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Research Article

Formulation and Evaluation of Candesartan Cilexetil Nanosuspension for Oral Drug Delivery System

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ABSTRACT

Nanocrystals are small particles whose size typically ranges from 1 to 100 nm. Nanocrystal-based nanosuspension is a drug delivery technique that involves incorporating drug nanocrystals into a nanosuspension matrix. The nanocrystal-based nanosuspension approach can be particularly useful for drugs with poor solubility or low bioavailability. The current study aimed to develop and characterize a nanocrystals-based nanosuspension of candesartan cilexetil (CC). In the preparation, CC-loaded nanosuspension was prepared to employ Eudragit RLPO and PVA as a stabilizer by using the solvent precipitation method. The drug content (%), DR (%) and zeta potential are performed for all formulations. The optimized formulation (CCSD2) had a particle size of 221 nm, a zeta potential of 30.4 mV, and a drug release rate of 95.58%, and it was used for further testing. The optimized formulation had a polydispersity index (PDI) of 0.218. SEM revealed drug nanocrystal agglomeration, which could have been caused by the water removal process. DSC showed a minor change in crystallinity, which could be attributed to the presence of lactose. The stability trial lasted 6 months. The solvent precipitation method is an efficient way to create a CC nanocrystal with lower particle size. DSC thermogram confirmed no interaction between the drug and excipients. The DR release study of the CC nanosizing method. CC are efficiently and successfully confined inside the polymer. CC and other class II medicines may therefore find a promising carrier in the nanocrystal method.

Introduction

Candesartan cilexetil (CC) represents an ester prodrug to candesartan (C) intended for promoting lipophilicity and enhancing penetration along absorption. CC has limited and irregular bioavailability, varying from 15 to 40%, with research evaluations revealing disparities and contradictions regarding its effectiveness as a potential hypertensive medication.[1] Several investigators attribute CC's poor or unpredictable bioavailability to the great lipophilicity along with poorly soluble in water, whereas some suggest it is due to early breakdown by esterase enzyme within the lumen of the bowel before digestion, resulting in low permeability primary molecule candesartan.[2]

CC becomes active after the degradation of its ester bond through gastrointestinal esterase molecules, producing the working compound candesartan, which is responsible for its antihypertensive effect. Following oral administration, CC itself was not detected in plasma; only the active metabolite candesartan was observed.[3]

It has been observed to ensure ester prodrugs, their biosynthesis ratio for active components are unexpected or varies based on the availability of ester enzymes within the system as well as variances in substrates accuracy, leading to pharmacology also toxicology differences. Numerous investigations have been conducted to improve the effectiveness of CC, utilizing both traditional methods like integration complexes to candesartan cilexetil

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alongside β-cyclodextrin, liqui-solid pills, or advanced nano medication delivery technologies consisting of solid lipid nanoparticles, nanoemulsions, self-emulsifying drug delivery systems, niosomes, and nanocrystals.[4]

CC, a BCS type II drug that has low solubility and high permeability and is classed mild acid having pKa of about 6, determining alkaline surroundings above its pKa could be a useful technique to improve its solubility and bioavailability. Recent studies have shown that incorporating tris with challenging drugs can significantly enhance dissolution rates, bioavailability, solubility, stability, and membrane permeability. Tris, a synthetic amines additive utilized as both buffers as well an alkalinizing substance in acid resistance therapy, has been shown to increase esterase activity, reduce bioavailability variability, and reduce changes to ester bond breakage throughout prodrug processing. The choice of an appropriate vehicle is critical to developing a successful nanosuspension preparation, as the carrier's qualities have a substantial impact on drug-dissolving characteristics. Literature indicates that incorporating either acidifiers or alkalinizes into nanosuspension formulations of weakly alkaline or acidic medicines having low water solubility can improve solubility rates by altering the pH of the surrounding within diffusion level. For instance, studies have demonstrated that the nanosuspension of telmisartan with PEG 6000, combined with various alkalinizes, resulted in a notable improvement in dissolution rates and, consequently, enhanced bioavailability. [5-10]

The objective of this endeavor is to improve the effectiveness to CC, a strong and targeted cardiovascular medication with a fluctuating and limited bioavailability, by creating nanocrystals by a simple nanosuspension method effectiveness of CC, a strong and targeted cardiovascular medication with fluctuating and limited bioavailability, by creating nanocrystals by a simple nanosuspension method. This approach involved utilizing tromethamine, an alkaline esterase activator carrier, to address the controversial effects of esterase enzymes and improve the drug's performance.

Pharmaceutical nanosuspensions are water dispersion containing undissolved medication particles that become stable with surfactants. In contrast, nanomaterials are medication vehicles in the form of polymeric or lipid colloids. If a medication ingredient possesses several limitations, like failure to produce salt, high molecular weight as well as dose, large log P, as well as a melting point, developing acceptable formulations becomes impossible. One important drawback to molecular complexation utilizing cyclodextrin in pharmaceutical preparations with their intrinsic propensity for improving preparation volume due to the substantial molecular weight of the complexing agent. Nanosuspensions address the particular medication administration challenges related to vigorous pharmaceutical ingredients (API) by keeping them in crystalline condition while allowing for greater medication loading during preparation progress. Accommodating big medication amounts with less dose volume offers further benefits in parenteral as well as ocular drug delivery systems since it reduces the need for toxic non-aqueous solvents along with extreme pH. Other benefits included improved stability, sustained drug release, greater efficacy via tissue targeting, minimal first-pass metabolism, as well as deep lung deposition.

Several investigations focused on creating nanosuspensions loaded with CC to enhance its oral bioavailability. To improve oral bioavailability, CC-loaded nanosuspensions were created utilizing several methods, such as high-pressure homogenization and ultrasonication. Research has indicated that candesartan cilexetil nanosuspensions have enhanced solubility, dissolving rate, and bioavailability in comparison to traditional formulations. Enhancing the stability and shelf-life of candesartan cilexetil nanosuspensions has also been studied through the application of stabilizers, such as polymers and surfactants. All things considered; CC-loaded nanosuspensions appear to potential new oral administration technique for the treatment of hypertension.[11]

This research investigated the effectiveness of SNEDDS in increasing candesartan cilexetil absorbed through the mouth via blocking intestinal P-glycoprotein carriers, however, elevated levels of surfactant posed a difficulty.[12] This research investigates the creation of high bioavailability drugs for hypertension therapy, with an emphasis on the gastro-retentive drug distribution systems.[13] CC was produced utilizing bioadhesive oral film, resulting in greater bioavailability along with a shorter elimination half-life due to a simple, sturdy, as well as rough fabrication technique.[14] Scientists created CC-loaded solid lipid nanoparticles (CC-SLNs) that enhance oral bioavailability along with long-term efficacy with candesartan cilexetil, a medicine utilized to treat hypertension as well as heart failure.[15] This study attempts to increase the solubility along with oral bioavailability of CC, a BCS type II drug, by creating a nanocrystalline preparation.[16] To create a novel pill dosage form for candesartan cilexetil having superior P-gp inhibition with direct compression. Naringin pills delivered 82% of the medicine within 30 minutes, enhanced oral bioavailability, which remained stable over 6 months.[17] The goal is to increase the strength, solubility, as well as dissolution of candesartan cilexetil with hypertension. Investigators developed nanoemulsions with transcutol HP, tween 80, and a poloxamer combination, with cinnamon oil. Such nanoemulsions demonstrated greater rates of release as well as amounts in both conventional tablet forms also simple medication powder.[18] By applying glyceryl monostearate along with Caproyl 90 to develop a nanostructured lipid carrier (NLP) to CC, a drug utilized to treat heart failure as well as hypertension, researchers

discovered that the CC-NLC elevated oral bioavailability via twofold, demonstrating the nanostructured lipid carriers may greatly enhance poorly water-soluble drugs.[19] The study intended to develop mucoadhesive oral tablets with polymers such as carbopol-934P, HPMC, Eudragit RLPO, as well as Na-CMC. In-vitro investigations revealed optimum release, demonstrating zero-order kinetics via a diffusion process. Additional *in-vivo* investigations will be necessary to determine the formulation's bioavailability performance.[20]

Materials And Methods

Dr. Reddy's Laboratories, Hyderabad, generously provided the candesartan Cilexetil. We purchased Eudragit (RLPO) from Evonik Lab in Mumbai. While methanol (HPLC grade) was acquired from LOBA Chemie Pvt. Ltd. The remaining compounds were all pure analytical grade.

Preparation of Nanosuspension by Solvent Evaporation Method

Screened solvents like acetone and methanol were used in the solvent evaporation approach for the nano-precipitation method. The loading of CC into the nanosuspension was done. Numerous polymers and surfactants, such as PVA and Eudragit (RLPO), were assessed as stabilizers both singly and in combination. By optimizing processing dimensions and attributes, such as stabilizer type, drug-to-surfactant ratio, solvent-to-antisolvent ratio, and rotating speed, the desired zeta potential (ZP) and particle size were reached. Sonication was performed utilizing an Ultrasonic Processor VC505 from Sonics & Materials Inc., USA, after nanoprecipitation in the combined procedure incorporating probe sonication. Five minutes of a five-second on, three-second-off, 25% amplitude pulses were used during the sonication. In the combination approach utilizing high-pressure homogenization (HPH), a suspension was prepared using the same nanoprecipitation technique and then HPH was applied using a Panda PLUS 1000 from GEA NiroSoavi, USA. After five cycles at 500 bar, the homogenization was carried out for fifteen cycles at 800 bar. During identical day also throughout the course of three successive days, each batch was made in triplicate (n = 3).[21]

Lyophilization of Candesartan Cilexetil-loaded Nanosuspension

A Christ Alpha 4D Plus lyophilizer from the USA was used to lyophilize an optimum batch to guarantee the stability of the nanosuspension. Two possible cryoprotectants that were examined were lactose and sodium starch glycolate. Initially, a 10% concentration of these cryoprotectants was introduced. After 48 hours of freezing at -80°C and thawing at ambient temperature, the nanosuspension underwent two cycles of freezing and thawing. Samples were first frozen at -45°C for lyophilization, and then they were dried both primary and secondary. Primary drying was started at -30°C and 250 mT of pressure. There were sporadic holds at -25°C for different lengths of time. Subsequent drying was carried out at 10°C for 300 minutes at 150 mT of pressure after the initial drying process.[22,23]

Characterization of Candesartan Cilexetil-loaded Nanosuspension

Particle size, poly-dispersity index and zeta potential

A particle sizer (HORIBA) based on dynamic scattering of light has been employed to calculate the polydispersity index (PDI) along with average particle size. With the same apparatus, zeta potential testing was also carried out. 1 mg of the lyophilized product was once more disposed of in 3 mL of Milli-Q water for analysis. To make sure the sample was spread evenly, vortexing was done as needed. Three measurements were taken for each.[24]

FTIR study

With the aid of a BRUKER Alpha II FTIR Spectrophotometer, FTIR spectra were acquired for a variety of materials, including pure medication, excipients, physical mixes, and improved formulation. After each sample, weighing around 5 mg, was examined, its spectra were collected between 400 and 4000 cm^{-1}. [25]

DSC study

The refined formulation, physical mixes, excipients, and pure pharmaceuticals were all subjected to differential scanning calorimetry (DSC) examination utilizing Shimadzu DSC-50 equipment that is based in Kyoto, Japan. Each sample was carefully weighed out to a volume of 40 mL in standard aluminum crucible pans. The lids were then sealed with tiny holes. The temperature range that was used to heat the samples was 0°C through 400°C, with a pace at 50°C each minute. The nitrogen environment was used for the analysis, and 40 mL per minute of flow was used. Double-sided adhesive tape was used to place the crucibles onto a steel platform in preparation for the analysis.[26]

XRD study

To assess the sample's physical condition, X-ray diffraction (XRD) was utilized. Cu/Ni radiation was used in an XRD instrument (Xpert MPD, Philips, Holland) to document the drug's XRD patterns. A scan rate of 2°/min was applied to the diffractograms, covering a range of 0° to 50° 2θ. Sample holders were filled with crushed samples that had been ground with a mortar and pestle before analysis. [27]

Determination of Encapsulation Efficiency

Three hours at 10,000 rpm and 7°C were spent centrifuging the formulation using a cooling centrifuge (24BL model, Remi, Mumbai, India). A UV-visible spectrophotometer (Shimadzu 1800, Japan) has been utilized to determine absorbance to the free medication concentration at 257 nm following the supernatant's separation. The entrapment

effectiveness of CC is determined by subtracting the quantity of released drug from the initial quantity of medication administered.[28] The entrapment efficiency (%EE) for each formulation was calculated with the help of the formula given below:

Entrapment Efficiency (EE%) = Initial Drug – Final drug / Initial Drug × 100

Stability Studies

To conduct stability experiments for the nanosuspension, the formulation was stored at 4°C, and samples were taken on 1, 2 and 3 months. Using the Zetasizer (HORIBA) and the previously outlined technique, the particle size as well as zeta potential for every sample have been measured. Three duplicates of each study were conducted. [29]

In-vitro Drug Release Study

The diffusion method of a bag for dialysis was used to study medication absorption *in-vitro* in both raw and powdered forms. Every dialysis bag has been produced as well as tightly packed with frozen powder or raw medication (dose equivalent: 2 mg). Each of these bags was subsequently placed under 250 cc of phosphate-buffered saline (pH 7.4) to preserve sink conditions. The temperature required for organizing was maintained above 37 ± 0.5°C using continual electromagnetic stirring at 100 rpm/min. At specific times, 5 mL of substance were removed *via* the receptor chamber and replaced with a new medium. A 48-hour discharge study was conducted. The amount of drug soluble has been measured using UV spectrophotometry at 241 nm, following a predetermined protocol. [30]

In-vivo Pharmacokinetics Study of Candesartan Cilexetil-loaded Nanosuspension

Protocol of experiments

Using the guidelines of the Association with Research, rat research is conducted to evaluate pharmacokinetic parameters like Cmax, Tmax, and AUC for prepared nanocrystal, nanocrystal-based nanosuspension and marketed formulation of candesartan. All animal procedures were authorized by the Institutional Animal Ethics Committee (IAEC) of the Dr. Shivajirao Kadam College of Pharmacy Kasabe Digraj, Sangli, Maharashtra (Protocol number: IAEC/21/DRSKCP/2023-24). Rats were split into three categories of nine rats each over a 12-hour fast. These rats, which weigh between 0.25 and 0.30 kg, are housed in a climate-controlled setting that has a 12-hour light-dark cycle and a constant temperature of 22/30°C. They have free access to water as well as normal food. The test group of rats was given a sedative, and an electric clipper was used to trim the hair on their abdomens. The free CC suspension is equivalent to 10 mg/kg of CC and is distributed in a 0.5% sodium carboxymethyl cellulose solution (1.0 mg/kg). The retro-orbital vein was utilized for drawing serial blood samples (0.3 mL) at 0.5, 1, 2, 4, 6 and 8 hours after injection.

All three groups were given the subsequent topical gel therapies:

- First group administered a dosage 8 mg/kg of optimized formulation of CC suspension (plane)
- The second group administered a dosage of 8 mg/kg of CC nanosuspension.
- The third group administered a dosage of 8 mg/kg of the marketed formulation of CC.

Blood Processing

Next, 200 µL blood samples were extracted through femur arteries at predetermined times until 24 hours after the injection. The samples were combined with heparin to impede the coagulation of blood. The sample was centrifuged at 5000 rpm for about 5 to 10 minutes to extract the plasma, and it was then stored at -20°C.

Chromatographic Analysis using HPLC

Utilizing ACN-5 mM sodium acetate (80:20, v/v) (pH adjusted to 3.5 using CH_3COOH as mobile phase with a flow speed of 0.8 mL/min), chromatographic separation of CAC has been accomplished on a Waters Reliant C18 column (250 × 4.6 mm, 5 µm) shielded via precolumn guard cartridge. The analyte was observed at 234 nm. The analytical column has been maintained over a temperature of 30°C.

Results

Particle Size, Poly-dispersity Index and Zeta potential

Prepared batches particle sizes varied from 203 to 264 nm. The biggest particle size was detected by the CCNS-9, while the smallest was detected by the CCNS-1 among the equipment employed. Particle size and processing variables, including polymer and surfactant content, were found to be correlated. With median particle sizes (less than 260 nm), the CCNS-3, CCNS-4, CCNS-5, CCNS-6, and CCNS-8 batches stood out as being appropriate for oral administration. Furthermore, as Table 28 illustrates, there was a direct correlation between average particle size and polymer content in the CCNS formulations, the result indicates that the concentration of drug-polymer ratio increases the practical size also increases.[31,32]

Zeta potential is an essential parameter in determining stability as well as surface charge for nanoparticulate systems. Larger magnitudes of zeta potential, whether positive or negative, generally enhance stability by promoting electrostatic repulsion amongst particles having similar charges, thus preventing aggregation. Zeta potential values for CC nanocrystals are presented in Table 1

and Fig. 1. The optimized formulation demonstrated a zeta potential of around 20.32, indicating improved stability. This stability is influenced by variations in polymer and surfactant concentrations.

Determination of Encapsulation Efficiency

Table 1 illustrates that with a larger ratio of polymer to drug, the drug encapsulation efficiency rise from 19.23 ± 2.11 to 92.69 ± 1.98%.

Compatibility Study by FTIR

It was necessary for the FTIR research to confirm the likelihood of a chemical bond interaction between the medicine and the formulation's excipients. In Fig. 2, the combined infrared spectra of the formulation, drug and polymer in physical combinations, polymer and drug, and pure drug were displayed. In purified CC, FTIR spectrum reveals several prominent peaks, such as symmetric C-O-C stretching about 1074.31 cm^{-1}, asymmetric C-O-C stretching about 1751.88 cm^{-1}, C-O stretching with in-plane bending about 1031.89 cm^{-1}, C=O stretching about 1751.88 cm^{-1}, and C-H out-of-plane bending about 745.50 cm^{-1}. [33]

Peaks around 3432.1 cm^{-1}, indicating the existence of tertiary amine, about 1731.4 cm^{-1} owing to C=O (ester), as well as about 1450.2 cm^{-1} owing to –CH3 bending were visible in FTIR spectra for Eudragit RLPO. Key peaks in the drug-polymer physical combination were found at 1728.6 cm^{-1} to C=O (ester), 1174.8 cm^{-1} to C–O stretching, 1604.1 cm^{-1} to C=C (aromatic stretching), 1453.1 cm^{-1} to –CH3 bending, 1728.6 cm^{-1} for the carboxyl group, and 2960.9 cm^{-1} for O–H (carboxylic acid). According to the formulation's spectrum, there are distinctive peaks at 3029. O-H axial deformation, which peaked in 2031, was the cause of the 62 & 65 cm^{-1} because of the C=O stretching (ketone), which peaks at 1423. Triazole, or N=N=N stretching, was the cause of the 65 cm^{-1} peak at 1485.28 cm^{-1} caused by the presence of N=N=N stretching (triazole), the peak at 1485.28 cm^{-1} caused by the presence of C-H bending vibration, peak about 1099.63 cm^{-1} was due to presence C-O stretching primary alcohol.

Differential Scanning Calorimetry

The properties of melting and re-crystallization of different substances are investigated using differential scanning calorimetry (DSC) and represented in Fig. 3. The CCNS-9 formulation, lactose, PVA, Eudragit RLPO, CC, and DSC thermograms were examined. A notable endothermic peak at 171.91°C was seen in candesartan cilexetil, suggesting that it is a crystalline substance. Melting endotherms were clearly visible for Eudragit RLPO at approximately 268.85°C and for PVA at 200 and 198.3°C. The physical mixture's thermal curve showed distinct melting point endotherms at 137.02, 138.32, and 224.89°C. Furthermore, a peak at 149.68°C was presented by the CCNS-2 formulation. The medicine and other ingredients in the formulation do not appear to interact significantly, according to these results.

XRD Study

The drug's distinctive peaks at 2θ values of 172.29, 122.12, 19.8, 21.6, and 26.08 were found by X-ray diffraction (XRD) examination, confirming its crystalline shape. As seen in Fig. 4, however, upon lyophilization, the intensity of these peaks dramatically dropped, suggesting that the medication had partially amorphized in the lyophilized sample. Several researchers have noted this post-lyophilization amorphization or partial amorphization of the active component. The stress created by the freeze-drying procedure, which entails the crystallization and sublimation of water, is most likely what caused this transition. Certain solutes have structural alterations that result in amorphization during the cooling phase when the solution hardens. [34]

Dissolution Study

Candesartan cilexetil-loaded nanosuspension with an optimized formulation achieved a 99% drug release at

Table 1: Physicochemical characterization of candesartan cilexetil loaded nanosuspension

Formulation batches	*Particle size (nm)*	*Polydispersity index*	*Zeta potential (mv)*	*Encapsulation efficiency (%)*
CCNS-1	203	0.698	13.01	19.23±2.11
CCNS-2	209	0.321	16.87	38.20±1.32
CCNS-3	211	0.546	19.32	42.32±1.96
CCNS-4	221	0.214	12.88	51.36±1.95
CCNS-5	234	0.587	13.65	65.78±1.96
CCNS-6	239	0.369	14.21	72.65±1.78
CCNS-7	243	0.215	18.14	82.65±1.63
CCNS-8	252	0.365	19.63	89.65±1.74
CCNS-9	264	0.365	20.32	92.69±1.98

CCNS- Candesartan Cilexetil loaded Nanosuspension, nm –Nanometer, mv-Millivolts, % -Percentage

Table 2: Kinetic profiles of *in-vitro* drug release of nanosuspension

Formulation Code	R^2 R^2 *n(slope)*			*Korsemeyer-Pappas equation*	
	Zero order	*First order*	*Higuchi kinetics*		
F1	0.9739 ± 0.12	0.9564 ± 0.21	0.9356 ± 0.55	0.8729 ± 0.68	0.4572 ± 0.91
F2	0.9898 ± 1.02	0.9522 ± 0.42	0.9564 ± 0.65	0.8878 ± 0.78	0.4251 ± 0.98
F3	0.9944 ± 0.23	0.9598 ± 0.74	0.9666 ± 0.12	0.9085 ± 0.84	0.4284 ± 0.75
F4	0.9987 ± 0.89	0.9897 ± 0.80	0.9598 ± 1.85	0.8863 ± 1.24	0.4363 ± 1.24
F5	0.9812 ± 0.41	0.9978 ± 1.23	0.9698 ± 0.74	0.8828 ± 1.45	0.4469 ± 1.10
F6	0.9897 ± 1.12	0.9987 ± 1.10	0.9789 ± 0.68	0.933 ± 1.14	0.4535 ± 0.67
F7	0.9822 ± 1.10	0.9632 ± 0.21	0.9898 ± 1.25	0.9468 ± 1.24	0.4785 ± 1.45
F8	0.9798 ± 1.30	0.9978 ± 0.41	0.9987 ± 1.41	0.9551 ± 1.66	0.4613 ± 1.12
F9	0.9932 ± 1.40	0.9878 ± 1.20	0.9899 ± 1.74	0.9253 ± 0.88	0.4374 ± 0.75

R^2 – Regression coefficient, n-Slope

Table 3: Results of *in-vivo* pharmacokinetics study

Parameters	*Candesartan cilexetil nanocrystal*	*Candesartan cilexetil nanosuspension*	*Marketed formulation of candesartan cilexetil*
AUC (µg/mL.hr)	3825.98 ± 39.85	6987.78 ± 21.98	4178.69 ± 18
C_{max} (µg/mL)	3.457 ± 19.36	20.789 ± 0.6549	6.985 ± 2.456
T_{max} (min.)	4 ± 0.2567	6 ± 0.45	5 ± 0.246

AUC- Area under the curve, C_{max} Maximum concentration, T_{max} – Maximum time, µg/mL.hr- Microgram per mililiter.hour, µg/mL- Microgram per milliliter, Min. – Minutes, ± - Standard deviation

60 minutes. The medication release amount has been very large in early intervals. Fig. 5 illustrates how the nanosuspension outperformed the solution in terms of drug release overall.

Drug Release Kinetics Study

Experimental *in-vitro* dissolution data, fitted in the Korsemeyer-Peppas model (log cumulative percentage to medication release vs. log time), yielded slopes and R2 values displayed in Table 2. *In-vitro* release kinetics for every CC-integrated nanosuspension was examined by the researchers.[35] The regression coefficient (r^2) using different kinetic equations is displayed in the table for each batch of nanosuspension. The best fit for *in-vitro* release through nanosuspension has been found to be the Korsemeyer-Peppas (KP) model, according to the examination of the kinetic data. Slopes of this model were used to construct diffusional release exponent 'n,' which varied from 0.655 to 0.850. Given that these values are all between 0.43 and 0.85, it appears that all formulations have Fickian release kinetics (Fig. 6). This suggests that nanosuspension integrated with candesartan and cilexetil intended for topical treatment follows a Fickian diffusion mechanism. Using this method resulted in a more uniform dispersion of candesartan cilexetil, as the *in-vitro* investigations showed (Table 2).

In-vivo Pharmacokinetics Study of Candesartan Cilexetil-loaded Nanosuspension.

This investigation involves a locally given nanosuspension (plane), CC-loaded nanosuspension compared *via* a commercially available CC preparation, as shown in Fig. 7 upon 6.12 minutes of retention, the chromatographic study revealed only one peak, designated as CC. The plasma levels of candesartan have been measured at various periods utilizing an HPLC method. AUC, Cmax, and Tmax are some of the variables measured on blood samples from each of the three groups (Table 3). Cmax values have been determined

Table 4: Stability study of optimized batch of nanosuspension formulation (CCNS-9)

Months	*Room temperature (4 ± 2°C)*		
	Particle size (nm)	*Zeta potential (mv)*	*Entrapment efficiency (%)*
0	221.2 ± 1.32	13.2	92.91 ± 1.23
1	217.38 ± 2.11	13.1	92.65 ± 1.73
2	228.77 ± 1.97	13.6	91.23 ± 1.65
3	222.5 ± 2.45	13.5	91.55 ± 2.98

Nm-Nanometer, mV-Millivolts, %-Percentage, °C- Degree Celsius, ± - Standard deviation

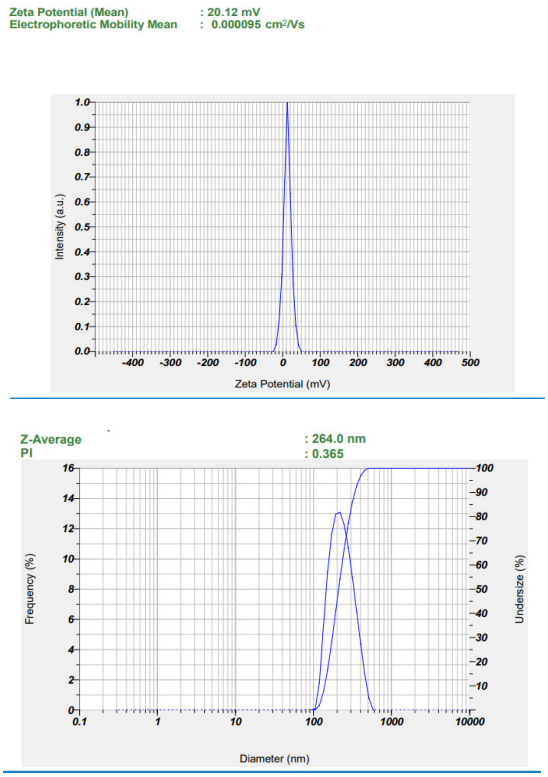

Fig. 1: Analysis report of nanocrystal (Batch RN-9) size distribution by intensity

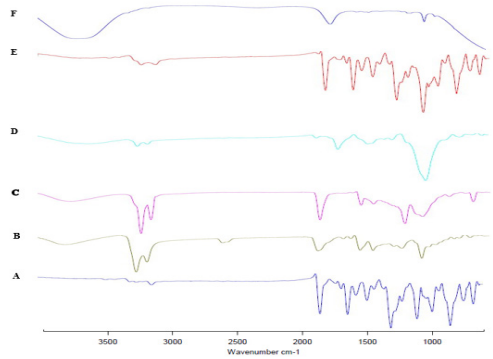

Fig. 2: FTIR layout of A) Candesartan cilexetil, B) Eudragit RLPO C) PVA D) Lactose E) Physical mixture (F) Formulation (CCNS-9)

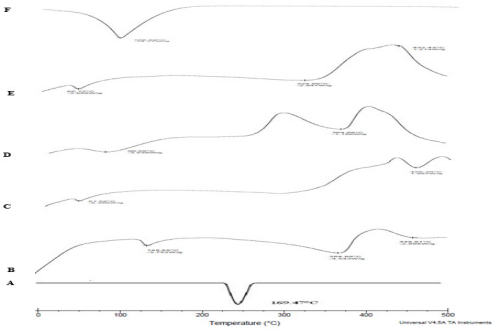

Fig. 3: DSC layout of A) Candesartan cilexetil, B) Eudragit RLPO C) PVA D) Lactose E) Physical mixture (F) Formulation (CCNS-9)

by Candesartan cilexetil optimum formulation (6.985 ± 0.456), commercial preparation (3.457 ± 19.36), and plane nanosuspension (2.789 ± 0.6549). The Tmax values were 4 ± 0.2567 minutes, 3 ± 0.246 minutes, or 2 ± 0.45 minutes, respectively. The optimized solution had an AUC value of 6987.78 ± 21.98 µg/mL.hr, while the marketed formulation had an AUC value of 4178.69 ± 18 µg/mL.hr. Ultimately, with the planar nanosuspension, its AUC value was 3825.98 ± 39.85 µg/mL.hr. The results obtained show the newly optimized Candesartan cilexetil formulation achieved the highest Tmax, Cmax, and AUC. Additionally, the Cmax of candesartan cilexetil-loaded nanosuspension was five times greater compared to that of plane nanosuspension along with twice that of the marketed preparation. Furthermore, the AUC revealed that the candesartan cilexetil formulation had increased bioavailability, as it was greater for the optimized dosage form than the alternative preparation.

Statistical Analysis

All results are analyzed by using sophisticated software (Design-Expert, version 11.0). It was clear by using the result that all of the dependent variables had *p-values* lower than 0.05 ($p < 0.05$). It was observed that the ZP, DR, and DC models F had values of 12.88, 90.42, and 87.11, respectively.[36] It highlights how important the model is. R-squared is a statistical degree to which the data agree with the fitted regression curve. In several regression instances, it is also known as the coefficient of prediction. In linear regression models, the goodness-of-fit measure known as R-squared is employed. The dependent variable's proportion of variance by the independent factors taken together is displayed.[37]

Stability Studies

For a period of three months, the drug nanosuspension did not change much in size at 4°C. Minimal increases in the size of particles were seen when sampling at different intervals. Nanosuspensions are frequently unstable due to Ostwald ripening, a phenomenon where larger crystallites grow at the expense of smaller ones. Physical instability is generally associated with a rise in size and which causes nanosuspension development into the micron range and sample non-homogeneity.[38] Look at Table 4. With just minor size increases, the nanosystem in this work demonstrated physical stability under storage conditions. Owing to the faster storage conditions, stability tests at 25°C showed a somewhat bigger rise in particle size compared to samples kept at 4°C. The larger kinetic energy at higher temperatures, which raises collision frequency and increases the likelihood of aggregation, is most likely the cause.[39]

Discussion

A measure of statistical significance called R-squared shows how well the results match the line to regression R-squared as an indicator of goodness to fit with models

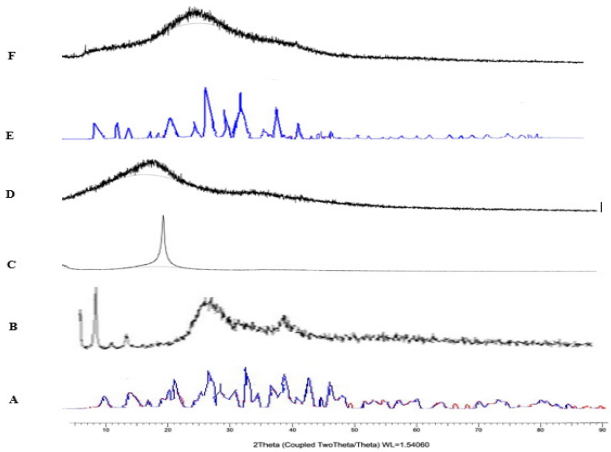

Fig. 4: XRD layout of A) Candesartan Cilexetil, B) Eudragit RLPO C) PVA D) Lactose E) Physical mixture (F) Formulation (CCNS-9)

for linear regression. In the framework of models of regression with numerous variables, this is often referred to as the degree of predictability and coefficient for several predictions. This statistic's value indicates what proportion of variance for the dependent variable will be determined by independent factors taken together. According to these findings, a greater amount of super disintegrant plus nanosuspension powder guarantees that the produced powder contains sufficient charge to stop vesicle agglomeration. Changes in concentration can influence particle size, with the super disintegrant playing a crucial role in the development of nanosuspension powder. According to reaction surface plots, particle size increases as the level of super disintegrant rises. Consequently, as the percentage of nanosuspension powder increases, the particle size also enlarges.

Three-dimensional graphs are made to measure the shift in the response surface for the measured responses. Based on the foregoing data on nanosuspension powder and disintegrant, it was determined that with an increase in concentration, created powder with increased drug absorption occurred. (Fig. 1A) Based on the foregoing data on nanosuspension powder and disintegrant, it was determined that with a rise in concentration, greater drug release was possible from the created powder. (Fig. 1B) Particle size may vary depending on the conc., and the disintegrant is crucial to the creation of nanosuspension powder. Zeta potential increases as the concentration of super disintegrants increases, according to the response surface plots. Consequently, zeta potential falls when nanosuspension powder concentration rises. (Fig. 1C).

When evaluating the degree of stability and electrical charge of nanoparticulate systems, zeta potential is an important consideration. Higher values of zeta potential, whether favorable or adverse, generally enhance stability by promoting electrostatic repulsion within particles with similar charges, thus preventing aggregation. Zeta potential values for CC nanocrystals are presented in Table 1. The

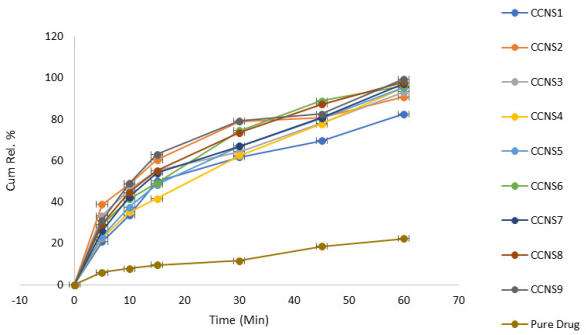

Fig. 5: Comparative drug release profile of all batches of nanosuspension

optimized formulation demonstrated a zeta potential of around 12.88, indicating improved stability. This stability is influenced by variations in polymer and surfactant concentrations.[26] The optimized formulation displayed a zeta potential of approximately 18.44, suggesting a formulation with enhanced stability, wherein alterations in polymer and surfactant. An *in-vitro* drug release study suggests that over a 10-hour period, the profile of release of the raw medication showed just a slight increase in the amount released. In contrast, throughout the same period, the manufacturing process demonstrated a steady and progressive rise in the release of drugs. In drug release kinetics, current topically applied CC-loaded nanocrystals follow the Fickian diffusion mechanism. According to in vitro research, using this technique produced CC which was distributed more evenly. Particle size determination experiments imply that the surface roughness of particles is greatly reduced by processing. Furthermore, Fig. 3 illustrates a strong correlation between the dimension

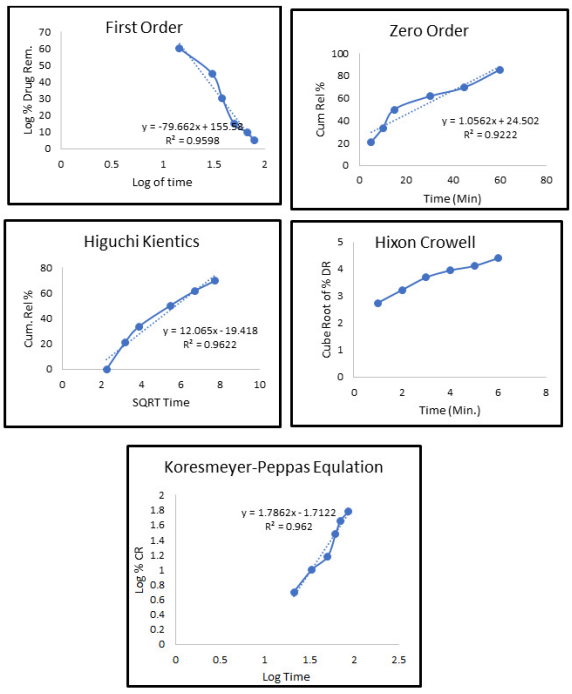

Fig. 6: Kinetic profiles of *in-vitro* drug release of nanosuspension

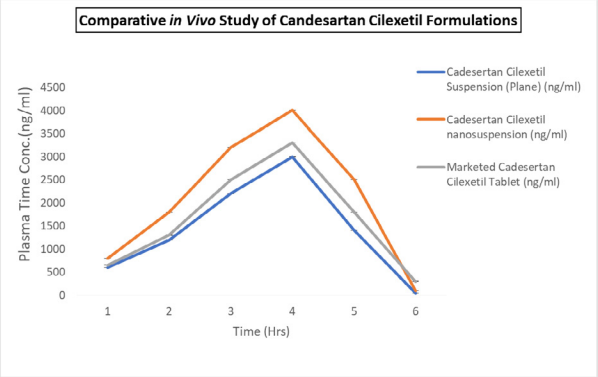

Fig. 7: Comparative *in-vivo* study of candesartan cilexetil formulations

of this nanoparticle as determined via dynamic light scattering DLS and the size detected by AFM. Studies on pharmacological nanocrystals, like the one involving amitriptyline hydrochloride, additionally demonstrate that smaller-sized nanoparticles possess a smoother surface than bigger agglomerates. Incompatibility studies, i.e., FTIR & DSC results findings suggest those there are not substantial interactions among medication as well as other components in the formulation.

In-vivo pharmacokinetic parameters determination, remarkably, the maximum concentration (Cmax) recorded using the CC-loaded nanosuspension was double as high compared with that of the CC suspensions plus more than five times higher in comparison to that of the commercially available CC tablet. Furthermore, CC nanosuspension's AUC was higher than both other formulations, suggesting better bioavailability.

Conclusion

Pharmaceutical experts continue to prioritize improving medicine absorption through permeability, dissolution, and solubility. Many *in-vivo* limitations, including lower bioavailability, increased dietary effects, higher inter-patient variability, and partial release from the dose form, are sometimes brought on by low drug solubility. Its solubility and permeability might be restricted by CC, a lipophilic compound that functions as a substrate for P-glycoprotein and is mainly insoluble in water. Lipophilic drugs like CC may benefit from extremely energetic unstructured develops, such as freezer-dried nanosuspension forms, whose solubility is increased by a carrier's hydrophilic forces and the dissolution of the crystal structure. Current work indicates ways to improve the high-energy solubility of amorphous forms in water.

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