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Formulation And In-Vitro Evaluation Of Candesartan Cilexetil Loaded Nanosponges For Solubility And Dissolution Enhancement

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ABSTRACT

Candesartan cilexetil (CC), a antihypertensive drug, a selective AT1 subtype angiotensine II receptor antagonist. CC is a BCS class II drug and has poor water solubility and dissolution rate, which results in a low bioavailability. In the present investigation an attempt has been made to increase the solubility of candesartan cilexetil by preparing nanosponges containing CC using ethyl cellulose and eudragit rs100 by emulsion solvent diffusion method. Nanosponges loaded with CC (CN1-CN8) were synthesized and thoroughly evaluated in terms of physicochemical attributes. Amongst all, CN3 was recognized as an optimized formulation with Particle Sizes (PS): Mean PS (756nm), Z-Average PS (1721.5nm), Poly Dispersity Index (0.844), Zeta potential (-21.1Mv), and Entrapment Efficiency (EE=85.06%).The prepared nanosponges containing CC were further characterized by FTIR, SEM image of optimized nanosponges revealed particles were spherical and spongy in nature. Saturation solubility of batches CN1-CN8 were performed and batch CN3 found to have highest solubility (10.22µg/ml). The dissolution of CN3 (78.57%) was also found to be improved as compared to pure CC (36.7%). Thus nanosponge technique may a potentially effective method for increasing solubility and dissolution rate of poorly water soluble drugs.

KEYWORDS Candesartan Cilexetil, Nanosponges, Emulsion Solvent Diffusion, solubility, dissolution

INTRODUCTION

Majority of drugs are poorly water soluble and are facing challenges in designing oral drug delivery system. The reduction of materials to nano size has significantly increased the effectiveness of such drugs.[1] Medical professionals have long struggled with getting medications to the right place in the body and managing their release to prevent overdose. This problem may be solved by developing a brand-new, complex molecule called a nanosponge (NS). A vast variety of different compounds can be contained in the tiny particles that make up nanosponges, which have cavities only a few nanometers across.[2] By altering the pharmacokinetic properties of the active ingredients, nanosponges can solubilize poorly water soluble drugs, provide extended release, and improve

medication bioavailability. Due to their internal hydrophobic chamber and exterior hydrophilic branching, nanosponges have the unmatched flexibility to load both hydrophilic and hydrophobic medicinal molecules. Nanosponges have a three-dimensional network or scaffold.[3] Angiotensin-receptor blockers (ARBs) like candesartan can be used alone or in combination with other medications to treat hypertension. It is given orally as the prodrug candesartan cilexetil, which quickly breaks down to become its active metabolite, candesartan, following absorption in the digestive tract. By inhibiting the renin-angiotensin-aldosterone system (RAAS), candesartan decreases blood pressure. It competes with angiotensin II for binding to the type-1 angiotensin II receptor (AT1) subtype and counteracts angiotensin II effects on blood pressure elevation. ARBs do not have the side effect of dry cough, unlike ACE inhibitors, which do. Treatment for hypertension, isolated systolic hypertension, left ventricular hypertrophy and diabetic nephropathy may involve candesartan. It can also be used as an alternate medication to treat heart failure, systolic dysfunction and other conditions. It is a BCS class II drug with a high permeability and low solubility. Candesartan Cilexetil has a poor oral bioavailability (15%) due to its extremely low aqueous solubility (5×10^{-5} mg/ml).[4] Therefore CC loaded nanosponges utilizing ethyl cellulose and eudragit rs100 were tried to prepare in the present work to increase its solubility.

MATERIALS AND METHODS

Candesartan cilexetil was obtained from Yarrow lab, Mumbai, ethyl cellulose, eudragit rs100, polyvinyl alcohol, dichloromethane, etc. were used of analytical grade.

FORMULATION OF CANDESARTAN CILEXETIL LOADED NANOSPONGES

Emulsion solvent diffusion method was used to formulate Candesartan Cilexetil loaded nanosponges by using ethyl cellulose and eudragit rs100. Various ratios of drug:polymer taken to formulate CC loaded nanosponges as in table 1.

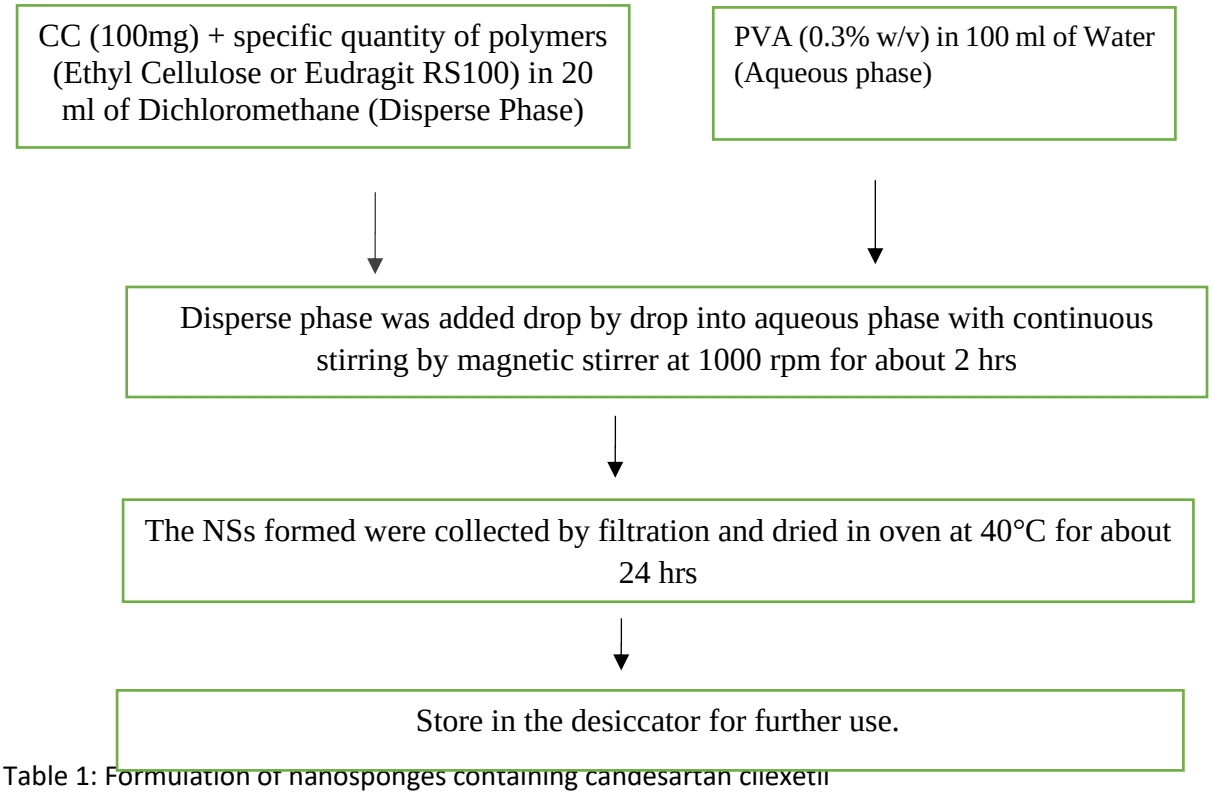

Table 1: Formulation of nanosponges containing candesartan cilexetil

Ingredients	Formulation code							
	CN1	CN2	CN3	CN4	CN5	CN6	CN7	CN8
Candesartan Cilexetil (mg)	100	100	100	100	100	100	100	100
Ethyl cellulose (mg)	50	100	150	200	-	-	-	-
Eudragit RS100 (mg)	-	-	-	-	50	100	150	200
Polyvinyl Alcohol (%w/v)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Dichloromethane (ml)	20	20	20	20	20	20	20	20
Water (ml)	100	100	100	100	100	100	100	100

Melting Point Determination: Melting point of CC is determined by using Digital Melting Point Apparatus (Labtronics pvt. Ltd., Haryana) and it is found to be 163.66°C.

Solubility: Solubility of CC was determined in different solvents such as ethanol, water, dichloromethane, DMSO & methanol and results depicted in Table 2.

Table 2: Solubility of candesartan cilexetil in different solvents

Sr. No.	Solvent	Slightly Soluble	Sparingly Soluble	Insoluble
1.	Dichloromethane	-	✓	-
2.	Ethanol	✓	-	-
3.	DMSO	-	✓	-
4.	Water	-	-	✓
5.	Methanol	-	✓	-

Construction of calibration curve

Preparation of stock solution

Pure CC 10 mg was precisely weighed and dissolved in 10 mL volumetric flask using methanol. The stock (primary) solution of candesartan cilexetil, with a concentration of 1000 µg/ml, was prepared by adding methanol to the volume until it reached the desired level. From this primary stock solution, prepared stock II of concentration 100 µg/ml. To create a working standard solution of 10, 20, 30, 40 and 50 µg/ml the aforementioned stock solution was further diluted using methanol. Solutions were scanned in range of 200 nm to 400 nm wavelength. Candesartan cilexetil maximal wavelength of absorption was discovered to be 258 nm. CC concentration was plotted on the X-axis, and their relative absorbances were plotted on the Y-axis, to create the calibration curve. [5]. In the calibration curve, linearity was obtained between 10 - 50 µg/ml concentration of CC and the regression value R^2 = 0.9994. Thus CC obeys Beer Lambert's Law and results are shown in Table 3 and Figure 1.

Table 3: Standard Calibration curve of Candesartan Cilexetil

Sr. No.	Concentration (µg/ml)	Absorbance at 258 nm
1.	10	0.32
2.	20	0.62
3.	30	0.93
4.	40	1.24
5.	50	1.56

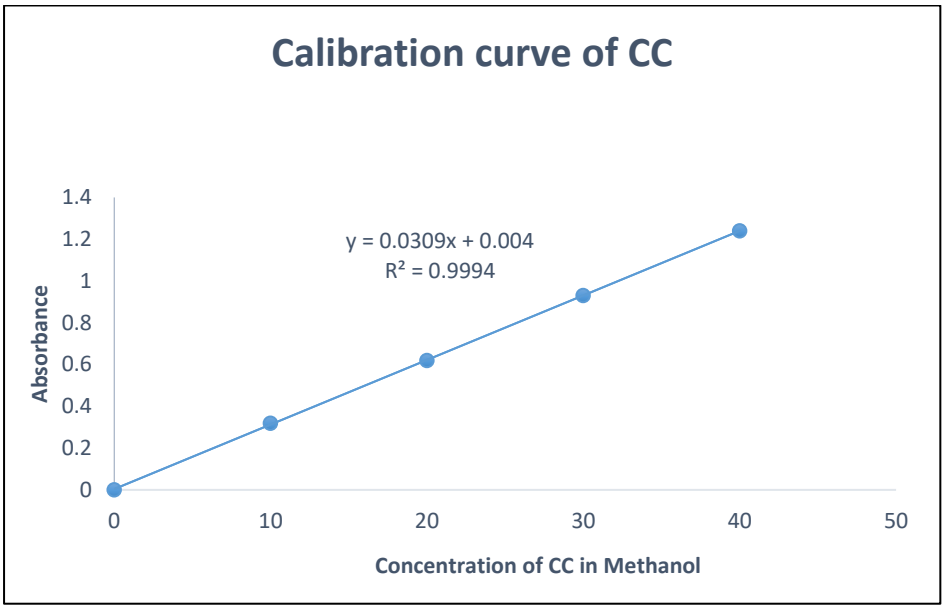

Figure1: Calibration curve of Candesartan Cilexetil

FTIR study for candesartan cilexetil and Excipients

Fourier transform-infrared spectroscopy (FT-IR) was used to conduct the drug and excipient compatibility assessments. After grinding the solid powder sample with 100 times the amount of potassium bromide in a mortar, the potassium bromide pellets were prepared using a KBr press. The spectra were obtained between 4000 and 400 cm-1 wavelength. [6]

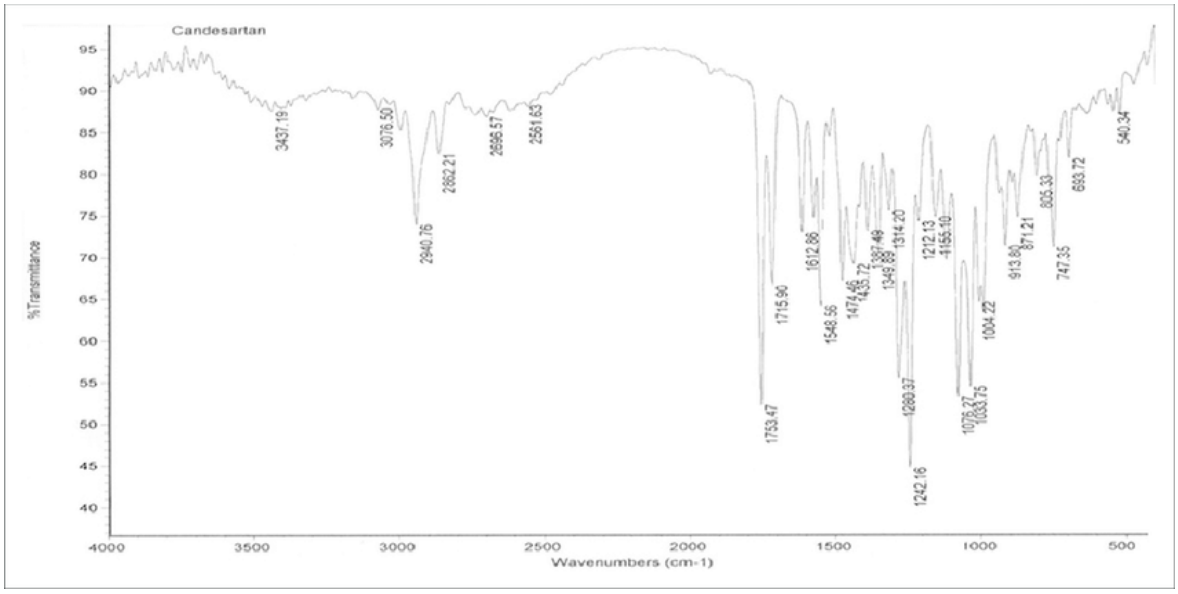

Figure 2**:** FTIR of candesartan cilexetil

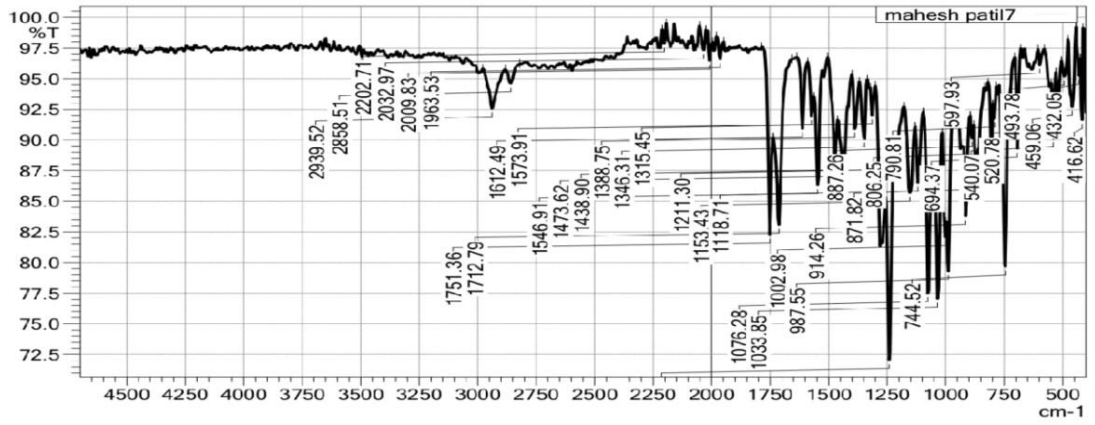

Figure 3: FTIR of physical mixture of candesartan cilexetil & polymers

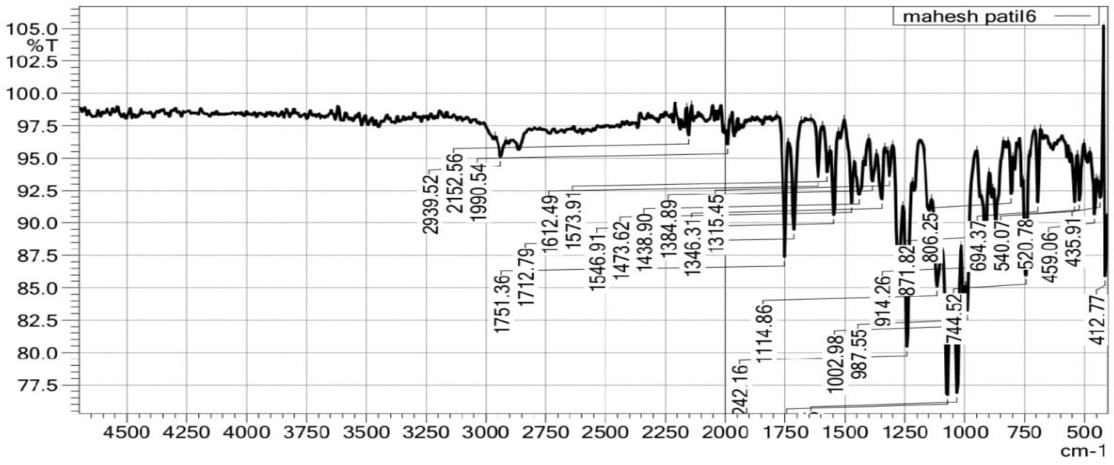

Figure 4: FTIR of candesartan cilexetil loaded nanosponges (CN3)

FTIR of CC, physical mixture of CC with polymers and optimized CC loaded nanosponges (CN3) reveals that the major functional groups responsible for pharmacological response were found to be unchanged therefore the CC is compatible with ethyl cellulose & eudragit rs100.

EVALUATION OF CANDESARTAN CILEXETIL LOADED NANOSPONGES

Production Yield [7]

Calculating the initial weight of raw materials and the final weight of nanosponges will give the production yield (PY).

$$\% PY = \frac{\text{Mass of prepared nanosponges}}{\text{Theoretical mass (polymer + drug)}} \times 100$$

The percentage yield was minimum for formulation CN8 (50%) and maximum for formulation CN3 (78.48%). From the results we can conclude that production yield is independent on polymers concentration. It can also be noted that the yield obtained while using ethyl cellulose as polymer is much higher when compared with eudragit rs100. The percentage yield of all formulations is depicted in Table 4.

Drug Entrapment Efficiency [8]

An indirect method was followed in order to measure the % Entrapment Efficiency (EE). The aqueous nanosponge dispersion was centrifuged (at 1200 rpm for 25 min) to separate the nanosponge particles, and the supernatant was then examined for the presence of free CC using UV spectroscopy at 258 nm. The % EE of CC loaded nanosponges were determined by using following formula.

$$\% EE = \frac{\text{(Initial amount of drug added - Drug amount in supernatant)}}{\text{Initial amount of drug added}} \times 100$$

The entrapment efficiency was found to be highest for CN3 formulation which is 85.06 % and the lowest entrapment of drug was found for CN4 formulation. The prepared nanosponges possess drug entrapment efficiency in the range of 69.80% - 85.06%. The EE of all formulations is depicted in Table.4

Table 4: Production yield and entrapment efficiency of nanosponges

Formulation	% P Y	% EE
CN1	67.8	75.25
CN2	57.8	76.42
CN3	78.48	85.06
CN4	71.56	69.80
CN5	72.13	82.91
CN6	51.25	82.93

CN7	62.8	79.74
CN8	50	76.97

Solubility study of candesartan cilexetil and candesartan cilexetil nanosponges

For determination of solubility known excess amount of Candesartan Cilexetil and Candesartan Cilexetil containing nanosponges were added in 10 ml glass vials containing distilled water. The vials were stirred for 24 hrs using magnetic stirrer at room temp. The samples were filtered using whatmann filter paper 0.43µ and analysed by UV spectrophotometer at 258 nm.

Table 05: Solubility of candesartan cilexetil and candesartan cilexetil nanosponges

Sr. No.	Sample	Solubility (µg/ml)	Solubility in folds
1	Candesartan Cilexetil	0.711	1
2	CN1	2.23	3.136
3	CN2	4.301	6.049
4	CN3	10.22	14.374
5	CN4	0.809	1.137
6	CN5	0.647	0.909
7	CN6	3.915	5.506
8	CN7	2.97	4.177
9	CN8	3.495	4.915

The solubility of Candesartan cilexetil containing nanosponges (CN3) found to be 10.22µg/ml which is 14.374 folds more as compared with pure Candesartan Cilexetil 0.711µg/ml.

Determination of Particle size, polydispersity index and zeta potential[9]

A Horiba SZ 100 instrument is used to disperse the CC-loaded nanosponges at 25°C, and dynamic light scattering, also known as photon correlation spectroscopy (PCS), is used to measure the particle size (z-averaged diameter) and polydispersity index (as a measure of the particle size distribution width). Prior to testing, all samples were diluted with ultra-pure water to obtain the proper scattering intensity. A disposable sized cuvette was filled with the nanosponge dispersion, diluted and put into the cuvette holder of the device for analysis. Air bubbles were removed from the capillary before measuring. Zeta potential is a measure of surface charge. Using a Zeta sizer (Horiba SZ 100 Instrument) with zeta cells, a polycarbonate cell with gold-plated electrodes and water as the sample preparation medium, one may determine the surface charge (electrophoretic mobility) of nanosponge. It is essential for the characterisation of stability of the nanosponges. Particle size (z-averaged diameter) ,polydispersity index and Zeta Potential was measured by using Horiba SZ-100 Zeta sizer.

Table 6: Particle size, polydispersity index and zeta potential of nanosponges

Formulation	Particle size (nm)		Zeta Potential

	Mean	Z- Average	Polydispersity index	
CN1	747.1	2842.3	0.581	-25
CN2	248.7	1601.9	1.16	-27.6
CN3	756	1721.5	0.844	-21.1
CN4	598	1939.1	0.571	-28.2
CN5	485.4	1067	0.874	-22.9
CN6	182	567.1	0.738	-18.8
CN7	194	605.7	0.494	-24.3
CN8	325.6	989.7	0.572	-22.2

Formulation CN3 was optimized based on the % EE, %PY, particle size, solubility study and zeta potential and was used for further research.

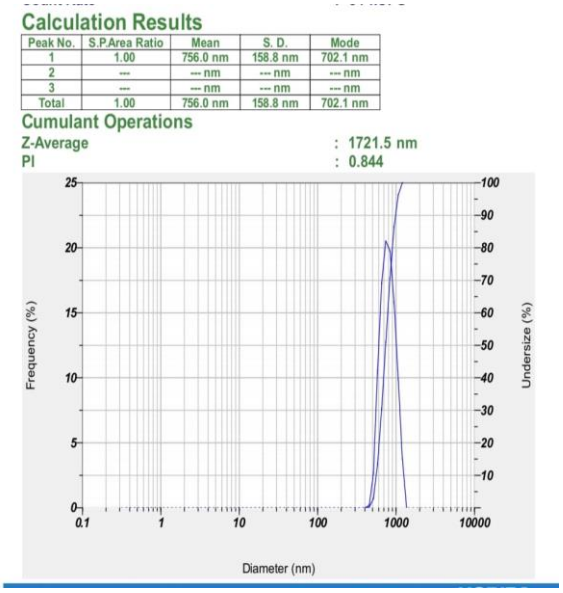

Figure 5: PS & PDI of CN3 NS

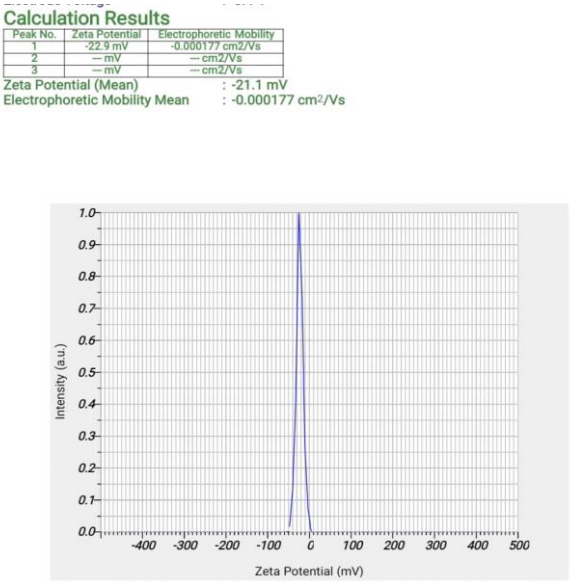

Figure 6: Zeta potential of CN3 NS

Surface morphology (SEM)[11]

The microscopic characteristics (shape & morphology) of the prepared Candesartan Cilexetil nanosponges were studied by SEM examination. Images of the nanosponge were captured using scanning electron microscopy (Shimadzu Analytical, Tokyo, Japan) at various magnifications, after they had been manufactured and thoroughly dried to reduce moisture content. Samples were placed on a glass slide that was held under vacuum and using a sputter coater unit that was operating at a 15 kV acceleration voltage, samples were then coated with a thin layer of gold.

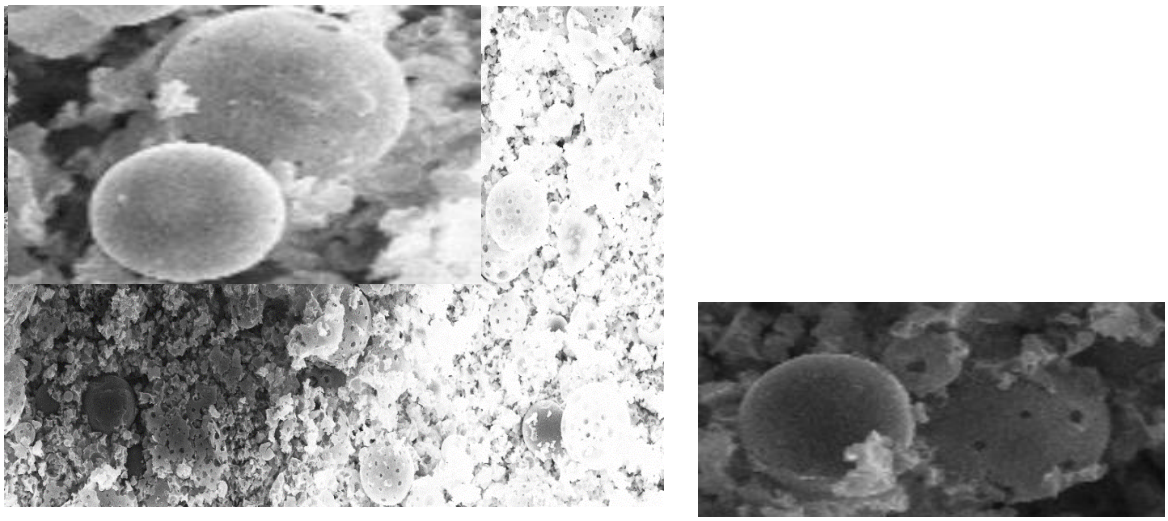

Fig.07: SEM images of candesartan cilexetil nanosponge (CN3)

The SEM images of CN3 Figure 7 showed the nanosponge was porous with a smooth surface morphology and spherical in shape. Due to evaporation of solvent, the nanosponge shell found to be smooth porous where outer surface was shiny smooth and inner surface was porous and spongy. The presence of pores was due to the impression of diffusion of the solvent dichloromethane.

In – vitro release study

The in-vitro release of Candesartan cilexetil from nanosponge was evaluated using the Dialysis Bag Diffusion method. 16 mg equivalent of CC loaded nanosponges were placed in the donor compartment of the dialysis bag and suspended in a 10 ml phosphate buffer pH 6.8. After that, the bag ends were sealed. The receptor compartment in which the dialysis bag was placed held 100ml of the buffer mixture and was kept at 37°C by rotating at 100 rpm. From the receptor compartment, an equal number of samples were taken out and replaced with the diffusion medium to maintain sink condition. The in vitro release of Candesartan cilexetil was detected spectrophotometrically at 258 nm (Table 7).

Table 07: In-vitro release study

Time (Minutes)	% Cumulative Release	
	Candesartan cilexetil	Candesartan cilexetil nanosponges (CN3)
30	4.73	22.15
60	8.46	25.18
90	12.21	28.22
120	14.40	35.80
150	14.60	47.94
180	18.66	52.49
210	23.06	58.56

240	23.97	61.59
270	25.64	64.62
300	27.45	66.7
330	29.9	69.02
360	33.61	74.21
390	36.7	78.57

The In-vitro release was found to be for Candesartan cilexetil (36.7%) and Candesartan cilexetil containing nanosponges (78.57%) at the end of 390 min (Table 7). The rate and extent of drug release found to be improved in candesartan cilexetil containing nanosponges as compared to pure candesartan cilexetil.

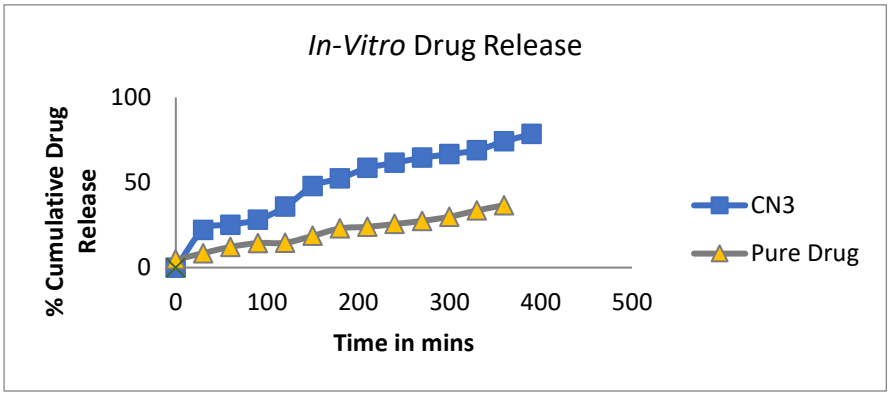

Figure 8: In-vitro drug release study

In-vitro drug release kinetics [13]

To evaluate the drug release mechanism, in-vitro release data were fitted into zero order, first-order, Higuchi, Hixon Crowell and Korsmeyer-Peppas kinetics models, and regression analysis was performed. In zero order kinetics, the rate of drug release is independent of concentration. In first order kinetics, the drug release rate is proportional to the concentration. Higuchi defined drug release from porous, insoluble matrix as a time dependent square root mechanism. The Korsmeyer-Peppas model illustrates how the proportion of drug release related to time exponentially. The data obtained from the in vitro release study was used to fit into kinetic models. This was done to find out the mechanism of drug release from candesartan cilexetil nanosponges. The preference of a certain mechanism was based on the coefficient of determination (R^2) for the parameters studied, where the highest coefficient of determination is preferred for the selection of the order of release (Table 8).

Table 8: Release kinetics of candisartan cilexetil containing nanosponges

Release Kinetics	X-axis	Y-axis	R^2	Linear Equation

Zero order equation	Time in mins	Cumulative % drug	0.972	y = 0.2095x + 13.211 R^2 = 0.972
First order equation	Time in mins	Log % cumulative drug release	0.976	y = -0.0017x + 1.9816 R^2 = 0.976
Higuchi release kinetic	Square root of time	Cumulative % drug release	0.9616	y = 4.0083x - 2.8956 R^2 = 0.9616
Hixon Crowell equation	Time in mins	Cube root of % drug release	0.9726	y = -0.0051x + 4.5481 R^2 = 0.9726
Korsmeyer Peppas equation	Log time	Log % cumulative drug release	0.9129	y = 0.5453x + 0.4692 R^2 = 0.9129

Since R^2 value is higher for first order equation for CN3, it is selected as the best fitted model and it indicates drug release is to be dose dependent.

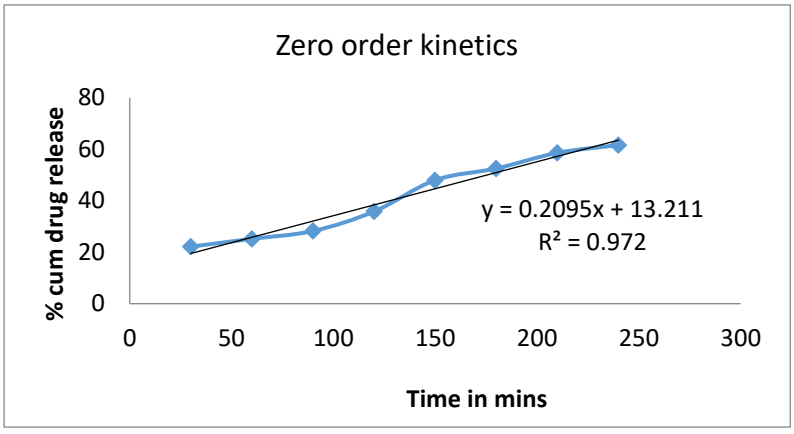

Figure 9: Zero order kinetics

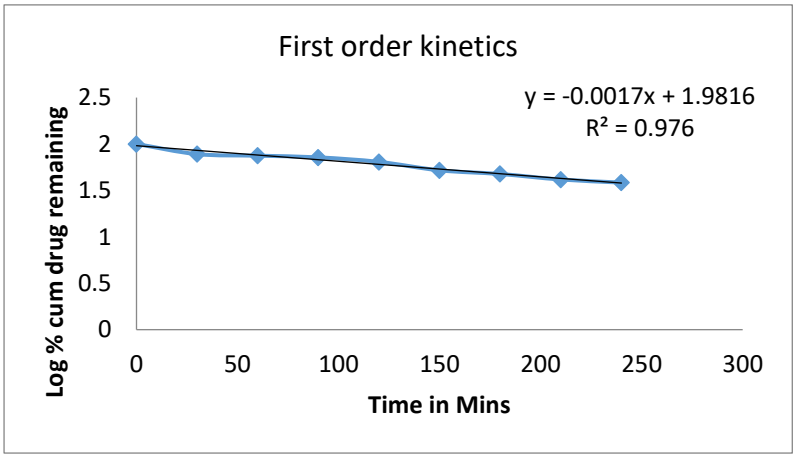

Figure 10: First order kinetics

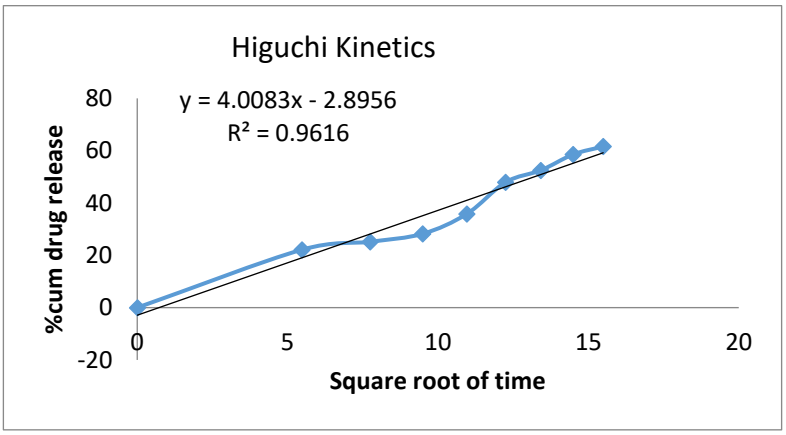

Figure 11: Higuchi kinetics

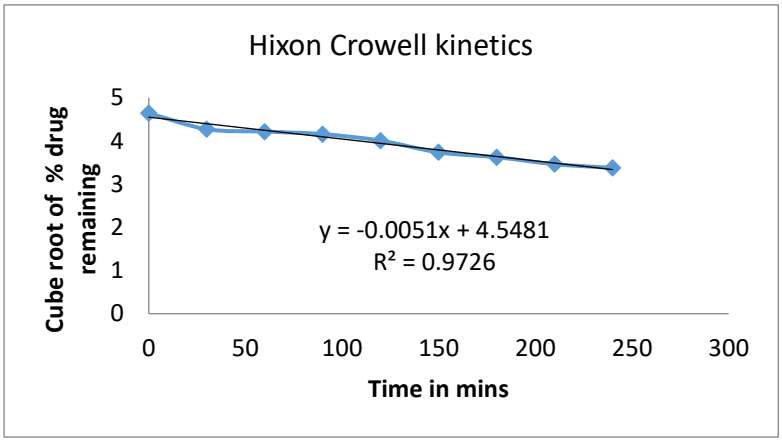

Figure 12: Hixson crowell kinetics

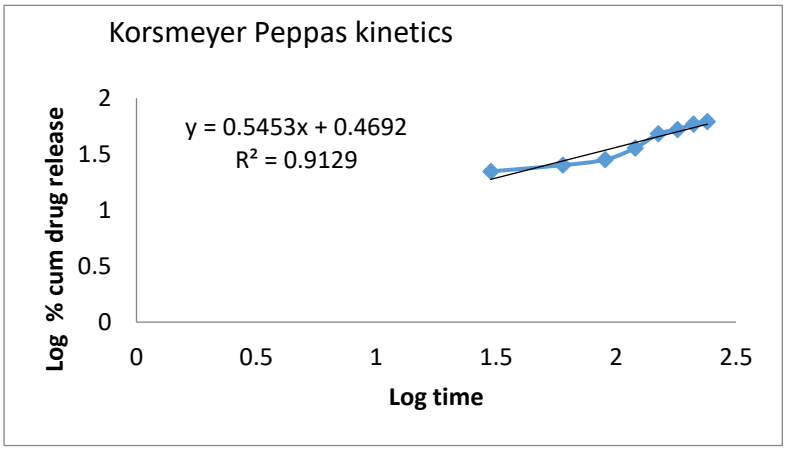

Fig.02 Prepared Nanosponges

Figure 13: Korsmeyer peppas kinetics

CONCLUSION

Ehtyl cellulose-based candesartan cilexetil-loaded nanosponges (CN3) have been successfully developed using emulsion-solvent diffusion method and was further evaluated by Particle Size (PS): Mean PS (756 nm), Z-Average PS (1721.5 nm), PDI (0.844), & EE (85.06%), FTIR, SEM, saturation solubility and in vitro drug release. Based on preliminary characterization, CC-loaded nanosponges (CN3) was considered as the optimized formulation and was evaluated for morphology, porosity, in vitro release and solubility determination. The optimized nanosponges (CN3) presented a better sustained release with improved rate and extent of solubility and dissolution. Hence, it was concluded that the developed nanosponges benefits from its nanosize, porous nature and may promise better therapeutic efficacy. However, further in vitro-in vivo correlation need to be done in this regard.

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Conflict of interest

The authors have declared no conflict of interest

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