A Theoretical and Experimental Analysis of the Complexation Process Between Progesterone and Cyclodextrin Nanosponge

Mehrdad Hadadian *, Behnam Mahdavi, Esmail Rezaei-Seresht

Department of Chemistry, Faculty of Science, Hakim Sabzevari University, Sabzevar, Iran

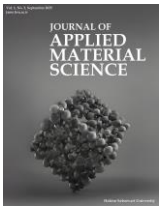
Editor's note: Cyclodextrin nanosponges are polymeric porous nanostructures that are widely utilized in encapsulation-based drug delivery systems. These nanosponges exhibit exceptional properties for encapsulating drugs such as progesterone. In this study, Hadadian et al. synthesized cyclodextrin nanosponges using hexamethylene diisocyanate as the crosslinker and investigated the complexation of progesterone within the nanosponge structure of these carriers. Their experimental and theoretical findings suggest the formation of a 1:2 (drug:host) molar ratio complex, indicating that hydrogen bonding is the primary driving force behind this phenomenon.

doi: 10.22034/jams.2025.210145

How to cite: M. Hadadian et al., *Journal of Applied Material Science*, **2025**, *1*, 210145.

JOURNAL OF
APPLIED
MATERIAL
SCIENCE
jams.hsu.ac.ir

Journal of Applied Material Science **2025**, *1*, 210145
How to cite: M. Hadadian et al., *Journal of Applied Material Science*, **2025**, *1*, 210145.
Journal homepage: **jams.hsu.ac.ir**



Original Research

A Theoretical and Experimental Analysis of the Complexation Process Between Progesterone and Cyclodextrin Nanosponge

Mehrdad Hadadian *, Behnam Mahdavi, Esmail Rezaei-Seresht

Department of Chemistry, Faculty of Science, Hakim Sabzevari University, Sabzevar, Iran

Abstract

Progesterone, a widely used steroid drug for the treatment of luteal phase deficiency, has very low water solubility and bioavailability. Cyclodextrin nanosponges are polymer-like materials used to enhance the aqueous solubility and bioavailability of lipophilic drugs. In this study, cyclodextrin nanosponges were synthesized using β-cyclodextrin and hexamethylene diisocyanate as a crosslinker; meanwhile, the complexation of progesterone within the nanosponge was investigated using Fourier transform infrared spectroscopy and X-ray diffraction analyses. Furthermore, the encapsulation efficiency and phase solubility of progesterone were assessed by UV-visible spectroscopy. Characterization techniques confirm the successful formation of the complex. The nanosponge was found to be able to encapsulate 70% of the drug and increase its aqueous solubility approximately 45 times. Moreover, phase solubility studies suggest the formation of a 1:2 (drug:host) molar ratio complex. Moreover, molecular docking simulation confirms the obtained experimental data and ensures the formation of a 1:2 complex by visualizing a hydrogen bond between the drug and host molecules.

Keywords: Drug delivery; Molecular docking; Phase solubility; Nanosponge; Cyclodextrin.

1. Introduction

Cyclodextrin nanosponges (CNS) are nanostructured polymers made up of hyperlinked cyclodextrins (CD) with unique superior properties [1]. These multifunctional materials, with their high porosity features, can encapsulate various organic and inorganic compounds while floating on aqueous media. In this regard, they are ideal candidates for biomedical applications by making inclusion or non-inclusion complexes with medications or for water filtration by removing pollutants from contaminated water [1]. Nanosponges take advantage of the beneficial features of pristine cyclodextrins, like increasing the water solubility of lipophilic substances, stabilizing degradable guests, conserving volatile ingredients, and prolonging drug release [2]; meanwhile, they show the efficacious properties of polymer-based materials, such as high thermal stability up to 300˚C [3]. Moreover, CNS is widely recognized as a safe material for drug delivery purposes, especially for steroidal drugs, as they could increase their water solubility [4].

Many approaches have been made to increase the water solubility of lipophilic drugs, and cyclodextrins are one of the most efficacious carriers for this purpose

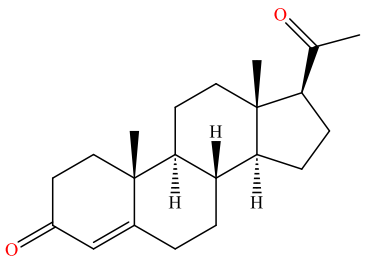
* Corresponding author.
Email address: hadadianmehrdad@gmail.com (M. Hadadian)

Received 7 June 2025
Revised 4 July 2025
Accepted 9 July 2025
Available online 11 July 2025

https://doi.org/10.22034/jams.2025.210145

[5, 6]. In this way, it is assumed that cyclodextrin-based nanosponges could show superior properties in encapsulating hydrophobic drugs, like higher encapsulation efficiency, biodegradability, thermal stability, and bioavailability.

Progesterone (Pro), a sex hormone of women, is produced by the adrenal gland to prepare the uterus for embryo implantation and sexual behavior or by the corpus luteum through the luteal phase (Scheme 1). Luteal phase deficiency (LPD) is when the level of progesterone in patients decreases below a sufficient level, resulting in an abnormal menstrual cycle, embryogenesis, and pregnancy [7]. It is suggested that a level of 10-20 ng/ml of progesterone is needed to suppress the LDP consequences. For this purpose, progesterone is commercialized to help patients in the reproductive technology cycle, with anovulatory bleeding, with secondary amenorrhea, and to prevent recurrent pregnancy loss. However, its low water solubility (11.9 µg/mL) hindered its transportation to the body organisms, resulting in limited absorption *via* the gastrointestinal tract and low oral bioavailability [8]. To confront this problem, many steroids are formulated as hydrogels using ethanol or microemulsions with the means of detergents [2]. For specific progesterone, it is formulated as a suspension in peanut oil, which could improve the intestinal absorption of the drug [8]. These formulations with their benefits have some drawbacks, including skin irritations, variable oral absorption, and hence, showing less impact on the treatment [2, 8].



Scheme 1. Molecular structure of progesterone.

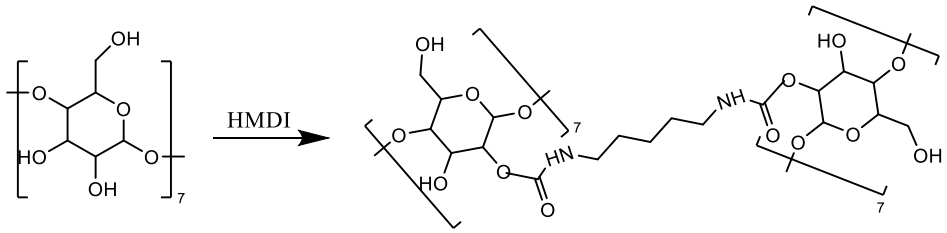
Many researchers have been making multiple efforts to increase the water solubility of lipophilic drugs using various methods, such as solid dispersion, salt formation, supercritical-fluid processing, micellar solubilization, hydrotrophy, solubilizers, and cyclodextrins [8, 9]. In recent years, cyclodextrins have found substantial interest because of their unusual physicochemical properties, especially when making an inclusion complex with drugs, increasing their bioavailability, solubility, and stability [10]. Meanwhile, CNSs have become of interest because of their capacity for delivering drugs to specific organs and increasing the effects of drugs. For example, several previous studies have declared that CNS could increase the stability, anti-inflammatory, anti-cancer, and anti-viral activity of active pharmaceutical forms [4, 5]. CNS could make inclusion complex with steroid drugs by encapsulating them within their nanocavities, similar to CDs, and non-inclusion complex in the tiny mesh-like spaces that have been formed after crosslinking [5]. For instance, the encapsulation of dexamethasone by CNS has been investigated, and the obtained results showed that the drug is entrapped within the nanocavities of CDs, as well as the porous matrix of CNS [11]. In addition, the encapsulation of curcumin within the CNS has been studied, which represents superior water solubility for curcumin, favoring its entry into the CNS [4]. Until recently, the complexation of progesterone within β-cyclodextrin (β-CD) has been widely studied, which has demonstrated the potential of β-CD to encapsulate this lipophilic drug for drug delivery and water purification applications [6, 12]. However, there is a huge gap in utilizing β-CD nanosponge for these purposes, except for a study by Matencio et al. that suggested a non-covalent nanosponge using the ball mill method [13].

This study aimed to fabricate a covalently crosslinked cyclodextrin nanosponge using hexamethylene diisocyanate (HMDI) as a crosslinker to increase the water solubility of progesterone by encapsulation. For the characterization, the FT-IR and XRD techniques have been used. The phase water solubility and drug loading studies were conducted spectroscopically. Moreover, the binding affinity of progesterone with cyclodextrin in 1:1 and 1:2 molar ratios has been assessed using molecular docking.

2. Experimental

2.1. Materials

Progesterone (Pro, purity >98%, $C_{21}H_{30}O_2$, MW: 314.46 g/mol) was purchased from Sigma-Aldrich. β-Cyclodextrin (β-CD, purity > 98%, $C_{42}H_{70}O_{35}$, MW: 1134.98 g/mol), dimethyl sulfoxide (DMSO), hexamethylene diisocyanate (HMDI, ≥ 99.0%), methanol (≥ 96.0%), and acetone (≥ 99.5%) were purchased from Merck (Germany). All other reagents and chemicals



Scheme 2. The synthesis route of cyclodextrin nanosponge.

used were of analytical grade and without additional purification.

2.2. Synthesis of β-Cyclodextrin Nanosponge (CNS)

Cyclodextrin nanosponge was prepared according to the literature with some modifications [14]. Firstly, 3.34 mmol of β-CD was dissolved in 16 mL of DMSO. Then, the temperature of the solution was increased to 40˚C, and 27.5 mmol of HMDI was added to the solution. For a further 4 hours, the reaction temperature was kept at 70˚C to form a gel-like CNS. Finally, the unreacted reagents were removed using Soxhlet extraction with acetone. Scheme 2 represents the synthesis route of NS.

A methanolic solution of progesterone (25 mg in 3 mL) was added to an aqueous dispersion of CNS (32 mg in 2 mL). Then, the mixture was put in an ultrasonic bath for 30 min at RT. Afterward, the mixture was permitted to stir under the same conditions. After 24 hours, the mixture was allowed to settle at 4 °C for 12 hours. Finally, the solid residue was separated using a centrifuge and washed three times with a mixture of deionized water and methanol to remove unreacted progesterone [2].

2.3. Measurements

FT-IR spectra of β-cyclodextrin, CNS, Pro, and Pro/CNS were obtained using a Shimadzu 8400 with KBr disc from 440 to 4000 cm^{-1}. The XRD patterns of β-CD, CNS, Pro, and Pro/CNS were obtained with a diffraction angle of 6° to 90° at a rate of 2°/min, and Cu Kα radiation at 45 kV and 40 mA, using the STOE PW2773.00 device. The spectroscopic method was utilized using a Jena Speko L2000 spectrophotometer at 200-800 nm.

Determination of the encapsulation efficiency (EE%): The encapsulation efficiency of the Pro/CNS complex was assessed using the spectroscopic method due to its cost-effectiveness, accessibility, simplicity, and high accuracy [15]. For this purpose, 30 mg of CNS was suspended in 2 mL of deionized water, and a 3 mL methanolic solution of Pro (20 mg) was added to the suspension, followed by the procedure mentioned in section 2.2.2. The residue was filtered using a nylon Cameo filter syringe (0.45 µm). The concentration of unloaded Pro was assessed spectrophotometrically at the maximum wavelength of Pro (258 nm) and compared with its calibration curve. The EE% was examined according to equation 1.

$$EE\% = \frac{amount\ of\ unloaded\ drug\ (mg)}{amount\ of\ feed\ drug\ (mg)} * 100 \qquad (1)$$

Phase solubility studies: The phase solubility of progesterone in aqueous media was assessed using the Higuchi-Connors method [2]. For this purpose, an excessive amount of progesterone was added to the vials containing various concentrations of CNS (0-10 mM), and the vials were shaken for 24 hours at 25 °C to reach an equilibrium. Then, the suspensions were passed through a nylon Cameo filter syringe (0.45 µm), and the concentration of unloaded drugs was calculated using a spectroscopic method at the maximum wavelength of 258 nm. The suspensions were diluted with methanol.

2.4. Molecular docking simulation

A molecular docking study is beneficial in rationalizing the experimental data. For this purpose, the PDB files of β-CD and progesterone were obtained from: https://pubchem.ncbi.nlm.nih.gov/. The energy of molecules was minimized using Gaussian 0.9W at the Hartree–Fock energy minimization at the 3-21G level. The energy minimization was performed in the gas phase, as it is recommended for organic molecules, while reducing computational complexity and retaining structural accuracy. Then, the minimized molecules

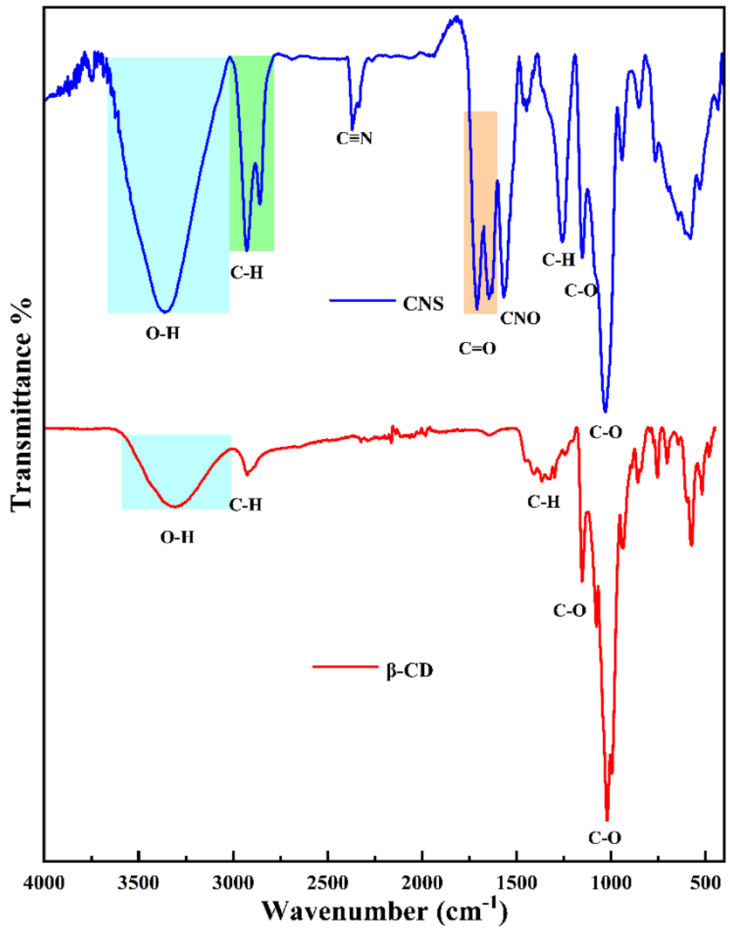


Figure 1. FT-IR spectra of β-CD and CNS.

were saved as an output file and converted to PDB format using GaussView 5.0. Cyclodextrin was assumed as the receptor and progesterone as the ligand in the Autodock Tools-1.5.7. The obtained data was visualized using Schrodinger PyMOL 3.1.1 software. For studying 1:2 drug/host complexation, the extracted β-CD was modeled into two β-CD in PyMOL, and its energy was minimized using Gaussian 0.9W.

For a 1:1 complex, β-CD was placed in the center of a grid box with dimensions of 40 Å × 40 Å × 40 Å with a grid spacing of 0.374 Å. For a 1:2 complex, a grid map with dimensions of 54Å×40Å×40Å and grid spacing of 0.374Å was provided. The molecular docking for both complexes was performed by LGA algorithms, and the number of runs was set to 150 with a population size of 150. These settings followed standard protocols to ensure the reproducibility and accuracy of docking.

3. Results and discussion

3.1. Physicochemical characterization of the prepared products

3.1.1. FT-IR analysis

Infrared spectroscopy is a reliable technique for assessing the stepwise formation of products by monitoring the spectra. Usually, the peak's presence, position, and shape can change after the formation of the host/guest complex, which is a key reason for the importance of FT-IR analysis in recognizing the final products [16]. The FT-IR spectra of β-CD and CNS are presented in Figure 1, and Pro and Pro/CNS in Figure 2. The spectrum of β-CD shows two characteristic peaks at 1023 and 1152 cm^{-1}, which are attributed to the C-O bonds. The peaks at 1345 and 2921 cm^{-1} are related to the

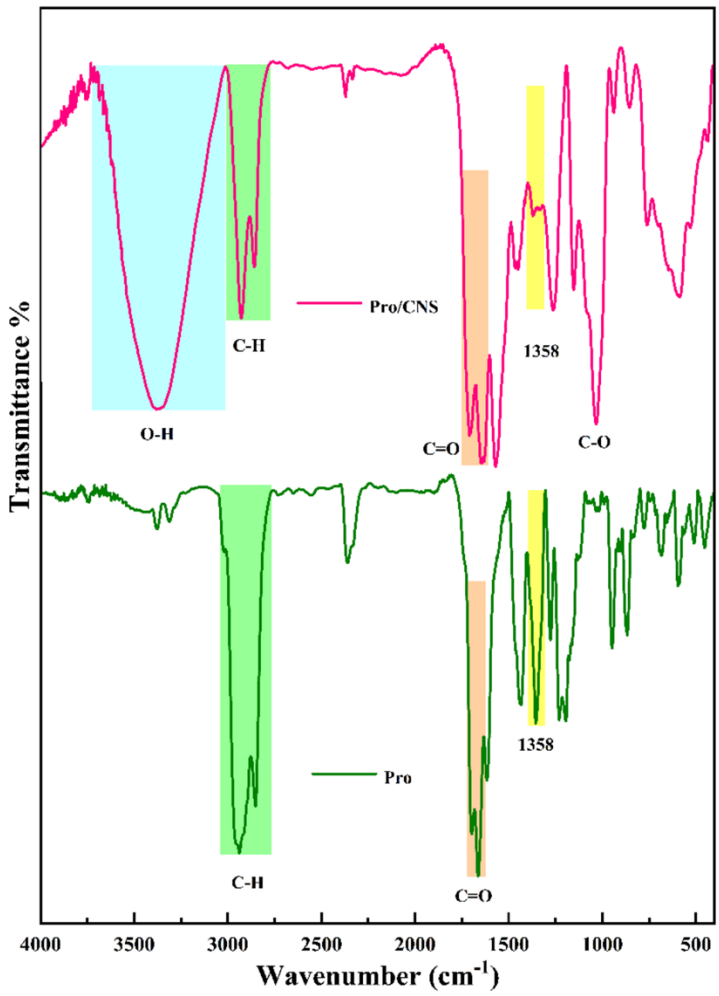


Figure 2. FT-IR spectra of Pro and Pro/CNS.

C-H bonds. Moreover, a broad peak at 3307 cm^{-1} relates to the abundant hydroxyl groups.

The peak at 938 cm^{-1} is assigned to the C-H stretching vibrational bands of the CD ring. The spectrum of CNS shows all the characteristic peaks of β-CD with some alteration in their positions (OH = 3362 cm^{-1}, C-O = 1032 cm^{-1}, C-H = 1259 cm^{-1}), along with two sharp peaks at 1647 and 1711 cm^{-1}, which are associated with the C=O groups variations, and a distinct peak of the CNO group at 1568 cm^{-1}. Besides, there is a sharp peak at 2370 cm^{-1} related to the nitrile group, indicating the presence of hexamethylene diisocyanate groups with one unreacted head, which could resonate and change from N=C=O group to the $N{\equiv}C{-}O^{-}$ group. In addition, the peaks between 700-1000 cm^{-1} relate to the deformation vibration bands of glucopyranose units [17].

Figure 2 represents the FT-IR spectrum of Pro, including two sharp peaks at 1664 and 1695 cm^{-1} associated with the C=O groups and a peak at 2927 related to the C-H groups. Moreover, the complexation of Pro with CNS modified the peaks' position, which confirmed the successful formation of the final product. In this regard, the peak at 1360 cm^{-1} recurred in both Pro and Pro/CNS; meanwhile, the C=O peaks are observable at 1711 and 1641 cm^{-1}, the C-H group at 2928 cm^{-1}, and the O-H group at 3362 cm^{-1}.

3.1.2. XRD analysis

The X-ray diffraction method provides additional evidence for the confirmation of the drug/host complex through peak disappearance, emergence, or reduction in the intensity of the peak. Figures 3 and 4 represent the

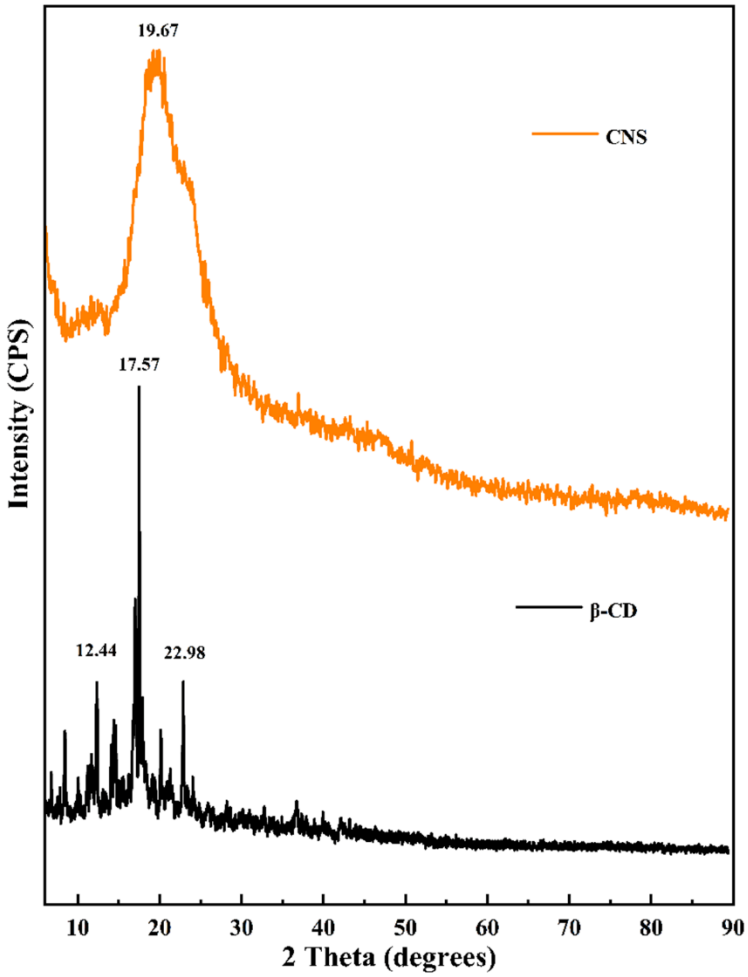


Figure 3. XRD patterns of β-CD and CNS.

XRD patterns of β-CD, CNS, Pro, and Pro/CNS. As can be seen in the diffractogram of β-CD, it has a crystalline structure with distinctive peaks at values of 2θ ~ 12.44°, 17.57°, and 22.98°. The XRD pattern of CNS shows a broad peak at the maximum intensity at 2θ ~ 19.67. It seems that the well-ordered crystalline structure of β-CD, which is evident by the sharp and intense peaks, was ameliorated upon polymerization, leading to an amorphous nanosponge. The polymerization process affects the XRD pattern by the disappearance or broadening of the sharp peaks of the ingredient and the appearance the broad humps, indicating the formation of an amorphous phase in the product. Similar observations are reported in previous research [18, 19]. Moreover, the X-ray spectrum of Pro recorded three sharp peaks at the values of 2θ ~ 11.48°, 15.92°, and 21.72°, indicating the crystalline structure. The XRD pattern of Pro/CNS shows the disappearance of Pro peaks and the appearance of some new peaks and peaks` modifications, which confirms the successful formation of the final product [4].

3.1.3. Drug loading study

To delve into the capability of the prepared CNS to encapsulate a hydrophobic drug, the co-loading study was run. For this purpose, the initial weight and the remaining amount of Pro were assessed using the UV-visible method and compared with the Pro calibration curve. According to equation 1, the EE% for Pro/CNS was calculated to be 70%. This noteworthy encapsulation efficiency for CNS could be attributed to various reasons, including entrapment of Pro into the pores of CNS with its high porosity features, hydrophobic interactions between the drug and

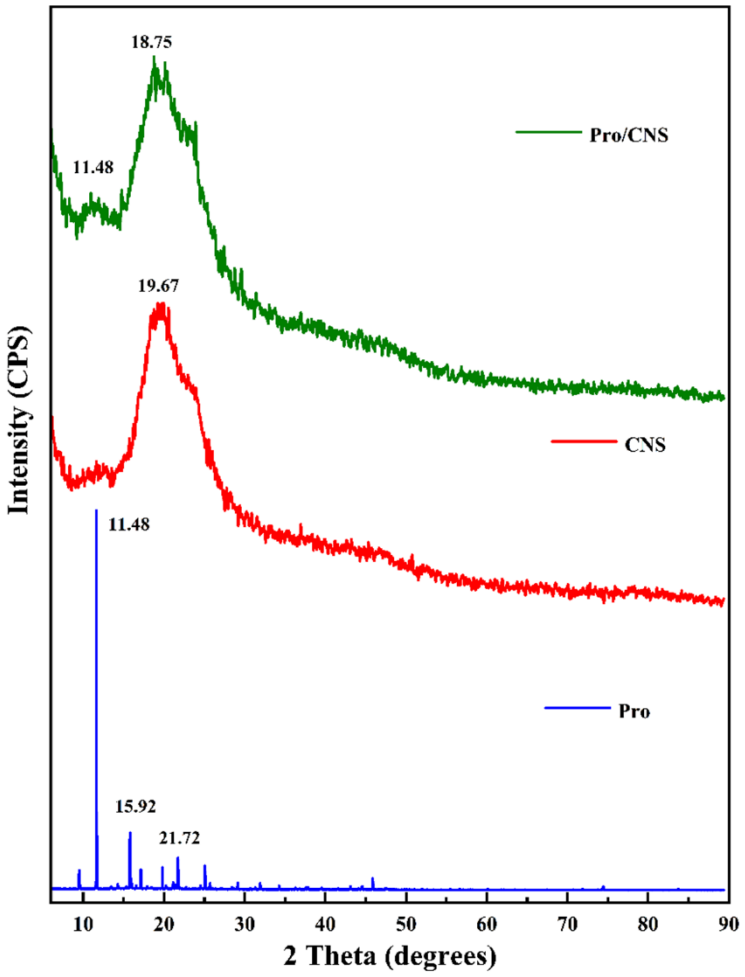


Figure 4. XRD patterns of Pro, CNS, and Pro/CNS.

cyclodextrin pores, and hydrogen bonding between the carbonyl groups of Pro and hydroxyl groups of CNS. Moreover, the gonane structure of Pro can fit perfectly into the cavity of cyclodextrin [16]. The prepared nanosponge showed higher encapsulation efficiency for progesterone compared to other carriers suggested for its delivery. For example, a microparticle of chitosan and tripolyphosphate showed an EE% of roundly 62% [20]. A magnetic nanoaggregate comprising Pluronic F-68 and β-cyclodextrin depicted a mean encapsulation efficiency of 49.68% for progesterone [21].

3.1.4. Phase solubility studies

The phase solubility of Pro/CNS was evaluated at RT and neutral pH. According to the Higuchi method, the phase solubility diagram is classified into two categories: A and B types, indicating complexes with high solubility and low solubility, respectively. Moreover, type-B diagrams can be subdivided into two categories, including Bs (limited solubility) and B_I (negligible solubility). On the other hand, three subtypes are derived from A-type diagrams. When the drug solubility increases in a linear slope as a function of the enhancement of CNS concentration, the related diagram is A_L. On the contrary, the A_P diagram exhibits diagrams with positive deviations and A_N for negative deviations. The phase solubility studies are so beneficial in optimizing CNS for the encapsulation of drugs to increase their solubility and designing drug delivery systems [16]. Figure 5 represents the phase solubility diagram of Pro/CNS in water. It seems that the aqueous solubility of Pro followed a linear increase as a function of CNS concentration, with a determination coefficient (R^2) of 0.868.

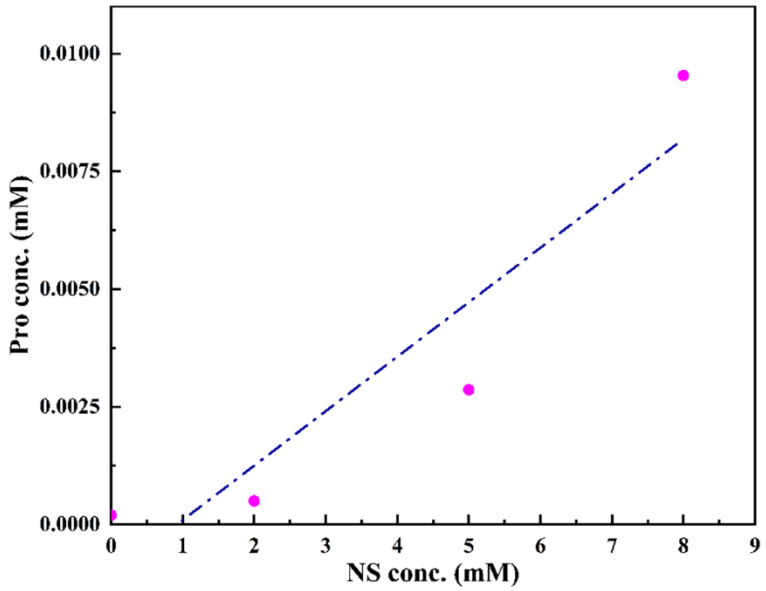


Figure 5. The phase solubility diagram of Pro/CNS in water and RT.

The phase solubility diagram of Pro/CNS depicts an A_P-type, indicating the formation of a complex in a molar ratio of 1:2 (Pro:CNS). This subtype suggests that abundant hydrogen bonding is formed between the host molecule and the solvent, and more water molecules are available to participate in the solute-solvent interactions, leading to positive deviation. In another way, this phenomenon can be interpreted by the observation that one drug molecule is surrounded by two CD molecules, which provide more oxygen and hydroxyl groups to make hydrogen bonds with the hydrogen of water [22]. In addition, the encapsulation of Pro within the CNS substantially increases the water solubility of this lipophilic drug. While the intrinsic aqueous solubility of Pro was calculated to be 0.2×10^{-3} mM, it increased to 9.5×10^{-3} mM with a CNS concentration of 8 mM. Table 1 compares the solubility of progesterone within different formulations that are reported in other literature and CNS, indicating better results for the Pro/CNS formulation.

3.2. Molecular docking study

Molecular docking simulation, by comparing the bond strength between molecules, provides deeper insight into the orientation and interaction of the drug with the cavity of cyclodextrins and also complements experimental data [25]. In this regard, the conformation with the lowest binding energy (ΔG) was chosen as the best conformation.

As the phase solubility studies suggest a 1:2 (drug/host) molar ratio complexation between drug and NS, we compared the 1:1 and 1:2 complexation to ensure the consistency of the obtained experimental data. The obtained result indicates that progesterone and NS make a complex in a 1:1 molar ratio with a binding energy of -

Table 1. Electrochemical parameters obtained from the polarization test on samples immersed in the saline solution

Delivery system	Carrier type	Preparation method	Route of administration	Solubility	Encapsulation efficiency (%)	Reference
Micelle	Brij®35	solvent evaporation	vaginal	20-fold	15%	[23]
Polymer	Polyvinylpyrrolidone	solvent evaporation	oral	1.4-fold	-	[24]
Polymer	Cyclodextrin-nanospone	Co-precipitation method	oral	45-fold	70%	This work

7.46 kcal/mol. Meanwhile, this value for the 1:2 complex was calculated to be -8.05 kcal/mol. These data reveal that the 1:2 complex is the stable product by recording a lower binding energy, which confirms the phase solubility studies` finding. The lower binding energy for the 1:2 complex is rooted in the interaction between the drug and host molecules.

Figure 6 represents the orientations and interactions between the Pro and cyclodextrins in 1:1 and 1:2 states. As can be seen, there is a hydrogen bond (blue dots) between the oxygen atom of pro and the hydroxyl group of cyclodextrin in the 1:2 complex, which is the main driving force for the formation of this complex. Moreover, this complexation could explain the high phase solubility of pro after encapsulation in CNS, as the whole of the pro molecule is entrapped within the two CD molecules, which are abundant in hydrogen and oxygen atoms, enabling them to make hydrogen bonds with water molecules. Apart from hydrogen bonds, various non-covalent interactions play a key role in the formation of the drug/host complexes, including dipole-dipole, electrostatic, and hydrophobic effects [26]. While the inner cavity of cyclodextrins is hydrophobic, the pro molecule could reside in this moiety due to the favorable enthalpy. Moreover, the pro with the negative dipole moment makes an electrostatic interaction with the positive dipole moment of cyclodextrin. These interactions are similar in both complexes, while hydrogen bonding makes the differences between them.

4. Conclusions

In this study, a complexation between progesterone and a nanosponge based on β-cyclodextrin has been prepared. The nanosponge was made using β-CD and HMDI as a crosslinker, where FT-IR and XRD analyses confirmed its formation. Moreover, similar characterization methods were used to ensure the preparation of the Pro/CNS complex. The encapsulation efficiency of Pro/CNS was found to be 70%. The phase

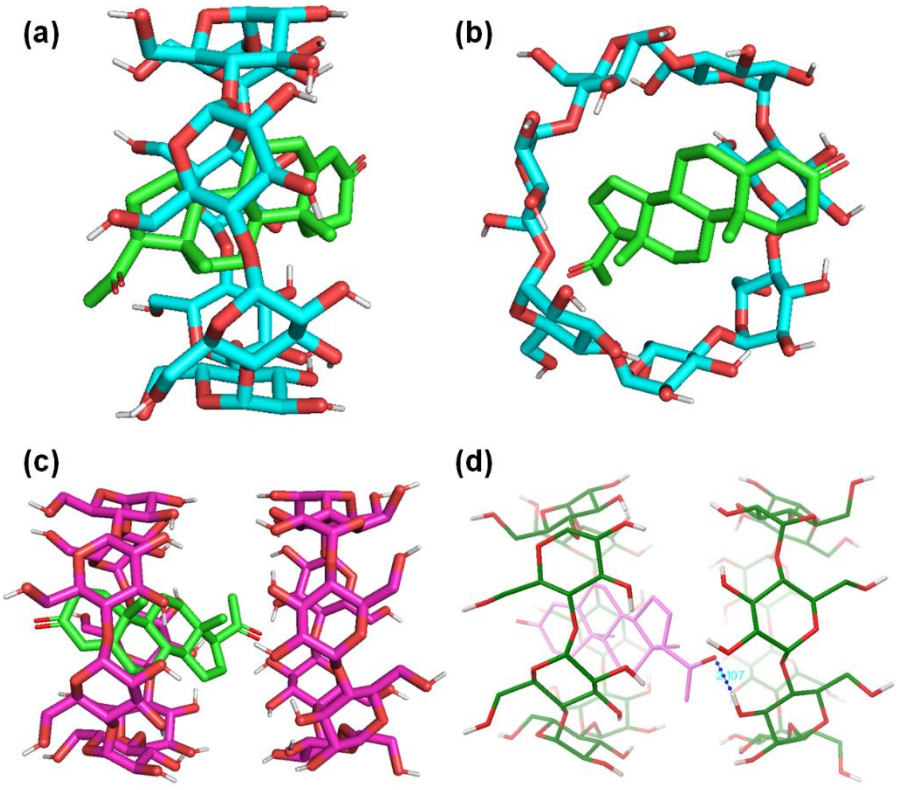


Figure 6. The 3D structures of (a and b) Pro/CNS (1:1) and (c and d) Pro/CNS (1:2) (hydrogen bonding in blue dots).

solubility findings indicated that a CNS concentration of 8 mM could increase the hydrophobicity of Pro roughly 45 times after the encapsulation process. Moreover, it suggested the formation of a 1:2 (Pro/CNS) complex, which was confirmed by molecular docking studies, indicating that hydrogen bonding is the key driving force for this phenomenon. The encapsulation of progesterone within cyclodextrin-nanosponge seems to be a promising strategy for increasing the hydrophobicity, bioavailability, and effectiveness of this drug, which is prescribed for multiple purposes. However, an *in vitro* drug release study should be conducted to check the behavior of this formulation in a physiological environment. Furthermore, an *in vivo* study is favored to assess the cytotoxicity of this formulation.

Conflict of Interest

The authors declare no conflict of interest.

References

1. B. Mognetti, et al. In vitro enhancement of anticancer activity of paclitaxel by a Cremophor free cyclodextrin-based nanosponge formulation. *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, 2012, *74*, 201.
2. M. Hadadian, B. Mahdavi, and E. Rezaei-Seresht. Exploring modified cyclodextrins for enhanced encapsulation and release of ethinyl estradiol: Physicochemical characterization and kinetic modeling. *Journal of Science: Advanced Materials and Devices*, **2025**, *10*, 100837.
3. A. Moin, et al. Design and formulation of polymeric nanosponge tablets with enhanced solubility for combination therapy. *RSC advances*, **2020**, *10*, 34869.
4. H. Mashaqbeh, R. Obaidat, and N. Al-Shar'i. Evaluation and characterization of curcumin-β-cyclodextrin and cyclodextrin-based nanosponge inclusion complexation. *Polymers*, **2021**, *13*, 4073.
5. S. Abou Taleb, et al. Quercitrin loaded cyclodextrin based nanosponge as a promising approach for management of lung cancer and COVID-19. *Journal of Drug Delivery Science and Technology*, **2022**, *77*, 103921.
6. M. Hadadian, R. Allahyari, and B. Mahdavi. A New Approach for Improving the Water Solubility of Progesterone by Encapsulation with Carboxymethyl-β-CD. *Organic Chemistry Research*, **2023**, *9*, 35.
7. T.B. Mesen and S.L. Young. Progesterone and the luteal phase: a requisite to reproduction. *Obstetrics and gynecology clinics of North America*, **2015**, *42*, 135.
8. V.K. Shankar, et al. Optimization of sulfobutyl-ether-β-cyclodextrin levels in oral formulations to enhance progesterone bioavailability. *International Journal of Pharmaceutics*, **2021**, *596*, 120212.
9. M. Hadadian, et al. Design, characterization, and in vitro evaluation of magnetic carboxymethylated β-cyclodextrin as a pH-sensitive carrier system for amantadine delivery: a novel approach for targeted drug delivery. *RSC advances*, **2025**, *15*, 446.
10. M. Hadadian, et al. Optoelectronic properties of β-Cyclodextrin compound and doped with Na: Comparative study by experimental and DFT approaches. *Journal of Molecular Structure*, **2024**, *1316*, 138862.
11. S. Swaminathan, et al. Structural evidence of differential forms of nanosponges of beta-cyclodextrin and its effect on solubilization of a model drug. *Journal of inclusion phenomena and macrocyclic chemistry*, **2013**, *76*, 201.
12. J.T. Hołaj-Krzak. Assessment of the usefulness of β-cyclodextrin in the removal of progesterone from the environment. *Science of The Total Environment*, **2024**, *950*, 174908.
13. A. Matencio, et al. Study of progesterone complexation in cyclodextrins and cyclodextrin-based nanosponges as an example of solvent-free complexation. *Journal of Drug Delivery Science and Technology*, **2024**, *98*, 105893.
14. P. Shende, et al. Acute and repeated dose toxicity studies of different β-cyclodextrin-based nanosponge formulations. *Journal of pharmaceutical sciences*, **2015**, *104*, 1856.
15. Y. Hanqi, et al. Development of a magnetic Carboxymethyl-β-Cyclodextrin nanocarrier for targeted sitagliptin delivery. *Journal of Molecular Liquids*, **2025**, *433*, 127872.
16. M. Hadadian, et al. Comparative study of β-cyclodextrin and carboxymethyl-β-cyclodextrin as effective drug delivery systems for oxymetholone: Design, preparation, characterization, phase solubility and in vitro drug release studies. *Journal of Science: Advanced Materials and Devices*, **2024**, *9*, 100751.
17. L. Nikolić, et al. The formulation of curcumin: 2-hydroxypropyl-β-cyclodextrin complex with smart hydrogel for prolonged release of curcumin. *Pharmaceutics*, **2023**, *15*, 382.
18. M. Ramirez-Ambrosi, et al. Encapsulation of apple polyphenols in β-CD nanosponges. *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, **2014**, *80*, 85.
19. J. Chen, et al. Characterization of curcumin/cyclodextrin polymer inclusion complex and investigation on its antioxidant and antiproliferative activities. *Molecules*, **2018**, *23*, 1179.

20. I.M. Helbling, et al. Preparation of TPP-crosslinked chitosan microparticles by spray drying for the controlled delivery of progesterone intended for estrus synchronization in cattle. *Pharmaceutical Research*, **2018**, *35*, 1.

21. D.M. Ragab, S. Rohani, and S. Consta. Controlled release of 5-fluorouracil and progesterone from magnetic nanoaggregates. *International journal of nanomedicine*, **2012**, *7*, 3167.

22. H.D. Williams, et al. Strategies to address low drug solubility in discovery and development. *Pharmacological reviews*, **2013**, *65*, 315.

23. A.S. Hassan, et al. Solubilization and enhancement of ex vivo vaginal delivery of progesterone using solid dispersions, inclusion complexes and micellar solubilization. *Current drug delivery*, **2018**, *15*, 110.

24. X. Chen, et al. Solubility improvement of progesterone from solid dispersions prepared by solvent evaporation and co-milling. *Polymers*, **2020**, *12*, 854.

25. F.M. Mady and U. Farghaly Aly. Experimental, molecular docking investigations and bioavailability study on the inclusion complexes of finasteride and cyclodextrins. *Drug design, development and therapy*, **2017**, *11*, 1681.

26. S.R. Arsad, et al. Theoretical and Molecular Docking Study of Ketoconazole on Heptakis (2, 3, 6-tri-O-methyl)-β-cyclodextrin as Chiral Selector. *Chirality*, **2016**, *28*, 209.