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Macrocycles in the Classroom – Making Biochemical Processes Understandable with the Help of Crown Ethers

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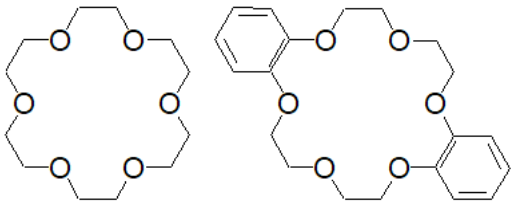
Abstract

Charles PEDERSEN discovered crown ethers rather by coincidence in the early 1960s. Crown ethers are macrocyclic polyethers that selectively bind alkali metal cations through molecular recognition, encapsulating them within their interior (cavity) via non-covalent interactions. This lipophilic encapsulation enables their transfer into organic solvents. Natural antibiotics like nonactin, which function as ionophores by transporting potassium ions across cell membranes, operate in a similar manner. The lipophilic encapsulation of cations by ionophores and the facilitated ionophore-mediated cation transport through membranes can be elegantly demonstrated in simple school experiments using structurally related crown ethers such as the non-toxic [18]crown-6 or dibenzo[18]crown-6. Model experiments for complexation, decomplexation and the combination of both – both of which take place during cell transport – are presented in the following article. The focus lies on the use of non-toxic solvents and potassium permanganate as a "model-salt", as it results in violet coloured solutions, making the processes in question easy to observe.

Keywords: *High School, biochemistry, demonstration, ionophores, transport properties*

1. Introduction

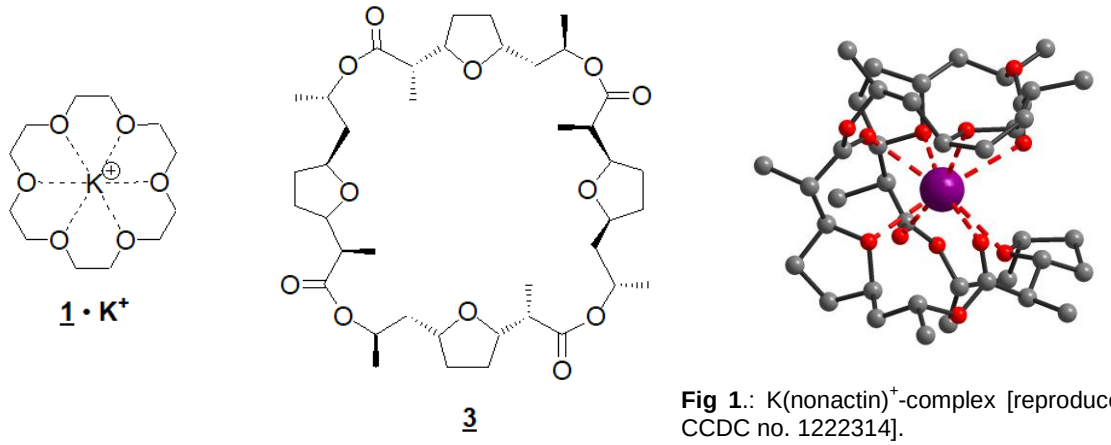
In 1987 Charles PEDERSEN, together with Donald CRAM and Jean-Marie LEHN, was awarded the Nobel Prize in Chemistry for the "development and use of molecules with structure-specific interactions of high selectivity": PEDERSEN discovered the macrocyclic polyethers, the so-called crown ethers, or more precisely their properties, rather accidentally in the early 1960s. LEHN and CRAM continued PEDERSEN's work to a certain extent and created further cyclic ethers based on the crown ethers. As an example, **1** and **2** represent two crown ether molecules: **1** shows a [18]crown-6 molecule (short: 18C6) and **2** a dibenzo[18]crown-6 molecule (short: DB18C6). Their names follow a simple nomenclature introduced by PEDERSEN, in which the number of ring atoms is counted and put in brackets, preceded by the common name "crown" and followed by the number of oxygen atoms. Additional substitutes (like benzyl rings fused to the ring) are placed in front of the name. [1] [2].



1 **2**

Crown ether molecules act as hosts, selectively binding cationic guests, especially alkali metal cations, within their hole or cavity. This was a novelty since especially sodium and potassium ions were reluctant to form complexes until then [1], [2]. Around the same time as PEDERSEN's first publication on crown ethers in 1967, it became known that some natural antibiotics were also able to bind selectively sodium and potassium cations that are essential for cell function. These natural substances include, for example, nonactin, whose similarity to crown ethers as macrocyclic polyethers

was quickly recognized [3] [4]. Just as [18]crown-6 can complex potassium ions (**1**·K^+), nonanctin (**3**) is also able to bind potassium ions in its interior [5] (see figure 1).



1 • K^+ **3**

Fig 1.: K(nonactin)$^+$-complex [reproduced from CCDC no. 1222314].

Both [18]crown-6 and nonactin molecules bind alkali metal cations via ion-dipole interactions in their hydrophilic cavity and thus wrap these cations in a kind of a lipid shell. This lipid shell is created by the outer surface of the complex formed by ethylene bridges (-CH_2-CH_2-) in the case of the [18]crown-6 molecules or the methyl groups and the backbone of the furan rings in the case of nonactin molecules (see figure 1). In this way, the complexed ions are masked and become fat-soluble, i.e. soluble in organic solvents in which alkali metal salts would otherwise not be soluble. The binding of the cations is selective: only those cations are effectively bound that fit into the hydrophilic cavity of a crown ether molecule or nonactin molecule [1], [2], [3], [5], [6], a process known as molecular recognition [7].
The effect of natural polyether antibiotics such as nonactin is based on the selective binding of biologically significant sodium and potassium ions: they transport these ions through the cell membrane (see figure 2) and in this way disrupt the sensitive sodium-potassium balance, which triggers cell death. Due to their ability to transport ions through the cell membrane, they are also referred to as *ion carriers* or *ionophores* [6] [7].

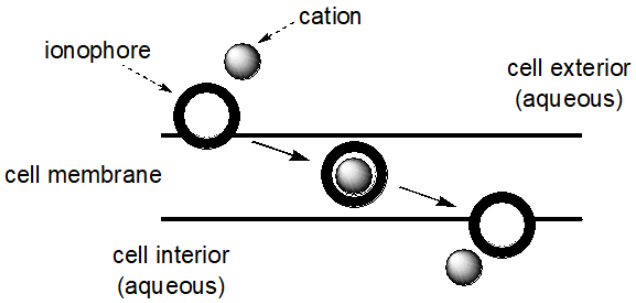


Fig. 2. ionophore-mediated cation transport through cell membrane (cf. [6]).

With their discovery, PEDERSEN, CRAM and LEHN founded the field of so-called supramolecular chemistry, in which the focus is no longer on the covalent bond between atoms, but on the interaction of molecules and/or ionic species with each other via non-covalent interactions such as hydrogen bonds, π- π interactions or ion-dipole interactions [8, 9]. Due to the similarity of crown ethers to natural ionophores, ionophores can be imitated using crown ethers, which offers great potential for educational purposes [10]: It is well known that biology can serve as a bridge to chemistry, allowing students to develop enthusiasm for the subject through contextualization [11].

2. Use in Education – Macrocycles in the Classroom

One reason why crown ethers have been barely considered in schools so far may be due to the rumour that crown ethers are toxic [9]. However, toxicological studies have shown that [18]crown-6 and also dibenzo[18]crown-6 – in contrast to many of their natural relatives – have a similar toxicity as aspirin®. Both crown ethers therefore require no more or less vigilance than other common chemicals

[9]. Another reason why crown ethers have not (yet) found their way into the classrooms may be due to the usage of chlorinated solvents such as chloroform (toxic) and dichloromethane (DCM; harmful to health) to conduct typical crow ether experiments [9]. In order to increase the acceptance of the use of crown ethers in schools, alternative solvents are required that show similar properties like chloroform and DCM in experiments but are less harmful. Anisole (phenyl methyl ether) and methyl benzoate (benzoic acid methyl ester) has proven to be good alternatives [12], as will be shown in the following.

(1) Complexation: The Lipophilic Masking of Cations

The process of complexation of alkali metal cations by [18]crown-6 or dibenzo[18]crown-6 molecules and therefore their lipophilic masking is standardly carried out using potassium permanganate ($KMnO_4$) in DCM or chloroform [9] [13]. These solvents can be substituted by anisole, which is neither toxic nor harmful to health. $KMnO_4$ sediments in anisole without dissolving (see figure 3, left). If a small spatula tip of [18]crown-6 or dibenzo[18]crown-6 (or a solution of these crown ethers in anisole) is added, the liquid will instantly turn into a purple solution like it does in DCM and chloroform [12] (see figure 3, right).

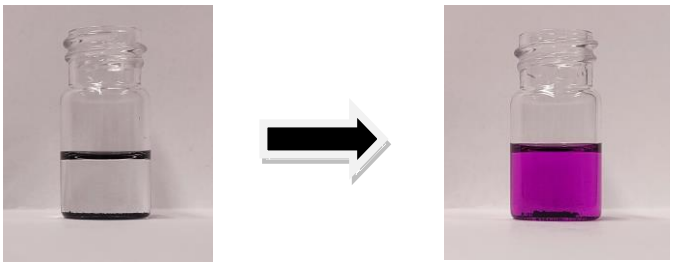


Fig. 3. left: sediment of $KMnO_4$ in anisole; right: solution of $KMnO_4$ after addition of a small volume of a solution of [18]crown-6 in anisole, with permission from [12].

At the particle level, crown ether molecules bind potassium cations in their cavity. The molecules can easily approach the salt lattice as they are themselves uncharged. In the hydrophilic interior of a [18]crown-6 molecule, a potassium ion is bonded sixfold by the ether oxygen atoms. In the complex, ethylene bonds ($-CH_2-CH_2-$) are twisted outwards, which are non-polar and lipophilic. In this way, a potassium ion is "lipophilically wrapped" and goes into solution. For charge compensation, the positive cation complex must be followed by an anion [13]. The salt is solubilized in this way. The process is easily visible due to the colour of permanganate solutions [14]. Using, e.g. [18]crown-6, the reaction equation is as follows:

$$KMnO_4(s) + 18C6(o) \rightleftharpoons K(18C6)MnO_4(o)$$

The index o in this equation indicates the organic phase [12].

(2) Decomplexation: The release of cations

As shown in figure 2, the complex of a ionophore molecule and an alkali metal cation is opened in the aqueous cell interior and the cation is released again. Crown ether molecule - alkali metal cation complexes are also broken down at the interface between water and an organic solvent: Water solvates the metal salt and thus extracts it into the aqueous phase [8]. The process of decomplexation of an ionophore molecule - metal cation - complex at the boundary to the aqueous cell interior can be demonstrated by using dibenzo[18]crown-6, $KMnO_4$ and anisole in the following way: First, both solutions of dibenzo[18]crown-6 and $KMnO_4$ are mixed together in anisole to obtain a violet-coloured anisole solution with c(DB18C6) = 3 mM. Then, 2 mL of a 1 M aqueous solution of calcium chloride ($CaCl_2$) is overlaid with 2 mL of this coloured anisole solution in a test tube (see figure 4, left). Since anisole and water have similar densities, $CaCl_2$ is used to increase the density of the aqueous phase and therefore to avoid the formation of an emulsion in the next step, when the biphasic system is gently shaken: While shaking $KMnO_4$ passes into the aqueous phase due to effective hydration of both the potassium and permanganate ions, resulting in decomplexation and an aqueous violet-coloured potassium permanganate solution. DB18C6 remains in the organic phase since it is insoluble in water (see figure 4, right) [12].

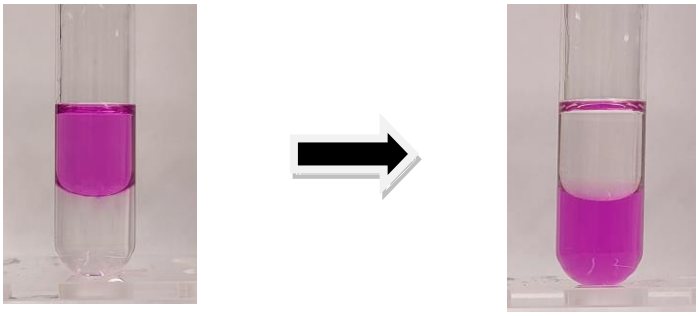


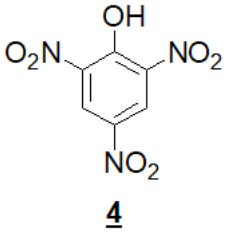
Fig. 4. on the left: K(DB18C6)MnO_4 in anisole on the top, water ($CaCl_2$(aq)) on the bottom; on the right after shaking; top: DB18C6 (insoluble in water), bottom: $KMnO_4$ (aq) (incl. $CaCl_2$(aq)), with permission from [12].

As can be seen in figure 4, the aqueous phase turns purple during shaking and the organic phase is decolorized. The reaction equation can be formulated as follows:

$$\mathrm{K(DB18C6)MnO_4(o)} \rightleftharpoons \mathrm{DB18C6(o)} + \mathrm{K^+(aq)} + \mathrm{MnO_4^-(aq)}$$

(3) Mimicking Ion Transport in a PRESSMAN *Cell*

Complexation and decomplexation take place sequentially during ionophore-mediated alkali metal cation transport from the aqueous cell exterior through the lipophilic cell membrane into the aqueous cell interior [6]. In order to simulate the process, DYSON [15] used a U-tube into which he poured chloroform and then filled one leg (here the left leg, cf. figure 5) with an aqueous solution of potassium picrate (potassium 2,4,6-trinitrophenolate) and the leg with water (here the right leg, cf. figure 5). The aqueous potassium picrate solution simulates the extracellular environment, the water-filled represents the intracellular space and the chloroform phase serves as the non-polar model for the lipophilic biomembrane [9][15][16]. The addition of an ionophore causes the potassium ions to be transported from the aqueous potassium picrate phase ("*donor phase*") through the chloroform barrier ("*liquid membrane*") into the aqueous phase in the other leg ("*receiving phase*"). The positive ionophore-potassium complex is accompanied by one picrate anion each for charge compensation. As picrate solutions are yellow, the process can be easily observed with the naked eye. The process is shown schematically in figure 5. Please note that metal picrate salts are salts of the toxic, highly explosive picric acid **4** and therefore are not allowed in schools [9].



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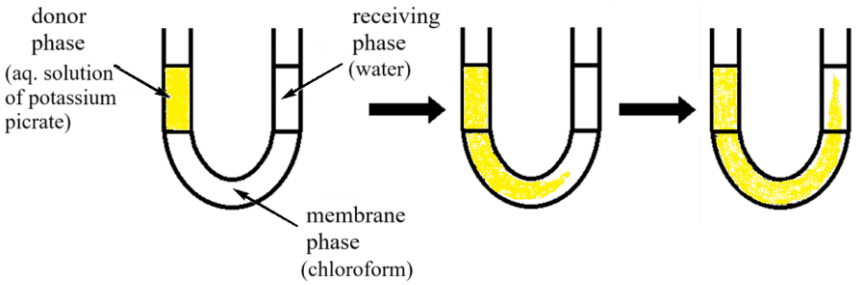

Fig. 5. Mimicking ion transport trough cell membranes in an U-tube PRESSMAN cell (left: without ionophore, middle and right: after addition of ionophore), resketched: cf. [16].

Such systems which use a liquid biomembrane to study ionophores are known as PRESSMANN cells, named after Berton PRESSMANN [17][18]. FRIEDRICH and OETKEN for example picked such a U-tube PRESSMANN cell to show cell membrane transport processes: In an experiment designed for schools they used [18]crown-6 as an ionophore, $KMnO_4$ and $KHCrO_4$ as potassium salts. They replaced chloroform with the less dangerous DCM. Since [18]crown-6 acts like an ionophore and since $KMnO_4$ and acidified potassium chromate (K_2CrO_4) solutions are coloured, the potassium transport mediated by the crown ether gets visible. Additionally, they accelerated the phase transfer by stirring the DCM phase in which they had inserted a stirring magnet. Nevertheless, the transfer of $KMnO_4$ from *donor* to *receiving phase* took about ten minutes [14]. DCM, still harmful to health, should also be substituted by an even less harmful solvent.

In order to develop a more school-friendly PRESSMANN cell that avoids the use of halogenated solvents, expensive glassware and relatively long reaction times, a *low-cost* U-tube was designed: For this purpose, four hot glue sticks (⌀ 11 mm) were glued together with a hot glue gun to form a 2x2 array. This array can easily be inserted into a (big) TicTac® box, resulting in a U-shaped gap (see figure 6, left). As the array is a little too large for the TicTac® box, it bulges the box slightly and thus ensures that the construction is tight. Chloroform or DCM normally used can be replaced by benzoic acid methyl ester (methyl benzoate): Methyl benzoate is used in perfumes, shampoos and cosmetics due to its pleasant odor [19]. As one of the few organic solvents, it has a higher density than water (ρ = 1.08 gcm-³) and can be used without restriction in student experiments.

For the experiment the modified TicTac® box is filled with 8 mL of methyl benzoate acting as the "biomembrane". One leg of the U-tube (here the right leg, cf. figure 6) is filled with 2.5 mL distilled water ("aqueous cell interior", *receiving phase*), the other leg is filled with 2.5 mL of a 0.1 M $KMnO_4$ solution ("aqueous cell exterior", *donor phase*). A small stirring magnet (10x6 mm) is placed to the side under the water phase (*receiving phase*). The modified TicTac® box is placed on a magnetic stirrer so that the magnet in the box is in the center of the magnetic stirrer. The glue sticks sticking out of the box can be fixed with a stand so that the box has the necessary stability. After the magnetic stirrer is switched on (stirring accelerates the phase transfer process), 1 ml of a solution of 18C6 in methyl benzoate (c = 0.4 M) is then injected with the help of a 1 mL syringe just below the $KMnO_4$(aq)/ methyl benzoate interface. Thereupon a salt transfer from the aqueous solution phase (*donor phase*) across the organic phase (model-biomembrane) to the water-phase (*receiving phase*) can be observed within ~ 1 min (see figure 6) [12, 14].

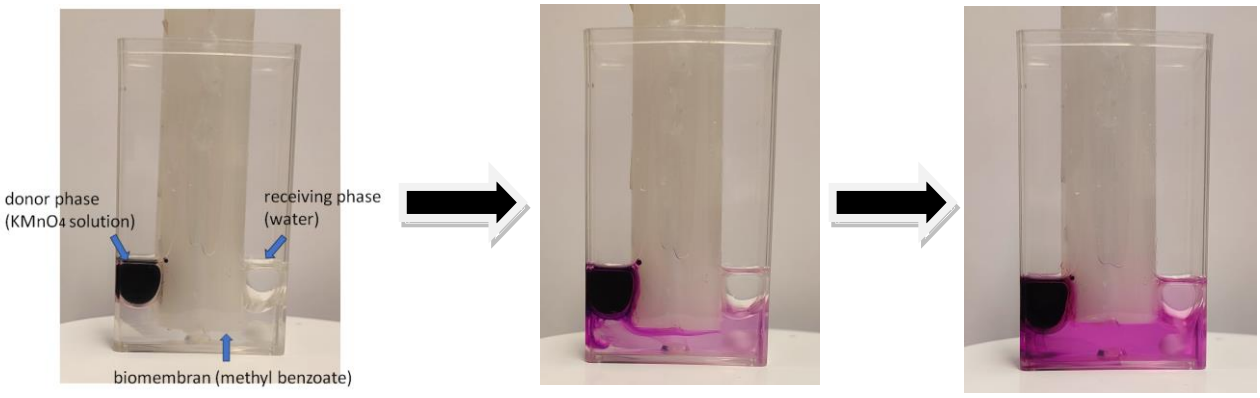

Fig. 6. Mimicking ionophore-mediatd potassium transport trough cell membranes in a low cost U tube PRESSMAN cell, with permission from [12].

A reaction equation composed of the previous equations can be set up as an overall reaction equation as follows:

$$K^+(aq) + MnO_4^-(aq) + 18C6(o) \rightleftharpoons K(18C6)MnO_4(o) \rightleftharpoons 18C6(o) + K^+(aq) + MnO_4^-(aq)$$

Note that 18C6 is also soluble in water and is therefore distributed between the aqueous phases and methyl benzoate. The distribution was omitted from the reaction equation for the sake of clarity. For the imitation of potassium ion transport through a biomembrane using a natural ionophore, see figure 2 again.

Outlook

The presented experiments offer a simple way to make the process of ion transport through membranes accessible for schools with the help of [18]crown-6 and dibenzo[18]crown-6. Further ideas for the use of crown ethers in education are currently being developed in our work-group. The aim for all the experimental developments is to foster references between chemistry and biology – as the use of biological contexts is demonstrably motivating for learners. In the long term, learning models and

materials are to be developed, tested and evaluated in collaboration with schools and students invited to the university laboratories.

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