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Candesertan niosomes-formulation and evaluation using Span 60 as non-ionic surafactant

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ABSTRACT

Candesartan is an angiotensin-receptor blocker (ARB) that may be used alone or with other agents to treat hypertension. The main aim of the research work is to formulate and evaluate Candesartan niosomes using Span 60 as a non-ionic surfactant. Niosomes are prepared using the ether injection method and are characterized for size, shape, entrapment efficiency and in vitro drug release. SEM studies have been carried out which shown that the niosomes are spherical in shape with smooth surface. All the vesicles appeared in the size range of 130- 170 nm. Percent entrapment efficiency has been carried out by dialysis method and found that increase in Span60 concentration increases the entrapment. Among all formulations, formulation F6 has shown highest entrapment efficiency 76.6%. Diffusion studies were carried out to study the drug release pattern from all formulations and are revealed that an increase in the concentration of surfactant decreases the drug release from niosomes, in all formulations prepared using span60. Stability studies were performed and the niosomes stored under refrigerated condition, showed greater stability. Formulation F6 was selected as an optimized formulation because of its good entrapment efficiency and slow drug release.

Keywords: Candesartan, niosomes, span60, ether injection method, entrapment,

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INTRODUCTION

Candesartan is an angiotensin II receptor antagonist and is used as a first line agent to treat uncomplicated hypertension, isolated systolic hypertension and left ventricular hypertrophy. It is poorly water soluble drug, and its absorption from oral route is also poor, as a result, failure in providing effective plasma drug profiles on conventional oral administration. The large dose and frequent administration of Candesartan may lead to hypotension [1].

Niosomes are microscopic lamellar structures of the size range between 10- 1000nm and consists of biodegradable, non-immunogenic and biocompatible surfactants. Niosomes (non-ionic surfactant based vesicles) are formed from the self assembly of non ionic amphiphiles in aqueous media resulting in closed bilayer structures. These structures are analogous to phospholipid vesicles (liposomes) and are able to encapsulate solutes are osmotically active and stable [2]. The low cost, greater stability and the resultant ease of storage of nonionic surfactants [3] has lead to the exploitation of these compounds as alternatives to phospholipids.

Span60 is having high phase transition temperature and low HLB (Hydrophilic Lipophilic Balance) so it will form vesicles of good size. One more reason for the selection of span 60 and that was the critical packing factor which is between 0.5 and 1 for this surfactant so it forms spherical vesicles. Span 60 having a higher Phase transition temperature, provides better entrapment [4]. Many researchers worked on formulating and evaluating niosomes using ether injection method [5-7].

The present study was aimed at formulating niosomes of Candesartan, optimizing the formulation, characterizing them and assessing *in vitro* performance of the system using a varying concentration of Span60 applying ether injection method.

EXPERIMENTAL SECTION

Materials

Candesartan obtained as a gift sample from Dr. Reddy's laboratories, Hyderabad. Cholesterol, Span60 and Tween20 purchased from Loba Chemicals, Mumbai. Solvents and other reagents were of analytical grade.

Solubility studies: The study was designed to select the suitable dissolution media. Solubility is the ability of a compound to dissolve in a liquid. It is defined as the amount of substance that passes into solution in order to achieve a saturated solution at a constant temperature and pressure. Excess drug (25mg) was added to 25ml of purified water, phosphate buffer 7.4, phosphate buffer with 0.5, 1.0 & 1.5 % tween 20 solutions taken in a series of 50ml stoppered conical flasks and the mixtures were shaken for 48hrs at 37°C on a rotary flask shaker. After 48hrs of shaking to achieve equilibrium, 2ml aliquots were withdrawn at 4h interval and filtered immediately. The filtered samples were diluted suitably assayed for Candesartan at 258nm against blanks prepared in the same solutions. Shaking was continued until the consecutive estimations were the same.

FTIR (Fourier transform infrared spectroscopy) studies:

IR spectroscopy also has its application in studies of drug – excipient interaction, contaminant analysis, etc. IR spectrum with the highest quality is acquired by KBr (pellet) method. Compatibility study of the drug with the excipients was determined by using FTIR. The sample powder of drug, excipients and mixture were prepared and placed on the glass plate and application of the infrared beam to record the spectra. The mixture spectra were compared with that of the original spectra.

Preparation of Candesartan niosomes:

Niosomes were prepared using the ether injection method [8-12]. The surfactant, cholesterol (5mg) and the drug (10mg) completely dissolve in the mixture of 10ml diethyl ether and methanol in the ratio of 1:1. This mixture was injected into the aqueous phase drop wise, which was heated at 60±2°C using 14 gauge needle with continuous stirring by placing water on the magnetic stirrer. Total six formulations were prepared by keeping the constant concentrations of drug and cholesterol and by varying the concentration of Span 60 from 2.5mg to 15mg. The composition of all formulations was given in Table 1.

Table 1: Formulae for formulation of Candesertan niosomes

Formulations	Drug (mg)	Cholesterol (mg)	Span 60 (mg)
F1	10	5	2.5
F2	10	5	5
F3	10	5	7.5
F4	10	5	10
F5	10	5	12.5
F6	10	5	15

Scanning electron microscopy:

The scanning electron microscope (SEM) is one of the most limited instruments widely applied to surface microstructure imaging. SEM is a type of electron microscopy that images the sample surface of a solid specimen by using a focused beam of high-energy electrons. Niosomes were characterized by SEM [13]. Niosomes containing Candesartan was taken in a cover glass and transferred on a specimen stub. Dried samples were coated with a platinum alloy to a thickness of 100° A using a sputter coater. After coating, scanning was done to examine the shape and size.

Particle size distribution:

The size of the formulation was analyzed by using a Zetasizer, Ver. 6.20 (Malvern Instrument Ltd). The formulation was placed in the sample holder and the particle size was measured [14].

Poly dispersibility index (PDI)

Polydispersity index [15] is a parameter to define the particle size distribution of nanoparticles obtained from photon correlation spectroscopic analysis. It is a dimensionless number extrapolated from the autocorrelation function and ranges from a value of 0.01for mono dispersed particles and up to values of 0.5-0.7. Samples with the very broad size distribution have polydispersity index values > 0.7.

Zeta potential
Zeta potential of the niosomes was measured using Malvern Zetasizer Ver. 6. 2. The Zeta analysis software produces a frequency spectrum from which the electrophoretic mobility hence the zeta potential calculated. The surface charge of the vesicles plays an important role in the *in vivo* performance of niosomes. The significance of zeta potential is that its value can be related to the stability of vesicular formulations. The zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in the dispersion system [16].

Entrapment efficiency:
Entrapment efficiency [17] of the niosomal dispersion can be done by separating the unentrapped drug by dialysis and the drug remained entrapped in niosomes is determined by complete vesicle disruption using 50% 2-propanol and analyzing the resultant solution by the appropriate assay method for the drug. Where,

$$\% \text{ Entrapment Efficiency} = \frac{\text{Total Drug} - \text{Diffused Drug} \times 100}{\text{Total Drug}}$$

***In vitro* release studies:**
In vitro release studies of all niosomes (10mg/ml) were performed using the exhaustive dialysis method [18,19] and the results were tabulated. Two side open ended glass tubes were taken and one side has been closed with semi permeable membrane. The fabricated tube was used as donor compartment, in which 5ml of suspension was taken and placed in receptor compartment containing 100 ml phosphate buffer pH 7.4 with tween20. The dialysis was carried out at 50 rpm at 37°C for 8hrs. Every hour 5ml of the sample was withdrawn and the same volume of fresh sample was replaced. The samples were analyzed using a UV spectrophotometer at 258nm.

Drug release kinetics:
The mechanism of Candesartan release from niosomal formulations was determined using the following mathematical models: zero-order kinetics (cumulative % release vs time), first-order kinetics (log % drug remaining vs time), Higuchi kinetics (cumulative % drug release vs. square root of time), Korsmeyer - Peppas (log cumulative % drug release vs log time) and Hixson-Crowel models (cubic root of drug remaining vs time). The r^2 and n values are calculated for the linear curves obtained by regression analysis of the above plots [20].

Stability studies:
The stability studies for best Candesartan niosomes formulation was carried out as per ICH guides for 3 months [21]. From this study, it was found that F6 formulation containing highest ratio of Span 60, has shown the desired release compared to other formulations and selected for stability studies. Formulated niosomes were divided into 3 groups. One group was kept in refrigeration (4°±2ºC). The second group was kept at room temperature (25°±2°C). The third group was kept at 40±2ºC and 60±5% RH. The niosomes were sampled at regular intervals of time (0,1,2,3 months), tested for percent drug retained. The results were shown in the Table 7

RESULTS AND DISCUSSION

The results of the solubility data were given in Table 2. The solubilities of tween20 in 1 and 2% were found to be 0.924 and 0.956 mg/ml, respectively, which may be sufficient to maintain sink conditions. As the solubilities of these two solutions are almost same, buffer with pH 7.4 and 1% tween20 was selected for the present work.

Table 2: Solubility data of Candesartan

Media	mg/ml
Purified Water	0.00151
Phosphate buffer pH-7.4	0.000672
Phosphate buffer pH-7.4with 0.5 % of Tween-20	0.335
Phosphate buffer pH-7.4 with 1 % of Tween-20	0.924
Phosphate buffer pH-7.4 with 1.5 % of Tween-20	0.956

FTIR spectrum for drug, excipients and combination of the drug and excipient were shown in Fig. 1 & 2. The characteristic peak for candesartan –OH stretching at 3400, -CH stretching at 2940 and 2861, -NH bending at 1716 and –OH bending at 1468 was also noticed in spectrum of drug with excipients. There is no appearance or disappearance of any characteristic peaks. This shows that there is no interaction between the drug and excipients used in the vesicle preparation.

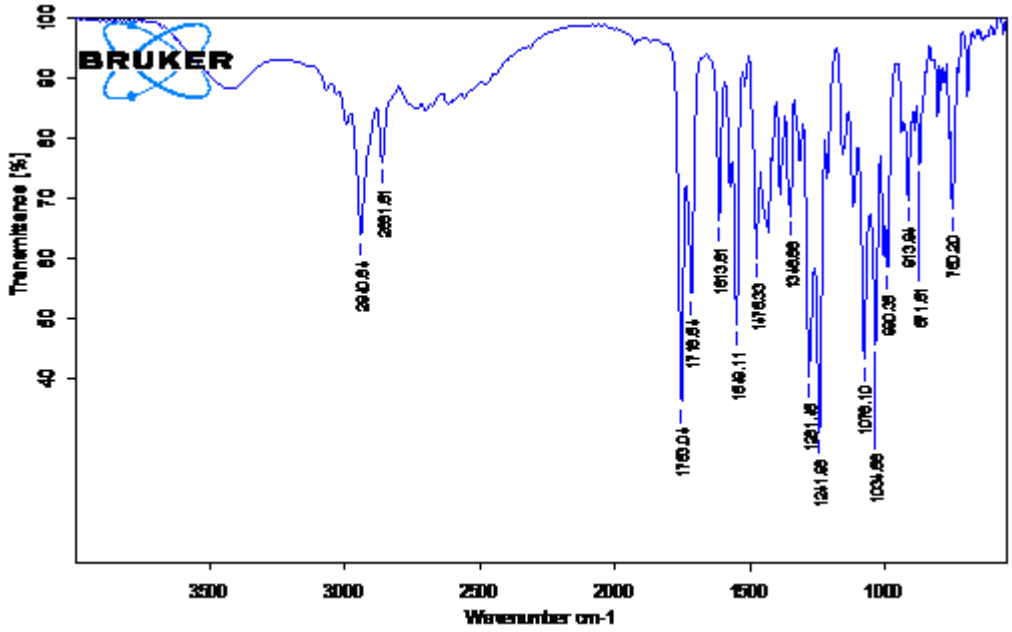

Fig. 1: FTIR spectra of Candesartan

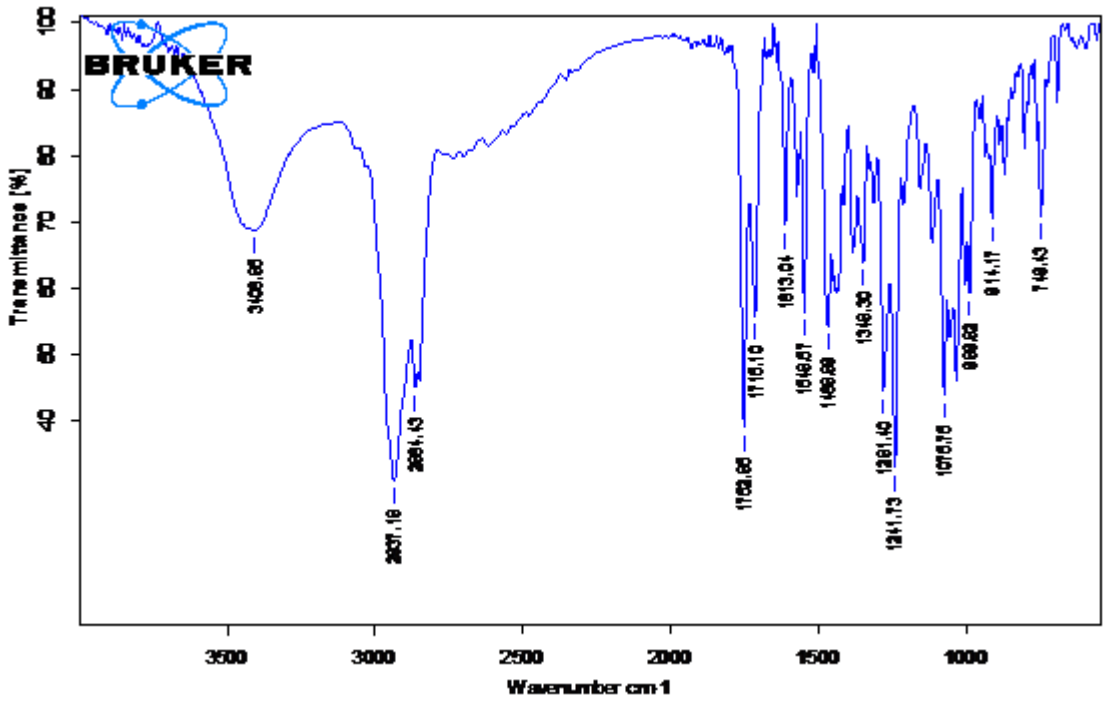

Fig. 2 : FTIR spectra of Candesartan, Cholesterol and Span 60 mixture

All developed formulations were characterized for surface morphology, particle size, entrapment efficiency and *in vitro* drug release. Candesartan niosomes were analyzed for their size and vesicle morphology by scanning electron microscope. SEM revealed that the niosomes were spherical. The following Fig.3 represents SEM images and Fig. 4 to 6 represents size distribution of Candesartan niosomes for the formulations F1, F3 and F6 respectively. The particle sizes for the formulation F1, F3 and F6 were given in Table 3.

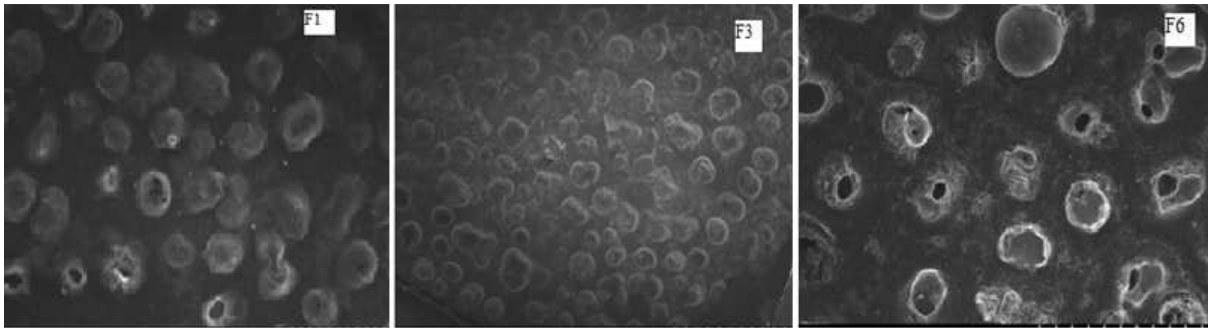

Fig. 3: SEM images of F1, F3 & F6 Formulation

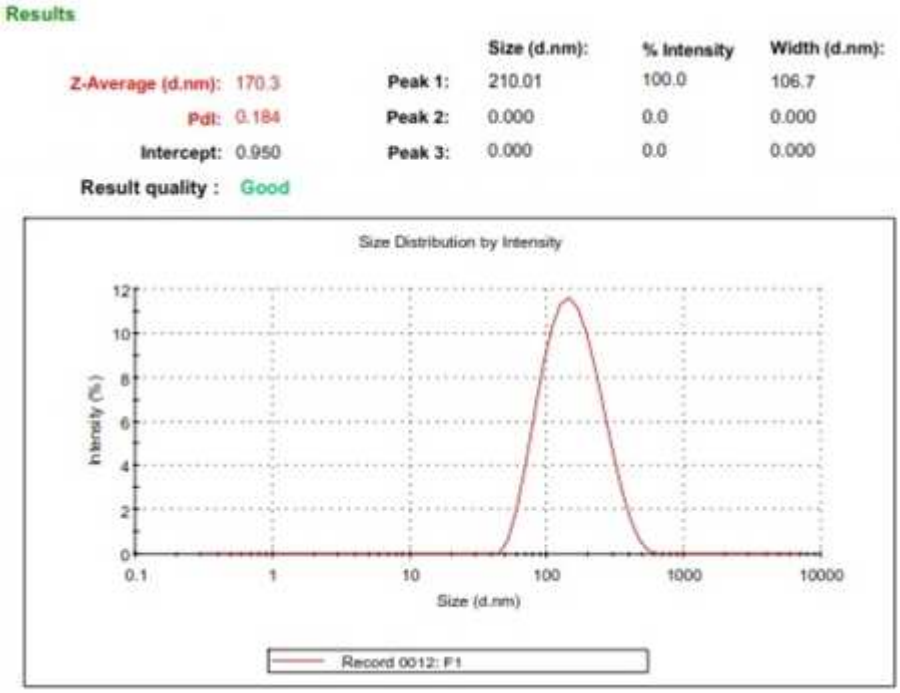

Fig. 4: Size distribution report for Formulation F1

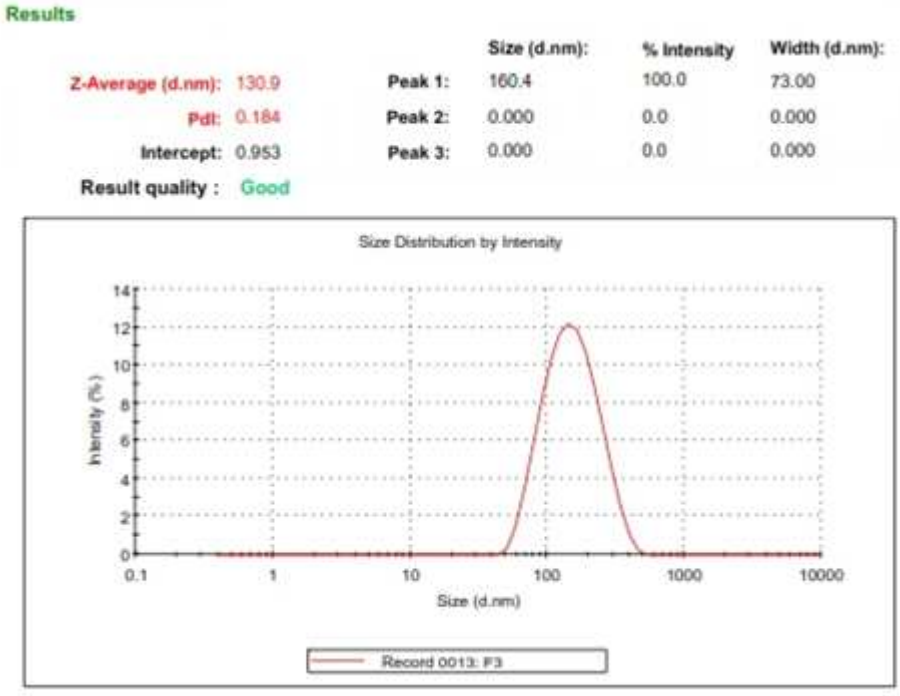

Fig. No.5: Size distribution report for Formulation F3

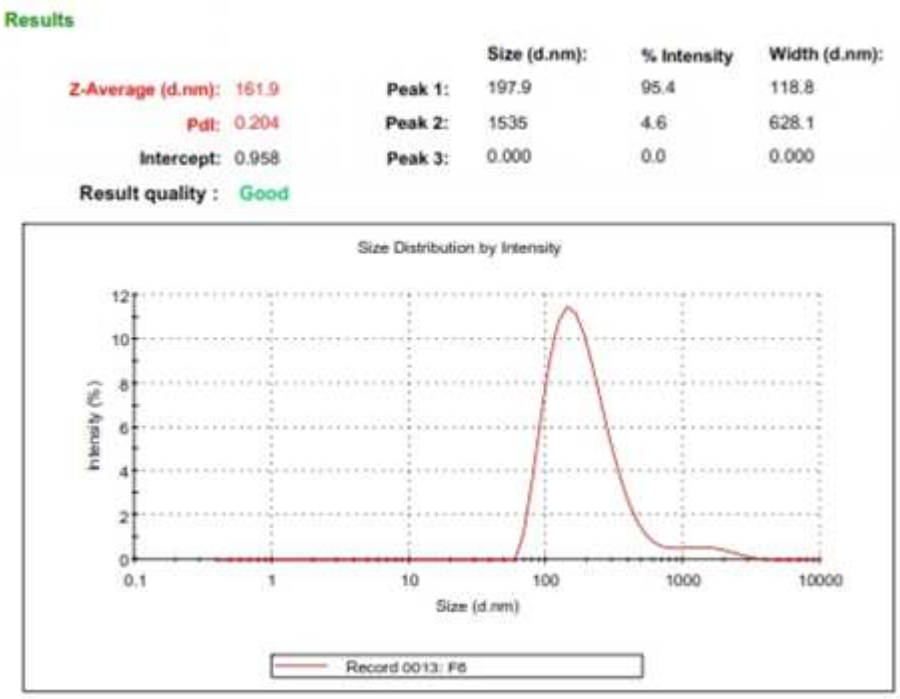

Fig. No. 6: Size distribution report for Formulation F6

Table 3: Particle size, PDI and zeta potential of museums

Formulation	Particle size (nm)	PDI	Zeta potential (mV)
F1	170.3	0.184	-35.6
F3	130.9	0.184	-35.6
F6	161.9	0.204	-35.6

From the size distribution reports as well as observations, it was comprehensible that the particle size of various niosomes prepared was found to be within narrow size range. Formulations have the size range between 130.9 and 170.3 nm as shown in Table 3. Increase in surfactant content did not change the size and stability of the niosomes.

The values from Table 4 indicate that the formulations had a very low PDI in the range of 0.184-0.204. From the particle size distribution data, it is evident that the particles were in the range between 130 – 170nm. The lower particle size and PDI may be because of the presence of surfactant which increases the surface tension between organic and aqueous phase and leads to the formation of smaller particle size. It also stabilizes newly generated surfaces and prevents aggregation of the particles as reported earlier [22].

The values of the zetapotential of Candesartan loaded niosomal formulations F1, F3 & F6 were found to be -35.6 mV which was shown in Table 3 and Fig. 7 to 9. The high negative surface charge on niosomes indicated higher stability because of the anticipated surface repulsion between similarly charged particles, hence inhibiting aggregation of the colloidal niosomal particles. Hence it was observed that all the formulations were sufficient to keep the particles stable.

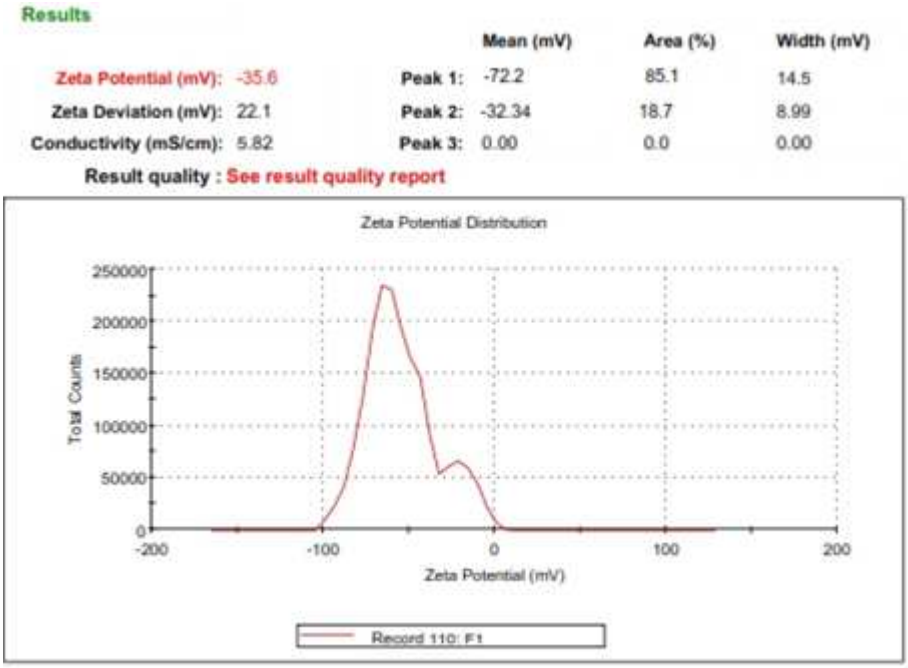

Fig.7: Zeta potential report of Formulation F1

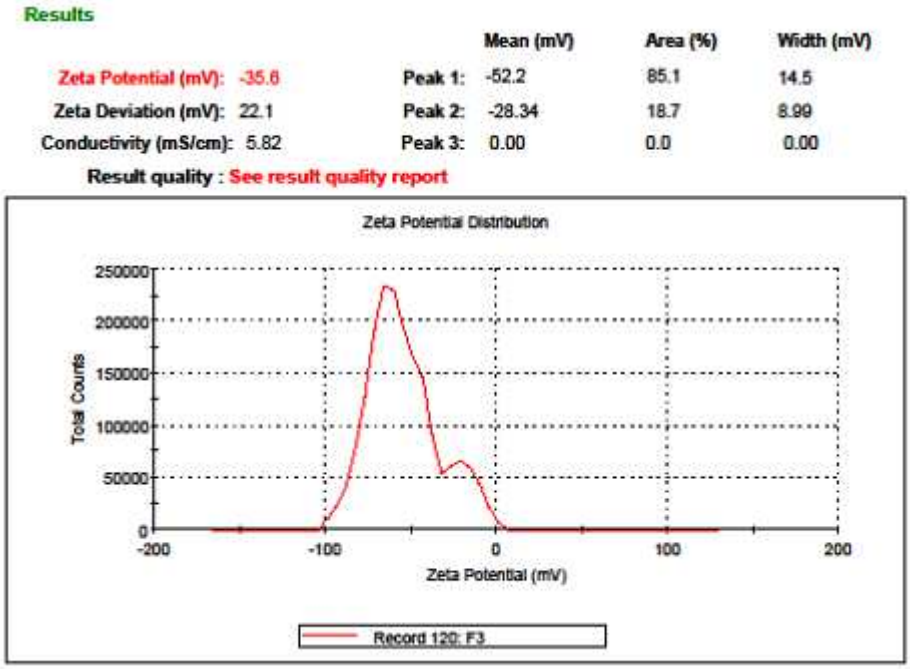

Fig.8: Zeta potential report of Formulation F3

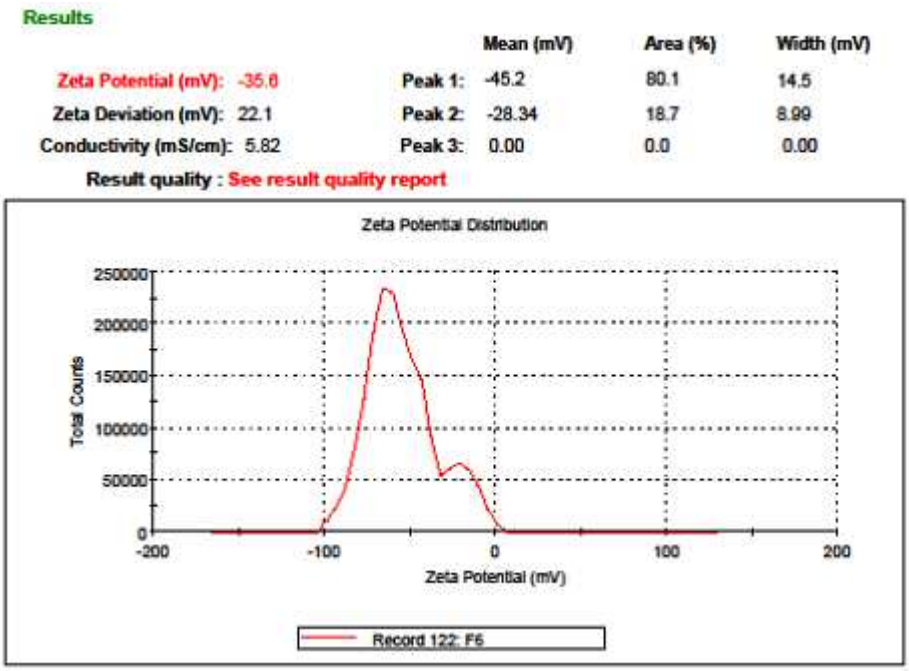

Fig. 9: Zeta potential report of Formulation F6

Table 4: Drug content in formulations

Formulation	Drug: Cholesterol: Surfactant	% Drug Content	% Entrapment Efficiency
F1	1 : 0.5 : 0.25	99.8 ± 0.06	33.7
F2	1 : 0.5 : 0.50	99.2 ± 0.08	52.6
F3	1 : 0.5 : 0.75	98.6 ± 0.07	69.3
F4	1 : 0.5 : 1	98.4 ± 0.11	72.5
F5	1 : 0.5 : 1.25	98.2 ± 0.01	74.3
F6	1 : 0.5 : 1.50	97.9 ± 0.06	76.6

The % entrapment efficiency in all formulations was found in the range of 33.7 – 76.6% (Table 4). The highest % entrapment efficiency was exhibited by F6 formulation and least was found in F1 formulation. Though the particle size of F6 formulation was similar to that of remaining formulations, it exhibited more % entrapment efficiency. This may be due to the formation of more number of niosomes per sq.mm area. Hence F6 can be considered as optimum formulation for the loading of the maximum amount of Candesartan in niosomal formulation.

The ability of the lamellar surfactant phase to accommodate drug, depends upon the structure of the surfactant phase. The higher entrapment may be due to the solid nature, hydrophobicity, and high-phase transition temperature of the Span60. The length of the alkyl chain influences the hydrophilic-lipophilic balance (HLB) value of the surfactant and the lower the HLB value of the surfactant, the lower will be the entrapment efficiency.

The *in vitro* drug release profiles were shown in the Fig. 10**.** Among the six formulations from F1 to F6 prepared by the ether injection method, F1 shows maximum drug release in 6 h. The order of percentage of drug release in 8 h was F1 >F2 > F3 > F4 > F5 >F6. Formulations F1, F2 and F3 have shown the complete release of the drug in 6, 7 and 8 h respectively. Formulation F4, F5 and F6 has shown the release of drug around 85, 79 and 77 percent.

The experimental studies showed that the rate of drug release depends on the percentage of drug entrapment efficiency. As the concentration of Span60 increases, entrapment efficiency of the formulation increased, and as entrapment of the drug was increased the release rate was lowered.

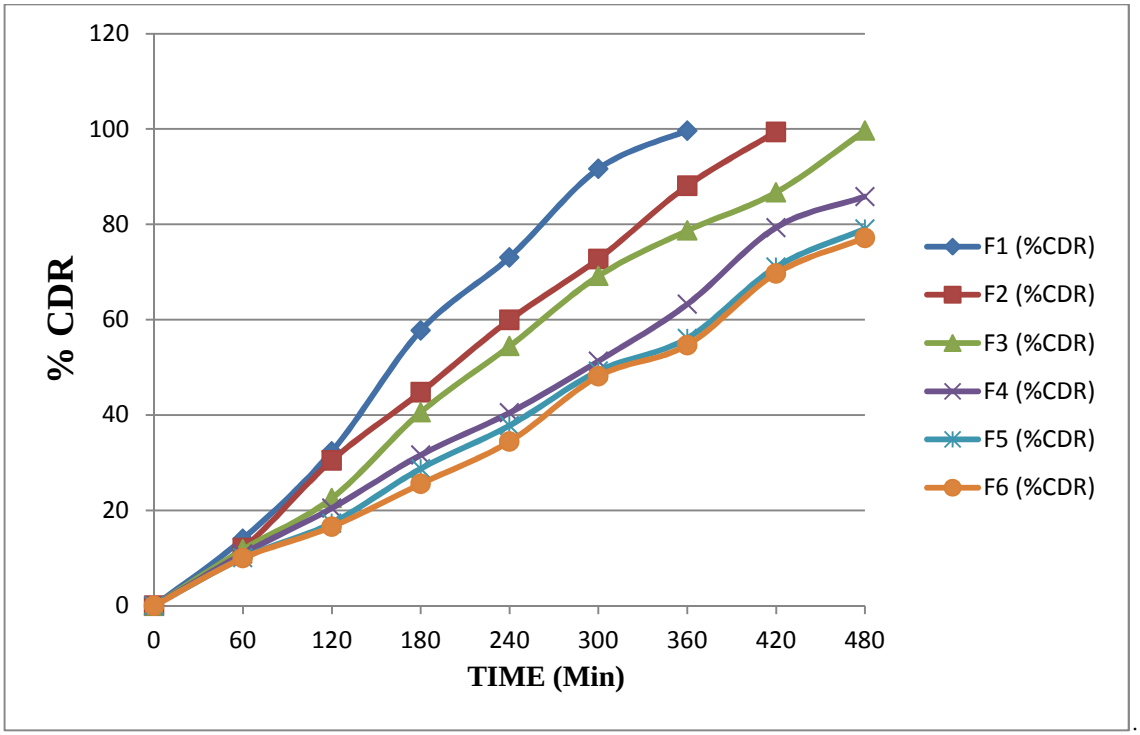

Fig.10: Candesartan *in vitro* release profiles from all formulations

In vitro drug release kinetics (Table 5) shows that in most of the formulated tablets, the r^2 values (0.997) were higher in zero-order models than in first-order (0.937) model, indicating that the drug release from most of the tablets was according to zero-order kinetics and thus showing that the drug release rate was independent of the residual concentration of drug. The mechanism of drug release from polymer-based matrices are complex and not completely understood. Some systems may be classified as either purely diffusion or erosion controlled, while most systems exhibit a combination of these mechanisms. The r^2-values (0.993) obtained for fitting the drug release data to the Higuchi equation indicated that the drug release mechanism from these tablets was diffusion controlled. By using Korsmeyer-Peppas equation, the *n* values obtained > 1 for all formulations. These values are characteristic of anomalous kinetics (non-Fickian) and super case-II transport, suggesting that more than one mechanism may be involved in release kinetics, referring to the combination of diffusion and erosion based drug release mechanism.

Table 5: *In vitro* drug release kinetics of all formulations

Formulation code	Zero order		First order		Higuchi		Korsmeyer-Peppas		Drug release mechanism
	r^2	Slope	r^2	Slope	r^2	Slope	r^2	Diffusion exponent (n)	
F1	0.988	0.294	0.759	0.005	0.912	0.048	0.988	1.124	Super case II
F2	0.997	0.241	0.722	0.004	0.993	6.888	0.992	1.068	Super case II
F3	0.992	0.213	0.676	0.003	0.990	6.380	0.992	1.049	Super case II
F4	0.995	0.181	0.913	0.001	0.965	5.458	0.997	1.007	Super case II
F5	0.996	0.165	0.939	0.001	0.969	4.992	0.995	1.015	Super case II
F6	0.993	0.163	0.937	0.001	0.957	4.927	0.989	1.019	Super case II

A stability study for the formulation F6 niosomes was carried out as per ICH guidelines for 3 months. Leakage of the drug from the prepared niosomes was analyzed in terms of percentage drug retained. At refrigerated condition the niosomal formulation F6 showed 81.25% at (4º±2ºC). At room temperature (25°±2°C) it showed 67.54% and at 40º±2ºC and 75%±5%RH it showed 51.32%. Further the drug release profile was also found to be good in all the three temperature conditions. From this study it was found to be good in all the three temperature conditions. From this study it was found that storage under refrigerator condition showed greater stability.

Table 6: Stability study of Formulation F6

Temperature	Drug retained (in %) after following months		
	01	02	03
4º±2ºC	95.82	88.36	81.25
25°±2°C	89.96	78.72	67.54
40º±2ºC and 75%±5%RH	85.32	72.36	51.32

CONCLUSION

Niosomes containing Candesertan were formulated using varying concentrations of a non-ionic surfactant Span60. From the above study it can be concluded that an increase in concentration of Span60 increased the entrapment of the drug in niosomes which caused to decrease in drug release. Formulation F6 which contained more amount of Span60 has shown more entrapment and slow release of the drug. The drug release followed the zero order with supercase II mechanism. The stability analysis suggested that niosomes stored under refrigeration were more stable comparatively with other storage conditions

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REFERENCES

[1] JB Shukla; SJ Patel. *Int J Pharm Pharm Sci.,* **2010,** 2 (4), 143-146.
[2] AJ Baillie; AT Florence; LR Hume; GT Muirhead; A Rogerson. *J Pharm Pharmacol.,* **1985,** 37 (12), 863 - 868.
[3] AT Florence. *Chemistry and Industry.,* **1993**, 24, 1000-1004.
[4] D Akhilesh; KB Bini; JV Kamath. *Int J Res Pharm Biomed Sci*., **2012,** 3(1), 6 – 12.
[5] R Parthibarajan; S Pradeep kumar; NL Gowri Shankar; I Balakishan; B Srinivas; V Bhagya laxmi; A Radha; K Sheshma. *Int J Pharm Pharm Sci.*, **2013**, 5(3), 604-611.
[6] TV Vaishnavi; B Sowjanya; V Umamaheshwara Rao. *Int J Pharm*., **2014,** 4 (1), 289-297.
[7] R Manivannan; A Balasubramaniam; R Senthilkumar; G Sandeep; S Sanaullah. *J Pharm Res.,* **2008,** 1 (2), 163-166.
[8] SP Vyas; RK Khar. Targeted and Control Drug Delivery, 1st Edition, CBS Publishers and Distributors, New Delhi, **2002**, 249-276.
[9] PM Satturwar; SV Fulzele; VS Nande; JN Khandare. *Ind J Pharm Sci.,* **2002**, 64(2), 155–158.
[10] A Rogerson; J Cummings; N Willmott; AT Florence. *J Pharm Pharmacol.,* **1988**, 40 (5), 337—342.
[11] D Deamer; AD Bhangham. *Biochimica et Biophysica Acta (BBA) - Nucleic Acids and Protein Synthesis.,* **1976**, 443 (3), 629-634.
[12] T Yoshioka; B Sternberg; AT Florence. *Int J Pharm.,* **1994**,105 (1), 1–6.
[13] EA Essa. *Asian J Pharm.,* **2010**, 4 (4), 227-233.
[14] A Elbary; HM El-laithy; MI Tadros. *Int J Pharm.,* **2008**, 357 (1-2), 189–198.
[15] M Nidhin; R Indumathy; KJ Sreeram; U Balachandran U. *Bull Mater Sci.,* **2008**, 31 (1), 93-96.
[16] Z Sezgin-Bayinder; N Yoksel. *AAPS Pharm Sci Tech.*, **2012**, 13 (3), 826–835.
[17] MN Azmin; AT Florence; RM Handjani-Vila; JF Stuart; G Vanlerberghe; JS Whittaker. *J Pharm Pharmacol*., **1985**., 37(4), 237-242.
[18] N Udupa; KS Chandraprakash; P Umadevi; GK Pillai. *Drug Dev Ind Pharm.,* **1993**, 19(11), 1331-1342.
[19] G Parthasarathi; N Udupa; P Umadevi; GK Pillai. *J Drug Target.,* **1994**, 2(2), 173-182.
[20] K Ruckmani; V Sankar. *AAPS PharmSciTech.,* **2010**, 11(3), 1119-1127.
[21] P Shivanand. *Int J Pharm World Res.,* **2010,** 1(3), 1-11.
[22] MA Schubert; CC Müller-Goymann. *Eur J Pharm Biopharm.,* **2003**, 55(1), 125-131.