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Original Article

QbD Based Development, Characterization and Pharmacokinetic Evaluation of Enzalutamide-Loaded Cyclodextrin Nano Sponges for Enhanced Drug Delivery

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ABSTRACT

Aim/Background: The aim of this study was to enhance the bioavailability of Enzalutamide by developing cross-linked cyclodextrin nanosponges loaded with the drug (EZL-CDNS). The objective of the work is to thoroughly investigate the impact of different molar ratios of DPC and cyclodextrin on the fundamental properties of EZL-CDNS, such as particle size, entrapment efficiency, and zeta potential. **Materials and Methods:** The Box-Behnken design methodology was employed to systematically investigate the factors affecting the properties of EZL-CDNS. Particle size, entrapment efficiency, and zeta potential were selected as response variables. The experimental design involved varying the molar ratios of DPC and cyclodextrin. The encapsulation of enzalutamide within the nanostructured carriers was confirmed using DSC and FTIR and morphology with SEM. **Results:** The EZL-CDNS exhibited a size distribution ranging from 295 to 471 nm, entrapment efficiency values spanning from 51.7% to 90.7%, and zeta potential values ranging from -15.3 to -28.1 mV. DSC and FTIR analyses confirmed the successful encapsulation of enzalutamide, leading to its transition into an amorphous state. SEM revealed the porous structure of EZL-CDNS. Importantly, *in vitro* drug release profiles demonstrated a significant improvement compared to pure EZL. Pharmacokinetic Modelling supported a zero-order release pattern, suggesting an enhanced bioavailability profile. EZL-CDNS exhibited a quicker onset of action, higher peak plasma Concentration (C_{max}), increased overall drug exposure, and the potential for a sustained release pattern. **Conclusion:** This study successfully developed and characterised EZL-CDNS with different DPC/cyclodextrin molar ratios. Nanostructured carriers had good size distribution, entrapment efficiency, and zeta potential. The increased *in vitro* drug release profile and pharmacokinetic modelling results suggest higher bioavailability and sustained release of enzalutamide. Further research is needed to confirm these findings and evaluate EZL-CDNS's clinical uses.

Keywords: Enzalutamide, Cyclodextrin, Nanosponges, Box-Behnken Design, Pharmacokinetics.

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INTRODUCTION

Enzalutamide is a novel androgen receptor inhibitor that has revolutionized the treatment of advanced metastatic Castration-Resistant Prostate Cancer (mCRPC).[1] Its mechanism of action, targeting the androgen receptor signalling pathway, has proven to be an effective strategy against prostate cancer growth.[2,3] Enzalutamide belongs to Biopharmaceutics Classification System (BCS) Class II, exhibits relatively low aqueous solubility, presenting a challenge in developing oral dosage forms. Its limited solubility may result in poor bioavailability, affecting drug absorption and systemic exposure.[4,5]

β-Cyclodextrin (βCD) is a cyclic oligosaccharide consisting of seven α-1,4-linked d-glucopyranosyl units.[6] The units are interconnected through the utilisation of different crosslinkers, including 1,4-butanediol diglycidyl ether, 2,6-naphthalene dicarboxylic acid, pyromellitic dianhydride, 6-hexamethylene diisocyanate, 1,1′-carbonyl diimidazole, and Diphenyl Carbonate (DPC).[7-9] This process results in the formation of β-Cyclodextrin Nano Sponges (β-CDNSs).

Cyclodextrins (CDs) are extensively employed in the pharmaceutical sector for the purposes of complexation, solubilization, stability enhancement, and the augmentation of dissolution and bioavailability of diverse pharmaceutical compounds.[10,11] Cyclodextrins (CDs) possess a Lewis base nature, which allows them to host guest supramolecular structures and improve their hydrophilic properties. The nanosponges formed by Cyclodextrins (CDs) exhibit hydrophilic and nanoporous characteristics, resulting in an augmented surface area.[12,13]

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The stability of β-Cyclodextrin Nano Sponges (β-CDNSs) is noteworthy, as they exhibit exceptional resistance to elevated temperatures, withstanding temperatures as high as 300°C. Furthermore, these CDNSs exhibit stability over a broad pH spectrum, ranging from 1 to 11. The fabrication of these nanosponges can be achieved through various techniques, including thermal desorption and ultrasound methods.[14]

The preparation of Cyclodextrin Nanosponges (CDNSs) of enzalutamide offers several pharmaceutical advantages. CDNSs can enhance enzalutamide's solubility and stability, leading to improved bioavailability and prolonged shelf life. They also allow for Sustained drug release, potentially reducing dosing frequency and improving patient compliance. CDNSs can be functionalized for targeted drug delivery, minimizing off-target effects and improving drug accumulation at specific sites. Furthermore, the adaptability of CDNS formulations offers a number of potential drug delivery system alternatives and suggests that CDNSs may improve the therapeutic efficacy of enzalutamide in treating metastatic prostate cancer and overcoming drug resistance.

Due to the benefits presented by cyclodextrin nanosponges and recognizing the limitations of enzalutamide, our team undertook efforts to develop enzalutamide-loaded nanosponges. We aimed to optimize the enzalutamide-loaded nanosponges utilizing Quality by Design (QbD) principles to ensure an efficient and effective formulation.

MATERIALS AND METHODS

Materials

Enzalutamide pure drug was gifted from RX Innovations Pvt. Ltd., Hyderabad. Cyclodextrin, Diphenyl carbonate and other organic solvents and reagents were procured from SRL Pvt. Ltd., Hyderabad. All the LC-MS grade solvents were procured from the Fischer scientific, Banglore, India.

Design of Experiments

The development of Enzalutamide-Loaded Cyclodextrin Nanosponges (EZL-CDNS) was conducted utilising a Box-Behnken design methodology.[15] This approach incorporated three distinct factors, namely the Crosslinker to Polymer ratio (A), Stirring speed (B), and Stirring time (C). The determination of the level ranges for each independent variable was based on preliminary tests and is outlined in Table 1. Seventeen model tests were randomly grouped using the Box-Behnken design model from Stat-Ease Design Expert® programme V13.1. In all experimental trials, the molar concentration of enzalutamide and the volume of the solvent were maintained at a constant level. Table 1 provides a comprehensive summary of the experimental settings employed in all model trials. The tests were carried out in accordance with the established design, and the results for the dependent variables, namely Particle size (Y1), Entrapment efficiency (Y2), and Zeta potential (Y3), were acquired and displayed in Table 2.

Synthesis of DPC crosslinked Cyclodextrin Nanosponges (DPC-CDNS)

Blank nanosponges were synthesized by crosslinking β-Cyclodextrin (βCD) with Diphenyl Carbonate (DPC). Different polymer-to-cross linker molar ratios (1:2 to 1:8) were taken as mentioned and allowed to react for 2-6 hr at around 90°C. To collect the resulting solid, the reaction mixture was kept aside to cool, and then filtered. Subsequently, the formed solid particles were broken down by gentle grinding, and Soxhlet installed extraction using ethanol for around 30 min to remove unreacted cross-linkers and other impurities. The solid material of obtained nanosponges (DPC-CDNS) were finally washed with alcohol and freeze dried.[16]

Loading of Enzalutamide (EZL) into DPC-CDNS

Enzalutamide and synthesized DPC-CDNS in defined weight ratios (1:1 w/w) were dispersed in dichloromethane and triturated and ultrasonicated for 15 min. The moist solid dispersion was then lyophilized, pulverized to obtain the Enzalutamide Loaded Cyclodextrin Nanosponges (EZL-CDNS) and stored in a glass-vial for further analysis.[17]

Characterization

Particle size, Polydispersity Index (PDI) and Zeta Potential (ZP) analysis

The particle size of all fabricated Enzalutamide Nano Sponges (EZL-CDNS) batches was analyzed by Malvern Instruments Ltd, Worcestershire, UK, to estimate the uniformity in particle size distribution and size range of the ENS. The ENS was appropriately diluted with distilled water before each analysis. The ZP of the same formulations was observed by a Zetasizer, Malvern Instruments Ltd, Worcestershire, UK. The device has functioned at a constant room temperature employing a clean disposable zeta cell. The average size, PDI and ZP of the ENS were evaluated and averaged after three observations.[18]

Encapsulation efficiency

For encapsulation studies, the dispersion of NSs was centrifuged at 20,000 rpm at 4°C for 30 min using a cold centrifuge (Sigma Labort Centrifuge GmbH, Germany). After centrifugation, the supernatant and the precipitation obtained were separated and analysed for the drug concentration using a UV spectrophotometer at wavelength 236 nm.[19]

The encapsulation efficiency was calculated using the following formula:

$$\%Encapsulation\ Efficiency = \frac{Amount\ of\ drug\ encapsulated}{Drug\ amount\ added\ initially\ in\ the\ formula} \times 100$$

$$\%Drug\ Loading = \frac{Amount\ of\ drug\ encapsulated}{Weight\ of\ Nanosponges} \times 100$$

DSC Studies

DSC curves of pure EZL, and DPC, β-CD and optimized EZL-CDNS were taken by thermo analytical technique using Scinco N650 (made in Korea). The sample (5 mg) were cramped into the hemispherical aluminium pan, individually, placed beside reference (empty-pan) in the chamber supplied with nitrogen (20 mL/min) and heated at a rate of 20°C in the temperature range of 50-250°C.[19]

FTIR Studies

The FTIR spectrums of pure EZL, β-CD, DPC and optimized EZL-CDNS were taken by triturating these samples with KBr (FTIR grade) and compressed into a transparent pellet using die. The scanning was done in the range of wavenumber 4000 to 400 cm^{-1}, each sample was passed through the IR intensity photons and fingerprints of functional groups present in the chemical entity was interpreted to propose the possible chemical interactions within the drug and β-CDNS.[19]

Surface Morphology

Scanning Electron Microscopy (SEM) of EZL-CDNS was performed with an SEM instrument (FEI QUANTA 200 SEM, USA) operating at 15, 20, and 30 kV for excitation. The samples are mounted on carbon tape and dried before gold coating to achieve approximately 300Å thickness. The prepared sample was then exposed to an electron beam operating at an excitation voltage of 10 kV and images were captured over a long period of time.[19]

In vitro Drug Release Profile

In vitro drug release studies of pure EZL and optimized EZL loaded DPC cross linked βCD NSPs were conducted using dialysis bag (Hi-media Mol. 12,000 Dalton) shake flask method. The suspension of samples was sealed in the dialysis membrane and bag is put-up in the conical flask with phosphate buffer pH 6.8 kept at temperature 37°C with continuous stirring at 100 rpm. At pre-determined time interval 1 mL sample is withdrawn from outer solution at each time interval and replaced by fresh PBS pH 6.8 medium. The aliquots were filtered and analyzed for drug release by using UV-spectrophotometer at 236 nm (Jasco V-630 Made in Japan). The experiment was done in triplicate. The drug release data were fitted to various kinetic models, including the zero order, first order, Higuchi, and Korsmeyer-Peppas models, by imposing relationships between the percentage of drug release per unit time, the percentage of log cumulative drug release per unit time, the percentage of log cumulative drug release per unit square root time, and the percentage of log cumulative drug release per unit log time, respectively. Based on the highest value of the correlation coefficient (R^2), the best-fit model for the release of the medication was selected. The value of the release-exponent, which suggests the mechanism of drug release, was computed using the slope and R^2 values of the plots.

In vivo Pharmacokinetics Study

In a comparative pharmacokinetic study, healthy adult male Wistar albino rats (n=6, weight 200-230 g) were employed to assess the pharmacokinetic profile of a newly synthesized optimized formulation of EZL-CDNS in comparison to a conventional EZL suspension. Rats were randomly allocated to one of two groups: the EZL-CDNS formulation (administered orally at 10 mg/kg) or the EZL suspension, which was dispersed in a 0.5% w/v carboxy methyl cellulose solution.[19] These animals, procured from Sai Life Sciences, were housed in recommended conditions with access to food and water. Ethical approval for the study was obtained from the Institutional Animal Ethics Committee (IAEC: 1292/ac09/ CPCSEA/2021/3). Following an overnight fast, blood samples (0.5 mL) were collected at specific time intervals (0, 0.25, 0.5, 1, 2, 4, 8, 12, 16, and 24 hr) after the administration of the respective formulations into pre-heparinized tubes. The collected blood samples were safely stored in deep freezers (maintained at -80±10°C) after centrifugation at 4500×g for 5 min to separate plasma from whole blood. The drug concentration in the plasma samples was determined using the LC-MS/MS method, following the protocol established by Kim *et al.* in their 2017 publication.[20] Non-compartmental pharmacokinetic analysis was conducted using the "Phoenix WinNonlin PK/PD Analysis Tool" by Certara, USA, to calculate various pharmacokinetic parameters.

Table 1: Summary of Experimental setting employed in the Box-Behnken design.

Factor	Name	Units	Minimum	Maximum	Mean	Std. Dev.
A	Crosslinker to Polymer ratio		2.00	8.00	5.00	2.12
B	Stirring speed	rpm	2000.00	4000.00	3000.00	707.11
C	Stirring time	Hrs	12.00	36.00	24.00	8.49
Response						
R1	Particle Size	nm				
R2	Entrapment efficiency	%				
R3	Zeta potential	mV				

Table 2: Summary of experimental results of dependent variables.

Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
	A: Crosslinker to Polymer ratio	B: Stirring speed	C: Stirring time	Particle Size	Entrapment efficiency	Zeta Potential
1	5	3000	24	295	87.6	-26.7
2	5	2000	12	425	58.7	-20.3
3	8	3000	12	471	67.4	-18.7
4	5	3000	24	309	90.7	-25.2
5	8	3000	36	377	54.6	-21.5
6	2	3000	36	365	70.2	-16.4
7	5	4000	36	374	53.3	-23.8
8	8	4000	24	402	55.9	-19.6
9	5	2000	36	328	70.5	-24.2
10	5	3000	24	301	87.1	-27.3
11	2	3000	12	380	64.2	-16.1
12	2	4000	24	349	59.9	-15.8
13	2	2000	24	362	63.2	-15.3
14	5	3000	24	320	89.3	-28.1
15	5	3000	24	298	86.7	-25.9
16	8	2000	24	407	51.7	-18.6
17	5	4000	12	365	69.4	-20.8

Table 3: Summary of analysis of variance of dependent variables.

Source	Particle Size		Entrapment efficiency		Zeta Potential	
	F-value	*p*-value	F-value	*p*-value	F-value	*p*-value
A-Crosslinker to Ploymer ratio	75.95	<0.0001	33.93	0.0006	26.30	0.0014
B-Stirring speed	1.93	0.2079	1.37	0.2806	0.3074	0.5965
C-Stirring time	72.96	<0.0001	5.37	0.0536	12.01	0.0105
AB	0.2406	0.6388	4.90	0.0624	0.0600	0.8135
AC	23.46	0.0019	30.81	0.0009	1.50	0.2601
BC	42.25	0.0003	67.85	<0.0001	0.1945	0.6725
A^2	160.37	<0.0001	318.98	<0.0001	181.97	<0.0001
B^2	39.81	0.0004	369.53	<0.0001	27.50	0.0012
C^2	118.86	<0.0001	130.83	<0.0001	12.49	0.0095
Lack of Fit	0.1983	0.8926	1.05	0.4634	0.5380	0.6811
R^2	0.9879		0.9934		0.9755	
Adjusted R^2	0.9723		0.9849		0.9440	
Predicted R^2	0.9584		0.9478		0.8601	
Adeq Precision	26.0870		27.1528		14.3637	

The results were expressed as mean±Standard Deviation (SD). Pharmacokinetic parameters, including elimination half-life ($T_{1/2}$), rate constant (kz), Mean Residence Time (MRT), peak plasma Concentration (C_{max}), time to reach peak concentration (T_{max}), Area under the Curve (AUC), and [(AUC_{0-24}) ($AUC_{0-\infty}$)], were estimated. A statistical comparison between the EZL-CDNS formulation and the standard EZL suspension was performed using an unpaired t-test, with statistical significance set at $p<0.05$.

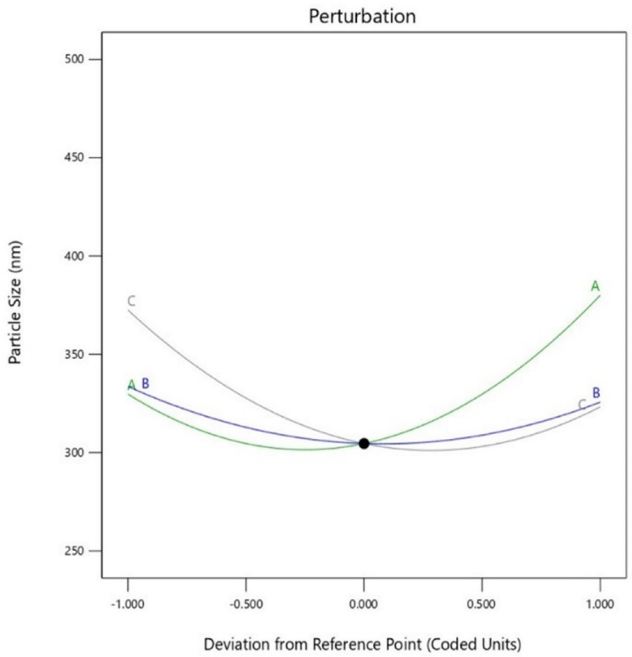

Figure 1a: Perturbation plot for particle size.

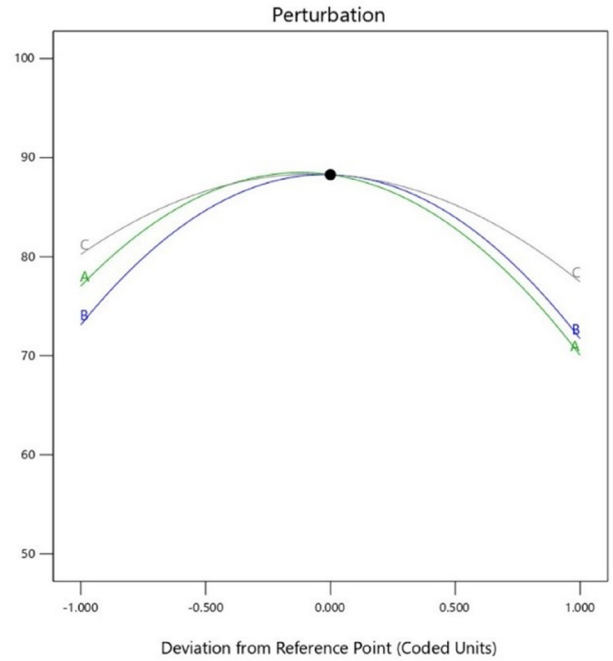

Figure 1b: Perturbation plot for entrapment efficiency.

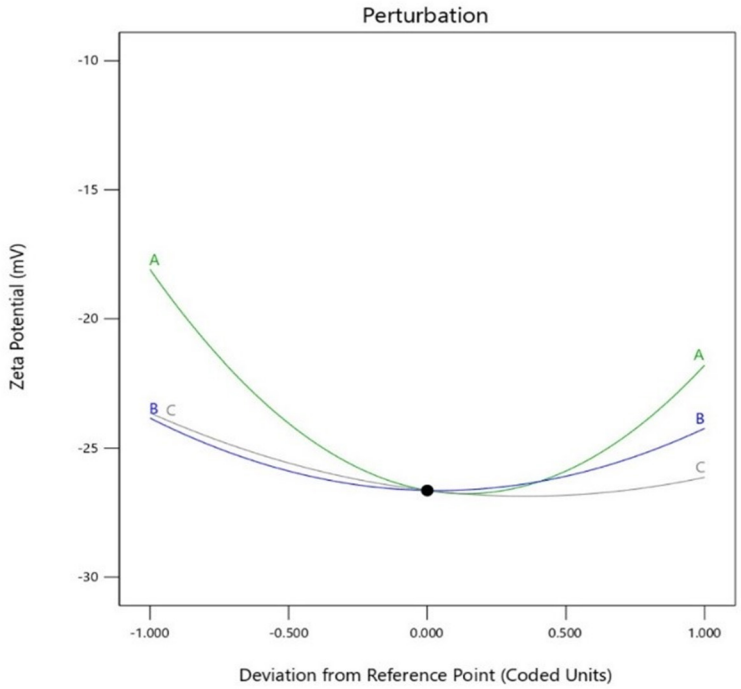

Figure 1c: Perturbation plot for zeta potential.

Figure 1a,1b,1c: Shows the perturbation plotsrepresenting the effective interaction between the responses all factors at a time.

RESULTS

Design of experiments

The Design of Experiments (DOE) was conducted to investigate the impact of various factors on the response variables, which is the particle size (R1), Entrapment efficiency (R2) and zeta potential (R3). All the responses were analysed using a quadratic model, and the results of the ANOVA are presented in the Table 3.

Particle Size (R1)

The particle size of the EZL-CDNS ranges from 295-471 nm. The model F-value of 63.31 indicates that the entire model of particle size is statistically significant. *p*-value less than 0.05 are considered statistically significant. From the results, we can see that the factors A (Crosslinker to Polymer ratio), C (Stirring time), and all the interaction terms (AC, BC) and quadratic terms (A^2, B^2, C^2) are statistically significant in influencing the particle size. The factors B (Stirring speed) and the interaction term AB, however, are not statistically significant at the 0.05 significance level. The perturbation plot (Figure 1a) showing the main effects of A, B, and C on the particle size (R1). The slopes in perturbation indicate that the factors A and C have profound effect on the determination of particle size of the nanosponges. The effect of independent variables on the particles further determined from the 3D response surface plots (Figure 2a). It discloses that the high levels of the Crosslinker to polymer ratio, stirring speed and

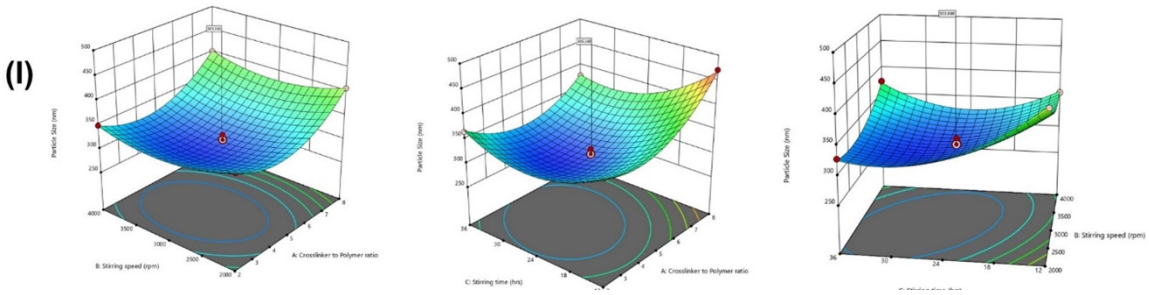

Figure 2a: 3D surface plots of interactions of factors A, B and C effect on particle size.

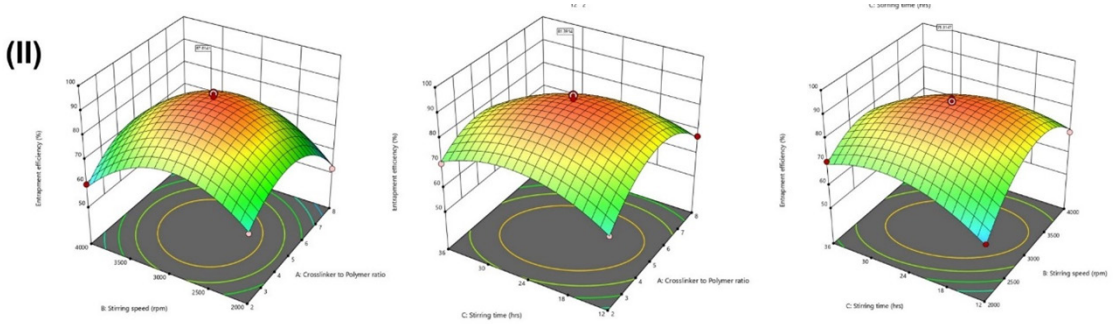

Figure 2b: 3D surface plots of interactions of factors A, B and C effect on entrapment efficiency.

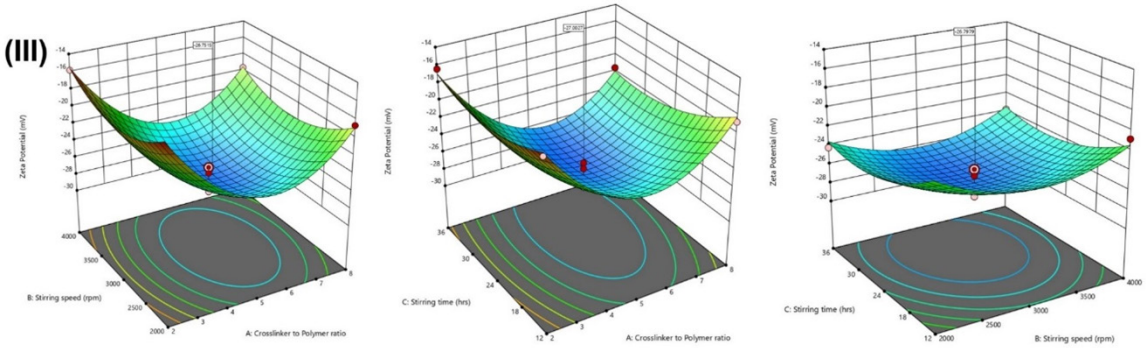

Figure 2c: 3D surface plots of interactions of factors A, B and C effect on zeta potential.

stirring time resulted in the negative effect i.e., increase in particle size of the nanosponges.

Entrapment efficiency (R2)

The entrapment efficiency of the developed nanosponges ranges from 51.7-90.7%. The Model F-value of 117.13 indicates that the overall model is highly significant. The factors A (Crosslinker to Polymer ratio), AC, BC, and all the quadratic terms (A^2, B^2, C^2) are statistically significant in influencing the entrapment efficiency. However, factors B (Stirring speed) and C (Stirring time) and the interaction term AB are not statistically significant at the 0.05 significance level and the perturbation plot (Figure 1b) further ascertains the positive impact of factor A. The 3D response surface plot (Figure 2b) discloses that the high levels of the Crosslinker to polymer ratio, stirring speed and stirring time resulted in the negative effect i.e., decrease in entrapment efficiency.

Zeta potential (R3)

The zeta potential of the developed nanosponges ranges from -15.3 to -28.1mV. The Model F-value of 30.99 indicates that the overall model is highly significant. Factors A (Crosslinker to Polymer ratio) and C (Stirring time), along with the quadratic terms A^2, B^2 and C^2, are all statistically significant in influencing the Zeta potential. However, factors B (Stirring speed) and the interaction terms AB, BC, and AC are not found to be statistically significant in this study. Further perturbation plot (Figure 1c) suggests the positive correlation of zetapotential with the factors A and C. The 3D response surface plot (Figure 2c) discloses that the high levels of the Crosslinker to polymer ratio, and stirring time resulted in the negative effect i.e., decrease in increase in zetapotential of the developed nano sponges.

Characterization Studies

Particle size, Polydispersity Index (PDI) and Zeta Potential (ZP) analysis

The EZL was encapsulated in β-CD NNs using DPC as a crosslinker. Total 17 batches of Four batches of EZL-loaded DPC-crosslinked βCD NS were prepared varying the molar concentration of (βCD: DPC; 1:2 to 1:8). The mean particles size, Polydispersity Index (PDI), ZP, and EE of the developed EZL-CDNS were determined in the range of 295-471 nm, 0.335-0.665, -15.3 to -28.1 mV, and 50.7-90.7% respectively (Table 2). The findings indicate that the size of the nanosponges is significantly influenced by the ratio of βCD: DPC. The findings demonstrated a direct correlation between crosslinker Concentration (DPC) and particle size. High ZP values indicate that the nanosponges would be stable because of stronger repulsive forces, which demonstrating that the supramolecular complexes will not assemble over time. Among these formulations, EZL-CDNS with βCD/DPC molar ratio of 1:4.5 was having better physical characteristics with mean size (304.6±10.06 nm), PDI (0.348±0.006) (Figure 3a), EE (88.28±1.67%), zeta potential (-26.64±1.13 mV) (Figure 3b), which was further evaluated for spectral analysis, *in vitro* release and pharmacokinetic studies.

DSC Studies

The comparative DSC spectra of pure EZL, βCD, DPC and optimized EZL-CDNS werbe shown in Figure 4a. Pure EZL showed an endothermic melting peak at 201°C, whereas EZL peak was completely disappeared in the EZL-CDNS, indicating the drug was molecularly dispersed in to the porous network of the NNs. Furthermore, this might show how EZL interacts with the Nano sponge structure through both the inclusion and non-inclusion phenomena. Crystalline drug was deformed to the amorphous nature inside the DPC crosslinked β-CD nano sponges.

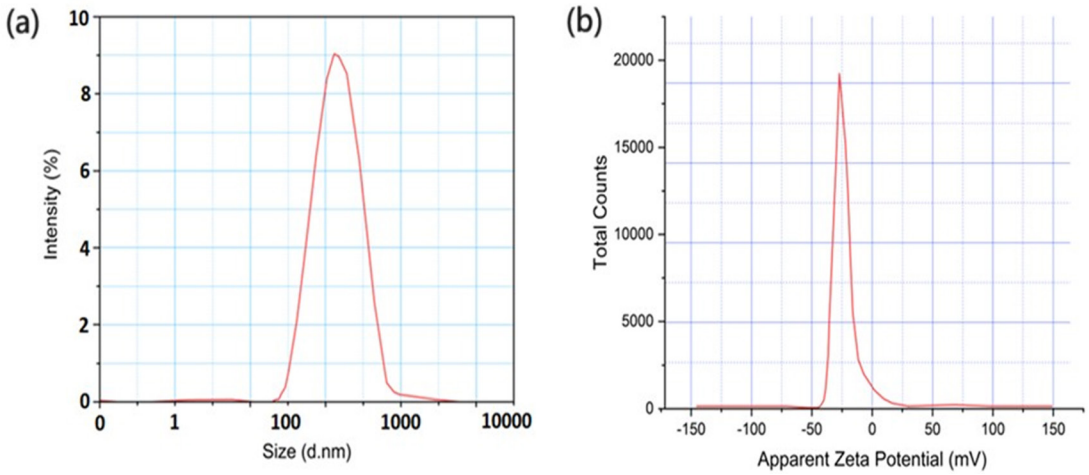

Figure 3: (A) PDI and (B) Zeta potential of optimized formulation.

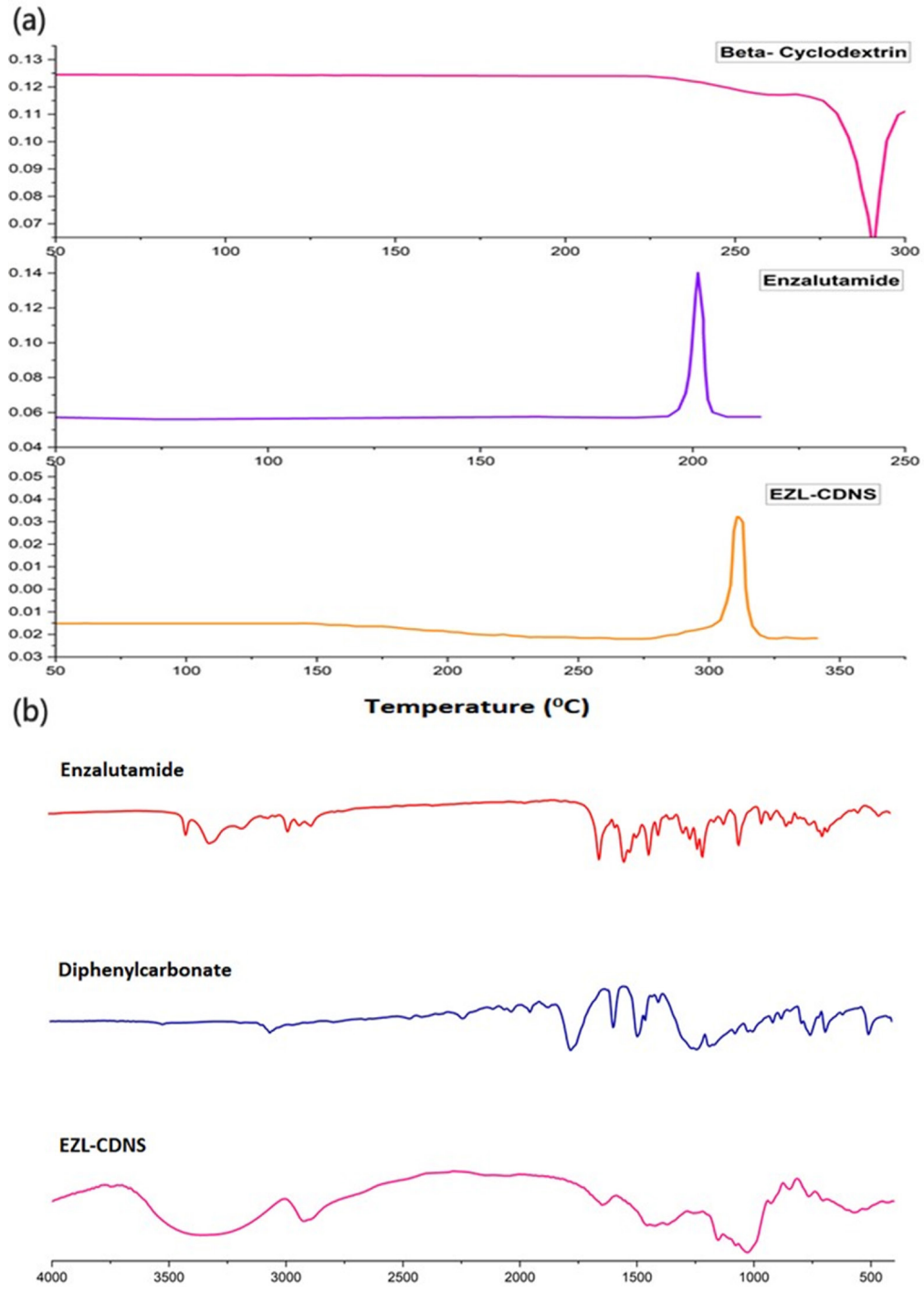

Figure 4: (a) DSC thermo grams of Beta cyclodextrin, Enzalutamide, EZL-CDNS. (b) IR spectra of Enzalutamide, diphenyl carbonate and optimized EZL-CDNS.

FTIR Studies

The FT-IR spectra of the Enzalutamide, diphenyl carbonate and optimized EZL-CDNS formulation were displayed in Figure 4b. The peak at 3430 cm^{-1} corresponds to the O-H stretching vibration of the Hydroxyl (-OH) group in beta cyclodextrin, and is unchanged in the physical mixture. The peak at 3287 cm^{-1} corresponds to the N-H stretching vibration of the Amide (-CONH-) group in enzalutamide, and is also unchanged in the physical mixture. The peaks at 3065 cm^{-1}, 2972 cm^{-1}, 2920 cm^{-1}, and 2879 cm^{-1} correspond to the stretching vibrations of the C-H bonds in the aromatic rings of diphenyl carbonate, and are also unchanged in the physical mixture. Similarly, the peak at 2135 cm^{-1}, which corresponds to the stretching vibration the Carbonyl (-CO-) groups of diphenyl carbonate, is unchanged. However, EZL peaks were disappeared in optimized EZL-CDNS that clearly evidenced complete encapsulation.

Surface morphology

The SEM studies examined the morphology of optimized EZL-CDNS. The image demonstrated the firm, rough surface and porous structure of the NSs formulation. This may be attributed to the EZL drug being encapsulated within the porous architecture of the Nano sponges.

In vitro Drug release Studies

The *in vitro* drug release study assesses the probable *in vivo* performance of dosage forms. The release data were presented as cumulative drug release vs time in hr in Table 4. The release profile of pure EZL and optimized EZL loaded DPC crosslinked βCD NSPs (EZL-CDNS) are shown in Figure 6. A significant ($p<0.05$) enhancement in release of EZL from optimized EZL-CDNS indicated as compared to the pure EZL (Figure 5a). The optimized EZL-CDNS exhibited a higher release of drug within 24 hr compared with pure EZL. The cross linked βCD present in the EZL-CDNS resulted in the production of porous, fluffy structure that has a greater capacity to speed up drug release and dissolution. The DPC crosslinked βCD nanosponge structure enhances CD's capacity to form particular complexes with guest molecules, which can be either strongly retained or released in a Sustained manner.

In vitro Drug Release Modelling

The *in vitro* drug release data were analysed using various pharmacokinetic models, including zero-order, first-order, Huguchi, Hixson-Crowell, and Korsmeyer-Peppas models. The key finding from this analysis was that the zero-order model provided an excellent fit to the release data, as indicated by the R^2 value 0.9987 (Figure 5b). This suggests that the release of enzalutamide from the cyclodextrin nano sponges closely follows a constant-rate pattern. In other words, the drug is released from the nano sponges at a consistent rate over time, without being influenced by the concentration of the drug remaining in the system. This can be advantageous in maintaining a steady and Sustained release of the drug, which may be desirable for certain therapeutic applications.

While the zero-order model was found to be highly effective in describing the release behaviour, the other models (first order, Huguchi, Hixson-Crowell, and Korsmeyer-Peppas) also provided reasonable fits to the data. However, they may not precisely capture the specific release behaviour observed in this study as effectively as the zero-order model. In summary, the pharmacokinetic analysis of the *in vitro* drug release data from cyclodextrin nano sponges revealed that the zero-order model best describes the release pattern of enzalutamide from the delivery system. This finding has implications for the design and optimization of drug delivery systems, as it suggests that a constant-rate release mechanism can be achieved using this technology.

Furthermore, the release exponent (n) value derived from the Korsmeyer-Peppas model is found to be 1.137 ($n>0.89$). This value strengthens the observation that the drug release mechanism aligns with case-II transport. Case-II transport signifies a release pattern characterized by a consistent, predictable, and uniform rate of drug release, resembling the characteristics of zero-order release.

In vivo Pharmacokinetics

The pharmacokinetic analysis results (Table 5) comparing Pure EZL (Enzalutamide) and EZL-CDNS Nanosponges reveal intriguing insights into the behaviour of these formulations in the body. One of the notable findings is the difference in T_{max}, the time to reach the maximum concentration, with EZL-CDNS achieving peak plasma concentration more rapidly than Pure EZL (2 hr vs. 4 hr). This suggests that the nanosponge formulation may enhance drug absorption, resulting in a quicker onset of action (Figure 6a and 6b). Moreover, EZL-CDNS exhibits a significantly higher C_{max} (peak plasma concentration) compared to Pure EZL (4.8825 vs 1.515), indicating improved drug solubility and bioavailability. This enhanced C_{max} implies a higher initial drug concentration in the bloodstream with the nano sponge formulation.

The AUC last (Area Under the Concentration-Time Curve from 0 to 24 hr) and AUCINF (Area Under the Concentration-Time Curve Extrapolated to Infinity) values are also substantially larger for EZL-CDNS (AUC last: 17.789 vs 74.2773; AUCINF: 17.8314 vs 78.234), signifying greater overall drug exposure over a 24-hr period and emphasizing the potential for a more sustained drug release profile. Additionally, the nanosponge formulation displays a smaller apparent volume of distribution (Vz_F_obs), which may restrict drug distribution to a more limited volume (6.80104 vs 3.30558) and potentially lead to higher drug concentrations in target tissues.

Furthermore, EZL-CDNS demonstrates a lower clearance rate (Cl_F_obs) compared to Pure EZL (0.56081 vs 0.12782), indicating a slower drug elimination rate and, consequently, prolonged drug exposure. Although there are differences in Ke (elimination rate constant) between the two formulations (EZL-CDNS: 0.229734949 vs. Pure EZL: 0.201213046), their half-lives ($T_{1/2}$) are comparable (EZL-CDNS: 8.405957422 vs. Pure EZL: 17.92540601), suggesting similar drug persistence in the body. The bioavailability of enzalutamide was enhanced by a factor of 4.175 when using EZL-CDNS in comparison to pure EZL.

DISCUSSION

This study focuses on the design, characterization, and evaluation of Enzalutamide-Loaded Cyclodextrin Nanosponges (EZL-CDNS). It provides detailed understanding of how different formulation factors affect the key properties of the nanosponges. The systematic utilisation of Design of Experiments (DOE)

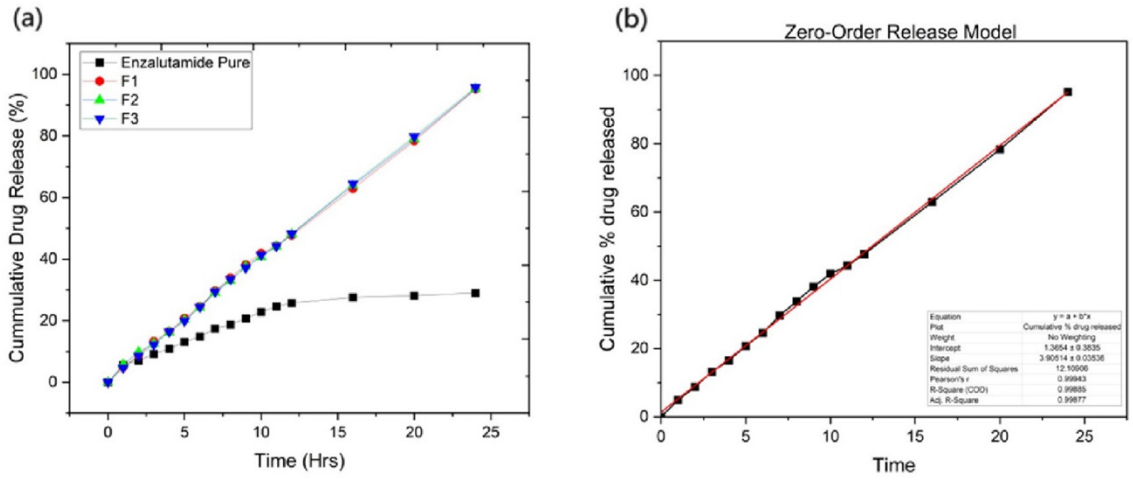

Figure 5: (a) Plot of % Cumulative drug release. (b) Zero-Order Plot of *in vitro* release kinetics.

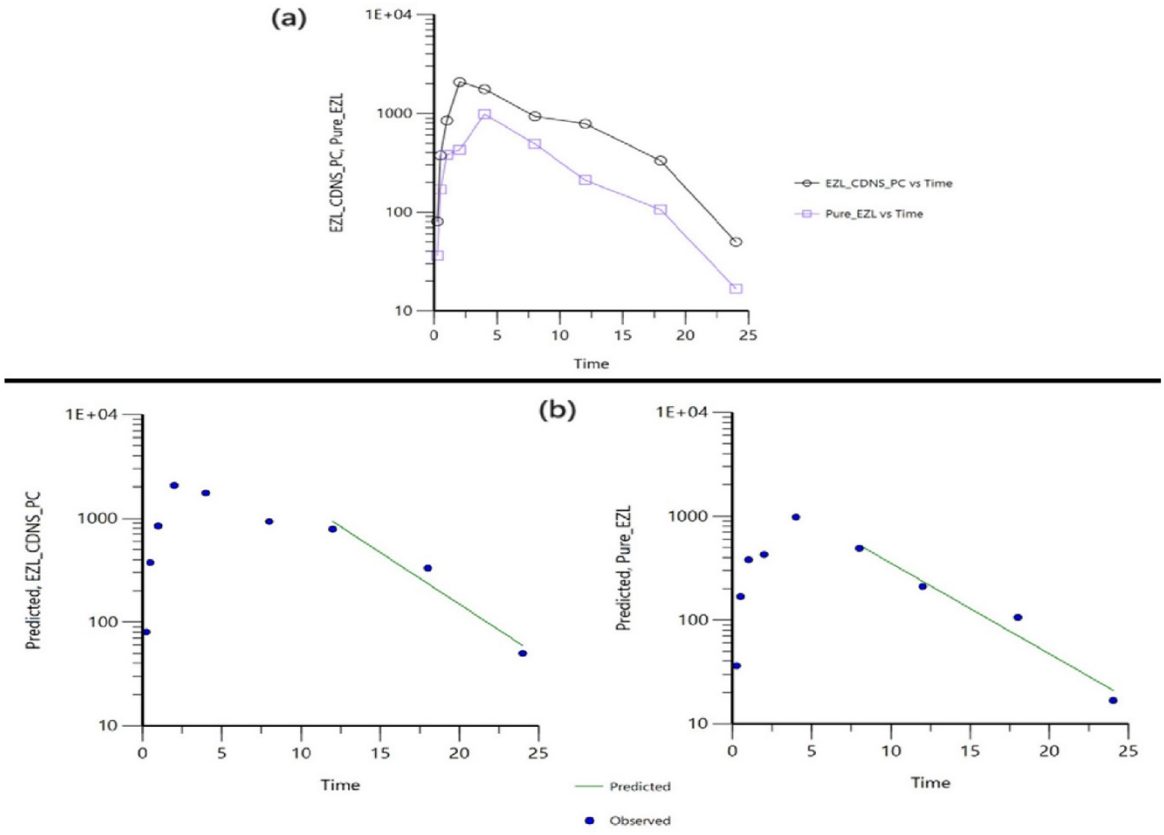

Figure 6: (a) Logarithmic Plot of Time Vs Plasma Concentration. (b) Observed and Predicted Y (Plasma Concentration) vs X (Time).

enabled a methodical exploration of particle size (R1), entrapment efficiency (R2), and zeta potential (R3), revealing critical elements that impact these response variables.

The study found that the particle size (R1) of EZL-CDNS ranged from 295 to 471 nm. The ratio of crosslinker to polymer (A), the duration of stirring (C), and their interactions had a substantial impact on the size of the particles. Higher values of these parameters were associated with an increase in particle size. Optimising the ratio of crosslinker to polymer and the duration of stirring are crucial factors in getting the desired features of the particles.

The entrapment efficiency (R2) varied between 51.7% and 90.7%, with the crosslinker to polymer ratio (A) and its interactions (AC, BC) having a substantial impact. The statistical analysis

Table 4: Dissolution profile of enzalutamide CDNS (optimized batches).

Time (h)	Percentage CDR			
	Control	F1	F2	F3
0	0	0	0	0
1	5.61	5.92	5.74	5.68
2	7.02	8.76	9.78	8.60
3	9.17	13.23	12.60	12.16
4	10.90	16.49	16.20	16.38
5	13.07	20.78	20.26	19.85
6	14.82	24.57	24.25	24.40
7	17.42	29.65	29.08	29.43
8	18.66	33.86	32.99	33.44
9	20.71	38.15	37.57	37.17
10	22.87	41.88	40.68	41.32
11	24.62	44.28	44.09	44.18
12	25.71	47.70	48.12	48.25
16	27.57	62.93	63.88	64.44
20	28.15	78.38	79.05	79.76
24	28.97	95.16	95.31	95.73

revealed that the stirring speed (B) and stirring time (C) did not have a significant impact. This highlights the crucial role of the crosslinker to polymer ratio in determining the entrapment efficiency. These findings emphasise the intricate equilibrium needed between formulation parameters to achieve efficient drug entrapment.

The Zeta potential (R3) varied between -15.3 and -28.1 mV, with both the crosslinker to polymer ratio (A) and stirring time (C) recognised as relevant variables. Elevated levels of these parameters were correlated with a reduction in zeta potential. The provided information is vital for comprehending the stability of EZL-CDNS, specifically in physiological situations, as it implies possible interactions among the nanosponges.

The procedure of formulating nanosponges by using different molar ratios of βCD/DPC resulted in nanosponges of diverse sizes, ranging from 295 to 471 nm. Significantly, a molar ratio of 1:4.5 between βCD and DPC was found to be the most favourable, demonstrating advantageous properties in relation to average size, Polydispersity Index (PDI), Entrapment Efficiency (EE), and zeta potential. The importance of the molar ratio in defining the physical characteristics of the nanosponges is highlighted.

The utilisation of analytical techniques, such as Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared Spectroscopy (FTIR), confirmed the achievement of drug encapsulation within the nanosponges. The absence of the exothermic melting peak observed in the DSC spectra of pure EZL

Table 5: Summary of P/K parameters of EZL-CDNS and Pure EZL.

Pharmacokinetic Parameters	EZL-CDNS	Pure EZL
	Mean±SD	Mean±SD
$t_{1/2}$ (hrs)	17.9254	8.40596
T_{max} (hrs)	2	4
C_{max} (µg/mL)	4.8825	1.515
AUC_{0-t}(µg/mL*h)	74.2773	17.789
$AUC_{0-\infty}$ (µg/mL*h)	78.234	17.8314
$AUMC_{0-\infty}$ (µg/mL*h^2)	1775.96	214.645
$MRT_{0-\infty}$ (hrs)	22.7006	12.0374
V_d[(mg)/(µg/mL)]	3.30558	6.80104
Cl[(mg)/(µg/mL)\h]	0.12782	0.56081
Elimination Rate constant (K_e)	0.23	0.2
Bioavailability (%)	417.5	100.00

indicates a shift from a crystalline to an amorphous state within the nanostructured carriers. FTIR spectra provided additional evidence of interactions among EZL, diphenyl carbonate, and the nanosponge structure, so confirming the concept of full encapsulation.

Scanning Electron Microscopy (SEM) analysis clearly validated the presence of pores and a rough surface in the optimised EZL-CDNS. This further validates the successful incorporation of the drug within the nanosponge matrix. The presence of this permeable structure is expected to play a role in the observed improvement in drug release during subsequent *in vitro* experiments.

The in vitro drug release experiments demonstrated a significant enhancement in the release pattern of EZL from the nanosponges in comparison to pure EZL. The DPC-crosslinked βCD nanosponges' porous shape enables a higher drug release capacity, indicating possible improvements in therapeutic efficacy. The zero-order model fitting and the release exponent (n) value obtained from the Korsmeyer-Peppas model demonstrated a consistent release rate pattern and a case-II transport mechanism, respectively.

Pharmacokinetic studies conducted in living organisms showed that EZL-CDNS outperformed pure EZL. The nanosponge formulation demonstrated accelerated start of action, elevated peak plasma Concentration (C_{max}), enhanced total drug exposure (AUC), and probable improvement in bioavailability. The extended drug release and prolonged duration of exposure, as indicated by the decreased clearance rate (Cl_F_obs) and increased AUC values, indicate promising potential for enhanced therapeutic results.

The findings support the use of nanosponges as a highly effective method for delivering enzalutamide, with benefits including precise release control, increased drug absorption, and improved drug distribution in the body. Although these results show potential, it is crucial to conduct additional investigations, specifically evaluating the effectiveness and safety, in order to successfully apply EZL-CDNS in a clinical setting. This study provides significant contributions to the field of nanotechnology-based drug delivery systems, establishing the foundation for the creation of enhanced formulations that have the potential to be used in clinical settings.

CONCLUSION

This study comprehensively analysed the design, characterization, drug release, and pharmacokinetics of Enzalutamide-Loaded Cyclodextrin Nano Sponges (EZL-CDNS). Using Design of Experiments (DOE), we assessed factors like crosslinker to polymer ratio and stirring time, revealing their significant impact on particle size, entrapment efficiency, and zeta potential. EZL-CDNS displayed a size range of 295-471 nm, entrapment efficiency between 51.7% and 90.7%, and zeta potential from -15.3 to -28.1 mV. The βCD/DPC molar ratio played a pivotal role in particle size modulation. DSC and FTIR affirmed successful drug encapsulation and interactions within the nano sponges. SEM imaging illustrated the porous EZL-CDNS structure, confirming drug integration. *In vitro* drug release demonstrated significantly improved profiles compared to pure EZL, thanks to the porous DPC-crosslinked βCD nano sponges. Pharmacokinetic modelling highlighted the zero-order release pattern and case-II transport. *In vivo* pharmacokinetics indicated superior EZL-CDNS performance with faster onset, higher concentration, increased exposure, and potential bioavailability enhancement. This study underscores nano sponges as promising drug delivery systems for optimizing enzalutamide's pharmacological properties, necessitating further efficacy and safety assessments for clinical translation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

EZL: Enzalutamide; **CDNS:** Cyclodextrin Nanosponges; **DPC:** Diphenyl carbonate; **DSC:** Differential scanning calorimetry; **FTIR:** Fourier transform infrared spectroscopy; **SEM:** Scanning electron microscopy.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval for the study was obtained from the Institutional Animal Ethics Committee (IAEC: 1292/ac09/ CPCSEA/2021/3).

SUMMARY

This work thoroughly investigated the design, characterisation, drug release, and pharmacokinetics of Enzalutamide-Loaded Cyclodextrin Nanosponges (EZL-CDNS). By employing Design of Experiments (DOE), crucial variables such as the ratio of crosslinker to polymer and the duration of stirring were determined, unveiling their substantial influence on particle size, entrapment efficiency, and zeta potential. The size of EZL-CDNS ranged from 295 to 471 nm, with entrapment efficiency ranging from 51.7% to 90.7%, and zeta potential ranging from -15.3 to -28.1 mV. The molar ratio of βCD to DPC was determined to be a critical factor that affects the size of the particles. The successful encapsulation of the drug and its interactions within the nanosponges were confirmed by the use of analytical techniques such as DSC and FTIR. This was further supported by SEM imaging, which showed the porous structure of EZL-CDNS. The drug release patterns seen *in vitro* showed substantial enhancement in comparison to pure EZL, which can be attributed to the porous DPC-crosslinked βCD nanosponges. The pharmacokinetic modelling revealed that EZL-CDNS follows a zero-order release pattern and case-II transport. *In vivo*

pharmacokinetics demonstrated that EZL-CDNS outperforms other drugs by exhibiting a faster onset, higher concentration, enhanced exposure, and probable augmentation of bioavailability. This study highlights the potential of nanosponges as effective drug delivery methods for enhancing the pharmacological characteristics of enzalutamide. It emphasises the need for additional evaluations of efficacy and safety to facilitate its clinical application.

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