# Self-assembly of amphiphilic anionic calix[4]arene and encapsulation of poorly soluble naproxen and flurbiprofen†

Lucia Barbera,[a] Giuseppe Gattuso,[a] Franz H. Kohnke,[a] Anna Notti,[a],* Sebastiano Pappalardo,[b] Melchiorre F. Parisi,[a] Ilenia Pisagatti,[a],* Salvatore Patanè,[c] Norberto Micali[d] and Valentina Villari[d]

[a]Dipartimento di Scienze Chimiche, Università di Messina, Viale F. Stagno d'Alcontres 31, 98166 Messina, Italy
[b]Dipartimento di Scienze Chimiche, Università di Catania, Viale A. Doria 6, 95125 Catania, Italy
[c]Dipartimento di Fisica e di Scienze della Terra, Viale F. Stagno d'Alcontres 31, 98166 Messina, Italy
[d] CNR-IPCF Istituto per i Processi Chimico-Fisici, Viale Viale F. Stagno d'Alcontres 37, 98158 Messina, Italy

## Electronic Supplementary Information

**General Experimental Methods**. ^{1}H and ^{13}C NMR spectra were recorded at room temperature in $D_2O$, at 500 and 125 MHz respectively. The solvent residual peak ($\delta_H$ 4.65 ppm) was used as an internal standard for ^{1}H NMR spectra, while the ^{13}C NMR spectrum was referenced to internal dioxane ($\delta_C$ 69.3 ppm). THF was dried by a standard method prior to use; other chemicals were reagent grade and were used without further purification. *p-tert*-Butylcalix[4]arene was synthesized according to a literature procedure.[1]

Diffusion Ordered NMR Spectroscopy (DOSY) and NOESY studies were performed on a Varian 500 MHz spectrometer equipped with a pulse-field gradient probe. DOSY spectra were recorded at 25 °C using a Doneshot pulse sequence.[2] Experimental parameters were optimized according to the sample under investigation. Diffusion gradients were incremented in 30 steps with gradient pulse amplitudes varying from 1.6 to 50 gauss/cm, the number of transients acquired for each increment ranging from 16 to 128, with a diffusion gradient length of 2–4 ms and diffusion delays in the 150–200 ms range. All measurements were performed in triplicate and the reported values are the mean ± standard deviation of the mean. The hydrodynamic radii ($R_h$) were obtained using the Stokes-Einstein equation: $D_{obs} = k_BT/6\pi\eta R_h$, where $k_B$ is the Boltzmann constant, T is the temperature (K) and $\eta$ is the viscosity of the solvent (Pa s).

The solubility of the drugs naproxen (**2**) and flurbiprofen (**3**) in $D_2O$ surfactant solution of different concentrations (2.5, 5.0 and 10.0 mM) was measured by NMR. A fixed amount of solid **2** or **3** (5 μmol) was added to 1 mL of the surfactant solution previously filtered through a 0.1 μm Millipore filter and the sample was equilibrated at room temperature for at least 24 h. The solutions were centrifugated (6000 rpm) for 10 minutes to remove undissolved solid. The "detectable" concentrations of the remaining calixarene and micelle-encapsulated naproxen/flurbiprofen in the supernatant were determined from the integral of selected peaks by using the quantitative qNMR software Varian Vnmrj 3.2.

2D NOESY experiments were carried out using a 500 ms mixing time, 16 transients for each increment (512 in total) and a relaxation time of 3 s.

UV solubility measurements were performed using a Varian Cary 50 Scan UV-Visible spectrophotometer. 2 mL of naproxen/surfactant solution ([**1**] = 5.0 mM) were prepared as described above. After centrifugation, one half of the supernatant solution was filtered through a 0.1 μm Millipore filter. Aliquots of filtered and unfiltered solutions (0.5 mL each) were allowed to stir for 24 h with 1 mL of $CHCl_3$. Finally 0.5 mL of the organic solutions were diluted with $CHCl_3$ (1 mL) and Naproxen concentration was determined by UV spectroscopy at $\lambda = 318$ nm (see table S2).

1. C. D. Gutsche and M. Iqbal, *Org. Synth.*, 1990, **68**, 234.
2. M. D. Pelta, G. A. Morris, M. J. Tschedroff and S. J. Hammon, *Magn. Reson. Chem.*, 2002, **40**, S147.

Dynamic light scattering (DLS) measurements were carried out using a 35 mW polarized He-Ne laser source ($\lambda$ = 632.8 nm). The polarized scattered light was collected at 90°, in a self-beating mode, using an Avalanche Photodiode (Single Photon Counting Module) by Excelitas Technologies. The signal was sent to a Malvern 4700 submicrometer particle analyzer system to measure the intensity autocorrelation function, $g_2(t)$. The intensity autocorrelation function is related to the scattered electric field correlation function $g_1(t)$ through the Siegert's relation: $g_1(t) \propto [g_2(t)-1]^{1/2}$.

Determination of the size distribution from the field correlation function $g_1(t)$ was obtained through a Laplace inversion:

$$g_1(t) = \int \tau A(\tau) \exp(-t/\tau) d(\ln \tau)$$

where $\tau = 1/(DQ^2)$, D and Q being the diffusion coefficient of the particle and the exchanged momentum of the scattered light, respectively. The diffusion coefficient D is related to the hydrodynamic radius through the Einstein-Stokes relation. A discrete multi-exponential non-negative least-squares fit (NNLS) was used to perform this inversion procedure.

The spectral amplitude $\tau A$ is related to the scattered intensity of each species (with radius R), which, in turn, depends on its number concentration, $n$, on the radius $R$ and on its form factor $P(QR)$. By using the Rayley-Gans form factor of a sphere it is possible to obtain the number distribution as:

$$n(R) = a \frac{\tau A}{[\sin(QR) - QR \cos(QR)]^2}$$

where $a$ is a constant giving the normalization to unity of the number-weighted size distribution.

Atomic force microscopy was performed using an Ntegra probe NanoLaboratory from NT-MDT working in tapping mode and employing a Vit-p as probe. The sample was prepared dropping 10 μL of a solution of **1** (0.1 μM) on the 110 surface of an electronic grade silicon wafer and the sample was dried under vacuum.

**Synthetic Procedure**. **Sodium *p-tert*-butyl-calix[4]arene-tetra-*O*-(4-butoxysulfonato) (1):**

*p-tert*-Butylcalix[4]arene (400 mg, 0.61 mmol), an excess of NaH (177.5 mg, 7.4 mmol) and 1,4-butane sultone (1 g, 7.4 mmol) were mixed in anhydrous THF (40 mL) and refluxed for 48 h. The mixture was allowed to cool to room temperature and MeOH (10 mL) was added. The precipitate was collected by filtration, washed with EtOH, dissolved in 10 mL of water and finally precipitated by the salting-out method with sodium acetate. After centrifugation, the solid was separated and refluxed in EtOH (40 mL) for 24 h. The white powder (508 mg, 64%) was collected by filtration. M.p. 305–310 °C (dec.). ^{1}H NMR (0.2 mM, D$_2$O) $\delta$ 0.97 (s, C(CH$_3$)$_3$, 36 H), 1.79–2.03 (m,

OCH$_2$(C$H_2$)$_2$, 16 H), 2.89 (t, $J$ = 7.9 Hz, SCH$_2$, 8 H), 3.13 and 4.31 (AX, $J$ = 12.5 Hz, ArCH$_2$Ar, 8 H), 3.83 (br t, OCH$_2$, 8 H), 6.87 (s, Ar, 8 H) ppm; $^{13}$C NMR (5.0 mM, D$_2$O) $\delta$ 23.7, 31.5, 33.2, 34.1, 36.4, 53.9, 77.3, 127.9, 136.9, 147.5, 156 ppm. Anal. Calcd for C$_{60}$H$_{84}$Na$_4$O$_{16}$S$_4$: C, 56.23; H, 6.61; S, 10.01; Found: C, 55.93; H, 6.61; S, 9.97.
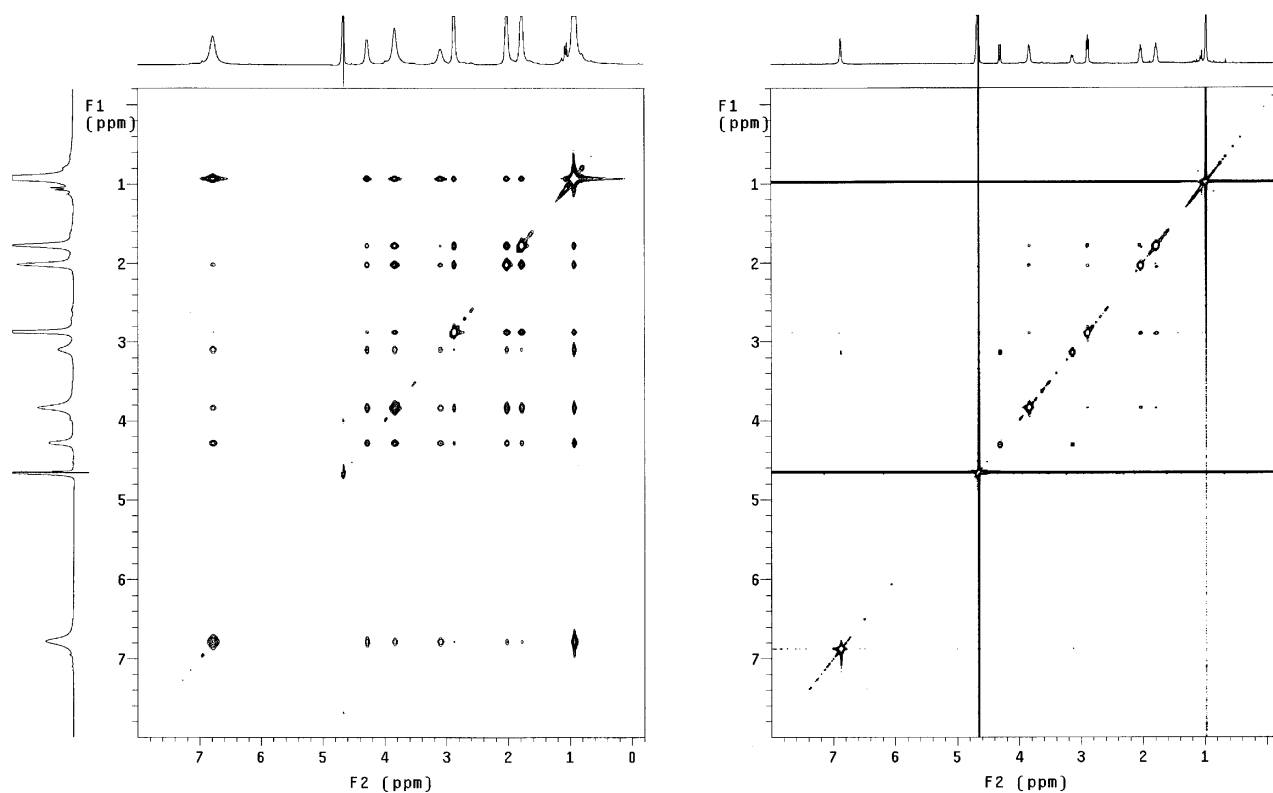
**Fig. S1**. 2D NOESY spectra of: (a) [**1**] = 5.0 mM, (b) [**1**] = 0.5 mM.
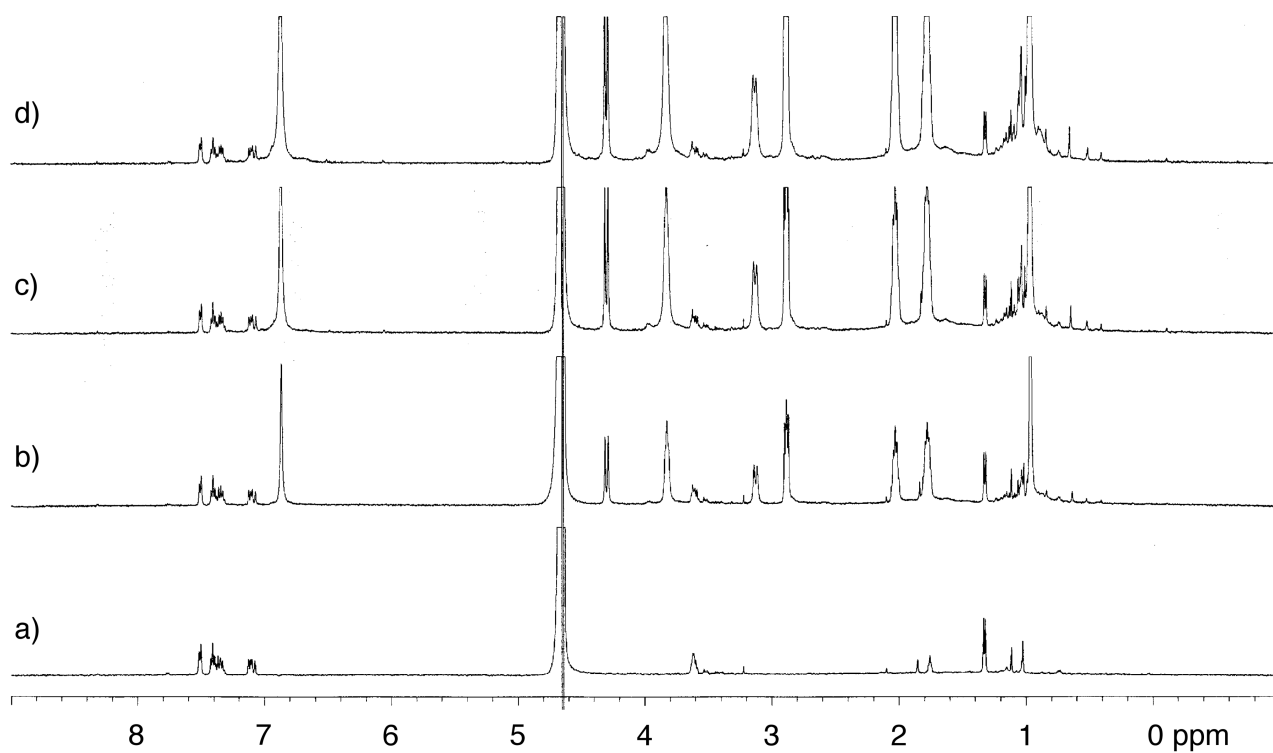


**Fig. S2**. Flurbiprofen (**3**) extraction experiments with **1** below the cmc: a) [**3**] = 0.13 mM; b) [**1**] = 0.25 mM, [**3**] = 0.13 mM; c) [**1**] = 0.5 mM, [**3**] = 0.13 mM; d) [**1**] = 0.75 mM, [**3**] = 0.13 mM.
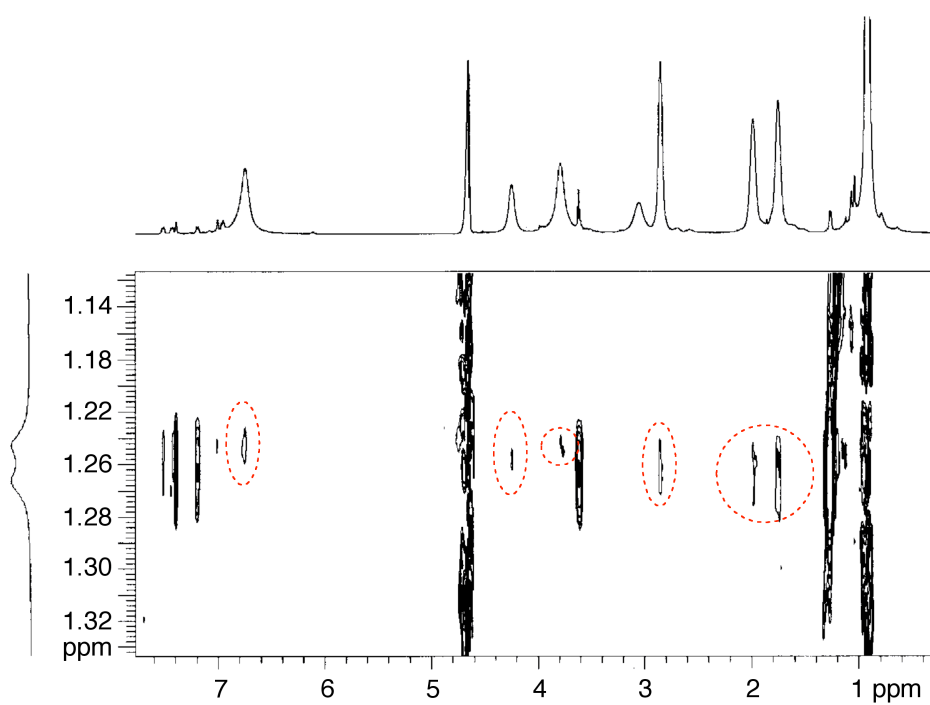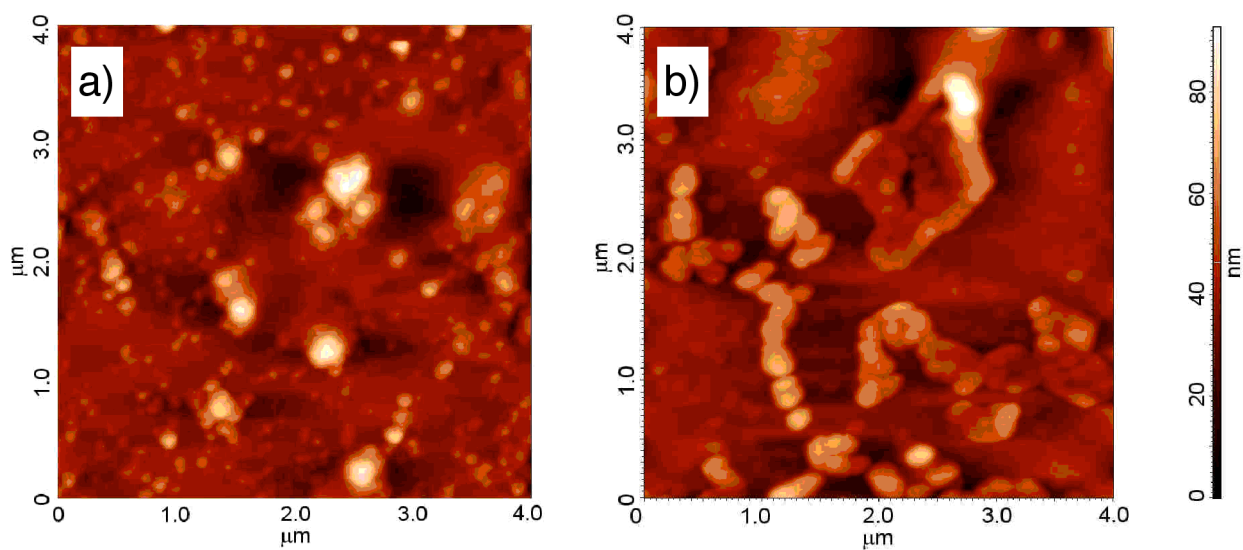
**Fig. S3**. Section of the 2D NOESY spectrum (500 MHz, 298 K) of a $D_2O$ solution of **1** and **2**.

**Table S1**. Diffusion coefficients ($D_{1,obs}$) of the species present at 298 K in $D_2O$ solutions of calixarene **1** at different concentrations. Data were calculated from DOSY experiments, using the $SCH_2$ resonance ($\delta$ = 2.89 ppm) as the probe signal.

| $C$ (mM) | $D_{1,obs} \times 10^{-10}$ ($m^2$/s) |
|---|---|
| 10 | 0.80 ± 0.03 |
| 7.5 | 0.88 ± 0.03 |
| 5 | 0.98 ± 0.01 |
| 4 | 1.14 ± 0.03 |
| 3 | 1.33 ± 0.08 |
| 2.8 | 1.38 ± 0.01 |
| 2.6 | 1.43 ± 0.03 |
| 2.5 | 1.48 ± 0.01 |
| 2.4 | 1.49 ± 0.01 |
| 2.3 | 1.54 ± 0.02 |
| 2.2 | 1.58 ± 0.02 |
| 2.1 | 1.62 ± 0.03 |
| 2.0 | 1.70 ± 0.03 |
| 1.9 | 1.76 ± 0.01 |
| 1.8 | 1.82 ± 0.02 |
| 1.7 | 1.88 ± 0.04 |
| 1.6 | 1.94 ± 0.04 |
| 1.5 | 2.01 ± 0.03 |
| 1.4 | 2.08 ± 0.02 |
| 1.3 | 2.16 ± 0.01 |
| 1.2 | 2.20 ± 0.02 |
| 1.1 | 2.28 ± 0.03 |
| 1.0 | 2.35 ± 0.02 |
| 0.9 | 2.39 ± 0.03 |
| 0.6 | 2.47 ± 0.02 |
| 0.4 | 2.48 ± 0.01 |
| 0.2 | 2.52 ± 0.01 |
| 0.1 | 2.52 ± 0.02 |

**Table S2**. UV and NMR determination of naproxen (**2**) solubility in a 5.0 mM micellar solution of **1**.

|  | UV (prior filtration) | UV (after filtration) | NMR |
|---|---|---|---|
| Dissolved naproxen |  |  |  |
| mM | 0.85 ± 0.03 | 0.63 ± 0.03 | 0.63 ± 0.05 |
| mg/mL | 0.196 ± 0.007 | 0.145 ± 0.07 | 0.145 ± 0.011 |



**Fig. S4**. AFM images of a) **1** + **2**; b) **1** + **3**.