REVIEW ARTICLE

Unleashing the potential of cyclodextrin-based nanosponges in management of colon cancer: A review

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Abstract: Colon cancer ranked second in terms of incidence in the world, yearly it shows an increase in the tendency of mortality. It is estimated that by the year 2035 total number of deaths will increase by 75 %. The treatment of cancer includes surgery, radiation therapy, and chemotherapy, however, they are limited due to their targeted therapeutic potential and side effects. In the case of a conventional drug delivery system, it is desired that the drug delivery system should be able to protect the therapeutic from degradation and safety target to the desired site. Cyclodextrin-based nanosponges (NS) are a versatile platform for colon cancer treatment, surpassing the limitations of conventional drug delivery systems. Nanosponge offers several advantages such as improved drug solubility, enhanced stability, targeted delivery, and potential for combination therapy due to its highly cross-linked structure. The fabrication of NS includes the use of biopolymers for the controlled release of drugs from pores through diffusion. These pores can efficiently encapsulate a wide range of therapeutic agents, enabling regulated release, and addressing challenges associated with poor solubility and limited stability of drugs used in the management of colon cancer. Additionally, their functionalization enables targeted drug delivery to colon cancer cells, minimizing off-target effects. Though several benefits are associated with NS, further research is needed to address regulatory considerations and scale up NS for translation into clinical practice. Overall, cyclodextrin-based NS holds promise in revolutionizing colon cancer treatment and improving patient outcomes.

Keywords: Cyclodextrin; Colon cancer; Drug delivery; Improved Solubility; Nanosponges.

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1. INTRODUCTION

Nanotechnology (NT) has tremendously enabled the exploration in development of nanomedicine, a subfield that is highly beneficial to healthcare. It discovers unique physical, chemical, and biological properties of drug material at the nanometre scale to diagnose, treat, or prevent diseases (Anjum *et al.*, 2021). In the last thirty years, the discipline of NT has been a crucial area of research, due to the unique chemical, electrical, optical, biological, and magnetic properties of nanomaterials (Sindhwani & Chan, 2021). NT has managed to attract significant attention because when NT joins hands with biotechnology, it gives birth to a platform that holds immense potential and importance for diversity in applications (Laouini *et al.*, 2021). Highly cross-linked structures, nanosponges, have been widely used in the last decade for various

therapeutic applications (Kumar & Rao, 2021). NS is defined as hydrophilic, water-insoluble, and supramolecular 3D-hyper-reticulated nanoporous structures with high stability over a wide range of temperatures and pH (Kumar *et al.*, 2020). Cyclodextrins (CDs) have been used in the synthesis of NS providing cooperative properties between the amphiphilicity and high surface area (Kumar & Rao, 2021). However, it should be stressed that the application of cyclodextrin in the fabrication of nanosponges (CDNSs) is still at an early stage.

CDs have been studied for over a century and widely used as a pharmaceutical excipient or being capable of incorporating therapeutic molecules into their central cavity (Hoti *et al.*, 2021; Kawano *et al.*, 2015). Currently, CDs are widely used in food products, textiles, toiletry, and various cosmetics as well as in certain medical products (Liu *et al.*, 2022; Sharma & Baldi, 2016). CDs complexation technique is mostly used to alter the solubility of drugs (Semalty, 2014). The solubility of drug drastically changes when it complexed with cyclodextrins due to the ability to encapsulate hydrophilic as well as lipophilic drugs

Colon cancer (CC) is the third most common cause of death in the world with a survival rate of only 10 % and majorly affects old ages (Basu *et al.*, 2024; Bhattacharya *et al.*, 2023; Bhattacharya *et al.*, 2024; Mohite *et al.;* Parihar *et al.*, 2024; Sahu *et al.*, 2024). It starts in the epithelial region and advances to polyp adenoma and then to carcinoma. The conditions correlated with chronic inflammation, mutagen exposure, and abdominal obesity (Pothuraju *et al.*, 2020). The survival rate of CC improves by employing adjuvant and non-adjuvant therapies, surgery, radiotherapy and chemotherapy alone. However, their efficiency is limited due to inherent susceptibility (Zeng *et al.*, 2018).

CC faces challenges in terms of effective management, particularly due to limitations encountered with conventional drug delivery systems (Kalaydina *et al.*, 2018). Issues such as poor solubility, limited stability, and inadequate targeting abilities hinder the therapeutic outcomes of conventional approaches. However, advancements in NT have opened up new possibilities for enhancing drug delivery in CC treatment. One such innovation is the utilization of CDNSs that offer unique advantages in improving the efficacy and safety of therapeutic agents. The use of CDNSs in the treatment of CC enhances drug solubility and bioavailability, targeted delivery to CC cells, prolonged, and controlled drug release, and protection of drugs from degradation with potential for combination therapy. By functionalizing the NS, they can be specifically targeted to CC cells, minimizing off-target effects, and reducing systemic toxicity. The NS are a novel class of colloidal systems having a unique appearance and highly cross-linked structure which enables the entrapment of drugs in the cavity. Moreover, the porous structure of NS enables sustained and controlled release of drugs, ensuring a continuous therapeutic effect. Additionally, the encapsulation of drugs within NS protects against degradation, improving their stability and shelf life. Furthermore, the ability of NS to encapsulate multiple drugs allows for combination therapy, targeting multiple pathways involved in CC progression simultaneously.

The present review aims to provide insight into the limitations encountered with conventional drug delivery systems in the management of CC that necessitate alternative approaches. CDNSs offer advantageous features such as enhanced solubility, targeted delivery, sustained release, and drug protection, making them a promising solution for improving CC treatment. Therefore, utilization of such NS as the carrier has the potential to overcome the challenges associated with conventional drug delivery systems with enhanced efficacy and safety of therapeutic agents in colon cancer management.

Cyclodextrin

Cyclodextrins are a family of cyclic oligosaccharides composed of glucose units that possess a unique molecular structure with a hydrophobic cavity and a hydrophilic outer surface (Fig. 1). This structural arrangement provides CDs several advantages, making them valuable in various applications, including drug delivery (Mura, 2020; Tiwari *et al.*, 2010).

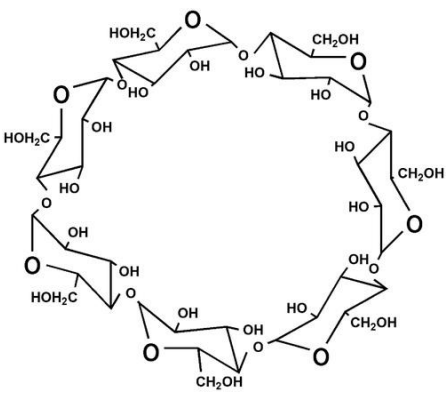

Figure 1. The torus-like shape of cyclodextrin.

One of the primary advantages of CD is its ability to improve the solubility of poorly soluble drugs (Archontaki *et al.,* 2002). CD can form inclusion complexes with hydrophobic drugs by encapsulating them within their hydrophobic cavities. This inclusion complex formation enhances the solubility of the drugs, allowing for their effective formulation into various dosage forms (Ahsan *et al.,* 2001). By improving the solubility, CD attenuates the bioavailability of drugs, ensuring that enough amount of the drug is available for absorption, which in turn enhances the therapeutic efficacy of the pharmaceutical formulation. CDs also offer the advantage of enhancing the stability of drugs (Arima *et al.,* 1998). By encapsulating drugs within their cavities, CD protects the drug molecules from degradation under environmental factors such as light, moisture, and oxygen. Moreover, this encapsulation improves the stability of the drugs, extending their shelf-life and maintaining their efficacy over a longer period. Furthermore, CDs mask the unpleasant tastes and odors of drugs, making them more palatable for patients. This property is particularly beneficial in the development of oral dosage forms, especially for pediatric and geriatric populations where patient compliance can be a challenge (Arias *et al.,* 2000). Additionally, CDs effectively mask the undesirable sensory attributes of drugs, increasing patient acceptance and adherence to the medication. In addition, CDs exhibit compatibility with various routes of administration. They can be incorporated into different pharmaceutical dosage forms, including oral tablets, capsules, creams, gels, and even parenteral formulations. This versatility allows for their use in diverse patient needs and preferences, ensuring that CDs can be employed in a wide range of pharmaceutical applications (Arias *et al.,* 2000). Importantly, CDs are considered safe and biocompatible. Besides, CDs are readily eliminated from the body without causing significant adverse effects, making them suitable for pharmaceutical applications and minimizing the potential for patient harm.

Limitation of native cyclodextrins

Cyclodextrins can include molecules of size and polarity compatible with its lipophilic inner cavity. Native cyclodextrins are not able to incorporate certain hydrophilic compounds or large molecules (Sherje *et al.,* 2017). One notable constraint is its limited solubility in water, which can hinder its effectiveness in aqueous formulations. This solubility issue may restrict its application in certain pharmaceutical and food formulations, where water solubility is a crucial factor. Additionally, plain cyclodextrin's relatively low complexation efficiency with certain guest molecules may limit its ability to encapsulate and stabilize specific compounds effectively. The structural rigidity of cyclodextrins can also pose challenges in accommodating larger or more flexible guest molecules, potentially restricting their inclusion in the cyclodextrin cavity. Moreover, the potential for retrogradation, wherein the inclusion complexes may revert to their original state over time, could impact the stability and shelf life of products incorporating plain cyclodextrin. Addressing these limitations has spurred research efforts focused on modifying cyclodextrins, such as derivatization or the development of novel derivatives, to overcome these challenges and enhance their overall utility in various applications (Puskás & Malanga, 2017; Rapp *et al.,* 2021).

2. FABRICATION TECHNIQUES NANOSPONGES

Nanosponges are highly cross-linked porous structures that encapsulate the drugs in the cavity. The maximum drug loading and porous nature are the desired characteristics of NS. Therefore, the selection of a suitable and effective method is preferred. NS can be synthesized by using different methods; some of the methods are listed as follows

Solvent method

This method involves adding a solution of polymer to an excess of the crosslinker, maintaining a temperature of 10 °C for 48 h. Further, the mixture is cooled, and excess water is added to it, which results in the formation of nanosponges. The prepared nanosponges were filtered under a vacuum and collected. Through a long-term Soxhlet extraction process with ethanol, the mixture is purified (Farsana *et al.,* 2021).

Suitable solvents, such as dimethylformamide and dimethyl sulfoxide which are polar aprotic solvents, were used in the process (Kartik Tiwari & Sankha Bhattacharya, 2022). To this, the polymer was added and properly blended in different ratios with crosslinker. Later crosslinked composition is left to react for 48 h at a temperature range of 100 °C or up to the solvent's reflux temperature. On completion of the reaction, the solution cooled

down until it reached room temperature followed by the addition of an excess quantity of double distilled water to obtain the product after vacuum filtration (Gadade & Pekamwar, 2020).

Naproxen sodium Nanosponges were developed by Ilyas *et al.*, utilizing the solvent diffusion method, and it was discovered that formulations had a diffusion rate equal to 89 % and a drug loading efficiency near 98 %. Additionally, they investigated stability studies, zeta potential, viscosity, and particle size. The Fourier Transform Infrared Spectroscopy results did not show any evidence of a drug-excipient interaction. The findings also demonstrated outstanding drug release characteristics and higher drug loading efficacy (Kartik Tiwari & Sankha Bhattacharya, 2022).

Ultrasound-assisted method

The ultrasound-assisted method is another technique to synthesize NS using polymer under an ultrasonic junction. The cross-linking process was initiated in the presence of ultrasonic waves for polymer and crosslinker at varied molar ratios, at a temperature of 90 °C and for a specified period. The temperature of the collected mixture reduced slowly after sonication, and the product split harshly to separate unreacted polymer and reagents with an excess volume of water. The washed solid was further purified with ethyl alcohol using Soxhlet extraction techniques. The filtered NSs acquired are vacuum-dried and processed correctly until further loading of drugs (Diego Marestoni *et al.*, 2020; K. Tiwari & S. Bhattacharya, 2022).

Melt method

Cyclodextrin-based nanosponges are also synthesized by a melt procedure. In this process, NS are obtained through crosslinking of different types of CDs with a carbonyl or a dicarboxylate compound as crosslinker. The different crosslinking agents dramatically modulate important parameters such as swellability and hydrophilicity/hydrophobicity of the nanoporous polymer. In the melt method, the crosslinker and polymers are transferred around the bottom flask in specific ratios and react at 100 °C. The reaction mixture was further allowed to cool down and washed with desired solvent to remove the unreacted polymer and phenol crystals followed by repeated washing with suitable solvents to remove unreacted excipients and by-products

Microwave Assisted Synthesis

This is the simplistic method for the synthesis of cyclodextrin-based NS using microwave irradiation that significantly reduces the reaction time (Singireddy *et al.*, 2016). These NS have a higher degree of crystallinity. Compared to the conventional heating method, microwave-assisted synthesis of NS showed a fourfold reduction in reaction time with homogeneous-sized particle distribution with uniform crystallinity (Sherje *et al.*, 2017).

Bubble electrospinning

A conventional and typical electrospinning configuration consists primarily of a syringe, a syringe pump, as defined in many literatures, a high-voltage power, and a grounded collector. However, one of the major limitations that limits their applications is the amount of output of nanofibers. In bubble electrospinning, polyvinyl alcohol can also be used as a polymer. By the addition of distilled water into it, the solution of polymer (10%) was organized, which was then moved at 80-90 °C for 2 h to obtain a one-phase mixture. It was then left to achieve at room temperature with the polymer solution and then used to prepare nanoporous fibers (Kartik Tiwari & Sankha Bhattacharya, 2022). (Fig. 2).

Drug loading process within nanosponges

The passive drug loading method is preferred in the case of β-cyclodextrin-based NS. Experimental investigation requires the weighed number of blank NS dispersed in the solution of the drug mixture followed by sonication to avoid aggregation. The resulting solution was further stirred for 24 h on a magnetic stirrer at a speed of 200 rpm and subsequently, the resulting mixtures are subjected to centrifugation. The drug-containing supernatant was separated and freeze-dried for 24 h to get porous drug-loaded NS. The structure of NS plays a very important role in the complexation with drugs. A study revealed that para-crystalline NS showed different loading capacities, compared to crystalline NS. While, in poorly crystalline nanosponges, the drug loading occurs as a mechanical mixture rather than an inclusion complex (Shringirishi *et al.*, 2014).

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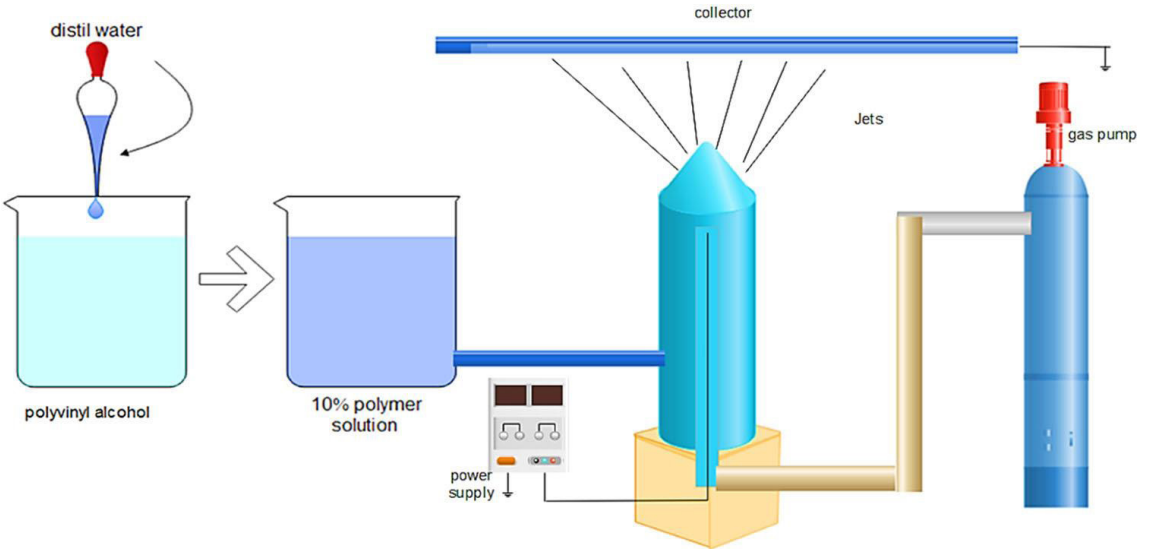

Figure 2. Schematic representation of bubble electrospinning method. Reproduce with permission from (Kartik Tiwari & Sankha Bhattacharya, 2022) under Creative Commons Attribution (CC BY 4.0) license.

3. CHARACTERIZATION TECHNIQUES FOR NANOSPONGES

Multiple physiochemical tests are required to test CDNSs strength, level of crosslinking, and rate of drug delivery that helps to evaluate the characteristic features of formulation. The following evaluation is deemed necessary to characterize the NS such as drug content, entrapment efficiency, morphological analysis, phase solubility, thermomechanical analysis, x-ray diffraction, functional group analysis, porosity, swelling index, in *vitro* release, and stability studies as given below.

Drug Content and Entrapment Efficiency

To determine drug loading, a high concentration of drug is dissolved in a suitable solvent considering the solubility of the drug, later CDNSs are suspended in the mixture. Subsequently, the dispersion is mixed and shaken at room temperature for a certain time period and then filtered to get the NS portion. This portion is then freeze-dried and drug content is quantified from the resultant mixture. Similarly to quantify the drug entrapment, the drug-loaded NS is mixed in drug-soluble liquid and sonicated for disrupting the complex within NSs hence causing the drug to dissolve in solvent and drug concentration in the solvent is estimated using analytical techniques through either UV-Vis spectroscopy or high-performance liquid chromatography (Lembo *et al.*, 2018).

Microscopic studies

The microscopic aspects such as morphology and surface topography of NSs, or the product are imaged using scanning electron microscopy or transmission electron microscopy. The difference in the crystallization state indicates the formation of inclusion complexes (Jilsha & Viswanad, 2013) (Fig. 3).

Phase solubility

The phase solubility technique investigates the effect of NS on drug solubility (Swaminathan *et al.*, 2007). Experimentally phase solubility determination requires excess drug addition to saturated solutions of suitable solvents. Thus, solubility study involves the addition of varying concentrations of blank nanosponges and continuing until equilibrium is obtained. Later the stability constant values obtained from the interaction between nanosponges and the drug can increase the solubility of poorly water-soluble drugs. Such studies have been carried out for itraconazole loaded cyclodextrin nanosponges prepared by Shankar and co-workers (Swaminathan *et al.*, 2007). Consequently, NS could markedly increase the solubility of molecules with very low aqueous solubility such as anticancer drugs, steroids, and anti-inflammatory drugs (Sawatdee *et al.*, 2016).

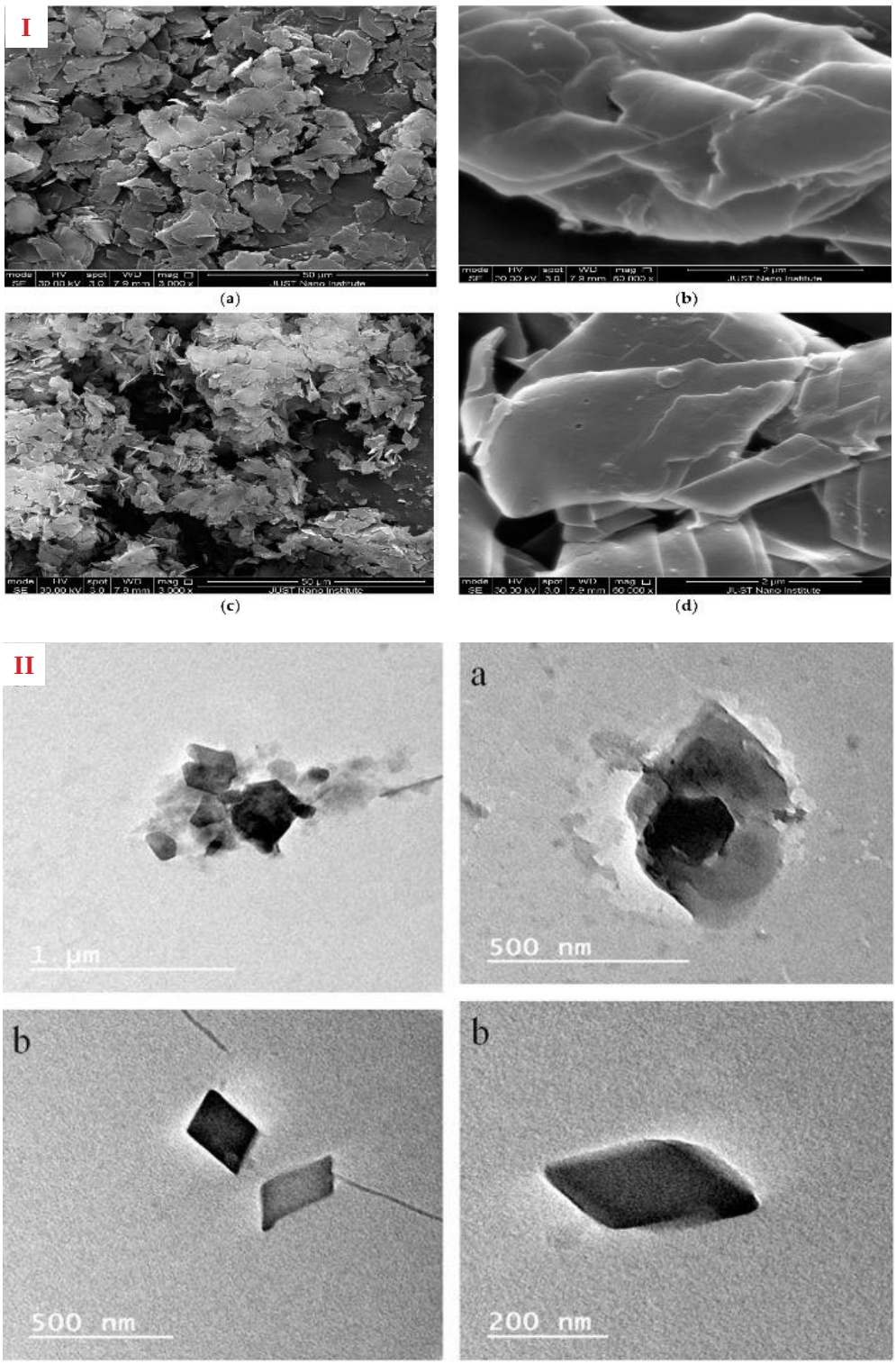

Figure 3. Scanning electron microscopy images of β-CDNS (I) [curcumin-loaded diphenycarbonate cross-linked NS (a); curcumin-loaded diphenycarbonate cross-linked NS (b); curcumin-β-CD complex (c); curcumin-β-CD complex (d)]. Transmission electron microscopy mages of β-CDNS -II [Blank NS (a) and Babchi Oil-Loaded β-CDNS (b)]. Reproduce with permission from (Kumar *et al.,* 2018; Mashaqbeh *et al.,* 2021) under Creative Commons Attribution (CC BY 4.0) license.

Thermo-analytical methods

Thermo-analytical methods determine whether the drug substance undergoes some change before the thermal degradation of the NSs, this change includes evaporation, decomposition, oxidation, or polymorphic transition of incorporated drug substance, which may indicate the complex formation. The thermogram obtained from differential scanning calorimetry, in case complex formed, broadening, shifting, and appearance of new peaks or disappearance of certain peaks are observed with changes in the weight loss (Jagtap *et al.*, 2019).

X-ray diffraction

X-ray powder diffraction (XRPD) has been used for evaluating the crystallinity of NS and its drug complexation capacity (Ahmed *et al.*, 2013a). Changes in crystallinity have a profound effect on drug loading, solubility, dissolution, and drug release kinetics. XRPD of β-CD shows an amorphous state while the NS may either be crystalline or para-crystalline (poorly crystalline) depending on processing conditions. Swaminathan and his colleagues report this phenomenon, CAM loaded CD-NS were prepared using two sets of conditions, namely, reacting β-CD and DPC at 90 °C, with and without using ultrasound. A crystalline product was obtained using the ultrasound-assisted method while a para-crystalline product was obtained without ultrasound. The XRPD data of the para-crystalline product showed an increase in the peak area while the intensity versus full width at half maximum ratio decreased (Ahmed *et al.*, 2013b). The XRPD patterns of crystalline NS at different β-CD/cross-linking agent ratios are shown in Fig. 4.

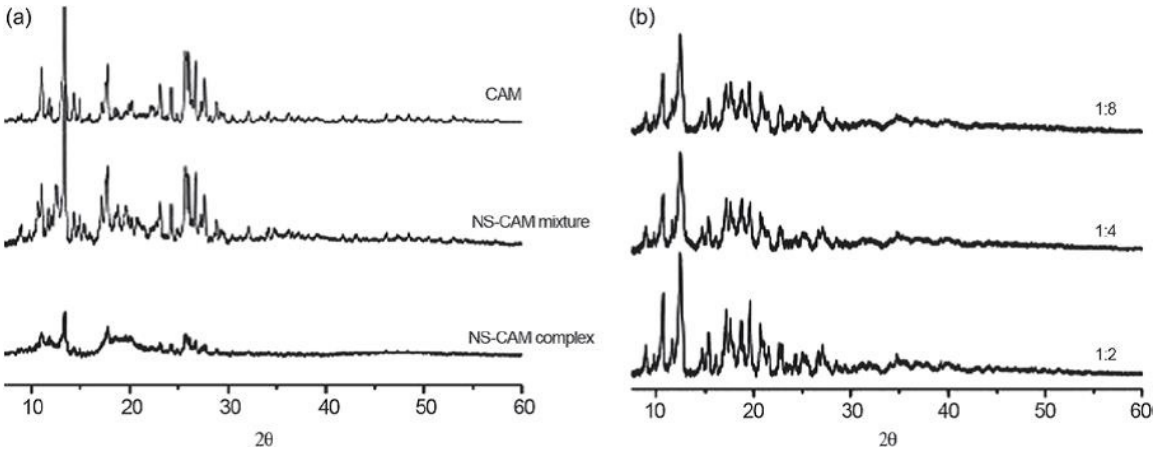

Figure 4. X-ray diagrams of crystalline camptothecin-loaded NS, compared with pure camptothecin-NS simulated physical mixture and plain camptothecin (a). X-ray diagrams of crystalline NS at different β-CD/cross-linking agent ratios (b). Reproduce with permission from (Swaminathan *et al.*, 2010), Righlink copyright clearance from.

Fourier transform-infrared spectroscopy

Fourier transform-infrared spectroscopy (FTIR) is the most important technique for structural elucidation, particularly functional group detection. Monomers get attached to form polymer during polymerization reaction where functional group peaks in the spectrum of FTIR are the characteristic indications of polymerisation. The range of 4000-650 cm^{-1} is used to take FTIR spectra of drug, polymer, drug-polymer physical mixture, neat NS, drug-loaded nanosponges and observed for any possible interaction. It also shows the hydrophilic and hydrophobic sites of nanosponges. The disappearance of any functional group peak in the case of hydrophobic drug is because of its inclusion in cyclodextrin/NS cavity (Kumar & Rao, 2022).

Raman spectroscopy

Raman spectroscopy is an extremely useful tool in molecular study as the intensity, width, and wavenumber of Raman peaks are quite responsive to confirmation, molecule environment and intermolecular reactions. Raman spectroscopy explores the elucidation of CDNS after entering a swollen form

from a dry state. Moreover, this technique also provides information about the illustration of the state of water and dissolved solute inside nanoporous NS architecture with meticulous importance to diffusion from the gelled condition. Dynamics of hydration are examined by analyzing vibration modes of decoupled O-H and C-H groups from bulk water background (Sherje *et al.*, 2017).

Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance spectroscopy techniques such as ^{13}C, ^{1}H, 2D NMR, and high-resolution magic angle spinning techniques help in understanding the structure of CD crosslinked polymers. The shift in chemical shift values (δ) indicates the transfer of proton among species in reaction and hence ascertains the structure of the NSs (Kartik Tiwari & Sankha Bhattacharya, 2022).

Particle size analysis

Mean particle size, zeta potential, and polydispersity index of nanosponges are analyzed using dynamic light scattering using a zetasizer instrument, at room temperature. Experimentally, the samples are dispersed in Milli-Q water to develop a monodisperse system and assessed. Zeta potential is a measure of the level of the surface charge and serves as an indicator of the relative magnitude of the repulsion force between colloidal particles in aqueous suspension. A high zeta potential prevents particle-particle agglomeration, whereas the zeta potential is greater than 30mV, then the dispersion is stable, which is measured using additional electrode in particle size equipment (Anwer *et al.*, 2022). Moreover, the mean hydrodynamic diameter and polydispersity index of the particles are calculated using the cumulated analysis after averaging the total measurements (Shringirishi *et al.*, 2014).

Void fraction/porosity, swelling, and water uptake

Void fraction investigations are performed to test the width of the nanoholes and nanopores that are developed after freeze-drying. Experimentally, a helium pycnometer measures the porosity of NS, since helium gas can invade associate- and intra-specific channels of fabric. The genuine amount of the fabric is measured by means of the helium uprooting cycle due to its permeable presence, nanosponges display more noteworthy porosity relative to the guardian polymer utilized to make the gadget (Prabhu *et al.*, 2020). The NS was developed using swellable polymers like polyamidoamine, the water uptake is quantified by soaking the prepared nanosponges in aqueous solvent (Prabhu *et al.*, 2020).

In-vitro release and release kinetics

The release behavior of the drug from NS is assessed using *in vitro* release study. Multi-compartment rotating cell in which donor compartment is filled with an aqueous dispersion of NS fortified with active moiety and receptor compartment filled with phosphate buffer of appropriate pH. The compartments are separated with a hydrophilic dialysis membrane and samples are withdrawn at a fixed time and replaced with fresh buffer to maintain the sink condition. Later using a suitable analytical technique the amount of drug is determined and drug release is calculated. Further, to investigate the mechanism of drug release from nanosponge the release data could be analyzed using Zero order, First order, Higuchi, Peppas, Hixon-Crowell, Kopcha and Makoid-Banakar models. The data set obtained is further used to estimate the parameters of a non-linear function that provides the closest fit between experimental observations and non-linear function (Kumar & Rao, 2022). Furthermore, the Nanosponges are tested for stability according to ICH guidelines (Venkateswarlu *et al.*, 2022). A summary of characterization performed over β-CDNS for various drugs is presented in Table 1.

4. FACTORS AFFECTING THE SYNTHESIS OF NANOSPONGE

Cross-linkers and polymer types

The performance and development of NSs are impacted by the type of polymer employed. The amount of cross-linker is responsible for getting the desired three-dimensional cross-linked structure (Table 2). Molecular nanocavities are transformed into three-dimensional nanoporous structures that are capable of cross-linkers. The ratio of polymer: crosslinker plays a vital role as they encapsulate the drug which affects the solubility of the drug (Kartik Tiwari & Sankha Bhattacharya, 2022). The use of different polymers for the preparation of nanosponges is reported in Table 2.

Drug	Polymer	Fabrication technique	Characterization	Critical attribute	Reference
Flutamide	cyclodextrin	Freeze-drying	Particle size, zeta potential, and morphology	Small size (82.53 ± 42.32 and 99.10 ± 22.15 nm) and high zeta potential (43.0 ± 9.89 and 24.1 ± 10.1 mV) could indicate the stabilization of NS suspension due to reduced aggregation probability of particles.	(Allahyari *et al.,* 2021)
			In-vitro release studies	*In-vitro* release pattern of FLT showed complete release in 180 min from NS complexes without burst effect. A dramatic increase observed in the rate and amount of drug release due to the wettability of flutamide. By comparing the release profiles of CDNS at different composition 1:2 and 1:4, experimentally observed that the release can be modified by changing the cross-linking degree of CD molecules.	
			Loading of flutamide into CDNSs	Moreover, NS (1:4) showed higher encapsulation of drug, compared with NS 1:2 might be due to a high cross-linker ratio and an increase in cavities number. The encapsulation capacity CDNSs might be affected by different factors such as the cross-linker ratio of CDNSs, size, and chemical structure of a guest molecule	
Econazole Nitrate (EN)	β-Cyclodextrin, N,N-carbonyldi imidazole	Melt method	Surface morphology	The scan electron microscopy of β-CD showed a compact structure whereas the of placebo NSs demonstrated a spongy structure of the formulation. In case of dug loaded NS the desired porosity not seen as the porosity was partly occupied by the drug in the drug-loaded nanosponges due to the formation of inclusion complexes	(Srivastava *et al.,* 2021)
			FTIR	The broadening of peaks of carboxylate stretching in structural analysis at 1535 cm^{-1} to higher wavelength suggested the development of hydrogen bonds between the groups of econazole nitrate and the hydroxyl groups of β-CD along with the desertion of the peaks at 3250 cm^{-1} (asymmetric O-H stretching) indicated entrapment of the drug into NS.	
			DSC	While the disappearance of peak disappeared in EN-CDNS signifying a change of the drug crystalline form into an amorphous state in the NS formulation demonstrating assimilation of the drug within the NS as inclusion or non-inclusion complexes.	

Drug	Polymer	Fabrication technique	Characterization	Critical attribute	Reference
Curcumin	β-CD	Freeze-drying method	XRD	The XRD pattern of EN showed a prominent sharp peak at 2θ angles 10.1, 17, 21.2, 26.8, and 29.1 which specify the crystalline nature. The XRD diffractogram of the drug in NS shows diffused peaks with low intensities indicating that the drug crystallinity was remarkably reduced indicating loading of drug into nanosponges in unstructured or solid-state solubilized form or disarrayed crystalline phase inside the polymeric matrix.	(Mashaqbeh *et al.*, 2021)
			Phase solubility studies	The solubility of curcumin enhanced as a feature of β-CD level increases, displaying the AN type of solubility phase profile. Meanwhile, the curcumin complex with NS exhibits the BS type of solubility phase diagram. Total curcumin solubility was enhanced by the formation of β-CD inclusion complexes up to 2.34-fold, compared to the inherent solubility	
			Molecular modeling	The molecular docking conferred that the interaction between cross linker and polymer which is desired for preparation of NS. The docking results showed that complexation schemes have comparable binding affinities with binding interactions at ratio of 1:1 complex.	
			In-vitro release profile	The in vitro release profile of curcumin loaded NSs showed an enhancement in curcumin release and curcumin was released from the NS4 sample faster than from the physical mixture and the raw curcumin.	
Vitamin D	β-cyclodextrin	Complexation method	Thermostability	The study demonstrates for the first time the ability of CDNS complexes to improve the thermostability, chemical, and biological function of Vit D3.	(Uberti *et al.*, 2023b)
6-Gingerol	CD	Complexation method	Thermogravimetric analysis	HP β-CD had two stages of thermal weight loss. The first stage demonstrated weight loss of 3.5% due to vaporization of residual water. While the second stage was the apparent thermal weight loss caused by HP β-CD decomposition, with 81.8% weight loss.	(Uberti *et al.*, 2023a)
			Scanning electron microscopy	The SEM micrograph quantified the morphological characters of the 6-Gingerol CDNS. Within the aqueous medium, a closed etho-niosomal bilayer developed that tends to minimize their surface free energy by the development of spherical trans-ethoniosomal vesicles.	

Drug	Polymer	Fabrication technique	Characterization	Critical attribute	Reference
Anticancer Hydrophobic Agents, Naringenin (NG)	hydroxypropyl-β-cyclodextrin	Cross linking method/ complexation method	zeta potential	The zeta potential reflects the charge and the stability of the CDNS. The higher zeta potential value of optimized formulation indicated the stability of the nano-dispersion within CDNS due to repulsive force between the trans-ethoniosomal nanovesicles and the presence of a high-energy barrier between them that inhibits their aggregation.	(Peimanfard *et al.,* 2022)
			Phase solubility studies	The Higuchi–Connors profile demonstrated that the complex formation of HPβ-CD with NG has a tremendous effect on the water solubility of NG, and a saturated concentration of HPβ-CD in water (almost 322 mM) could increase the NG aqueous solubility by about 2375-fold.	
			^{1}H NMR and ^{13}C cross-polarization Magic angle spinning solid-state NMR	NMR spectroscopy has been widely used to study CD complexes, the results obtained from the experimental ^{1}H NMR spectra and the Higuchi–Connors method suggested that HPβ-CD formed a host–guest complex with NG through an inclusion phenomenon.	

Table 1. Summary of characterization performed β-CDNS for various drugs.

Cross-linkers	Diarylcarbonates, carbonyldiimidazoles, pyromellitic anhydride, carboxylic acid dianhydrides, glutaraldehyde, epichloridrine, 2,2-bis (acrylamide) acetic acid, Diphenyl carbonate, and di-isocyanates
	Polyvinyl alcohol, ethyl cellulose, Hyper-cross-linked polystyrene, cyclodextrins and its derivatives, including methyl-CD, alkyloxy carbonyl-CD, and 2-hydroxy propyl-CD, as well as copolymers such poly (allylvalerolactone-allylvalerolactone), poly(allylvalerolactone-allylvalerolactoneoxepanedione)

Table 2. Chemicals frequently used in the synthesis of NS.

Epichlorohydrin is used as a cross-linker to develop hydrophilic NSs. An effective drug carrier can be employed in formulations for immediate release with such an NS, which also improves drug absorption across biological barriers. Diphenylcarbonate treatment can create a hydrophobic NS. Carbonyldiimidazoles pyromellitic anhydride, diisocyanates as cross-linkers, and they could serve as a vehicle for the sustained release drug delivery of hydrophilic medicines, such as proteins and peptides. Considering recent investigations, various examples of polymers employed with NS preparation methods are presented in Table 3 along with prospective applications.

Polymer	Fabrication technique	Size	Use	References
β-CD and different crosslinkers	Cross-linking using polymers	Not quoted	Removing toxic molecules from the body	(Varan *et al.,* 2020)
CD and their derivatives	Simple thermal desorption, extraction with solvents and/or use of microwave and ultrasound techniques (diphenylcarbonate or pyromellitic anhydride as crosslinkers)	Below 500 nm	Solubility enhancement, cytotoxicity, hemolytic, antifungal, antiviral activity	(Cavalli *et al.,* 2010; Osmani *et al.,* 2018b)
β-CD and copolyvidonum	Simple thermal desorption, extraction with solvents and/or use of microwaves and ultrasounds	Not quoted	Saturation solubility study	(Mele *et al.,* 2011)
β-cyclodextrin with diphenyl carbonate (DPC), pyromellitic dianhydride (PMDA) and epichlorohydrin (EP)	Simple thermal desorption, extraction with solvents	166-673 nm	herbicide	(Liu *et al.)*
β-Cyclodextrin-based carbamate nanosponges	Cross-linking using polymers	Not quoted	NSAIDS	(Pawar *et al.,* 2019)

Table 3. Cyclodextrin Nanosponges prepared by diverse methods and their potential applications.

Type of drugs and medium used for crosslinking

The solvent system employed demonstrates a significant impact on the production of NS, in addition to the type and nature of the polymer and cross-linker utilized. To encapsulate the maximum quantity of drug crosslinked with nanostructure, the physiochemical properties of drug were considered. Moreover, a molecular weight of less than 500 Da is a basic prerequisite and preferred for encapsulation.

To be properly entrapped within nanocavities, drug molecules are required to comply with specific properties such as simple to trap molecules, molecular mass between 100 and 400 Da, and fewer than five condensed rings. Additionally, the molecules should possess a melting point of around or less than 250 °C with solubility in water of not more than 10 mg/mL (Osmani *et al.*, 2018a). Higher melting points of pharmaceuticals make it difficult to develop stable complexes between medicines and niacin due to the need to maintain higher stability constant values after loading in the NS. Moreover, the loading of the drug is significantly impacted by a greater drug melting point. Additionally, due to the stiffness of the compound's structural makeup, melting of compounds at higher temperatures results in reduced drug loading. A hydrophilic media forces organic guest molecules into the hydrophobic cavities, while an organic solvent tends to release the organic molecules trapped in NS. Therefore, the medium has a significant impact on how targeted compounds interact with NS cavities. Furthermore, optimized physical and chemical interactions, such as structural characteristics, size, mutual polarity matching, and hydrophobic environment drive the strong attraction between host and guest molecules (Osmani *et al.*, 2018a).

Degree of substitution

The quantity, position, and type of the substituent on the polymeric molecule demonstrate a significant impact on a NSs capacity to complex (Jagtap *et al.*, 2019). As the β-CD derivatives are widely available in major three forms due to variations in the functional groups present on the surface of CD derivatives, the kind of substitution is essentially different. Different types of complexed material (β-CD NSs, CD-carbonate NSs, and CD-carbamate NSs,) can be developed with different functional groups when they are complexed together using cross-linker. The number of substitutions and cross-linking are directly proportional to one another, which shows that exhibiting more substituents may increase the likelihood of higher levels of cross-linking, which can produce highly porous NSs as a result of more linkages between polymers and the establishment of a mesh-like network. In addition, the various conditions of system production also significantly affect

the position of substitution. This might be because the functional group on the parent compound may occupy a different location as a result of a change in the production method, new materials with different physicochemical properties are. developed. For instance, if produced under different production conditions, samples of hydroxypropyl β-cyclodextrin with the same degree of substitution might not demonstrate similar physicochemical characteristics. This could be explained by the likelihood that the hydroxypropyl groups on the parent CD molecule reside in different locations. Thus, the degree of polymer substitution is crucial and has a considerable impact on the ultimate quality of NSs, as shown by the production process and material purity.

Swaminathan and co-worker utilized ultrasonography and loaded Camptothecin (CAM) within NSs in three different molar ratios of 1:2, 1:4 and 1:8 (β-CD: cross-linker). Experimental investigation demonstrated high drug loading, compared to the traditional method (reflux heating), which showed just 10% w/w with para-crystalline NS. Additionally, the type of NS employed had a significant impact on the release kinetics of CAM after complexation in NS. This may be attributable to optimal levels of cross-linking and greatest drug loading. Whereas a low drug loading at the 1:8 molar ratio was most likely caused by inadequate NS network development as a result of the steric obstructions mentioned above (Ahmed *et al.,* 2013a).

Complexation temperature

As was previously noted, the type and nature of the polymer define the type of NSs that are to be manufactured; several varieties of NSs can be constructed and developed based on the polymer used. Several prominent instances of NS include silicon NS particles, titanium-based NSs, CD-based NSs and hyper-cross-linked polystyrene NSs. Among all the different forms of NSs, CD-based NSs have attracted the most attention and have therefore been extensively explored.

Temperature

The complexation of the drug is greatly impacted by temperature changes, due to a potential reduction in drug NSs contact forces, Vander Waals forces, and hydrophobic forces with rising temperature and apparent stability of the NS complex that diminishes with temperature change.

Fabrication techniques

Drug/NSs complexation plagues the drug loading in NSs, which depends on the strategy used to entrap the drug inside of them. The physiochemical properties of the medicine and chemicals also reduce the method's productivity. In most instances, the potency of the drug's complexation was discovered by drying up (Allahyari *et al.,* 2021). As a result, β-CD is typically chosen over other ingredients when making NS. There are numerous methods for fabricating NS using various suitable grades of polymeric substance are covered in the next section.

5. CYCLODEXTRIN BASED NANOSPONGES FOR THERAPY AND DIAGNOSTIC APPLICATIONS

The use of CDNS is widespread in various therapeutic applications and can modify the physiological properties of drugs for various ailments. Saeideh and his colleagues developed the CDNS of flutamide (FLT) with solubility improvement ability as a novel delivery system. The CDNS at different ratios was introduced as a non-toxic delivery system for FLT with dissolution rate improvement characteristics. However, the number of cavities in nanosponge structure might affect the loading percentage and dissolution rate of FLT (Allahyari *et al.,* 2021). Hadeia and co-workers examined curcumin's complexation stability and solubilization with β-CD and β-CDNS. NSs were fabricated through cross-linking of β-CD with different molar ratios of diphenyl carbonate. Experimentally developed β-CD complexes enhanced curcumin solubility up to 2.34 fold, compared to the inherent solubility and 2.95-fold increment in curcumin solubility when loaded in β-CDNSs. Interestingly, the stability constant for curcumin NS was (4972.90 M^{-1}), which was 10 times higher than that for the β-CD complex, where the value was 487.34 M^{-1}. The study results indicated a decrease in the complexation efficiency and solubilization effect with the increased cross-linker amount. Cyclodextrin polymers and CDNSs have been widely investigated for improving the drug bioavailability. The investigation indicated the negative effect of further increasing the molar ratio of diphenyl carbonate by more than 1:4 of β-CD: cross-linker (Mashaqbeh *et al.,* 2021). Currently, there is a need for the development effective drug delivery system for colon cancer. The NS-based drug delivery system helps

to overcome the side effects of conventional drug delivery systems. Novel broad spectrum was used for treatment but, they show instability in gastric pH to overcome these problem encapsulations this type of drug is a better alternative. Nisin is a polycyclic antibacterial peptide widely used for the treatment of colon cancer; however, demonstrates degradation and remains unstable at gastric pH to overcome this problem Yousef, and co-workers developed two different types of CDNSs using carbonyl di-imidazole and pyro-metallic dianhydride. The anticancer activity was compared using MCF-7 cells other physiochemical properties and release kinetics were successfully studied. The important aspect for nisin is stability which was studied using tricin-SDS-PAGE electrophoresis. The cell line studies revealed significant cell damage by nisin with improvement in stability (Fig. 5) (Khazaei Monfared *et al.*, 2022).

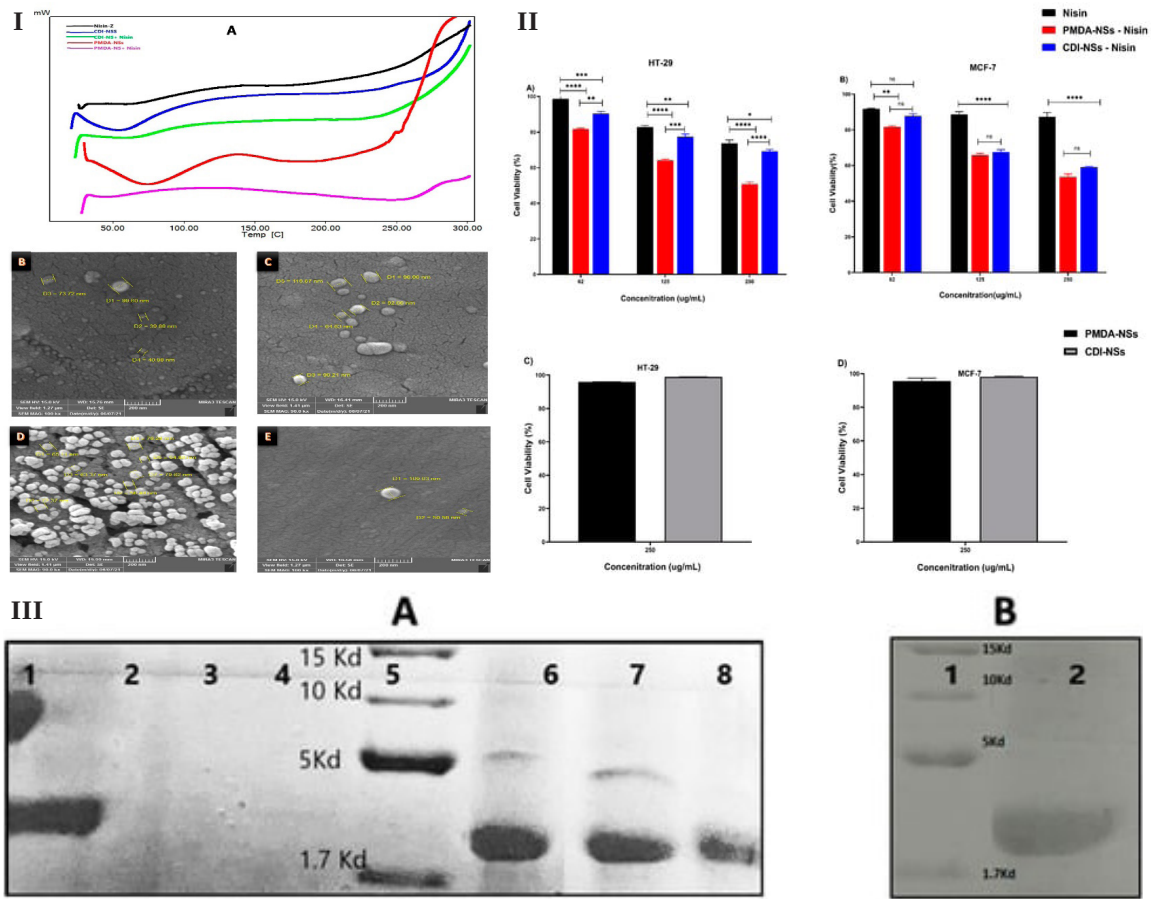

Figure 5. Microscopical characterization of nisin loaded NS (I). Cell viability results for colon cancer (HT-29) and breast cancer (MCF-7) cells exposed to nisin-Z and loaded on NS (II-A,B) and plain nanosponges (II-C,D) for 24 h. Tricine-SDS-PAGE analysis of nisin with different treatments. Lane 1: Nisin (4 mg/mL); Lane 2: Nisin (4 mg/mL) and pepsin (7.5 mg/mL); Lane 3: pyromellitic dianhydride-NSs (15 mg/mL); Lane 4: carbonyl diimidazole-NSs (15 mg/mL); Lane 5: Marker; Lane 6: Nisin and pyromellitic dianhydride-NS; Lane 7: Nisin and carbonyl diimidazole-NSs; Lane 8; Nisin, carbonyl diimidazole-NSs and pepsin (7.5 mg/mL) (III-A). Lane 1: Marker; Lane 2: Nisin, pyromellitic dianhydride-NSs and pepsin (7.5 mg/mL) (III-B). Reproduce with permission from (Khazaei Monfared *et al.,* 2022) under Creative Commons Attribution (CC BY-4.0) license.

Hamid Shah et al (2023) reported the anticancer activity of *S. nigrum* extract encapsulated in β-cyclodextrin and cross-linked with arabinoxylan was examined by Hamid Saeed Shah *et al.*, using the MCF-7 breast cancer cell line. It has been reported that different medications and biomolecules can be carried on nanosponges. Nanosponges with a morphological spherical, smooth, and 226 nm size were

 https://doi.org/10.37819/nanofab.009.1823

created. The SN extract had enhanced anticancer potential as demonstrated by its greater bioavailability and stability in the target site, as revealed by cytotoxic studies. Additionally, in vivo, anticancer activity showed that the SN extract that was encapsulated caused a decrease in tumor volume and weight, which increased the likelihood of survival by up to 85%. The current study's findings validated the delivery of synthetic and natural anticancer medicines using cyclodextrin-based nanosponges to increase their respective bioavailability and stability (Shah *et al.*, 2023).

Ferulic acid (FA) was effectively encapsulated into NSs by Atefe Rezaei *et al.*, (2019), which led to a notable increase in FA solubility of up to 15 times when compared to the free form of FA. FTIR, XRD, and DSC physicochemical and structural characterizations showed that FA was appropriately encapsulated into the NS structures. The MTT assay demonstrated that FA-NS had a greater antiproliferation effect against MCF7 and 4T1 breast cancer cell lines than free FA did. FA was released from NSs slowly and under control, according to in vitro release data. According to our findings, FA can be delivered by CD-NS, a viable nano-delivery method that may improve FA's cytotoxicity, solubility, and anticancer potential (Rezaei *et al.*, 2019).

Two different CD-NS formulations were created by Sally Abou Taleb *et al.*, to load QCT and improve its aqueous solubility to increase its biological activities, specifically its anti-proliferative and anti-SARS-CoV-2 properties. With PS in the nanosize range, the produced QCT loaded CD-NS showed a high EE% of QCT (94.17-99.31%). FT-IR spectroscopy demonstrated that QCT-loaded CD-NSs were formed. The results of the in vitro release investigation showed that the created formulations' QCT release was enhanced, suggesting better solubilization. The IC50 of free QCT against the lung cancer cell line A549 was 1.57-5.35 times greater than that of the developed QCT-loaded CD-NS formulations. In terms of SARS-CoV-2 activity, the IC50 values of free QCT were 5.95-26.95 times greater than those of the developed QCT loaded CD-NS formulations. It is noteworthy that QCT-loaded CD-NS using 2-HPβCD (QCT-HPBCD/DPC 1:3) outperformed QCT-loaded CD-NS using βCD (QCT-BCD/DPC 1:3) in terms of the entrapped QCT's anti-proliferative and anti-SARS-CoV-2 activities in vitro. This could be explained by 2-HPβCD's superior wetting ability and increased water solubility over βCD (Abou Taleb *et al.*, 2022).

The cytotoxicity investigation demonstrated that the formulations incorporating CAM were more cytotoxic than pure CAM, and the in vitro experiments suggested delayed and prolonged drug release over 24 hours. In 2012, Mognetti and colleagues synthesized β-cyclodextrin nanosponges loaded with paclitaxel, a colloidal system that is stable in water and prevents paclitaxel from recrystallizing. According to the in vitro release trials, there was no early burst impact and full drug release was achieved in two hours. By decreasing the paclitaxel IC50 and increasing the amount of paclitaxel that entered cancer cells, the administration of paclitaxel via nanosponges improved its pharmacological action (Mognetti *et al.*, 2012).

6. FUTURE PERSPECTIVES

The future perspectives of CDNSs in CC treatment offer exciting possibilities for advancements in personalized medicine, integration with emerging technologies, and overcoming challenges for widespread implementation. One significant future perspective is the application of personalized medicine in CC treatment. Researchers can focus on developing CDNS that are tailored to deliver specific therapeutic agents based on individual patient characteristics. By considering factors such as tumor heterogeneity and molecular profiling, NS can be designed to optimize treatment outcomes and minimize side effects, leading to more precise and effective colon cancer therapy. The integration of CDNSs with emerging technologies holds immense potential. Future studies can explore the combination of NSs with imaging modalities, such as magnetic resonance imaging or near-infrared imaging, to enable real-time monitoring of drug delivery and tumor response. This integration can provide valuable insights into drug distribution and effectiveness, enabling clinicians to adjust treatment plans accordingly. Additionally, incorporating stimuli-responsive materials into nanosponges can allow for on-demand drug release triggered by specific physiological or environmental cues, further enhancing the therapeutic efficacy and reducing systemic toxicity.

Another promising future perspective is the use of cyclodextrin-based NSs as carriers for novel therapeutic agents. Researchers can focus on encapsulating emerging targeted therapies, such as small interfering RNA (siRNA) or gene-editing

tools, within NS. This approach can facilitate the efficient delivery of these agents to CC cells, enabling precise and targeted treatment modalities that hold promise for revolutionizing colon cancer therapy. To fully realize the potential of CDNSs in CC treatment, it is crucial to address challenges related to scalability, manufacturing processes, and regulatory considerations. Future research efforts should be directed toward developing scalable production methods for nanosponges, ensuring reproducibility, and establishing robust quality control protocols. Additionally, researchers and regulatory agencies need to collaborate to address regulatory requirements and obtain necessary approvals for clinical use, enabling the widespread implementation of these innovative nanosponges in colon cancer therapy.

7. CONCLUSION

Cyclodextrin-based NSs show great promise in treating CC in the future. Exciting opportunities to improve treatment outcomes arise from integrating personalized medicine, combining it with emerging technologies, and using them as carriers for novel therapeutic agents. Colon cancer treatment can move towards personalized medicine by tailoring nanosponges to match individual patient characteristics, which can enhance therapy and decrease side effects. By combining NSs with imaging modalities and stimuli-responsive materials, it is possible to monitor drug delivery in real-time and have precise control over drug release. Moreover, by encapsulating emerging targeted therapies, we can expand treatment options and open doors for precision medicine approaches. Cyclodextrin-based NSs widespread adoption in clinical practice causes addressing scalability, manufacturing processes, and regulatory considerations. To revolutionize colon cancer therapy for better patient outcomes, it is crucial to continue researching and developing these nanosponges.

Abbreviations

CC: Colon cancer; BCD: β-cyclodextrin; NS: nanosponges; BCDNS: β-cyclodextrin nanosponges; NT: Nanotechnology.

Declaration of Competing Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization, PM; Methodology, SM and AP; Validation, PM and SS; Formal analysis, Resources, SM; Data curation, SM and AP; writing original draft preparation, SM and AP; Writing review and editing, PM and SS.; Supervision, PM; Project administration, PM, and SS;. All authors have read and agreed to the published version of the manuscript.

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