DOI: 10.1070/RCR4595

Macrocyclic cavitands cucurbit[*n*]urils: prospects for application in biochemistry, medicine and nanotechnology

Olga A. Gerasko,[a] Ekaterina A. Kovalenko,[a] Vladimir P. Fedin [a, b]

[a] *Nikolaev Institute of Inorganic Chemistry, Siberian Branch of the Russian Academy of Sciences prosp. Akademika Lavrentieva 3, 630090 Novosibirsk, Russian Federation*
[b] *Novosibirsk State University ul. Pirogova 2, 630090 Novosibirsk, Russian Federation*

The prospects of using the organic macrocyclic cavitands cucurbit[*n*]urils (CB[*n*]) and their derivatives in biochemistry, medicine and nanotechnology are considered. A combination of CB[*n*] characteristics, such as a rigid highly symmetrical structure, polarized hydrophilic portals, a rather large intramolecular hydrophobic cavity, as well as high resistance to thermolysis and corrosive media and low toxicity, account for a wide range of unique opportunities for the deliberate design of new functional materials, which may find application in various areas of modern chemistry and new technologies. Inclusion compounds of CB[*n*] with biologically active molecules demonstrate a high potential for the design of a new generation of prolonged action pharmaceuticals. The review presents the prospects for the application of CB[*n*] to manufacture unique materials, such as CB[*n*]-containing vesicles, films and surfaces, suitable for immobilization of various molecules and nanoparticles on their surface and for the separation of complex mixtures. Potential applications of CB[*n*]-modified electrodes and hydrogels are analyzed, and the use of CB[*n*] in proton-conducting materials and materials for the gas sorption and separation are discussed.
The bibliography includes 164 references.

Contents

I.	Introduction	796
II.	Cucurbit[*n*]urils as nanocontainers for biologically active agents	797
III.	Functionalization of cucurbit[*n*]urils	802
IV.	Functionalized cucurbit[*n*]uril-based polymer surfaces	803
V.	Methods of attaching cucurbit[*n*]urils to the surface	804
VI.	Immobilization on cucurbit[*n*]uril-containing surfaces	805
VII.	Cucurbit[*n*]uril-containing surfaces for separation of mixtures	806
VIII.	Cucurbit[*n*]uril-modified electrodes for analytical applications	807
IX.	Cucurbit[*n*]urils and metal nanoparticles	807
X.	Cucurbit[*n*]uril-based hydrogels	810
XI.	Cucurbit[*n*]urils for gas sorption and separation	812
XII.	Proton conductivity of cucurbit[*n*]urils	813

O.A.Gerasko. Doctor of Chemical Sciences, Leading Researcher and Scientific Secretary of the NIIC SB RAS.
Telephone: +7(383)330–9486, e-mail: olager@niic.nsc.ru
Current research interests: supramolecular and coordination chemistry, macrocyclic cavitands, inclusion compounds.
E.A.Kovalenko. Candidate of Chemical Sciences, Senior Researcher at the same Institute.
Telephone: +7(383)316–5845, e-mail: e.a.kovalenko@niic.nsc.ru
Current research interests: supramolecular chemistry, nanostructures, biomolecules, metal complexes, inclusion compounds, NMR spectroscopy, mass spectrometry, X-ray diffraction.

V.P.Fedin. Corresponding Member of the Russian Academy of Sciences, Doctor of Chemical Sciences, Professor, Director of the same Institute, Head of the Chair of Inorganic Chemistry at the Department of Natural Sciences at NSU.
Telephone: +7(383)330–9490, e-mail: cluster@niic.nsc.ru
Current research interests: inorganic, coordination and supramolecular chemistry, clusters, coordination polymers, macrocyclic cavitands — synthesis, structure and properties.

Received 27 August 2015
Translation: T.N.Safonova

© 2016 Russian Academy of Sciences and Turpion Ltd.

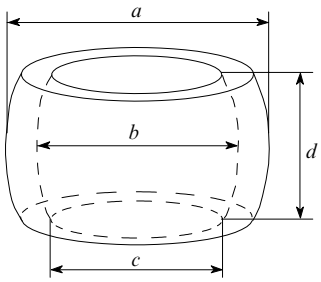
I. Introduction

Cucurbit[*n*]urils ($C_{6n}H_{6n}N_{4n}O_{2n}$, CB[*n*], $n = 5-10$) is a trivial name of organic cavitands comprising *n* glycoluril units linked by $2n$ methylene bridges (Fig. 1).[1–4] These molecules have a rather rigid highly symmetrical macrocyclic structure and an intramolecular hydrophobic cavity accessible through two hydrophilic portals formed by *n* carbonyl groups. The name of cucurbit[*n*]urils is derived from the resemblance of these molecules to the *Cucurbitaceae* family of plants, including pumpkins. The sizes of CB[*n*] molecules are comparable with those of cyclodextrins and calixarenes (Table 1).[5]

A guest species encapsulated within a barrel-shaped cavity of CB[*n*] can be completely immersed inside the cavitand, as opposed to the most of inclusion compounds of calixarenes and cyclodextrins, in which guest molecules are only partially included in the bowl-shaped cavity.[6] This leads to an increase in the stability of adducts with CB[*n*] compared to the above-mentioned cavitands. An important distinguishing feature of CB[*n*] is their structural rigidity. Thus, these compounds virtually retain their shape upon the inclusion of different guests and, consequently, exhibit higher selectivity in the formation of inclusion compounds.

A guest molecule binds to the CB[*n*] molecule mainly through noncovalent interactions with the hydrophobic cavity of the host. Cucurbit[*n*]urils have rather high negative charges on the portal carbonyl oxygen atoms and also some negative charge on the inner surface of the cavity.[7] Therefore, electrostatic ion-dipole interactions between the guest and the host are the driving force for the formation of inclusion compounds, resulting in that CB[*n*] preferably bind to neutral and positively charged guests. In these cases, the formation constants of inclusion compounds with CB[*n*] are $\sim 10^4$ litre mol^{-1} and higher, these values being larger than the corresponding constants for inclusion compounds with cyclodextrins.[5] Of special notice are superstable inclusion compounds with cucurbit[*n*]urils characterized by the formation constant of $10^{12}-10^{13}$ litre mol^{-1}; in the record case, 10^{17} litre mol^{-1} (see Ref. 8). These values are comparable with or even higher than the corresponding constant for the biotin–avidin interaction, which is the strongest noncovalent interaction known in nature. The presence of the intramolecular hydrophobic cavity along with the polarized portal carbonyl groups of cucurbit[*n*]urils are responsible for the highly specific formation of host–guest compounds.

Table 1. Geometric parameters of cucurbit[*n*]uril molecules.[5]



Parameter	CB[5]	CB[6]	CB[7]	CB[8]	CB[10]
Outer diameter (*a*) /Å	13.1	14.4	16.0	17.5	18.7–21.0
Cavity diameter (*b*) /Å	4.4	5.8	7.3	8.8	10.7–12.6
Portal diameter (*c*) /Å	2.4	3.9	5.4	6.9	9.0–11.0
Height (*d*) /Å	9.1	9.1	9.1	9.1	9.1
Cavity volume /Å^3	82	164	279	479	870

Cucurbit[*n*]urils are colourless crystalline compounds, which are insoluble or poorly soluble in water and organic solvents, but are readily soluble in some acids (for example, in HCl, H_2SO_4, CF_3SO_3H, HCO_2H) and aqueous solutions of many metal salts. Cucurbit[*n*]urils are thermally stable and do not decompose upon heating up to 370 °C. These compounds are characterized by high resistance to corrosive media (hot concentrated acids and alkalis). Cucurbit[*n*]urils with $n = 5-8$ are commercially available reagents.

The synthesis, structures and properties of cucurbit[*n*]urils and their inclusion compounds, as well as the thermodynamics and kinetics of formation of host–guest complexes, are summarized in a series of reviews, including our earlier reviews.[5, 7, 9–19]

The present review focuses on the applied aspects of the chemistry of cucurbit[*n*]urils. An analysis is given for the prospects of applying these macrocycles, primarily in medicine for the drug delivery, as well as for the design of unique materials, such as functionalized surfaces for immo-

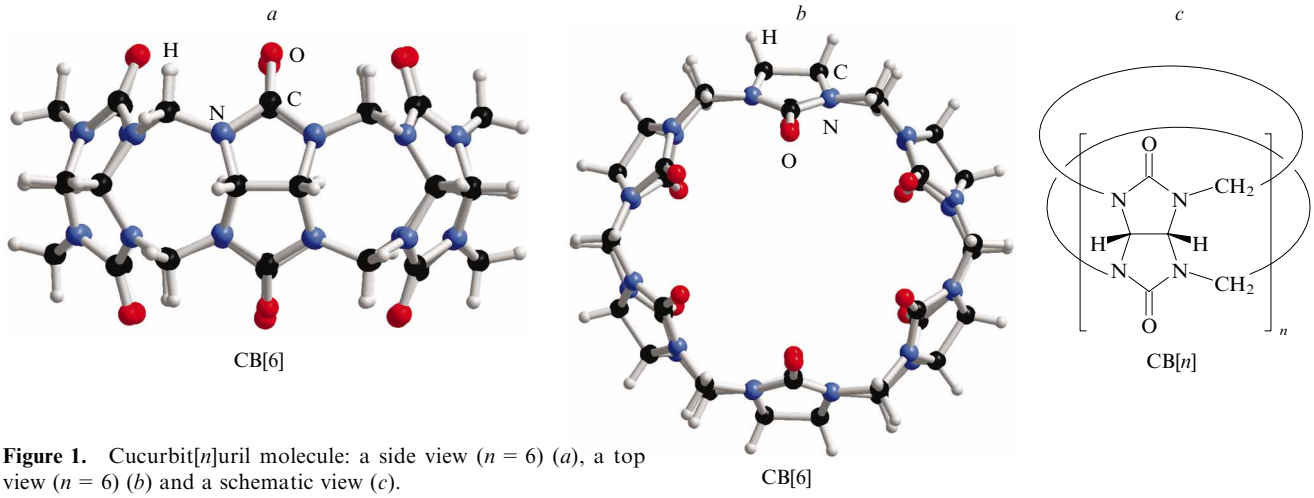


Figure 1. Cucurbit[*n*]uril molecule: a side view ($n = 6$) (*a*), a top view ($n = 6$) (*b*) and a schematic view (*c*).

bilization of molecules and separation of complex mixtures, chemical sensors and biosensors, hydrogels, proton-conducting materials and modified electrodes for analytical applications. The opportunities of using cucurbit[*n*]urils for stabilization of metal nanoparticles and for the gas sorption and separation are discussed.

II. Cucurbit[*n*]urils as nanocontainers for biologically active agents

The use of CB[*n*] as molecular containers for the delivery of biologically active agents is one of the most interesting and important fields of their application. At the present time, supramolecular structures are widely used in pharmacology as transporters of different molecules (peptides, oligonucleotides, amino acids, antigens, antibiotics, cytostatics, genes and so on) for the targeted delivery of an active substance, for an increase in the duration of the therapeutic effect and for the protection of drugs against biodegradation in the bloodstream or the gastrointestinal tract, which can significantly reduce the drug dose. Cyclodextrins, calixarenes and crown ethers are extensively studied as molecular containers. By varying the size and chemical nature of the macrocycle, one can control the degree of host–guest binding and the rate of the drug release. This is an obvious advantage of macrocyclic molecules over micelles and liposomes, which can also include guest molecules.

Since 2004 when it was first reported that CB[*n*] can be used for the drug delivery,[20] the number of publications devoted to investigations of these macrocyclic cavitands in order to apply them in pharmacology has been rapidly growing.[12, 13, 15] The prospects of using CB[*n*] as molecular containers are determined by such key properties of these molecules as the ability (in particular, as inclusion compounds) to penetrate cell membranes, low toxicity and the absence of a substantial effect of CB[*n*] on the activity of the encapsulated drug molecule. Cucurbit[*n*]urils have a substantially lower solubility in water compared to cyclodextrins and calixarenes; however, due to high salt and acid concentrations of such biological media as the blood plasma, saliva, gastric and intestinal juice, the solubility of CB[*n*] reaches the concentrations required for pharmaceutics.[3] Due to stability of CB[*n*] in a wide pH range (1.3–9.0) characteristic of the gastrointestinal tract, these compounds are ideal candidates for oral administration.[21]

II.1. Cucurbit[*n*]urils as nontoxic nanocontainers

In order to be applied in clinical practice as molecular containers for the drug delivery, CB[*n*] should be no less safe than the cavitands that are already in use (for example, cyclodextrins and calixarenes) and should ideally reduce the severity of side effects of the administered medications. Therefore, the cytotoxicity (the ability to cause abnormal changes in the cells) and toxicity (the type and degree of the severity of side effects) of CB[*n*] have been extensively investigated.

Recent studies both *in vitro* and *in vivo* have demonstrated that CB[*n*] are quite safe. The lack of cytotoxicity of CB[*n*] ($n = 5, 7$) up to millimolar concentrations was shown by the analysis of the growth of normal and cancer cells of animal and human origin: normal cell lines (human embryonic kidney, HEK293; human hepatocytes, HepG2; murine macrophage, RAW264.7) and cancer cell lines (human lung cancer, A549; human ovarian carcinoma, SKOV-3; human melanoma, SKMEL-2; human brain cancer, XF-498; human colon cancer, HCT-15).[21–24] The lack of cucurbit[*n*]uril cytotoxicity does not mean that there is no uptake of these compounds into cells. The fact that CB[*n*] cross the cell membrane was established by monitoring labelled (*i.e.*, with a luminescent guest molecule included in the cavity) cavitands. Thus, CB[6] loaded with a spermine conjugate crosses the liver cancer cell membrane,[25] CB[7] penetrates the murine macrophage cell membrane,[22] and the inclusion complexes of the macrocycles CB[7] and CB[8] with the dyes acridine orange and pyronine, respectively, cross the murine embryonic cell membrane.[23, 26]

Cucurbit[*n*]urils were shown to be relatively nontoxic *in vivo*. The maximum tolerated dose (MTD) of CB[7] after the intravenous injection into mice was 250 mg per kg of body weight; after the oral administration of CB[7] and CB[8], MTD was up to 600 mg kg^{-1} (which was attributed to low adsorption of cucurbit[*n*]urils in the gastrointestinal tract).[21] For comparison, the lethal dose of β-cyclodextrins used in clinical practice for mice and rats is 0.3–18.8 g kg^{-1} depending on the mode of administration; thiacalixarenes are nontoxic up to a concentration of 100 mg kg^{-1}, and their lethal dose is ~400 mg per kg of body weight.[13] Assays performed on zebrafish models showed that CB[7] exhibits also measureable cardiotoxicity and neurotoxicity at a concentration of $\geqslant 0.50$ mmol $litre^{-1}$ in solution and has virtually no effect on hepatotoxicity at a concentration of $\leqslant 0.75$ mmol $litre^{-1}$ although the extended exposure (2 days) of zebrafish to solutions of CB[7] in the concentration range of 0.5–0.75 mmol $litre^{-1}$ can induce the mortality of tested fish. Taking into account that the concentrations of CB[7] in solutions used for the complexation with drugs and their delivery are $\leqslant 0.25$ mmol $litre^{-1}$, the overall toxicity profile of CB[7] can be considered as safe.[27]

Therefore, CB[*n*] have a low toxicity at doses, which are much higher than those required for the application in pharmacology, and would be highly competitive in the near future with such molecular containers as cyclodextrins that are widely used for the drug delivery.

II.2. Encapsulation of biologically active molecules in cucurbit[*n*]urils

II.2.a. Encapsulation of metal complexes

Some metal complexes possessing anticancer activity are often highly toxic for the human body. The encapsulation in nanocapsules is one way to substantially reduce toxicity and increase the efficiency of biologically active agents conventionally used in medicine, as well as to modify the properties of the available drug dosage forms. The host molecule should prevent the guest complex from rapid decomposition, reduce its toxicity and serve to transport the active component in the body.

Most of studies on the application of CB[*n*] as molecular containers for drugs deal with inclusion compounds of platinum complexes. Such macromolecules as liposomes and polymers are commonly used to deliver platinum agents. Only a few host–guest complexes with cyclodextrins are known as molecular containers; however, the encapsulation in these macrocycles sometimes completely blocks anticancer activity of the agent.[28]

Biologically active platinum complexes contain amine ligands and more labile ligands, for example, chloride or carboxylate ligands. Once these complexes enter the body,

they can interact with nucleophiles (adenosine and guanosine in DNA, cysteine and methionine in proteins and peptides), which can replace the ligands thus deactivating the drugs. The encapsulation of platinum complexes in CB[*n*] provides their spatial protection from the nucleophilic attack, although the guest complex can be only partially included in the cavity.

The binding of metal complexes to CB[*n*] occurs mainly through noncovalent interactions between guest ligands and the hydrophobic cavity of the host. According to the X-ray diffraction data, in the inclusion complex of oxaliplatin [$Pt(C_8H_{14}N_2O_4]$, **1**) with CB[7] of the composition $\{\mathbf{1}@CB[7]\}\cdot 8\,H_2O$, a part of the diaminocyclohexane ligand of the complex is immersed inside the cavity (Fig. 2).[24] The amino groups of this ligand are located at the portals and are hydrogen bonded to oxygen atoms, thus additionally stabilizing the structure. The platinum atom is located above the portal plane and is sterically protected by seven carbonyl oxygen atoms, which prevent access by nucleophiles.

In order to examine the effect of inclusion in CB[7], the reactions of $\{\mathbf{1}@CB[7]\}\cdot 8\,H_2O$ with guanosine and L-methionine as model compounds of sulfur-containing enzymes were studied. The latter form Pt–S bonds with anticancer drugs, thus causing side effects (nephrotoxicity and ototoxicity). The lower reactivity of the included guest molecule in these reactions compared to the free complex (3 and 15 times lower, respectively) indicates that the inclusion in CB[7] reduces undesirable side effects caused by the binding of the platinum complex to enzymes. However, in this case the inclusion compound exhibits, apart from lower toxicity, 5–10 times lower anticancer activity compared to free oxaliplatin.[24]

The encapsulation of another platinum complex — cisplatin [*cis*-$[Pt(NH_3)_2Cl_2]$, **2**] — in CB[7] (see Ref. 29) also reduces side effects. As opposed to the free drug,

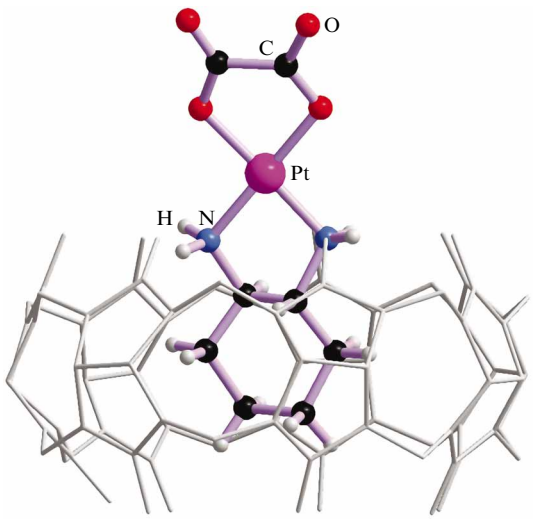
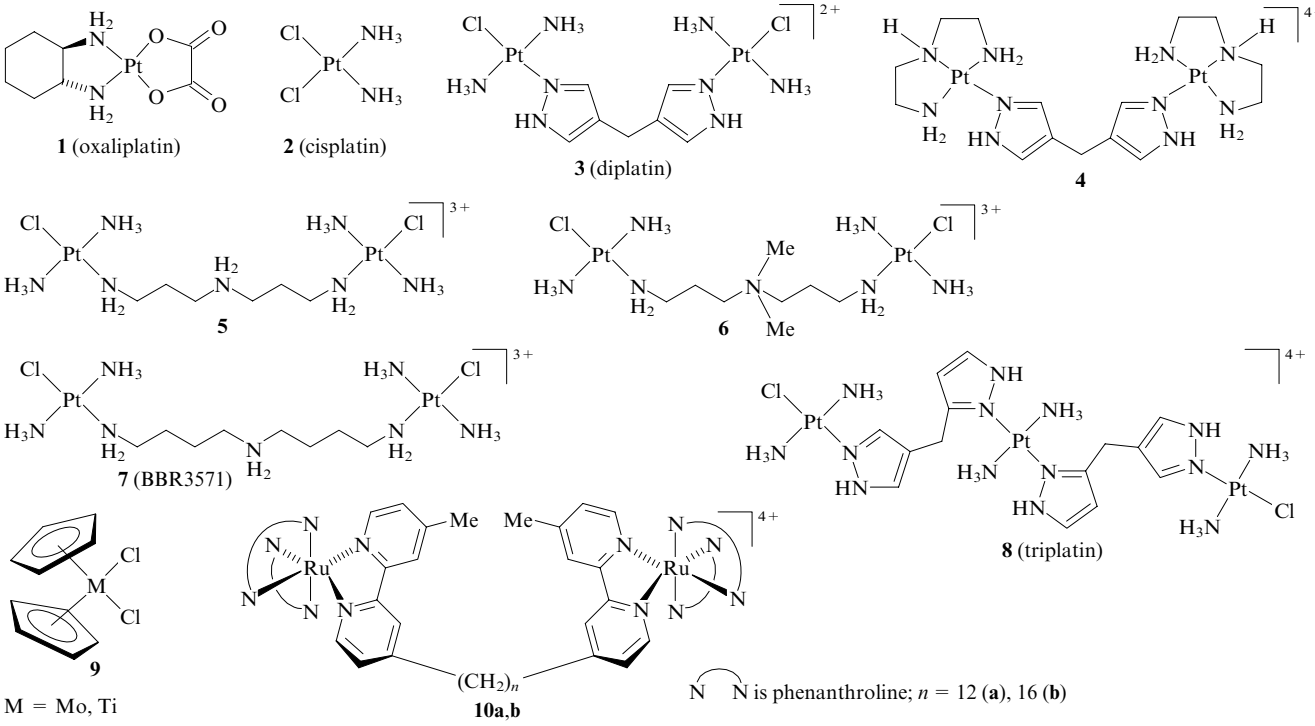


Figure 2. Inclusion compound of the anticancer drug oxaliplatin with CB[7] $\{\mathbf{1}@CB[7]\}\cdot 8\,H_2O$.[24]

inclusion compounds can cross the cell membrane (for example, ovarian cancer cell membrane)[2] and, what is more surprising, cisplatin included in CB[7] is active when delivered orally, a route of administration not previously thought possible for the free drug.[13]

Polynuclear platinum complexes are much more active than mononuclear oxaliplatin and cisplatin and are characterized by higher levels of cellular uptake, but they are more toxic and undergo rather rapid decomposition and deactivation in the body. Therefore, the encapsulation of these complexes in molecular containers is a challenge. Dinuclear and trinuclear platinum complexes — $[(trans\text{-}Pt(NH_3)_2Cl)_2(\mu\text{-dpzm})]^{2+}$ (dipla-

Structures 1–10



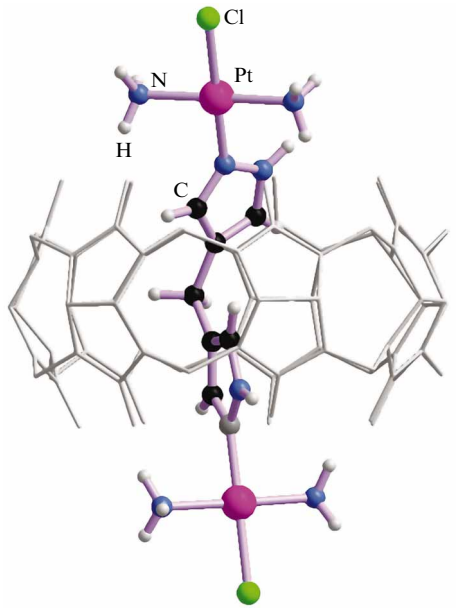


Figure 3. Inclusion compound of the anticancer drug diplatin with CB[7] $\{\mathbf{3}@CB[7]\}Cl_2 \cdot 5.5\,H_2O$.[30]

tin, **3**),[20,30] $[\{Pt(dien)\}_2(\mu\text{-dpzm})]^{4+}$ (**4**),[20] *trans*-$[\{Pt(NH_3)_2Cl\}_2(\mu\text{-}NH_2(CH_2)_3NH_2(CH_2)_3NH_2)]^{3+}$ (**5**),[31] *trans*-$[\{Pt(NH_3)_2Cl\}_2(\mu\text{-}NH_2(CH_2)_3NMe_2(CH_2)_3NH_2)]^{3+}$ **6**),[31] *trans*-$[\{Pt(NH_3)_2Cl\}_2(\mu\text{-}NH_2(CH_2)_4NH_2(CH_2)_4NH_2)]^{3+}$ (BBR3571, **7**)[28] and $[\{trans\text{-}Pt(NH_3)_2Cl\}_2\{trans\text{-}Pt(\mu\text{-dpzm})_2(NH_3)_2\}]^{4+}$ (triplatin, **8**)[32] — form stable inclusion compounds with CB[7] and CB[8], the bridging dipyrazolylmethane ligand (dpzm) (Fig. 3) or a hydrocarbon chain is included in the cavity.[20,28,30–32]

The encapsulation of polynuclear complexes in CB[7] and CB[8] slows down their reactions with nucleophiles (for example, the reaction of cysteine with the inclusion compound of complex **6** with CB[7] is 8–9 times slower) and has only a slight effect on the anticancer activity of the drugs.[20,28,31,33,34] Thus, the IC_{50} (half maximal inhibitory concentration) values of free diplatin (**3**) for the murine leukemia cell lines L1210 and L1210/DDP are 3.8 and 8.8 μmol litre^{-1}, respectively. Upon encapsulation in CB[7], these values are 2.6 and 16.5 μmol litre^{-1}, respectively, which is considered as a insignificant change.[20] In experiments with the cell line L1210, the IC_{50} for the free binuclear complex BBR3571 (**7**) is equal to that for its inclusion compound with CB[7] (0.0115 μmol litre^{-1}); in experiments with the cells L1210/DDP, only a slight change in cytotoxicity was observed, the IC_{50} values being 0.0075 and 0.009 μmol litre^{-1} for the free and encapsulated complex, respectively.[34]

A decrease in the rate of the reaction with nucleophiles leads to a considerable reduction in side effects of the drugs. The inclusion of the complex BBR3571 (**7**) in CB[7] makes it possible to increase the maximum tolerated dose of the drug from 0.1 to 0.45 mg kg^{-1} (*in vivo* experiments in mice), and the encapsulated platinum complex is as active as the injected free drug up to doses of 0.27 mg kg^{-1}.[28]

The association constants of the inclusion compounds of platinum complexes with CB[*n*] (except for cisplatin) are sufficiently high for the use of these cavitands for the drug delivery. Usually, for a host–guest complex to remain stable in biological media, the constants should be at least 10^5 litre mol^{-1} (see Ref. 28). For inclusion compounds of oxaliplatin and diplatin with CB[7], the binding constants are 2.1×10^5 and 2.3×10^5 litre mol^{-1}, respectively, whereas this value for cisplatin is as low as 530 litre mol^{-1} (see Refs 24 and 28). By using CB[*n*] of different sizes, it is possible to control the rate of exchange between the encapsulated and free complex, thus varying the activity and toxicity of the inclusion compound: Generally, the rate of exchange is higher for inclusion compounds with CB[8] compared to that with CB[7] due to a larger diameter of the portals.

Importantly, the inclusion of platinum complexes in CB[*n*] enhances the thermal stability. The inclusion compound of oxaliplatin with CB[7] $\{\mathbf{1}@CB[7]\} \cdot 8\,H_2O$ is resistant to high temperature and pressure and is stable in air for more than a year, whereas this complex in the free state decomposes upon storage at room temperature for >6 h.[24] Studies by thermogravimetric analysis and differential scanning calorimetry (DSC) demonstrated that diplatin encapsulated in CB[7] does not decompose up to 290 °C as opposed to the free complex, which begins to decompose at 190 °C.[30] This is evidence that the inclusion compound $\{\mathbf{3}@CB[7]\}Cl_2 \cdot 5.5\,H_2O$ can be heated for a long period of time to remove water of crystallization. This is important for the drug production.

Cyclopentadienyl complexes of molybdenum and titanium **9**, which are known to have anticancer activity, also form inclusion compounds with CB[7] and CB[8].[35] In aqueous solutions, the starting complexes Cp_2MCl_2 (Cp = η^5-C_5H_5; M = Mo, Ti) are hydrolyzed and, according to ^{1}H NMR spectroscopic data consistent with the results of molecular modelling, the $[Cp_2Mo(H_2O)(OH)]^+$ and $[Cp_2Ti(OH)_2]$ forms are predominantly included in CB[7]. In the presence of CB[7], the rate of hydrolysis of bis(cyclopentadienyl)dichlorotitanium decreases. The treatment of cancer cells with the adduct of CB[7] and $[Cp_2Mo(H_2O)(OH)]^+$ resulted in an increase in the antiproliferative effect due, apparently, to an increase in the solubility of the guest in a physiological medium upon its inclusion in CB[7] and the ability of the inclusion complex to cross membranes.

The binuclear ruthenium complexes $(\Delta\Delta\text{-}[\{Ru(phen)_2\}_2(\mu\text{-bbn})]^{4+}$ (**10a**,**b**) [phen is 1,10-phenanthroline, bbn is 1,*n*-bis[4-(4′-methyl-2,2′-bipyridine)]alkane, *n* = 12 (**a**) and 16 (**b**)] display significant antibacterial properties, but they immediately react with serum proteins, resulting in a decrease in their activity.[36] These complexes form host–guest compounds with CB[*n*] of large sizes, namely, with CB[10], due to the inclusion of the bridging $(CH_2)_n$ ligand in the cavity (NMR spectroscopic data and quantum chemical calculations).[36,37] However, in this case the encapsulation does not prevent the reaction of the complex with proteins. Thus, a decrease in the activity is equal for both free ruthenium complexes **10a**,**b** and those encapsulated in CB[10].

Therefore, cucurbit[*n*]urils hold considerable promise as nanocontainers for the delivery of biologically active metal complexes since the encapsulation usually decreases the degree of decomposition of the complexes and reduces side effects of the drugs, with only a slight change in antitumour activity. Drugs containing inclusion compounds of CB[7] with oxaliplatin,[38] as well as inclusion compounds of poly-

nuclear platinum, palladium and gold complexes with CB[*n*] ($n = 5-12$)[34] are covered by patents.

II.2.b. Encapsulation of organic drugs

The ability of CB[*n*] to act as nanocontainers was demonstrated for a wide range of biologically active organic molecules — drugs of different pharmacological groups. These are the well-known fever reducer paracetamol, the anaesthetics procaine, prilocaine, tetracaine, procainamide and dibucaine, vitamin B_{12}, adrenaline, a series of antifungal and anthelmintic benzimidazoles, the alkaloids palmatine, dehydrocoridaline and berberine, the antituberculosis drugs pyrazinamide and isoniazid, the β-adrenergic receptor blocker atenolol, the antidiabetic drug glibenclamide, the mydriatic drug tropicamide and so on.[13,15]

The encapsulation of organic molecules in CB[*n*] occurs through hydrophobic interactions between the molecules (or their functional groups) and the cavity, as, for example, in the inclusion compound of coumarin (**11**) of the composition $\{2(\mathbf{11})@CB[8]\} \cdot 4\,H_2SO_4 \cdot 12\,H_2O$.[39] In some cases, the structure is additionally stabilized by hydrogen bonds and ion–dipole interactions with the portals. For instance, in the inclusion compound of adrenaline (in the protonated form H-**12**) with CB[6] of the composition $\{2(\text{H-}\mathbf{12})@CB[6]\}Cl_2 \cdot 15\,H_2O$, the methyl groups of two guest molecules are included in the cavity and the protonated amino groups bind to the CB[6] portals through hydrogen bonds and ion–dipole interactions (Fig. 4).[40]

The encapsulation usually has no effect on the properties of the drugs. For example, the biological activity of the free antituberculosis drug ethambutol (**13**) against infected murine macrophage-like cells RAW264.7 was compared with the activity of this drug encapsulated in CB[7], and no substantial differences in the ability of the drug to kill mycobacteria were revealed.[22] Besides, the inclusion of thiabendazole in this macrocycle even enhances the antifungal activity of the drug.[41]

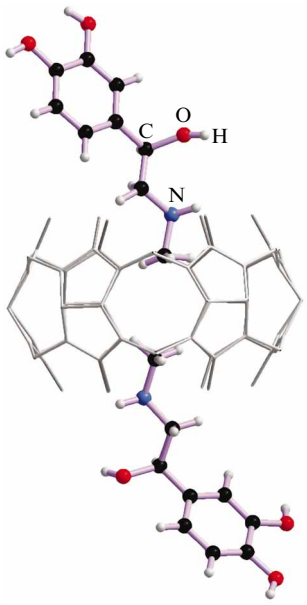
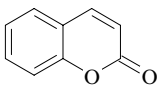


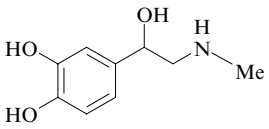
Figure 4. Inclusion compound of two adrenaline molecules with CB[6] $\{2(\text{H-}\mathbf{12})@CB[6]\}Cl_2 \cdot 15\,H_2O$.[40]

The encapsulation of organic molecules in CB[*n*], for example, such molecules as isoniazid (**14**), camptothecin (**15**), paracetamol (**16**), memantine (**17**) and atenolol (**18**), leads to a substantial increase in thermal stability of these drugs (as for the above-described platinum complexes).[42–45] The DSC study showed that the heating of the inclusion compounds of isoniazid and pyrazinamide with CB[7] was

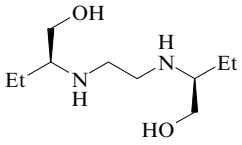
Structures 11–22



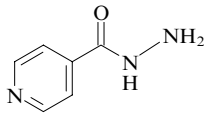
11 (coumarin, anticoagulant)



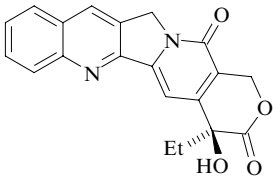
12 (adrenaline, vasoconstrictor, bronchodilator agent)



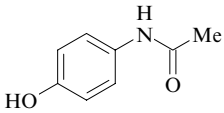
13 (ethambutol, antituberculosis drug)



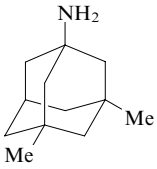
14 (isoniazid, antituberculosis drug)



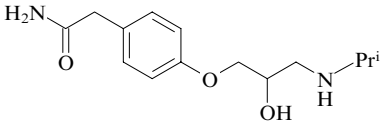
15 (camptothecin, antitumour drug)



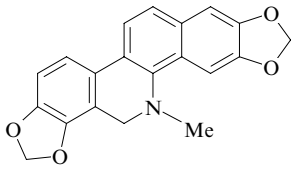
16 (paracetamol, analgesic)



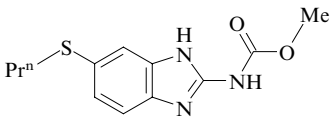
17 (memantine, *N*-methyl D-aspartate receptor inhibitor)



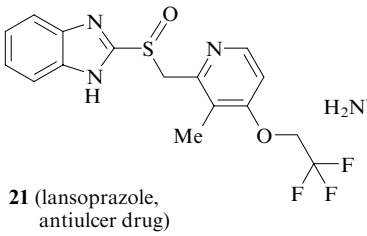
18 (atenolol, β-adrenergic receptor blocker)



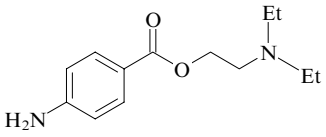
19 (sanguinarine, alkaloid)



20 (albendazole, antihelminthic drug)



21 (lansoprazole, antiulcer drug)



22 (procaine, anaesthetic)

not accompanied by melting or decomposition up to 280 °C, whereas these drugs in the free state already melt at 171 and 189 °C, respectively.[42]

The inclusion of drugs in CB[*n*] can, to a certain extent, solve the problem of chemical degradation of the drugs in the body. For instance, the encapsulation in CB[6] and CB[7] prevents the acylation of isoniazid (**14**), which is easily catalyzed in the human body by the acetyl coenzyme A.[46] The encapsulation of the alkaloid sanguinarine (**19**) in CB[7] protects the drug from nucleophilic attack and hinders the photooxidation.[47] The binding of vitamin B_{12} and the coenzyme B_{12} to CB[7] (through inclusion of the dimethylbenzimidazole ligand in the cavity) stabilizes their active form.[48]

Another important property of cucurbit[*n*]urils is their ability to enhance solubility of drugs in aqueous media. This accelerates the uptake of drugs into cells, thus providing their higher activity. Many drugs are therapeutically active only at millimolar concentrations. Besides, drugs administered orally should be dissolved in a rather small gastric volume at substantially higher concentrations prior to their dilution to therapeutic concentrations once they reach the target organs of the body. The inclusion in CB[*n*] enhances the solubility of benzimidazole drugs, such as albendazole (**20**), carbendazim, thiabendazole, fuberidazole, omeprazole and lansoprazole (**21**),[49–52] the anticancer drugs gefitinib and camptothecin (**15**),[43] the local anaesthetic drugs procaine (**22**), tetracaine, prilocaine and dibucaine.[53] For example, the addition of CB[6], CB[7] or CB[8] to albendazole increases the solubility of the latter in water from 3 μmol litre^{-1} to 2–7 mmol litre^{-1} (2000 times!);[49] the solubility of 10-hydroxycamptothecin (HCPT) increases 1170 times upon the inclusion in CB[7].[51] An increase in the solubility is attributed to an increase in the protonation constants of functional groups of biomolecules due to the presence of carbonyl groups at the portals of CB[*n*].[43, 51, 53]

II.2.c. Encapsulation of amino acids, peptides and proteins

The design of artificial receptors for the molecular recognition of peptides and proteins in aqueous solutions is a challenge in biochemistry, and the use of CB[*n*] for this purpose has great prospects.[14]

Studies by different physicochemical methods, including X-ray diffraction, showed that amino acids can form 1 : 1 or 2 : 1 supramolecular adducts with CB[*n*] either through hydrogen bonds with the portals of the macrocycle [for example, in the adduct of tryptophan (Trp) and CB[6], see Fig. 5] or through the formation of inclusion compounds [for example, in the adduct of L-tyrosine (L-Tyr) and CB[8], see Fig. 6)].[54–56] The amino acids Phe (phenylalanine), Trp and Tyr (or small peptides containing these amino acids) most efficiently and selectively bind to CB[7] and CB[8], particularly if these acids are at the N-terminus of the polypeptide chain.[14, 56, 57] The aromatic moiety of these amino acids is included in the hydrophobic cavity, the interactions between the amino groups and the portals of the macrocycle contributing to stabilization. The efficiency of the peptide binding to CB[8] is influenced also by amino acids adjacent to Phe, Trp and Tyr.[58]

The use of CB[*n*] for the selective molecular recognition of peptides and proteins was convincingly demonstrated for the CB[7]–insulin system.[59] The cavitand CB[7] binds to insulin through an interaction with terminal phenylalanine (the association constant $K_a = 1.5 \times 10^6$ litre mol^{-1}).

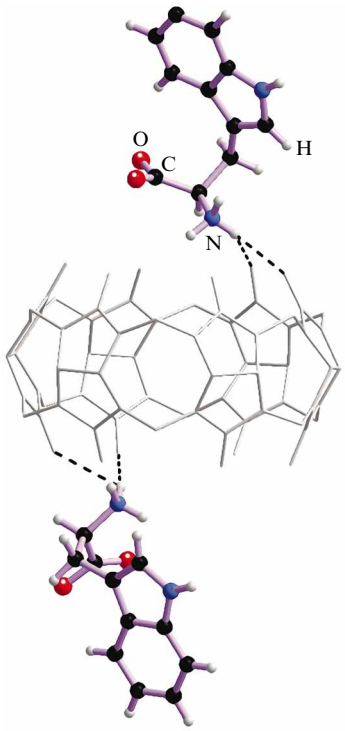


Figure 5. Supramolecular adduct of tryptophan and CB[6] (2 : 1) of the composition H[(Cl@CB[6])·2HTrp]Cl·9 H_2O.[54]
The tryptophan molecule is protonated.

According to the X-ray diffraction data, the aromatic moiety of Phe is fully included within the cavity. However, the binding of insulin occurs only if the Phe residue is sterically accessible to CB[7]. Thus, the insulin molecule was found to adopt another conformation, in which the terminal Phe residue is located closer to the other part of the protein molecule (the ability to unfold like a thread at the end of a ball is typical of terminal amino acid residues). This spatial

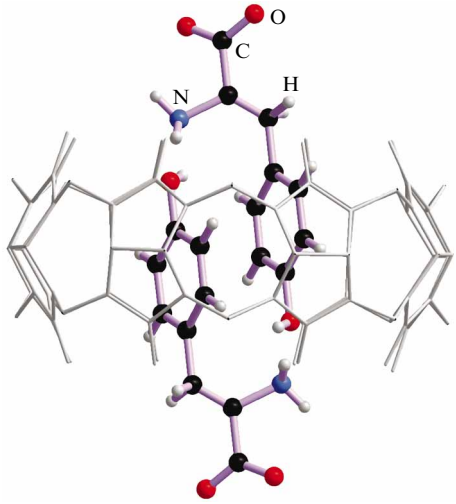


Figure 6. Inclusion compound of two L-tyrosine molecules with CB[8] {2(L-Tyr)@CB[8]}·27 H_2O.[56]
The tyrosine molecule is as the zwitterion.

arrangement does not allow this insulin molecule to form an inclusion compound with CB[7].

The terminal Phe residue is responsible for the highly selective binding of CB[7] to insulin compared to protein molecules lacking terminal Phe (50–100 times higher). The ability of CB[7] to bind insulin was shown to have a potential for the therapy of Alzheimer's disease.[60] The inclusion of phenylalanine residues in cucurbit[7]uril prevents the formation of insoluble amyloids from Phe-containing protein chains, which is believed to be the cause of this disease.

The sizes of the CB[8] cavity are suited to include two guest molecules. The ternary complex of CB[8] with methyl viologen ($CH_3N^+C_{10}H_8N^+CH_3$, MV) and a Trp-containing peptide was the first example of the use of macrocycles for molecular recognition.[61] This complex is formed only if the indole group of tryptophan is at the N-terminus. In this case, the electrostatic interaction between two guests provides a high association constant of MV@CB[8] and Trp ($K_a = 1.3 \times 10^5$ litre mol^{-1}). More recently, this system was applied to separate peptides on a CB[8]-modified gold surface (see Section VII).

The association constants of amino acids and CB[*n*] are usually in the range of 10^3–10^6 litre mol^{-1}, but they may be higher. Thus, in the case of inclusion of two Trp or Phe molecules in CB[8], these constants are 6.9×10^7 or 1.1×10^8 litre^2 mol^{-2}, respectively.[62] This high affinity plays a key role in the application of CB[*n*] in biological systems, because the target peptides and proteins are often present at low concentrations.

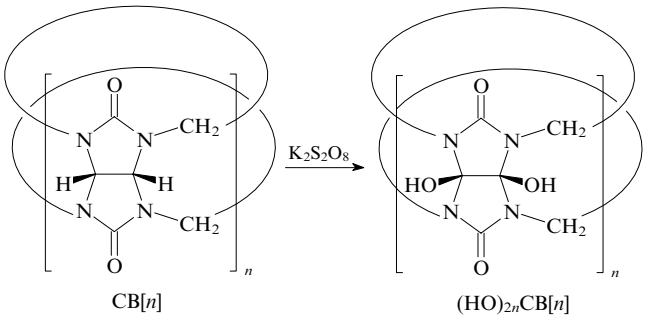
The inclusion of peptides in CB[*n*] protects the former from exposure to an environment, thus hindering the hydrolysis by leucine aminopeptidase, trypsin and other enzymes of the gastrointestinal tract,[63] which is important because of a wide use of peptides in medicine, for example, as antiviral drugs.

Therefore, the formation of inclusion compounds of drug molecules with CB[*n*] has been extensively studied. Cucurbit[*n*]urils demonstrate a high potential as drug delivery systems. Thus, these compounds perform a protective function, prevent the decomposition of drug molecules and enhance their solubility, having virtually no effect on the biological properties. However, some problems remain to be solved. For instance, the metabolism of CB[*n*] in the body is poorly understood. The *in vivo* studies were carried out primarily in mice over a rather short period of time and, consequently, the effect of long-term application (chronic effect) of CB[*n*] is unknown. Interactions of CB[*n*] with chemicals used in pharmaceutics for the manufacture of different dosage forms (tablets, capsules and so on) also require further investigations. These and many other problems should be studied in detail in order to benefit fully from the advantages of cucurbit[*n*]urils to use them in medicine and pharmacology.

III. Functionalization of cucurbit[*n*]urils

Low solubility of cucurbit[*n*]urils in water and organic solvents has long been one of the factors that interfere with the application of these cavitands for the design of new functional materials. A great deal of attention has been given to the development of methods for the production of soluble CB[*n*] derivatives.[9] Nowadays, this problem has been practically solved by introducing various functional substituents although it should be noted that cucurbit[*n*]uril derivatives are still much more difficult to synthesize than, for example, calix[*n*]arene derivatives. The research team headed by Professor K. Kim[25,64] has developed and successfully implemented an approach to the synthesis of perhydroxycucurbit[*n*]urils of the composition $(HO)_{2n}$CB[*n*] ($n = 5$–8). The heating of an aqueous solution of $K_2S_2O_8$ with CB[*n*] leads to the complete replacement of CH hydrogen atoms at the equatorial positions of the CB[*n*] molecule by hydroxy groups (Scheme 1).[64] The perhydroxycucurbit[*n*]urils thus synthesized are readily soluble in DMSO and DMF and, what is more important, hydroxy groups can be easily replaced with other groups by means of conventional chemical methods. For example, the reaction of $(HO)_{2n}$CB[*n*] ($n = 5$, 6) with allyl bromide affords perallyloxycucurbit[*n*]uril $(H_2C{=}CHCH_2O)_{2n}$CB[*n*], which is a convenient reagent for further transformations due to its good solubility in organic solvents along with the presence of reactive functional groups (Scheme 2).

Scheme 1

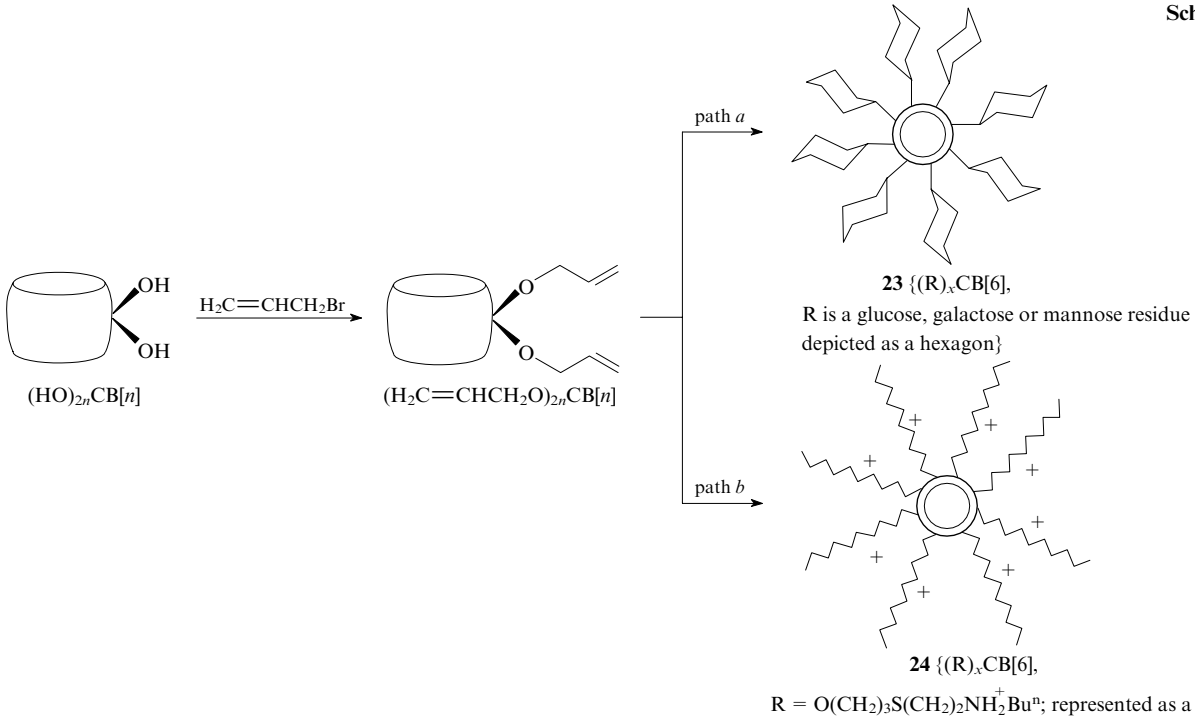


CB[*n*] $(HO)_{2n}$CB[*n*]

The approaches to the synthesis of modified CB[*n*] {hereinafter referred to as $(R)_x$CB[*n*]} containing required functional groups, which are soluble in different solvents, have paved the way to their diverse applications, including their use as nanocontainers for the drug delivery. For example, $(R)_x$CB[6] **23**, in which all peripheral carbon atoms are bound to carbohydrate residues (glucose, galactose, mannose) (see Scheme 2, path *a*), exhibits high selectivity and enhanced affinity for certain proteins.[25] This fact was attributed to the formation of numerous contacts between the protein molecules and carbohydrates on the CB[*n*] surface. These interactions play a key role in cellular processes and are responsible for the delivery of substances to the target cell *via* receptor-mediated endocytosis. In view of a wide range of drugs that form inclusion compounds with CB[*n*] (see Section II), these modified CB[*n*] hold considerable promise for the targeted drug delivery and other therapeutic applications.

The modified cucurbituril $(R)_x$CB[6] **24** surrounded on the periphery by amphiphilic moieties can serve as a model of an antibiotic. The hydrophobic part of peripheral molecules comprises an unbranched hydrocarbon chain, and the hydrophilic part is a positively charged protonated amino group (see Scheme 2, path *b*).[65] The antibacterial action of known amphiphilic peptides is associated with the bacterial membrane disruption due to electrostatic and hydrophobic interactions between the side chains of peptides and the negatively charged bacterial membranes. Like peptide antibiotics, modified cucurbit[6]uril **24** disrupts cell membranes with high activity and selectivity (with respect to mamma-

Scheme 2



lian cells). It should be noted that cucurbit[*n*]urils have an advantage over peptides in that they are resistant to enzymatic degradation, which is attractive for the use of these cavitands in clinical practice.

IV. Functionalized cucurbit[*n*]uril-based polymer surfaces

The self-assembly of functionalized CB[6] through interactions between side groups gives rise to polymer surfaces, including nanocapsules (vesicles). The formation of nanocapsules is a rare example of the irreversible covalent self-assembly of molecular components into nanometre-scale objects. The nanocapsules are produced in high yield from a mixture of $(R)_xCB[6]$ and the additional reagent H_2L, the latter molecules acting as bridges. This method does not require any template, which is advantageous over known methods for the synthesis of polymer surfaces (Fig. 7). Nanocapsules are formed, for example, under ultrasonic irradiation of a mixture of perallyloxycucurbit[6]uril $(H_2C{=}CHCH_2O)_{12}CB[6]$ and 2-[2-(2-methoxyethoxy)-ethoxy]ethanethiol[66] or under UV irradiation of a mixture of $(H_2C = CHCH_2O)_{12}CB[6]$ and dithiols [$H_2L = HS(CH_2)_2(OCH_2CH_2)_nSH$ ($n = 2$, 4), $HSCH_2CH_2SH$].[67,68] The nanocapsules thus formed are stable and have a rather narrow size distribution. The size is, on the average, ~100 nm and depends on the solvent used in the synthesis and the length of the bridging molecule.

These objects have attracted great interest because of the prospects of their use as nanocontainers for the drug delivery. In addition to the inclusion of drugs containing amino groups in the cavity of CB[6] molecules that form a shell of the capsule, here a radically different pattern is implemented — the inclusion of the drug in the nanocapsule itself. For instance, the hydrophobic molecule of the anticancer drug paclitaxel (PTX) can be encapsulated in the cavity of a vesicle composed of $(R)_xCB[6]$ [$R = O(CH_2)_3S(CH_2)_6OH$], the characteristics of the inclusion efficiency being comparable with those for other polymer capsules used for this drug.[69] One of important requirements to nanocontainers is the opportunity to control drug release after the drug delivery to the cell in response to external stimuli, *e.g.*, pH, heating, intracellular environment. The use of a hydrocarbon chain bearing a disulfide group ($L = \text{-}NHC(O)(CH_2)_2S{-}S(CH_2)_2C(O)NH\text{-}$) as a bridging molecule between $(R)_xCB[6]$ makes nanocapsules responsive to the intracellular reductive environment.[70]

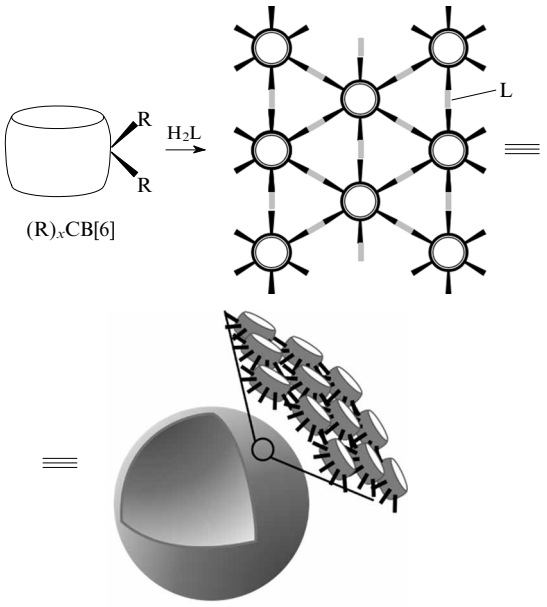


Figure 7. Formation of polymer surfaces through the bridging of modified cucurbit[6]urils $(R)_xCB[6]$ by molecules (L).

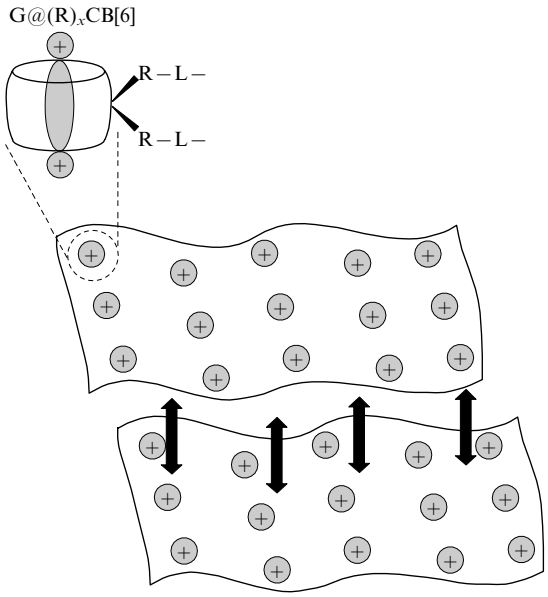


Figure 8. Inclusion of a charged guest (G) in $(R)_xCB[6]$ leads to the separation of 2D polymer sheets by electostatic repulsion [R = $CH_2CH{=}CH_2$, $H_2L = HS(CH_2)_2SH$, $G = NH_3^+(CH_2)_3NH_2^+(CH_2)_4NH_2^+(CH_2)_3NH_3^+$].[72]

Under reducing conditions, S–S bonds are cleaved and nanocapsules are disrupted to release the drug.

Of particular interest are temperature-responsive nanocapsules based on CB[6] and bridging poly(*N*-isopropylacrylamide), which is a temperature-sensitive polymer widely used due to its ability to undergo a reversible phase transition at 32 °C.[71] The temperature responsiveness of this nanocapsule can be controlled by including guests, for example, n-butylamine, in the cavity of the constituent CB[6] molecules.

The study of the mechanism of nanocapsule formation showed that small 2D polymer patches are initially formed in solution and then they are turned into hollow spheres.[68] A new CB[6]-containing nanomaterial — planar 2D polymer sheets with macroscopic dimensions — was prepared by thoroughly selecting the bridging molecules and the solvent to prevent the folding (Fig. 8).[72] The surface thickness of the new CB[6]-containing material is 2.8 nm, which corresponds to ~2–3 layers of CB[6] molecules. An ingenious method was developed for the preparation of single-monomer-thick 2D polymer surfaces. Protonated spermine $[NH_3^+(CH_2)_3NH_2^+(CH_2)_4NH_2^+(CH_2)_3NH_3^+]$ added to the initial reaction mixture is included in the cavity of $(R)_xCB[6]$, resulting in the mutual electrostatic repulsion between the layers. This is the first example of the production of monolayer polymer surfaces in solution. Such ultrathin monolayer polymers (for example, graphene) are of great interest because of their unique properties. However, simple and effective methods for the preparation of such materials are lacking.

V. Methods of attaching cucurbit[*n*]urils to the surface

The attachment of receptors to the surface is of great importance in many areas of application, particularly for the manufacture of chemical sensors and biosensors. There are the following three main methods for binding CB[*n*] to the surface: 1) through noncovalent interactions between the portal carbonyl groups and the surface (usually, the gold surface); 2) through noncovalent interactions between the guest molecule and the cavity of CB[*n*], the other part of the guest molecule being bound to the surface; 3) through covalent binding of functionalized $(R)_xCB[n]$ molecules to the surface (Fig. 9).

A simple, facile and effective method for the attachment of CB[*n*] ($n = 6–8$) to the surface without pre-modification of either the macrocycles or the substrate was proposed in 2008.[73] The immersion of a gold electrode in saturated aqueous solutions of CB[*n*] results in the formation of monolayers of CB[*n*] molecules through noncovalent interactions between the carbonyl groups and the gold electrode surface (see Fig. 9 *a*). Due to multiple C=O···Au interactions, the gold coating is rather stable. Thus, the washing of the electrode with different solvents does not lead to desorption of CB[*n*].

The second method of attaching CB[*n*] to the surface is based on the ability of these macrocycles to form stable inclusion complexes. In this case, an additional guest molecule serves as a linker. One moiety of this guest molecule can form a stable host–guest complex with CB[*n*], whereas the other one can bind to the surface (see Fig. 9 *b*). For instance, the immobilization on the gold surface can be performed using inclusion compounds of guest molecules with CB[*n*], in which the moiety that is not included in the cavity bears a sulfur-containing group capable of forming strong S–Au linkages.[74–77]

Another method for anchoring cucurbiturils on the surface was made possible due the development of the facile synthesis of functionalized CB[*n*]. Perallyloxycucurbit[6]uril $(H_2C{=}CHCH_2O)_xCB[6]$ was attached to the modified glass surface through the binding of allyloxy groups to $(CH_2)_3SH$ groups anchored on the substrate (see Fig. 9 *c*).[64]

The hydrophobic cavity in surface-immobilized CB[*n*] remains accessible to guest molecules and retains the unique recognition ability. Therefore, such materials hold great promise for the application in diverse fields: in separation

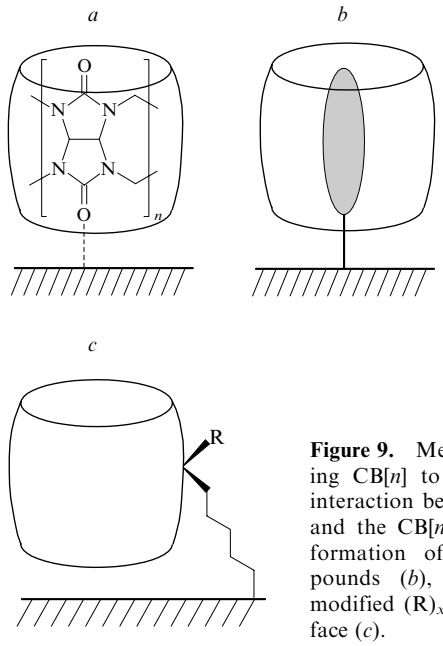


Figure 9. Methods of attaching CB[*n*] to the surface: the interaction between the surface and the CB[*n*] portals (*a*), the formation of inclusion compounds (*b*), the binding of modified $(R)_xCB[n]$ to the surface (*c*).

and extraction processes, chromatography, catalysis, drug delivery, construction of artificial ion channels, design of sensors and biochips and so on. The use of CB[*n*]-containing materials for the further surface modification and the separation of complex mixtures is covered in the literature in most detail.

VI. Immobilization on cucurbit[*n*]uril-containing surfaces

VI.1. Methods of material immobilization

The immobilization on the CB[*n*]-containing surface is based on the ability of CB[*n*] to form highly stable inclusion compounds, for example, of CB[7] with ferrocene derivatives (Fc) or of CB[8] with two guests to form highly stable ternary inclusion complexes (Fig. 10). Guest molecules, which decorate the surface of the material to be adhered, are included in the cavities of the macrocycles of the CB[*n*]-containing substrate and both surfaces are rather tightly stuck together through multiple host–guest interactions.

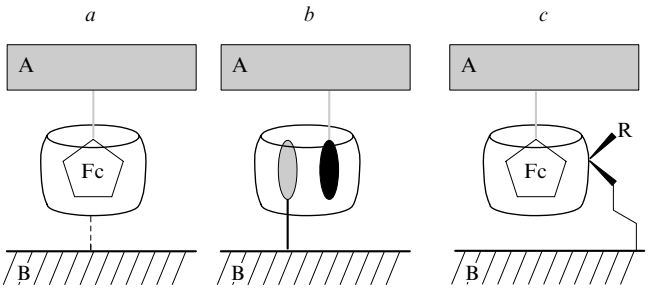


Figure 10. Immobilization of materials (A) on CB[*n*]-containing surfaces (B) through the formation of inclusion compounds with CB[7] (*a*, *c*) and CB[8] (*b*).

The size and shape complementarity between the rigid host and guest molecules is responsible for high values ($10^{12}-10^{13}$ litre mol^{-1}) of the association constants for inclusion compounds of different substituted ferrocenes FcR (R = COO^-, CH_2OH, $CH_2NHMe_2^+$, $CH_2NMe_2^+$, *etc.*) with CB[7].[11,78–80] Recently, it has been found[8] that this constant for the inclusion compound of the diamantane-4,9-diammonium cation with CB[7] is as high as 7.2×10^{17} litre mol^{-1}. These values of association constants are comparable with or even higher than the corresponding constant for the biotin–avidin interaction ($10^{13}-10^{15}$ litre mol^{-1}), which is the strongest noncovalent interaction known in nature.[81] The biotin–avidin pair is widely used for the surface immobilization of biomolecules. However, this system has some drawbacks. For example, it is denatured by organic solvents or at elevated temperatures.

One of the most beautiful examples of the utilization of a strong interaction between CB[*n*] and a guest molecule that demonstrates the above-mentioned effect at the macro level refers to so-called 'supramolecular velcros'.[82,83] Two substrates functionalized with CB[7] and ferrocene, respectively, being immersed in water, were stuck together using fingers, with a contact area of as small as 1 cm^2; nevertheless, due to the formation of multiple strong host–guest interactions, such small pieces can hold a weight of 2 kg in water (Fig. 11 *a*). After drying in air, adhesive forces are enhanced, and the holding power increases to 4 kg (see Fig. 11 *b*). This value is substantially larger than the lap shear adhesion strength of commercial Velcro widely used in light industry (1.12 and 0.74 MPa for the 'supramolecular velcro' Fc@CB[7] and commercial Velcro, respectively). The adhesion of the substrates is mechanically reversible and chemically switchable (thus, Fc@CB[7] can be unfastened by means of an interaction between CB[7] and the guest with a higher association constant for the formation of inclusion complexes, for example, with adamantane). In addition, the manufacture of 'velcros' in an aqueous solution solves the major challenge in the synthesis of stuck surfaces in an aqueous medium — the exclusion of water molecules between the adhesive and adherent surfaces.

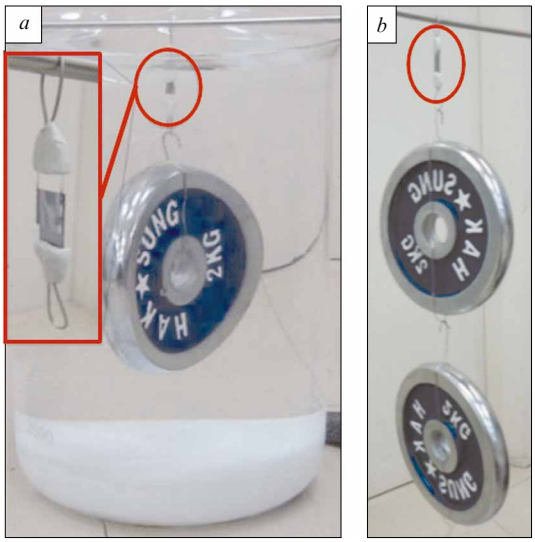


Figure 11. Formation of 'supramolecular velcros' based on Fc@CB[7] from two substrates (1 cm^2) functionalized with CB[7] and Fc, respectively, that are adhered with each other under water (the inset in Fig. *a*).[82]
The holding power of the substrates stuck together is 2 kg in water (*a*) and up to 4 kg in the air after drying (*b*).

The immobilization on the CB[8]-containing surface occurs through the formation of ternary inclusion complexes (see Fig. 10 *b*). The inclusion of two organic molecules — electron-deficient (for example, methyl viologen) and electron-donating (for example, 2,6-dihydroxynaphthalene or 1,4-dihydroxybenzene) — in CB[8] provides a tight contact between these molecules inside the cavity, resulting in a strong charge-transfer interaction (the association constant is $10^{10}-10^{11}$ litre^2 mol^{-2}), which is much stronger compared to that when these organic molecules are not included in the cavity.[74,84] In these cases, the CB[8] molecule is often referred to as a molecular 'handcuff'. Therefore, one of the surfaces to be adhered should contain an inclusion complex of a macrocycles with a guest, for example, an electron-deficient molecule, whereas the adherent surface should contain molecules of another guest acting as an electron donor.

VI.2. Immobilization of biomolecules

Biochips play an important role in modern diagnostic techniques. A method commonly used for producing biochips is based on the immobilization of biomolecules on the

surface with the retention of their biological activity. A great advantage of supramolecular immobilization over the covalent binding of biomolecules to the surface is the formation of noncovalent bonds, which can be more easily broken, if it is necessary, to detach biomolecules from the surface.

The immobilization of biomolecules on the CB[*n*]-modified surface was proposed for the first time by the research team headed by Professor K. Kim in 2007.[85] A ferrocene molecule bound to the protein glucose oxidase, which is a well-known sensor for glucose, was employed as a guest. Perallyloxycucurbit[7]uril molecules anchored to a gold substrate served as hosts (see Fig. 10 *c*). The immobilization of the protein was implemented by the direct immersion of this substrate in a solution of Fc glucose oxidase. It was shown that in this case, the properties of glucose oxidase as a sensor for glucose are maintained.

Yellow fluorescent protein (YFP), which is well-known as a fluorescent label in cell and molecular biology, was immobilized on the surface using supramolecular inclusion compounds with CB[7] and CB[8]. In the former case, monolayers of CB[7] molecules anchored to a gold substrate (hosts) were prepared by spontaneous adsorption of these macrocycles on the surface.[86] Upon the immobilization of Fc-YFP on the CB[7]-attached Au surface (see Fig. 10 *a*), the protein molecules form a monolayer; other protein molecules bound to the surface in any other mode are absent in the space between the anchored protein molecules. In the latter case, where CB[8] was used, YFP monolayers were formed through the inclusion of the appropriate moieties of two guests in the cavity (see Fig. 10 *b*): viologen-functionalized SiO_2 nanoparticles (or glass surface) and the 2-naphthyloxy-functionalized protein YFP.[87] The protein-modified surface was rather stable, as evidenced by the fact that only a slight decrease in the fluorescence was observed over several days.

An important feature of such inclusion compounds is the reversible encapsulation of guests and, correspondingly, the reversible immobilization of biomolecules. The treatment of the CB[7]-Au/Fc-YFP surface with a $FcCH_2NMe_3I$ solution, which has a higher association constant with CB[7] compared to that of Fc-YFP, results in the displacement of the latter from the cavity and the release of the protein.[86] In the case of the system with CB[8], YFP is released upon the reduction of viologen, resulting in the destruction of the guest charge-transfer complex, and the subsequent oxidation of viologen returns the system to the initial state.[87]

The surface immobilization with the RGD peptide (RGD is arginylglycylaspartic acid; the amino acid motif of the RGD peptide is responsible for the recognition and protein – protein interactions in cells) through the formation of inclusion compounds with CB[7] and CB[8] was successfully used for the spatial resolution of cell adhesion. The RGD peptide was attached to the CB[7]-Au surface by including Fc-RGD in the cavity of CB[7];[88] and attached to the CB[8] surface, *via* the CB[8] 'handcuff' that links the appropriate moieties of two guests: a viologen-functionalized hydrocarbon chain (which is bound to the gold substrate surface *via* the thiol group at the other end of the chain) and the tryptophan-functionalized RGD peptide.[89] Arrays of gold stripes on glass substrates rather than solid gold substrates were employed in the cited works. The interaction between the RGD-peptide-modified surface and HUVEC cells (HUVEC are human umbilical vein endothelial cells) results in that the adhesion occurred only at gold stripes, thus providing the spatial resolution of cell adhesion according to the pattern specified by the gold array. This supramolecular control of the cell distribution is of great importance for the design of biomaterials for cell engineering.

The 'handcuff' effect was successfully used for the immobilization of living organisms — bacterial cells.[90] *E. coli* outer membrane proteins modified with a tryptophan-containing peptide were attached to the gold stripes pre-immobilized with a CB[8] monolayer (by inserting a methyl viologen chain containing the terminal thiol group into CB[8]). It is important that bacteria retain activity upon this supramolecular immobilization.

VII. Cucurbit[*n*]uril-containing surfaces for separation of mixtures

Due to the unique recognition properties, CB[*n*]-modified surfaces hold promise for the separation of complex mixtures through the selective binding only of such components that form stable inclusion compounds with these cavitands. Examples of the use of the CB[6]-modified silica surface as the stationary phase in high-performance liquid chromatographic separation of alkaloids are available in the literature.[91 – 93]

The CB[8]-containing surface has proved to be particularly efficient for the peptide separation.[94] The modified gold substrate surface consists of CB[8] containing a viologen molecule in the cavity (Fig. 12). Due to the effect of a CB[8] 'handcuff', the treatment with a mixture of peptides results in the selective immobilization of tryptophan-containing peptides on the surface. The electrochemical reduction of viologen triggers the release of the anchored peptides from the surface, the process being reversible and reproducible. The problem of protein purification has attracted great interest in the past decades. Therefore, procedures using CB[*n*]-immobilized surfaces hold great promise since they do not require, as opposed to conventional methods, large amounts of eluents and additional ligands.

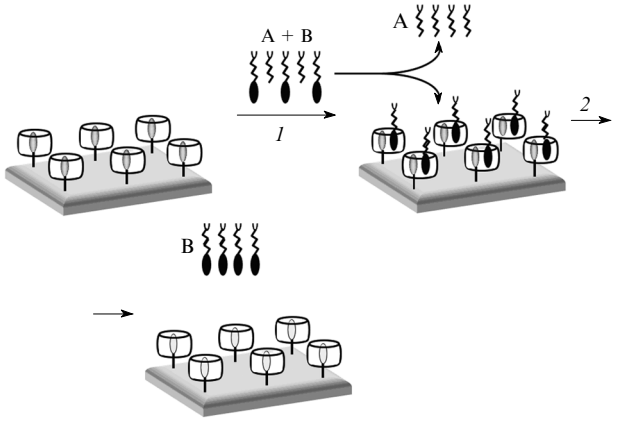


Figure 12. Use of the CB[8]-immobilized surface modified with viologen for the separation of peptides A and B.[94]
(*1*) Selective inclusion of peptide B and release of peptide A; (*2*) electrochemical reduction of viologen included in the cavity of CB[8] and release of peptide B.

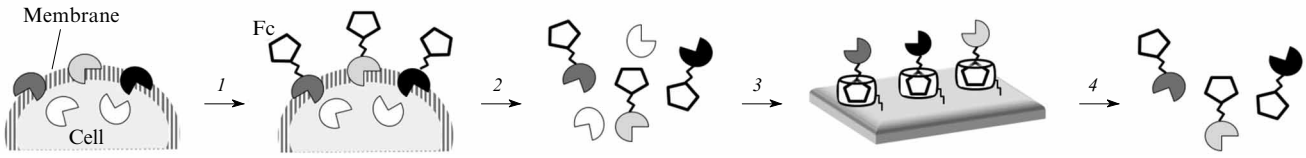


Figure 13. Use of CB[7] for the selective isolation of cell membrane proteins.[95]
(*1*) Functionalization of cell membrane proteins with ferrocene; (*2*) cell disruption; (*3*) selective anchoring of Fc-labelled proteins to the CB[7]-immobilized surface; (*4*) release of proteins.

The ability of CB[7] to form highly stable inclusion complexes with ferrocene derivatives served as the basis for the selective isolation of cell membrane proteins (Fig. 13).[95] The separation and identification of these proteins is a challenge in modern proteomics concerned with functions of proteins and their interactions in living organisms. In the state-of-the-art analysis of proteins in the cell membrane, streptavidin is commonly used as a receptor for the extraction of biotinylated protein molecules. The use of CB[7]-modified Sepharose granules (Sepharose is a polysaccharide for gel filtration of high-molecular-mass compounds) and Fc-labelled membrane proteins showed the efficient protein trapping comparable with that provided by the streptavidin – biotin system. The proteins extracted by this method are free from biotinylated by-products. Another advantage of CB[*n*] is that proteins trapped by CB[7] can easily be isolated from Sepharose granules by the treatment with a $FcCH_2NMe_3I$ solution at room temperature, as opposed to the extraction from streptavidin-modified granules, which requires much more drastic conditions.

Therefore, the synthetic but biocompatible cucurbit[*n*]uril-based host – guest system may be a useful alternative for membrane proteomics, as well as for other biological and biotechnological applications.

VIII. Cucurbit[*n*]uril-modified electrodes for analytical applications

The attachment of CB[*n*] to the surface of different electrodes is of particular interest for analytical applications. Mono- or multilayer CB[*n*]-immobilized surfaces can be prepared, for example, by the immersion of a gold electrode in an aqueous CB[*n*] solution,[73, 96] the adsorption of CB[5] on a mercury electrode in a Na_2SO_4 solution,[97] the coating of a glassy carbon or any other electrode with a mixture of CB[*n*] and Nafion or polyvinyl chloride (PVC)[98 – 102] or with a graphene – CB[*n*] suspension.[103] Using semipermeable CB[6]/PVC membranes as ionic conductors on the electrode surface, it is possible to perform highly efficient electrochemical studies of insoluble substrates and, in the authors' opinion,[98] in some cases such membranes can easily replace widely used Nafion and carbon-paste electrodes.

Electrodes modified with CB[7] and CB[8] are of interest as electrochemical sensors. The ability of CB[*n*] to be involved in host – guest interactions provides high sensitivity and selectivity of the method. Furthermore, no additional pre-treatment of the sample is required. This was demonstrated by an example of the detection of tryptophan using a CB[8]/Nafion-modified glassy carbon electrode.[99]

Electrodes modified with a suspension of graphene with CB[7] or CB[8] combine the molecular recognition properties of CB[*n*] and high electrical conductivity of graphene, which allows the electrochemical detection of biomolecules. For example, tryptophan was detected in a solution at a concentration of 0.69×10^{-7} mol litre^{-1} using a graphene – CB[8] electrode.[103]

An interesting use of a CB[8]-modified electrode as an electrochemical transducer in a flow injection analysis was proposed.[101] This electrode was utilized for the determination of dopamine (a neurotransmitter released in the human and animal brains) in blood serum samples, the method being characterized by a rather low detection limit, selectivity and reproducibility.

IX. Cucurbit[*n*]urils and metal nanoparticles

Nanoparticles (1 – 100 nm) have properties substantially different from those observed in the bulk, including the crystal lattice parameters, electronic structure, magnetic, optical, catalytic and other properties. Metals in a highly dispersed state are often active in such reactions, in which bulk metals are barely active, if at all. For example, although bulk gold is known as one of the most inert metals, its dispersions with a particle size of $\leqslant 5 - 10$ nm exhibit higher catalytic activity in the oxidation of CO than metallic palladium and platinum conventionally used as catalysts for this reaction.[104] Gold nanoparticles have attracted attention due to their high chemical stability and low toxicity. Commercial silver nanoparticle-based biologically active additives exhibit antibacterial, antiviral and fungicidal activities. The major obstacle to the use of metal nanoparticles is their tendency to form agglomerates, resulting in an increase in the particle size and, consequently, in a decrease in their activity. Nanoparticles can be stabilized against agglomeration by chemically anchoring the particles on the surface or by means of surrounding by different protecting reagents (encapsulation), such as surfactants, dendrimers and polymers.[105, 106]

IX.1. Cucurbit[*n*]urils and gold nanoparticles

Cucurbit[n]urils were used for the first time to stabilize nanoparticles in 2007. Thus, the reduction of chloroauric acid with sodium borohydride in the presence of CB[7] produced gold nanoparticles (Au-NP).[107] The stabilization of gold nanoparticles by cucurbit[*n*]urils is usually attributed to the fact that nanoparticles are surrounded by molecules of the macrocycle through interactions between the portal oxygen atoms and the gold surface (see Fig. 9 *a*). This prevents agglomeration. Thus, the CB[*n*] – Au-NP particles are stable over several months.

The possibility of the formation of gold nanoparticles inside the cavity of cucurbit[*n*]urils was mentioned in a number of publications. The reduction of $HAuCl_4$ in the

presence of CB[7] gave two types of nanoparticles with diameters in the ranges of 0.4–1.2 and 4–9 nm.[107, 108] The authors hypothesized that smaller nanoparticles are located inside the cavity of CB[7]. In the case of CB[5] and CB[6], the formation of nanoparticles of these sizes was not observed, which is consistent with much higher stability of the CB[7]–Au-NP system compared to CB[5]–Au-NP and CB[6]–Au-NP. Thus, the former system remains unchanged in the presence of cyanide anions and gold is not extracted with toluene and is not precipitated upon heating. It was shown that, upon photolysis, CB[7]–Au-NP nanoparticles (~1 nm) cause a 7-fold increase in the rate of dimerization of phenylacetylene to 1,3-diphenylbutadiene.[109] The electronic structure and microstructure of the CB[7]–Au-NP nanoparticles prepared by this method were studied by X-ray absorption spectroscopy (Au L_3-edge EXAFS, XANES and HERFD).† These studies showed that gold nanoparticles are characterized by 0.01–0.02 Å smaller interatomic distances compared to bulk gold. Specific properties of atoms on the surface of such nanoparticles may be associated with structural disorder, deformations and strain, which increase with a decrease in the particle size.[110]

In the case of the stabilization of nanoparticles through interactions between the cucurbit[*n*]uril portals and the gold surface, the cavitand molecule can be attached to Au-NP by either one (1 : 1) or both portals (1 : 2). In the latter case, this gives rise to supramolecular aggregates, in which Au-NP are linked together to form chains (1D) or three-dimensional (3D) structures (Fig. 14).[111] The formation of such aggregate is a unique situation and is not observed for other macrocycles (for example, cyclodextrins or calixarenes). The stability of aggregates is influenced by the presence of sodium cations in the reaction solution. These ions block (through the coordination of the cation) the free portal of CB[*n*] (n = 5–8) bound to Au-NP (1 : 1), thus hindering the further aggregation of nanoparticles.[112]

The CB[*n*]–Au-NP aggregates with a repeatable, fixed (the value is strictly specified by the height of the CB[*n*] molecule, *i.e.*, ~0.9 nm) and rigid nanojunction proved to be convenient objects for the SERS (surface-enhanced Raman scattering, giant Raman scattering) method. Conventional SERS experiments are performed using molecules adsorbed on specially prepared rough metal surfaces or on small (10–100 nm) metal particles. The effect is manifested as an increase in the intensity of the lines due to the enhancement of the electromagnetic field between tightly connected nanoparticles (up to 10^{14} times for colloidal gold), thus making it possible to sense single molecules.[113] The SERS spectra of CB[*n*]–Au-NP (n = 5–8) show an enhancement of the signals of both CB[*n*] itself and molecules included in the cavity of these cavitands. For example, the inclusion of methyl viologen in CB[6] led to the enhancement of the SERS signals by about 10^8; the inclusion of ferrocene in CB[7] resulted in the SERS enhancement up to 10^9.[114–117]

The unique ability of cucurbit[*n*]urils to act as synthetic molecular vessels, in which bimolecular reactions between the specially selected guests proceed with high regio- and stereoselectivity, was successively employed in the design of SERS nanoreactors.[118] The SERS method can be used to monitor in real time the photoisomerization and photodimerization of stilbene inside the cavities of CB[7] and CB[8], respectively.

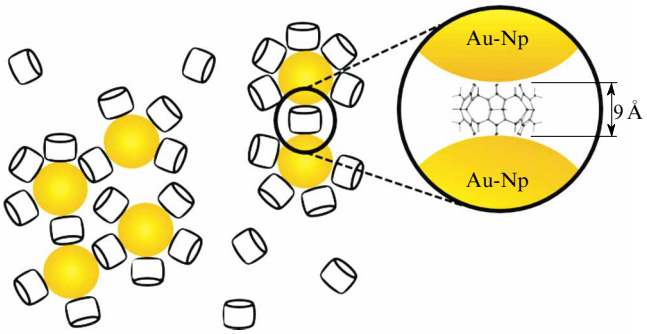


Figure 14. Formation of supramolecular aggregates through the interaction between gold nanoparticles and CB[*n*] portals.[111]

An original strategy was proposed for the synthesis of gold nanoparticle chains with CB[7] junctions.[119] The spontaneous 3D aggregation of nanoparticles is redirected into chain growth by the electrokinetic control of the interactions between Au-NP and CB[7] at the interface of a nanoporous polycarbonate membrane (Fig. 15). One-dimensional (1D) or branched (2D) chains are formed depending on the supply rate of CB[7] molecules to a compartment containing nanoparticles (Fig. 16). The chains, in which nanoparticles are at an equal specified distance from each other (in this case, the value is determined by the height of the CB[*n*] molecule, ~0.9 nm), are of great interest due to the unique plasmonic and electronic properties.

The CB[*n*]–Au-NP aggregates can be employed as optical probes for the detection of proteins by enzyme-linked immunoassay (based on the specific antigen–antibody reaction).[120] It is well known that CB[*n*] portals are bound to alkylammonium ions through ion–dipole interactions.[5] The enzyme urease was chosen as a label in enzyme-linked immunoassay. This protein is responsible

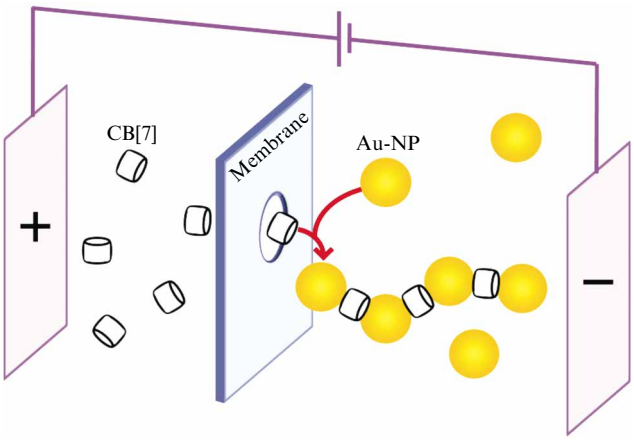


Figure 15. Assembly of supramolecular Au-NP–CB[7] chains based on the electrokinetic control of the interactions between CB[7] and gold nanoparticles at the interface of a nanoporous membrane.[119]

† HERFD is high-energy resolution fluorescence detected spin-polarized X-ray absorption spectroscopy.

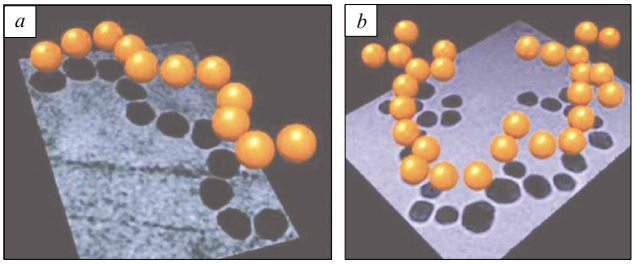


Figure 16. Images of one-dimensional (1D) (*a*) and branched (2D) (*b*) CB[7]–Au-NP chains calculated from cryogenic transmission electron microscopy (TEM) data.[119]

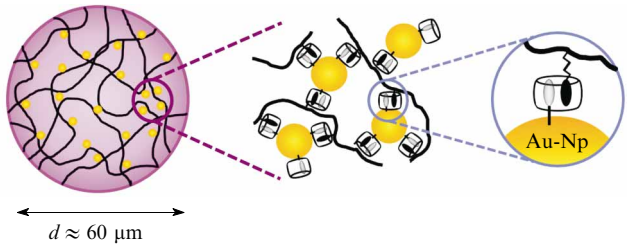


Figure 17. Microcapsule with a shell comprised of a polymer containing gold nanoparticles (held together by CB[8] as a supramolecular 'handcuff') dispersed in the polymer network.[123]

for the biological generation of ammonium ions. Urease-labelled antibodies were added to a CB[7]–Au-NP-containing solution of the antigen (mouse IgG) to be assayed. Ammonium ions that are produced due to the enzymatic activity of urease successfully competed with nanoparticles for CB[7] portals, CB[7]–Au-NP aggregates were disrupted, resulting in a shift of the line in the surface plasmon resonance spectrum proportionally to the IgG concentration. The use of the non-biological CB[*n*] molecule reduces the risk of false signals in the spectrum. Taking into account this fact, along with a good detection limit and a broad dynamic range of detectable concentrations characteristic of enzyme-linked immunoassay, this approach can be considered as alternative to colorimetric assay techniques for the determination of proteins.

The ability of CB[8] to form stable inclusion complexes is utilized both for the attachment of nanoparticles to the surface or organic polymers (see Fig. 10 *b*) and for adhesion of nanoparticles to each other.[74, 121–124] A stable materials containing gold nanoparticles on a silicon substrate was produced by immobilization of naphthyloxy-functionalized gold nanoparticles onto the viologen-functionalized surface using CB[8] as a supramolecular 'handcuff'.[74] This method can be applied to attach nanoparticles at definite sites on the surface and provides a tool to reversibly control the binding events. Therefore, the materials prepared by this method are of interest for integration into photonic devices, chemical and biological sensors and single molecule detectors.

The formation of inclusion compounds (methyl viologen–*trans*-4-hydroxyazobenzene)@CB[8] underlies the preparation of unique colloidal systems containing large particles (SiO_2 microspheres with a diameter of ~280 nm, TEM data) decorated with smaller gold nanoparticles at the surface.[121] The reversible assembly and diassembly of gold nanoparticles is provided by the ability of the *trans*-4-hydroxyazabenzene molecule to undergo, under light irradiation, a reversible transformation to the *cis* form, which cannot form inclusion compounds with CB[8].

Polymer films with a controlled dispersion of gold nanoparticles were assembled from a 2-naphthyloxy-functionalized organic copolymer and viologen-functionalized Au-NP held together by CB[8] as a supramolecular 'handcuff'.[122] This system (CB[8]–2-naphthyloxy-copolymer–methyl viologen-Au-NP) served as a platform for the unique technique for the fabrication of monodisperse microcapsules (diameter ~60 μm), the shell of which is comprised of a polymer containing gold nanoparticles dispersed in the polymer network and which contain water in the cavities (Fig. 17).[123] Due to the formation of a highly stable inclusion compound with CB[8], the microcapsules are resistant to heating to 100 °C. This technique can be applied to insert different compounds in the cavity of the capsule. The latter can be released by destroying the capsule shell (reversible encapsulation of guests in CB[8]). Due to the presence of gold nanoparticles, the microcapsules exhibit strong plasmonic properties and can be used as a SERS substrate for encapsulated materials.

IX.2. Cucurbit[*n*]urils and nanoparticles of other metals and their compounds

The stabilization of nanoparticles of other metals with cucurbit[*n*]urils has received less attention in the literature as compared with gold nanoparticles. Silver nanoparticles were synthesized by the reduction of an $AgNO_3$ aqueous solution with sodium borohydride.[125] The cucurbiturils CB[5] and CB[6] induce the rapid formation of aggregates with a diameter of >13 nm followed by their sedimentation, whereas CB[7] and CB[8] facilitate the formation of nanoparticles with a narrow size distribution and with diameters of ~5.3 and ~3.7 nm, respectively. The computer modelling (in terms of the density functional theory with the hybrid functional B3LYP) provided evidence that there are interactions between silver nanoparticles and the oxygen atoms of CB[*n*] portals, although the IR spectra do not display a shift of the C=O stretching band characteristic of coordination. Silver bromide nanoparticles CB[*n*]–AgBr-NP (n = 5, 7) were obtained in the presence of trace amounts of bromide anions in the reaction solution of silver nitrate and CB[*n*].[126] The irradiation of these nanoparticles with an electron beam produced in an electron microscope leads to the partial reduction of silver and the formation of so-called Janus particles composed of two components with different composition (AgBr and Ag). Cucurbit[*n*]uril molecules are bound to the surface of both silver and AgBr (the diameter of Janus particles is ~25 and ~10 nm in the case of CB[5] and CB[7], respectively) (Fig. 18). First investigations of the possibility of using silver nanoparticles stabilized with cucurbit[*n*]urils in biomedicine demonstrated that these nanoparticles hold promise for cancer therapy. Thus, the cytotoxicity of CB[7]–Ag-NP against breast adenocarcinoma cells and bronchiolar lung cancer cell attests to higher efficiency of these nanoparticles compared to the currently used glutathione-stabilized silver nanoparticles.[127]

Cucurbit[*n*]uril-stabilized palladium nanoparticles were synthesized by the reduction of a $PdCl_2$ aqueous solution with sodium borohydride or other reducing agents in the

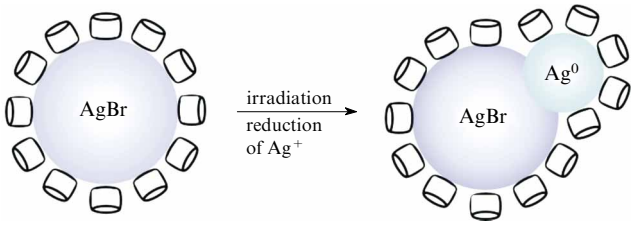


Figure 18. Stabilization of nanoparticles composed of two components with different composition (AgBr and Ag) with CB[5] or CB[7] molecules.[126]

presence of CB[*n*] ($n = 5-8$) ($\sim$3.3 nm for CB[6] and $\sim$2.5 nm for CB[7]).[128–132] The CB[*n*]–Pd-NP system proved to be a highly efficient catalyst for reactions commonly used in organic chemistry, such as the Suzuki and Heck reactions, direct C–H functionalization of fluorinated aromatic hydrocarbons, reduction of 4-nitrophenol and so on.

The reduction of an aqueous solution of H_2PtCl_6 with ethylene glycol or ascorbic acid in the presence of CB[6] gives rise to platinum nanoparticles ($\sim$5 nm).[133] The CB[6]–Pt-NP nanoparticles exhibit higher activity in the methanol electrooxidation than the commercial Pt/C catalyst and they are also more resistant to inhibition due to the ability of CB[6] to include small molecules, thus facilitating the methanol and CO oxidation.

A nanocapsule composed of CB[6] molecules linked together can be decorated with Pd, Au or Pt nanoparticles.[134] Advantages of this material are high stability and dispersibility in aqueous solutions, a specified nanoparticle size, as well as (for a Pd-containing system), excellent catalytic activity and recyclability in C–C and C–N bond forming reactions in an aqueous medium.

The size of Fe_3O_4 nanoparticles (redution with sodium acetate; the diameter is 150–500 nm) can be controlled by varying the CB[*n*] ($n = 5-8$) to $FeCl_3$ molar ratio in solution.[135] Such biocompatible (tested in the HEK293 cell line) magnetic nanoparticles are of interest for various medical applications.

Stabilization of ZnO nanoparticles, which are produced from a $Zn(NO_3)_2$ aqueous solution by the addition of NaOH in the presence of CB[7], is a facile route to this semiconductor with a particle size of $\sim$150 nm.[136]

The reaction of $Pb(NO_3)_2$, KI and CB[7] gave PbI_2 nanodiscs with a diameter of $\sim$5–34 nm and a thickness of $\sim$0.7 nm.[137] The disc thickness is comparable with the layer thickness in the crystal structure of lead iodide. It was hypothesized that CB[7] prevents the packing of PbI_2 layers, which restricts the growth of nanoparticles in the *z* direction.

The thermal decomposition of supramolecular inclusion compounds is an example of the unusual application of CB[*n*] ($n = 7, 8$) for the preparation of nanoparticles.[138, 139] In the inclusion compound of the copper acetate complex $[Cu_2(OAc)_4(H_2O)_2]$ with CB[7], the binuclear complex is located between two cavitands, two opposite acetate ligands being included in the cavities of CB[7].[138] The heating of this compound to 600 °C affords CuO nanoparticles having a much narrower particle size distribution and a smaller diameter ($\sim$4–6 nm) compared to CuO nanoparticles, which were prepared by heating copper acetate in the absence of cucurbit[7]uril.

Therefore, the simple one-pot reduction of metal salts in an aqueous solution in the presence of CB[*n*] is most often used to stabilize metal nanoparticles with cucurbit[*n*]urils. The size of the nanoparticles thus formed depends on the ratio of the reagents and is, on the average, $\leqslant$10 nm. The interaction between CB[*n*] and metal particles prevents their agglomeration, but it does not block the properties specific to nanoparticles, as exemplified by the cytotoxicity of CB[7]–Ag-NP or the catalytic activity of CB[6]–Pd-NP, CB[7]–Pd-NP and CB[6]–Pt-NP. All this is evidence that CB[*n*]-containing nanoparticles hold promise in medicine and catalysis. The CB[*n*]–Au-NP aggregates, in which nanoparticles are kept at a fixed distance from each other, are convenient objects for the SERS detection of molecules included in CB[*n*]. Taking into account a huge range of guest molecules for cucurbit[*n*]urils, these cavitands are highly promising for the design of chemical sensors and biosensors based on SERS detection.

X. Cucurbit[*n*]uril-based hydrogels

The similarity of hydrogels — three-dimensional frameworks formed by cross-linking of polymers — to soft biological tissues makes them increasingly important for the drug delivery, tissue engineering and other biomedical applications. Supramolecular hydrogels formed through noncovalent binding of polymers show rapid responsiveness to external stimuli, *e.g.* changes in the concentrations of the reagents, solvent, pH, temperature, illumination and so on. Systems based on natural and synthetic receptors (streptavidin–biotin, cyclodextrin–adamantane, *etc.*) are used for the supramolecular assembly of polymers. However, the application of the former receptors is limited by difficulties in performing chemical modification and producing these compounds on an industrial scale; besides, the mechanical properties of the resulting materials are in some cases unsatisfactory because of low binding constants of guest molecules to cyclodextrins.[140–142] Cucurbit[*n*]urils are attractive for the preparation of hydrogels because of their ability to form stable inclusion compounds in an aqueous medium. Thus, polymers bearing appropriate functional groups can be linked together by the encapsulation of these groups in the cavity of CB[8].

Upon the addition of CB[8], a colourless aqueous solution containing two polymers — a polymer bearing methyl viologen moieties in side chains (polymer A) and a polymer bearing 2-naphthoxy groups (polymer B) — is transformed into a viscous dark-red hydrogel, the colour of which is associated with visible light absorbance on account of the formation of a charge-transfer complex in the cavity of CB[8] (Fig. 19).[143] [Copolymers of *N*-(4-vinylbenzyl)-*N*′-methyl-4,4′-bipyridinium chloride iodide with *N*-hydroxyacrylamide, 4-vinylbenzyltrimethylammonium chloride or sodium styrene sulfonate prepared by free radical polymerization were used as the polymer A; a copolymer of *N*-(2-napthoxyethyl)acrylamide with dimetylacrylamide prepared by the chain-transfer radical polymerization was used as the polymer B.] Upon the addition of CB[8], the viscosity of the medium increases more than 10^3-fold. The process is thermally reversible, the viscosity and pore size depend on the CB[8] concentration and thermal treatment of the hydrogel (Fig. 20). The mechanical

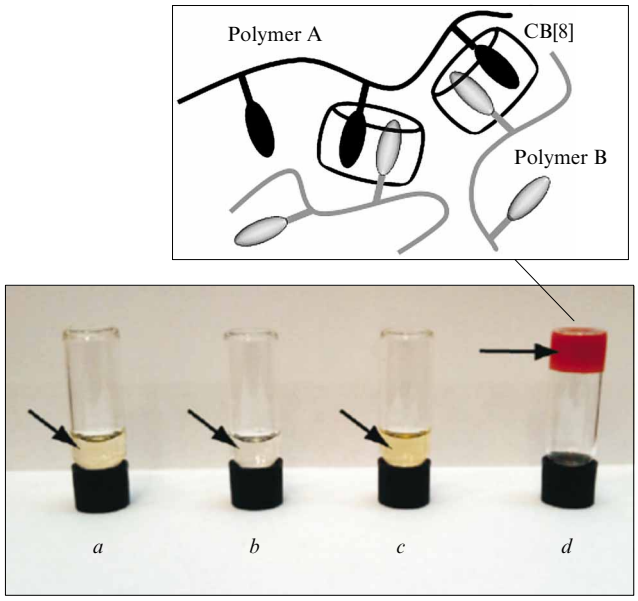


Figure 19. Hydrogel formation through the association of polymers *via* surpamolecular CB[8] 'handcuff'.[143]
(*a*) Polymer A bearing naphthoxy groups; (*b*) polymer B bearing methyl viologen moieties; (*c*) a mixture of polymers A + B; (*d*) the addition of CB[8] to a mixture of polymers A + B.

properties and viscosity of the resulting material are similar to those of supramolecular hydrogels used in practice.

CB[*n*]-Modified hydrogels with extremely high water content (up to 99.7%) were prepared from commercially available precursors — cellulose and poly(vinyl alcohol).[144, 145] The starting compounds were functionalized with moieties (naphthalene and methyl viologen, respectively) that form inclusion compounds with CB[8]. The resulting material retains high elasticity even at an extremely high water content, undergoes rapid self-healing after damage caused by deformation, displays responsiveness to changes in the temperature, chemical potential and to the presence of competing guests in the solution. The protein release characteristics of the hydrogel were investigated.[145] The sustained drug release is of great importance in the therapy of different diseases and makes it possible to replace the regular drug therapy with a single injection of a drug having a prolonged duration of action. Complete and extremely sustained (over the course of 160 days) release of bovine serum albumin from the above-described hydrogel containing only 1.5% polymeric constituents far surpasses the corresponding characteristics for other known hydrogels.

Supramolecular hydrogel based on CB[8] can also be prepared by the inclusion of two guests of the same type in the cavitand. The viologen groups of the copolymer of acrylamide with *N*-(4-vinylbenzyl)-4,4′-bipyridinium dichloride form a 1 : 2 inclusion compound with CB[8] only in an alkaline medium (in the presence of NaOH).[146] This hydrogel is responsive to changes in the temperature (reversible gel–sol transition takes place) and the CB[8] concentration. In an acidic medium, hydrogel of the 1 : 2 composition was produced *via* the inclusion of diethylaminobenzyl groups of functionalized chitosan in CB[8].[147] The distinguishing features of this hydrogel are high pH sensitivity and a very low rate of release, in an acidic medium, of the anticancer drug 5-fluorouracil embedded into this hydrogel. Therefore, this hydrogel holds promise for the pH-controlled drug delivery.

Smaller cucurbit[*n*]uril, CB[6], is also used for the preparation of hydrogels.[148, 149] Biocompatible hydrogel based on hyaluronic acid (polysaccharide that is present in human tissues) was prepared *via* the supramolecular assembly of two polymers — hyaluronic acid modified with CB[6] and hyaluronic acid modified with aminohexylamino groups (Fig. 21).[148] The reaction proceeds *in situ* in NIH3T3 fibroblast cells upon the addition of modified polymers without additional reagents or stimuli. Hydrogels with different physical and mechanical properties can be

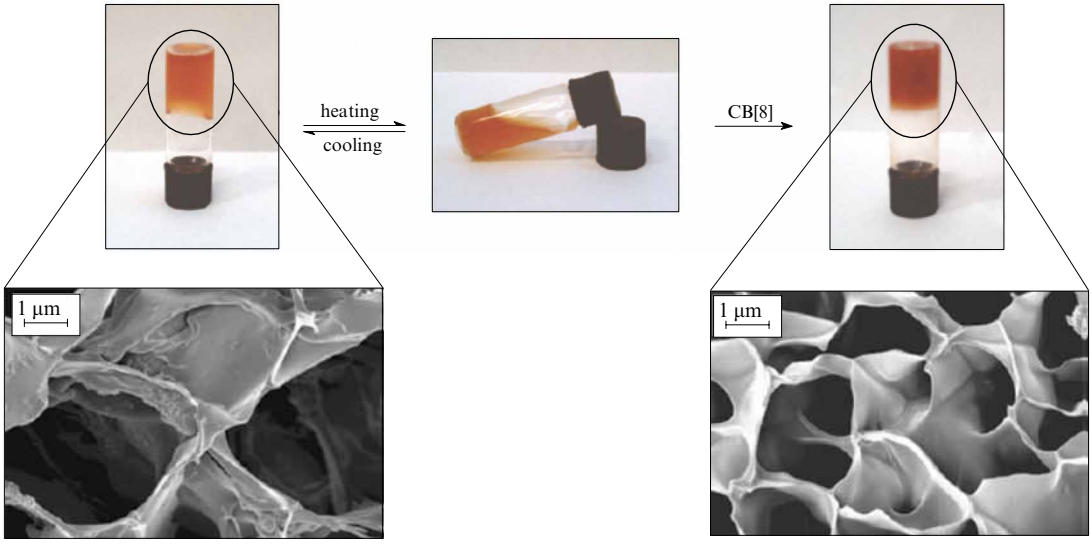


Figure 20. Dependence of the viscosity and pore size of the surpamolecular CB[8]-containing hydrogel on the thermal treatment and CB[8] concentration.[143]

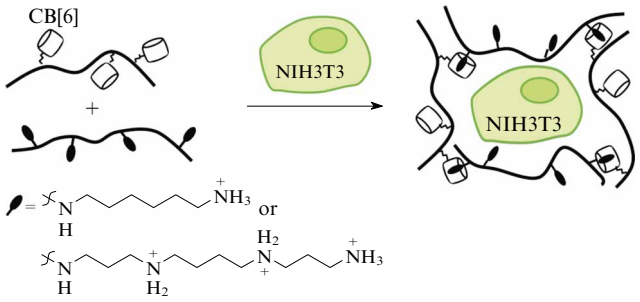


Figure 21. Formation of NIH3T3 cell-entrapped hydrogel *via* the supramolecular assembly of hyaluronic acid modified with CB[6] and of hyaluronic acid modified with polyamine.[148]

produced by replacing diaminohexane moieties with other groups, which can also form inclusion compounds with CB[6] (for example, spermine moieties). Park *et al.*[148] showed that in the case of subcutaneous injections of the starting polymers in mouse tissues, hydrogel was formed within a few minutes post-injection and it remained stable and kept its shape for longer than 2 weeks. It was also demonstrated that the finished hydrogel can be modified. For example, after the insertion of a solution of CB[6] modified with fluorescein isothiocyanate in the hydrogel, the fluorescence of the dye was observed *in vivo* for 11 days, whereas no fluorescence of carboxyfluorescein bound to CB[6] was observed in 1 day (Fig. 22). These results attest to the feasibility of the use of this hydrogel for cell therapy and 3D tissue engineering.

As opposed to other homologues, CB[7] is by itself able to form hydrogels in aqueous solutions of mineral acids due to rather strong interactions between the portals and the hydroxonium cations.[150] The hydrogel formation is thermally reversible, sensitive to changes in pH and is blocked in the presence of alkali metal cations. The inclusion of diaminostilbene in CB[7] leads to the controlled reversible gel–sol transition depending on the *trans* or *cis* configuration of the guest.

The hydrogel formation was observed upon cooling of an aqueous solution of CB[6] and n-butylammonium tosylate. In this case, hydrophobic, $\pi-\pi$ and van der Waals interactions between the tosylate moieties and CB[6] are responsible for the gel formation.[151]

Therefore, taking into account the simplicity of the synthesis of hydrogels based on cucurbit[*n*]urils, their rapid responsiveness to external stimuli, high elasticity, controlled viscosity, biocompatibility and the ability to provide sustained drug release, hydrogels can be considered as promising candidates for biomedical and other applications.

XI. Cucurbit[*n*]urils for gas sorption and separation

Microporous materials efficient for sorption and separation of different gases are mostly polymeric compounds, such as zeolites, metal-organic structures and porous organic polymers. These materials are chemically stable and have a high specific surface area. Meanwhile, examples of porous materials derived from discrete organic molecules linked together through multiple van der Waals interactions to form a framework are scarce. Such supramolecular frameworks are usually not quite stable and do not retain porosity after the removal of guest molecules. However, the possibility of controlling the properties of materials, including porosity, *via* different packing modes of large molecules in the solid material has attracted ever-increasing attention.

A porous material based on cucurbit[*n*]uril molecules as a framework was obtained by the recrystallization of CB[6] from hydrochloric acid.[152,153] In the material with the composition $CB[6]\cdot 0.5\,HCl\cdot 11\,H_2O$, the CB[6] molecules form a honeycomb-like structure comprising channels $\sim$6 Å in diameter through van der Waals interactions and C–H···O hydrogen bonds between adjacent macrocycles. The inner cavities of CB[6] and the channels are filled with water and HCl molecules, which can be almost completely removed upon heating to 200 °C, the supramolecular framework composed of cucurbit[6]uril molecules being retained. The surface area (Brunauer–Emmett–Teller method using nitrogen adsorption, 77 K) of this 'activated' CB[6] is 210 $m^2\ g^{-1}$, and the pore volume is 0.13 $cm^3\ g^{-1}$. These

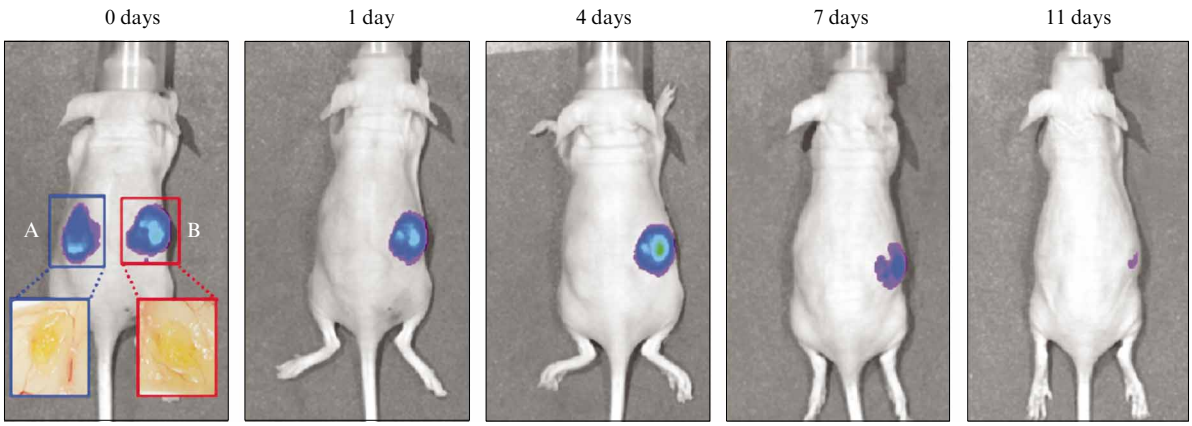


Figure 22. *In situ* formation of supramolecular CB[6]-containing hydrogel after subcutaneous injections in mouse tissues and the observation of fluorescence of the modifying dye.[148]
The labels A and B refer to carboxyfluorescein and fluorescein isothiocyanate, respectively, bound to CB[6].

values are comparable with the data for the metal-organic polymers $Mg(HCO_2)_2$ and $Mn(HCO_2)_2$ (284 and 0.14, 297 and 0.13, respectively [154, 155]). This microporous material is thermally and chemically stable and has high sorption characteristics with respect to acetylene (52 $cm^3\ g^{-1}$ at 298 K, 1 atm) and CO_2 (45 $cm^3\ g^{-1}$ at 298 K, 1 atm), which are also comparable with the corresponding values for a number of metal-organic coordination polymers. The most important and interesting property of this material is its highly selective sorption of carbon dioxide. The X-ray diffraction study of the structure of CB[6] with adsorbed CO_2 showed that carbon dioxide molecules are located both in intermolecular channels [held by hydrogen bonds and(or) dipole–quadrupole interactions with the CB[6] molecule] and inside the cavity (Fig. 23). It was hypothesized that these multiple surpamolecular interactions are responsible for a high enthalpy of CO_2 adsorption (33.0 kJ mol^{-1}). The selectivity of CO_2/CH_4 adsorption (Henry constant is 14.8) is attributed to the absence of interactions between methane molecules and the portal oxygen atoms.

The recrystallization of CB[6] from hydrochloric acid in an aqueous ethanol solution afforded three structurally different forms of CB[6], in the crystal structures of which the macrocycles are packed in layers.[156] One layered structure retains the supramolecular framework after the removal of guest molecules, and this 'activated' form adsorbs 76 $cm^3\ g^{-1}$ (298 K, 1 atm) of carbon dioxide, which corresponds to 15 wt.%. This value is substantially higher than the corresponding characteristics of any porous molecular material.

In the amorphous state, a material based on CB[*n*] larger than CB[7] also has high thermal stability and porosity and is able to selectively adsorb CO_2 (50 $cm^3\ g^{-1}$, 10 mass %, 297 K, 1 atm) and SO_2 (105 $cm^3\ g^{-1}$, 30 mass %, 297 K, 1 atm).[157] High adsorption characteristics toward SO_2 are attributed to a strong dipole–dipole interaction between these polar molecules and the CB[7] portals. The enthalpy of CO_2 adsorption (40.0 kJ mol^{-1}) is substantially higher than the corresponding values for porous organic materials and is comparable with the values for some metal-organic polymers.

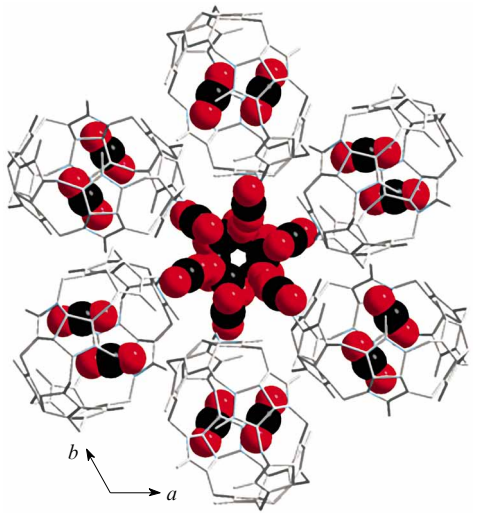


Figure 23. Arrangement of carbon dioxide molecules in intermolecular channels and inside the cavity of the cavitands in the structure of CB[6] with adsorbed CO_2.[153]

Therefore, porous materials based on CB[*n*] are highly promising for gas sorption and separation. The presence of both intramolecular and intermolecular cavities in the structures of these materials provides high adsorption performance, and the interactions with the portal oxygen atoms are responsible for the selectivity of separation of different gas molecules. The selective adsorption properties of CB[*n*] make it useful for the separation of CO_2 in metallurgical industry, processing technologies for CO_2 removal from natural gas (separation of CO_2 and CH_4) and so on. Of particular interest is the use of CB[*n*] in materials for the purification of flue gases from SO_2 taking into account that most of metal-organic and porous organic polymers are sensitive to water, which is the main component of combustion products of carbon-containing fuels.[157]

XII. Proton conductivity of cucurbit[*n*]urils

The fact that organic molecular materials can exhibit proton-conducting properties was demonstrated for the first time for CB[*n*]-based porous organic materials.[158] The conductivity value of these materials is comparable with that of known proton-conducting materials, for example, metal-organic coordination polymers. Materials having high proton conductivity have attracted great attention because of their potential applications as solid electrolytes, catalysts, proton sensors, proton-conducting membranes in fuel cells and so on. In the compounds $CB[6]\cdot 1.1\,HCl\cdot 11.3\,H_2O$, $CB[6]\cdot 1.2\,H_2SO_4\cdot 6.4\,H_2O$ and $CB[8]\cdot 6.8\,HCO_2H\cdot 13\,H_2O$, which were prepared by the recrystallization of CB[*n*] from hydrochloric, sulfuric and formic acids, respectively, the macrocycles form a honeycomb-like structure comprising channels ~7.5 Å in diameter, which are filled with acid and water molecules linked to each other by hydrogen bonds. The proton conductivity depends on the nature of the acid that fills the channels and is 1.1×10^{-3}, 1.3×10^{-3} and 1.3×10^{-4} S cm^{-1} (298 K), respectively. These values are comparable with the largest value found for coordination polymers (8×10^{-3} S cm^{-1}).[159] Although these values are smaller that those for the widely used organic proton-conducting material Nafion, single-crystal studies of CB[*n*]-based compounds showed that the latter have highly anisotropic proton conductivity (the conductivity along the channels is much higher than that in the perpendicular direction). The compound $CB[6]\cdot 1.2\,H_2SO_4\cdot 6.4\,H_2O$ containing sulfuric acid in the channels shows the highest anisotropy among known polymer materials ($\sigma_{\parallel}:\sigma_{\perp}=8600$). Therefore, the latter compound has potential applications in devices with highly directed proton conductivity.

* * *

More than a century ago, Robert Behrend and co-workers,[1] when studying the condensation of glycoluril and formaldehyde, discovered a new compound, which was later named cucurbituril. The second 'discovery' of cucurbituril was made in 1981, when Mock and co-workers [2] determined the structure of this compound by X-ray diffraction. Recent advances in the chemistry of these macrocycles, particularly with the availability of homologues of different sizes, as well as of soluble functionalized derivatives, disclosed various

potential applications of cucurbit[*n*]urils. Although the chemistry of inclusion compounds of these cavitands has been studied for a relatively short period, researchers already obtained results significant for medicine. Thus, the inclusion in CB[*n*] leads to a substantial decrease in toxicity of platinum complexes, enhances anticancer activity of metal cyclopentadienyl complexes and ensures the controlled release of biologically active guest molecules. All this gives promise that cucurbit[*n*]urils have a considerable potential for the design of a new generation of long-acting pharmaceuticals. Due to a wide range of applications of CB[*n*] in various materials with unique properties — polymers, nanoparticles, hydrogels and so on — these macrocycles are very attractive for researchers. In just one year, 2015, several reviews concerning the chemistry of cucurbit[*n*]urils appeared, most of them being published during the preparation of the present review for publication.[16, 160 – 164] Taking into account growing interest in the prospects of the use of cucurbit[*n*]urils, it would be expected that these compounds will occupy a proper place among new functional materials in the foreseeable future.

This review has been written with the financial support of the Council on Grants at the President of the Russian Federation (Project NSh-7178.2016.3).

References

1. R Behrend, E Meyer, F Rusche *Justus Liebigs Ann. Chem.* **339** 1 (1905)
2. W A Freeman, W L Mock, N-Y Shih *J. Am. Chem. Soc.* **103** 7367 (1981)
3. J Kim, I-S Jung, S-Y Kim, E Lee, J-K Kang, S Sakamoto, K Yamaguchi, K Kim *J. Am. Chem. Soc.* **122** 540 (2000)
4. A Day, A P Arnold, R J Blanch, B Snushall *J. Org. Chem.* **66** 8094 (2001)
5. J Lagona, P Mukhopadhyay, S Chakrabarti, L Isaacs *Angew. Chem., Int. Ed.* **44** 4844 (2005)
6. A R Mustafina, V V Skripacheva, A I Konovalov *Russ. Chem. Rev.* **76** 917 (2007)
7. J W Lee, S Samal, N Selvapalam, H-J Kim, K Kim *Acc. Chem. Res.* **36** 621 (2003)
8. L Cao, M Šekutor, P Y Zavalij, K Mlinarić-Majerski, R Glaser, L Isaacs *Angew. Chem., Int. Ed.* **53** 988 (2014)
9. K Kim, N Selvapalam, Y H Ko, K M Park, D Kim, J Kim *Chem. Soc. Rev.* **36** 267 (2007)
10. L Isaacs *Chem. Commun.* 619 (2009)
11. Y H Ko, I Hwang, D-W Lee, K Kim *Isr. J. Chem.* **51** 506 (2011)
12. N Saleh, I Ghosh, W M Nau, in *Supramolecular Systems in Biomedical Fields* (Ed. H-J Schneider) (Cambridge: Royal Society of Chemistry, 2013) p. 164
13. S Walker, R Oun, F J McInnes, N J Wheate *Isr. J. Chem.* **51** 616 (2011)
14. A R Urbach, V Ramalingam *Isr. J. Chem.* **51** 664 (2011)
15. E Masson, X Ling, R Joseph, L Kyeremeh-Mensah, X Lu *RSC Adv.* **2** 1213 (2012)
16. K I Assaf, W M Nau *Chem. Soc. Rev.* **44** 394 (2015)
17. O A Gerasko, D G Samsonenko, V P Fedin *Russ. Chem. Rev.* **71** 741 (2002)
18. O A Gerasko, M N Sokolov, V P Fedin *Pure Appl. Chem.* **76** 1633 (2004)
19. O A Gerasko, V P Fedin *Russ. J. Inorg. Chem.* **56** 2025 (2011)
20. N J Wheate, A I Day, R J Blanch, A P Arnold, C Cullinane, J G Collins *Chem. Commun.* 1424 (2004)
21. V D Uzunova, C Cullinane, K Brix, W M Nau, A I Day *Org. Biomol. Chem.* **8** 2037 (2010)
22. G Hettiarachchi, D Nguyen, J Wu, D Lucas, D Ma, L Isaacs, V Briken *PLoS ONE* **5** e10514 (2010)
23. P Montes-Navajas, M González-Béjar, J C Scaiano, H García *Photochem. Photobiol. Sci.* **8** 1743 (2009)
24. Y J Jeon, S-Y Kim, Y H Ko, S Sakamoto, K Yamaguchi, K Kim *Org. Biomol. Chem.* **3** 2122 (2005)
25. J Kim, Y Ahn, K M Park, Y Kim, Y H Ko, D H Oh, K Kim *Angew. Chem., Int. Ed.* **46** 7393 (2007)
26. S Mecozzi, J Rebek Jr *Chem. – Eur. J.* **4** 1016 (1998)
27. H Chen, J Y W Chan, X Yang, I W Wyman, D Bardelang, D H Macartney, S M Y Lee, R Wang *RSC Adv.* **5** 30067 (2015)
28. N J Wheate *J. Inorg. Biochem.* **102** 2060 (2008)
29. J A Plumb, B Venugopal, R Oun, N Gomez-Roman, Y Kawazoe, N S Venkataramanan, N J Wheate *Metallomics* **4** 561 (2012)
30. A R Kennedy, A J Florence, F J McInnes, N J Wheate *Dalton Trans.* 7695 (2009)
31. Y Zhao, M S Bali, C Cullinane, A I Day, J G Collins *Dalton Trans.* 5190 (2009)
32. N J Wheate, D P Buck, A I Day, J G Collins *Dalton Trans.* 451 (2006)
33. M S Bali, D P Buck, A J Coe, A I Day, J G Collins *Dalton Trans.* 5337 (2006)
34. Patent WO 2005068469 (2005)
35. D P Buck, P M Abeysinghe, C Cullinane, A I Day, J G Collins, M M Harding *Dalton Trans.* 2328 (2008)
36. F Li, M Feterl, J M Warner, A I Day, F R Keene, J G Collins *Dalton Trans.* **42** 8868 (2013)
37. M J Pisani, Y Zhao, L Wallace, C E Woodward, F R Keene, A I Day, J G Collins *Dalton Trans.* **39** 2078 (2010)
38. Patent WO 2003024978 (2003)
39. R Wang, D Bardelang, M Waite, K A Udachin, D M Leek, K Yu, C I Ratcliffe, J A Ripmeester *Org. Biomol. Chem.* **7** 2435 (2009)
40. O Danylyuk, V P Fedin, V Sashuk *Chem. Commun.* **49** 1859 (2013)
41. Q Liu, Q Tang, Y-Y Xi, Y Huang, X Xiao, Z Tao, S-F Xue, Q-J Zhu, J-X Zhang, G Wei *Supramol. Chem.* **27** 386 (2015)
42. N J Wheate, V Vora, N G Anthony, F J McInnes *J. Inclusion Phenom. Macrocycl. Chem.* **68** 359 (2010)
43. K Gavvala, A Sengupta, P Hazra *ChemPhysChem* **14** 532 (2013)
44. F J McInnes, N G Anthony, A R Kennedy, N J Wheate *Org. Biomol. Chem.* **8** 765 (2010)
45. N Saleh, M A Meetani, L Al-Kaabi, I Ghosh, W M Nau *Supramol. Chem.* **23** 650 (2011)
46. H Cong, C-R Li, S-F Xue, Z Tao, Q-J Zhu, G Wei *Org. Biomol. Chem.* **9** 1041 (2011)
47. Z Miskolczy, M Megyesi, G Tárkányi, R Mizsei, L Biczók *Org. Biomol. Chem.* **9** 1061 (2011)
48. R Wang, B C MacGillivray, D H Macartney *Dalton Trans.* 3584 (2009)
49. Y Zhao, D P Buck, D L Morris, M H Pourgholami, A I Day, J G Collins *Org. Biomol. Chem.* **6** 4509 (2008)
50. Y Huang, Q-H Hu, G-X Song, Z Tao, S-F Xue, Q-J Zhu, Q-d Zhou, G Wei *RSC Adv.* **4** 3348 (2014)
51. Y Lü, L-f Yan, N Dong *Spectrosc. Spectr. Anal.* **34** 1610 (2014)
52. N Saleh, A L Koner, W M Nau *Angew. Chem., Int. Ed.* **47** 5398 (2008)
53. I W Wyman, D H Macartney *Org. Biomol. Chem.* **8** 247 (2010)
54. O Danylyuk, V P Fedin *Cryst. Growth Des.* **12** 550 (2012)
55. E A Kovalenko, D A Mainichev *Appl. Magn. Reson.* **46** 281 (2015)
56. J-M Yi, Y-Q Zhang, H Cong, S-F Xue, Z Tao *J. Mol. Struct.* **933** 112 (2009)

57. J W Lee, H H L Lee, Y H Ko, K Kim, H I Kim *J. Phys. Chem. B* **119** 4628 (2015)
58. L C Smith, D G Leach, B E Blaylock, O A Ali, A R Urbach *J. Am. Chem. Soc.* **137** 3663 (2015)
59. J M Chinai, A B Taylor, L M Ryno, N D Hargreaves, C A Morris, P J Hart, A R Urbach *J. Am. Chem. Soc.* **133** 8810 (2011)
60. H H Lee, T S Choi, S J C Lee, J W Lee, J Park, Y H Ko, W J Kim, K Kim, H I Kim *Angew. Chem., Int. Ed.* **53** 7461 (2014)
61. M E Bush, N D Bouley, A R Urbach *J. Am. Chem. Soc.* **127** 14511 (2005)
62. P Rajgariah, A R Urbach *J. Inclusion Phenom. Macrocycl. Chem.* **62** 251 (2008)
63. A Hennig, G Ghale, W M Nau *Chem. Commun.* 1614 (2007)
64. S Y Jon, N Selvapalam, D H Oh, J-K Kang, S-Y Kim, Y J Jeon, J W Lee, K Kim *J. Am. Chem. Soc.* **125** 10186 (2003)
65. J Choi, J Kim, K Kim, S-T Yang, J-I Kim, S Jon *Chem. Commun.* 1151 (2007)
66. H-K Lee, K M Park, Y J Jeon, D Kim, D H Oh, H S Kim, C K Park, K Kim *J. Am. Chem. Soc.* **127** 5006 (2005)
67. D Kim, E Kim, J Kim, K M Park, K Baek, M Jung, Y H Ko, W Sung, H S Kim, J H Suh, C G Park, O S Na, D-k Lee, K E Lee, S S Han, K Kim *Angew. Chem., Int. Ed.* **46** 3471 (2007)
68. D Kim, E Kim, J Lee, S Hong, W Sung, N Lim, C G Park, K Kim *J. Am. Chem. Soc.* **132** 9908 (2010)
69. K M Park, K Suh, H Jung, D-W Lee, Y Ahn, J Kim, K Baek, K Kim *Chem. Commun.* 71 (2009)
70. E Kim, D Kim, H Jung, J Lee, S Paul, N Selvapalam, Y Yang, N Lim, C G Park, K Kim *Angew. Chem., Int. Ed.* **49** 4405 (2010)
71. X Huang, N Zhang, L Ban, H Su *Macromol. Rapid Commun.* **36** 311 (2015)
72. K Baek, G Yun, Y Kim, D Kim, R Hota, I Hwang, D Xu, Y H Ko, G H Gu, J H Suh, C G Park, B J Sung, K Kim *J. Am. Chem. Soc.* **135** 6523 (2013)
73. Q An, G Li, C Tao, Y Li, Y Wu, W Zhang *Chem. Commun.* 1989 (2008)
74. F Tian, N Cheng, N Nouvel, J Geng, O A Scherman *Langmuir* **26** 5323 (2010)
75. K Kim, W S Jeon, J-K Kang, J W Lee, S Y Jon, T Kim, K Kim *Angew. Chem., Int. Ed.* **42** 2293 (2003)
76. K Kim, D Kim, J W Lee, Y H Ko, K Kim *Chem. Commun.* 848 (2004)
77. X Ma, Y Xue, L Dai, A Urbas, Q Li *Eur. J. Inorg. Chem.* 2682 (2013)
78. W S Jeon, K Moon, S H Park, H Chun, Y H Ko, J Y Lee, E S Lee, S Samal, N Selvapalam, M V Rekharsky, V Sindelar, D Sobransingh, Y Inoue, A E Kaifer, K Kim *J. Am. Chem. Soc.* **127** 12984 (2005)
79. M V Rekharsky, T Mori, C Yang, Y H Ko, N Selvapalam, H Kim, D Sobransingh, A E Kaifer, S Liu, L Isaacs, W Chen, S Moghaddam, M K Gilson, K Kim, Y Inoue *Proc. Natl. Acad. Sci. USA* **104** 20737 (2007)
80. S Yi, W Li, D Nieto, I Cuadrado, A E Kaifer *Org. Biomol. Chem.* **11** 287 (2013)
81. M J Swamy *Biochem. Mol. Biol. Int.* **36** 219 (1995)
82. Y Ahn, Y Jang, N Selvapalam, G Yun, K Kim *Angew. Chem., Int. Ed.* **52** 3140 (2013)
83. A E Kaifer *ChemPhysChem* **14** 1107 (2013)
84. H-J Kim, J Heo, W S Jeon, E Lee, J Kim, S Sakamoto, K Yamaguchi, K Kim *Angew. Chem., Int. Ed.* **40** 1526 (2001)
85. I Hwang, K Baek, M Jung, Y Kim, K M Park, D-W Lee, N Selvapalam, K Kim *J. Am. Chem. Soc.* **129** 4170 (2007)
86. J F Young, H D Nguyen, L Yang, J Huskens, P Jonkheijm, L Brunsveld *ChemBioChem.* **11** 180 (2010)
87. A González-Campo, M Brasch, D A Uhlenheuer, A Gómez-Casado, L Yang, L Brunsveld, J Huskens, P Jonkheijm *Langmuir* **28** 16364 (2012)
88. P Neirynck, J Brinkmann, Q An, D W J van der Schaft, L-G Milroy, P Jonkheijm, L Brunsveld *Chem. Commun.* **49** 3679 (2013)
89. Q An, J Brinkmann, J Huskens, S Krabbenborg, J de Boer, P Jonkheijm *Angew. Chem., Int. Ed.* **51** 12233 (2012)
90. S Sankaran, M C Kiren, P Jonkheijm *ACS Nano* **9** 3579 (2015)
91. S-M Liu, L Xu, C-T Wu, Y-Q Feng *Talanta* **64** 929 (2004)
92. E R Nagarajan, D H Oh, N Selvapalam, Y H Ko, K M Park, K Kim *Tetrahedron Lett.* **47** 2073 (2006)
93. L Ma, S Liu, Q Wang, L Yao, L Xu *J. Sep. Sci.* **38** 1082 (2015)
94. F Tian, M Cziferszky, D Jiao, K Wahlström, J Geng, O A Scherman *Langmuir* **27** 1387 (2011)
95. D-W Lee, K M Park, M Banerjee, S H Ha, T Lee, K Suh, S Paul, H Jung, J Kim, N Selvapalam, S H Ryu, K Kim *Nat. Chem.* **3** 154 (2011)
96. E Blanco, C Quintana, L Hernández, P Hernández *Electroanalysis* **25** 263 (2013)
97. E V Stenina, L N Sviridova *Mendeleev Commun.* **25** 59 (2015)
98. H D Correia, G J-F Demets *Electrochem. Commun.* **11** 1928 (2009)
99. M del Pozo, P Hernández, L Hernández, C Quintana *J. Mater. Chem.* **21** 13657 (2011)
100. M del Pozo, E Blanco, E Fatás, P Hernández, C Quintana *Analyst* **137** 4302 (2012)
101. M del Pozo, J Mejtas, P Hernández, C Quintana *Sensors Actuators B: Chem.* **193** 62 (2014)
102. M C Tadini, M A Balbino, I C Eleoterio, L S de Oliveira, L G Dias, G J-F Demets, M F de Oliveira *Electrochim. Acta* **121** 188 (2014)
103. M Buaki-Sogo, M del Pozo, P Hernández, H García, C Quintana *Talanta* **101** 135 (2012)
104. V I Bukhtiyarov *Russ. Chem. Rev.* **76** 553 (2007)
105. X Huang, S Tang, H Zhang, Z Zhou, N Zheng *J. Am. Chem. Soc.* **131** 13916 (2009)
106. S -W Kim, J Park, Y Jang, Y Chung, S Hwang, T Hyeon, Y W Kim *Nano Lett.* **3** 1289 (2003)
107. A Corma, H García, P Montes-Navajas, A Primo, J J Calvino, S Trasobares *Chem. – Eur. J.* **13** 6359 (2007)
108. P Montes-Navajas, L C Damonte, H García *ChemPhysChem* **10** 812 (2009)
109. P Montes-Navajas, H García *J. Phys. Chem. C* **114** 18847 (2010)
110. S B Erenburg, S V Trubina, E A Kovalenko, O A Gerasko, V I Zaikovskii, K Kvashnina, S G Nikitenko *J. Exp. Theor. Phys. Lett.* **97** 285 (2013) [*Pis'ma v Zh. Eksp. Teor. Fiz.* **97** 326 (2013)]
111. T-C Lee, O A Scherman *Chem. Commun.* **46** 2438 (2010)
112. T-C Lee, O A Scherman *Chem. – Eur. J.* **18** 1628 (2012)
113. A M Schwartzberg, C D Grant, A Wolcott, C E Talley, T R Huser, R Bogomolni, J Z Zhang *J. Phys. Chem. B* **108** 19191 (2004)
114. R W Taylor, T-C Lee, O A Scherman, R Esteban, J Aizpurua, F M Huang, J J Baumberg, S Mahajan *ACS Nano* **5** 3878 (2011)
115. S Mahajan, T-C Lee, F Biedermann, J T Hugall, J J Baumberg, O A Scherman *Phys. Chem. Chem. Phys.* **112** 10429 (2010)
116. C-a Tao, Q An, W Zhu, H Yang, W Li, C Lin, D Xu, G Li *Chem. Commun.* **47** 9867 (2011)
117. J C Fraire, V N S Ocello, L G Allende, A V Veglia, E A Coronado *J. Phys. Chem. C* **119** 8876 (2015)
118. R W Taylor, R J Coulston, F Biedermann, S Mahajan, J J Baumberg, O A Scherman *Nano Lett.* **13** 5985 (2013)

119. N Hmsken, R W Taylor, D Zigah, J-C Taveau, O Lambert, O A Scherman, J J Baumberg, A Kuhn *Nano Lett.* **13** 6016 (2013)
120. R de la Rica, A H Velders *Small* **7** 66 (2011)
121. Y Lan, Y Wu, A Karas, O A Scherman *Angew. Chem., Int. Ed.* **53** 2166 (2014)
122. R J Coulston, S T Jones, T-C Lee, E A Appel, O A Scherman *Chem. Commun.* **47** 164 (2011)
123. J Zhang, R J Coulston, S T Jones, J Geng, O A Scherman, C Abell *Science* **335** 690 (2012)
124. S T Jones, J M Zayed, O A Scherman *Nanoscale* **5** 5299 (2013)
125. X Lu, E Masson *Langmuir* **27** 3051 (2011)
126. G Loget, T C Lee, R W Taylor, S Mahajan, O Nicoletti, S T Jones, R J Coulston, V Lapeyre, P Garrigue, P A Midgley, O A Scherman, J J Baumberg, A Kuhn *Small* **8** 2698 (2012)
127. T Premkumar, Y Lee, K E Geckeler *Chem. – Eur. J.* **16** 11563 (2010)
128. M Cao, J Lin, H Yang, R Cao *Chem. Commun.* **46** 5088 (2010)
129. K Karami, N H Naeini *Appl. Organomet. Chem.* **29** 33 (2015)
130. M Cao, D Wu, W Su, R Cao *J. Catal.* **321** 62 (2015)
131. M Cao, Y Wei, S Gao, R Cao *Catal. Sci. Technol.* **2** 156 (2012)
132. T Premkumar, K E Geckeler *Mater. Chem. Phys.* **148** 772 (2014)
133. M Cao, D Wu, S Gao, R Cao *Chem. – Eur. J.* **18** 12978 (2012)
134. G Yun, Z Hassan, J Lee, J Kim, N-S Lee, N H Kim, K Baek, I Hwang, C G Park, K Kim *Angew. Chem., Int. Ed.* **53** 6414 (2014)
135. X-L Qiu, Y Zhou, X-Y Jin, A-D Qi, Y-W Yang *J. Mater. Chem. C* **3** 3517 (2015)
136. L F S Da Silva, F Leroux, C Taviot-Guého, J B Valim, G J-F Demets *Synth. React. Inorg. Met.-Org. Nano-Met. Chem.* **43** 1078 (2013)
137. E M S dos Santos, L S Pereira, G J-F Demets *J. Braz. Chem. Soc.* **22** 1595 (2011)
138. T Premkumar, K E Geckeler *Small* **2** 616 (2006)
139. V V Bakovets, V A Nadolinnii, E A Kovalenko, P E Plyusnin, I P Dolgovesova, V I Zaikovskii *J. Solid State Chem.* **221** 202 (2015)
140. M Ehrbar, R Schoenmakers, E H Christen, M Fussenegger, W Weber *Nat. Mater.* **7** 800 (2008)
141. C Koopmans, H Ritter *Macromolecules* **41** 7418 (2008)
142. Q Wang, J L Mynar, M Yoshida, E Lee, M Lee, K Okuro, K Kinbara, T Aida *Nature (London)* **463** 339 (2010)
143. E A Appel, F Biedermann, U Rauwald, S T Jones, J M Zayed, O A Scherman *J. Am. Chem. Soc.* **132** 14251 (2010)
144. E A Appel, X J Loh, S T Jones, F Biedermann, C A Dreiss, O A Scherman *J. Am. Chem. Soc.* **134** 11767 (2012)
145. E A Appel, X J Loh, S T Jones, C A Dreiss, O A Scherman *Biomaterials* **33** 4646 (2012)
146. H Yang, H Chen, Y Tan *RSC Adv.* **3** 3031 (2013)
147. Y Lin, L Li, G Li *Carbohydr. Polym.* **92** 429 (2013)
148. K M Park, J-A Yang, H Jung, J Yeom, J S Park, K-H Park, A S Hoffman, S K Hahn, K Kim *ACS Nano* **6** 2960 (2012)
149. H Jung, J S Park, J Yeom, N Selvapalam, K M Park, K Oh, J-A Yang, K H Park, S K Hahn, K Kim *Biomacromolecules* **15** 707 (2014)
150. I Hwang, W S Jeon, H-J Kim, D Kim, H Kim, N Selvapalam, N Fujita, S Shinkai, K Kim *Angew. Chem., Int. Ed.* **46** 210 (2007)
151. H Yang, Y Tan, Y Wang *Soft Matter* **5** 3511 (2009)
152. S Lim, H Kim, N Selvapalam, K-J Kim, S J Cho, G Seo, K Kim *Angew. Chem., Int. Ed.* **47** 3352 (2008)
153. H Kim, Y Kim, M Yoon, S Lim, S M Park, G Seo, K Kim *J. Am. Chem. Soc.* **132** 12200 (2010)
154. D G Samsonenko, H Kim, Y Sun, G-H Kim, H-S Lee, K Kim *Chem. – Asian J.* **2** 484 (2007)
155. D N Dybtsev, H Chun, S H Yoon, D Kim, K Kim *J. Am. Chem. Soc.* **126** 32 (2004)
156. J Tian, Jian Liu, Jun Liu, P K Thallapally *CrystEngComm* **15** 1528 (2013)
157. J Tian, S Ma, P K Thallapally, D Fowler, B P McGrail, J L Atwood *Chem. Commun.* **47** 7626 (2011)
158. M Yoon, K Suh, H Kim, Y Kim, N Selvapalam, K Kim *Angew. Chem., Int. Ed.* **50** 7870 (2011)
159. M Sadakiyo, T Yamada, H Kitagawa *J. Am. Chem. Soc.* **131** 9906 (2009)
160. A A Elbashir, H Y Aboul-Enein *Crit. Rev. Anal. Chem.* **45** 52 (2015)
161. D Shetty, J K Khedkar, K M Park, K Kim *Chem. Soc. Rev.* **44** 8747 (2015)
162. N Zhang, X Huang, L Ban, H Su *Prog. Chem.* **27** 192 (2015)
163. S J Barrow, S Kasera, M J Rowland, J del Barrio, O A Scherman *Chem. Rev.* **115** 12320 (2015)
164. E N Ushakov, S P Gromov *Russ. Chem. Rev.* **84** 787 (2015)