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Confinement and Step-Wise Reopening of Channels in an Artificial Cell/Inorganic Capsule: A ^{7}Li NMR Study

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In previous contributions we have demonstrated that the anionic porous molybdenum oxide based capsule(s) $[Li_n\subset\{(Mo^{VI})Mo^{VI}{}_5O_{21}(H_2O)_6\}_{12}\{Mo^{V}{}_2O_4(SO_4)\}_{30}]^{72-n}$ (**1a**; $n\approx5$) of the compound $[(CH_3)_2NH_2]_{44}Li_{28-n}\cdot\mathbf{1a}\cdot\approx250H_2O$ (**1**; Figure 1) can be considered as models for cellular cation transport,[1] in particular with respect to the exchange of lithium ions between the interior of the capsules and the surrounding solution.[2,3] Additionally, we could show that appropriate cationic organic species, such as formamidinium cations ($FA\cdot H^+$), can act as "corks"/guests; that is, they are able to close, in a supramolecular fashion, the pores exhibiting crown-ether function, and separate the interior from the exterior.[4] From ^{7}Li NMR spectra it has been deduced that separate signals can be observed for the various lithium species present. However, the characteristics of the internal lithium cations (Li^+ confined in the cavity of the capsule) were only deduced indirectly, because Li^+ ions are involved in exchange processes (Figure 1, bottom). Here, we present an NMR study that allows for a precise interpretation of the processes associated with the exchange and confinement of capsule associated lithium cations. In particular we show that stepwise re-opening of the pores (partial release of the formamidinium plugs) is possible by addition of defined amounts of water, an incident which models ligand-gated ion channels.

A first indication of the differentiation between external and internal lithium cations arises from the study of the diffusion behaviour of the system as depicted by the ^{7}Li NMR signals. If a solution of **1** (preparation according to reference [1]) in dimethyl sulfoxide (DMSO) and treated with

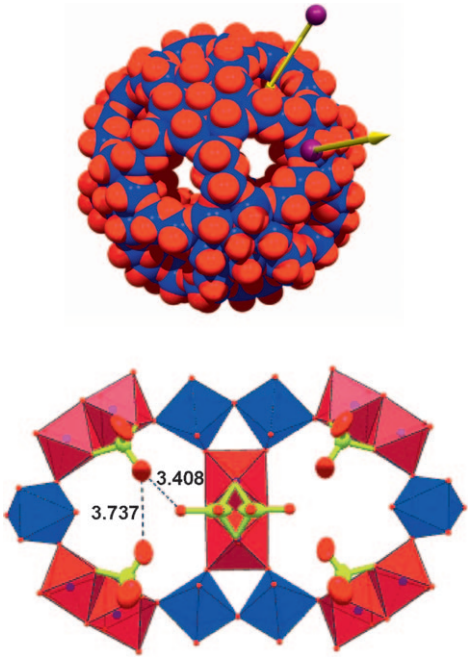

Figure 1. Top: Schematic space-filling representation of the uptake and release of cations (counterion transport) through the pores of the highly charged anionic capsule **1a** (Mo blue, O red). Bottom: View of two of the 20 pores of **1a** (MoO_6 octahedra of the pentagonal units blue and of the $\{Mo_2\}$ type linker groups red; for details see reference [1]). The disorder of the sulfates observed by X-ray crystallography (S yellow, O red) comes about by the (not directly observable) Li^+ ions.[1]

$FA\cdot HCl$ is investigated in a ^{7}Li DOSY experiment,[7] a substantial difference in the diffusion coefficients of encapsulated lithium cations moving with the capsule ($Li^+\subset\mathbf{1a}$) as compared to solvated Li^+ ($[Li(dmso)_n(H_2O)_{4-n}]^+$) is to be expected. This is in fact observed (Figure 2). The Stokes ("hydrodynamic") radius obtained for **1a** from the diffusion coefficient ($9.3\times10^{-11}\,m^2s^{-1}$) is ≈1.2 nm. Keeping in mind the ambiguities with the calibration of the diffusion experiments, the determined value is in reasonable agreement to $r=1.5$ nm obtained from the single-crystal X-ray structure analysis of **1**.[1]

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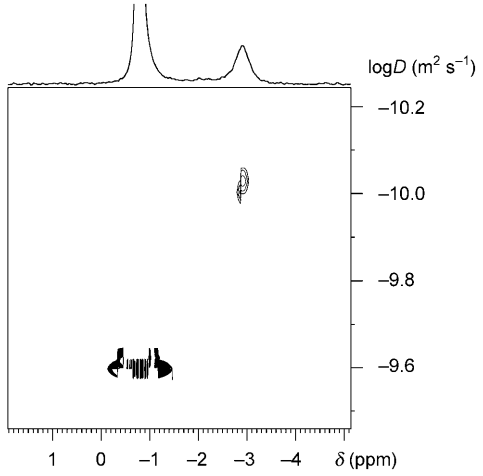

Figure 2. ^{7}Li DOSY spectrum of dried compound **1** dissolved in dry DMSO plus 16 μL of water and FA·HCl. Concentrations: c(**1**) = 1 mM, c(H_2O) = 0.9 M, c(FA·HCl) = 15 mM. The encapsulated Li^+ (trace at the upper right) moves more slowly than solvated Li^+ (trace at lower left).

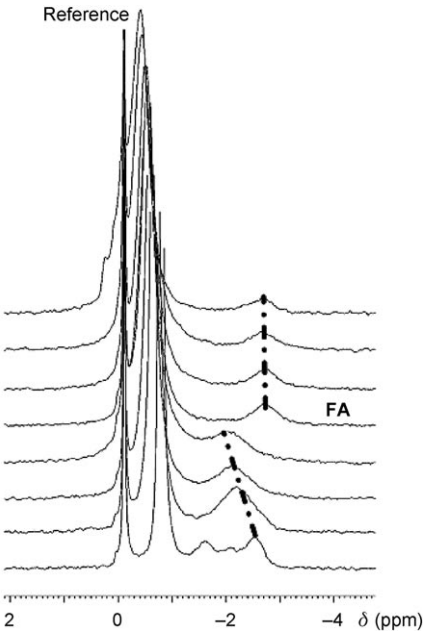

Figure 3. ^{7}Li NMR spectra of **1** dissolved in DMSO (c(**1**) = 1 mM). The sharp signal at 0 ppm is an external reference (aqueous LiCl), the signal at about −0.8 ppm corresponds to "free" Li^+ of **1**, that is, $[Li(dmso)_n(H_2O)_{4-n}]^+$, and the broad low-frequency signal(s) to Li^+ associated with the capsule. The bottom trace represents the situation prior to addition of the shift reagent $[Dy(PPP)_2]^{7-}$, the three successive traces correspond to the scenarios due to the addition of increasing amounts of $[Dy(PPP)_2]^{7-}$. The four upper traces describe the situation in the presence of the formamidinium plugs.

Although this spectrum clearly shows that the diffusion of Li^+ ions confined to the capsule is dominated by the mobility of the capsule, the exact location of the Li^+ ions with respect to the capsule cannot be extracted from this experiment. This information is obtained from the ^{7}Li NMR spectra of solutions of **1** in DMSO with an added lanthanide shift reagent. In biochemical studies, $[Dy(PPP)_2]^{7-}$ (PPP = triphosphate(5−)) is commonly employed to distinguish between intracellular and extracellular sodium[5] and lithium ions.[6] We have performed a corresponding experiment with **1** dissolved in DMSO with and without FA·HCl (Figure 3). The results can be summarised as follows: After treatment of solutions of **1** in DMSO with $[Dy(PPP)_2]^{7-}$, the high-frequency signal for external Li^+ ($[Li(dmso)_n(H_2O)_{4-n}]^+$) and the low-frequency signals for $Li^+\subset$**1a** are shifted to higher frequency and are evidently broadened. If this solution is treated with the formamidinium plugs, followed by addition of the shift reagent, the signal for $[Li(dmso)_n(H_2O)_{4-n}]^+$ still moves downfield, but the low-frequency signal(s) corresponding to Li^+ ions associated with the capsule are not affected by further addition of $[Dy(PPP)_2]^{7-}$. Thus, the low frequency signal(s) have to be due to Li^+ ions, which are physically separated from the shift reagent by the plugs. This insensitivity to the shift reagent also excludes that, in the plugged capsule, the low-frequency signals correspond to Li^+ in close contact with the surface of the capsule; that is, there is no competition between Li^+ and FA^+ for binding to the pores' outside opening. Consequently, all of the Li^+ ions confined to the capsule are located in its interior. In this context it is also of interest to note that there is just one (though broad) characteristic signal for $Li^+\subset$**1a** for the plugged capsule at about −3.1 ppm (slightly concentration and solvent dependent), the highest ^{7}Li shielding so far observed in these systems.

Additional information has been obtained from T_1 relaxation measurements.[7] In a standard solution (**1** dissolved in untreated DMSO; with a small amount of water intrinsically supplied by the solvent, and water of crystallisation of **1**), the T_1 values for the various types of Li^+ sites in the system do not differ substantially, reflecting an intermediate status corresponding to the exchange of Li^+ at external and internal capsule sites. If **1** is dissolved in dried DMSO, and this solution treated with a defined amount of water (16 μL per 1 mL of DMSO solution, corresponding to c(H_2O) = 0.9 M), the low-frequency signal for $Li^+\subset$capsule at approximately −3.1 ppm exhibits a relaxation time clearly shorter than that for externally solvated Li^+ ions. In this medium with low water contents, Li^+ is dislocated from internal to external sites by *slow* exchange; thus, several of the Li^+ ions remain associated with the capsule, experiencing fast relaxation (Figure 4). Adding more water increases the mobility of these Li^+ ions. The T_1 values for external and internal Li^+ consequently approach each other until the exchange is accelerated to the extent at which the relaxation behaviour is equilibrated. Addition of formamidinium cations blocks the pores, leaving behind internal Li^+ sites with even shorter relaxation times than in the partially open system, in which residual water acts as a mediator of transport. These short T_1 values correspond to Li^+ bonded to sulfate groups at the inner surface of the capsule. This site in the completely blocked system, characterised by the broad $\delta = -3.1$ ppm signal, should be considered the genuine site of location of Li^+ ions within the capsule.

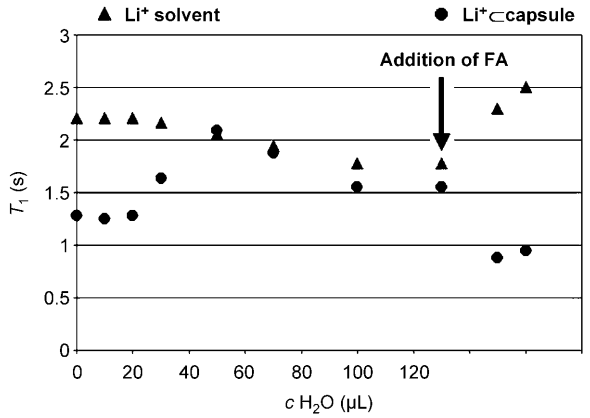

Figure 4. Dependence of T_1 values of the ^{7}Li resonances with respect to increasing amounts of water (20 μL of water correspond to a concentration $c(H_2O) \approx 1.1$ M) added to a solution of **1** in DMSO (1 mM) before and after the addition of formamidinium hydrochloride (c(FA) = 60 mM, $c(H_2O) \approx 6.6$ M for the last three data points). At low $c(H_2O)$, the different Li^+ still have well-defined T_1 values, while these values equilibrate at higher $c(H_2O)$ due to increasing exchange.

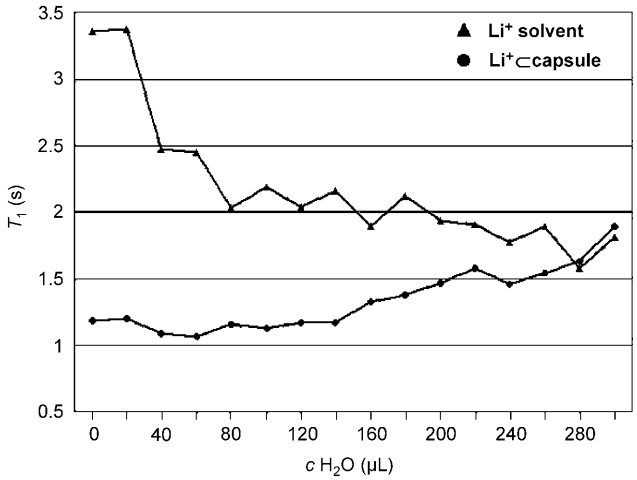

Figure 6. T_1 variations on addition of water to solutions of **1** in DMSO plugged with formamidinium, showing that with increasing amounts of water, T_1 values for external Li^+ (upper line) and internal Li^+ (lower line) approach each other. c(**1**) = 1 mM ($c(Li^+)$ = 28 mM); 20 μL of H_2O correspond to $c(H_2O) \approx 1.1$ M.

On stepwise addition of [Cr(acac)$_3$] (acac = acetylacetonate(1−)), as an alternative relaxation agent, to a solution of the plugged capsule an efficient reduction in T_1 for external Li^+ is observed (Figure 5), but almost no influence upon internal Li^+, just supporting the above statements, according to which the internal Li^+ ions are "protected" from the influence exerted by the relaxation additive. This result also represents a nice proof for the stability of the capsule.

After this demonstration of the efficacy of the encapsulation process, the question arises if it is possible to open the pores again and make the internal cations available for new "events", for example, for the subsequent exchange with Na^+.[3] This can in fact be achieved by adding water to the solution (Figure 6). With the first amounts of water added, the T_1 of the internal Li^+ remain unaffected, while with increasing amounts of water T_1 increases and more or less ends up at the T_1 of the external Li, indicating an increasing exchange between internal and external Li^+; that is, increasing release of the formamidinium plugs from the outer pore openings of the capsule.

In conclusion, we have shown that, under conditions for which exchange between internal and external Li^+ is prevented by plugging the capsule's 20 pores with formamidinium cations on the basis of supramolecular chemistry, internal Li^+ is defined by one broad NMR signal at low frequency (high magnetic field). The complete confinement of Li^+, that is, the positioning (with no option for exchange) at an internal site, is convincingly supported by the fact that neither its chemical shift nor its relaxation are influenced by paramagnetic reagents added to the solution. However, opening the pores by addition of water changes the scenario completely and leads again to an exchange of Li^+ ions. Hence, the spin-lattice relaxation time T_1 is a very sensitive parameter to study ion-exchange phenomena in this type of porous capsule systems. Potential applications lie in the use of the plugged capsules with Li^+ incorporated as a contrast agent for imaging intracellular situations and cellular surroundings by ^{7}Li magnetic resonance imaging.[6] The results also prove nicely the stability of the investigated capsule under well-defined conditions.

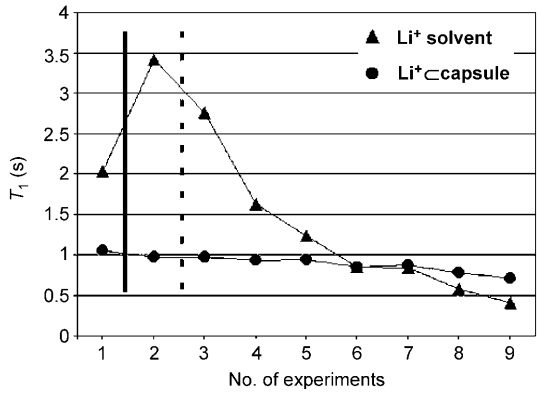

Figure 5. Influence of the relaxation reagent [Cr(acac)$_3$] on the T_1 values of DMSO solutions of **1**. Vertical bars indicate the starting points for the addition of formamidinium hydrochloride (full line) and the relaxation reagent (broken line). The running numbers at the abscissa refer to successive experiments: 1: original solution, c(**1**) = 1 mM; 2: after addition of FA·HCl (c(FA) = 15 mM); 3–9: addition of increasing μg amounts of [Cr(acac)$_3$].

Keywords: ion transport · nanocapsules · NMR spectroscopy · polyoxometalates · supramolecular chemistry

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[7] The ^{7}Li-DOSY experiment was performed on a Bruker AVANCE-400 spectrometer by using a standard BBO-probehead with a Z-gradient. The ledbpgp2s-pulse-sequence[8] was applied. The T_1 measurements were performed on a VARIAN-GEMINI-2000 spectrometer by using the standard VARIAN pulse sequence and T_1 calculation software. The mean error was less than 0.2 s throughout all of the measurements.

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