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Aqueous solubilization of furosemide by supramolecular complexation with 4-sulphonic calix[n]arenes

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Abstract

The solubilization of the practically water insoluble drug furosemide by guest:host inclusion complexation with 4-sulphonic calix[n]arenes has been reported. The 4-sulphonic calix[n]arenes are water-soluble phenolic cyclooligomers that form inclusion complexes with neutral molecules. The solubility of furosemide in acidic (pH < 4) aqueous solutions containing increasing concentrations of the calixarenes was determined at 30° C and the concentration of furosemide in solution was determined by HPLC. Results showed that the molecular size of the 4-sulphonic calix[n]arenes and the concentration of the calix[n]arenes significantly influenced the increase in the solubility of furosemide. 4-Sulphonic calix[6]arene improved the solubility of furosemide the most (± 104%) followed by 4-sulphonic calix[8]-arene (± 84–102%), while 4-sulphonic calix[4]arene increased the solubility of furosemide the least (± 73–81%). The increase in furosemide solubility afforded by the calixarenes was most probably the result of the incorporation of the non-polar portions of the furosemide molecule into the non-polar cavities of the calixarenes similar to furosemide:cyclodextrin complexes. The driving force for this interaction was the reduction in the non-polar–water interfacial surface area when the furosemide (guest) molecules were inserted into the 4-sulphonic calix[n]arenes (host).

Introduction

Furosemide, 5-(aminosulfonyl)-4-chloro-2-((2-furanyl-methyl)amino) benzoic acid (Figure 1), is a loop diuretic that is used orally to adjust the volume and/or composition of body fluids in a variety of situations, including hypertension, heart failure, renal failure, nephritic syndrome, and cirrhosis (Reynolds 1989). Furosemide is practically insoluble but its solubility increases with an increase in pH (Al-Obaid et al 1989; Reynolds 1989).

The oral bioavailability of furosemide is very poor due to insufficient aqueous solubility at gastrointestinal pH, making solubility the rate-determining step in the gastric absorption of furosemide (Chungi et al 1979; Hammarlund et al 1984). To overcome the poor bioavailability of furosemide various techniques have been used to increase its aqueous solubility, including cyclodextrin complexation (Özdemir & Ordu 1998; Ammar et al 1999; Spamer et al 2002; Vlachou & Papaioannou 2003). The experimental results of those studies showed a significant increase in the solubility and dissolution rate of furosemide when complexed with 2-hydroxypropyl-β-cyclodextrin (50% increase in dissolution), α-cyclodextrin (55%), β-cyclodextrin (70%), γ-cyclodextrin (76%), heptakis (2,6-di-O-methyl)-β-cyclodextrin (76%), and heptakis (2,3,6-tri-O-methyl)-β-cyclodextrin (54%).

Since cyclodextrins are able to encapsulate furosemide into their hydrophobic cavities (Özdemir & Ordu 1998; Ammar et al 1999; Spamer et al 2002; Vlachou & Papaioannou 2003), other supramolecular host compounds might also form host–guest complexes with furosemide. Along with the cyclodextrins and crown ethers, calixarenes are the third major class of supramolecular host systems (Gutsche 1989, 1998). The calixarenes (Figure 1) are a class of cyclooligomers formed via a phenol-formaldehyde condensation. They exist in a 'cup'-like shape with a defined upper and lower rim and a central annulus. Their rigid conformation enables calixarenes to act as host molecules because of their preformed hydrophobic cavities. Due to their ability to form host–guest type complexes

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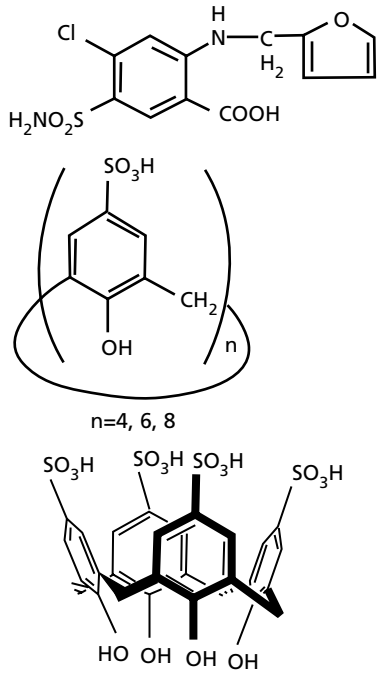

Figure 1 Molecular structures of furosemide and the 4-sulphonic calix[n]arenes.

with a variety of organic or inorganic compounds, the calixarenes have received increasing attention during the last two decades (Gutsche 1989,1998; Ferguson et al 1991; Zhang & Cao 2001). In addition, the ease with which various functional groups can be introduced to modify either the upper or the lower rim of the 'cup' makes it easy to change the affinity of these cyclooligomers towards target molecules and/or increase the solubility of the calixarenes (Shinkai 1986; Shinkai et al 1986, 1988). One such modified calixarene, the water-soluble 4-sulphonic calix[n]arenes, may selectively include various guests according to their size and hydrophobicity in a manner similar to cyclodextrins (Szejtli 1982; Shuette et al 1992; Zhang et al 1996).

To date only one study has reported on the effect of the water-soluble 4-sulphonic calix[n]arenes on the solubility of drugs (Millership 2001). Those results showed that depending on the size of the calixarene and the pH of the solution, the sulphonated calixarenes significantly increased the aqueous solubility of the water insoluble drug testosterone. To explore further the properties of drug:calixarene complexes, the aim of this study was to investigate the effect of three water-soluble 4-sulphonic calix[n]arenes on the aqueous solubility of furosemide at pH values close to that of the gastric environment.

Materials and Methods

Materials

Furosemide (Figure 1, $C_{12}H_{11}ClN_2O_5S$, MW = 331) was purchased from Spectrum Chemical Company (Gardena, CA). 4-Sulphonic calix[4]arene hydrate ($C_{28}H_{24}O_{16}S_4$ 9 H_2O), 4-sulphonic calix[6]arene hydrate ($C_{42}H_{36}O_{24}S_6$ 13 H_2O), and 4-sulphonic calix[8]arene hydrate ($C_{56}H_{48}O_{32}S_8$ 21 H_2O) were purchased from Acros Organics (Geel, Belgium). The molecular structures of the calix[n]arenes are given in Figure 1. HPLC-grade methanol was used for chromatographic assay of furosemide (Spectrum Chemical Company). For the solubility studies and HPLC analysis deionized water was used (Nanopure, Barnstead International, Dubuque, IA). All the other chemicals and solvents were of analytical reagent grade and used as received.

Thermogravimetric analysis

Thermogravimetric (TG) analysis was performed on the calix[n]arenes to determine the amount of crystalline water contained in the crystal structures (Table 1). TG analysis traces were measured with a Hi-Res Modulated TGA 2950 (TA Instruments, New Castle, DE). Samples weighing approximately 5 mg were heated at 20 K min^{-1} under nitrogen gas flow of 35 mL min^{-1}.

Differential scanning calorimetry (DSC)

DSC thermograms were recorded with a DSC 2920 modulated DSC (TA Instruments, New Castle, DE). Indium (mp 156.6°C) and tin (mp 231.9°C) were used to calibrate the instruments. A mass, not exceeding 3 mg, was measured into aluminum pans. DSC curves were obtained under a nitrogen purge of 20–30 mL min^{-1} at a heating rate of 3 K min^{-1}. DSC scans were recorded for furosemide, the calixarenes and 1:1 w/w furosemide:calixarene mixtures prepared by grinding in a mortar with a pestle for 30 min. Changes in the melting temperatures determined as extrapolated onset temperatures, defined as the point of transition, were used as indications of complex formation.

HPLC analysis of furosemide

The HPLC method used in this study complied with specifications for precision, accuracy, selectivity, linearity, and ruggedness as required by the USP XXIV (USP 2000). Analysis was carried out with an automated high-performance liquid chromatograph (AS1000 autosampler

Table 1 Structural properties of the 4-sulphonic calix[n]arenes.

Calix[n]arene	Molecular weight	Crystal H_2O (%)*	Reported H_2O (%)$^+$
4	745	18 ± 2	20
6	1117	17 ± 2	14
8	1489	20 ± 4	–

*Measured by thermogravimetric analysis, n = 5. $^+$Arena et al (1992), Zhang et al (1996).

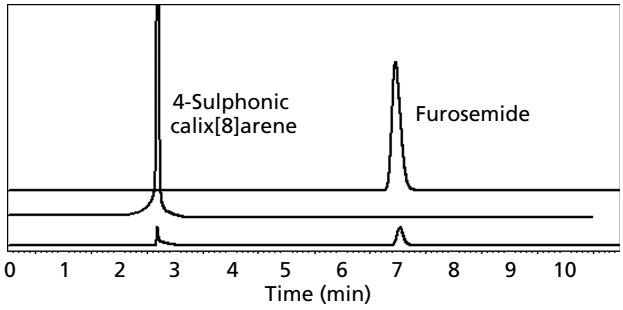

Figure 2 HPLC chromatograms of furosemide and 4-sulphonic calix[n]arenes and furosemide in the presence of the 4-sulphonic calix[n]arenes.

and P2000 pump, Thermo Separation Products, Waltham, MA) with a UV detector (UV3000 detector) set at 276 nm. Furosemide eluted on a Supelco Discovery RP Amide C_{16} column (25 cm × 4.6 mm, 5 μm, Bellefonate, PA) after 7 min (Figure 2) using a mobile phase of water:acetonitrile:acetic acid (60:40:1 v/v); flow rate 1.0 mL min^{-1}; injection volume 20 μL. Results were the mean of three analysis and solutions were prepared in the dark to prevent furosemide photodegradation.

Solubility measurements

The solubility of furosemide in water at pH 1, 2 or 3 containing increasing concentrations of the 4-sulphonic calix[n]arenes (0.0001–0.008 M) was measured according to the rotating bottle method (Higuchi & Connors 1965). pH was adjusted with hydrochloric acid. An excess of furosemide powder was added to screw-capped amber vials containing 3 mL solvent and increasing amounts of the 4-sulphonic calix[n]arenes. The vials were rotated at 60 rev min^{-1} and kept at 30 ± 0.5°C until no further increase in the solubility of furosemide was observed. The solutions were passed through 0.45-μm cellulose acetate filters (Osmonics Inc., Minnetonka, MN), the filtered liquids were collected and the concentration of furosemide in solution determined by HPLC. Phase solubility diagrams were constructed by plotting the molar concentration of furosemide dissolved (solubility) vs molar concentration of calix[n]arenes. From these diagrams, the stability constants for the complexation of furosemide with calix[n]arenes were calculated (Higuchi & Connors 1965).

Statistical analysis

The water content of three calix[n]arenes (n = 4, 6, or 8) with five replicates, and the percentage increase in solubility of furosemide (three replicates) in the presence of 0.008 M calix[n]arenes (n = 4, 6, or 8) were evaluated using a one-way analysis of variance (SAS Institute, Inc., Cary, NC). In both cases, post-hoc comparisons of the means of individual groups were performed using Duncan's test. A significance level of $P < 0.05$ denoted significance in all cases.

Results

Preliminary evaluation of the possibility of complex formation between furosemide and the 4-sulphonic calix[n]arenes was done by studying drug–calixarene mixtures by DSC. The results of this experiment (Figure 3) clearly showed changes in the DSC thermogram of furosemide in the presence of the three calixarenes. Furosemide started to melt with decomposition at 212–213°C ($\Delta H = 103.8$ J g^{-1}). The calixarenes exhibited a typical broad endothermic peak between 50 and 175°C assigned to its dehydration as it was determined by the weight loss registered by TG analysis. The dehydrated compounds melt at approximately 250–270°C. As seen in Figure 3, the peak representing the endo/exothermic process for furosemide melting/decomposition at approximately 210–215°C disappeared in the case of the solid binary complexes prepared by grinding. These results can be explained on the basis of an interaction between the drug and the cyclodextrin, indicating the complexation of furosemide with the 4-sulphonic calix[n]arenes.

Using the DSC results as a base, the interaction of furosemide with the 4-sulphonic calix[n]arenes was determined from the increase in aqueous solubility of the drug with an increase in calixarene concentration (Figures 4–6). Since the 4-sulphonic calix[n]arenes were hydrated, the

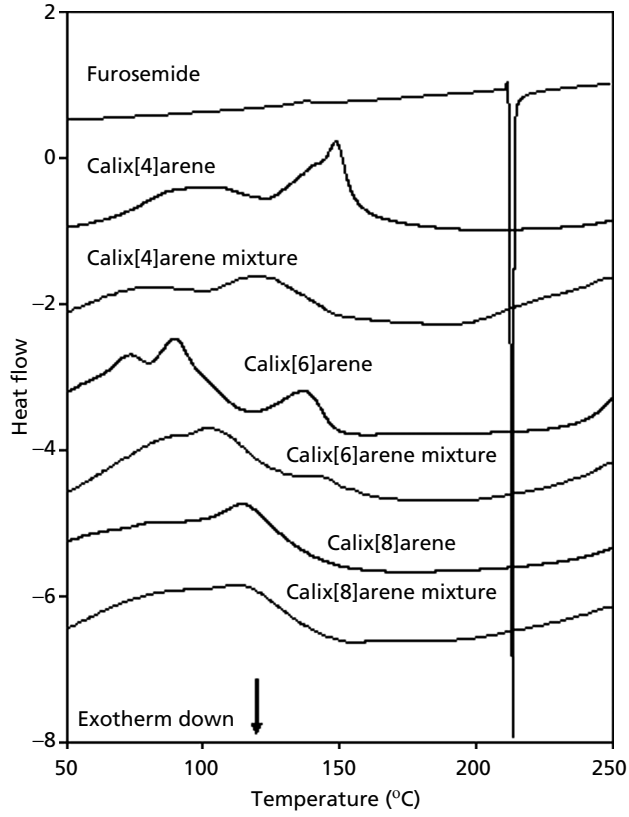

Figure 3 DSC thermograms of furosemide, the 4-sulphonic calix[n]arenes, and furosemide: calixarene mixtures prepared in a mortar and pestle.

water content of the powdered calixarenes (Table 1) was determined by TG analysis. There was no significant difference between the water content of all three calixarenes ($P > 0.05$). The water content of the three calix[n]arenes with n = 4, 6 and 8 repeating phenolic units was taken into account when reporting the molar concentrations of the calixarene solutions. In this study the aqueous solubility of the calixarenes was not measured, but the 4-sulphonic calix[n]arenes were water-soluble and their aqueous solubilities were at least 0.1 M (Gutsche 1989). Within the concentration ranges used in this study, adjusting the pH of the solutions to pH 1–3 did not cause any visible precipitation of the calixarenes from solution. Preliminary determination of the solubility of furosemide confirmed that the drug was practically insoluble in water (38 μg mL^{-1} at 30°C). The solubility was even lower at pH 1–3 (11–31 μg mL^{-1}). However, as reported by other researchers, the solubility increased significantly with an increase in pH above 3 (102 μg mL^{-1} at pH 4 up to 22 mg mL^{-1} at pH 8) (Al-Obaid et al 1989).

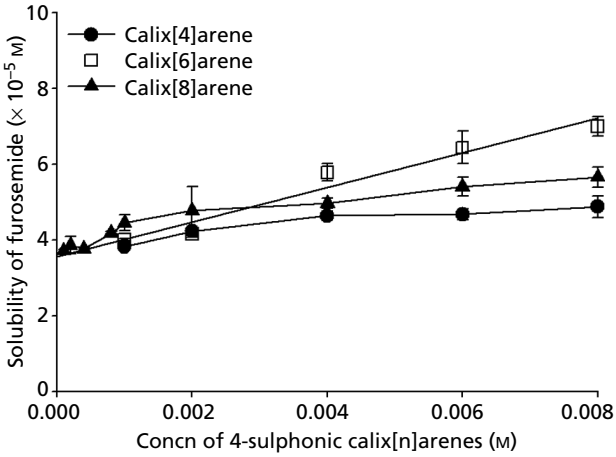

Figure 4 Phase solubility diagrams measured at 30 ± 0.5°C and pH 1 of furosemide in the presence of increasing concentrations of 4-sulphonic calix[n]arenes.

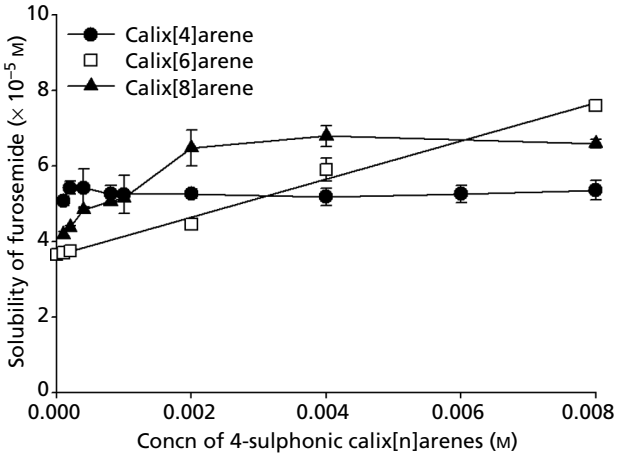

Figure 5 Phase solubility diagrams measured at 30 ± 0.5°C and pH 2 of furosemide in the presence of increasing concentrations of 4-sulphonic calix[n]arenes.

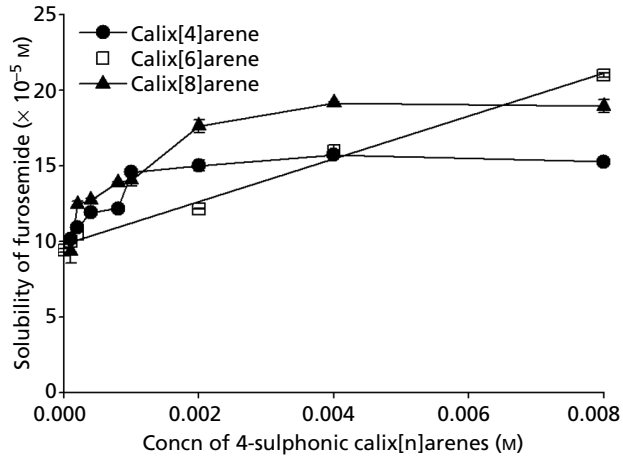

Figure 6 Phase solubility diagrams measured at 30 ± 0.5°C and pH 3 of furosemide in the presence of increasing concentrations of 4-sulphonic calix[n]arenes.

As shown in Table 2, the largest increase in solubility, mean ± 104%, was seen with increasing concentrations of 4-sulphonic calix[6]arene at pH 1, 2 and 3. At pH 1 and the highest calixarene concentration, 0.008 M, the order in which the 4-sulphonic calix[n]arenes increased the solubility of furosemide was n = 6 > n = 8 = n = 4 (Table 2). At pH 2, the order was n = 6 > n = 8 > n = 4. At pH 3, the order was n = 6 = n = 8 > n = 4. Overall, 4-sulphonic calix[6]arene increased the solubility of furosemide the most, while 4-sulphonic calix[4]arene increased the solubility the least.

The increased solubility of furosemide in the presence of increasing 4-sulphonic calix[n]arenes concentrations indicated that calixarenes might form complexes with furosemide. Phase-solubility diagrams, such as those shown in Figures 4–6, are generally used to study the possible molecular interactions by which the solubilizing agents might increase the solubility of drugs (Higuchi & Pisano 1964). The almost linear increase in solubility by 4-sulphonic calix[6]arene seen in these graphs represents Type A_L phase solubility profiles (Higuchi & Connors 1965). This

Table 2 The percentage increase in the aqueous solubility of furosemide when mixed with 0.008 M of the 4-sulphoniccalix[n]arenes (three replicates).

pH	Increase in solubility (%)		
	Calix[4]arene	Calix[6]arene	Calix[8]arene
1	73 ± 8	104 ± 8	84 ± 8
2	73 ± 7	104 ± 4	86 ± 3
3	81 ± 1	104 ± 1	102 ± 5

type of phase solubility diagram could be due to one or more molecular interactions between the furosemide and the calixarenes that leads to the formation of distinct chemical species, which may be referred to as soluble furosemide:4-sulphonic calix[n]arene complexes. Good linear fits for calix[6]arene indicated that the complexes must be of the first order in calix[n]arene concentration and since all the slopes of the Type A_L diagrams were less than unity it can be assumed that 1:1 complexes were formed.

The solubility profiles for the 4-sulphonic calix[4]arene and 4-sulphonic calix[8]arene were of the Type A_N diagrams, the origin of which is uncertain. It may be associated with an alteration in the effective nature of the solvent in the presence of these calixarenes, thus leading to a change in the complex formation constant (Higuchi & Connors 1965).

Since the phase solubility profiles fitted typical Type A_L and A_N with possible 1:1 complexes, stability constants (Table 3) were calculated according to the equation:

$$K_{st} = \text{slope}/S_0(1 - \text{slope})$$

where S_0 is the solubility of furosemide in the absence of calix[n]arenes. These stability constants are empirical parameters, which describe approximately the increase in apparent solubility of furosemide in the presence of the 4-sulphonic calix[n]arenes. Based on the calculated stability constants all three 4-sulphonic calix[n]arenes formed stable complexes with furosemide at all three pH values because the K_{st} values were between 104 and 5212 M^{-1}. These K_{st} values (Table 3) were similar to those reported for furosemide:cyclodextrin complexes such as: 2110 M^{-1} for hydroxypropyl-β-cyclodextrin (Vlachou & Papaioannou 2003); 824 M^{-1} for β-cyclodextrin (Özdemir & Ordu 1998); 89 M^{-1} for γ-cyclodextrin (Ammar et al 1999); 117 M^{-1} for heptakis (2,6-di-O-methyl)-β-cyclodextrin (Ammar et al 1999); and 75 M^{-1} for heptakis (2,3,6-tri-O-methyl)-β-cyclodextrin (Ammar et al 1999).

Discussion

The solubility of furosemide was increased significantly when the concentrations of the three 4-sulphonic calix[n]arenes in the aqueous solutions were increased (pH < 4), and so it was clear that the calixarenes must in some way have interacted with furosemide to form more soluble furosemide:calixarene associations. The increase in solubility was not due to micellar aggregation of the calixarenes around the furosemide molecules because the concentrations of 4-sulphonic calix[n]arenes used in this study were below the critical micelle concentrations (Tao & Barra 1998). The increase in solubility was not due to an increase in the percent ionized drug because furosemide ($pK_a = 3.9$) was only slightly ionized (0.1–11%) between pH 1–3.

From the literature, it is known that the water-soluble 4-sulphonic calix[n]arenes form complexes with small organic molecules afforded by weak interaction forces including hydrogen bonding, π–π interactions, electrostatic interactions, or dipole–dipole moments (Arena et al 1992, 2000; Zhang et al 1997). With the help of one or a combination of these forces furosemide most probably formed non-covalent inclusion complexes with the 4-sulphonic calix[n]arenes similar to the complexes it forms with cyclodextrins (Özdemir & Ordu 1998; Ammar et al 1999; Spamer et al 2002; Vlachou & Papaioannou 2003). This is possible because the hydrophobic and hydrophilic properties and the inner cavity diameters are similar to that of the α-, β-, γ-cyclodextrins (Szejtli 1982; Gutsche 1989; Zhang et al 1997). The cavity diameter of 4-sulphonic calix[4]arene (3.0 Å) is slightly smaller than that of α-cyclodextrins (5.7 Å), the inner cavity diameter of the calix[6]arene (7.6 Å) is comparable with that of β-cyclodextrin (7.8 Å), while that of the calix[8]arene (11.7 Å) is slightly larger than that of γ-cyclodextrin (9.5 Å).

Furosemide is a hydrophobic molecule that can form hydrophobic bonds between itself and non-polar hydrocarbon molecules or portions of cyclodextrin molecules (Spamer et al 2002). Although these forces are small, large solvent–solvent intermolecular attractive forces enhance them in polar media such as water (Higuchi & Connors 1965). In aqueous solutions, the hydrophobic furosemide molecule is therefore "squeezed out" of the polar phase by its high internal pressure leading to an appreciable degree of interaction between furosemide and the non-polar hydrophobic cavity of calixarenes. Although hydrophobic interactions are limited by the cavity size of calixarenes, Shinkai et al (1988) showed that 'host-size selectivity' does exist in host–guest-type complexation with calixarenes. In accordance with the inner cavity diameter mentioned earlier, it is expected that the larger calix[6]arene and calix[8]arene cavity would geometrically be more suited for a closer and stronger interaction with furosemide than the smaller calix[4]arene cavity. This was confirmed by the higher solubility afforded by 4-sulphonic calix[6]arene and calix[8]arene compared with 4-sulphonic calix[4]arene (Figures 4–6). Solubility differences as a function of cavity size suggested that based on the molecular structure of furosemide, the complexes between the sulphonic calixarenes and this molecule could be classified as intermolecular complexes as suggested by Vicens & Böhmer (1991).

Table 3 Stability constants (K_{st} values (s.d.)) estimated from phase solubility profiles for furosemide in the presence of 4-sulphonic calix[n]arenes.

pH	1			2			3		
Calix[n]arene	4	6	8	4	6	8	4	6	8
K_{st} (M^{-1})	746 (56)	1370 (101)	1669 (120)	2117 (205)	1441 (30)	3093 (200)	420 (20)	104 (12)	5212 (493)

After considering all these possibilities, and based on the properties of furosemide and the calixarenes molecules (Figure 1), and the solubility increase, it is proposed that the major contributing factor to the complexation of the drug with the calixarenes was that when in acidic aqueous solutions the non-polar furosemide molecules were squeezed out of the water. When squeezed out of the water these molecules were forced to either aggregate or to move into structures that minimized their contact with water (Yalkowsky 1999). This meant that the furosemide molecules were driven into the non-polar cavities of the 4-sulphonic calix[n]arenes.

Conclusion

Solubility studies showed that the molecular size of the 4-sulphonic calix[n]arenes and the concentration of the calix[n]arenes significantly influenced the increase in the solubility of furosemide achieved by furosemide:4-sulphonic calix[n]arene complexation at low pH in aqueous solutions. Overall, 4-sulphonic calix[6]arene improved the solubility of furosemide the most (±104%). This was followed by 4-sulphonic calix[8]arene (±84–102%) while 4-sulphonic calix[4]arene increased the solubility of furosemide the least (±73–81%). The driving force for the increase in furosemide solubility afforded by the calixarenes was most probably the reduction in the non-polar water interfacial surface area because more and more of the non-polar portions of the furosemide molecules (guest) were inserted into the non-polar cavities of the 4-sulphonic calix[n]arenes (host).

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