Cucurbiturils as Molecular Containers:
The Mechanism of Complexation of Small Guests, the Effects of the
Inclusion on their Photophysical Properties, and Potential
Applications

A thesis presented by

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to

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To my family and friends
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Abstract

In this thesis the mechanism of complexation of small guest by the cavity of cucurbiturils has been investigated, as well as the effect provoked by the inclusion on their photophysical properties. Special attention has been paid to potential applications of these host-guest systems.

Detailed mechanistic studies on the host-guest complexation of organic cations inside the core of cucurbit[6]uril (CB6) based on kinetic and thermodynamic considerations have been accomplished. The binding constants and complexation rate constants for organic amine and ammonium compounds were found to vary in a contrasting manner with pH, due to a different complexation mechanism and stability of the host-guest system. The studies as a function of guest size indicated that the effective container volume of the CB6 cavity is approximately 100 Å³. It is suggested that larger guests are excluded for two reasons: a high activation barrier for ingression imposed by the tight CB6 portals and a destabilization of the complex due to steric repulsion inside.

The association of metal cations at the portals and the CB6 molecular recognition capacity has been explored. When the concentration of the inorganic salt present (necessary to dissolve CB6) was increased, the binding constant of the guest decreased due to a competitive binding of the metal ion and the ammonium site of the guest with the ureido carbonyl portals of CB6. Other factors such as the
size of the cation have been studied, and indicate that the guest binding constants decrease with an increase in cation binding constant.

The “inner phase” of cucurbiturils – in particular the larger cucurbit[7]uril (CB7) – and Cram’s hemicarcerands have been investigated by studying the polarizability inside them. For this purpose, the azoalkane 2,3-diazabicyclo[2.2.2]oct-2-ene (DBO) and biacetyl were included as guest molecules, and bulk polarizability-dependent solvatochromic shifts were measured in comparison with different solvents. The extreme polarizability obtained for CB7 (0.12) and hemicarcerands (0.45) reinforce the hypothesis that these cavities may behave like a new phase of matter.

The possibilities of CBn have been expanded by using CB7 as a chemical tool to discriminate between different fluorescence quenching mechanisms in the interaction between DBO and nucleotides, establishing exciplex formation and hydrogen abstraction as the main mechanisms depending on the base. “Through-distance” quenching mechanisms common for most of the cases described in the literature could therefore be disregarded.

Finally, potential applications of the CB7•DBO host-guest system have been considered based on its extremely long fluorescence lifetime in solution – more than 1 µs in aerated water. Its use as a sensor in applications based on long-lived fluorescence in screening assays, e.g., for metal ions, has been evaluated.
1. Introduction

1.1. Supramolecular Chemistry

“Supramolecular chemistry is the chemistry of the intermolecular bond, covering the structure and functions of the entities formed by association of two or more chemical species.”\(^1\) It is connected with the ability for recognition, selective binding, rigidity, constrictions, and tensions, as well as dynamics, reorientations, transport, and transformations.\(^2\) Hydrogen bonding, ion pairing, \(\pi-\pi\) dispersion interactions, van-der-Waals attractions, ion-dipole coupling, and solvophobic phenomena are the intermolecular forces holding molecular complexes together.\(^3,4\) Most life processes are based on intermolecular recognition and interactions, \textit{e.g.}, the transportation of ions,\(^5\) such as sodium and potassium, enzymatic catalysis, antigen-antibody complexes, etc. Life depends on these weak, delicate interactions, which require much less energy than covalent bonds require.\(^6\) These forces involve binding energies smaller than the 50–100 kcal mol\(^{-1}\) of covalent bonds, and simultaneous interaction of multiple sites is often required to accumulate a binding energy in solution of 2–20 kcal mol\(^{-1}\).\(^4\)

Since Charles J. Pedersen described the first crown ethers, and their ability to form strong complexes with the alkali metal ions,\(^7-9\) host-guest chemistry has become one of the most rapidly expanding areas within supramolecular chemistry, in particular, and within chemical research, in general. The pursuit of many
scientists has been to create and learn from simple model complexes that reproduce particular properties and behavior observed in natural processes. Supramolecular complexes are relevant to different fields such as biochemistry, pharmacology, organic chemistry, and to practical applications such as sensors, catalysis, removal of contaminants, and nuclear waste treatment. Along this line, different types of macrocycles and molecular receptors have been described: e.g., podands, corands, cryptands, spherands, cyclophanes, cavitands, carcerands, hemicarcerands, calixarenes, and cyclodextrins (see Figures below). They are distinguished from one another by the size and shape of the binding cavity, their openings, and their flexibility.² My research in the present dissertation has focused on a single, particularly rigid, and water-soluble member of the family: cucurbituril.
Supramolecular Hosts and Molecular Containers. I.

Crown Ether

Corand

Cryptand

Cyclophane

Spherand

Cyclodextrin

Cucurbituril
Supramolecular Hosts and Molecular Containers. II.
References


(5) The Nobel Prize is being awarded in 2003 for discoveries concerning channels in cell membranes with one half of the prize to Peter Agre, for the discovery of water channels, and the other half of the prize to Roderick MacKinnon for structural and mechanistic studies of ion channels. Such channels are classical structures in supramolecular chemistry.


1.2. Cucurbiturils as Molecular Containers

In 1905 Behrend, Meyer, and Rusche described the acidic condensation between an excess of formaldehyde and bis(ureido)glycoluril, producing a crystalline precipitate which was exceedingly stable towards a number of potent reagents. Unfortunately, they did not possess the appropriate methods at that time to properly characterize this substance. It was not until the early 1980s that Freeman, Mock, and Shih repeated the synthesis, describing the product as the hexameric macropolycyclic structure of composition $\text{C}_3\text{N}_2\text{O}_{12}$, now known as cucurbit[6]uril (CB6):\(^{11}\)

![CB6](image)

Cucurbit[6]uril, whose name originates from its resemblance to a pumpkin (botanical family: "Cucurbitaceae"), is a slightly water-soluble molecular container compound with molecular recognition properties. It encloses a 5.5 Å wide and 6.0 Å high cavity devoid of hydrogens and non-bonded electron pairs, and is able to
accept nonpolar organic residues through the release of high-energy water and potentially, stabilizing van-der-Waals interactions. The guest enters the cavity through two carbonyl rims acting as “portals”, which possess partial negative charge densities. Thus, the portals can accommodate positively charged compounds such as ammonium salts, hydronium ions, metal cations of different charge, and hydrogen donors such as amines and alcohols.

The CB6 complex with a cyclohexylmethylammonium ion

The portals, and the possibility of “closing” them by adding metal ions, place cucurbiturils in an intermediate position between “closed” structures such as carciplexes and hemicarciplexes and other “open” host systems with unobstructed openings like cyclodextrins and calixarenes. These properties make CB6 an attractive object for kinetic and thermodynamic studies.
After the identification of CB6, new homologues and derivatives have appeared. In the year 2000, Kim et al. isolated cucurbit[5]uril (CB5), cucurbit[7]uril (CB7), and cucurbit[8]uril (CB8)\textsuperscript{12} as by-products of the same synthesis of CB6. The larger host CB7 was of great interest for the present dissertation.

In 1992, Flinn et al. reported the synthesis of decamethylcucurbit[5]uril (1),\textsuperscript{13} a first derivative from the cucurbituril series, soluble in organic media, from the
reaction of dimethyl-glycoluril and formaldehyde. In 2001 Zhao et al. obtained two additional derivatives, CB5 and CB6 with five and six fused cyclohexane rings respectively (2), by using cyclohexanoglycoluril and formaldehyde, both soluble in water as well as in organic media. In 2002, Isobe et al. obtained CB6 with two phenyl groups situated on the same glycoluril unit (3), thereby providing the first asymmetric derivative. In 2003, Jon et al. reported the synthesis of perhydroxycucurbit[n]urils, expanding the utilization of cucurbit[n]urils (4).

Cucurbit[n]urils (CBn) have been successfully employed in catalytic processes, as components in the construction of supramolecular switches,
polyrotaxanes,\textsuperscript{20,21} catenanes,\textsuperscript{22} and charge-transfer complexes.\textsuperscript{23} They have also been employed for the removal of contaminants from air and water,\textsuperscript{24} as well as in the assembly of fluorescent solids.\textsuperscript{25} The research groups of Kim \textit{et al.} and Buschmann and coworkers have made major contribution to the application of cucurbituril in supramolecular chemistry.

The general objective of this work has been to explore the intrinsic properties and the potential use of different cucurbituril derivatives, to study the factors affecting the formation of host-guest complexes, and to investigate the influence of the cavity on the chemical and physical properties of the guest moieties.
References


1.3. **Scope of Dissertation**

My undertaking in the expanding field of supramolecular chemistry was in the first stages of my Ph.D. focused on qualitatively and quantitatively describing the complexation of small organic molecules with CB6, with the final goal of introducing into the host core 1-(aminomethyl)-2,3-diazabicyclo[2.2.2]oct-2-ene (DBOA or Fluorazophore-A), a long-lived fluorescent probe. The achievement of this complexation would allow our group not only to explore the multiple, potential applications of such a “nano-flashlamp,” but also to explain specific details in the mechanism of fluorescence quenching of the singlet state of DBOA by nucleotides and amino acids. The spacing imposed by the host walls would help differentiate between “through-distance” and “contact” quenching mechanisms, of crucial importance in the development of a new fluorescence screening assay methods based on the measurement of the kinetics of end-to-end collision in biopolymers, including peptides and oligonucleotides.

However, careful kinetic and thermodynamic studies on the complexation of cyclohexylmethylamine and CB6 in the presence of inorganic salts revealed the impossibility of introducing DBOA into the CB6 core, and led to the use of the larger homologue CB7. This larger molecular container allowed the complexation not only of DBOA, but also of the parent fluorazophore 2,3-diazabicyclo[2.2.2]oct-2-ene (DBO or Fluorazophore-P), which can be prepared by a simpler procedure.
The fluorescent host-guest system CB7•DBO possesses quite interesting properties, such as a fluorescence lifetime similar to the gas phase (> 1 μs), and resistance to “contact” quenchers and partially to “through-distance” quenchers. The potential uses of the complex became more evident after we noticed the strong fluorescence response of CB7•DBO to the association with metal ions.

The achievements obtained during my Ph.D. are reflected in six papers, four already published, one in press, and the latest submitted for publication. Two additional papers are now in preparation, and some new projects have been initiated to explore all the possibilities that this long-lived fluorescent host-guest system may offer.

The practical realization of the present thesis has required the use of several experimental spectroscopic techniques covering different fields. Temperature- and time-dependent $^1$H- and $^{13}$C-NMR spectroscopy were extensively used in the detailed kinetic and thermodynamic studies of the host-guest complexation mechanisms, while a thorough UV-spectroscopic compilation was done for the study of polarizabilities inside molecular containers. In the case of the study on DBO fluorescence quenching by nucleotides, steady-state as well as time-resolved fluorescence spectroscopy measurements were performed. This was also the case for studies on the metal/CB7•DBO association efficiency, its sensor ability, and the DBO photodecomposition. In the latter, gas chromatography–mass spectrometry
was used as a technique to elucidate the product identity and the product yield ratios.

Computational simulations have been performed at different levels in the studies concerned with the kinetics and thermodynamics of host-guest complexations, from \textit{ab initio} and semi-empirical methods for the conformational energy optimizations to molecular mechanics methods for the molecular dynamics calculations. Finally, different fitting procedures and algorithms were used extensively in the processing of the experimental results.

Several compounds have been synthesized in the course of these investigations, including the fluorazophore DBO and the cucurbituril oligomers. For the latter, the synthetic procedure was directed towards the optimization of the CB7 yield, thereby reducing the laborious purification process described in the literature. Furthermore, the project on the nucleotides required the synthesis of several nucleobase derivatives which are not commercially available.

All of my projects have been incorporated into the global research framework of Prof. Nau’s group, being closely related to projects of other group members. The basic mechanistic and polarizability studies are directly connected with the cyclodextrin and calixarene studies currently in progress by Mr. Huseyin Bakirci, and with other studies previously undertaken by Dr. Xiangyang Zhang. The studies on the quenching capacity in the presence or absence of CB7 form part of the required background on projects related to biopolymer flexibility and enzyme-
substrate recognition, undertaken by Ms. Gabriela Gramlich, Mr. Fang Huang, Mr. Harekrushna Sahoo, Mrs. Xiaojuan Wang, and Prof. Dr. Robert R. Hudgins (now at York University, Toronto).
2. Complexation Mechanism


The inclusion of small organic molecules into the cavity of cucurbituril oligomers and the exploration of potential applications of such host-guest complexes require a thorough understanding of the complexation mechanisms. In this project the pH dependence of the rate constants for ingestion ($k_{\text{ingress}}$) and egression ($k_{\text{egress}}$) of cyclohexylmethylamine (c6) into the CB6 cavity was analyzed and compared to those of smaller cycloalkylmethylamines. $^1$H- and $^{13}$C-NMR spectroscopy were used to quantify the amounts of free and complexed guest under various conditions, and the experimental data obtained were then modeled and interpreted by different mathematical fitting procedures according to various kinetic models.

The results show that the complex is destabilized at low pH values ($< 2$), at which protonation of CB6 occurs, thereby reducing the driving force for
complexation. The complex is also destabilized at high pH values (> 11), at which the ammonium complex is converted to the amine, which lacks the stabilizing ion-dipole interactions with the CB6 ureido-carbonyl groups. In between these two extreme pH values, $K$ reaches a plateau value of 170 M$^{-1}$. The $k_{\text{ingress}}$ and $k_{\text{egress}}$ values are also affected by the pH, covering a range of more than two orders of magnitude.

However, $k_{\text{ingress}}$ proved to be unrelated to the driving force of complexation; although the amine form has a lower binding constant, its $k_{\text{ingress}}$ value is higher. We have suggested the reaction mechanism in Scheme 1, where the amine enters by a direct penetration of the organic residue into the nonpolar cavity.

Scheme 1

![Scheme 1 Diagram]
This pathway is unfavorable for the ammonium ion, which rapidly forms an intermediate *association* complex, in which the ammonium site coordinates with the ureido-carbonyl rim while the organic residue is still exposed to the aqueous phase. The subsequent rate-determining ingressiion of the organic residue occurs in a flip-flop manner, namely through a different transition state. The transition state for conversion of the association complex to the inclusion complex requires a significant distortion of the portal, which renders this pathway slower than for the amine, but avoids a complete loss of the stabilizing ion-dipole interactions since it allows the ammonium ion to remain at least partly coordinated with the portal.
2.2. The Mechanism of Host-Guest Complexation by Cucurbit[6]uril

*Corresponds to:* Márquez, C.; Hudgins, R. R.; Nau, W. M. *submitted for publication.*

In continuation of the pH-dependent complexation behavior, I have concentrated on the thermodynamic factors affecting the formation of host-guest systems between CB6 and small organic molecules. Careful studies on the effects of temperature, and on the size and concentration of metal ion were performed. Finally, by comparing the results obtained experimentally with computational simulations, I was able to provide a feasible explanation for the inability of CB6 to locate DBOA in its core.

The investigations performed show that the behavior of the CB6•cyclohexylmethylamine complex (CB6•c6) with respect to temperature is that expected for a bulky guest; the binding constant becomes smaller with increasing temperature. The temperature dependence study shows that enthalpy stabilizes the complex, while entropic factors destabilize it.

The complexation process is also affected when sodium ions are present. Increasing the concentration of the salt decreases the binding constant due to a decrease in the ingression rate constant, while the egression rate constant remains unaffected. The cation size effect on the binding constant of the guest has also been
investigated. The binding constant decreases with an increase in the cation size and, therefore, in its association strength.

Studies on the effect of guest size indicate that the accessible volume of the CB6 cavity is ~100 Å³; large guests will be excluded due to steric repulsion. Between the two spherical guests 2,3-diazabicyclo[2.2.1]hept-2-ene (DBH) and DBO – calculated volumes: 96 Å³ and 111 Å³, respectively –, DBH rapidly forms a bound complex (1300 M⁻¹), while DBO does not form a complex even after several months, and neither at elevated temperatures.

Molecular mechanics calculations of the complexation kinetics and thermodynamics were performed for different guests. Qualitative agreement was found between experimental and calculated activation energies for complexation as a function of both guest size and the protonation state.
3. Fluorescent Host-Guest Systems

3.1. 2,3-Diazabicyclo[2.2.2]oct-2-ene in Supramolecular Chemistry

The azoalkane 2,3-diazabicyclo[2.2.2]oct-2-ene (DBO or Fluorazophore-P) is a fluorophore with a weak n,π* absorption in the near UV (λ_{max} = 365 nm in H_{2}O). It fluoresces in water with a high quantum yield (ca. 20%) over a broad spectral range (λ_{max} = 430 nm). DBO has distinct properties that have stimulated the study of its interaction with molecular containers.

![DBO Structure](image)

DBO

The fluorescence lifetime (τ) of DBO is exceedingly long, i.e., up to 1 μs in the gas phase and 325 ns in aerated water, and it responds strongly to the chemical environment. DBO fluorescence is quenched mainly through aborted hydrogen abstraction via a conical intersection, or through exciplex formation, but can also be quenched through energy and electron transfer.

Relatively few organic molecules display lifetimes in this long time regime, with azoalkanes derived from DBO displaying the longest fluorescence lifetime in
solution. The record for the longest fluorescence lifetimes of an organic chromophore in solution lies currently at 1.03 µs (in aerated H₂O!) and is held by the supramolecular complex CB7•DBO prepared in this thesis.

In addition to these features, DBO has a small spherical volume, good solubility in water, and high photostability, all of which favor its use as a fluorescent probe in host-guest systems. Several articles have been published on the behavior of DBO inside other containers. For instance, investigations have been done with zeolites, and cyclodextrins. The disposition of the guest inside the cavity, the complexation kinetics, the quenching propensity of the cavity, and the metal ion recognition capacity have been main objectives in these investigations.


References


3.2. Polarizabilities Inside Molecular Containers


– This article was selected for presentation as

the cover picture for the printed version of the journal –

The inclusion of DBO into the core of CB7 allowed me to study the inner phase of molecular containers by calculating the polarizabilities inside their cavities. The study was not only focused on cucurbiturils, but also extended to hemicarcerands, for which biacetyl was previously used as guest, and its spectroscopic properties described. This work is valuable since polarizability is considered an important factor in determining the binding strength of host-guest complexes. Moreover, it has been proposed that the polarizability of a host assists supramolecular biomimetic catalysis.

Solvatochromic studies on DBO and biacetyl in the gas phase and in eleven solvents revealed that the absorption and fluorescence maximum, the extinction coefficient, and the oscillator strength, all depend on the bulk polarizability of the solvent. A relation with the solvent polarity was not observed.

The dependence of the oscillator strength is most pronounced (*Figure 1*), since it varies systematically over one order of magnitude. These solvatochromic effects
render DBO an excellent molecular probe of polarizability of the chemical 
environment. The oscillator strength of DBO is much lower inside the CB7 cavity, 
50% lower than in water. This observation provides spectroscopic evidence that the 
environment sensed by DBO inside CB7 is characterized by a polarizability (0.12), 
even lower than that of perfluorohexane (0.16). The opposite is true for 
hemicarcerands, which possess electron-rich π systems with high electron density 
inside the cavity. A high polarizability (0.45) has been observed in hemicarcerands 
– by using biacetyl as a phosphorescence probe –, larger than that of diiodomethane 
(0.40).

![Contour plots of the combined absorption spectra of (a) DBO and (b) biacetyl as a function of the solvent polarizability (P). Shown on the top are the resulting correlations of the inverse oscillator strength (1/f), i.e., the integrated absorption intensity, versus bulk polarizability.](image)

**Figure 1.** Contour plots of the combined absorption spectra of (a) DBO and (b) biacetyl as a function of the solvent polarizability (P). Shown on the top are the resulting correlations of the inverse oscillator strength (1/f), *i.e.*, the integrated absorption intensity, *versus* bulk polarizability.
Molecules included in the cavities of molecular containers may therefore experience unusually low or high polarizabilities, depending on the host system. Cram’s hypothesis that these cavities may behave like a new phase of matter has therefore been confirmed through unexpected, extreme polarizabilities.
4. Potential Applications

4.1. Selective Fluorescence Quenching of 2,3-Diazabicyclo[2.2.2]oct-2-ene by Nucleotides


It appeared indispensable for future applications of DBO, for studies of oligonucleotide flexibility for instance, to provide compelling *experimental* evidence that the fluorescence quenching of DBO by nucleotides requires a close molecular contact, either in the form of a hydrogen-transfer transition state or an exciplex, and that quenching does not occur through-space or through-solvent. The latter applies for quenching mechanisms such as energy and electron transfer.

I have used the now-available technology for encapsulating DBO in the core of CB7 to quantitatively form (\(K \approx 4 \times 10^5 \text{ M}^{-1}\)) a supramolecular host-guest complex (CB7•DBO), which excludes contact between the (excited) chromophore and potential quenchers. This supramolecular inclusion prevents excited-state hydrogen abstraction or exciplex formation, while maintaining the possibility, perhaps at a reduced rate, of through-space electron or energy transfer over a moderate distance (ca. 5–8 Å) through the walls of the supramolecular cage.
None of the nucleotides, which are efficient quenchers of free singlet-excited DBO, caused any quenching of the long-lived excited CB7•DBO complex in aerated 0.2 M Na$_2$SO$_4$ solution even at 0.2 M concentrations. This is surprising, as the fluorescence lifetime of the CB7•DBO complex (up to 1 µs) is much longer as a consequence of the shielding from quenching by water and oxygen than for DBO in aerated water (325 ns), thus providing an improved dynamic range to observe quenching. The absence of quenching suggests, for all cases studied (dGMP, dAMP, dTMP, dCMP, and UMP), that the quenching rate constants are lower than $5 \times 10^5$ M$^{-1}$s$^{-1}$ (see Figure 2 for example).

\[ k_q = 5 \times 10^8 \text{ M}^{-1}\text{s}^{-1} \]

\[ k_q < 5 \times 10^5 \text{ M}^{-1}\text{s}^{-1} \]

Figure 2. Fluorescence quenching rate constants for the singlet state of DBO by dGMP in aerated aqueous solution in the absence and presence of CB7.
These results demonstrate experimentally that the quenching of DBO by nucleotides requires close molecular contact (hydrogen transfer, exciplex formation) and does not occur over moderate distance through space, thereby excluding electron and energy transfer as quenching mechanisms.
4.2. Cucurbiturils: Molecular Nanocapsules for Time-Resolved Fluorescence-based Assays


The large binding constant of $4 \times 10^5 \text{M}^{-1}$ along with the protection of DBO inside CB7 from external “contact” quenchers, in particular nucleotides or amino acids, allow the use of CB7 as an enhancer in time-resolved fluorescence-based assays, e.g., to screen enzymatic activity or inhibition by using DBO-labeled peptides as substrates. The contribution of the molecular container is two-fold; on one hand CB7 extends the lifetime of DBO, and on the other hand, it protects the fluorescence against possible quenchers. For example, the following DBO-labeled peptide possesses a lifetime of 14 ns, contrasting with the DBO lifetime in aerated aqueous solution (325 ns). The peptide is flexible enough to allow the approach of the quencher, attached to the other end of the chain, therefore reducing its fluorescence lifetime.
When the DBO group is protected by the use of CB7, the fluorescence lifetime increases to 1030 ns, since contact between the probe and the quencher is no longer possible.

This protection of the fluorescent probe from the external environment is of interest for screening assays where fluorescent probes are employed to signal molecular events, such as the inhibition of an enzyme by a library of potential
drugs. Short-lived emission is ubiquitous and may stem from other additives, sample impurities, biological components, scattered light, the solvent, or sample container materials of cuvettes and microplates. The shorter-lived components can be eliminated from detection through a time-gate, such that the emission from the long-lived fluorescent label (which serves as signaling unit) can be selectively detected (Figure 3). This dramatically reduces the background noise during the measurement.

![Figure 3](image)

**Figure 3.** Comparison of the fluorescence decay of a long-lived fluorescent probe ($\tau = 1.0 \, \mu s$) with that of a shorter-lived component ($\tau = 10 \, ns$); the shorter-lived component is $10^6$ times more intense (relative pre-exponential factors). A suitable time gate for use in a time-resolved assay is shown at 200 ns, improving the signal-to-background ratio by more than eight orders of magnitude.
The incorporation of CB7 expands the lifetime of the guest in aerated water to a value otherwise only achieved in the gas phase. The resulting CB7•DBO complex becomes a true nanomolecular device; it can be used as a “nano-flashlamp” in biological environments due to its exceedingly long-lived emission.
5. **Ongoing Projects**

The potential of the complex CB7•DBO is enormous if the appearance of new CBn derivatives is taken into consideration. The CBn family now contains derivatives soluble in organic media or able to be attached to larger molecules. However, any investigation on possible applications requires an initial, detailed understanding of the model compound CB7•DBO itself.

Three relatively new projects I have recently addressed explore potential applications of the CB7•DBO complex to metal detection and transport, and catalysis.

Two projects under investigation have focused on the complex photophysical and photochemical properties of CB7•DBO in the presence of metal ions. The potential use of CBn to bind heavy metals and transport them, or to imitate natural processes such as catalysis by metallo-enzymes motivated these studies.

The other new project reinforces the results published on polarizabilities inside molecular containers, and includes the study of other properties apart from the oscillator strength, such as the natural fluorescence lifetime, and other containers able to include DBO or other luminescent probes.

Influence of Associated Metal Ions on its Photophysical Properties


Cucurbiturils are inexpensive and easy to synthesize; in a single-step synthesis all the oligomers are available. Different research groups are considering the industrial use of cucurbiturils as metal cation scavengers or carriers. The inclusion of a fluorescent probe such as DBO permits the incorporation of a sensitive technique such as fluorescence spectroscopy in these investigations. Furthermore, the complex CB7•DBO could be used as a metal sensor resembling the ionophores described in the literature. In this project I have explored the ternary association complexes formed after the addition of metal cations, and attempted to define the most important factors to be considered in these systems. It was a comprehensive project that has required several different techniques such as UV spectroscopy, steady-state and time-resolved fluorescence spectroscopy, and EPR and NMR spectroscopy, among others.

In this investigation I have observed that the fluorescent host-guest system CB7•DBO is sensitive to metal ions, specifically (but not exclusively) to transition
metals. The presence of metal ions alters the photophysical properties of the azoalkane; in general, the absorption maximum undergoes a blue shift, the magnitude of which depends dramatically on the ion (see Figure 4 for example). The Ni$^{2+}$, Co$^{2+}$, and Fe$^{2+}$ ions show the strongest shifts.

Figure 4. Hypso- and hyperchromic effects are observed on the absorption spectra of CB7•DBO (2 mM) upon addition of CoCl$_2$ (up to 5 mM). The inset shows the absence of an effect of the addition in the spectrum of free DBO.
Figure 5. A hyperchromic effect is observed on the absorption spectra of CB7•DBO (2 mM) after addition of Tl$_2$SO$_4$ (up to 5 mM). The inset shows the absence of an effect of the addition in the spectrum of free DBO.

The lifetime of CB7•DBO is reduced or increased depending by the ion and its quenching ability, with a maximum of 1 µs observed for the alkalis (Na$^+$, K$^+$, and Rb$^+$), similar to the value measured in the gas phase and a minimum of no fluorescence detected for specific metal cations (Ag$^+$, Tl$^+$, or Co$^{2+}$). Meanwhile, the fluorescence intensity is reduced when any kind of metal is present, depending directly on its quenching capacity, with no apparent band shift in the fluorescence. Electron and energy transfer are the relevant mechanisms for the quenching of DBO fluorescence by the metal ions, even when included in CB7.

The metal ion association constant is slightly correlated with the ionic radii, which needs to match the rigid CB7 portal. The highest value obtained when no
specific interactions with DBO are present, \textit{i.e.}, for alkali and alkaline-earth ions, is with metal ions possessing a radius of $\sim 1.1$ Å. The association depends more on the metal ion charge; in other words, the CB7•DBO system is able to differentiate between oxidation states of the same metal. For instance, the binding constant for complexation of the first metal ion is three fold larger for Fe$^{3+}$ than for Fe$^{2+}$.

Careful EPR and time-resolved fluorescence studies probed the influence of the ions in the CB7 portals on the association strength of the second ion in the other portal. The distance between both ions is not large enough to avoid an anti-ferromagnetic effect on the paramagnetic ions. In general, the metals are in their high-spin configuration, and in their more stable coordination geometries. Interestingly, EPR and UV spectrophotometry experiments also demonstrated a \textit{forced covalent interaction} between DBO and the transition metals, only observed when CB7 is present (the thermodynamic constants for this interaction were roughly estimated, showing a preference for complexation with Co$^{2+}$). CB7, with a hydrophobic cavity, partially desolvates the ion, and imposes a reduced distance to the fluorophore, placing an azo nitrogen in the first coordination sphere of the metal. This situation illustrates how volume constrictions can favor covalent interactions that are thermodynamically unstable under “normal” conditions, \textit{i.e.}, in homogeneous solution.

Corresponds to: Márquez, C.; Nau, W. M. in preparation.

The possibility of substituting DBO with other organic molecules displays the system CB7•guest•2Mn+ a model for metallo-enzymes described in the literature. The associated metal could be strained by the presence of CB7 and the guest, both acting as ligands, and favoring electrochemical processes. However, the strain imposed can also affect the ligands, facilitating their possible transformation. Therefore, a molecular container can become an environment in which the chemistry of single molecules under special conditions can be investigated. This exploratory study makes use of this concept, where DBO is forced to photodecompose in the restrictive inner core of CB7 in the presence of different metal ions, comparing the results with the results in the absence of the host. The effect of metals can be observed through the variation of the final product distribution, i.e., the ration 1,5-hexadiene:bicyclo[2.2.0]hexane (HD:BCH).
It has been reported in the literature how intersystem crossing (ISC, see Scheme 2) in the biradical intermediates of DBO denitrogenation drastically affects the final product distribution. However, factors affecting the stability of the biradicals, independent of their spin multiplicity have not been adequately investigated, although it has been reported that radical-stabilizing substituents such
as phenyl or ethenyl groups at the bridgehead of DBO shift the distribution completely to the side of HD. For instance, interactions of external atoms with cyclohexane-1,4-diazenyl (CHA) and cyclohexane-1,4-diyl (CHD) intermediates able to extend their lifetime may be responsible for the improved HD yield in the photolysis of DBO inside cavitands such as β-cyclodextrins or CB7.

For the system CB7•DBO•2Mn+, the heavy atom effect appears insignificant. For instance, no difference is observed when Na+ is replaced by Cs+ (see Table 1), contrasting with the faujasite type zeolite systems, where an increase of 10% in product yield for HD was reported. However, when Mn+ is a transition metal ion a strong effect is observed, with a maximum ratio of 92:8 for Co2+, similar to the 100:0 obtained when phenyl or ethenyl groups are present on the bridgehead-carbons of DBO. These high ratios are only reached when CB7 is present, and they decrease to values close to that of the photolysis of free DBO in water when CB7 is absent. *Forced covalent interactions* between the metals and the CHD diradicals could be responsible for the extended stability of the diazenyl diradical, resulting in an increase of HD, which is dependent on the strength of the interaction. Other intrinsic properties of the metals such as spin-orbit coupling or spin multiplicity have no significant effects in the photodecomposition of the CB7•DBO systems.
<table>
<thead>
<tr>
<th>Salt</th>
<th>$k_q/10^7$ M$^{-1}$s$^{-1}$</th>
<th>HD:BCH</th>
<th>HD:BCH$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>No salt</td>
<td></td>
<td>61:39</td>
<td></td>
</tr>
<tr>
<td>Na$_2$SO$_4$</td>
<td>$&lt; 0.05$</td>
<td>71:29</td>
<td></td>
</tr>
<tr>
<td>K$_2$SO$_4$</td>
<td>$&lt; 0.05$</td>
<td>72:28</td>
<td>70:30</td>
</tr>
<tr>
<td>Rb$_2$SO$_4$</td>
<td>$&lt; 0.05$</td>
<td>71:29</td>
<td></td>
</tr>
<tr>
<td>Cs$_2$SO$_4$</td>
<td>$&lt; 0.05$</td>
<td>71:29</td>
<td></td>
</tr>
<tr>
<td>Mg$_2$SO$_4$</td>
<td>$&lt; 0.05$</td>
<td>72:28</td>
<td></td>
</tr>
<tr>
<td>CaCl$_2$</td>
<td>$&lt; 0.05$</td>
<td>71:29</td>
<td>71:29</td>
</tr>
<tr>
<td>SrCl$_2$</td>
<td>$&lt; 0.05$</td>
<td>72:28</td>
<td></td>
</tr>
<tr>
<td>CrCl$_2$</td>
<td>90</td>
<td>71:29</td>
<td></td>
</tr>
<tr>
<td>MnCl$_2$</td>
<td>9.5</td>
<td>77:23</td>
<td>70:30</td>
</tr>
<tr>
<td>FeCl$_2$</td>
<td>130</td>
<td>85:15</td>
<td></td>
</tr>
<tr>
<td>CoCl$_2$</td>
<td>220</td>
<td>92:8</td>
<td>75:25</td>
</tr>
<tr>
<td>NiCl$_2$</td>
<td>75</td>
<td>83:17</td>
<td>73:27</td>
</tr>
<tr>
<td>CuCl$_2$</td>
<td>120</td>
<td>77:23</td>
<td></td>
</tr>
<tr>
<td>ZnCl$_2$</td>
<td>$&lt; 0.05$</td>
<td>71:29</td>
<td>72:28</td>
</tr>
<tr>
<td>Cr(NO$_3$)$_3$</td>
<td>115</td>
<td>71:29</td>
<td></td>
</tr>
<tr>
<td>Tl$_2$SO$_4$</td>
<td>550</td>
<td>78:22</td>
<td>78:22</td>
</tr>
<tr>
<td>(NH$_4$)$_2$SO$_4$</td>
<td>$&lt; 0.05$</td>
<td>65:35</td>
<td></td>
</tr>
<tr>
<td>CdSO$_4$</td>
<td>2.0</td>
<td>73:27</td>
<td></td>
</tr>
<tr>
<td>H$_2$SO$_4$</td>
<td></td>
<td>70:30</td>
<td>69:31</td>
</tr>
</tbody>
</table>

**Table 1.** Photodecomposition of the CB7•DBO•2M$^{n+}$ host-guest system, where M$^{n+}$ represents the metal ions corresponding to the salts listed. DBO fluorescence quenching rate constant by the metal ions, product distribution, and product distribution –marked with *– when CB7 is not present.
5.3. Additional Evidence Related to Polarizabilities Inside Molecular Containers

The polarizability inside molecular containers is important for understanding certain catalytic processes. The former project on polarizabilities (Section 3.2) showed that molecular containers can possess extreme polarizability values. Thus, researchers will choose among them, depending on the catalytic process desired. However, this emerging idea requires first a confirmation of our previous experimental results. I have thus planned to obtain the polarizability value of CB7 by a different experimental procedure, and contrast the results with those derived from the oscillator strength. The new investigation is based on the following theoretical concepts.

When an electric field ($\xi$) generated by a nearby ion or molecule ($^1\text{DBO}^*$) distorts the electron distribution of a polarizable molecule (solvent), it gives rise to an electric dipole in it. The magnitude of the induced dipole moment ($\mu$) is proportional to the strength of the field, with the molecular polarizability ($\alpha$) being the proportionality constant (Equation 1).

$$\mu = \alpha \xi \quad (1)$$

In my previous project I related the inverse of the oscillator strength ($f$) for the electronic transition n-$\pi^*$ of the fluorazophore DBO (Equation 2) to the bulk polarizability ($P$) defined by the classical Claussius–Mosotti or Lorentz–Lorentz
relations *(Equation 3)*. This relationship is based on the time-dependent quantum mechanical relation between \( f \) and \( \mu \), and the assumption that the differences in the proportionality factor between the *bulk* and the *molecular* polarizability are not significant for the solvents studied.

\[
f = 4.32 \times 10^{-9} \int_{\text{Band}} \varepsilon(\tilde{\nu}) d\tilde{\nu} \quad (2)
\]

\[
P = \frac{n^2 - 1}{n^2 + 2} = \frac{N}{3\varepsilon_o V} \alpha \quad (3)
\]

The linear relationship obtained shows that the bulk polarizability of the medium can therefore be chosen instead of the molecular one, connecting a microscopic property related to the induced dipole moment such as the oscillator strength \( (f) \) with a macroscopic and easily accessible one such as the refractive index \( (n) \).

From a first glance, the experimental assignment of the *macroscopic* bulk polarizabilities for the CB7 and hemicarcerand cores is contradictory with the fact that the cores are *microscopic* environments. However, a simple visualization of the microscopic disposition (Scheme 2) shows how the cavitand reproduces the solvent cell. The effect of the cavity on the DBO oscillator strength can be compared with the effect of the solvent cell, where the only solvent molecules interacting with the encapsulated DBO are the closest ones. The assumption that macroscopic properties apply for the solvent cell must therefore also apply to the core of the molecular container.
In this project, new experiments can be designed in order to establish if the linearity previously observed is maintained between the natural lifetime measured in different solvents and inside the cavitands. The data can be then compare with the values reported in my former publication. This can be done on the basis of the expected relationship deduced from the Einstein coefficients of absorption and spontaneous emission.

The Einstein coefficient for absorption ($B$) is related to the transition dipole moment ($Equation 4$), which means that there is also a relationship between $B$ and $f$. Similarly, the Einstein coefficient for spontaneous emission ($A$) is related to the same transition dipole moment ($Equation 5$), and therefore to $f$. 
\[ B = \frac{2\pi\mu^2}{3\hbar^2} \]  \hspace{1cm} (4)

\[ A = \frac{4}{3c^3\hbar} \omega^3 \mu^2 \]  \hspace{1cm} (5)

Since \( A \) is simply the reciprocal of the radiative natural lifetime of the excited state, a direct relation must be expected between the bulk polarizability and the fluorescence rate constant \( (k_f) \). If that is the case, the polarizability values for CB7 and hemicarcerands obtained this way should be consistent with those assigned previously by means of the oscillator strength.

This project will require careful measurement of the fluorescence quantum yields in different environments, which is far from trivial.
6. Summary of Former Projects


Before joining the group of Prof. Werner M. Nau, I participated in investigations of the synthesis of charge-transfer complexes based on the structure of pyridinophanes supporting both donor and acceptor molecules. The part that Ms. María Lorenzo and I shared consisted of the preparation of pyridine derivatives with functional groups for the attachment of the pyridines to larger molecules.

To this end, the synthesis of substituted 2-dicyanomethylene-1,2-dihydropyridines and 2-dicyanomethylene-2,3,4,5-tetrahydropyridine was accomplished from malononitrile dimer and propenenitriles. The dicyanomethylene part of the molecule was to be photooxidized to the acid form, allowing the attachment of this pyridine to a larger structure using both the amino and the acid functional groups.
By a different approach, the amalgam (Na•Hg) reduction of some 4-substituted-2-amino-3,5-dicyano-6-methoxypyridines resulted in the formation of 3,4-dihydropyridines in high yields. The posterior synthesis of C-4 deuterium labeled dihydropyridine by this procedure has elucidated the role played by these dihydropyridines in the requisite oxidation path for the synthesis of pyridines from malononitrile and benzylidenemalononitriles.
The same amalgam (Na•Hg) reduction process was applied to a larger pyridine containing a derivative of triethylene glycol, becoming an unprecedented method for synthesis of oxygen-bridged \([n](2,5)\)pyridinophane dihydro-analogues.
7. List of Publications

Complexation Mechanism


Fluorescent Host-Guest Systems

3. Polarizabilities Inside Molecular Containers


Potential Applications

4. Selective Fluorescence Quenching of 2,3-Diazabicyclo[2.2.2]oct-2-ene by Nucleotides

5. **Exploiting Long-Lived Molecular Fluorescence**

Nau, W. M.; Huang, F.; Wang, X.; Bakirci, H; Gramlich, G.; Márquez, C.


6. **Cucurbiturils: Molecular Nanocapsules for Time-Resolved Fluorescence-based Assays**


**Ongoing Projects**


   Influence of Associated Metal Ions on its Photophysical Properties


Márquez, C.; Nau, W. M. *in preparation.*
Former Projects

9. **Synthesis and Structure of New Substituted 2-Dicyanomethylene-1,2-dihydropyridines**

10. **Amalgam (Na•Hg) Reduction of some 4-Substituted-2-amino-3,5-dicyano-6-methoxypyridines. New Evidence Regarding the Oxidation Step in their Synthesis**

8. List of Presentations at Conferences

1. Social Rules Governing Molecular Tête-à-Tête
   C. Márquez and W. M. Nau
   GSA Graduate Colloquium, International University Bremen
   Bremen (Germany), 2003

2. Understanding Host-Guest Systems
   H. Bakirci, C. Márquez, and W. M. Nau
   National Research Program 47 Meeting
   Bern (Switzerland), 2003
   – This poster was selected as one of the three best presentations of the NRP47 meeting –

3. Influence of the Binding of Metal Cations on an Exceptionally Fluorescent Host-Guest Compound
   C. Márquez and W. M. Nau
   XX International Conference on Photochemistry
   Moscow (Russia), 2001

4. Exceptional Protection of a Fluorescent Probe by Supramolecular Inclusion
   C. Márquez and W. M. Nau
   Joint Meeting of the Italian, French, and Swiss Photochemistry Group
   Lausanne (Switzerland), 2001
5. **Fluoreszenzsonden schützen, ohne sie einzusperren**
   C. Márquez and W. M. Nau
   Minisymposium Photochemie
   Oberjoch (Germany), 2000

6. **Interaction of DNA with a Long-Lived Fluorescent Probe**
   C. Márquez, U. Pischel, and W. M. Nau
   EPA Graduate Student Symposium in Photochemistry
   Fribourg (Switzerland), 2000

7. **Photoreaction of Ethyl 6-Amino-3,5-dicyano-2-pyridincarboxylates Derivatives with DMF**
   C. Márquez, M. Lorenzo, and L. Fuentes
   IV Spanish Congress of Photochemistry.
   Gandía (Spain), 1999

8. **Synthesis and Structural Studies of 1,1’,3,3’-Tetramethyl-bisimidazolidin-2-yl**
   C. Márquez, M. Lorenzo, and L. Fuentes
   Alicante (Spain), 1997

9. **Amalgam (Na•Hg) Reduction of 2-Amino-3,5- dicyano-6-methoxypyridines**
   M. Lorenzo, C. Márquez, and L. Fuentes
Alicante (Spain), 1997

10. **Photosensitized Autooxidation of Ethylenediamine**
C. Márquez, M. Lorenzo, and L. Fuentes
III Spanish Congress of Photochemistry.
El Escorial (Spain), 1995

11. **Photooxidation of Ethylenediamine**
C. Márquez, M. Lorenzo, and L. Fuentes
XXV Biennial Reunion of the Royal Chemistry Society of Spain.
Vitoria (Spain), 1994
9. Curriculum Vitae

Personal details

Name César Augusto
Surname Márquez González
Date of birth 16.11.1967
Nationality Spanish

University Education

1985-1990 Degree in Organic Chemistry by Alcalá University (Spain).
1990-1994 Member of the Photochemistry Research Group of Alcalá University within the Project: “Photoinduced Synthesis of Charge Transfer Complexes”, included in the Program of New Technologies of the Comunidad de Madrid (Spain).
1995-1998 Lecturer of the Specialization Course at Alcalá University: “Photochemistry and Lasers. Industrial, Medical, and Biological Applications”.
1999-2003 Ph.D. Student at the University of Basel under the supervision of Prof. Dr. Werner M. Nau.

2003 Ph.D. Student at the International University Bremen under the supervision of Prof. Dr. Werner M. Nau.

**Attended Lectures during Graduate Studies**

- Physical-Chemistry of Drugs
- Design and Synthesis of Drugs A. Design of Drugs
- Design and Synthesis of Drugs B. Strategies in Pharmacological Synthesis
- Design and Synthesis of Drugs C. Techniques in the Synthesis of Drugs
- Design and Synthesis of Drugs D. Industrial Synthesis of Drugs
- Applications of Nuclear Magnetic Resonance
- Photochemistry
- Photochemistry and Photobiology
- Deutsch als Fremdsprache