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Biocompatibility of cucurbit[*n*]urils

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Abstract

This review considers the biological properties of nitrogen-containing macrocyclic glycoluryl derivatives – cucurbit[*n*]urils (CB[*n*]). Currently, the use of biomaterials that come into contact with the internal environment of the body is widespread in medicine. Of particular interest is the possibility of giving biocompatible biomaterials new properties by impregnating them with various chemical compounds, fine-tuning these properties for each specific task. One example of such customization is the use of cucurbit[*n*]urils. Cucurbit[*n*]urils are a class of macrocyclic organic compounds that are hollow molecules in a "barrel" shape. Their structure is formed from glycoluryl (a urea derivative) and formaldehyde linked into a cyclic framework. The cavity of cucurbit[*n*]urils is hydrophobic and the entrances are hydrophilic, allowing them to "capture" and retain guest molecules. They are chemically stable, resistant to acids and high temperatures, and able to select guest molecules based on their size, shape and chemical nature. The presented review focuses on the properties of cucurbit[*n*]urils, their biological activity, *in vitro* and *in vivo* toxicity, their potential use in medicine as antidotes, for disease diagnosis, and targeted drug delivery. General information about cucurbit[*n*]urils as a basis for creating biomaterials for medical purposes and targeted drug delivery agents is considered. Generalized experimental data on the toxicity of cucurbit[*n*]urils both *in vitro* and *in vivo* are overviewed.

Key findings

- CB[*n*] has moved from the category of laboratory innovations to the category of promising tools for nanomedicine.
- A thorough understanding of the structure-biocompatibility relationship allows for the active design of safe and functional systems rather than passive observation of their properties.
- The next decade will likely witness the first clinical trials based on CB[*n*], opening a new chapter in supramolecular therapeutics, where control over molecular interactions will be key to the development of future drugs.

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Supplementary information
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Sustainable Development Goals
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1. Introduction

Modern medicine and pharmacology are on the threshold of a paradigm shift driven by nanotechnology and, in particular, supramolecular chemistry. In the search for highly efficient and safe platforms for drug delivery, diagnostics, and theranostics, the scientific community has turned its attention to a class of macrocyclic compounds known for their unique ability to selectively bind various molecules. Among these, the cucurbit[*n*]urils (CB[*n*]) family stands out as one of the most promising and multifunctional candidates.

CB[*n*] are rigid macrocyclic structures consisting of repeating glycoluril units linked by methylene bridges. Their unique "barrel-shaped" architecture forms an internal hydrophobic cavity capable of accommodating various organic molecules, ions, and gases, and two identical polarized portals flanked by carbonyl groups. This determines their outstanding properties as molecular hosts: high affinity for a wide range of guests, stabilization of labile compounds, and the ability to control chemical and biological processes at the molecular level [1–3].

Initially studied in the context of fundamental supramolecular chemistry, CB[*n*] quickly became the focus of biomedical researchers. CB[*n*] have demonstrated promising potential for reducing side effects through encapsulation and controlled release of active molecules and for detoxification through selective binding and removal of toxic substances from the body. Furthermore, there is significant potential for their use in increasing the solubility and biostability of hydrophobic and easily degradable drugs, as well as for creating theranostic systems that combine diagnostic and therapeutic modules in a single platform.

However, the path to clinical application of any new material lies through a comprehensive study of its biocompatibility—a complex characteristic reflecting the ability to interact with living systems without causing adverse reactions. For CB[*n*], this question is central and multifaceted. Their potent interactions with biological targets not only possess therapeutic potential but also pose as a potential source of toxicity. Key aspects requiring in-depth analysis include cytotoxicity, hemocompatibility, immunogenicity, and pharmacokinetics. Moreover, the biocompatibility of CB[*n*] is not universal; it depends on their structure. The size of the molecule, determined by the number of repeating units *n* (from 5 to 10 or more), directly influences the cavity size, the portal diameter, and, consequently, the mechanism of interaction with biological membranes, proteins, and nucleic acids. For example, while CB[7] exhibits low acute toxicity and is rapidly eliminated via the kidneys, larger homologues such as CB[8] may exhibit more pronounced effects due to their stronger binding capacity [1, 2].

The aim of this review is to systematize and critically analyze current scientific data on the biocompatibility of the CB[*n*] family. This article will examine in detail the influence of CB[*n*] structural parameters on their toxicological profile, mechanisms of interaction with cells and biomolecules, and functionalization strategies to minimize adverse effects. Particular attention will be paid to the potential use of biocompatible CB[*n*] derivatives in the development of new therapeutic and diagnostic agents, which defines their role as key players in the medicine of the future. The presented review of scientific articles on the biocompatibility of CB[*n*] covers the period from 2004 to 2024.

2. CB[*n*] as a basis for the creation of biomaterials for medical application

2.1 General information about representatives of the CB[*n*] family

One of the most recognizable effects in supramolecular chemistry is the macrocyclic host-guest interaction. One such macrocyclic host, cyclodextrin, has a long history as an excipient and solubilizing agent in pharmaceutical practice. The non-covalent interaction between host molecules, such as cyclodextrin or CB[*n*], and small hydrophobic guest molecules has also been used for cross-linking polymeric precursors to produce supramolecular biomaterials. Polymers commonly used as biomaterials, such as hyaluronic acid and polyacrylamide, can be grafted with either a host molecule or a complementary guest molecule to produce cross-linked hydrogel materials when the host and guest polymers are mixed, imparting self-healing and shear-thinning properties to these materials.

The subject of the present review is the biocompatibility of cucurbit[*n*]urils (CB[*n*])]). CB[*n*] are a family of macrocyclic molecules consisting of repeating glycoluril units linked in pairs by methylene bridges (–CH_2–) and the general molecular formula $(C_6H_6N_4O_2)_n$. Currently known homologues include cucurbit[5]uril (CB[5]), cucurbit[6]uril (CB[6]), cucurbit[7]uril (CB[7]), cucurbit[8]uril (CB[8]) and cucurbit[10]uril (CB[10]). A comparative analysis of the homologues of the cucurbit[n]urils family is presented in Table 1, and the chemical structures of some homologues are shown in Figure 1 [1,2].

Table 1 Comparative characteristics of homologues of the CB[*n*] family [1,2].

Homologous	Number of links (*n*)	Approximate internal diameter (Å)	Approximate portal diameter (Å)
CB[5]	5	~2.4 Å	~4.2 Å
CB[6]	6	~3.9 Å	~5.8 Å
CB[7]	7	~5.4 Å	~7.3 Å
CB[8]	8	~6.9 Å	~8.8 Å
CB[10]	10	~10.5 Å	~11.5 Å

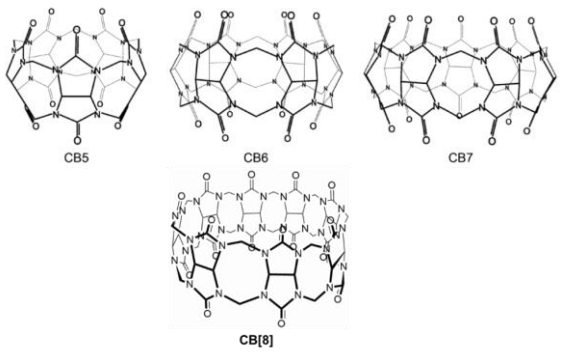

Figure 1 Molecular structures of some CB[*n*] homologues.

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CB[*n*] is a macrocyclic hexamer of glycoluril with a methylene bridge. This molecule is named so because of its external resemblance to pumpkin (Cucurbita) [1–3]. A record-breaking binding affinity for guest molecules within its hydrophobic cavity has also been discovered, thus opening possibilities for using these compounds as containers [1]. Therefore, over the years, the fundamental properties of CB[6]—high affinity, highly selective, and constrictive binding interactions—have led to their application in wastewater purification, as artificial enzymes, and as molecular switches (Figure 2) [4].

Cucurbit[*n*]urils have a strong tendency to coordinate various particles, making thema convenient starting reagent for the synthesis of supramolecular compounds. For example, the portal oxygen atoms of CB[*n*] possesses an extremely high capacity for binding metal ions [1].

More recently, the CB[*n*] family has expanded to include homologues (CB[5]–CB[10]), derivatives, and analogues. Their sizes exceed the range of compound encapsulation available for α-, β-, and γ-cyclodextrins. Scientific findings from fundamental research on non-covalent interactions have practical significance in a wide range of applications, including chromatographic stationary phases, isolation of pollutants from solutions, development of catalysts, chemical sensors, novel drugs, and medical biomaterials [1,4,5].

Among all CB[*n*], CB[5] has the smallest cavity volume, and CB[10] has the largest cavity volume. One remarkable feature of the CB[*n*] family is their thermal stability [1]. Thermogravimetric analysis measurements show that thermal stability exceeds 370 °C in an inert atmosphere [6]. CB[6] and CB[8] have low solubility in pure water, but their solubility increases in the presence of ammonium, alkali, and alkaline earth metal ions due to complex formation between the metal and the carbonyl portals. CB[5] and CB[7] have somewhat greater solubility in aqueous environments than CB[6] and CB[8] [1,7].

Cucurbit[*n*]urils consist of *n* glycoluril units linked to each other by methylene linkers [1]. The number of repeating glycoluril units determines the cavity size and the nature of guest encapsulation [7]. CB[*n*] cavities are hydrophobic, while the portals with their carbonyl rims exhibit dipolar properties [1,7].

The general scheme of synthesis and separation of CB[*n*] homologues is presented in Figure 3 [1–3].

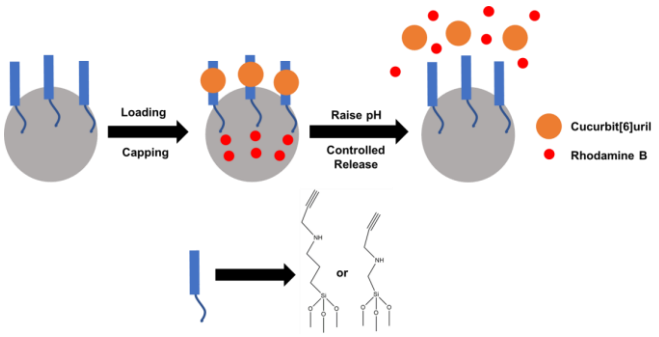

Figure 2 Graphical representations of active supramolecular nanowires [5].

CB[5] is the smallest homologue of the CB[*n*] family. It is the least used due to its small cavity volume. The portal diameter of CB[5] is 2.4 Å with a cavity volume of 82 Å^3. Although the portal opening is small, the cavity is rigid and slightly larger in diameter than the portals; CB[5] encapsulates only a few small guest molecules [8,9]. CB[5] binds well with H^+, alkali, alkaline earth metals, and ammonium cations via portal interaction, as well as with some gas molecules (N_2, O_2, N_2O, CO, CO_2, some noble gases, etc.). Furthermore, some solvent molecules (methanol and acetonitrile) can also be encapsulated within the CB[5] cavity [4,7,10]. Since the structure of CB[5] is highly symmetric, it is used as a building block for supramolecular systems. CB[5] has also been used as a coating on Au surfaces and as a binding agent to hold gold nanoparticles together due to its portal interaction [1,6,11].

CB[6] is the most common representative of CB[*n*]. The portal diameter of CB[6] is 3.9 Å. Similar to CB[5], the CB[6] portal readily binds H^+, alkali and alkaline earth metals, as well as transition metals and lanthanides in their cationic forms. CB[6] also forms a stable complex with aliphatic amines due to the presence of an induced partial negative charge by the carbonyl oxygen atoms on each of the portals. This leads to ion-dipole interactions, where protonated amines tend to interact strongly with the portals [8].

CB[6] binds most strongly to pentane and hexane α,ω-functionalized diammonium ions. It also binds to guest molecules based on imidazolium [1,6,12]. However, the stability of the diammonium guest bound in CB[6] is highly dependent on the environment [13,14]. Stability in the solution phase is due to the combined effect of hydrogen bonds from water molecules and ion-dipole interactions, but in the gas phase only ion-dipole interaction exists. Thus, in the gas phase, the guest must undergo a structural distortion from its original conformation to achieve the ion-dipole interaction, which is not required in the solution phase. The overall binding affinity of CB[6] for protonated aliphatic amines has led to the creation of various rotaxanes and pseudorotaxanes, where alkylamines act as axles and CB[6] acts as wheels [2,13].

CB[6] binds differently to catecholamines such as adrenaline and isoprenaline. The crystal structure of the CB[6]-adrenaline complex transforms from needle-like to prismatic over several days.

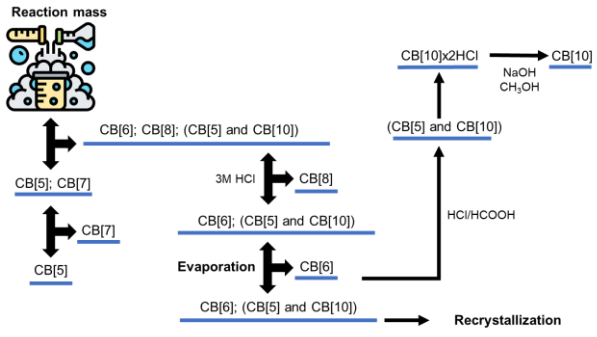

Figure 3 Scheme of synthesis and separation of CB[*n*] homologues [1–3].

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The reason for the crystal structure transformation is related to the kinetics and thermodynamics of the binding process [1,7,14].

CB[6] is also known to modulate chemical reactions within its cavities. There is a possibility of using an inclusion complex of halogen, such as I_2-CB[6], for catalytic reactions and bromination of benzene, and formation of bromohydrin using the Br_2-CB[6] complex. This complex can facilitate the electrophilic bromination of benzene [1,2,15].

CB[7] is the third member of the CB[*n*] family with a larger cavity size than CB[5] and CB[6]. Therefore, it is capable of binding larger guest molecules. CB[7] has a portal diameter of 5.4 Å and a cavity diameter of 7.3 Å, resulting in a volume of 279 Å^3. The internal cavity volume is large enough to accommodate three-dimensional molecules such as ferrocene, bicyclooctanes, naphthalene, stilbene, viologen, o-carborane, cobaltocenium, and adamantylamine derivatives with very high binding constants (log$K$ > 12). The high-energy expulsion of water molecules acts as part of the driving force for the high binding values of CB[7] [15,16].

A feature that makes CB[7] widely used as a host molecule in CB[*n*] chemistry is its solubility in water compared to other family members [15]. CB[7] exhibits a solubility in water of 30 mM compared to the 0.01 mM solubility of CB[8] in water. Solubility combined with the large cavity size makes CB[7] one of the widely used host molecules among the CB[*n*] family [17]. Solubility factors also play a role in biological applications, such as drug encapsulation and enhanced solubility of water-insoluble drugs. CB[7] binds to metal complexes and metal complex-based preparations. For example, cytotoxic platinum-based drugs encapsulated in CB[7] have potential for cancer treatment with reduced toxicity [17–19], Figure 3.

The application of CB[7] in biomedical research is not limited to drug delivery alone. The solubility of CB[7] at therapeutic levels has pushed the use of this host molecule into various toxicological studies—from *in vitro* studies to *in vivo* studies in mice and zebrafish [20,21]. CB[7] has also found unique applications in areas such as supramolecular energy transfer, catalysis, and underwater adhesives.

CB[7] can also be used in capillary electrophoresis as a stationary phase for the separation of positional isomers [3]. CB[8] has a cavity volume of 479 Å^3, which is 1.7 times larger than that of CB[7].

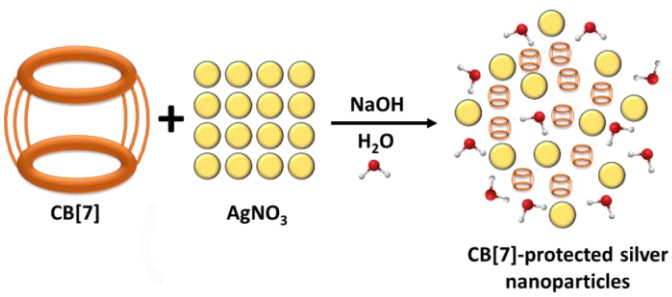

Figure 4 Schematic illustration of the formation and stabilization of well-dispersed AgNPs [19].

The large cavity facilitates the encapsulation of amphiphilic positively charged guest molecules, including ferrocene and adamantamine. CB[8] binds molecules such as cyclen and cyclam, thus forming a complex of one macrocycle inside another [16]. CB[8] also has the ability to bind larger molecules such as fullerene. In this case, it forms a complex with two fullerene molecules, each bound externally to the portals of CB[8] [22].

Long-chain alkylammonium salts carrying long aliphatic chains bind inside the CB[8] cavity in a U-shaped conformation. This structural form was confirmed by ^{1}H NMR studies, which clearly showed that the methylene protons in the long chain are bound within the cavity [22].

CB[8] exhibits the unique ability to bind two aryl guest molecules within its large cavity. There are a number of compounds that demonstrate double encapsulation within the CB[8] cavity, such as naphthyl derivatives, coumarin, N-phenylpiperazine, 9-aminoacridinium, bipyridinium salts, anthracene derivatives, berberine, neutral red, 9-aminoacridinium, p-methylaminobenzonitrile, phenylpyridinium derivatives, and some peptides containing tryptophan and phenylalanine residues [14,17].

Macrocyclic systems derived from glycoluril and its derivatives, such as CB[*n*] and bambusuril, serve as ideal starting reagents for the surface modification of porous materials. CB[*n*] demonstrate an excellent ability to form host-guest complexes with cationic molecules and boast a wider range of molecular cavity sizes [7]. CB[*n*] exhibits an inherent ability to selectively accept various organic guest molecules through a range of interaction mechanisms, including hydrophobic interactions, hydrogen bonds, van der Waals forces, π–π stacking, and ion-dipole effects, leading to the formation of inclusion complexes [27–30].

Unlike many other macrocyclic carriers, CB[*n*] possesses an exceptionally rigid structure, making the determination of cavity parameters particularly interesting. For example, all variants of CB[*n*] (n = 5–8 and 10) have the same height (d = 9.1 Å), but differ significantly in the cavity width. CB[5] has an inner diameter of 4.4 Å, while CB[8] has twice that at 8.8 Å [31]. This feature allows for the accommodation of drug molecules of different sizes within the CB[*n*] cavity, thereby expanding the possibilities of their application in various fields. Antitumor, antibacterial, anticholinergic drugs, antioxidants, neurotransmitters, cholinesterase reactivators, and other compounds can be encapsulated in the CB[*n*] cavity [32]. Utilizing these properties allows researchers to impart various necessary characteristics to materials. Currently, methods for modifying the surface of porous materials by impregnation with biologically active compounds, including macromolecular ones, are widely used in various fields [32]. These techniques allow for precise control over the release kinetics of antibiotics, pharmaceuticals, biologically active substances, and cells, opening broad possibilities for personalized therapeutic intervention and biomedical applications [23].

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CB[10] is a homologue that represents an inclusion complex encapsulating CB[5] inside. CB[10] has the largest cavity among the CB[*n*] family members, with an estimated cavity volume of 870 Å^3. During encapsulation, a significant change in the shape of the CB[10] cavity occurred. The observed cavity shape was elliptical. This demonstrated the flexibility of CB[10] to accommodate guests without altering their photophysical and electrochemical properties [33].

2.2 Application of CB[*n*] in medicine and biology

Despite the large amount of data available on CB[*n*], their biocompatibility is insufficiently studied [10]. A comprehensive assessment of their various types of toxicity is necessary for risk analysis of the application of these compounds as pharmaceuticals. Their peculiar structure makes them ideal candidates for performing supramolecular functions such as catalysis, molecular and biomolecular recognition, sensing, self-assembly, and threading to form interpenetrating architectures. Due to their synthetic versatility, CB[*n*] are useful platforms for designing more complex structures for the self-assembly of supramolecular polymers and applications in biomimetic chemistry [34,35].

For example, it is possible to create special structures based on CB[*n*] – so-called water-soluble molecular tweezers (MT) [4]. These structures are capable of forming complexes with the cationic side chains of lysine and arginine within their cavities. Complex formation is driven by secondary interactions between the cationic parts and the electron-rich aromatic cavity of the MT. In this process, toxic protein aggregates are dissolved and redirected into amorphous, benign aggregates. Thus, MTs can be considered promising candidates for therapy in the early stages of neurodegenerative diseases [34,36].

Cucurbit[*n*]urils are ideal candidates for applications in medicinal chemistry and chemical biology due to their low toxicity and host-guest properties. In particular, CB[*n*] are capable of encapsulating drugs for their targeted delivery and controlled release [10].

The constant search for new biomedical applications has led to the creation of metal-calix[4]arene complexes capable of inhibiting the growth of bacteria, fungi, and cancer tumor cells. In this case, the biological activity is attributed more to the inorganic ions than to the calixarene ligand [16,34].

2.3 Targeted drug delivery

Creating a "magic bullet" that can specifically target a diseased cell and selectively deliver a therapeutic agent is a very complex challenge. Recently, researchers have focused their efforts on creating new systems based on CB[*n*] that can deliver drugs to the target site. Targeting can be achieved by appropriate functionalization of the carrier system with functional groups that are recognized by specific receptors present on the cell surface [3,10].

The incorporation of these functional groups into the system can be achieved by covalent or non-covalent attachment of such groups to the self-assembling delivery carrier [38]. A promising example is the synthesis of a functionalized CB[6] derivative that assembles into vesicles [3]. The vesicle surface can be altered through non-covalent interactions of labeled alkylammonium guests with the free cavities of CB[6] [10]. When the vesicle surface is modified with a thiourea conjugate of α-mannose-spermidine and mixed with a solution containing concanavalin A (ConA), immediate aggregation with the lectin, which exhibits specificity for α-mannose, is observed. When a galactose derivative is used instead of mannose, no aggregation is observed [7].

The resulting system could potentially be applied to diseases where mannose receptors are overexpressed. Functionalized CB[6] was also used to produce nanoparticles loaded with Nile Red (NR, as a model hydrophobic drug) and modified with folic acid conjugated to spermidine [3].

The folic acid-enriched system showed efficient uptake of the dye by HeLa cells, whose surface has overexpressed folate receptors. A negative control with nanoparticles lacking folic acid resulted in no or minimal dye uptake. After endocytosis, Nile Red is released, which can be detected by confocal laser scanning microscopy. Galactose-functionalized CB[6] can also be used to demonstrate receptor-mediated galactose endocytosis in hepatocellular carcinoma HepG2 cells [3].

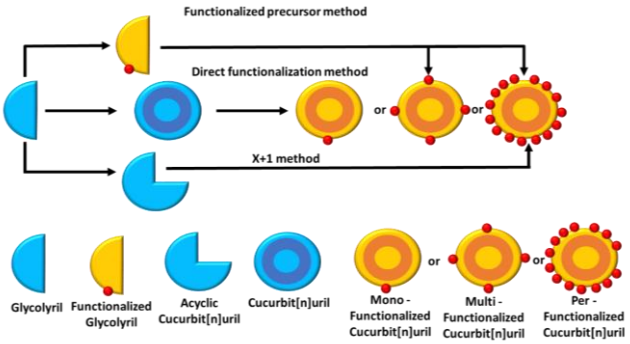

Figure 5 Schematic representation of three main strategies for the synthesis of functionalized CB[*n*] [3, 32].

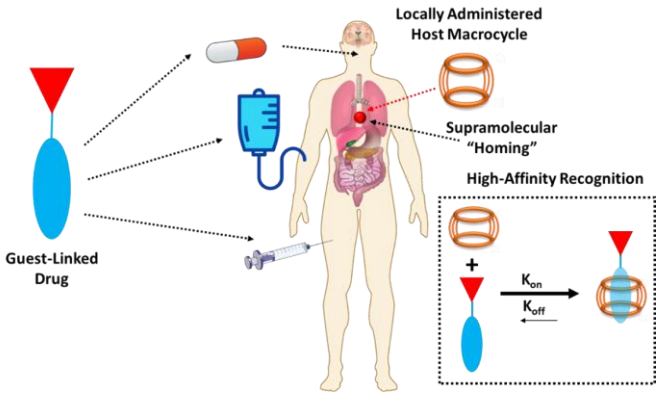

Figure 6 Schematic illustration of supramolecular homing of guest-attached small molecules based on affinity for locally applied host macrocycles [37].

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The same CB[6]-galactose conjugate was used for the non-covalent encapsulation of dextran-spermine conjugates into asialoglycoprotein receptor (ASGPR)-containing hepatocytes. This model was also employed to demonstrate the viability of a non-toxic and biocompatible receptor-mediated gene delivery system [3]. A polymer nanocapsule consisting of CB[6] with a disulfide bridge was. Treatment with dithiothreitol cleaves the disulfide bond and ruptures the nanocapsule, releasing the pre-loaded dye [3]. The potential application of this system for targeted drug delivery was illustrated by encapsulating a galactose-spermidine conjugate within the CB[*n*] cavity and presenting the galactose fragment on the surface of the nanocapsules. Carboxyfluorescein was used as an imaging probe. After incubation with hepatocellular carcinoma HepG2 cells, a change in fluorescence inside the cells was observed, indicating cellular uptake of the entire system [3]. Controlled targeted release of doxorubicin *in vitro* in HeLa cells was reported based on the same principle [39].

The stability of such a system in biological media was demonstrated both *in vitro* and *in vivo*. Recently, a CB[7]-PEG copolymer was developed as a drug carrier [38, 40], Figure 7. The CB[7] cavity is capable of encapsulating the anticancer drug oxaliplatin. The resulting supramolecular polymer material exhibited low cytotoxicity towards normal cells. However, the cytotoxicity of the encapsulated oxaliplatin was restored in cancer cells. The release of the anticancer drug is associated with the high concentration of spermine in cancer cells, which acts as a competitive guest. Thus, spermine specifically triggers the release of the complexed drug [41].

Another model of an antitumor agent is trimetazidine. This drug is highly toxic by nature and non-specifically affects both tumor and normal cells. When encapsulated in CB[7], the cytotoxicity of trimetazidine towards normal cells was significantly reduced. However, for tumor cells, the overexpressed spermine displaces the encapsulated trimetazidine from the complex, thereby allowing the restoration of its cytotoxicity towards tumor cells [42].

2.4 Complex Formation Involving CB[*n*]

CB[*n*] can form complexes with various molecules. Among these molecules can be drugs, pharmaceutical agents, and other biologically active molecules. Drug molecules that have been studied to date for their complexation with CB[*n*] include antitumor, antipathogenic, antagonistic agents, vitamins, hormones, enzyme inhibitors, neurotransmitters, neuromuscular blockers, antituberculosis agents, local anesthetics, and others [15, 38-42], Figure 8.

Sanguinarine, which possesses antioxidant, antitumor, antibacterial, and anti-inflammatory properties, forms a stable complex with CB[7]. Furthermore, sanguinarine in the complex was stabilized against photoirradiation compared to the free drug [43].

CB[7] forms a stable host-guest complex with berberine, an antimicrobial agent. The binding can be monitored by the change in berberine fluorescence upon complexation. The fluorescence of berberine was enhanced 500-fold upon complexation with CB[7]. In CB[8], unlike CB[7], two units of berberine can be encapsulated [43]. The antimicrobial alkaloid coptisine also forms complexes with CB[*n*], which is also reflected in fluorescence changes [44]. CB[7] binds to isoquinoline alkaloids, namely palmatine and dehydrocorydaline, with binding affinities of $2.4 \cdot 10^6$ and $3.2 \cdot 10^4$ M^{-1}, respectively [44]. A sharp fluorescence enhancement upon complexation with CB[7] can be observed with the naked eye [45].

Mitoxantrone, an anthracenedione antitumor agent used to treat certain cancers, forms a 2:1 host-guest complex with CB[8]. Complexation increases the uptake of mitoxantrone by mouse breast tumor cells and reduces its toxicity [46].

Encapsulation of an anticancer platinum drug was also reported [19]. Oxaliplatin forms a 1:1 inclusion complex with CB[7] in aqueous solution with a *Ka* value of $2.3 \cdot 10^6$ M^{-1}. Complexation within the CB[7] cavity leads to increased stability of the complex [19]. The CB[7]-oxaliplatin complex exhibited jointly enhanced antitumor activity compared to oxaliplatin alone [41].

Phenanthriplatin, an anticancer drug, also forms complexes with CB[7] [4,41]. CB[7] accommodates one molecule of phenanthriplatin, whereas the larger cavity of CB[8] can simultaneously bind two molecules. Release of phenanthriplatin can be achieved by the addition of cations [34,36].

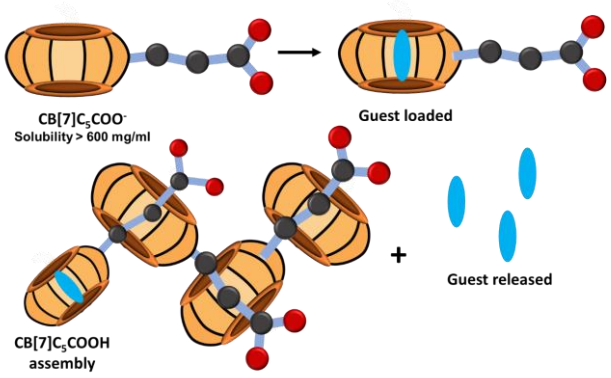

Figure 7 Schematic illustration of the chemical structure of CB[7]C5COO- and its pH-induced cargo release [37].

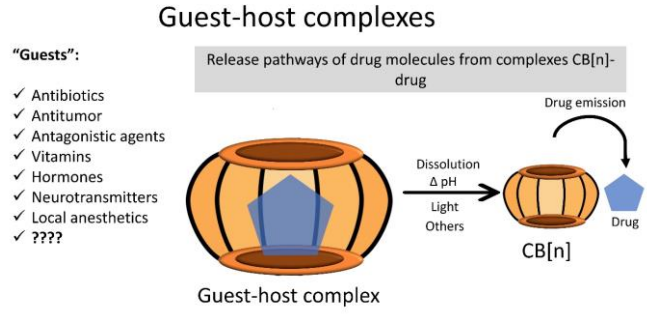

Figure 8 Some groups of drugs capable of forming complex compounds with CB[in] and factors inducing their release from the "Guest-host" complex [38-42].

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NMR studies show that cisplatin forms an inclusion complex with CB[7], while cis-$[PtCl(NH_3)_2(H_2O)]^+$ binds only to the portals [18].

Formation of a 1:1 CB[7]-riboflavin complex in aqueous solution (Ka = $1.25 \cdot 10^4$ M^{-1}) was reported [47]. Coumarin, an anticoagulant, was found to form stable inclusion complexes with CB[7] and CB[8] in aqueous solution [21,46]. Thus, it can be concluded that CB[n] have high complex-forming activity. Furthermore, complex formation can radically alter the properties of the original compounds.

To shift the dynamic chemical equilibrium towards the bound drug in solution, it is necessary to adjust the host concentration so that the drug substances are predominantly present in their complexed forms [48]. The affinity of CB[n] for guest binding is exceptionally high, facilitating the preparation of solutions containing the complexed drug even at relatively low (excess) concentrations of CB[n] [49].

The simplest, safest, most convenient, and most common route of drug administration is oral. Thus, the development and production of CB-drug complexes in tablet form is essential [10,49,53]. These tablets cannot be made solely from CB-drug complexes, but several pharmaceutical adjuvants must be included in the formulation [48,50,51].

The compatibility of CB[6] with other excipients was confirmed by various methods. CB[6] in a dosage form containing hydroxypropyl methylcellulose (HPMC) and sodium carboxymethyl cellulose (NaCMC) was successfully used as an intranasal drug form. The use of CB[7] in the dosage form was found to have an additional advantage: CB[7] prevents the mutual conversion of crystalline drug polymorphs and allows them to retain an amorphous structure in the resulting complex [47,49]. It should also be noted that CB[7] does not affect the surface area and pore size distribution, which is useful for processing and determining reliable composition [51,52].

Recently, a so-called "Trojan antibiotic" was created from a host-guest complex of CB[7] and a bolalipid with glycosylamine headgroups at both ends. Similar to a bacterial wall, this supramolecular ensemble has a surface entirely modified with sugar-like components. This Trojan antibiotic is harmless to a wide range of bacteria under weak alkaline conditions in daylight. But at pH 4.0 and under UV irradiation at 365 nm, it becomes a powerful bactericide against both Gram-positive and Gram-negative flora. The MIC_{50} of the Trojan antibiotic is 10 times lower than that of conventional drugs [4].

2.5 Application of CB[n] for disease diagnosis

CB[n] bind well to amino acids, peptides, proteins, biomolecules (e.g., neurotransmitters), and dyes, indicating applicability for extremely precise biosensing applications at sub-nanomolar concentrations [4,16,54].

The sequence recognition property of CB[7] can be transferred from recognition applications to separation applications. For example, monofunctionalized CB[7] was coated onto a solid Sepharose resin to separate proteins (human growth hormone and native insulin) in complex mixtures [4,42].

Gene therapy is a method of delivering genetic material into specific cells of patients to treat genetic diseases. It has attracted widespread attention due to its superior therapeutic effect on Parkinson's disease, severe combined immunodeficiency, and other genetic diseases. The improvement of gene delivery technology has long been a focus. When CB[n] connects to a DNA strand, the presence of CB[n] can lead to the loss of activity of some targets, thereby disrupting normal physiological activity. Stimulating the release of DNA from the CB[n] cavity under specific conditions and at the right time to restore activity is called a controlled drug release strategy. Zhou et al. connected CB[7] to 5-formylcytosine (5fC) sites on DNA, masking the presence of 5fC. Thus, supramolecular active reactive probes (SARPs) cannot digest them. However, when 5fC was replaced by amantadine in CB[7], it reverted to its original properties, allowing the DNA strand to be digested by SARP. Researchers have also combined photostimulation with DNA delivery. Du et al. modified DNA-loaded polyethylenimine (PEI) by attaching it to CB[7], and CB[7] was also attached to a metal sphere capable of responding to light [21].

The photothermal capability of the metal sphere upon light exposure makes it easier to penetrate the cell and release its DNA, increasing the DNA transfection rate. Furthermore, targeted delivery of gene delivery vectors has been shown to be possible through modification of CB[n]—a process similar to the targeted drug delivery process described above. Many existing gene delivery methods have drawbacks. Polyethylenimine (PEI) is recognized as a vector with good gene delivery efficiency, but it was shown to possess a certain degree of cytotoxicity [21]. Additionally, since polycationic gene vectors are generally prone to hemolysis, the hemolysis of the PEI/CB[7] vector was also examined. Thus, the intervention of CB[7] can help reduce the side effects of PEI, and this does not affect the efficiency of gene delivery (Figure 9). Therefore, CB[n] have potential significance for improving gene transfection efficiency and enabling targeted gene therapy, representing a novel method in this regard.

2.6 Toxicity of CB[n] *in vitro*

Any potential biological or medical application depends on the cytotoxicity and biocompatibility of the substances used. A comprehensive assessment of selective toxicity is necessary to analyze its hazard as a pharmaceutical. One property of pharmaceutical substances is hematotoxicity – a type of selective damage to the function of blood cells or their cellular components. The most significant functions of blood cells are oxygen transport, hemostasis, and providing immunity. Hemolysis of erythrocytes occurs due to their dysfunction, cell death through direct damage to the cell's cytoplasmic membrane, or induction of cellular apoptosis as a manifestation of hematotoxicity.

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Apoptosis is the process of programmed cell death. Nanoparticles can exert toxic effects on cells. Nanoparticles are capable of inducing apoptosis through various mechanisms, such as activation of mitochondrial pathways, enhanced expression of caspases-3, -8, and -9, as well as other signaling pathways. CB[7] can induce apoptosis in human keratinocyte HaCaT cells and the HepG2 cell line via activation of the caspase pathway [56,57]. The number of apoptotic cells depends on the concentration of CB[7]. A high concentration of CB[7] causes apoptosis in more than 50% of cells. It can be noted that CB[*n*] can increase the level of early apoptosis associated with the appearance of phosphatidylserine on the cell surface. Even in this case, the integrity of the cell membrane was not compromised [58].

Very good biocompatibility of CB[5] and CB[6] was demonstrated. The viability of HaCaT keratinocytes after incubation with these compounds did not change even at concentrations up to 15 mg/mL for CB[6]. Furthermore, no hemolytic effect on erythrocytes was observed after incubation with CB[6], unlike the structural analogues cyclodextrins.

However, CB[7] exhibited significant cytotoxic effects on HaCaT keratinocytes and caused apoptotic cell death. In accordance with these findings, CB[5] and CB[6] may be useful in the biomedical field as drug delivery systems due to their good biocompatibility combined with excellent host-guest complexation properties [57,59].

Hemolysis with albumin is associated not so much with the toxic effect of the CB[*n*] themselves, but with the mediated interaction of CB[*n*] with components of the medium. CB[7] is capable of forming complexes with amino acids, peptides, and proteins. For example, CB[7] and CB[8] can bind to albumin. CB[7] at the used concentration interacts with amino acid residues of albumin and possibly enhances its delivery to erythrocytes, which may cause cell death. Possibly, the enhancement of hemolysis in the presence of CB[7] requires further study. Since the effect was not detected in serum containing equivalent concentrations of albumin, it is possible that a similar result will be observed only in experimental *in vitro* studies. It should be noted that at concentrations from 2 mM to 0.01 mM, CB[*n*] appears to be safe for these cells at concentrations up to 0.3 mM. Concentrations of CB[*n*] above 0.3 mM may not be required for drug delivery systems. The absence of such an effect for CB[6] may be due to the fact that CB[6] (unlike other CB[*n*]) has a small cavity and different chemical and biological properties for effective binding with biologically active molecules. Thus, CB[n] do not directly damage cells but may affect components of the cellular microenvironment [56,58].

CB[*n*] have great potential for future biomedical applications due to their specific complex-forming properties. This requires excellent biocompatibility, which is insufficiently studied. To date, little is known about the cytotoxic properties of CB[*n*], particularly on human keratinocytes.

Another study describes the cytotoxicity of CB[*n*] for the HaCaT cell line. The viability and proliferation of HaCaT keratinocytes incubated for 24 h with increasing concentrations of CB[5], CB[6], and CB[7] were determined by cellular ATP content. A dose-dependent decrease in cell viability was observed. The cytotoxicity of CB[n] appears to be structure-dependent. CB[6] had little effect on cell viability even at concentrations up to 30 mg/mL (IC_{50} not determined). Incubation with CB[7] led to a cytotoxic effect on HaCaT keratinocytes after 24 h of incubation at concentrations of 3.75 mg/mL with a calculated IC_{50} value of 3.5 mg/mL. The following order of cell compatibility was observed: CB[6] > CB[5] > CB[7] [22,41,42]. Nevertheless, it can be assumed that the biological property of CB[*n*] can be altered through complexation with other molecules.

Thus, comprehensive research on the specific application of complex agents based on CB[n] is of great importance [63–65].

Macrocyclic compounds are often preferred over alternative drug delivery systems such as dendrimers, liposomes, micelles, carbon nanotubes, hydrogels, and polymers [66]. This preference is due to a number of advantages they possess. Macrocyclic compounds generally exhibit increased stability and allow for control over drug release rates [67,68].

According to the literature, all CB[*n*] exhibits low toxicity towards various cell types [65]. Studies have shown that CB[*n*] does not cause hemolysis even at high concentrations (1 mM) under phosphate-buffered saline (PBS) conditions.

Nevertheless, it was demonstrated that CB[*n*] has the ability to increase the level of early apoptosis, which is associated with the presence of phosphatidylserine on the cell surface. Notably, even under these conditions, the integrity of the cell membrane was not compromised. Furthermore, CB[7] caused hemolysis in the presence of albumin, although not under PBS conditions, except for CB[7] at a concentration of 2 mM. It is worth noting that CB[7], along with other homologues (except CB[5] and CB[6]), possesses the ability to bind cholesterol molecules. Thus, the hemolytic impact induced by CB[*n*] may be a mirror reflection of the effects observed with cyclodextrins [66].

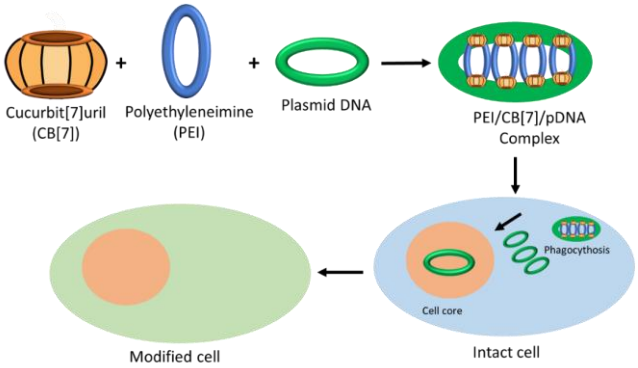

Figure 9 Use of CB[7] for gene delivery [21].

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Hemolysis in the presence of an albumin medium is primarily explained not by the direct toxic action of the CB[*n*] themselves, but by the indirect interaction of CB[*n*] with components of the medium. It has been established that CB[7] has the ability to form complexes with amino acids, peptides, and proteins [67]. However, due to its lower solubility, CB[8] was used at lower concentrations compared to CB[7]. Consequently, CB[7] at this concentration may interact with amino acid residues in albumin. Presumably, this interaction facilitates the delivery of CB[7] to erythrocytes, leading to cell death. The increased hemolytic activity observed in the presence of CB[7] requires further study. Notably, this effect was not manifested in serum with an equivalent albumin concentration, suggesting that a similar result may only appear under experimental *in vitro* conditions.

It should be noted that in previous studies [56], concentrations of CB[*n*] in the range from 2 to 0.01 mM were used, with CB[*n*] being well tolerated by cells at concentrations up to 0.3 mM. Concentrations exceeding 0.3 mM may not be required for drug delivery systems. The absence of such an effect for CB[6] is likely due to its smaller cavity size and distinctive chemical and biological properties, allowing for effective binding with biologically active molecules in the experimental medium [56].

Thus, the use of nitrogen-containing macrocyclic compounds for the surface modification of porous materials pursues two main goals: enhancing biocompatibility and creating medical biomaterials with the capability for controlled drug release. Therefore, CB[*n*] become unique and intriguing molecules for creating biocompatible materials that find multifaceted applications in various fields. Continued research in this area may lead to a further expansion of their horizons of use and the discovery of new breakthroughs.

2.7 Toxicity of CB[*n*] *in vivo*

The safe dose of CB[*n*] for animals depends on the size and type of CB[*n*] and the method of its administration. The lowest maximum tolerated dose of CB[7] is 250 mg/kg when administered by injection. Assuming a human weight of 75 kg, this corresponds to a concentration of approximately 3.2 mM. To put the CB[*n*] toxicity results into perspective, they are compared with the known safe macrocycle, β-cyclodextrin, which has no neuro-, myo-, or cardiotoxic side effects [66]. For example (see Figure 10), CB[7] is administered orally after ingestion of PQ. In the stomach or intestines, PQ retains CB[7], preventing further intestinal damage and reducing PQ absorption and distribution in tissues. Most of the pesticide is excreted from the body as PQ-CB[7] complexes [21].

Regarding compounds and nanomaterials based on CB[*n*], recent demonstrations have shown the non-toxicity of CB[*n*] molecules with median effective dose (ED_{50}) levels greater than 100 μM against human lung and ovarian cancer cells [3].

Viability testing of CB[7] *in vitro* using the MTT assay on CHO-K1 cells showed no significant cytotoxicity up to 1 mM at an incubation time of 3 hours; after 48 h of incubation, an IC_{50} value of 0.53 mM was determined [68].

Due to the low solubility of CB[8], precise determination of its toxicity level is difficult. However, 20 μM CB[8] caused a slight decrease in cell viability (86%) after 48 hours of incubation. A single oral dose of CB[7] and CB[8] as a mixture in equal proportions showed no toxicity up to 600 mg/kg [68]. CB[5] and CB[7] were also tested for toxicity and biological activity. They were found to be non-toxic within the desired concentration range [59].

As a model for studying the neurotoxicity of macrocycles, the unmyelinated mouse sciatic nerve preparation was chosen. The process of removing the sheath involved careful removal of the outer epineurial tissue surrounding the nerve, allowing the macrocycles direct access to the nerve cells [42,69].

Neurotoxicity is one of the major dose-limiting side effects of cisplatin. Studies have shown that cisplatin mainly accumulates in the dorsal root ganglion (DRG) neurons and predominantly causes sensory neuropathy, characterized by severe loss of proprioception (body movement), tingling sensation, and numbness of the feet [18], Figure 11.

The effect of CB[7] on the neurotoxicity of cisplatin was investigated by analyzing and comparing the effect of 1 mM cisplatin and cisplatin-CB[7] on the nCAP amplitude. The results show that there is no statistical difference in the neurotoxicity of cisplatin compared to cisplatin-CB[7].

After 80 min, cisplatin reduced the *n*CAP amplitude by 13 ± 4.7%, indicating some neurotoxicity, but not at the level typically observed clinically.

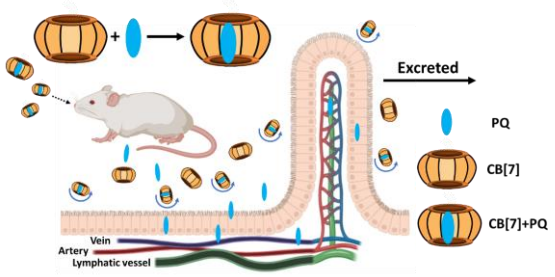

Figure 10 Proposed function of CB[7] as a treatment for paraquat (PQ) pesticide poisoning.

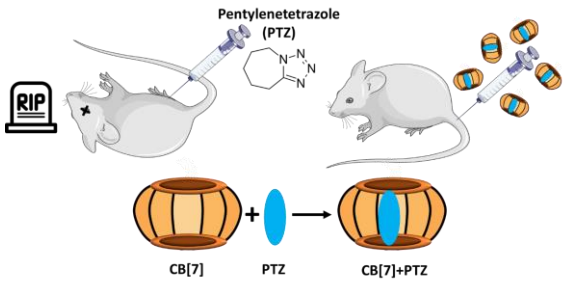

Figure 11 Schematic showing the reduction in neurotoxicity of pentylenetetrazole (PTZ) by CB[7] administration to mice [21].

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The reason for the lower toxicity is most likely that the sciatic nerve consists mainly of neuronal axons with fewer DRGs, which are predominantly located in the spinal cord. Therefore, the slight neurotoxicity caused by cisplatin in this experiment is due to small amounts of DRG. The results for cisplatin-CB[7] are similar to those for free cisplatin, with a reduction in nCAP amplitude of 22.7 ± 6.6%. Thus, the results show that CB[7] does not have a neuroprotective effect on cisplatin [18].

The myotoxic activity of macrocycles was investigated on the isolated chick biventer cervicis neuromuscular tissue preparation. Under ex vivo conditions, the muscle can be made to contract through chemical or electrical stimulation. For chemical stimulation, the addition of exogenous acetylcholine (ACh) or KCl leads to muscle contraction. ACh acts by binding to nicotinic receptors located on the muscle membrane, causing depolarization followed by contraction (postsynaptic effect). Potassium chloride causes depolarization of the muscle membrane, leading to the release of calcium into the synaptic cleft (the area between the nerve and muscle). Calcium then binds to neuronal receptors, leading to the release of ACh from the neuron, ultimately causing muscle contraction (presynaptic effect) [18].

Baseline results for muscle contraction strength were determined using both electrical and chemical stimulation. The neuromuscular tissue was then exposed to macrocycles, and after two hours the muscle contraction strength was determined again. Macrocycles are myotoxic if they demonstrate a statistically significant increase or decrease in muscle contraction strength compared to baseline results. An increase in contraction strength due to exogenous ACh indicates that the test compound may have an anticholinesterase effect; cholinesterase is an enzyme located in the synaptic cleft that terminates signal transmission by breaking down acetylcholine activity, thereby prolonging the enhancing effect of ACh. Increasing the lifetime of ACh would synergistically enhance and prolong the response to KCl [18,21,59].

Overall, the greatest myotoxic effect was observed upon exposure of the neuromuscular system to CB[7]. A significant reduction in activity is evident for KCl (p = 0.05) and electrical stimulation (p = 0.01); this indicates that CB[7] may bind to and block the nicotinic receptors of the postsynaptic muscle, thereby impeding the depolarization capacity of the membrane. A slight reduction in twitches upon electrical stimulation is also observed after exposure to CB[6] (p = 0.3), although this reduction is significantly smaller in magnitude compared to CB[7]. Given that β-cyclodextrin also leads to a reduction in electrically stimulated contraction (p = 0.9) and that it is not a known myotoxic compound, the results may indicate that the electrical stimulation results for CB[6] and CB[7] are statistically reduced. However, this may not lead to toxicity *in vivo* [42,68,69].

The effect of CB[7] on the myotoxicity of cisplatin was investigated by analyzing and comparing the effect of cisplatin and cisplatin-CB[7] on the response of neuromuscular chemical and electrical stimulation.

The results show that cisplatin induces significant myotoxic activity when measured using both chemical and electrical stimulation. Free cisplatin increased the strength of neuromuscular contraction to ACh and KCl by (96 ± 32%, p = 0.005) and (121 ± 3%, p = 0.001) respectively, and reduced the strength of its electrically stimulated contraction by 96 ± 4% (p = 0.001), which is consistent with the peripheral neuropathy known to occur with cisplatin chemotherapy. The results also suggest that cisplatin may possess anticholinesterase activity, as shown in previous studies, and that this may also partially contribute to the neurotoxic side effects of cisplatin. CB[7] exhibited a myoprotective effect, as cisplatin-CB[7] reduced electrically stimulated muscle contraction by only 36 ± 12% (p = 0.04); this is a 60% reduction in the myotoxic activity of free cisplatin [18].

As a model for studying the cardiotoxic activity of macrocycles, rat atria were used. In untreated atria (control), the results show that by the end of the experiment the left atrium was more stable than the right, as no changes were observed in either its rate or strength of contraction. In contrast, after two hours, the rate and strength of contraction of the right atrium increased by 8 ± 1% and 16 ± 1.9%, respectively.

All macrocycles caused more significant changes in contraction strength compared to contraction rate. Changes in contraction strength are observed within the first 15-30 min after macrocycle exposure, whereas a longer period of time (30–45 min) is required for a change in contraction rate.

A few clinical studies have reported cardiotoxic effects of cisplatin. Toxicity manifests as atrial or ventricular arrhythmias, tachycardia, bradycardia, and conduction disorders. Most of these heart problems are reported to be clinically asymptomatic and occur within hours of drug infusion [18].

The protective effect of CB[7] on the cardiotoxic activity of cisplatin was investigated using free cisplatin and cisplatin-CB[7] in the unstimulated right atrium. The right atrium was chosen for this study because cisplatin did not exhibit cardiotoxic activity in the left atrium.

The results show that cisplatin induced cardiotoxic activity, reducing both the rate and strength of contraction by 68.8 ± 8.4% (p = 0.07) and 53.7 ± 17.0% (p = 0.2) respectively. Furthermore, cisplatin caused a gradual and sharp increase in atrial contraction strength by 250% within the first 60 min, after which it decreased by 54% by the end of the experiment. Upon encapsulation by CB[7], the cardiotoxic activity of cisplatin was significantly reduced; cisplatin-CB[7] caused only an 11 ± 5.6% (p = 0.2) reduction in atrial contraction rate and increased contraction strength by only 27 % ± 18.0 (p = 0.2). In addition to reducing the magnitude of the change in contraction rate, CB[7] also stabilized the contraction strength compared to free cisplatin. After 60 min, contraction strength increased by only

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27 ± 18.0% and remained at this level throughout the experiment [66].

All CB[*n*] did not exhibit neurotoxic activity during the test period, as there was no statistically measurable difference between the nCAP changes caused by CB[*n*] compared to changes caused by β-cyclodextrin or control. In the myotoxicity study, CB[7] demonstrated the greatest myotoxic activity, causing the greatest reduction in electrically stimulated neuromuscular contractions, while Motor2 and β-cyclodextrin exhibited the least myotoxic activity [66]. All the macrocycles exhibited cardiotoxic activity by preferentially reducing atrial contraction strength compared to the change in its contraction rate, with Motor2 being the most cardiotoxic [18,21].

The effect of drug encapsulation by CB[7] on these specific tissue toxicities was tested using the platinum-based anticancer drug cisplatin. While free cisplatin exhibited toxicity towards all three tissue types, upon encapsulation by CB[7], the extent of its myo- and cardiotoxic activity was significantly reduced, while no statistical changes in toxicity were observed in neurotoxicity studies [18,70,71]. Overall, although some toxicity of macrocycles towards myo- and cardiomyocytes was observed, these experiments represent very high doses with direct access to tissues. In clinical settings for humans, the dose used would be 2–10 times lower. For example, when used as a protective agent for cisplatin, the actual dose of CB[7] would be from 80 to 160 μM (assuming a cisplatin dose of 60 to 120 mg/m^2). is excreted through the kidneys, and the dose to which nerves, muscles, and the heart are exposed would be significantly lower still. However, the results require further study of CB[*n*] toxicity to establish their safety [18].

As follows from the data indicated in [50], the effect of intravenous administration was investigated only for CB[7] due to the limited solubility and lack of a noticeable cytotoxic effect of the larger homologue CB[8]. In particular, the effect of a single intravenous dose of CB[7] on mouse body weight at doses up to 300 mg kg^{-1} was investigated. No toxicity (assessed and defined here as 10 percent weight loss) was observed up to the level of 200 mg kg^{-1}. However, at higher doses and rapid administration, mice went into a shock-like state immediately after administration. Thus, the maximum tolerated dose of CB[7] was set at 250 mg kg^{-1} with slow intravenous administration of the compound. The influence of CB[7] on mouse body weight over several days at the minimum toxic dose of 250 mg kg^{-1}, administered by slow intravenous route. The effects observed upon intravenous single administration *in vivo* suggest that CB[7] possesses very low acute toxicity at a dose of 250 mg kg^{-1}. Importantly, all mice that received slow intravenous infusion began to recover after 5–8 days. The recovery of the mice may indicate the excretion or metabolism of CB[7] from the body. As a drug delivery vehicle or biological tool, this is certainly a desirable characteristic. Potential pharmaceutical applications of CB[7] (as well as CB[8]) would have to compete with those already established with β-cyclodextrin (β-CD), which has an equally large cavity and can include similarly large drugs. Intuitively, one would expect higher toxicity from the former synthetic carrier compared to the latter, which is of natural origin and based on α-D-glucose. Indeed, the synthetic macrocycle CB[7] exhibits somewhat lower intravenous tolerability than β-CD, for which an LD_{50} value of about 790 mg kg^{-1} was recorded. However, the difference is surprisingly small, especially considering that the maximum tolerated dose (MTD) of CB[7] (250 mg kg^{-1}) is not equivalent to the LD_{50} value, but corresponds to a sublethal effect on long-term growth and recovery, which typically lies much lower than the LD_{50}. Moreover, any applications based on supramolecular drug encapsulation will depend on the host association constants, which are typically much higher for CB[7] (10^4–10^7 M^{-1}) than for β-CD (10^1-10^4 M^{-1}). Consequently, a smaller amount of CB[7] would be required to effectively bind a given amount of drug [59]. For example, to achieve the same solubility of the drug albendazole, a 6-times greater concentration of β-CD is required than that of CB[7]. Furthermore, these two macrocycles differ substantially in selectivity and binding preferences. Accordingly, they could be used complementarily for binding, for example, cationic (for CB[7]) and hydrophobic residues (for β-CD) in peptide drugs [72].

2.8 Immunotoxicity of CB[*n*]

The bioavailability of many drugs is low due to poor solubility. CB[7] has good solubility in aqueous environments, while CB[6] and CB[8] are poorly soluble. However, in the presence of a number of ions that are abundant in biological media, the solubility of CB[6] increases significantly [10].

It is currently known that CB[*n*] demonstrate low toxicity in numerous *in vitro* and *in vivo* studies. The application of CB[n] at very high doses may cause myotoxicity and neurotoxicity, but at standard concentrations used in complexes with pharmaceuticals the signs of toxicity are absent [73].

However, in addition to immunotoxicity, the possible immunomodulatory properties of delivery systems must also be studied, as immunostimulatory or immunosuppressive effects of the system could either enhance or weaken the effect of the delivered drug. Studying the possible immunomodulatory effect of nanoscale cavitands is important for the development of new delivery systems and should be considered when assessing indications and contraindications for the treatment of various diseases [74].

It has been shown that under *in vitro* conditions CB[*n*] have almost no immunosuppressive effect, with the exception of a slight reduction in MHC class II cell surface receptor (HLA-DR) expression and production of reactive oxygen species by T-helpers in stimulated cultures. However, for macrocyclic cavitands and for CB[*n*], it has been shown that some properties manifest only under *in vivo* conditions [75].

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When assessing the effect of cucurbit[7]uril on blood parameters after three intraperitoneal injections over one week in laboratory animals, a decrease in the number of leukocytes in the blood of mice after CB[6] injections from 7013 ± 1437 to 3400 ± 461 cells/μL was revealed. The normal number of leukocytes in mice ranges from 2000 to 10000 per μL. Thus, the observed decrease in leukocyte count is within the normal range. CB[6] did not cause a statistically significant decrease in the relative numbers of leukocyte subpopulations, such as lymphocytes, monocytes, and neutrophils. However, there was a trend towards a decrease in neutrophil count after the third injection in animals administered CB[6]. CB[7] and CB[8] did not affect the leukocyte count in mice after three injections. Changes in platelet, erythrocyte, and monocyte counts, as well as in a number of other parameters such as mean corpuscular hemoglobin, were not detected [66, 69].

Assessment of the immunotoxic action of CB[*n*] on central and peripheral organs of the immune system was carried out using the method of determining lactate dehydrogenase (LDH) activity. It was found that triple intraperitoneal administration of CB[*n*] at high concentrations does not have a toxic effect on mouse bone marrow cells. However, it was shown that administration of CB[7] can lead to toxic damage to spleen cells, as a weak toxic effect on splenocytes was detected. It is known that CB[*n*] possess myotoxicity and neurotoxicity, so the increase in serum LDH level is most likely associated with toxic action on muscles and the nervous system, rather than on the immune system [70,71].

After CB[6] injections, a decrease in CD4+ T-helpers and a simultaneous increase in the proportion of CD19+ B-lymphocytes were observed in the peripheral blood compared to the control. CB[7] and CB[8] did not affect the subpopulation composition of peripheral blood lymphocytes.

Thus, it was established that CB[6] and CB[7] can increase the percentage of B-cells in immune system organs, both in the spleen and in the bone marrow. Treatment with CB[8] leads to an increase in the relative number of B-cells and cytotoxic T-lymphocytes in the spleen of mice. Administration of CB[6] reduced the level of polyphosphoric acid (PPA) in the spleen of laboratory animals compared to the control [70].

Intraperitoneal administration of CB[*n*] to mice did not lead to changes in blood parameters and did not cause death of immune cells, with the exception of spleen cells upon treatment with CB[7]. It should be noted that high concentrations of CB[*n*] were used in this study; when used for drug delivery, such high concentrations are unlikely to be required.

Thus, CB[*n*] possess almost no immunotoxicity, with the exception of a cytotoxic effect on spleen cells *in vivo* upon administration of CB[7] at a high dose, as well as a trend towards an increase in the number of apoptotic cells in cultures grown in the presence of CB[8]. The obtained results may indicate the immunological safety of using CB[*n*] for drug delivery. It was also shown that different homologues affect the immune system differently. Based on the obtained *in vitro* data, CB[8] causes a decrease in IFN-γ level [42], indicating its possible immunosuppressive effect. Since CB[8] is capable of suppressing cytokine production, the possible immunosuppressive effect requires further study. If immunosuppressive activity is confirmed, CB[8] could be used as a basis for delivery systems of various immunosuppressive drugs. However, when assessing cellular and humoral immune responses in animals after intragastric administration of CB[8], no immunosuppression was observed.

In the case of CB[6], its stimulating effect on B-cells and the humoral immune response was demonstrated, as it increased the expression of HLA-DR molecules on B-lymphocytes [21], the level of spontaneous interleukin IL-4 production (by 1.5 times), and also the *in vivo* relative number of B-cells in immune system organs. At the same time, the intensity of the humoral immune response to a specific antigen after CB[6] administration decreased, which requires investigating the mechanisms of this process. Possibly, the strong non-specific stimulation of B-lymphocytes did not allow the cells to respond to antigen stimulation.

CB[7] exhibited mild immunostimulatory properties, increasing the relative number of B-cells in mice and suppressing the production of interleukin IL-10 in *in vitro* culture. These properties make CB[7] an excellent basis for drug delivery systems for the treatment of infectious and tumor diseases. Complexation of drugs with CB[7] enhances their antitumor activity. Extensive data suggest that the clinical success of chemotherapy is due not only to toxicity to tumor cells but also to the restoration of immune surveillance, which has received extremely little attention in previous preclinical and clinical studies. Immune cells are an important component of the tumor microenvironment, contributing to immunosuppression towards tumor structures, as well as influencing therapy efficacy. Delivery systems and their components can reduce the number of immune suppressor cells (Tregs, myeloid suppressors, etc.) in the tumor microenvironment, affect the expression of checkpoint inhibitors in tumor tissue, enhance antigen uptake and maturation of dendritic cells, and stimulate immune cells involved in the antitumor immune response. Furthermore, the supramolecular delivery system can enhance the antitumor effect of the drug through the immune system by inducing immunogenic tumor cell death. Impact on the tumor microenvironment and induction of immunogenic cell death have been shown for antitumor drug delivery systems based on macrocyclic components, namely cyclodextrin and pillararene [73, 74].

In summary, the biomedical application of cucurbiturils holds significant promise for the future. However, despite impressive progress in this area, many applications are currently in the preclinical stage of research. Further toxicokinetic studies and scaling up of synthetic methods for functionalized derivatives are necessary for clinical translation.

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It should be noted that the field of biomedical application of CB[*n*] is currently actively developing, and new applications are constantly emerging. Table 2 illustrates the current areas of biomedical application of CB[*n*] for 2023–2025.

3. Limitations

It should be noted that despite the outstanding potential of CB[*n*] as a platform for the creation of drug delivery systems, diagnostics and theranostic agents, their translation into clinical practice currently faces a number of fundamental and applied challenges. The most significant barrier is the current insufficient study of the *in vivo* toxicological profile of various CB[*n*] homologues. Studies demonstrate a pronounced dependence of cytotoxicity on the macrocycle size; for example, CB[5] and CB[6] exhibit relatively low cytotoxicity *in vitro* over a wide range of concentrations. CB[7] is often considered to be the optimal balance between cavity size and biocompatibility; however, the data vary depending on the type of cell lines. Larger homologues, such as CB[8], exhibit increased toxicity, which is associated with their stronger interaction with cellular membranes and the potential ability to disrupt their integrity. It should also be noted that the practical application of CB[*n*] is limited by their suboptimal pharmacokinetic characteristics. Low-molecular-weight homologues (CB[5]-CB[7]) are rapidly cleared from the systemic circulation via the kidneys, limiting their circulation time and therapeutic window. The lack of selective accumulation in target tissues (e.g., tumors) necessitates high doses, increasing the risk of systemic side effects. This problem is compounded by CB[*n*]'s high ability to form complexes with endogenous molecules (e.g., choline, spermidine), which could theoretically alter their distribution and reduce efficacy. CB[*n*]'s high affinity for protonated amines and quaternary ammonium compounds, a key advantage, becomes a disadvantage *in vivo*. Blood plasma and the intracellular environment contain a vast number of potential guest molecules (amino acids, polyamines, neurotransmitters), which can compete with the target drug for binding to the CB[*n*] cavity. This could presumably lead to instability of host-guest complexes and premature release of the active substance.

Another potential obstacle is the potential immunogenicity of CB[*n*]. Currently, the potential immunogenicity of CB[*n*] has been poorly studied. Being synthetic macrocycles, they can be recognized by the immune system as foreign agents, initiating humoral or cellular immune responses. This can lead to rapid clearance, antibody formation, and allergic reactions upon repeated administration. Despite impressive advances in fundamental research on CB[*n*] complexes with biologically active molecules, their path to clinical practice remains challenging. Overcoming these limitations requires consolidated efforts in the fields of supramolecular chemistry, toxicology, pharmacology, and materials science. Key areas of future research should include the targeted design of CB[*n*] with reduced toxicity and improved pharmacokinetic profile, the development of targeting strategies, and a comprehensive study of their long-term effects on the body *in vivo*.

4. Conclusions

Although the original CB[*n*] molecule is a century old, the chemistry of the CB[*n*] family is experiencing rapid growth. Recent developments, including the synthesis of homologues and derivatives, have injected dynamism into CB[*n*] chemistry, as evidenced by the increased interest in this field in recent years. In terms of practical applications, the field of functional materials and devices based on supramolecules is still in its infancy, and its prospects are enormous. With such promise, the future of CB[*n*] chemistry appears brighter than ever. The CB[*n*] family represents a unique set of macrocyclic hosts, where each homologue (from CB[5] to CB[10]) has its own characteristic size and properties. This "molecular lineup" allows researchers to tailor the ideal receptor to a specific guest molecule, making them indispensable tools in supramolecular chemistry, nanotechnology, biology, and medicine.

Table 2 Current biomedical applications of CB[*n*].

Homologous	Biomedical application	Potential mechanism	References
CB[6]	There are currently no specific biomedical applications.	Strong binding of diamines and cations.	[2-5, 8, 10, 13, 16, 19, 22, 23, 29]
CB[7]	1. Increasing drug bioavailability. 2. Reducing side effects 3. Antidote therapy	1. Formation of inclusion complexes with hydrophobic drugs, dramatically increasing their solubility and stability. 2. Binding and neutralization of cytotoxic drugs (cisplatin) for kidney protection. 3. Potential decontamination agent for chemical weapons.	[2-5, 8, 10, 14-23, 29-43, 47, 49, 51, 59, 70-75]
CB[8]	1. Theranostic platforms 2. Creation of "smart" hydrogels 3. Control over protein interactions 4. Genes delivery	1. Simultaneous incorporation of a therapeutic agent (drug, siRNA) and a diagnostic label (fluorophore) into a single cavity. 2. Formation of cross-links in polymer networks through ternary host-guest-guest complexes, creation of injectable self-healing hydrogels. 3. Reversible protein dimerization using modified guests to control cellular signaling pathways.	[2-5, 8, 10, 16, 20, 24, 26, 29-39, 42, 47, 49, 59, 70-75]

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From stabilizing unstable compounds to creating smart materials and drug delivery systems, the potential applications of each homologue continue to be actively explored.

This review clearly demonstrates that the CB[*n*] (CB[*n*]) family represents a unique class of macrocyclic hosts with outstanding potential for biomedical applications. While their biocompatibility is not absolute, it is predictable and controllable, opening the way for the targeted design of safe and effective therapeutic and diagnostic platforms.

The biocompatibility of CB[*n*]s directly correlates with their size and charge. CB[7] exhibits an optimal balance: it is sufficient to incorporate a wide range of bioactive molecules, yet it does not induce significant hemolytic activity and exhibits low acute toxicity *in vivo* due to its rapid elimination via the kidneys without accumulation in major organs. The potential cytotoxicity of CB[*n*], primarily for CB[6] and above, is related to their ability to disrupt the integrity of cell membranes (especially red blood cells) through strong interactions with phospholipids and cholesterol, as well as to induce oxidative stress. However, these effects are dose-dependent.

Strategies for functionalizing the portals and outer surface of CB[*n*] with hydrophilic groups (e.g., hydroxyl groups) dramatically increase their solubility in physiological environments and reduce the negative impact on membranes, while simultaneously providing a targeting tool.

Based on established principles of biocompatibility, several of the most realistic and promising avenues for further research can be identified: targeted delivery and controlled release of drugs, theranostics, detoxification, and protection from adverse drug effects.

However, despite optimistic forecasts, the path of this class of substances to clinical practice requires addressing a number of challenges. A complete understanding of the long-term distribution, metabolism, and elimination pathways of both CB[*n*] and their complexes in vivo is essential. Cost-effective and environmentally friendly methods for synthesizing CB[*n*] and their derivatives in quantities required for preclinical and clinical trials are needed, as is a thorough assessment of the potential immune response to CB[*n*] during chronic administration.

CB[*n*] has moved from the category of laboratory innovations to the category of promising tools for nanomedicine. A thorough understanding of the structure-biocompatibility relationship allows for the active design of safe and functional systems rather than passive observation of their properties. The next decade will likely witness the first clinical trials based on CB[*n*], opening a new chapter in supramolecular therapeutics, where control over molecular interactions will be key to the development of future drugs.

List of designations and abbreviations

CB[6], CB[7], CB[8], CB[10] – cucurbit[*n*]urils (*n* = 6, 7, 8, 10);
AgNPs – silver nanoparticles;
NMR – nuclear magnetic resonance;
MTs – water-soluble molecular tweezers;
NR – Nile Red dye as a model hydrophobic drug;
HeLa – immortalized cervical cancer cell line;
ASGPR – asialoglycoprotein receptor;
HepG2 – hepatocellular carcinoma cell line;
CB[7]-PEG – copolymer of CB[7] and polyethylene glycol;
HPMC – hydroxypropyl methylcellulose;
NaCMC – sodium carboxymethyl cellulose;
MIC_{50} - minimum inhibitory concentration at which the growth of 50% of the test strain population is suppressed;
5fC – 5-formylcytosine;
SARPs – supramolecular active reactive probes;
PEI – polyethylenimine;
HaCaT – immortalized human epidermal keratinocyte line;
CyDs – cyclodextrin;
PQ – pesticide "Paraquat";
CHO-K1 – immortalized Chinese hamster ovary epithelial cell line;
nCAP – nerve junction action potential;
PTZ – pentylenetetrazole;
DRG – dorsal root ganglia of neurons;
ACh – exogenous acetylcholine;
LDH – lactate dehydrogenase;
PPA – polyphosphoric acid;
HLA-DR – major histocompatibility complex class II protein;
PBS – phosphate-buffered saline.

Supplementary materials

This manuscript contains supplementary materials, which are available on the corresponding online page.

Data availability statement

Data sharing is not applicable to this article, as no new data were generated.

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Conflict of interest

The authors declare no conflict of interest.

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