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# *Para*-Carboxy Modified Amphiphilic Calixarene, Self-Assembly and Interactions with Pharmaceutically-Relevant Molecules

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Dedicated to Professor Daniel Belluš on the occasion of his 70th birthday

*Abstract:* The self-assembly properties of the amphiphilic 5,11,17,23-tetra-carboxy-25,26,27,28-tetradodecyloxycalix[4]arene have been investigated at the air–water interface as monomolecular Langmuir layers and in water. The interactions of this amphiphile with salicylic acid (SA), acetyl-salicylic acid (ASA) and acetaminophene (APAP) have been studied at the air–water interface by means of the Langmuir balance technique. It has been demonstrated that the calix-arene molecules, when self-assembled as Langmuir monolayers, have the ability to interact with all the tested compounds. While APAP causes a stabilization of the monolayer, ASA and SA cause a slight loss of stability and a drastic change of the compressibility of the monolayer. The study of the self-assembly properties of the title compound in water revealed that this amphiphile can be self-assembled as solid lipid nanoparticles (SLNs). The atomic force microscopy investigations of the colloidal suspension, spread on a solid surface and dried, revealed the coexistence of the SLNs with layered structures.

**Keywords:** Acetyl-salicylic acid · Acetaminophene · Amphiphile · Calixarene · Salicylic acid · Solid lipid nanoparticle

## Introduction

Calixarenes are most probably one of the most important class of macrocyclic compounds used in supramolecular chemistry.[1] Produced by the base-catalyzed reaction of *para*-substituted phenols and formaldehyde, the number of the phenolic units forming the macrocycle can be tuned by changing the experimental conditions. The possibility to control the chemical modification of either the upper or the lower rim of the macrocycle opened up the possibility to produce a broad range of calixarene derivatives that have been used for various applications including artificial enzymes,[2] non-linear optic material,[3] sensors,[4] catalysts,[5] protein[1] or nucleic acid ligands,[6] to name but a few. The possibility to graft on the macrocycle both hydrophobic groups and aliphatic moieties allows the production of amphiphilic systems that have been shown to possess self-assembly properties at interfaces as Langmuir or Langmuir Blodgett films, self-assembled monolayers (SAMs),[7] in water as vesicular[8] or nanoparticulate systems[9] and in the solid-state.[9] Langmuir monolayers and Langmuir Blodgett films of calix-arenes have been demonstrated to possess molecular recognition properties of bio-molecules (nucleosides,[10] nucleotides,[11] amino acids,[12] proteins[13]), small organic molecules[14] and ions.[15] The rigidity of the calix[4]arene (*i.e.* possessing four phenolic units) and the relative rigidity of the calix[6]arene make these macrocycles attractive to control the orientation of the chemical units grafted on the macrocycle. In fact, most of the amphiphilic calixarenes developed are based on these macrocycles. More recently, the group of Coleman developed new calixarene-based amphiphiles using bigger macrocycles containing eight or nine units.[9,16]

We have recently reported on the synthesis, self-assembly and molecular recognition properties of *para*-amino amphiphilic calix[4]arenes.[17] We have demonstrated that this poly-cationic molecule has the ability to self-assemble at the air–water interface as stable insoluble monolayers which can serve as a charged surface for binding DNA. In the present study, we report on the self-assembly properties of a poly-anionic amphiphilic calixarenes and its ability to self-assemble at the air–water interface and in water. The interactions of these self-assembled systems with three pharmaceutically and environmentally relevant molecules, namely salicylic acid (SA), acetyl-salicylic acid (ASA) and acetaminophene (APAP) (*cf.* Fig. 1) are described.

## Experimental

### *General*

All chemicals were purchased from Sigma-Aldrich (Switzerland) and used without further purification.

5,11,17,23-tetra-carboxy-25,26,27,28-tetradodecyloxycalix[4]arene (**1**) was produced following the previously reported method,[18] the analytical data measured ($^1$H NMR, $^{13}$C NMR and MALDI-TOF) were found to be in perfect agreement with that reported.

### *Self-assembly in Water*

To 1.5 ml of a solution of **1** in THF (5 mg/ml), under magnetic stirring, was

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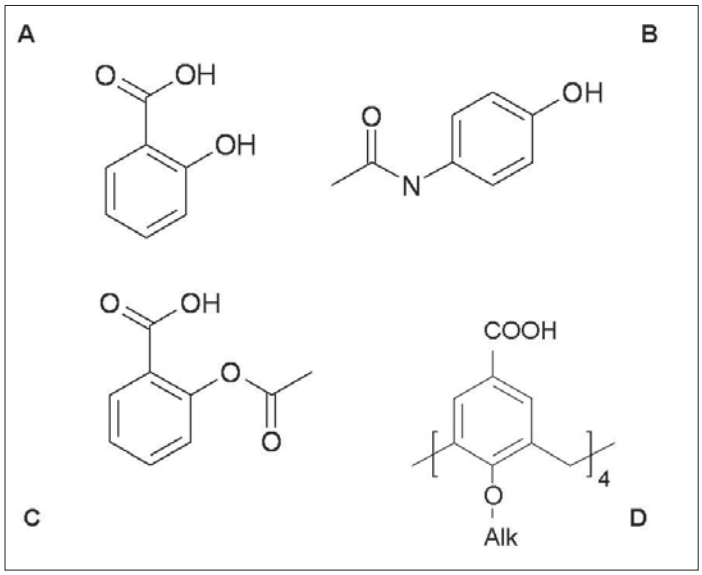


Fig. 1. Chemical formulae of salicylic acid (**A**), acetaminophene (**B**), acetyl-salicylic acid (**C**) and 5,11,17,23-tetra-carboxy-25,26,27,28-tetradodecyloxy-calix[4]arene (**1**) (**D**) on a pure water subphase.

added 50 ml of water at a flow rate of approximately 500 ml/min. The resulting suspension is stirred for an additional minute and the THF evaporated under reduced pressure at 40 °C.

### *Particle Size and ζ-Potential Measurements*

Photon correlation spectroscopy experiments were carried out using a Zetasizer Nano ZS system (Malvern Instruments Ltd., UK). The samples were prepared diluting the calixarene suspension produced as described above to a concentration of 50 mg/l in water, at 298 K.

### *Langmuir Monolayer Studies*

Langmuir film experiments were carried out using a Nima 112D system (Nima Technology, Coventry, UK). The trough and the barriers were cleaned with analytical grade chloroform and water. Surface tension was monitored using a Wilhelmy plate system. Compressions were performed continuously at a speed rate of 5 $cm^2min^{-1}$. The drug-containing subphases were prepared by dissolving the appropriate amount in pure water. Compressions without monolayer spread on the surface were performed in order to ensure the absence of surface-active molecules in the sub-phase; in no case was a relevant change in surface tension observed. Spreading solutions were prepared by dissolving **1** in chloroform at a concentration of 1 mg/ml. 5 µl of this solution was spread on the aqueous subphase using a Hamilton gas-tight microsyringe, 20 min were allowed for total evaporation of the solvent and equilibration of the amphiphiles at the interface.

### *Atomic Force Microscopy*

The sample was prepared by spreading 10 µl of the calixarene colloidal suspension at a concentration of 300 mg/l on freshly cleaved mica surface; and dried overnight at room temperature. Imaging was carried out in non-contact mode in air using a NTegra Prima system (NT-MDT, Moscow, Russia) equipped with a silicon rectangular cantilevers with a resonant frequency of 250 kHz (NT-MDT). Prior to the measurements, the system was calibrated using a calibration grid.

### *Scanning Electron Microscopy (SEM)*

SEM samples were prepared spreading 10 µl of the calixarene colloidal suspension on freshly cleaved mica surfaces and dried at room temperature. After Au-Pd sputter coating, imaging was carried using a Supra 40V system (Carl Zeiss, Switzerland) at an accelerating voltage of 10 kV using an in-lens detector.

## Results and Discussion

Compound **1** was produced *via* hydrolysis of the corresponding tetra-nitrile compound using the methods described by Gutsche[18a] and Shuker.[18b] The Langmuir isotherm of **1** has been measured on a pure water subphase and is presented in Fig. 2. It shows an apparent molecular area of 139 $Å^2$ and a collapse pressure of 52 $mNm^{-1}$. In addition to the liquid-expanded to liquid-condensed phase transition, an additional kink is observed at a pressure of 38 $mNm^{-1}$. These values are in good agreement with those reported by Ströbel *et al.*,[8d] the slight shift observed in the isotherm might be due to different experimental conditions (*e.g.* compression rate). The kink observed was also reported and attributed to a reorganization–relaxation of the amphiphiles within the monolayer.

The isotherms of **1**, on subphases containing salicylic acid (SA), acetyl-salicylic acid (ASA) and acetaminophene (APAP) at a concentration of 10 mM have been measured and are presented in Fig. 3; the

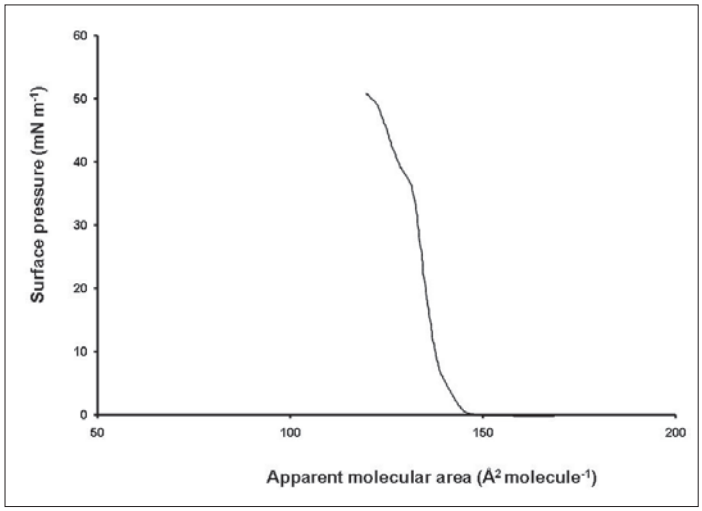


Fig. 2. Compression isotherm of **1** on a pure water subphase.

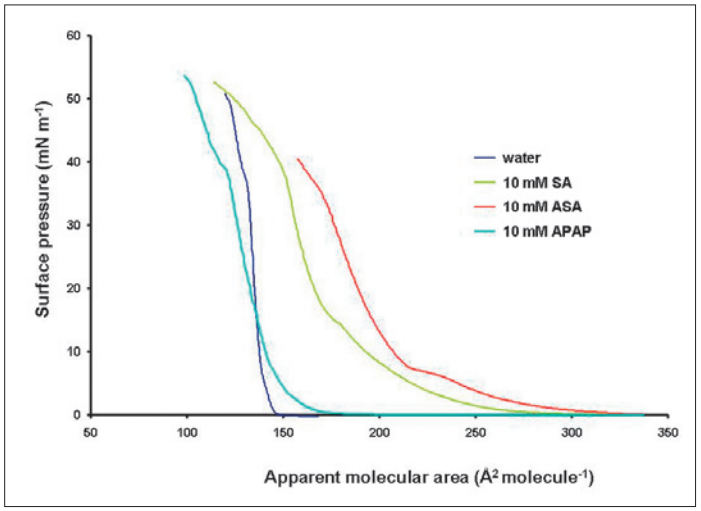


Fig. 3. Compression isotherm of **1** subphases containing 10 mM SA. ASA, APAP.

Table 1. Characteristic values extracted from isotherms of **1** subphases of 10 mM of acetyl-salicylic acid (ASA), salicylic acid (SA) and acetaminophene (APAP). $\Pi_{coll}$ and $A_{coll}$ represent the surface pressure and the surface area at the collapse of the monolayer, $A_{10}$, $A_1$ and $A_0$ represent apparent molecular areas for surface tensions of 10, 1 and 0 mN.m$^{-1}$ respectively; $A_{lim}$ the extrapolation of the linear part of the isotherm on the × axis; $\Pi_{coll}$ is given in mN m$^{-1}$, the other values are in Å$^2$ molecule$^{-1}$.

| Sub-phase | $\Pi_{coll}$ | $A_{coll}$ | $A_{10}$ | $A_1$ | $A_0$ | $A_{lim}$ |
|---|---|---|---|---|---|---|
| – | 50 | 120 | 138 | 144 | 146 | 148 |
| ASA | 40 | 157 | 207 | 312 | 328 | 212 |
| SA | 38 | 151 | 192 | 257 | 287 | 176 |
| APAP | 53 | 151 | 140 | 151 | 163 | 158 |

characteristic values of the measured isotherms are presented in Table 2.

From these results it can be seen that APAP has only a slight effect on the compression isotherm of **1** causing a shift of the collapse area from 120 Å$^2$ molecule$^{-1}$ to 151, and $A_0$ from 146 to 163 while the collapse pressure increases from 50 to 53 mN m$^{-1}$. This suggests that at this concentration there is a stabilization effect due to the interaction of APAP with the monolayer. In the case of ASA and SA, the main effect observed is a drastic expansion of the monolayer revealed for example by an $A_1$ value shifting from 144 Å$^2$ molecule$^{-1}$ to 287 and 328, for ASA and SA respectively. In both cases, the monolayer stability is decreased with collapse pressure value dropping from 50 mN m$^{-1}$ to 40 and 38 mN m$^{-1}$ respectively. This could be attributed to the fact that the interactions of the calixarene-based amphiphile with the molecules dissolved in the sub-phase cause an expansion of the monolayer due either to the inclusion of the aromatic moiety of the drug in the cavity of the calixarene causing a higher change density at the surface, or at the interaction of one drug molecule with more than two calixarenes, as already observed with DNA.[17] Co-crystallization of **1** with these guest molecules is expected to help the understanding of the observed results at the air–water interface.

Ströbel *et al.* have demonstrated, using cryo-transmission electron microscopy, that **1** forms a mixture of large and small vesicles, distorted vesicles, and isolated lamellae. We have previously demonstrated that a wide range of amphiphilic calixarenes can be assembled as stable solid lipid nanoparticles, in the absence of an additional stabilizing surfactant, using a different method based on nano-precipitation.[19] In the present work, this method has been applied to **1.** Photon correlation spectroscopy carried out on the colloidal suspension revealed that the polydispersity of the sample is high which prevented us from measuring a consistent size distribution. In order to assess the composition of the samples, the suspension was spread on a mica slide and imaged by atomic force microscopy and scanning electron microscopy. The results are presented in Fig. 4.

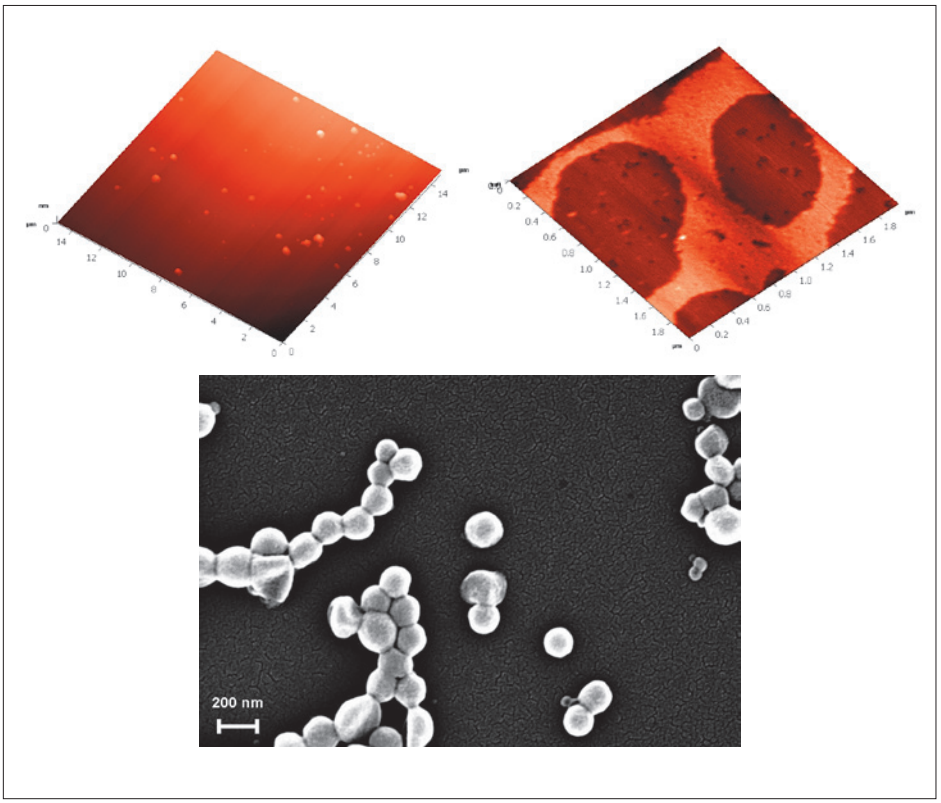


Fig. 4. AFM images of a suspension of **1** spread on a mica surface (top) at scan ranges of 15 (left) and 2 μm (right) and SEM images of **1**-based SLNs.

The AFM investigations revealed the co-existence of two different species at the surface of the same sample. The first ones are round shaped objects of around 200 nm in diameter, quite heterogeneous in size, exhibiting only a moderate flattening which can be compared to that observed for calixarene-based SLNs and which is in contrast with the imaging of liposomes directly dried on a surface which typically shows a pronounced flattening. The SEM observation confirmed the solid structure of the particles which can stand the high vacuum necessary for the coating of the sample and its imaging. The second type of structure observed was a much more unexpected layered system. Showing a height of 1.5–2 nm, the layers look very similar to supported lipid bilayers. Nevertheless, any hypothesis regarding the structure of these layers would be highly speculative and many more additional experiments are needed.

## Conclusion

We have demonstrated that the title compound, when self-assembled as Langmuir monolayers, possesses the ability to interact with aromatic drugs, namely salicylic acid, acetyl-salicylic acid and acetaminophene. The nanoprecipitation method used for this amphiphile yield a heterogeneous mixture composed of SLNs and layer-forming structures, presumably liposomes.

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