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ORIGINAL ARTICLE **OPEN ACCESS**

Design and Development of Novel Controlled-Release Azilsartan Medoxomil Loaded Provesicular Powder

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ABSTRACT

The present study involved Design and Development of novel controlled-release Azilsartan medoxomil loaded provesicular powder (Azil). The provesicular powder of Azilsartan Medoxomil was prepared with a carrier, Cholesterol, span 60 by rotary flask evaporation technique &was evaluated for various physicochemical properties such as solubility, drug entrapment efficiency, surface morphology and analysis were carried out using Fourier transform infrared spectroscopy, Differential scanning calorimetry and powder X-ray diffraction. Theoptimized Formulation batch F6 was selected based on 100% cumulative drug release in 9 hours& showed99.95% CDR in 9 hours while drug entrapment efficiency was 93.00%. The mechanism of the drug release rate kinetics of the Batch F6 followed the Korsmeyer-Peppas. Thus, it can be concluded that Span 60 based provesicular powder, enhanced the solubility 20 folds while Cholesterol and Maltodextrin proved an effective carrier for developing controlled release provesicular powder.

Keywords: *Azilsartan medoxomil, Span 60, Provesicular powder, Solubility.*

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INTRODUCTION

A number of novel drug delivery systems using colloidal particulate carriers had emerged, encompassing various routes of administration, to achieve controlled and targeted drug delivery. Colloidal particulate carriers such as liposomes [1] or niosomes [2] have distinct advantages over conventional dosage forms. Liposomes consist mainly of phospholipids, prepared from double-chain phospholipids (neutral or charged) [3]. Niosomes are analogous to liposomes and also serve as promising drug carriers [4]. Niosomes offer an alternative to phospholipid vesicles. The hydrated mixtures of cholesterol and non-ionic surfactants give rise to these lipid vesicles [5]. These carriers can act as drug reservoirs, and modification of their composition or surface can adjust the drug release rate and/or the affinity for the target site. As a drug carrier, encapsulating a sufficient amount of the drug is one of the most desirable properties for niosomes usage [6-7]. The uniqueness of these lipid vesicles relies on their ability to carry hydrophilic drugs by encapsulation and hydrophobic drugs by partitioning within their lipid domains [8]. Niosomes behave like liposomes in vivo as they are able to prolong the circulation of encapsulated drug altering its organ distribution and metabolic stability [9] or to increase the contact time of drug with the applied tissue in topical preparations [10]. Niosomes proved to be an alternate to liposomes because they pose less problems related to chemical stability and are low cost substitutes. However, niosome dispersions may exhibit physical instability problems during storage like vesicle aggregation, fusion, leaking, or hydrolysis of encapsulated drugs, which could affect the shelf life of the dispersion [11]. Pro-niosomes are liquid crystalline compact niosomal hybrid which could be converted into niosomes upon hydration with water. Niosomes are water-soluble carrier particles, and these are dried to form niosomal dispersion on brief agitation in hot aqueous media. This dehydrated product is pro-niosome. The

niosomes which are obtained are more correlative to conventional niosomes and of higher size uniformity. The additional merits associated with pro-niosomes are low toxicity owing to their non-ionic nature, no requirements of special precautions, and conditions for formulation and preparations [12-13].
Proniosomes are microscopic lamellar structures. They combine a non-ionic surfactant and cholesterol followed by hydration in aqueous media. The surfactant molecule directs themselves such that the hydrophilic ends of the non-ionic surfactant orient outward, while the hydrophobic ends are in the opposite direction to form the bilayer. Both the liposomes and proniosomes are made from bilayer, but the liposome's bilayer is made up from phospholipid whereas proniosome's bilayer is made up from non-ionic surface-active agents. Formation of unilamellar or multilamellar proniosome also depends on the method of preparation [14-15]. The niosome is made of a surfactant bilayer with its hydrophilic ends exposed on the outside and inside of the vesicles while the hydrophobic chains (both) face each other within the bilayer. Because of this reason, the proniosomes hold both hydrophilic as well as hydrophobic drugs. Hydrophilic drugs hold within the space enclosed in the vesicle and the hydrophobic drugs are embedded within the bilayer [16-19].

MATERIAL AND METHODS

Material

Azilsartan medoxomil was obtained from USV limited, Mumbai. Cholesterol(CH), Maltodextrin & surfactants like Span 40,60,80 was purchased from Research Lab, Mumbai.

Methods

Preparation of provesicular powder of azilsartan medoxomil with cholesterol
The lipid mixture (250 µM) were prepared comprising of Spans(SA) and cholesterol(CH) in different molar ratios (175:75, 150:100, and 125:125, respectively). Specifically 30mg of drug was dissolved in 20 ml(1:1v/v) of chloroform and methanol. The previous solution was transferred to round bottom flask formerly groomed to as carrier (1 g of carrier per mole of surfactant) maltodextrin slurry. The mixture constituent was dried under vaccum using rotary flash evaporator at 45oC under reduced pressure 600 mmHg. Then dry provesicular powder was obtained by extrusion with an extruder.

Table No 1 Composition of the prepared Azilsartan medoxomil proniosomes

Formulation code	SA/CH (molar ratio)	Surfactant			Cholesterol	Azilsartan Medoxomil	Maltodextrin
		Span40	Span60	Span80			
F1	175:75	70.45	-	-	29.01	40	175
F2	150:100	60.39	-	-	38.67	40	150
F3	125:125	50.32	-	-	48.33	40	125
F4	175:75	-	75.35	-	29.01	40	175
F5	150:100	-	64.54	-	38.67	40	150
F6	125:125	-	53.82	-	48.33	40	125
F7	175:75	-	-	75.01	29.01	40	175
F8	150:100	-	-	64.29	38.67	40	150
F9	125:125	-	-	53.57	48.33	40	125

Characterizations

Saturation solubility study

The quantitative estimation of solubility was determined by shake flask method. The AZM and provesicular powder were suspended in dolphin orbital flask shaker containing 25ml distilled water at 37^0c. The samples were stirred at 100rpm for 48hr. Then aliquots from each withdrawal were filter through 0.45µm membrane filter. The filtrate after suitable dilutions absorbance was determined using a UV- spectrophotometer (1650 PC, Shimadzu, Kyoto, Japan) at 249 nm.

Fourier Transform Infrared(FT-IR)

The functional group of AzilsartanMedoxomil was determined by FT-IR spectrophotometer The potassium bromide (KBr) disks with sample were prepared using electrically operated KBr Press Model HP-15. About 1 mg of sample was triturated with about 5 mg of dry KBr and then pressed into the disks. The FTIR spectrum was recorded using Jasco 4100 (TOKYO, JAPAN) with IR resolution software. The scanning range was 4000-400 cm^{-1}.

Drug entrapment efficiency study of azilprovesicular powder.

The provesicular powder 10mg suspended in 100ml ethanol followed by sonication and further mixture constituent was filter through 0.45 µm membrane filter. Thereafter filtrate was digested in suitable medium and analyzed by UV spectrophotometrically (1650 PC, Shimadzu, Kyoto, Japan) at 249nm and AZM content was determined using following equation,

$$\%\text{Drug loading} = \frac{\text{Weight of drug in provesicular powder}}{\text{Weight of provesicular powder}} \times 100 \text{.....(1)}$$

Surface morphology study

Surface morphology study of optimized batch of Azil provesicular powder was done by SEM analysis. surface characteristics of Azil provesicular powder was observed by using a JEOL scanning electron microscope (Model: JSM 6360 A, Japan). SEM was the most commonly used method for characterizing particulate delivery system. SEM was used to determine surface topography, texture and to examine the morphology of fractured surface.

Differential scanning calorimetry (DSC)

The termal properties of AZM, polymer, & optimized provesicular power batch were investigated using differential scanning colorimetry equipped with an intra-cooler (DSC METTLER STAR^e SW 12.10, Switzerland). The analysis was carried out on an approximately 2-5 mg of sample sealed in standard pierced aluminium pans (Al -Crucibles, 40 Al). An empty aluminium pan used as reference. An inert atmosphere was maintained by purging nitrogen gas at a flow rate of 25 ml/min at a scanning rate of 10°C/min from 40°C to 300°C.

Powder X-ray diffraction

The X-ray diffraction study of azilsartan medoxomil and its provesicular powders were investigated using a Philips PW3710 analytical X-ray diffractometer with Cu K 2α rays. A voltage of 40 kV and a current of 25mA were used. The samples were scanned for 2Θ values from 5^0 to 50^0. Diffraction patterns of azilsartan, medoxomil and its provesicular powders in Span 60 were recorded.

***In-vitro* dissolution of azil provesicular powder**

The release of AZM from the developed provesicular powder batches were monitored separately using USP II apparatus. The known amount of AZM (40mg) was placed in phosphate buffer pH 7.4(900ml) at 37o C and stirred at 50rpm. Aliquots were withdrawn at definite time point filter (0.45µm) and replenish equal volume of fresh medium. Then estimation concentration of AZM was performed by using UV spectrophotometrically (PC 1600 Shimadzu, Kyoto, Japan) at 249 nm.

Stability study

The selected formulations were stored at refrigerator (4 °C±2) at ambient room temperature (25 °C±2) in sealed glass vials for a period of 3 months. At the end of the storage period in vitro drug release was carried out for proniosome-derived niosomes. The dissolution profiles of stored proniosomes were compared to non-stored ones according to the model-independent mathematical approach of Moore and Flanner. The similarity factor (f2) was calculated according to the following equation:

$$f_2 = 50.\log \{[1+(1/n) \sum_{t=1}^{n} (R_t - T_t)^2]^{-0.5}.100\}$$

where n is the number of sampling points, and Rt and Tt are the mean percent released from the reference (non-stored) and the test (stored), respectively, at time t. f2 represents a logarithmic transformation of the sum of squared error of differences between the reference and products. A value of 100 for f2 suggests that the test and reference dissolution profiles are identical. Values between 50 and 100 indicate that the release profiles are similar, whereas smaller values imply an increase in dissimilarity between release profiles over all time points.

Result and discussion:

Solubility studies

Pure drug azilsartan showed the 0.0035 mg/ml saturation solubility. In the saturation solubility study, it was found that the span based provesicular powder increased the solubility of azilsartan up to 20 times. Azilprovesicular powder showed the maximum saturation solubility of 0.060 mg/ml. Saturation solubility of plain drug and its Provesicular powder batches

Table No 2 saturation solubility study

Drug	F1	F2	F3	F4	F5	F6	F7	F8	F9
0.0035	0.038	0.041	0.031	0.049	0.051	0.060	0.041	0.038	0.035

Entrapment efficiency

The percentage entrapment efficiency of formulated provesicular AZM was shown in table 1. The batch F6 showed the 93% of entrapment efficiency. It was observed that span 60 and presence for cholesterol increased entrapment efficiency of AZM. Another reason for enhanced entrapment efficiency may be due to plasticizer crosslinking which leads to increase in holding capacity.

Table No 3 The % Entrapment efficiency of azilsartan provesicular powder

Batches	% Entrapment efficiency
F1	87.30%
F2	81.10%
F3	85.03%
F4	90.28%
F5	91.50%
F6	93.00%
F7	86.90%
F8	88.85%
F9	83.39%

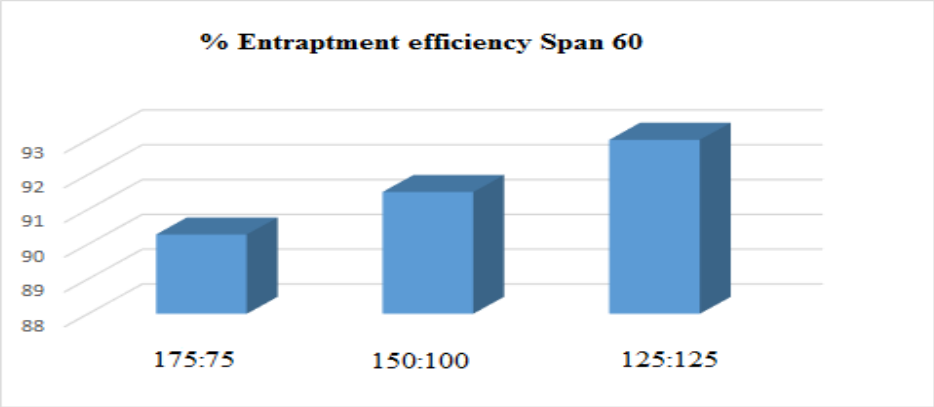


Fig 1: % Entrapment efficiency Span 60 (% Entrapment efficiency of provesicular powders)

Surface morphology

Surface morphology of optimized provesicular powder batch Azil was studied using SEM and computer-controlled image analysis software. SEM images confirmed that the particles were slightly smooth to rough in texture and irregular shape having diameter less than 50 µm.

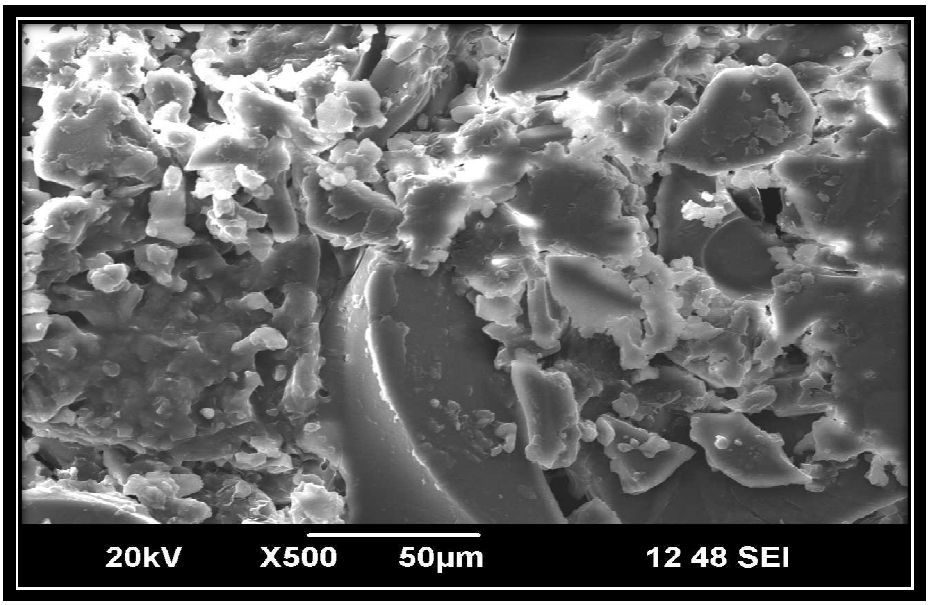


Fig 2: SEM image of azil provesicular batch 6

IR interpretation of azilsartan medoxmil

Table No 4 Fourier Transform Infrared(FT-IR)

Sr. no.	Functional group	Ranges(cm^1)	Observed Ranges(cm^1)
1	Ester C=O	1750-1680	1691.63
2	Hydroxy O-H	3400-2400	3317
3	Ester C-O	1300-1000	1045.45
4	Aromatic C=C	1600-1450	1550.82
5	Alkene =C-H	1000-675	748.41
6	C=N	1690-1640	1697
7	Amine	1360-1080	1278.85

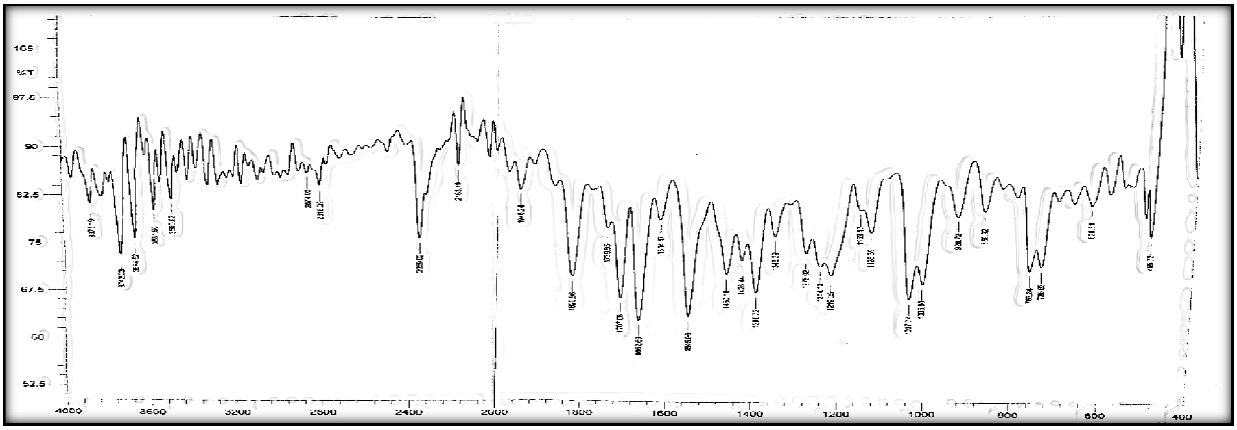


Fig 3: IR spectra of azilsartan medoxomil

FTIR studies were conducted to determine compatibility between drug and excipients. FTIR spectra and characteristic peaks of pure drug Azilsartan medoxomil, Maltodextrin and optimized formulation were obtained respectively. Azilsartan FTIR spectra showed characteristic peaks at 1691.63cm^{-1}, 3317 cm^{-1}, 1045.45 cm^{-1}, 1550.82cm^{-1}, 748.41cm^{-1}, 1647cm^{-1}, 1278.85 cm^{-1}. All the characteristic peaks of Azilsartan medoxomil were found and no new bands were observed in FTIR spectrum of optimized proniosomal formulation confirming no considerable interaction between drug and all other excipients in the optimized proniosomal formulation

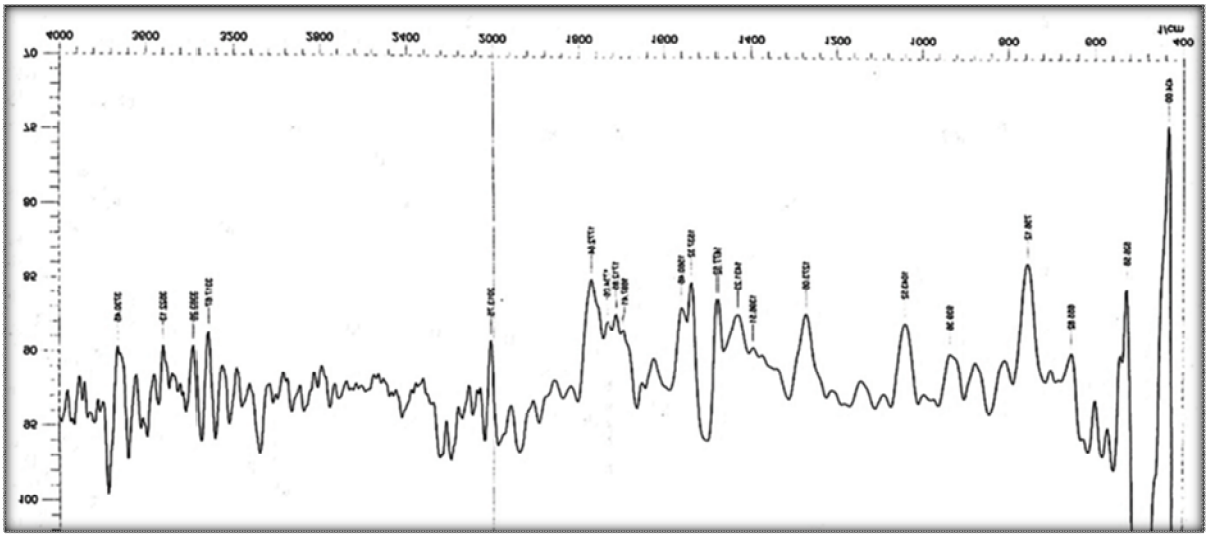


Fig 4:IR spectra of optimized azilprovesicular powder

Differential scanning calorimetry (DSC)

DSC an reliable method to detect drug interaction indicated by presence of new peak, change in shape of peak and area. The DSC thermogram of Azilsartan medoxomil showed a sharp melting endothermic peak

at 219.85°C indicating crystalline nature of the drug. The optimized provesicular powder batch (Azil) showed no distinct melting endotherm peak for drug. The formation of amorphous provesicular powder attributed to molecular interaction between drug and polymer. These indicate that drug exists in amorphous state in provesicular powder. The disappearance of the sharp melting endotherm in the DSC scan of provesicular powder suggested that the drug has been converted to the amorphous form during the provesicular powder process.The DSC thermogram of cholesterol showed a sharp melting endothermic peak at 150°C and span 60 shows endothermic peak at 54.63°C.

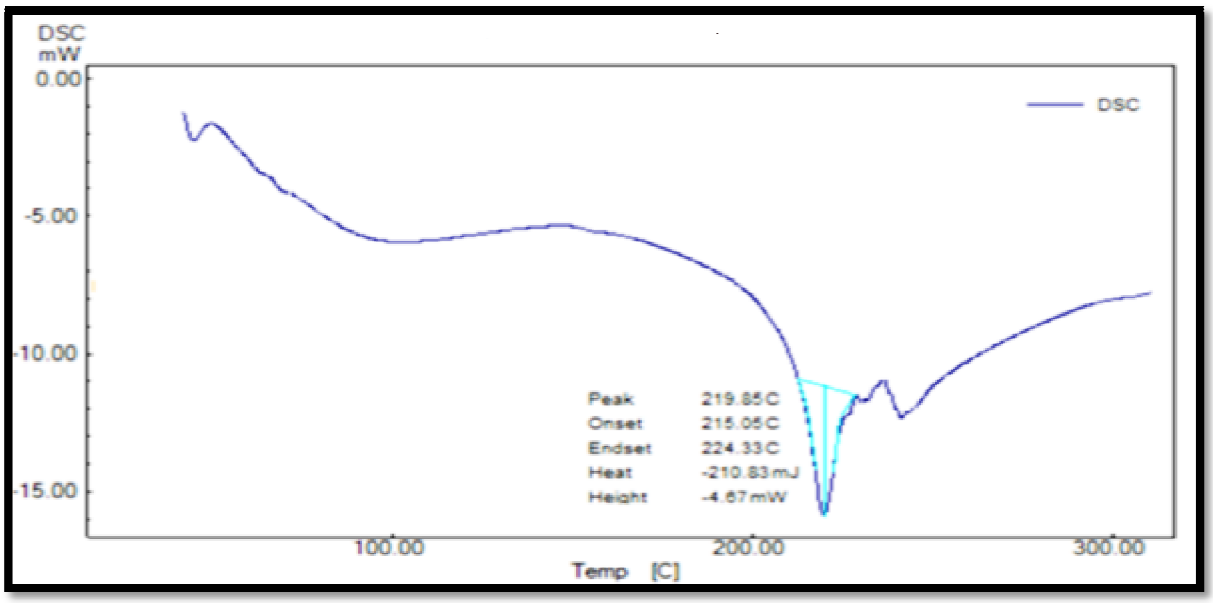


Fig 5: DSC of Azilsartan medoxomil

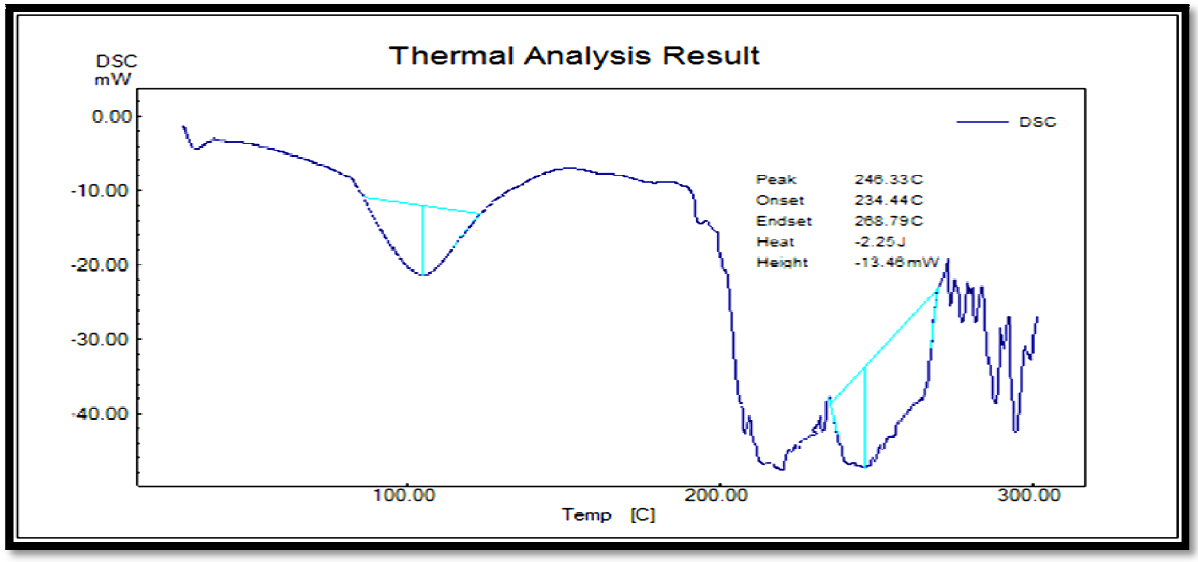


Fig 6: DSC of optimized Provesicular powder batch (Azil)

5.6 Powder X-ray diffraction(XRD)

The X-ray diffractograms of Azilsartanmedoxomil and Azilprovesicular powder showed in Figure 7 & 8 respectively. The X-ray diffractogram of Azilsartanmedoxomil showed sharp multiple peaks, indicating the crystalline nature of the Azilsartan Medoxomil. It showed sharp peak at diffraction angle (2θ) value of 23.69, 21.61, 26.85, 25.24, 20.53, 19.50, 18.12, 18.44, 15.04, and 15.53 indicating crystalline nature of azilsartan medoxomil. In case of provesicular powder (Azilprovesicular powder batch 6) diffractogram, the characteristic peaks of azilsartan medoxomil were disappeared due to conversion of crystalline dug into amorphous form.

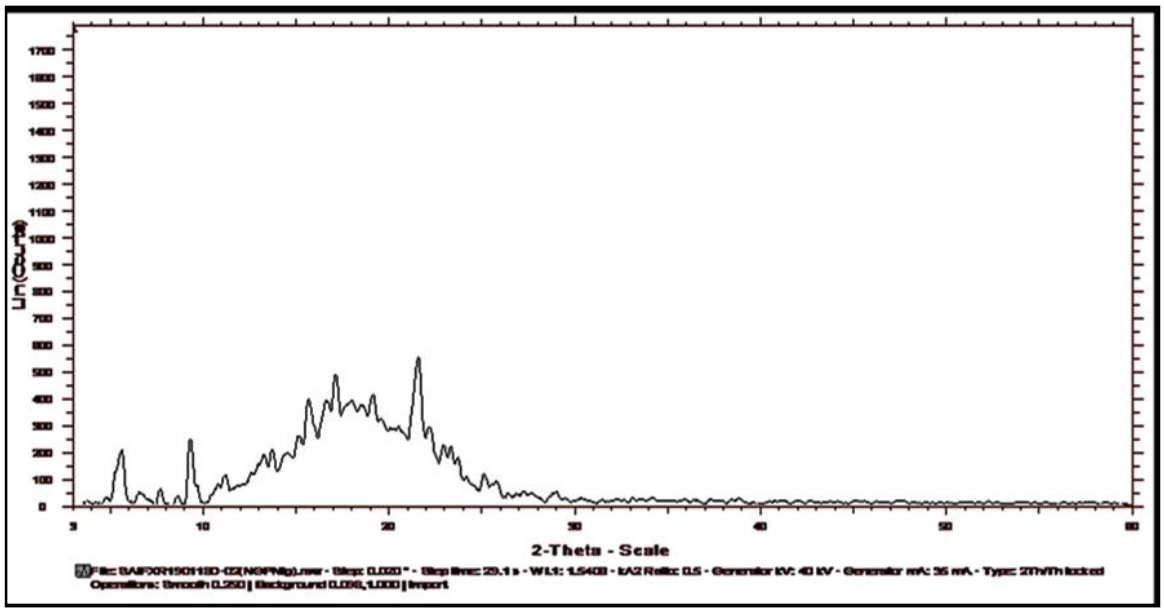


Fig 7: PXRD of Azilsartan medoxomil

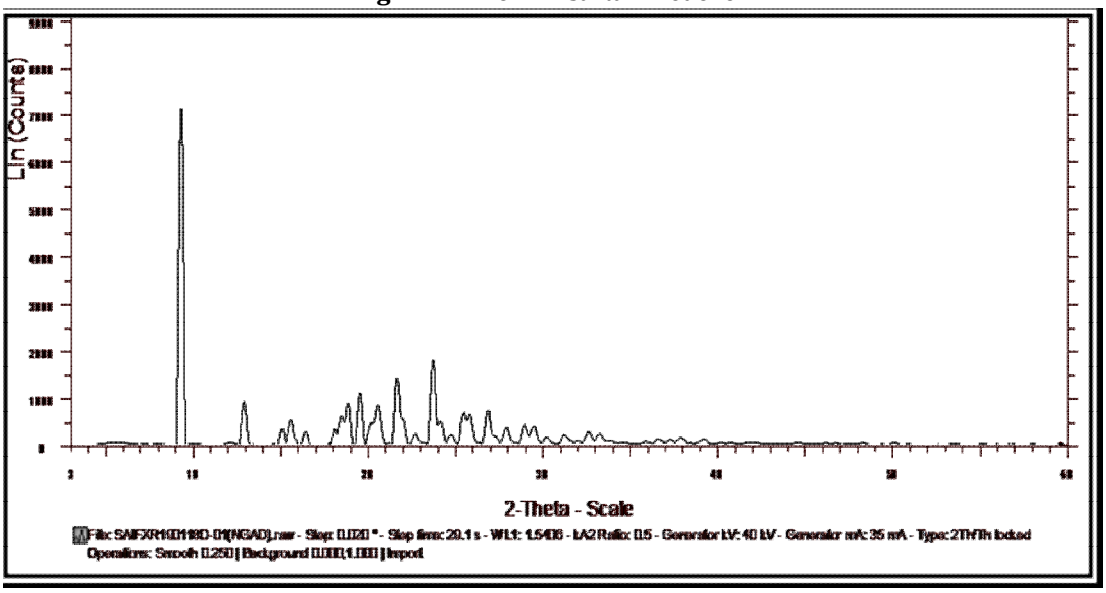


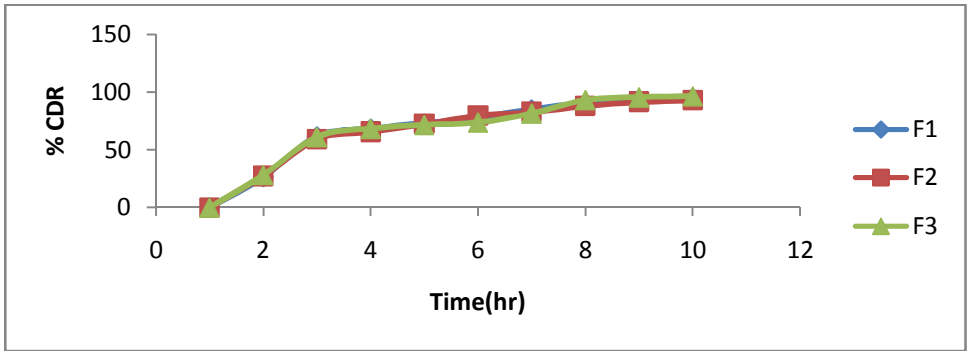
Fig 8: PXRD of optimized Provesicular powder batch (Azil)

***In-vitro* dissolution study of Azilsartan provesicular powder:**

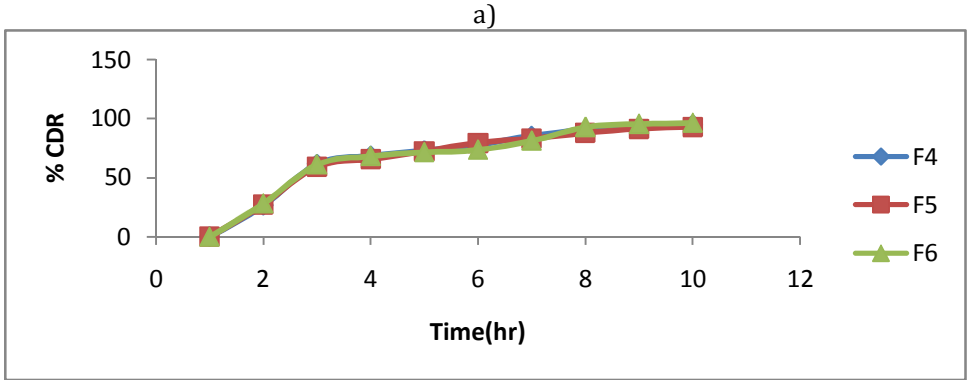
The in-vitro release profile of AZM from the proniosomal powder was investigated over 9 h.In-vitro release study shows in-vivo release behavior predication. During the first hour, the drug release was less than 30% probably because of the slow diffusion of drug from proniosomal powder then it increased till the 9th hour. The dissolution data of drug provesicular powder showed that batch F6 released was found to be 99.55 in 9 hrs, at 37^0c ± 0.5^0c in phosphate buffer pH 6.8 respectively. Remaining batches were unable to retarded the drug release up to 9hr. So, it was concluded that Batch F6 showed required release pattern.

Table No 5 Dissolution data of azilsartan provesicular powder batches (F1- F9)

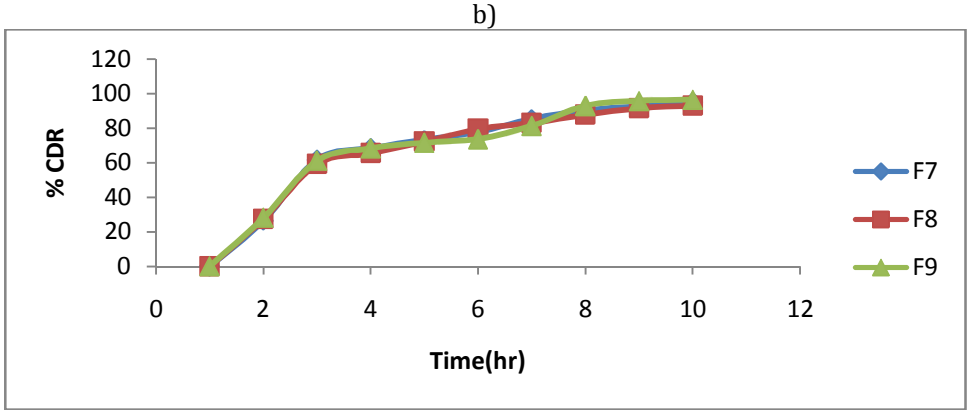
Time in hr.	% Cumulative drug release								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	26.08	26.59	26.08	27.87	26.33	26.33	26.38	27.36	28.12
2	51.14	63.66	60.59	61.36	64.94	59.33	61.68	59.32	61.11
3	57.84	67.05	68.32	69.09	68.33	71.39	68.33	65.51	68.33
4	59.44	74.02	76.06	76.83	73.00	79.13	73.00	72.23	71.47
5	68.44	76.91	83.30	85.60	79.73	85.09	77.68	79.46	73.59
6	76.95	82.87	87.73	89.53	85.18	91.32	85.17	82.87	81.34
7	96.47	91.40	94.22	95.50	95.49	94.23	90.30	87.81	92.92
8	97.32	94.31	95.60	97.65	96.36	98.17	92.26	91.49	95.58
9	97.94	96.20	97.49	98.27	98.51	99.55	94.92	92.87	96.45



a)



b)



c)

Fig 9 Dissolution profile of batch F 1-3 b) batch F 4-6 c) batch F 7-9

Stability Study

Stability of the provesicular powder of AZM was studied at different conditions of temperature (4±0.5 and 25±0.5±°C)to study the influence of storage on the drug assay and particle size. The provesicular powder was found to be most stable at 4±0.5°C (3 months) conditions. Stability study carried out as per ICH Q1 C stability testing for new dosage form for 3 months of duration.

Table No 6 Stability Study

	Storage condition
Short term	$25^0C \pm 2^0C$/ 65% RH± 5% RH
Short term	$4^0C \pm 2^0C$

DISCUSSION

The saturation solubility study indicated that the span based provesicular powder increased the solubility of azilsartan medoxomil up to 20 times.The surface analysis was done by the SEM& the images confirmed that the particles were slightly smooth to rough in texture and irregular shape having diameter less than 50 µm. The I.R study showed that there were no considerable interactions between the drug and all other excipients in the optimized proniosomal formulation. DSC scan of provesicular powder suggested that the drug has been converted to the amorphous form during the provesicular powder process. The X-ray diffractograms confirms that the characteristic peaks of azilsartan medoxomil were disappeared due to conversion of crystalline dug into amorphous form. The F6 showed required release pattern i.e., retarded the drug release up to 9 hours and showed maximum of 99.55% in 9 hours.

CONCLUSION

In conclusion, the provesicular powder of Azilsartan medoxomil with Span 60 (Azil) showed a 20-fold increase in the solubility and may increase bioavailability of Azilsartan medoxomil. This may be due to highly crystalline nature of Azilsartan medoxomil converted into amorphous form successfully. Further, provesicular powder developed using rotary flask evaporator, Span 60 and cholesterol was showed better and desired drug release pattern i.e., 99.95 % in 9 hours. It followed Korsmeyer-peppas release kinetics mechanism. Formulation of drug provesicular powder in controlled release provesicular powder is good approach for enhancing the solubility and bioavailability, reduce dose frequency, drug side effects, enhance patient compliance and protect the drug from inactivation, premature degradation and unwanted immunological or pharmacological effect.

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