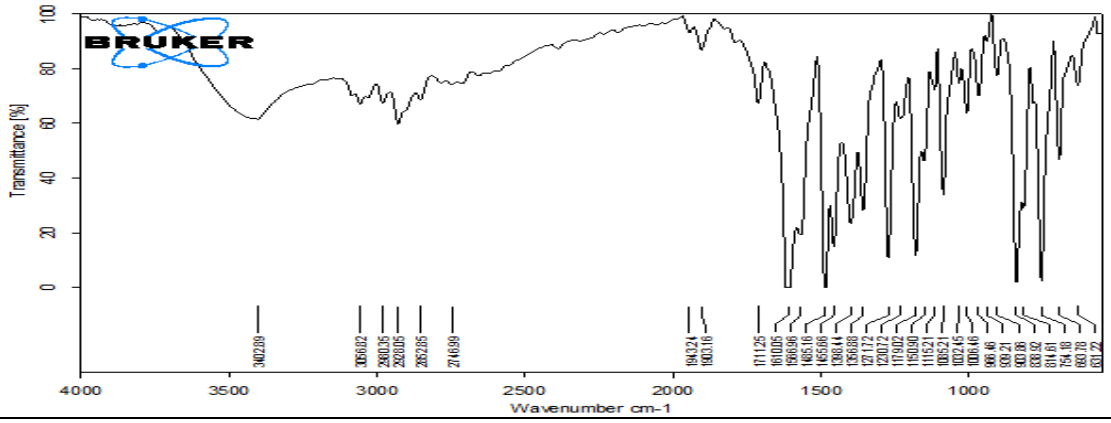


Fig. 10. IR spectrum of Olmesartan Optimised Formulation



Determination of melting point

The melting point of Olmesartanwas found to be in range of 177° C which was determined by capillary method.

Saturation Solubility

Saturation solubility was carried out at 25^0C using Methanol, Ethanol, 0.1N HCL, 6.8 phosphate buffer, and 7.4pH buffer. From the above conducted solubility studies in various buffers we can say that pH 6.8 phosphate buffer has more solubility when compared to other buffer solutions.So Ph 6.8 buffer is used as dissolution medium,based upon the solubility studies on organic solvents methanol has more solubility than others so methanol was used in the nanosuspension formulation.

Determination of absorption maximum (λmax)

Determination of Olmesartanλ-max was done in pH 6.8 buffer medium for accurate quantitative assessment of drug dissolution rate. The linearity was found to be in the range of 5-30 µg/ml in acetone, pH 6.8 buffer. The regression value was closer to 1 indicating the method obeyed Beer-lamberts' law.

Drug excipient compatibility

Drug and excipient compatibility was confirmed by comparing spectra of FT-IR analysis of pure drug with that of various excipients used in the formulation. Form the drug excipient compatibility studies we observe that there are no interactions between the pure drug (Olmesartan) and optimized formulation (Olmesartan+ excipients) which indicates there are no physical changes.

Entrapment efficacy

The entrapment efficacy of the formulated Nanosuspension was found to be in the range of 83.23%-97.26% respectively. The entrapment efficacy of formulation F1 was found to be 83.23±0.26%, formulation F2 was found to be 85.15±1.1%, formulation F3 was found to be 87.45±0.56%, formulation F4 was found to be 89.63±0.14%, formulation F5 was found to be 88.23±0.56%, formulation F6 was found to be 97.14±0.47%, formulation F7 was found to be 93.26±0.59%, formulation F8 was found to be 95.15±0.47%, formulation F9 was found to be 97.26±0.63%.

Zeta Potential

The measurement itself is a particle electrophoresis, the particle velocity is determined via the doppler shift of the laser light scattered by the moving particles. The field strength applied was 20 V/cm. The electrophoretic mobility was converted to the zeta potential in mV using the Helmholtz-Smoluchowski equation. At standard measuring conditions (room temperature of 25°C, water) this equation can be simplified to the multiplication of the measured electrophoretic mobility (µm/cm per V/cm) by a factor of 12.8, yielding the ZP in mV.

Average particle size of nanosuspension of optimized formulations (F6) was found to be having maximum particles at a range of 118 nm.

From the above invitro studies we can say that increase in the polymer concentration of polymers decrease in the dissolution time of all the formulations.

From the above invitro studies we can say that at low polymer concentrations the drug release time was increased.so F6 is considered as optimized formulation as it shows drug release with in 30mins.

Among all the three stabilizers we have used F6 containing PVP K90 at 0.75% concentration releases maximum drug release at the end of 30 mins when compared to the formulations prepared by using Poloxamer 184 and PVA.

Increase in the stabilizer concentration of PVP K90 shows 98.65% of drug release, so the formulations prepared by using PVP K90 releases more drug release at the end of 30mins than the other stabilizers.

The drug release from the Nanosuspension was explained by using mathematical model equations such as zero order, first order, and equation methods. Based on the regression values it was concluded that the optimized formulation F6 follows first order kinetics, indicating concentration dependent drug release.

CONCLUSION

In present investigation Nanosuspensions of Olmesartan was prepared by Emulsification solvent evaporation method. The Nano suspensions are novel promising target and controlled released dosage form which is gaining importance because of ease of manufacturing and diversified applications. The present trend of pharmaceutical research lies in the usage of biodegradable polymer because of its availability and low toxicity. Nanosuspension containing drug was prepared by emulsification solvent evaporation method by using combinations of Tween 80, poloxamer 184, PVP-K90, PVA, methanol and quantity sufficient water). Estimation of Olmesartan was carried out spectrophotometrically at 246nm. The Nanosuspension were evaluated for parameters such as drug content uniformity, scanning electron microscopy, particle size analysis, zeta potential, in-vitro release, drug excipient interactions (FTIR). The stability data was also subjected to statistical analysis. The melting point of Olmesartan was found to be in range of 177°C which was determined by capillary method. Saturation solubility was carried out at 25^0C using 0.1N HCL, 6.8 phosphate buffer, 7.4 pH buffer, methanol & ethanol. Form the drug excipient compatibility studies we observe that

there are no interactions between the pure drug (Olmesartan) and optimized formulation (Olmesartan+ excipients) which indicates there are no physical changes.

The entrapment efficacy of formulation F1 was found to be 83.23±0.26%, formulation F2 was found to be 85.15±1.1%, formulation F3 was found to be 87.45±0.56%, formulation F4 was found to be 89.63±0.14%, formulation F5 was found to be 88.23±0.56%, formulation F6 was found to be 97.14±0.47%, formulation F7 was found to be 93.26±0.59%, formulation F8 was found to be 95.15±0.47%, formulation F9 was found to be 97.26±0.63%. Zeta potential value for the optimized formulation (F6) was found to -7mv which was found to be within the acceptable limits. Average particle size of nanosuspension of optimized formulations (F6) was found to be 118nm. From the invitro studies we can say that formulation F6 shows best drug release of 98.65% within 30 minutes where as all the other formulations didn't release the drug.

The drug release from the Nanosuspension was explained by the using mathematical model equations such as zero order, first order, and equation methods. Based on the regression values it was concluded that the optimized formulation F6 follows first order kinetics.

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