Cyclodextrin-based nanosponges: a versatile platform for cancer nanotherapeutics development

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Abstract

Nanosponges (NSs) are a new age branched cyclodextrin (CD) polymeric systems exhibiting tremendous potential in pharmaceutical, agroscience, and biomedical applications. Over the past decade, different varieties of NS based on the type of CD and the crosslinker have been developed tailored for specific applications. NS technology has been instrumental in achieving solubilization, stabilization, sustained release, enhancement of activity, permeability enhancement, protein delivery, ocular delivery, stimuli sensitive drug release, enhancement of bioavailability, etc. There is a major explosion of research in the area of NS-aided cancer therapeutics. A wide of anticancer molecules both from a pharmacological and physicochemical perspective have been developed as NS formulations by several groups including ours. Our objective in this review is to capture a systematic and comprehensive snapshot of the state-of-the-art of NS-aided cancer therapeutics reported so far. This review will provide an ideal platform for both the formulation scientists working on new polymeric/drug development and cancer biologists/scientists to understand the current nanotechnologies in CD-based NS-aided cancer therapeutics. The scope of the review is limited to small molecules and CD-based NS. The review covers in detail the problems associated with anticancer small molecules, and the solution provided by CD-based NS specifically for camptothecin, curcumin, paclitaxel, tamoxifen, resveratrol, quercetin, oxygen-NS, temozolomide, doxorubicin, and 5-Fluorouracil.

INTRODUCTION

Nanomedicine and Cancer

Nanotherapeutics research has been at the center stage of the cancer therapeutic drug design and discovery for over three decades. 'In the year 2000, when they look back at this age, they will wonder why it was not until the year 1960 that anybody began seriously to move in this direction,' opined Richard Feynman, in his famous 1959 talk, 'There's plenty of room at the Bottom,' at the annual meeting of the American Physical Society at the California Institute of Technology (Caltech) referring to the staggeringly small world that is below—which today we know as the world of nanotechnology. Reportedly, it was in this lecture (later published in Ref 1) that the seeds of nanomedicine were first sown.[2] Despite the rapid inroads, high publication volumes, and significant government spending on nanomedicine, experts in the field have expressed concerns about the future of nanomedicine and the bench to bedside transition of promising technologies.[2–4] Albeit with mixed results, over the years, researchers have harnessed the benefits of nanotechnology to cancer therapeutics extensively. Nanoparticles—delivering small packets (100–500 nm) of these drug molecules—can utilize the enhanced permeation and retention (EPR) effect to the fullest extent through extravasation via gaps in hyper permeable tumor vasculature.[7] Another silver lining in the otherwise dark cloud that researchers have utilized for tumor targeting is the lower pH and increased temperature of the tumor environment as compared with the normal tissues. Stimuli sensitive drug delivery using nanoparticles help in targeting of the drug encapsulated nanoparticles to the tumor site. Active targeting on

the other hand, utilizes the technique of attaching ligands to the nanoparticles wherein the ligand recognizes and binds to the over expressed tumor-associated antigens or receptors.[8]

Cyclodextrins (CDs) have the ability to improve the physicochemical properties of the drug by leaps and bounds. It also presents a unique opportunity for cancer therapeutics through its marriage to nanotechnology for overcoming most of the abovementioned barriers. Readers are directed to two of the most recent reviews for further reading on the various drug delivery systems explored so far.[5,9] A novel drug delivery system known as 'Nanosponges' (NSs) based on CDs was developed by researchers about a decade ago for circumventing the potential issues in drug delivery and cancer therapeutics. The objective of this review is to present a comprehensive snapshot of the development, properties, and applications of NSs of CD in cancer therapeutics. To maintain the focus of the review, the scope of this review is limited only to the research based on small molecule therapies in cancer.

CD Polymers and NS Evolution

Recent advances in nanotechnology have demonstrated the applications of supramolecular assembly from simple monomeric blocks for housing drug molecules. CDs—owing to its typical toroidal structure of inner hydrophobic and outer hydrophilic orientation—presents a unique opportunity for the drug molecules to interact and complex. They are widely used in the pharmaceutical, food, textile, and home-based consumer products owing to its binding capacity for small molecules with phenyl rings. However, native CDs typically do not form viable complexes with hydrophilic molecules, or macromolecules, and most commonly used CD form—β-CD—has poor water solubility which limits their complexation abilities. Moreover, β-CD cannot be injected intravenously as they are known to complex with cholesterol, thereby leading to nephrotoxicity. To overcome these potential problems of native CDs, several structural modifications have been explored (reviewed in Ref 5). Several approaches were reported decades ago, wherein researchers utilized crosslinking of CDs with dialdehydes, epoxides, epichlorohydrin, and diacyl chlorides (reviewed in Ref 10). To the best of our knowledge, the term 'nanosponges' was first coined by DeQuan Li and Min Ma in the year 2000, when they crosslinked β-CD with organic diisocyanates for water purification purposes.[11] They demonstrated that water contaminants such as p-chlorophenol—albeit present in trace amounts (ppb)—were completely removed by the NS treatment. However, it was Trotta and collaborators, who first published proof of concept NS results with model drugs.[12,13] They also improvised the synthetic procedure by replacing the potentially toxic diisocyanates with carbonate compounds. This work was then followed by a plethora of research publications in the area of NS-based drug delivery from Trotta, Cavalli, Vavia, Swaminathan, Torne, Ansari and coauthors.[14–27]

Till date, several researchers, including our groups have summarized the current state of the art of NS in drug delivery;[5,10,28–34] however, to the best of our knowledge, a very comprehensive review such as the current one for NS application carved out for cancer therapeutics has not been published yet (at least up to the date of submission of this review).

CD-based NSs: Development and Key

Concepts

CD-based NS are a novel class of hyper-branched polymers extensively studied in the past decade.[10,12,26,27,31,34–36] NS-based systems have emerged as one of the leading polymeric carriers based on CDs to be explored recently as seen from the explosion in the number of research and review articles. Several recent reviews summarize the development, characterization, and applications aspects of NS in a great detail. This review will highlight the key aspects of NS development; however, readers are directed to some of the

recently published reviews for in-depth understanding of the NS basics.[5,10,28–34] A recent EU report highlighted the use of CD NSs as a promising innovative system for drug delivery applications.[37] The scope of this review is confined to the applications of NS in modulating anticancer drug properties.

NS are highly porous, hyperbranched CD-based polymers that have shown to possess a unique ability of forming a nanosuspension upon dispersion in water, resembling sponges microscopically.[10,31,34] Figure 1 presents a schematic structure of NS. Reports indicate that NS may form both inclusion and noninclusion complexes with drug molecules, thereby providing greater interaction sites and thereby greater drug loading as compared with native CDs.[10,15,31] Over the years, NS has been extensively explored for solubilization, chemical stabilization, permeability enhancement, ocular delivery, potentiation of cytotoxicity, sustained release/drug release modulation, reduction of toxicity, protein delivery, and others (refer Refs 10,28,29,31,34). Figure 2 highlights the key applications of NS in cancer therapeutics. Use of NS offers replacement of potentially toxic organic solvents as in the case of conventional polymeric nanoparticles with aqueous media. Moreover CDs—the backbone of NS—are widely recognized and used in several pharmaceutical products, and enjoy a 'generally regarded as safe (GRAS)' status, therefore, NS presents a promising carrier also in terms of future regulatory approvals of drug products.

Researchers have obtained NS using a reaction of CDs with crosslinkers such as carbonyldiimidazole, diphenyl carbonate, hexamethylene diisocyanate,[38] and pyromellitic anhydride. In a one-pot single step synthetic reaction often lasting just for a few hours.[13] Primary hydroxyl groups are involved in the formation of the nanoporous crosslinked networks as indicated by in-depth Fourier transform infrared spectroscopy (FTIR), solid state-nuclear magnetic resonance (NMR), and Raman studies.[39]

Variations in synthetic procedures have yielded interesting characteristics of NS that has further helped to understand the NS structure.[40] For example, a crystalline structure was obtained by the use of ultrasound which yielded a higher loading and better properties for the encapsulated drugs.[20,21,40] Moreover, the crosslinker amounts and types can be varied to obtain the desired type of NS required for tailoring the drug release of molecules—a property that makes NS an invaluable tool for cancer therapeutics. Additionally, presence of free OH groups in the backbone structure makes NS a promising target for further functionalization by succinic anhydrides, yielding a special type of NS with surface charges (F-NS).[10,19,22,34,41] This could potentially be also be leveraged for layer-by-layer surface engineering of the NS for precise, tailored drug release.[10,34,36]

In the past decade, NS have been extensively characterized to ascertain its applications in cancer drug delivery and other pharmaceutical applica-tions.[10,21,27,33,34,36,38,42] For brevity, the authors are presenting only key highlights in this review. NS are thermally stable up to 300°C and autoclave-compliant. X-ray powder diffraction (XRD) coupled with high resolution transmission electron microscopy (TEM) confirmed the presence of the hexagonal geometry of CD in NS, which was also used to tease apart the crystalline and paracrystalline forms of NS. In most studies, the particle sizes obtained range between 200 and 500 nm, with a low polydispersity index (PI). The conventional NS has a negative sur-face charge, in the order of −20 to −40 mV zeta potential, thereby lending stability to the nanosuspension. NS was found to be stable releasing a small amount of free CD molecules at 60°C after 2 h in acidic conditions. However, it remained intact in basic conditions. Dynamic vapor sorption studies demonstrated that NS retained its crystal structure after several programmed cycles absorption and desorption.[42] Further, the elastic properties were of NS were determined by analysis of the spectral modification of the Boson peak and Brillouin frequency.[43] Readers are directed to a series of recently published papers by several of our collaborators for an in-depth understanding on the structural, morphological, thermal, and physical characterization of NS using FTIR, light scattering, neutron scattering, and Raman spectroscopic studies.[39,43–50]

Extensive in vitro cell line toxicity (evaluated on MCF-7, HT-29, Vero, HCPC-I cell lines), hemolytic activity assessment, and preclinical safety/toxicity assessments have been performed on NS.[10,20,27,34,35,51] Hemolytic

activity revealed the nonhemolytic/blood compatible nature of NS. NS was localized in the perinuclear spaces after incubation with VERO and CaCo2 cell lines.[19,31,36] In their preliminary studies, Swaminathan[35] and Vavia[27] showed that NS did not show any apparent signs of toxicity in mice via oral administration. Subsequently, recently, Shende et al.[51] carried out acute and repeated dose toxicity studies on rats, further demonstrating the safe nature of NS. They found that the maximally tolerated dose of NS was 2000 mg/kg. Repeated dose studies for 28 days on rats did not show any signs of toxicity as the hematological and biochemical parameters were unaltered.

Drug Delivery of Cancer Therapeutics

Using NSs

Camptothecin

Camptothecin (CAM)—a pentacyclic quinolone plant alkaloid isolated and characterized by Wall et al.[52]—is a potent anticancer agent obtained from the oriental tree Camptotheca acuminata, acting through the inhibition of topoisomerase I during the S-phase of the cell cycle.[53] Despite a great start owing to its unprecedented antitumor activity, it failed to live to its expectations due to problems associated with serious side effects. It was only in the early 1990s that the interest was rekindled when it was found that CAM acted by binding to the topoisomerase I-DNA complex, thereby leading to accumulation of DNA strand breaks upon replication, causing cell death.[54] CAM and its derivatives have found applications against a wide spectrum of human malignancies such as lung, prostrate, breast, colon, stomach, ovarian carcinomas, melanoma, lymphomas, and sarco-mas.[55,56] Despite its cytotoxic potency and wide range of applications, its clinical utility is marred by developmental issues due to its poor aqueous solubility, serious side effects such as myelosuppression and haemorrhagic cystitis (reviewed in Ref 57) and opening of the lactone ring at physiological pH to yield the inactive carboxylate form.[58,59] Moreover, due to the ring opening, new charged drug species with low molecular diffusivity and poor permeability are formed thereby further reducing the bioavailability.[60]

Several structural and formulation-based modifications have been explored in the past two decades to overcome these aforementioned problems of CAM. CAM analogues such as topotecan and irinotecan have enjoyed decent success rates since their discovery.[61]

Although these compounds show a better pharmacological profile and a greater efficacy compared to CAM, either necessitate continuous infusion or frequent administrations to achieve effective levels.[62–64] Extensive research has also been carried out to design drug delivery systems for the insoluble lactone form of CAM and its derivatives. Some representative examples include (but are not limiting to) entrapment into liposomes, microspheres, polymeric/lipidic/iron-oxide nanoparticles, composite systems, complexation with lipids/CDs/derivatives of CDs/polymer conjugates, and preparation of macromolecular prodrugs.[65–76]

NS structure offered a great opportunity for CAM stabilization and sustained delivery. Swaminathan et al. were the first to explore the applications of NS for solubilization, stabilization, and sustained release of CAM.[20] Three compositions of CAM NS (F1:2,F1:4, and F1:8) were designed and evaluated using different crosslinker amounts (such as 1:2, 1:4, and 1:8 on a molar basis of the CD:diphenyl carbonate crosslinker). The authors also studied a new paracrystalline form of NS for suitability in improving the physicochemical properties of CAM. The authors postulated that the crystal lattice structure of NS was critical for optimizing the properties of CAM as the channels or cavities running through the NS crystal structure acted as additional drug-binding sites for CAM molecules, and when the crystal structure is absent, the hypothesized preferential sites cease to exist. This was further supported by their findings of higher drug loading in case of crystalline

NS (37% w/w) as compared to 10% w/w in case of CAM compositions with paracrystalline NS of the same CD to crosslinker ratio. CAM was loaded up to 21, 37, and 13% w/w in formulations F1:2, F1:4, and F1:8, respectively, suggesting that the degree of crosslinking may also play an important role in tailoring the loading and drug release. The characteristic CAM peaks in FTIR were broadened or shifted in the formulations suggesting interaction of CAM and NS at a molecular level. Differential Scanning Calorimetry (DSC) thermograms emphatically supported the author's conclusions of formation of CAM inclusion complexes. TEM studies confirmed the uniform spherical shape of NS compositions. The particle sizes of the CAM-loaded NS compositions were between 450 and 600 nm with low polydispersity indices. Figure 3 shows a pictorial representation of the NS formulation of CAM. The zeta potentials obtained for all the compositions were in the range of −20 to −25 mV—high enough to produce physically stable nanosuspensions. The in vitro studies revealed a slow and sustained CAM release over a period of 24 h, wherein not more than 25% of the total drug was released at the end of 24 h. The NS compositions appeared to protect the lactone ring structure of CAM in physiological conditions for 24 h with about 80% w/w of intact lactone ring present as compared to a mere 20% w/w of plain CAM after 24 h (Figure 4). Preliminary cytotoxic results on HT29 cell lines presented in the paper showed that the cytotoxicity studies on HT29 cells showed that most of the NS compositions were significantly more cytotoxic than plain CAM after 24 h.[20] Further, Minelli et al. in a comprehensive study[77] validated these aforementioned findings of NS CAM compositions by successfully demonstrating a higher in vitro antitumor efficacy of CAM NS formulations as compared to CAM alone in androgen refractory models of prostate cancer viz. DU145 and PC-3, and in androgen sensitive model LNCaP. The authors demonstrated a progressive time-dependent increase in the intracellular content of CAM over a period of 6 h treatment of CAM-NS formulation on PC-3 cancer cell line thereby corroborating the previous findings of achieving physico-chemical stabilization of CAM in physiological conditions by NS complexation. A semi-quantitative estimation of Topoisomerase-I activity in PC-3 cells treated with CAM NS formulation and CAM alone revealed a greater inhibitory activity in case of NS formulation suggesting a possible enhancement in its anticancer efficacy. The authors showed using cell proliferation and clonogenic assays that the PC-3 cells showed greater sensitivity to free CAM as compared to the DU145 cells. Moreover, both the cells responded better to the CAM encapsulated within NS, as compared to the free NS or free CAM. Figure 5 represents the results discussed. The authors analyzed the phosphorylation status of the histone H2A.X on Ser 139 using Western Blot analyses. They demonstrated that after 24 h of treatment with 1–10 nM CAM NS, a substantial induction of H2A. X phosphorylation in both PC-3 and DU145 cells occurred; however, the same treatment with CAM did not have any effect. Moreover, immunofluorescence experiments revealed nuclear localization of P-H2A.X only in CAM NS-treated PC-3 cells. Cell cycle arrest studies revealed that the CAM NS formulation successfully caused cell cycle arrest even at a low concentration of 1 nM, at which the 'as-is' drug was ineffective. Similar promising results for cell proliferation, viability, and clonogenicity were obtained by the authors for CAM NS formulations in the androgen receptor expressing LNCaP cells. The authors found that LNCaP cells displayed minimal susceptibility to CAM as compared with CAM NS, which substantiate antitumor activity. In all these studies, a lower dose of CAM encapsulated as NS formulations were required for same or higher activity as compared to plain CAM. Moreover, CN-CPT inhibited AR expression at 10 nM, whereas CPT was inactive at these dose levels. The authors indicated that the CAM NS formulation could potentially serve as a potent drug delivery option for prostate cancer pending further in-depth studies.

Curcumin

Curcumin—a hydrophobic polyphenolic phytochemical—has poor aqueous solubility at acidic and neutral pH, but is soluble at alkaline pH (reviewed in Ref 78). It is a major constituent of turmeric powder—a common Indian spice. Besides being a potent antioxidant, cardioprotective, neuro-protective, antidiabetic, anti-inflammatory, and anti-atherosclerotic agent (a nonexclusive list), curcumin has been extensively reported as a promising anticancer agent.[78,79] Several studies suggest that curcumin may be a promising drug for

treatment of several types of cancers such as breast cancer, colon cancer, kidney cancer, leukaemia, liver cancer, and prostate cancer (reviewed in Ref 78). Curcumin exerts its anticancer activities via its effects on the nuclear factor-κ B (NF-κB), tumor necrosis factor α (TNF-α), interleukins, interferon γ (IFNγ), c-Jun N-terminal kinase, cyclooxygenases, mammalian target of rapamycin (m-TOR), protein kinase C (PKC), mitogen-activated protein kinase (MAPK), peroxisome proliferator-activated receptor γ (PPARγ) etc.[78,79] It inhibits cell proliferation and metastasis along with inducing apoptosis by modulating these aforementioned pathways. It has also been postulated that due to its pleiotropic properties, curcumin may be more effective than a single-pathway targeted therapy.[79] Despite its wide array of applications, its bench-to-bedside transition is plagued with a plethora of formulation challenges.[80–85] It is well documented that curcumin exhibits solubility related poor oral bioavailability and poor gastrointestinal absorption and undergoes extensive metabolism.[78,86] Coupled with that, it also undergoes degradation at physiological pH values.[83] Extensive formulation research efforts have been made toward mitigating these problems of curcumin delivery (reviewed in Ref 80). One such approach was the use of NS by Darandale et al.[17] Darandale et al. formulated and characterized β-CD NS-curcumin formulations as a potent anticancer drug delivery option. The authors demonstrated over 50 times enhancement in solubility of curcumin as compared to plain curcumin and about four times in comparison to the β-CD complex of curcumin. They speculate that the higher solubility is a result of inclusion complexation of curcumin with NS and also crosslinking of CD molecules provides a favorable environment for the drug molecules to orient. The formulations had an average particle size of about 487 nm, with a PI of 0.476 showing a unimodal particle size distribution with narrow range. The zeta potential values were about −27 mV, considered sufficiently high for formation of a stable suspension. Major characteristic peaks of curcumin were either completely masked or shifted, and some of the NS peaks shifted suggesting interactions at a molecular level. None of the characteristic peaks of the curcumin crystalline structure were retained in the NS formulation suggesting that the encapsulated curcumin is in amorphous form or is solubilized in the NS polymeric matrix. The authors also postulate that as the drug is in amorphous state, it can easily diffuse through the polymeric NS matrix providing a con-trolled release. In DSC, the characteristic peak of cur-cumin at 176°C was not obtained suggesting that it formed an inclusion complex. The authors obtained in vitro sustained release of curcumin over a period of about 2 days. The formulations were nonhemolytic up to a concentration of 2 mg/mL. Cytotoxicity studies conducted on MCF-7 cells using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay showed that curcumin formulation has a comparable toxicity to curcumin thereby suggesting that the molecular structure remains unchanged after the formulation.[17]

Paclitaxel

Paclitaxel (Taxol) is a versatile small molecule anticancer diterpenoid originally isolated from the tree bark Taxus brevifolia. Paclitaxel is reported to possess strong anticancer activity against ovarian, breast, nonsmall cell lung cancer, Kaposi's sarcoma, pancreatic cancer, and head and neck tumors (reviewed in Ref 87). Paclitaxel acts via its activity on microtubules, during the mitotic phase of the cell division wherein it promotes polymerization of the tubulin proteins and stabilizes the microtubules making them dysfunctional. Trends (2001–2013) indicate that the market for the drug formulations crossed a whopping figure of $30 billion, making it one of the best-selling antitumor molecules (covered in Ref 34). Similar to the other potent anticancer molecules discussed before, Paclitaxel delivery is also ridden with formulation challenges. It is a highly lipophilic molecule with a very poor aqueous solubility (<0.5 mg/L), dissolution, and oral bioavailability.[88] Paclitaxel lacks potentially ionizable functional groups, therefore the common methodologies of solubility enhancement such as salt formation and prodrugs are not feasible.[87] Paclitaxel also undergoes first pass metabolism in liver and intestine via the cytochrome P450 pathway. It is also known to be effluxed by cellular P-glycoproteins, thereby further limiting the intracellular concentration and efficacy. One of the most widely used formulations of paclitaxel for clinical application consisted of a

solubilized form of the drug in a 1:1 v/v mixture of polyoxyethylated castor oil (Cremophor EL) and dehydrated alcohol for intravenous applications.[25,87,88] Cremophor EL is reported to be associated with severe side effects and hypersensitivity reactions such as bronchospasms, hyperlipidemia, neurotoxicity, hypotension, vasodilatation, and labored breathing.[25,34,87] It can also lead to leaching of common plasticizer excipients from the formulation container. To possibly avoid some of the reactions, it is recommended that the solutions must be diluted about 5–20 times in physiological solutions before administration. Hence, the stability and precipitation at site are the major concerns.

About a decade ago, the US FDA approved the use of albumin bound paclitaxel nanoparticles (Abraxane™) with a particle size of ~130 nm that showed a greater specificity toward solid tumors as compared to the Cremophor formulations.[87] Several other alternative and innovative approaches have been explored by researchers including emulsions, liposomes, CD nanoparticles, polymeric nanoparticles, solid lipid nanoparticles, nanocapsules, combination of CDs and polymeric/lipidic nanoparticles, and other nanoparticles.[87–92]

Torne et al. first explored NSs (using diphenyl carbonate as the crosslinker) for oral delivery of paclitaxel in 2010 by studying the potential of NS for enhancement of bioavailability.[25] Paclitaxel-loaded NS were prepared in a ratio of 1:1 w/w using freeze drying and characterized for size distribution, pay load, and encapsulation efficiency. Paclitaxel NS had a monomodal distribution (PI < 0.2) with a size of ~350 ± 25 nm. The encapsulation efficiency was above 99%, and about 1.7% of the drug crystallized after 24 h at 7 ± 2°C. They evaluated the bioavailability of NS formulation (dispersed in phosphate-buffered saline (PBS), pH 7.4) (10 mg/kg) with Taxol® (10 mg/kg) via both the oral and intravenous route in Sprague Dawley rats. The relative oral bioavailability of paclitaxel administered in NS formulation was 2.56 in comparison to the Taxol®. The area of the plasma concentration time curve was increased by about threefold in comparison with the control group (<0.05). The mean absolute bioavailability of paclitaxel from the NS formulation administered was also increased by 2.5-folds as compared to Taxol®. The PK results are presented in Figure 6. Along the enhancement of bioavailability, the NS formulation also mitigated the potential complications of use of Cremophor formulation, as NS completely replaced both ethanol and Cremophor for solubilizing paclitaxel. They further sought to perform an in-depth characterization of the NS formulation and also study the in vitro cytotoxicity in MCF-7 cell line.[93] NSs interacted at a molecular level with paclitaxel, as suggested by the FTIR, DSC, and NMR studies. The authors observed major changes in the fingerprint region of paclitaxel after formulation with NS. They also observed a shift in one of the NS peaks related to the OH stretching interacting with the secondary amide group of Paclitaxel. The encapsulation was further evidenced by the masking of DSC endotherms of paclitaxel by NS. Significant proton shifts were observed by the authors in the ^{1}H NMR studies carried out on paclitaxel and NS. They observed an involvement of distant groups which suggested the involvement of multiple CD cavities/molecules as obtained by NS synthesis. The freeze-dried particles presented a spongy morphology and spherical shape. The formulations were stable over a period of 6 months as observed at 25°C, 60% RH and 40°C, 75% RH as there was no aggregation after redispersion of the formulation.

In vitro studies corroborated the previous studies as release kinetics was directly proportional to the crosslinking densities. The drug was completely released over a period of 2 h under sink conditions in PBS at 37°C suggesting uniform solubilization of paclitaxel in the formulation, as the physical mixture of paclitaxel with NS did not show uniform and complete release. The paclitaxel NS formulation showed about 10% better inhibition of MCF-7 cell proliferation as compared to ‘as-is’ paclitaxel at all concentrations studied at both 24 and 48 h time points, thereby showing that the NS formulations may be more cytotoxic than the plain drug.[93]

Mognetti et al. further continued the quest for an elusive surfactant (Cremophor) free formulation of paclitaxel by carrying out further in vitro efficacy investigations on carbonyl diimidazole (CDI) cross-linked CD NSs of paclitaxel[94] with an aim to better understand the enhanced efficacy of NS formulations. The authors

obtained similar conclusions as the previous studies in terms of formulation and characterization of paclitaxel formulations.[25,93,94] Briefly, FTIR and DSC suggested molecular dispersion of paclitaxel in NS. NS had an average diameter of about 400 nm with a low polydisperisty index. TEM revealed spherical shape before and after formulation. NS synthesized with CDI solubilized paclitaxel efficiently with 1 mL of 1.5% w/v NS solubilized about 2 mg of Paclitaxel (~2.3 mM paclitaxel) as compared to a 0.2–0.9 μM solution of free paclitaxel. The nanosuspensions showed good physical stability over a period of 6 months, while not showing signs of crystallization of aggregation. Moreover, it was also found that the NS formulation could be diluted without the inherent problem of drug crystallization as seen in case of the marketed Cremophor-based formulation.[87,94] The drug release over a period of 2 h was complete and uniform without any burst effect suggesting molecular encapsulation and corroborating the previous studies. The formulation showed negligible hemolytic activity suggesting that it was safe via injectable route. In vitro cytotoxicity studies on AT84 cell lines conclusively showed the promising cytotoxic effects of NS-based formulations of paclitaxel. After 48 h, the NS formulation significantly inhibited cell proliferation at a much lower concentration (31.5 nM, $P < 0.01$) as compared to 'as-is' paclitaxel (125 nM, $P < 0.001$). Moreover, after 72 h, NS formulation significantly inhibited cell proliferation at an even lower concentration (1.9 nM, $P < 0.001$) as compared to 'as-is' paclitaxel (62.5 nM, $P < 0.001$). Figure 7 captures the cytotoxicity results. The authors studied the internalization behavior by incubating AT84 cells with FITC-loaded NS and found that the particles were accumulated in the cytoplasm with a sporadic perinuclear behavior. Moreover, paclitaxel NS formulation also led to about 18 times higher intracellular paclitaxel concentration per cell as compared to 'as-is' paclitaxel.[94]

Tamoxifen

Tamoxifen—a selective estrogen receptor modulator—is effective against early and advanced estrogen positive breast cancer.[95] Tamoxifen being a weak base and having low aqueous solubility (5.9 mg/L) necessitates the use of a citrate salt for obtaining a slightly better soluble form. As expected, the salt formation alone is not sufficient for obtaining the most effective tamoxifen delivery. Moreover it shows a remarkable intra- and interpatient variation in bioavailability when administered orally.[96] It is also a low dose therapeutic with a fairly long dosing regimen. It is known to cause severe side effects such as endometrial carcinoma, liver cancer, venous thrombosis, pulmonary emboli, and ocular toxicities—all these dependent on the dose and con-centration at the site (from Ref 24). Solubilization and subsequent modulated uniform release may likely help circumvent the current problems of tamoxifen delivery.[24,96–99] Extensive research has been carried out on tamoxifen delivery using novel nanocarriers such as nanostructured lipid carrier, chitosan nanoparticles, polymeric nanoparticles, liposome targeting, ethosomes, amphiphilic CD nanoparticles, lecithin/chitosan controlled release (CR) systems, liposomes, thiolated alginate-albumin nanoparticles, albumin-based nanoparticles to highlight a few recent research works.[96–104] Torne et al.[24] explored β-CD-based NSs for oral delivery of tamoxifen. CDI-based NSs were utilized to formulate three main tamoxifen formulations with varying crosslinking densities (F1:2,F1:4, and F1:8). Solubility was enhanced several folds by the use of NS (F1:4 and F1:8) as compared to the 'as-is' drug with about 5 mg NS solubilizing about 2.2 mg tamoxifen.[24,34] In vitro release indicated a rapid and complete release of the drug, while directly correlating with the crosslinking density of NS. Almost complete drug release occurred at around 2 h time period. Formulation particles were about 400–600 nm with a low PI. Similar to paclitaxel, an interaction of the free OH group of NS and the amide group of tamoxifen was indicated by the FTIR studies. DSC and XRD further lend support to corroborate the inclusion of tamoxifen in NS matrix. Cytotoxicity studies on MCF-7 cell line indicated that the NS formulation was more cytotoxic and showed greater inhibition of proliferation as compared to the 'as-is' drug. Oral bioavailability studies of tamoxifen NS formulation in comparison with the tamoxifen citrate on Sprague–Dawley rats (2 mg/mL tamoxifen, 4 mg/kg) showed that the NS formulation enhanced the bioavailability of tamoxifen by about 1.45-folds.[24] The results are shown in Figure 8.

Resveratrol

Resveratrol—a nonflavonoid, poly phenolic molecule—is found in commonly consumed food items such as grapes, peanuts, pistachios, and blue-berries. It is widely recognized as a powerful antioxidant, anti-inflammatory agent, and an anticancer agent (reviewed in Ref 105). Despite the plethora of research data available and the broad range of healthcare applications, the clinical use of resveratrol is somewhat limited by the short biological half-life, labile nature,[106] and extensive and rapid metabolism followed by elimination (reviewed in Ref 105,107). Moreover, some studies have indicated that the oral bioavailability of resveratrol is very negligible serving as the coup de grace to its further clinical development.[105,108,109] Despite the large number of studies, the knowledge on the free resveratrol plasma level and bioavailability still remains murky (reviewed in Ref 110). Several efforts have been undertaken by researchers worldwide to overcome the hydrophobicity-related problems in resveratrol delivery, notably niosomes, polymeric nanoparticles, dendrimers, nanovesicles, solid lipid nanoparticles, lipid-core nanocapsules, self-emulsifying drug delivery system, CD-based delivery systems along with others have been explored (a nonexhaustive list).[111–117]

Ansari et al. used CDI-based NS in an attempt to improve the solubility, stability, and topical skin permeability of resveratrol.[15] They optimized two formulations of NS with varying degrees of crosslinking (1:2 and 1:4, molar basis of CD: CDI). Solubility of resveratrol was enhanced by about 33-folds (F1:2) and by about 48-folds (F1:4) as compared to the 'as-is' drug. The drug was loaded to a greater extent in F1:4 as compared to F1:2. Average particle size of 400–500 nm was obtained with a low PI. The major changes in the FTIR fingerprint region of resveratrol (900–1400 cm^{-1}) and the O-H stretching peak shift of NS suggested molecular interaction of NS with resveratrol. DSC and XRD further corroborated the inclusion observation of FTIR, wherein the drug endotherm in DSC diminished in intensity, while the long range crystalline peaks of resveratrol in X-ray spectrum changed drastically to broad peaks suggesting amorphization. Resveratrol release was more uniform and complete from the F1:4 formulation as compared to the F1:2 or 'as-is' resveratrol symbolizing solubilization by NS. More than 50% of resveratrol was intact in F1:4 as compared to about less than 10% 'as-is' drug under ultraviolet (UV) irradiation suggesting enhanced photostability of resveratrol upon formulation with NS. Formulation F1:4 exhibited a higher degree of cytotoxicity as compared to 'as-is' drug in HCPC-I cells, which was both dose and time dependent. A twofold higher in vitro rabbit mucosa accumulation of resveratrol from F1:4 was observed by the authors as compared to the drug dispersed in a hydro alcoholic mixture (1:1 v/v). These results are shown in Figure 9. They also observed a higher permeation of resveratrol across pig skin via the NS formulation as compared to the drug dispersed in a hydro alcoholic mixture.[15]

Quercetin

Quercetin—a dietary flavonoid found in vegetables, fruits, red wine, and seeds—has shown an enormous potential as a chemoprevention agent.[118] The most common supplement and active form of quercetin is an aglycone form exhibiting very poor oral bioavailability (>2% in humans) which in turn is related to poor solubility and thus poor dissolution in the gastrointestinal tract.[14,119,120] Moreover, it also undergoes extensive first-pass metabolism which additionally hampers the oral delivery.[121] Being a BCS class II compound, it has an aqueous solubility of about 7.7 µg/mL, which was reported to be enhanced by utilizing nanoparticles and solid dispersions to only about 0.4 mg/mL.[121,122] Hence, there is a clear need for further improving the physicochemical properties of quercetin.[14,121] Some of other novel technologies utilized by researchers to overcome the problems associated with quercetin delivery are solid lipid nanoparticles, polymeric nanoparticles, sodium hyaluronate-chitosan multilayered liposomes, SNEDDS, biodegradable nanoparticles, polymeric microparticulate systems, nanocrystals, NLC, lipid nanoemulsions etc.[121,123–130]

Recently, Anandam et al. explored diphenyl carbonate-based NS for improving quercetin delivery.[14] They prepared five different types of quercetin NS formulations (utilizing NS with different cross-linking densities

ranging from 1:2 to 1:10, molar ratios of CD: diphenyl carbonate) for their studies. Solubility of quercetin was enhanced by about 20-folds as compared to the 'as-is' drug after molecular inclusion in NS. With about 40–45% w/w quercetin loading, F1:4 and F1:6 stood out as the optimum formulae. Peak shifts and peak broadening in the FTIR spectra-finger print region of quercetin after formulation with NS suggested interactions at molecular level. Further, using Raman spectroscopy, the authors observed that important markers of quercetin were substantially masked or shifted after complexation with NS, which directly corroborated their FTIR findings. DSC and XRD findings further lend support to the molecular inclusion complexation of quercetin with NS. In DSC, the authors observed a complete disappearance of the drug endotherm in the NS formulations. Distinct peaks in XRD spectra were masked after formulation with NS, suggesting the amorphization of the drug post complexation with NS. As the study utilized a specific size fraction of NS pretreated for formulation (mean size of NS <100 nm), the particle size of the formulations were also found to be in the range of 40–95 nm—lower than the previous reports by Trotta et al., Vavia et al., and Swaminathan et al.[15,19,20,22,24,25,93,94] The particles were spherical, uniformly dispersed, and showed a tight size distribution with a low PI and sufficiently high negative zeta potential. The NS formulation released quercetin in a significantly faster rate as compared to the 'as-is' drug with about 92–98% of drug release within 24 h as compared to about less than 5% of 'as-is' quercetin. More than 50% of the drug degraded after 6 h incubation with simulated intestinal fluid, as compared to <10% in case of NS formulations. Similarly about 22% of drug degraded upon irradiation with light as compared to about less than 12% with NS formulations. Both the studies confirmed enhancement in physico-chemical stability and photostability of quercetin upon NS formulation, in line with previous reports on stabilization of other labile molecules by NS.[20]

They further evaluated the in vitro antioxidant activities of quercetin NS formulations in comparison with quercetin. The DPPH (2,2-diphenyl-1-picrylhy-drazyl) radical-scavenging activity—a key parameter symbolizing antioxidant potential—showed that NS formulations of quercetin exhibited about 500- to 850-fold higher activity as compared with 'as-is' quercetin (F1:4: 569-fold, F1:6: 824-fold). Similarly, antisuperoxide formation assay revealed about 550-to 725-fold higher activity of NS formulation as compared with 'as-is' quercetin (F1:4: 556-fold, F1:6: 723-fold), while the superoxide anion-scavenging activity painted a similar picture wherein there was an enhancement of about 600- to 1200-fold activity by NS formulations (F1:4: 612-fold, F1:6: 1234-fold). The authors conclusively showed a higher metal chelating activity for the NS formulations of quercetin as compared to the 'as-is' quercetin.[14] These promising results are presented as Figure 10.

NS Formulations Delivering Oxygen

Cavalli et al. published the first studies exploring the gas holding capacity and delivery of NSs as a potential tool in hypoxia associated with tumors.[16] Oxygen deficiency leads to hypoxia that plays a key role in resistance to therapies. Moreover, it is also known to promote tumor progression and development of an aggressive phenotype.[131] Disease-free survival for patients with hypoxic cervical cancers or soft tissue sarcomas has been poor.[131] Considering the potential of NS in drug encapsulation and controlled release, Cavalli et al. synthesized three different types of CDI-based NS (α, β, and γ NSs) using the respective CD molecules. Suspensions of NS were homogenized with high shear (using Ultra-turrax®) at 24,000 rpm and 2 min in vials which were then sealed and saturated with oxygen using oxygen purge (concentration of free O_2 up to 35 mg/L). They evaluated the stability of oxygen-encapsulated NS stored at 25°C by measuring the free O_2 level and NS sizes. They further evaluated the cytotoxicity using MTT assay on Vero cells. A 5 mg/mL NS aqueous dispersion was injected into small Teflon vial containing saline in hypoxic conditions at constant temperature to study the in vitro O_2 release pattern with an in-line oximeter. An in-house customized device was used for studying the permeation of O_2, wherein two compartments were separated by a silicon membrane. The

surface area values for NS were in the range of 40 and 50 m^2/g. NS were spherical with particle sizes in the range of 400 and 550 nm with a narrow distribution and high negative zeta potential value (−30 mV). The O_2 formulations were nontoxic to the Vero cells. The formulation suspensions did not aggregate and showed good physical stability in sealed vials at 25°C for 15 days. Oxygen was released at a rapid rate (6 mg/L) in the initial phase as seen by the in vitro release studies results followed by a slower and sustained release. They found that ultrasound increased the O_2 release by about 58% from the β-CD NS formulation. They validated the findings in comparison with plain O_2 without the NS carrier. Oxygen permeation studies showed that the β-CD NS formulation exhibited a higher permeation as compared to their α- and γ-CD counterparts. Oxygen permeation from the β-CD NS formulation improved by about 192% with the use of ultrasound, however, presented an initial O_2 spike. The authors then explored a Pluronic®-based hydrogel system of the β-CD NS formulation which provided a uniform, sustained release of O_2 without the spike (with or without ultrasound).[16]

Continuing the research further, Trotta et al. utilized a modified methodology for engineering the O_2-loaded NS.[26] They included sodium chloride, PEG 400, and decafluoropentane in the formulation mix along with NS and water to further improve the O_2 loading, storage, and delivery. They engineered two formulations of β-CD NS and one of α-CD NS. The physical characteristics obtained were similar to the previous study. These formulations were nonhemolytic and safe to be injected in vivo as evaluated by in vitro hemolytic activity studies. Sustained O_2 release for up to 60 min was obtained by NS formulations. Ultrasound had a positive effect on O_2 release. A Pluronic®-based hydrogel coating led to a lowering of O_2 release. Ultrasound brought about a 30% increase in the permeation rate of O_2 from the β-CD NS formulation.[26]

Temozolomide

Jain et al. explored diphenyl carbonate-based β-CD NS for development, characterization, and in vitro cytotoxicity assessment of temozolomide as a potential brain tumor therapy.[18] Temozolomide has been used as a first-line therapy for the treatment of gliomas after its surgical resection.[132] They present considerable challenges clinically by having a short half-life of 1.8 h and protein binding of 15%, thereby necessitating repeated dosing.[132,133] Implantable microspheres, polymeric nanoparticles, solid lipid nanoparticles, nanostructured lipidic capsules, magnetic nanoparticles along with other nanoparticles have shown promise in targeting temozolomide to the tumor site.[132,134–139] The authors explore NSs with a similar long-term goal.[18] They confirmed the structure of NS using NMR spectroscopy. They evaluated the drug interaction and loading using solution state studies wherein a shift in the original wavelength of the drug suggested interaction of hydrophobic groups. The complexation and encapsulation within NS was validated using FTIR, DSC, and XRD studies, as the drug peaks were either shifted or masked after formulation with NS. In vitro release indicated a slower release in case of NS-based formulations of temozolomide. Cytotoxicity of the NS formulations was comparable to that of free drug however, there were changes observed in the morphology of U-373 cells which were distorted or degenerated after treatment. The formulations developed thus were touted to be a potential delivery system for brain delivery.[18]

Delivery of Water Soluble and Sparingly Soluble Anticancer Molecules

To test the broad range of applications on NS, Trotta, Cavalli, and coworkers evaluated the properties of NS for delivering water soluble and sparingly soluble anticancer molecules such as doxorubicin and 5-Fluorouracil (5-FU), respectively.[10,12,140] Doxorubicin is one of the most commonly used molecules for treating cancers of the breast, bladder, stomach, lung ovaries, thyroid, and soft tissues.[141] Although water soluble, doxorubicin poses severe dose-limiting cardiotoxicity concerns.[141] Doxorubicin hydrochloride injection was the first liposomal anticancer product to receive regulatory approval. In the recent years, much of the

research efforts have been focused toward exploring nanotechnology tools for reducing the cardiotoxicity and increasing the specificity of doxorubicin, such as modified liposomes, polymeric nanoparticles, PEGylated dendrimers, lipid nanoparticles, magnetic gold nanoparticles, niosomes, microemulsions, doxorubicin protein nanoparticles to highlight a few recent research efforts.[141–149] Cavalli, Trotta, and coworkers utilized NS for the first time for modulating the release of doxorubicin.[10,12] Doxorubicin was loaded in NS (loading of 20% w/w) as indicated by aforementioned techniques. The authors found that Doxorubicin was released in a slow and sustained manner after incorporation in NS. In vitro release studies suggested that doxorubicin was released in a pH-dependent manner at a very slow rate of about 1% at pH 1.2 after 2 h. At a pH of 7.4, doxorubicin release was about 29% after 3 h. This also indicated a protective effect of NS on doxorubicin in acidic environment, potentially as seen that in the stomach, where NS appeared to shield the drug and release it at a higher pH of 7.4, similar to that of duodenum and the intestine. In-depth studies are needed to validate this selective release behavior conclusively.[10,12]

Further, Cavalli et al. improved the properties of 5-FU by using NSs of γ-CD. 5-FU is the most preferred drug in the treatment of oropharyngeal cancer, colorectal cancer, stomach cancer, and cervical cancer. It is a highly polar drug with pka values of 8.0 and 13.0.[150] It is poorly absorbed orally with erratic bioavailability. It exhibits low terminal half-life (8–20 min) via the parenteral route and is eliminated rapidly.[150] It is also reported to be photosensitive and is known to cause severe side effects when given by the intravenous route.[10,34,140,150] Research to mitigate these problems of 5-FU delivery has utilized innovative approaches such as gellan gum microbeads, chitosan-polycarbophil interpolyelectrolyte complex, mastic gum-based systems, albumin nanoparticles, solid lipid nanoparticles, and other conventional polymeric nanoparticles.[150–155]

In their studies, Cavalli et.al loaded up to 30% of 5-FU in γ-CD NS.[140] The drug release was modulated by the NS matrix and only about 60% drug was released after 2 h. Importantly, they reported that NS protected 5-FU for 6 months and exhibited unchanged cytotoxicity against MCF-7 cells.[140]

FUTURE DIRECTIONS AND CONCLUSIONS

Several groups including ours are working on further improving the NS properties and expanding the application basket of NS. Our efforts are now focused on structural modification of NS to make it more adaptable to the tumor conditions either by functionalization or via internal and external stimuli. A new group of NS known as the pyro-NS is currently being evaluated for improving delivery of cis-platin and doxorubicin, and we have found that these systems show tremendous encapsulation efficiency for both the drugs likely due to the additional electrostatic interactions between the protonated amine groups of the drug molecules with the carboxylic groups of the pyro-NS (reviewed in Ref 34). Owing to this added interaction, we have been able to achieve better sustained release profiles as compared to carbonate crosslinked NS in our ongoing unpublished results. We are also exploring stimuli-sensitive NS such as Glutathione (GSH)/GSH disulfide-responsive NS for targeted intracellular drug release in tumors.[156,157] Intracellular concentration of GSH is about threefolds higher than the extracellular levels in chemoresistant cells; hence, NS with GSH/GSH-disulfide switches can be easily turned on after the intracellular delivery thereby leading to cleavage of the disulfide bond and subsequent targeted release of the encapsulated drugs. NS offers an additional advantage of multiple interacting sites for drug loading. The crosslinking density and the synthesis can be tuned to modulate the drug release based on the stimuli. In our initial studies, we presented promising results with a series of disulfide-containing NS (with varying amounts of the sensitive disulfide bridges) nanosized by high pressure homogenization (average size ~200 nm).[156] These GSH-NS showed good swelling capacity without causing any cytotoxic effects on several tested cancer cell lines.[34] We tested the stimuli-dependent in vitro release of two model drugs viz. CAM and doxorubicin by modulating the levels of GSH in the donor compartment. Interestingly, the hypothesis was validated by our preliminary results. Initial in vivo animal studies also suggested that these nanocarriers were nontoxic. Further, research is being directed toward

development of simpler and faster methods for NS synthesis.[38] Molecular imprinting of NS with drugs could be another direction worth considering, wherein the drug could be intercalated in the NS reticulate structure during synthesis thereby leading to a much slower release. Work is underway in synthesis and characterization of PEGylated NS, soluble NS, and cationic NS for a myriad of applications. Research will also likely also continue in the direction of exploring various routes of administration such as intraocular, intratumoral, topical, parenteral, buccal, and nasal-to-brain. We have presented a bird's eye view of the key applications of NSs in cancer therapeutics for quick reference in Figure 11.

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Conflict of interest: The authors have declared no conflicts of interest for this article.

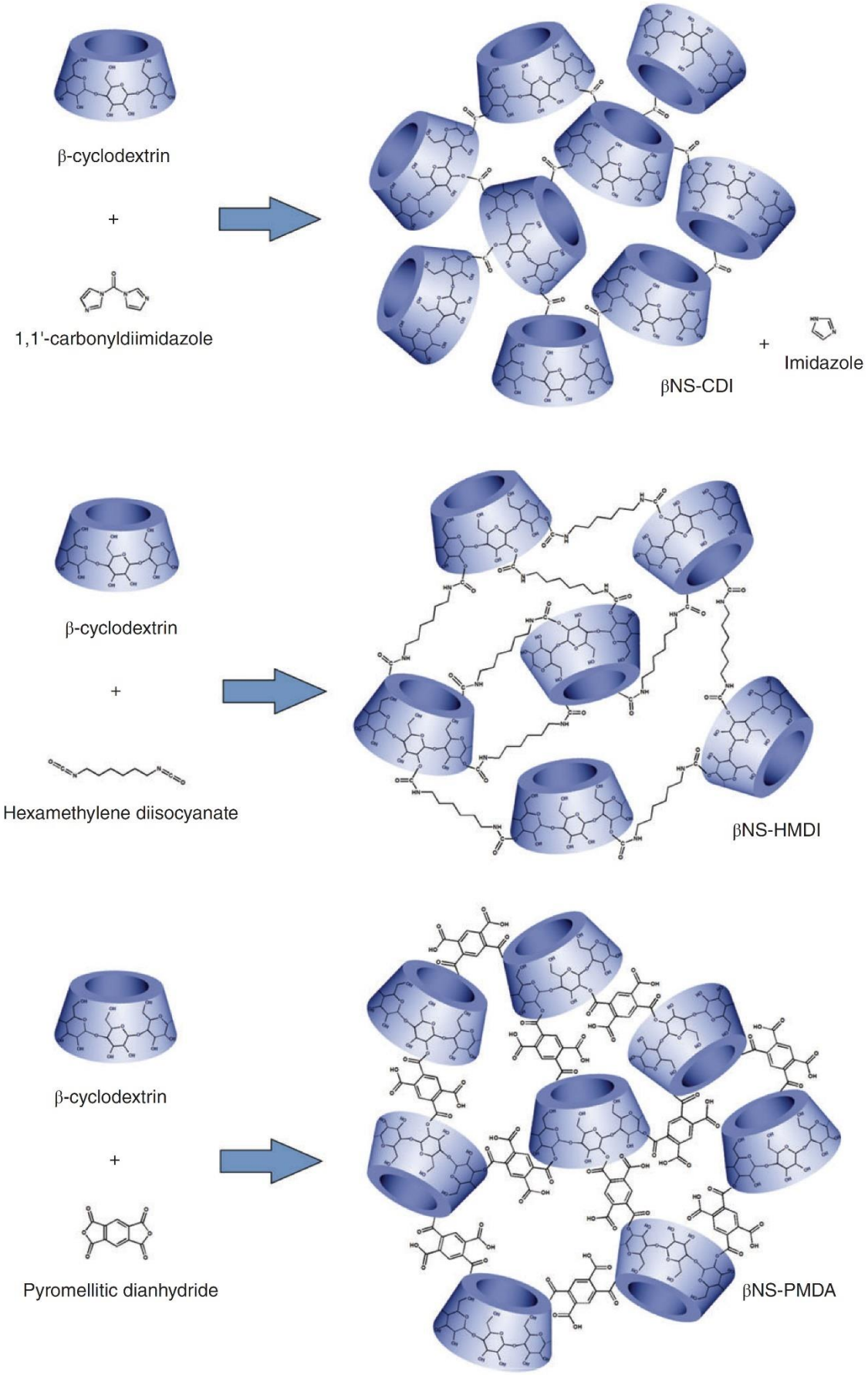

Figure 1. Schematic structure of cyclodextrin nanosponge. (Reprinted with permission from Ref 51. Copyright 2015 Wiley Periodicals, Inc. and the American Pharmacists Association)

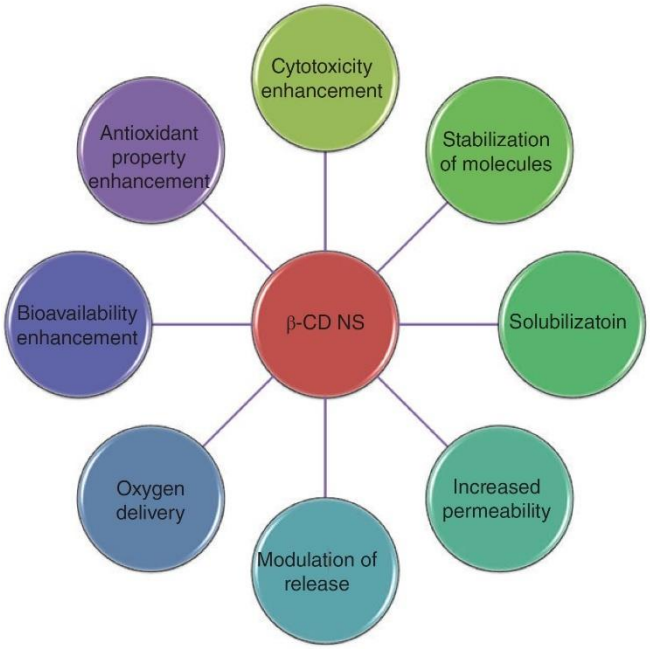

Figure 2. General applications of cyclodextrin nanosponge in cancer therapeutics presented in a schematic form.

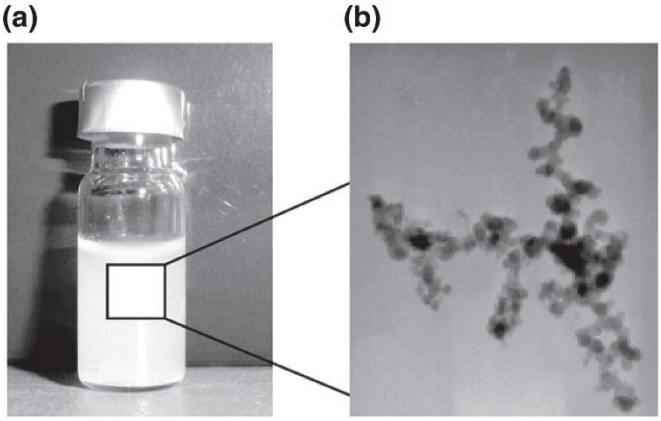

Figure 3. Camptothecin-loaded β-cyclodextrin nanosponge. (a) Camptothecin-loaded β-cyclodextrin nanosponge (CN-CPT) provides a stable aqueous nanosuspension with low polydispersity index. (b) Transmission electron microscopy (TEM) images of CN-CPT: TEM showed a spherical morphology and corroborated the particle size results. (Reprinted with permission from Ref 77. Copyright 2002 Elsevier B.V.)

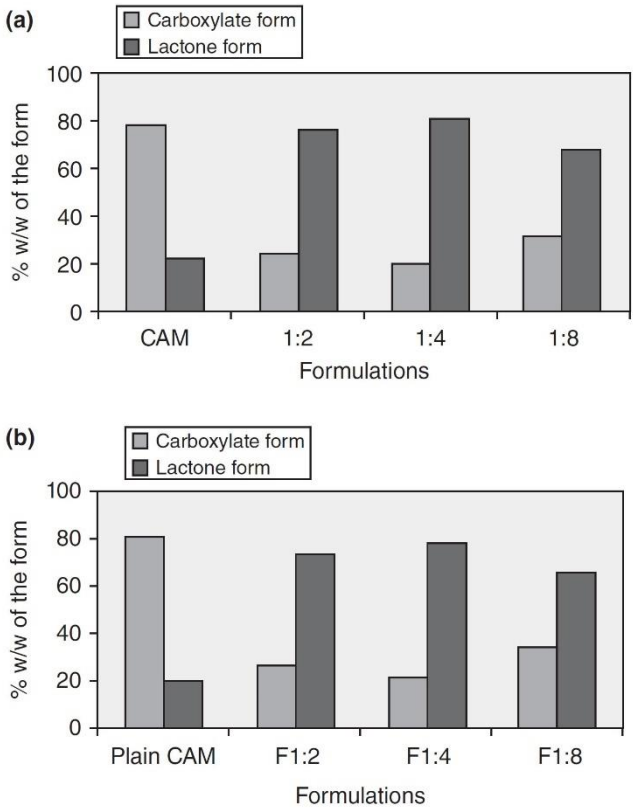

Figure 4. Plasma stability of the camptothecin (CAM) formulations: (a) Effect of various nanosponge on the stability of CAM complexes in PBS over a period of 24 h, (b) Plasma stability of CAM complexes over a period of 24 h. (Reprinted with permission from Ref 20. Copyright 2010 Elsevier B.V.)

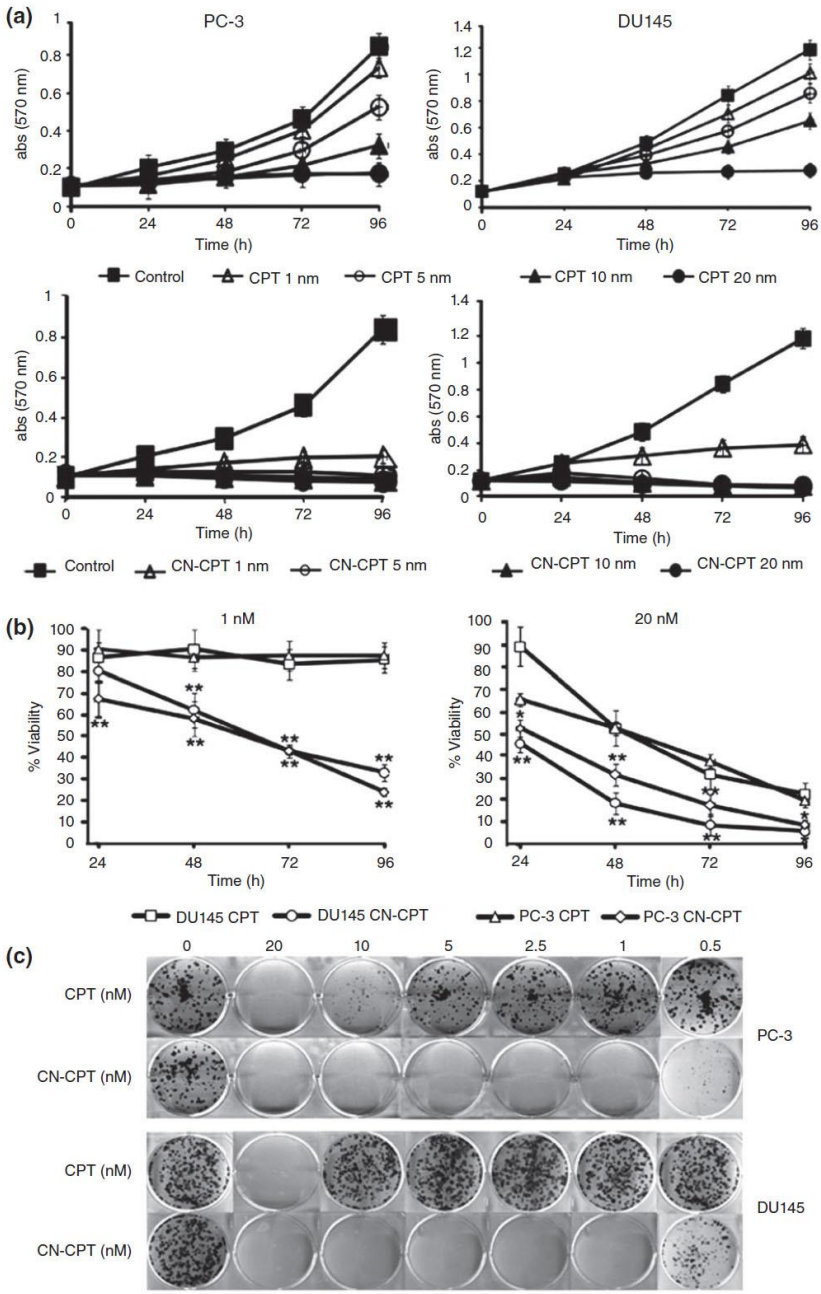

Figure 5. Inhibition of proliferation and clonogenicity following CPT and CN-CPT treatment. (a) Effect of CPT and CN-CPT on PC-3 and DU145 cell lines proliferation was tested by MTT assay. Cells (800/well) were treated with increasing concentrations of CPT and CN-CPT for 24–96 h and the result was expressed as absorbance at 570 nm. Values are expressed as mean ± SD; each group n = 8, experiments in triplicate. (b) Effect of CPT and CN-CPT on PC-3 and DU145 cell lines viability. Data were expressed as the percentage of cells viability versus control. One-way ANOVA and the Dunnett's test revealed statistically significance differences (*p <0.05; **p *0.01) of CN-CPT versus CPT treated cells. (c) Effect of CPT and CN-CPT on prostate cancer cells clonogenicity was tested by colony forming assay. DU145 and PC-3 cells (500/well) were seeded in six well plates and treated with both compounds at the indicated concentrations for 72 h. Cells were cultured for 10 days and subsequently fixed and stained with crystal violet. (Reprinted with permission from Ref 77. Copyright 2012 Elsevier B.V.)

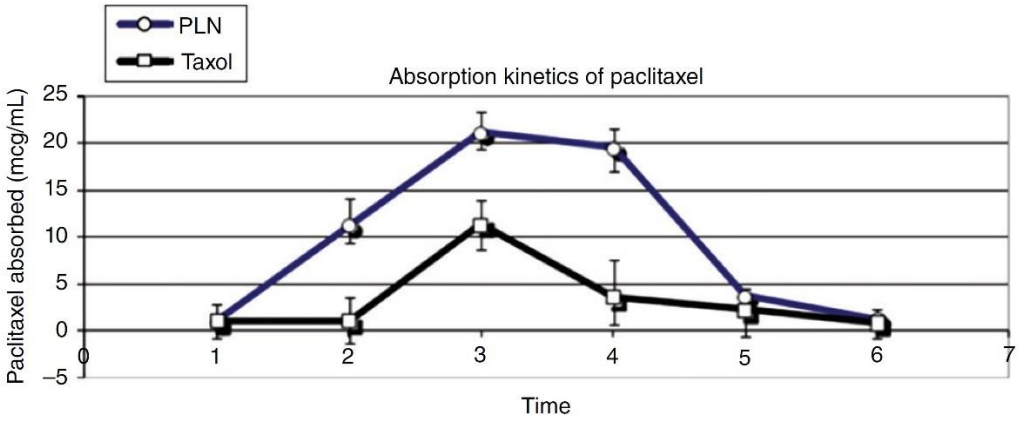

Figure 6. Absorption kinetics of paclitaxel. (Reprinted with permission from Ref 25. Copyright 2010 Informa UK Ltd)

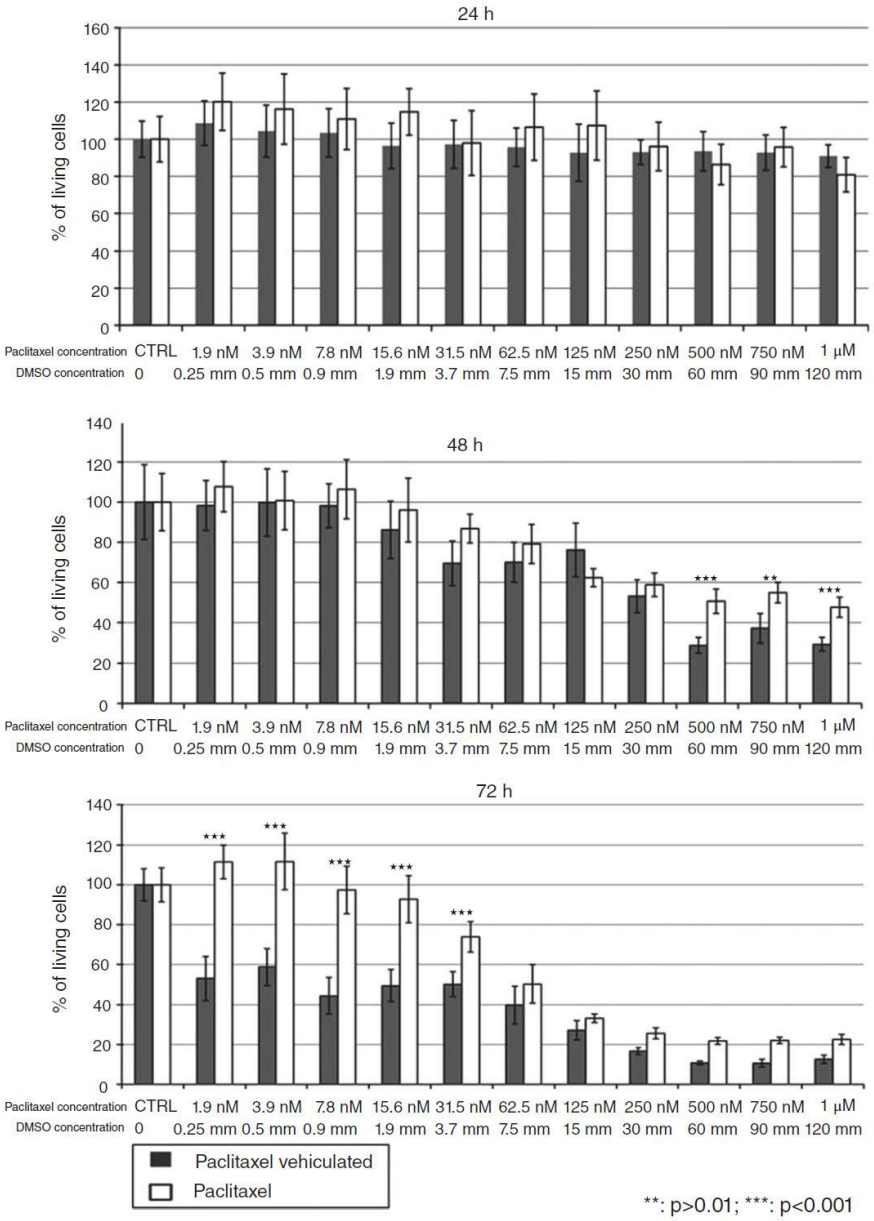

Figure 7. Nanosponge-vehiculated paclitaxel. Toxicity of paclitaxel-loaded nanosponges dissolved in DMSO compared to plain paclitaxel at three different time points. **P = 0.01; ***P = 0.001. Significance against internal control as described in the text. (Reprinted with permission from Ref 94. Copyright 2012 Springer Science+Business Media B.V.)

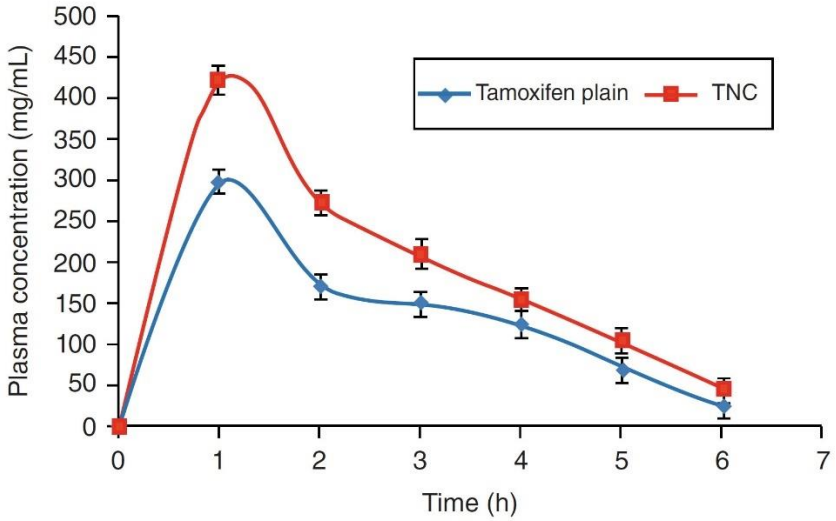

Figure 8. Oral pharmacokinetic profiles of tamoxifen citrate and tamoxifen nanosponge formulations. (Reprinted with permission from Ref 24. Copyright 2013 Informa Healthcare USA, Inc.)

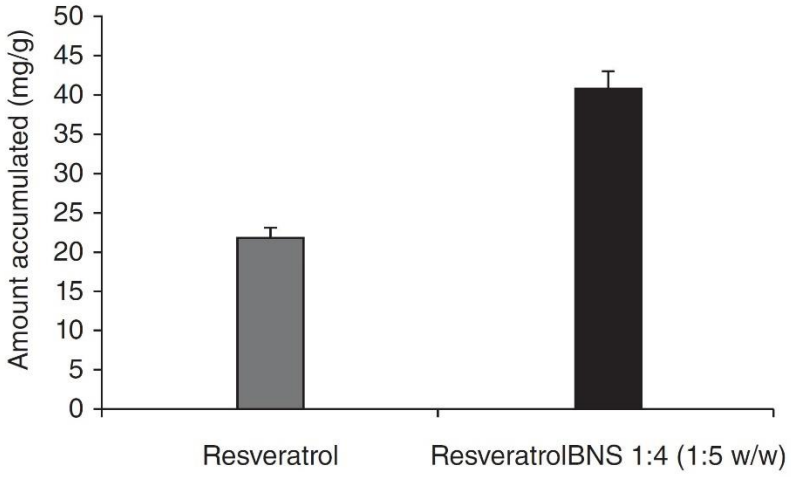

Figure 9. Accumulation study of resveratrol and complex in rabbit mucosa. (Reprinted with permission from Ref 15. Copyright 2011 American Association of Pharmaceutical Scientists)

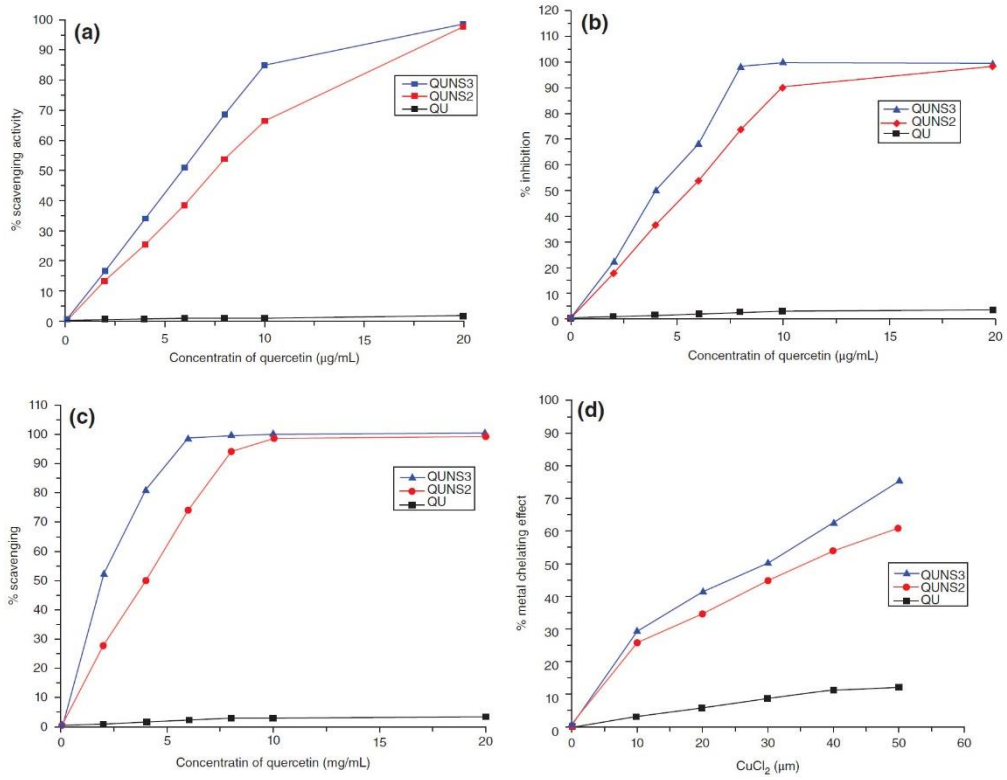

Figure 10. Effect of quercetin nanosponge in: (a) DPPH radical-scavenging activity, (b) antisuperoxide anion formation, (c) superoxide anion-scavenging activity, (d) metal chelating activity. (Reprinted with permission from Ref 14. Copyright 2014 Springer Science+Business Media New York).

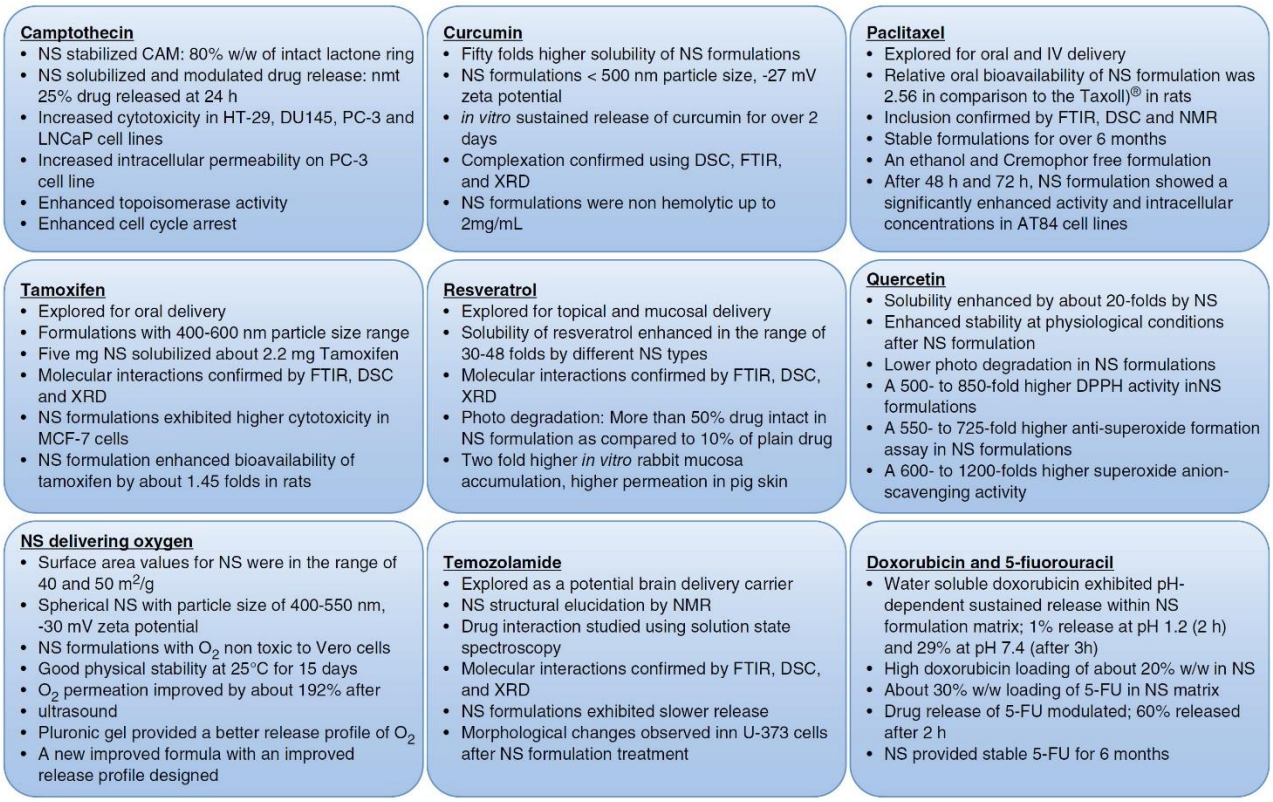

Figure 11. Nanosponge-aided delivery for cancer therapeutics in a nutshell.