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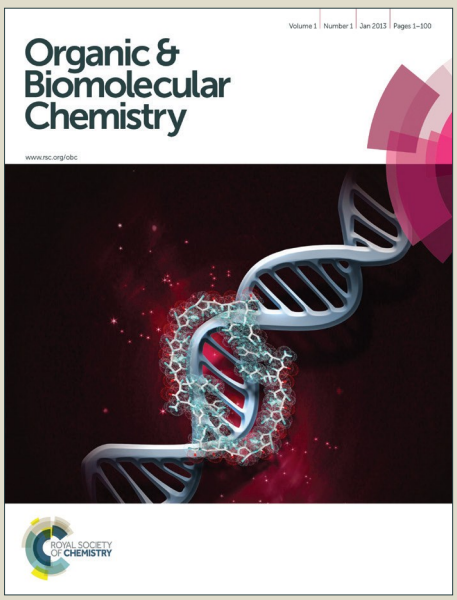

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Journal Name

COMMUNICATION

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

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Supramolecular aggregates formed through the association of an amphiphilic tetra-*O*-butylsolfonato calix[4]arene 1 were investigated in aqueous solution by a combination of different techniques (NMR, DLS and AFM). The ability of the micellar aggregates of calixarene 1 to increase the solubility of poorly water-soluble drugs was studied.

The controlled self-assembly of synthetic amphiphiles has attracted wide interest for its potential in many biotechnological applications. The use of tailor-made surfactants is pivotal to the study of natural self-assembled structures (e.g. bio-membranes) and to the understanding of their structural transition during biological processes. Specially designed amphiphiles can provide useful information on the different non-covalent contributions (hydrophobic, hydrogen bonding, van der Waals, π-stacking) involved in the formation of a given type of micelle, vescicle or bilayer.[1] In addition, surfactant formulations are finding growing applications as drug delivery systems in the clinical field, owing to their ability to encapsulate and transport a hydrophobic pharmaceutically active drug to its therapeutic target. In this context, the correlation between different surfactant aggregation modes and the resulting interactions with a given substrate is of particular importance. Micellar systems, when used as aqueous carriers,[2] have several key advantages over other delivery systems,[3] such as soluble polymers and liposomes, including reduced size, high-loading capacity as well as control over their assembly/disassembly, and hence over their loading and releasing abilities, which are in turn driven by their critical micelle concentration (cmc).

Calixarene-based surfactants are a relatively new class of amphiphiles which, in addition to the usual hydrophobic and hydrophilic properties, offer additional inclusion capabilities thanks to the presence of the aromatic cavity.[4] Moreover, by changing the nature of the polar moieties[5] and/or their position on the calixarene macrocycle (i.e., on the upper or lower rim),[6] and/or the size of the calix[*n*]arene core and/or the length of the hydrophobic portion,[7] it is possible to obtain a range of tailor-made amphiphiles with different cmcs and aggregation properties. When compared with conventional surfactants, amphiphilic calixarenes show lower cmc values and the ability to self-assemble into structurally persistent micelles.[8] Their recognition properties in water,[9] low cytotoxicity and lack of haemolytic effects make them promising candidates for medical and pharmaceutical applications.[6,10] As a consequence, charged or neutral amphiphilic calixarenes have recently been investigated because of their ability to form micelles to be used in drug delivery.[11]

We recently reported that lower rim alkanesulfonate calix[5]arene micelles are able to encapsulate biologically relevant amines in water despite their intrinsically hydrophilic character.[12] In this communication we wish to describe the aggregation properties of a homologous amphiphilic calix[4]arene[13] and, down the line, the ability of the resultant micellar aggregates to solubiliee poorly water-soluble anti-inflammatory drugs, naproxen (**2**) and flurbiprofen (**3**).[14]

Amphiphilic calix[4]arene **1** was obtained in 64% yield from *p-tert*-butylcalix[4]arene and 1,4-butanesultone by adapting a procedure previously reported[15] for the larger calix[5]arene derivative.[9e] The ^{1}H NMR spectrum of **1**, recorded at a low concentration ([**1**] = 0.2 mM, D_2O) displays a set of sharp resonances typical of a monomeric species adopting a *cone* conformation (Fig. 1a).[16]

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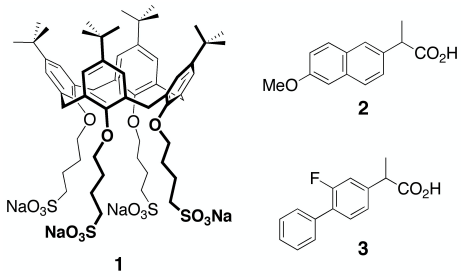
† Electronic Supplementary Information (ESI) available: General experimental methods, synthetic procedures, NOESY spectra, Self-diffusion coefficients of calixarene **1**, UV data, AFM images. See DOI: 10.1039/x0xx00000x



Upon increasing the concentration to 1.4 mM (Fig. 1b), the resonances broaden, indicating that an aggregation process is taking place, and thus confirming the surfactant nature of **1**. At this concentration, a second broad signal for the aromatic protons appears at δ 6.78 ppm (see arrows in Fig. 1 b,c), whose intensity increases until it becomes the predominant species in solution (for [**1**] ≥ 4.0 mM, Fig. 1c,d), and can be assigned to the formation of a micellar aggregate. The concomitant presence of two close but distinct sets of aromatic signals indicates a slow exchange on the NMR time-scale between the two species.

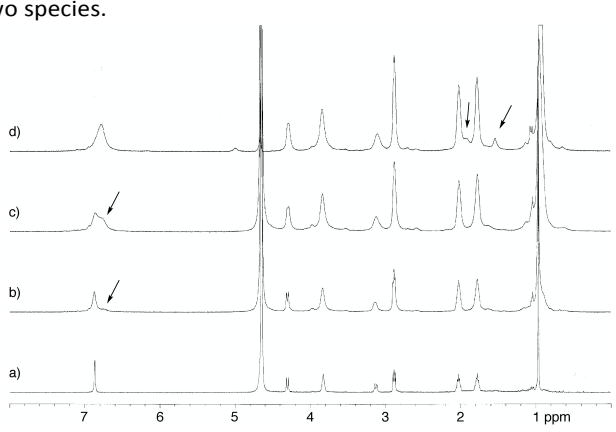


Fig. 1 ^{1}H NMR (500 MHz, 298 K, D_2O) spectra of calix[4]arene **1** at different concentrations: (a) 0.2 mM, (b) 1.4 mM, (c) 2.3 mM and (d) 4 mM.

The critical micellar concentration for **1** in D_2O was determined by diffusion-ordered NMR (DOSY)[17] studies, using calixarene solutions in the 10.0–0.10 mM concentration range. A cmc of 1.15 mM‡ was estimated from the intersection point of the two lines best fitting the experimental data points obtained by plotting the self-diffusion coefficient ($D_{\mathbf{1},obs}$) *vs* the inverse of the concentration of calixarene **1** (Fig. 2).

A closer inspection of the concentration ranges slightly below and above the cmc (see insert in Fig. 2) showed the presence of two distinct break points (at 0.83 and 1.41 mM, respectively), reasonably ascribable to two distinct aggregation phenomena of **1**. The former (0.83 mM) likely refers to the initial formation of small oligomers (premicelles)[18] seen –on the NMR time-scale– in a fast-exchange regime with the monomers. The latter (1.41 mM), on the other hand, most probably indicates the onset of micellar aggregation (Fig. 1b).

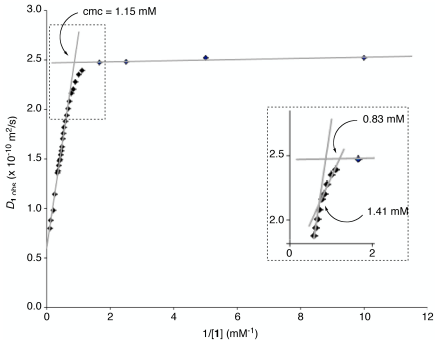


Fig. 2 Plot of the self-diffusion coefficients ($D_{\mathbf{1},obs}$) *vs* the inverse of the concentration of calixarene **1** in D_2O at 25 °C.

As the concentration of **1** increases further (above 3.0 mM), new peaks appear in the ^{1}H NMR spectrum (see arrows in Fig. 1d) and, accordingly, new aggregates form as the result of an additional structural transition (micelle-micelle or micelle-vescicles).§ The hydrodynamic radii (R_h) for the monomer (7.9 Å) and the micelle (24.9 Å) were conveniently obtained from the $D_{\mathbf{1},obs}$ values determined below ([**1**] = 0.1 mM) and above ([**1**] = 10.0 mM) the cmc, using the Stokes-Einstein equation.

Additional evidence corroborating micelle formation was obtained from 2D NOESY studies (see ESI, Fig. S1†). The spectrum of a 5.0 mM solution of **1** shows NOE cross-peaks for all the calixarene resonances. Interestingly, there are cross-peaks indicating the proximity of the upper-rim *tert*-butyl hydrogen atoms and the lower-rim butanesulfonate residues of adjacent calixarene molecules. Conversely, the absence of cross-peaks in the spectrum of **1** in the monomeric form (0.5 mM) demonstrates the intermolecular origin of the NOEs mentioned earlier (i.e., [**1**] = 5.0 mM), suggesting that in the micellar aggregate the calixarene units are probably arranged in a staggered fashion (Fig. 3).[12,19]

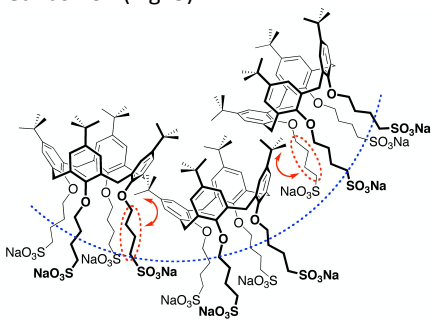


Fig. 3 Schematic representation of staggered calixarene molecules within a micelle. Arrows indicate relevant NOE cross-peaks.

As already observed for the parent calix[5]arene,[9e] NMR quantitative analysis (see ESI†) of D_2O solutions of calixarene **1** below the cmc showed an apparent discrepancy between the nominal and the detectable concentrations of monomer in solution (for example, for [**1**] = 0.2 mM, a concentration of [**1**] = 0.1 mM was calculated).

In line with previous observations on the tendency of amphiphilic calixarene-type surfactants to aggregate below the cmc, in large infinite structures ("open model"[19]), in the case of **1**, dynamic light scattering (DLS) experiments (Fig. 4a) below

the estimated cmc revealed the presence of two populations of very large aggregates[11a,20] with hydrodynamic radii of approximately 100 and 300 nm. The number fraction of the 300 nm aggregates was found to be about 0.03.[21] When 0.5 and 5.0 mM aqueous solutions of **1** were pre-filtered through a 0.45 μm millipore membrane, the 100 nm aggregates became even more predominant, with the number fraction of the larger aggregates (300 nm) decreasing to about 0.01 and becoming almost negligible in the two solutions, respectively (Fig. 4b). Remarkably, no smaller micellar aggregates (i.e., in the 2 nm range) were visible by DLS either before or after filtration. It then follows that a combined use of DOSY –for the smaller aggregates– and DLS –for the larger ones, "transparent" to NMR[22]– is necessary to get a full picture of the aggregating behaviour of amphiphilic molecules such as **1**.[23] Our data indicate that calixarene **1** self-assembles in premicelles and very large aggregates below the cmc.

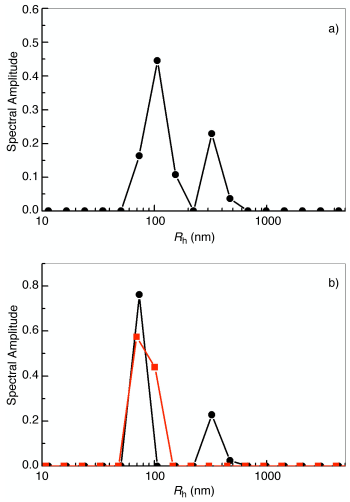


Fig. 4 Size distributions of the calixarene **1** aggregates in aqueous solutions: (a) [**1**] = 0.5 mM; (b) [**1**] = 0.5 and 5.0 mM (black and red trace, respectively) after filtration through a 0.45 μm millipore filter.

Atomic force microscopy (AFM) measurements provided information on the morphology of these aggregates, showing that calixarene **1** tends to form complex structures, most of them having an average diameter of 100–200 nm, though many smaller particles (*ca*. 50 nm) are also visible (Fig. 5).

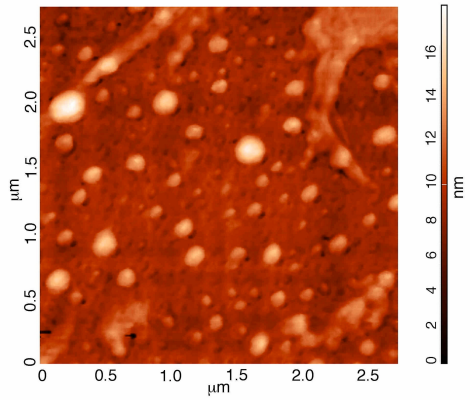


Fig. 5 AFM topography image of calixarene **1**.

In order to test the potential of amphiphilic calix[4]arene **1** to act as a molecular carrier upon micelle formation, we decided to investigate its ability to increase the water solubility of the nonsteroidal anti-inflammatory hydrophobic drugs naproxen (**2**) and flurbiprofen (**3**). The ^{1}H NMR spectra of pure naproxen and flurbiprofen in D_2O prior to and after segregation within a 5.0 mM micellar solution of **1** (see below for the extraction conditions used) are shown in Fig. 6. In both cases under examination, all the resonances of **2** and **3** underwent significant complexation-induced shifts, as a result of an interaction between the drug and the micellar environment provided by **1**. On the other hand, a parallel extraction experiment, carried out below the cmc (see ESI, Fig. S2†), ruled out the formation of inclusion complexes between **1** (as a monomer) and the two drug molecules.

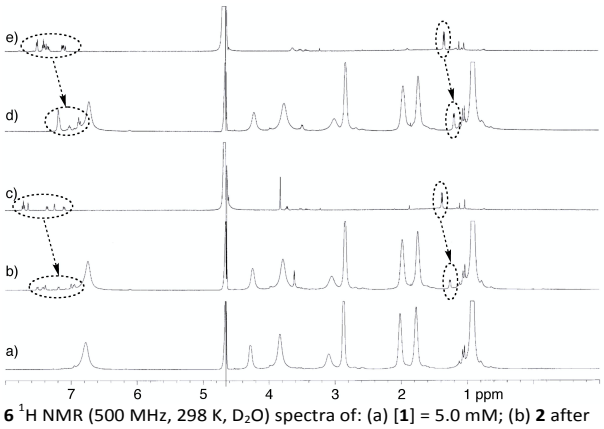


Fig. 6 ^{1}H NMR (500 MHz, 298 K, D_2O) spectra of: (a) [**1**] = 5.0 mM; (b) **2** after extraction with a 5.0 mM solution of **1**; (c) [2] = 0.14 mM; (d) **3** after extraction with a 5.0 mM solution of **1** and (e) [**3**] = 0.13 mM.

2D NOESY NMR experiments, were used to shed light on the location and orientation of the drugs within the micelles. The α-CH_3 group of **2** shows NOE interactions with the hydrogen atoms of both the aromatic and the aliphatic moieties of **1** (Fig. S2†). Flurbiprofen (**3**) displays stronger correlation peaks, with the aromatic signal at δ 7.19 ppm showing diagnostic contacts with all the calixarene resonances (red circles in Fig. 7). Cross-peaks are consistent with the aromatic unit of **3** and the lower-rim aliphatic moieties of **1** being in close proximity. These findings suggest that naproxen and flurbiprofen molecules most likely reside within the micellar palisade of **1**, oriented in such a way that the aromatic ring of the drug points towards the interior of the micelle, where they can experience favourable π-π interactions with the calixarene outer faces, while the carboxylic acid moiety points in the opposite direction, towards the bulk.

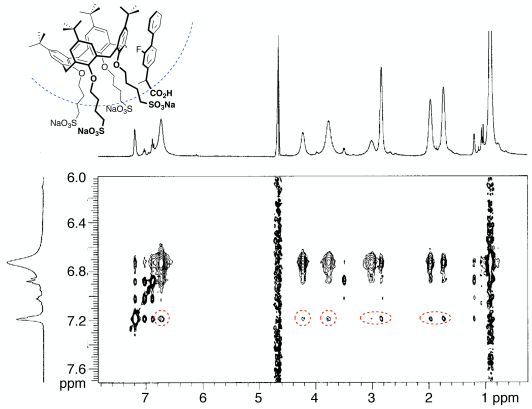


Fig. 7 Section of the 2D NOESY spectrum (500 MHz, 298 K) of a D_2O solution of **1** and **3**.

To estimate the degree of solubilisation of the two drugs by the calixarene micellar solutions, three different solutions of **1** with concentrations above the cmc (2.5, 5.0 and 10.0 mM) were employed for solid-liquid extraction experiments with fixed aliquot drug samples (5 mmoles). After 24 h stirring at room temperature, the resulting suspensions were centrifuged to remove the undissolved **2** or **3**, as well as any large calixarene/drug aggregates formed, and then the concentrations of the calixarene and naproxen/flurbiprofen present in the supernatant were determined by a quantitative NMR protocol (see ESI†).

Data in Fig. 8 shows the amount of naproxen and flurbiprofen solubilised as a function of the surfactant concentration. The sparing water solubility of these drugs[24] significantly improves –a linear correlation is observed– with increasing calixarene concentration. Molar solubilisation capacities[24a] of 0.20 and 0.51, for naproxen and flurbiprofen respectively, were derived from the slope of the linear regression.

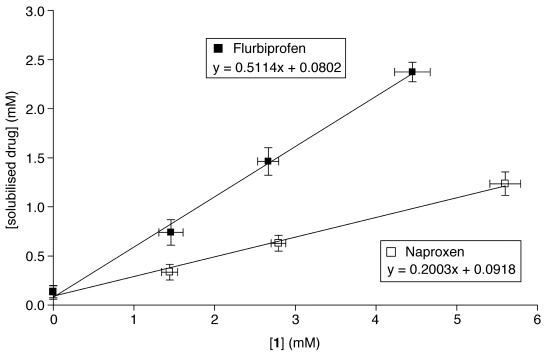


Fig. 8 Plots of drugs *vs* calixarene concentrations determined by quantitative NMR.

To the best of our knowledge, these results compare well with those reported for other anionic surfactants.[24a,25,26] It is a matter of fact that the extent of segregation of molecules bearing acid moieties inside anionic micelles depends on the pK_a of the target molecule. That is, the lower the pK_a of the target molecule, the stronger the repulsion between the acidic guest (in the deprotonated form) and the anionic head-groups of the micelles is expected to be, thus decreasing the solubilisation efficiency.[24a] On the other hand, it is known that the pK_a of lipophilic carboxyl acids may increase in the hydrophobic medium of micelles with respect to aqueous solution.[27] Naproxen and flurbiprofen (pK_a *ca*. 4.2–4.8)[28] are not fully ionized in an aqueous solution of calixarene **1** (pH 5.4) and, as a result, the undissociated form can easily be incorporated inside the anionic micelles.

Given that quantitative NMR measurements cannot indicate the molar fraction of drug molecules segregated inside very large aggregates (R_h = 100–300 nm) –as these are "NMR invisible"– the total concentration of naproxen dissolved in an aqueous 5.0 mM micellar solution of **1** was also investigated by UV spectroscopy. To this end, to avoid the potential overlapping of calixarene and naproxen absorbance bands during the analysis in an aqueous solution, naproxen was extracted in $CHCl_3$ and its concentration spectrophotometrically determined, in the absence of **1**, before and after filtration through 0.1 µm Millipore filters (see ESI†). Data obtained (Table S2†) for the filtered solutions ([**2**] = 0.63 ± 0.03 mM) are in excellent agreement with the NMR measurements, whereas slightly higher values were found for the unfiltered ones ([**2**] = 0.85 ± 0.03 mM), thus indicating that the bigger aggregates of **1** are also able to encapsulate the drugs under investigation.

The affinity of drugs **2** and **3** for the micellar environment of calixarene **1** was also demonstrated by means of DOSY measurements. Data in Table 1 shows that the diffusion coefficients of naproxen and flurbiprofen decrease from 5.47–5.66 × 10^{-10} m^2/s (for the free species) to values closer to those of the calixarene micellar aggregates. Moreover, the diffusion coefficients of calixarene **1** in drug-encapsulated micelles ($D_{\mathbf{1},drug}$; Table 1) are even smaller than that measured for **1** on its own (D_1).¶ These results clearly demonstrate that the translational mobility of **2** and **3** is considerably reduced as a result of their binding to the micelle, and that upon drug encapsulation micellar aggregates of calixarene **1** grow in size. These findings were further confirmed by AFM investigations (see ESI, Fig. S4†).

Table 1. Diffusion coefficients (× 10^{-10} m^2/s) of drugs **2** and **3** on their own (D_{drug}), after extraction with a 5.0 mM solution of **1** ($D_{drug,1}$), and **1** after binding the drug ($D_{\mathbf{1},drug}$)

	D_{drug}	$D_{drug,1}$	$D_{\mathbf{1},drug}$
2	5.47 ± 0.08	1.58 ± 0.04	0.98 ± 0.02 (D_1 = 1.38 ± 0.01 at 2.8 mM)
3	5.66 ± 0.07	1.29 ± 0.02	1.01 ± 0.02 (D_1 = 1.48±0.01 at 2.5 mM)

Conclusions

The aggregation properties of the amphiphilic *p-tert*-butylcalix[4]arene tetra-*O*-butylsulfonate (**1**) have been evaluated by means of complementary techniques (NMR, DLS, AFM) to reveal the presence of differently-sized aggregates even below the cmc. The lypophilic environment created by the micellar aggregates greatly enhances the solubility of poorly water-soluble drugs such as naproxen and flurbiprofen. This study indicates that calixarene-type surfactants have interesting aggregation features, which could be exploited

further in the mimicking of natural surfactants and in the development of novel drug delivery systems.

Acknowledgments

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Notes and references

‡ A cmc of 1.76 mM was determined by surface tension by another group.[13]

§ Due to the low solubility of calixarene **1** for [**1**] > 10 mM, the newly-formed aggregate could not be studied by NMR.

¶ D_1 values refer to D_2O solutions of calixarene **1** at concentrations equivalent to those measured (by quantitative NMR) in the supernatant of the solid-liquid extraction experiments mentioned earlier (i.e., [**1**] = 2.5 and 2.8 mM in the cases of **2** and **3** respectively).

1 (*a*) J. Voskuhl and B. J. Ravoo, *Chem. Soc. Rev.,* 2009, **38**, 495–505; (*b*) I. W. Hamley and V. Castelletto, *Angew. Chem. Int. Ed*., 2007, **46**, 4442–4455; (*c*) J. A. Zasadzinski, E. Kisak and C. Evans, *Curr. Opin. Colloid Interface Sci.,* 2001, **55**, 85–90.

2 (*a*) C. O. Rangel-Yagui, A. Pessoa-Jr and L. Costa-Tavares, *J. Pharm. Pharmceut. Sci.*, 2005, **8**, 147–163; (*b*) A. S. Narang, D. Delmarre and D. Gao, *Int. J. Pharm*., 2007, **345**, 9–25.

3 V. R. Vemula, V. Lagishetty and S. Lingala, *Int. J. Pharm. Sci. Rev. Res*., 2010, **5**, 41–51.

4 (*a*) S. Shinkai, S. Mori, T. Tsubaki, T. Sone and O. Manabe, *Tetrahedron Lett.*, 1984, **25**, 5315–5318; (*b*) S. Shinkai, *Pure Appl. Chem*, 1986, **58**, 1523–1528.

5 M. Strobel, K. Kita-Tokarczyk, A. Taubert, C. Vebert, P. A. Heiney, M. Chami and W. Meier, *Adv. Funct. Mater.*, 2006, **16**, 252–259.

6 (*a*) F. Perret and A. W. Coleman, *Chem. Commun.*, 2011, **47**, 7303–7319; (*b*) E. V. Ukhatskaya, S. V. Kurkov, S. A. Matthews and T. Loftsson, *J. Pharm. Sci.*, 2013, **102**, 3485–3512.

7 (*a*) N. Basílio, L. Garcia-Rio and M. Martín-Pastor, *Langmuir*, 2012, **28**, 2404–2414; (*b*) N. Basílio and L. Garcia-Rio, *Chem. Phys. Chem.,* 2012, **13**, 2368–2376.

8 M. Kellermann, W. Bauer, A. Hirsch, B. Schade, K. Ludwing and C. Bottcher, *Angew. Chem., Int. Ed.,* 2004, **43**, 2959–2962.

9 (*a*) C. Gaeta, T. Caruso, M. Mincolelli, F. Troisi, E. Vasca and P. Neri, *Tetrahedron*, 2008, **64**, 5370–5378; (*b*) O. Bistri, B. Colasson and O. Reinaud, *Chem. Sci.*, 2012, **3**, 811–818; (*c*) Q. Li, D.-S. Guo, H. Qian and Y. Liu, *Eur. J. Org. Chem.*, 2012, 3962–3971; (*d*) E. A. Andreyko, P. L. Padnya, R. R. Daminova and I. I. Stoikov, *RSC Adv.,* 2014, **4**, 3556–3565; (*e*) G. Gattuso, A. Notti, S. Pappalardo, M. F. Parisi and I. Pisagatti, *Supramol. Chem.*, 2014, **26**, 597–600; (*f*) D.-S. Guo and Y. Liu, *Acc. Chem. Res.*, 2014, **47**, 1925–1934.

10 (*a*) E. da Silva, A. N. Lazar and A. W. Coleman, *J. Drug Deliv. Sci. Technol.*, 2004, **14**, 3–20; (*b*) O. Danylyuk and K. Suwinska, *Chem. Commun*., 2009, 5799–5813.

11 (*a*) E. V. Ukhatskaya, S. V. Kurkov, S. A. Matthews, A. El Fagui, C. Amiel, F. Dalmas and T. Loftsson, *Int. J. Pharm.,* 2010, **402**, 10–19; (*b*) G. M. L. Consoli, G. Granata and C. Geraci*, Org. Biomol. Chem.*, 2011, **9**, 6491–6495; (*c*) H. Huang, D.-M. Li, W. Wang, Y.-C. Chen, K. Khan, S. Song and Y.-S Zheng, *Org. Biomol. Chem.*, 2012, **10**, 729–735; (*d*) Y.-X. Wang, D.-S. Guo, Y. Cao and Y. Liu, *RSC Adv*., 2013, **3**, 8058–8063; (*e*) L. Gallego-Yerga, M. Lomazzi, F. Sansone, C. Ortiz Mellet, A. Casnati and J. M. García Fernández, *Chem. Commun*., 2014, **50**, 7440–7443.

12 G. Gattuso, A. Notti, S. Pappalardo, M. F. Parisi, I. Pisagatti and S. Patanè, *New J. Chem.,* 2014, **38**, 5983–5990.

13 W. Ding, W.-q. Bai, G.-m. Qu, L. Ma and T. Yu, *Applied Chemical Industry*, 2007, **36**, 1092–1095.

14 C. J. Friel and M. C. Lu, in *Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry*, eds. J. M. Beale and J. H. Block, Lippincott Williams & Wilkins, Philadelphia, 2011, ch. 24.

15 G. Gattuso, A. Notti, A. Pappalardo, S. Pappalardo, M. F. Parisi and F. Puntoriero, *Tetrahedron Lett.*, 2013, **54**, 188–191.

16 (*a*) C. D. Gutsche, Calixarenes - An Introduction, in Monographs in Supramolecular Chemistry, ed. J. F. Stoddart, The Royal Society of Chemistry, Cambridge, 2nd edn., 2008, vol. 6; (*b*) C. Jaime, J. de Mendoza, P. Prados, P. M. Nieto and C. Sanchez, *J. Org. Chem*., 1991, **56**, 3372–3376.

17 Y. Cohen, L. Avram and L. Frish, *Angew. Chem. Int. Ed.*, 2005, **44**, 520–554.

18 X. Cui, S. Mao, M. Liu, H. Yuan and Y. Du, *Langmuir*, 2008, **24**, 10771–10775.

19 P. S. Denkova, L. Van Lokeren, I. Verbruggen and R. Willem, *J. Phys. Chem B,* 2008, **112**, 10935–10941.

20 (*a*) L. Ya. Zakharova, Y. R. Kudriashova, N. M. Selivanova, M. A. Voronin, A. R. Ibragimova, S. E. Solovieva, A. T. Gubaidullin, A. I. Litvinov, I. R. Nizameev, M. K. Kadirov, Y. G. Galyametdinov, I. S. Antipin and A. I. Konovalov, *J. Membr. Sci.,* 2010, **364**, 90–101; (*b*) T. N. Pashirova, E. M. Gibadullina, A. R. Burilov, R. R. Kashapov, E. P. Zhiltsova, V. V. Syakaev, W. D. Habicher, M. H. Rümmeli, S. K. Latypov, A. I. Konovalev and L. Ya. Zakharova, *RSC Adv.*, 2014, **4**, 9912–9919.

21 A. D'Urso, D. A. Cristaldi, M. E. Fragalà, G. Gattuso, A. Pappalardo, V. Villari, N. Micali, S. Pappalardo, M. F. Parisi and R. Purrello, *Chem.-Eur. J.*, 2010, **16**, 10439–10446.

22 G. M. L. Consoli, G. Granata, R. Lo Nigro, G. Malandrino and C. Geraci, *Langmuir*, 2008, **24**, 6194–6200.

23 L.-H. Zhuang, K.-H. Yu, G.-W. Wang and C. Yao, *J. Colloid Interface Sci*., 2007, **408**, 94–100.

24 (*a*) P. A. Bhat, G. M. Rather and A. A. Dar, *J. Phys. Chem. B*, 2009, **113**, 997–1006; (*b*) J. Zhang, Y. Tozuka, H. Uchiyama, K. Higashi, K. Moribe, H. Takeuchi and K. Yamamoto, *J. Pharm. Sci.*, 2011, **100**, 4421–4431.

25 P. Dutta, J. Dey, V. Perumal and M. Mandal, *Int. J. Pharm.*, 2011, **407**, 207–216.

26 For an example of non-ionic calixarene-based amphiphile used by Zheng and co-workers to enhance the solubility of naproxen see ref. 11c.

27 (*a*) O. A. El Seoud, *Adv. Colloid Interface Sci.*, 1989, **30**, 1–30; (*b*) L. Dupont-Leclercq, S. Giroux, B. Henry and P. Rubini, Langmuir, 2007, **23**, 10463–10470.

28 (a) M. Shalaeva, J. Kenseth, F. Lombardo and A. Bastin, *J. Pharm. Sci.*, 2008, **97**, 2581–2606; (*b*) M. Meloun, S. Bordovská and L. Galla, *J. Pharm. Biomed. Anal.*, 2007, **45**, 552–564.