ORIGINAL ARTICLE

Solubilizing effect of the *p*-phosphonate calix[*n*]arenes towards poorly soluble drug molecules such as nifedipine, niclosamide and furosemide

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Abstract This article reports the solubilization of the practically water insoluble drug molecules such as nifedipine, niclosamide and furosemide by guest:host inclusion complexation with *p*-phosphonates calix[*n*]arenes as drug soluble agent. This complexation studies were carried out by using the phase solubility technique. From the obtained results, it was observed that the solubility of drug molecules was significantly increased in the presence of calix[*n*]arene host molecules. The increase in solubility of drugs by the calix[*n*]arene was most probably due to inclusion complexation between drug molecules and cavities of the calixarene skeleton similar to drug:cyclodextrin complexes.

Keywords Calixarene · Drug · Nifedipine · Niclosamide · Furosemide

Introduction

Molecular design of the host systems based on calixarenes which can form molecular complexation is a focus of interest and research activity within supramolecular chemistry [1–3]. Calixarenes represent, along with the crown ethers [4] and the cyclodextrins [5], one of the three major groups of synthetic macrocyclic host molecules in supramolecular chemistry. The importance of calixarenes has been entirely recognized since pioneering studies of

Gutsche [1, 6]. This can be largely attributed to the fact that they are attractive host molecules that can be easily functionalized into terms bearing suitable binding sites for target guest species [7]. Calixarenes are cyclic oligomers made of several phenolic units bounded with methylene bridges and can easily be used as building blocks for the design and synthesis of more sophisticated supramolecular structures [8]. Calixarenes are sparingly soluble in aqueous media and this property is the major problem for the calixarene use in biopharmaceutical applications. To overcome these limitations water-soluble groups containing positive or negative charges such as amine [9], phosphonate [10] and sulphonate [11] groups or with neutral groups such as sulfonamides [12], sugars [13], and polyoxyethylene [14] can be located on the lower or upper rim of calixarene skeleton.

Furosemide 5-(aminosulfonyl)-4-chloro-2-[(2-furanyl methyl) amino] benzoic acid, niclosamide 5-chloro-*N*-2-chloro-4-nitrophenyl-2-hydroxybenzamide and nifedipine 3,5-dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate are poorly water-soluble drug molecules and used as loop diuretic, anthelmintic and calcium-channel blocker, respectively [15–17]. The main problem of these molecules is poor aqueous solubility. Commonly used technique to increase the solubility of poorly water-soluble drugs is by supramolecular complexation [18, 19]. Among the macromolecules used to solubilize drugs the cyclodextrins are the most widely used. Cyclodextrins are a family of three major well-known cyclic oligosaccharides. The negligible cytotoxic effects of cyclodextrins are an important attribute in application of drug carriers [20]. Calixarenes may selectively include various guests according to their size and hydrophobicity in a manner similar to cyclodextrins [21–23]. Although FDA has currently not approved the use of calixarenes in medicines to date the calixarenes have showed neither toxicity nor immune responses.[21] Studies of

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calixarenes on human fibroblast cells using inhibition of cell growth have been showed that calixarene derivatives have the same level toxicity as glucose [24]. Furthermore, it has been observed that calix[4]arene phosphonic acid derivative showed no effects on the cell growth of human fibroblasts [22]. On the other hand, more recently it has been observed that solid lipid nano-particles based on amphipihilic calix[4]arenes show zero haemolytic effects at concentrations ranging up to 300 mg/L [25]. This situation is in contrast to the behaviour of the solid lipid nano-particles based on amphipihilic cyclodextrins which show significant haemolytic effects [26]. Therefore, this situation increases interest their use instead of cyclodextrins in the biopharmaceutical applications beyond their current use for the complex forming agents to remove molecules from the environment [27–29].

To date although several works about the effect of the water-soluble *p*-sulphonic calix[*n*]arenes on the solubility of drugs has been reported [30–32]. The inclusion behaviors of water-soluble *p*-phosphonate calix[*n*]arene receptors towards nifedipine, niclosamide and furosemide have not been explored so far by means of phase solubility process. In our previous study [33], it was observed that *p*-phosphonate calix[*n*]arene receptors effectively transported poorly soluble drug molecules from the organic phase to the aqueous phase. This study showed that a possible interaction occurred between calixarene backbone and drug molecules. Therefore, the aim of the present study was to explore the use of water-soluble *p*-phosphonate calix[*n*]arene as drug solubilizing agents towards nifedipine, niclosamide and furosemide drug molecules using phase solubility process.

Results and discussion

Synthesis

Calix[*n*]arenes **1–4** containing phosphonate groups at the upper rim, have been synthesized by the reaction with corresponding chloromethylated derivatives of calix[*n*]arenes which was synthesized according to modified literature procedure [10, 33] and trimethyl phosphite in chloroform at reflux (Scheme 1). Phosphorylated calix[*n*]arene was easily converted their corresponding water-soluble *p*-phosphonate derivatives **1–4** by following our previously published literature procedure [33].

Phase solubility studies

Furosemide

Furosemide is a derivative from the anthranilic acid, whose structure is presented below (Fig. 1), represents a powerful loop diuretic that is widely used in the treatment of hypertension and edema. It is usually commercialized as tablets or parenteral solutions. The orally bioavailability of furosemide is very poor due to aqueous solubility at gastrointestinal pH, making solubility the rate-determining step in the gastric absorption of furosemide [32]. Several techniques have been used to increase its aqueous solubility, including cyclodextrin complexation [34, 35]. Obtained results show that furosemide drug molecules are encapsulated into hydrophobic cavity of cyclodextrins and a significant increase in the solubility and dissolution rate of furosemide. Also, calixarene compounds might form host–guest complexes with furosemide. Therefore, we have performed some preliminary evaluations to investigate solubilizing agent properties of calixarene phosphonate hosts **1–4** toward guest molecule furosemide by phase solubility process. From the solubility studies, calixarene phosphonates **1–4** was found to be an effective guest molecule for practically water insoluble furosemide (38 μg mL^{-1} at 30 °C in water).

Obtained results showed that both the molecular size and the concentration of the *p*-phosphonate calixarenes significantly influenced the increase in the solubility of furosemide. The largest increase in solubility from (4.44 ± 0.03) × 10^{-3} and (2.80 ± 0.03) × 10^{-3} M to (12.20 ± 0.03) × 10^{-3} and (11.10 ± 0.03) × 10^{-3} M was observed at 0.007 M calixarene compound **2** and **3** in water, respectively (Fig. 2; Table 1). This is not surprisingly results. Because in our previous liquid phase extraction study [33], furosemide drug molecules were transported around 25% extraction percentage to the aqueous phase. However, there was no significant difference in the increase in solubility of furosemide caused by calix[4]arene **1** and calix[8]arene **4**. Comparing the *p*-phosphonate calix[*n*]arenes **1–4**, the calix[4]arene derivative **2** having *tert* butyl groups, gave better results for furosemide drug molecules. Cavity of the calixarene **2** with *tert* butyl groups is large enough to include furosemide. This situation is accordance with similar literature results. Because, the structural changes in the calixarenes as removing the *para* substituents affect the molecular interactions [36]. Generally, removal of *tert* butyl groups at the *para* position decreased the molecular interaction of calixarenes significantly. Furthermore, *p*-H-calix[4]arene compounds indicated greater flexibility than in analogues with *p-tert* butyl substituents and this situation is effect the inclusion complexation behaviour of calixarene skeleton [37]. On the other hand, the increased solubility of furosemide indicated that the calixarenes must interact with furosemide to form more soluble nifedipine–calixarene complexes. The almost linear increase in solubility diagram of furosemide as shown Fig. 2 represents Type A$_L$ phase solubility profiles indicating the formation of 1:1 furosemide:calixarene complexes [32].

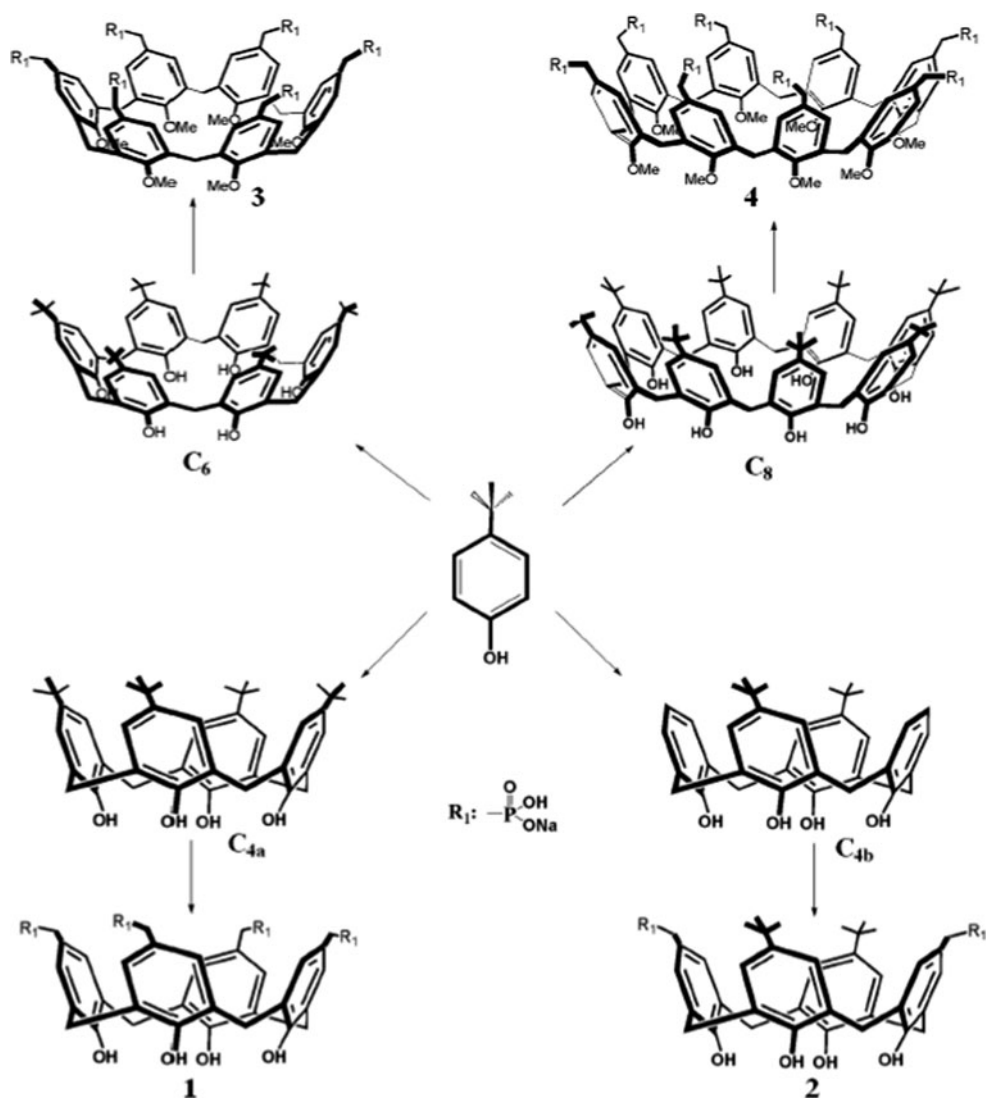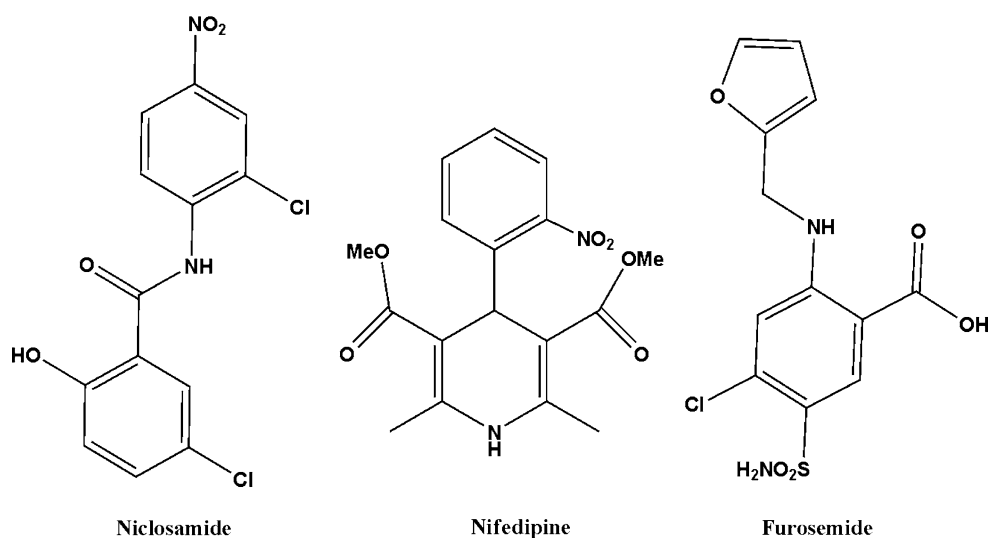**Scheme 1** Synthetic pathway and structures of the studied *p*-phosphonate calix[*n*]arene receptors **1–4**
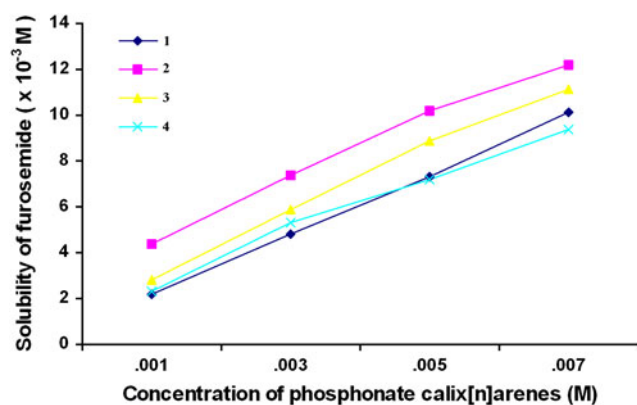

Fig. 1 Structures of the drug molecules

Niclosamide   Nifedipine   Furosemide

Fig. 2 Phase solubility diagrams of furosemide by host receptors **1–4**

Fig. 3 Phase solubility profiles

Table 1 Concentration values of furosemide with increasing calixarene concentrations in water

Compounds	Concentration values (mol L^{-1} in water)			
	0.001[a]	0.003[a]	0.005[a]	0.007[a]
1	2.2 ± 0.03[b]	4.8 ± 0.03	7.3 ± 0.04	10.1 ± 0.04
2	4.4 ± 0.03	7.4 ± 0.03	10.2 ± 0.03	12.2 ± 0.03
3	2.8 ± 0.03	5.9 ± 0.03	8.9 ± 0.04	11.1 ± 0.04
4	2.3 ± 0.03	5.3 ± 0.03	7.2 ± 0.04	9.3 ± 0.04

[a] Concentration values of calixarenes **1–4**

[b] Averages and standard deviations calculated for data obtained from three or four independent solubility experiments

Higuchi and Connors [38] have classified complexes based on their effect on solubility of substrate as shown in Fig. 3. A type phase solubility profiles are obtained when the solubility of the drug increases with increasing ligand concentration. A_L model shows the association constant of $K_{1:1}$ indicating one molecule of drug forms a complex with one molecule of ligand and a linear relationship exhibits. Type A_P system indicates that one molecule of drug forms a complex with two molecule of ligand and a positive deviation from linearity is obtained. Also A_N type profile, which is the least encountered system, shows a negative deviation which indicates a decreasing with increasing ligand concentrations [39]. Generally, the most common stoichiometry of drug/calixarene inclusion complexes is 1:1, and is often studied by the phase solubility studies. B type phase solubility profiles indicate formation of complexes with limited solubility in the aqueous complexation medium. This interaction is attributed to the weak interaction forces including hydrogen bonding, π–π interactions, dipole–dipole bonding or electrostatic interaction between hydrophobic cavity or phosphonate groups of receptors and phenyl, furan ring or substituted group of furosemide. With the help of one or a combination of these forces furosemide most
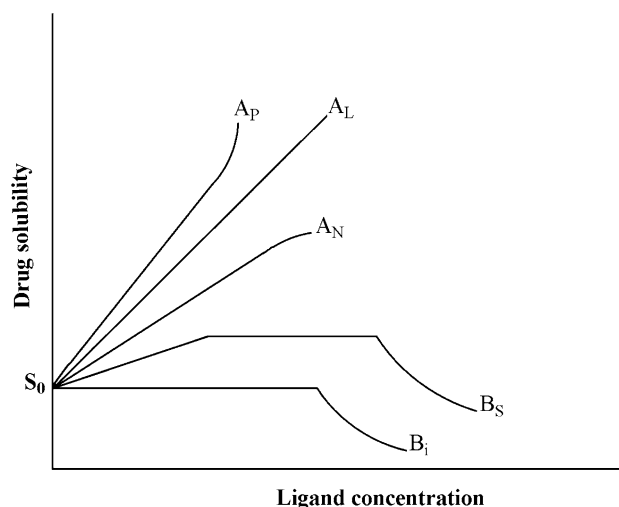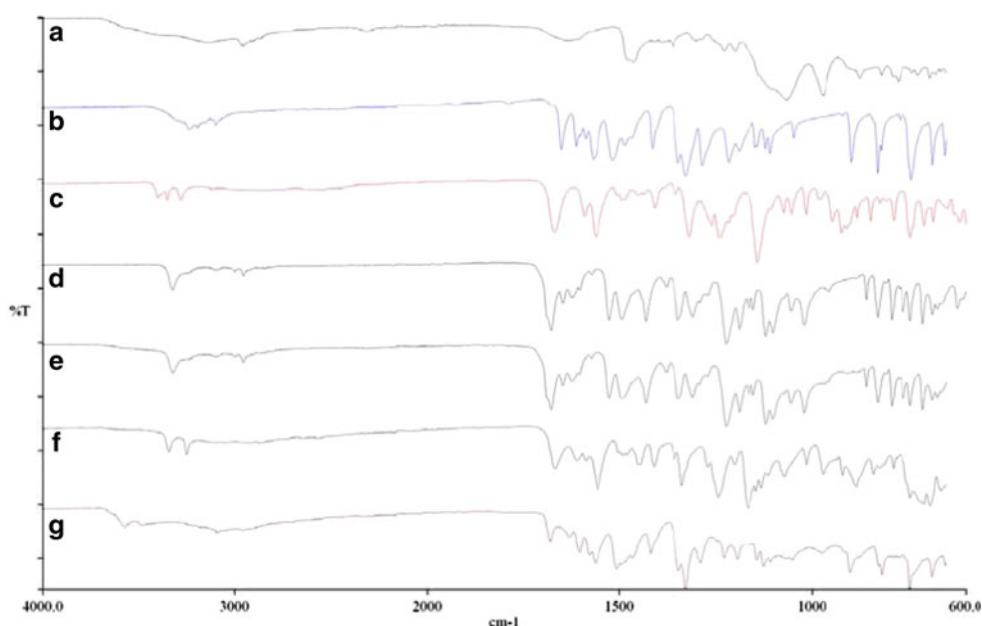
probably formed non-covalent inclusion complexes with the calixarene receptors **1–4** similar to the complexes it forms with 4-sulphonic calix[n]arenes [32]. ^{1}H NMR and FTIR spectra of the solid inclusion complex of furosemide:calixarene **2** was used to clarify the possible interaction between furosemide drug molecule and cavity of calixarene compound **2**. FTIR (ATR) analysis supported the complex formation between calixarene **2** and furosemide. FTIR spectra of furosemide and furosemide:calixarene **2** inclusion complex were given in Fig. 4. The characteristic absorption bands of furosemide, C=O stretching of the carboxylic acid group at 1,668 cm^{-1}, N–H bending at 1,591 and 1,561 cm^{-1} as well as the S=O stretching of the sulfonamide group at 1,317 cm^{-1} (asymmetric) and 1,139 cm^{-1} (symmetric) shifted to different wavenumber positions in the furosemide:calixarene **2** complex spectrum. The C=O and N–H absorption bands shifted to lower frequencies (1666, 1588 and 1557 cm^{-1}, respectively) that could be explained by intermolecular hydrogen bonding between cavity of calixarene **2** and furosemide. In contrast the frequency of both S=O absorption bands shifted to higher frequency values (1,337 and 1,165 cm^{-1}, respectively). The reason might be the interruption of intermolecular hydrogen bonding between furosemide molecules due to interaction of furosemide with the calixarene. And also, the spectrum of solid inclusion complex did not show any new peaks which indicates that no new chemical bonds were formed in the complex. This situation is accordance with literature results [40, 41] of inclusion complex of furosemide drug molecule with macrocyclic ligands by supramolecular complexation. From the FTIR (ATR) results it can be concluded that a complexation between furosemide and calixarene **2** occurred by hydrogen bonding. Furthermore, in the ^{1}H NMR spectra of the furosemide in DMSO-d_6 the characteristic absorptions of aromatic protons of furosemide were

Fig. 4 FTIR (ATR) spectra of:
a calixarene **2**, **b** niclosamide,
c furosemide, **d** nifedipine,
e nifedipine:**2** inclusion
complex, **f** furosemide:**2**
inclusion complex,
g niclosamide:**2** inclusion
complex

observed in the range of 7.0–8.4 ppm. After complexation this signals become more or less shifted in the ^{1}H NMR (D$_2$O) spectra of the furosemide drug molecule. While the ^{1}H NMR spectra of the furosemide showed two singlet peak assigned to aromatic hydrogen protons around 8.55 and 7.21 ppm (DMSO-d_6), the same peaks were observed at 8.24 and 6.85 ppm (respectively) in ^{1}H NMR spectra of inclusion complex of furosemide:calixarene **2** (D$_2$O). Also, ^{1}H NMR data of the furosemide in DMSO-d_6 showed two doublets at 6.34 and 6.26 ppm, attributed to the furan ring protons of furosemide. After complexation the same peaks were seen at 6.27 and 6.22 ppm in the ^{1}H NMR (D$_2$O). The slight difference of the furan protons of furosemide drug molecule in inclusion complex showed that the complexation did not occur close to the furan group. As a result, both NMR and FTIR spectra showed that phenyl group of furosemide is included in the cavity of calixarene skeleton **2**.

Nifedipine

Nifedipine as a L-type calcium-channel blocker is used extensively for the clinical management of a number of cardiovascular diseases such as essential hypertension, congestive heart failure, and cerebral ischemia [17]. A major pharmaceutical problem associated with nifedipine is its poor aqueous solubility, 5–6 μg/cm^3 over a pH range of 2–10, which may account for its highly variable bioavailability in humans [42]. Obtained solubility results showed that nifedipine drug molecule could be dissolved by *p*-phosphonate calixarene receptors **1–4** in water. Solubility of nifedipine was changed the some extend with calixarenes receptors **1** and **3** but did significantly increase with calixarene receptor **2** and **4**. From the experimental

results, it was observed that *p*-phosphonate water soluble calix[4]arene derivative having two *tert* butyl groups **2** was effective host molecule for nifedipine in water (Table 2). And also from the previous extraction study [33], it was clear that maximum extraction percentage occurred 43.3% for *p*-phosphonate calix[8]arene receptor toward nifedipine drug molecules. This is probably due to large enough inner cavity of the calixarene with *tert* butyl groups for nifedipine. Comparing the inner cavity size of the receptors **2** and **4**, it is expected that the larger calix[8]arene cavity would geometrically be more suited for a closer and stronger interaction with nifedipine than the smaller calix[4]arene cavity as mentioned literature data [30]. However, calix[8]arenes are more flexible than the calix[4]arenes. This situation may comparatively affected the dissolution of nifedipine by calix[8]arene receptor **4**. Furthermore, phosphate groups of calix[*n*]arenes could involve hydrogen bonding between the calix[*n*]arenes and substituted groups of nifedipine. Because calixarenes with phosphoryl (P=O) groups are capable of binding effectively different cations and organic molecules with hydrogen bond donors [43]. From the phase solubility profiles of the nifedipine, a linear increase in solubility of nifedipine as shown Fig. 5 represents Type A$_L$ phase solubility profiles attributable the formation of 1:1 nifedipine:calixarene complexes In the same time, weak interaction forces such as π–π interactions, dipole–dipole bonding and/or electrostatic attraction as mention above for furosemide may be another important contributions to the interaction between receptors **1–4** and nifedipine. The FTIR (ATR) spectra of nifedipine and the inclusion complex calixarene:nifedipine were almost identical with respect to the both position and intensity of the bands. The FTIR spectrum of nifedipine (Fig. 4)

showed absorption bands originating from (N=O) at 1,525 cm^{-1} (C=O) at 1,677 cm^{-1} and (N–H) at 3,322 cm^{-1} that are directly related to its structure. Carbonyl stretching and N–H absorption bands of nifedipine drug molecule appear as broad shoulder in the inclusion complex shifted from 1,677 to 1,676 cm^{-1} and 3,322 to 3,320 cm^{-1} which indicates carbonyl groups of nifedipine structure are included in the cavity of calixarene skeleton **2**. The spectrum of solid inclusion complex did not show any new peaks which indicates that no new chemical bonds are formed in the complex. However, it was clear that some of IR absorption peaks in inclusion complex was different from that of the corresponding pure samples, the shape and location of the bands of nifedipine was shifted to some extent [44].

Niclosamide

Niclosamide is active against most tapeworms, including the beef tapeworm, the dwarf tapeworm, and the dog tapeworm [45]. This drug is also used as a molluscicide for the treatment of water in schistosomiasis control programs [31]. Niclosamide is practically insoluble (230 ng/mL), which may severely limit its efficacy [33]. Comparing the solubilizing efficiency of calix[*n*]arenes **1–4**, it was seen that *p*-phosphonate water-soluble calix[6]arene derivative **3**

was an effective molecular receptor for niclosamide. From the phase solubility experiments, the increase in solubility of niclosamide from (4.30 ± 0.03) × 10^{-6} to (13.61 ± 0.03) × 10^{-6} M was observed at 0.007 M calix[6]arene compound **3** in water (Fig. 6; Table 3). Niclosamide is a highly hydrophobic molecule as nifedipine and furosemide. Therefore, similar interactions to dissolve the drug molecule between *p*-phosphonate calixarene receptors **1–4** and niclosamide drug molecule was probably occurred as mentioned both nifedipine and furosemide. Furthermore, in our previous extraction study it was observed that *p*-phosphonate calix[6]arene receptor was extracted the niclosamide drug molecules around 38% extraction percentage from the organic phase to the aqueous phase. It is expected that the larger cavities would geometrically be more suited for a stronger interaction with niclosamide [31]. However, calix[8]arene **4** have less effect than calix[4]arenes **1** and **2**. This situation might be attributable to the both rigid dimension of calix[4]arene and flexible structure of calix[8]arenes with large cavity are most optimal for the niclosamide molecule, because "host-size selectivity" does exist in host–guest-type complexation with calixarenes [22]. Hydrogen bonding and weak interaction forces as π–π interactions and dipole–dipole bonding are the most important forces responsible for the

Table 2 Concentration values of nifedipine with increasing calixarene concentrations in water

Compounds	Concentration values (mol L^{-1} in water)			
	0.001^a	0.003^a	0.005^a	0.007^a
1	4.4 ± 0.04^b	5.6 ± 0.04	6.5 ± 0.04	7.1 ± 0.03
2	5.5 ± 0.04	8.2 ± 0.04	10.2 ± 0.04	12.2 ± 0.04
3	4.3 ± 0.03	5.3 ± 0.03	5.9 ± 0.03	6.6 ± 0.04
4	4.6 ± 0.03	6.5 ± 0.03	8.3 ± 0.04	9.6 ± 0.04

^{a} Concentration values of calixarenes **1–4**

^{b} Averages and standard deviations calculated for data obtained from three or four independent solubility experiments
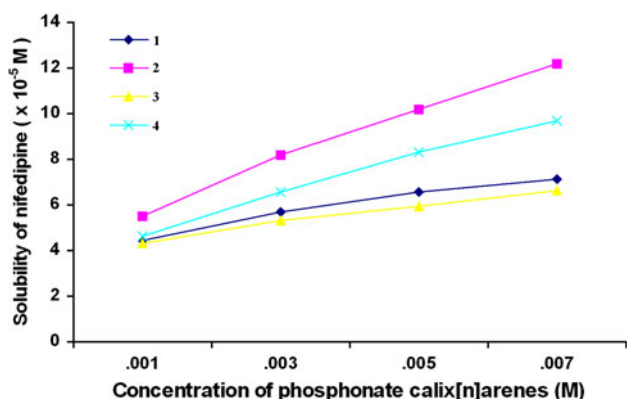

Fig. 6 Phase solubility diagrams of niclosamide by host receptors **1–4**

Table 3 Concentration values of niclosamide with increasing calixarene concentrations in water

Compounds	Concentration values (mol L^{-1} in water)			
	0.001^a	0.003^a	0.005^a	0.007^a
1	4.4 ± 0.03^b	5.6 ± 0.03	7.8 ± 0.04	8.9 ± 0.04
2	3.4 ± 0.03	6.5 ± 0.03	10.1 ± 0.04	12.8 ± 0.04
3	4.3 ± 0.03	7.3 ± 0.03	10.9 ± 0.04	13.6 ± 0.04
4	4.6 ± 0.03	5.5 ± 0.03	7.3 ± 0.04	8.5 ± 0.04

^{a} Concentration values of calixarenes **1–4**

^{b} Averages and standard deviations calculated for data obtained from three or four independent solubility experiments

Fig. 5 Phase solubility diagrams of nifedipine by host receptors **1–4**
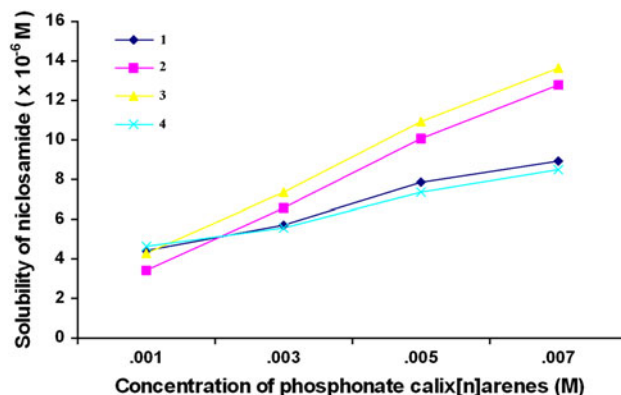
possible interaction of niclosamide with calix[*n*]arene receptors **1–4**. Furthermore, *p*-phosphonate calix[*n*]arenes **1–4** contains four, six or eight (P=O) groups, suggesting that the complexation mechanism could involve hydrogen bonding between the calixarenes and the substitute group of niclosamide. FTIR (ATR) analysis supported the complex formation between calixarene **2** and niclosamide. The characteristic absorption bands of niclosamide, C=O stretching of the drug at 1,651 cm^{-1}, N–H bending at 1,588 and 1,568 cm^{-1}, C–N and C–O stretching at 1,284 and 1,216 (respectively) as well as the NO$_2$ stretching of the niclosamide at 1,515 cm^{-1} shifted to different wave number positions in the niclosamide:calixarene **2** complex spectrum (Fig. 4). The C=O and N–H absorption bands shifted to higher frequencies (1,679 and 1,604 cm^{-1}, respectively). And also, C–N and C–O stretching bands shifted to higher frequencies (1,288 and 1,228 cm^{-1}, respectively) that indicates nitro phenyl group of niclosamide drug molecule is included in the cavity of calixarene skeleton **2** by intermolecular hydrogen bonding between cavity of calixarene **2** and niclosamide. In contrast the frequency of NO$_2$ absorption bands of drug broadened and shifted to lower frequency value (1,506 cm^{-1}). And also, the spectrum of solid inclusion complex did not show any new peaks which indicates that no new chemical bonds were formed in the complex. In the ^{1}H NMR spectra of niclosamide, while the characteristic absorptions of aromatic peaks (nitro phenyl) of niclosamide were seen around 8.2–8.5 ppm (CDCl$_3$), the same peaks shifted and seen around 7.9–8.1 ppm in D$_2$O after inclusion complexation of the niclosamide:calixaren **2**. Especially, the slight difference of the phenolic ring protons of niclosamide around 7.1 and 7.5 ppm showed that the complexation did not occur close to this aromatic group.

Conclusions

In the course of this study, aqueous solubility studies of niclosamide, furosemide and nifedipine drug molecules were performed in water by host:guest complexation. Obtained results showed that the molecular size or the concentration of the calix[*n*]arenes significantly influenced the increase in the solubility of drug molecules. Furthermore, phase solubility profiles of drugs revealed that *p*-phosphonate calix[*n*]arene receptors **1–4** might be useful host molecules as drug solubilizing agents towards niclosamide, furosemide and nifedipine. Especially, comparing the drug molecules, it was observed that these receptors **1–4** improved the solubility of furosemide the most. It could be concluded that the solubility properties of drug molecules by host–guest complexation depends on the structural properties of the water-soluble p-phosphonate

calixarene such as hydrophobic inner cavity diameters, hydrogen binding ability, stability or rigidity and also depends on ion–dipole attraction or electrostatic interaction between calixarenes and drug molecules.

Experimental

General

All starting materials and reagents used were of standard analytical grade from Alfa Aesar, Merck and/or Aldrich and some of them were used without further purification. Analytical thin layer chromatography (TLC) was performed using Merck prepared plates (silica gel 60 F$_{254}$). All reactions, unless otherwise noted, were conducted under a nitrogen atmosphere. The drying agent used was anhydrous MgSO$_4$. All aqueous solutions were prepared with deionized water that had been passed through a Millipore milli-Q Plus water purification system. ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a Varian 400 MHz spectrometer in CDCl$_3$ or D$_2$O. Melting points were determined on an Electrothermal 9100 apparatus in a sealed capillary and are uncorrected. IR spectra were obtained on a Perkin Elmer spectrum 100 FTIR spectrometer (ATR). UV–Vis spectra were obtained on a Shimadzu 160A UV–Vis spectrophotometer. Elemental analyses were performed using a Leco CHNS-932 analyzer. A Crison MicropH 2002 digital pH meter was used for the pH measurements. 5,11,17,23-tetrakis(hydroxyphosphonoyl)methyl-25,26,27,28-tetrahydroxycalix[4]arene **1**, 5,17-bis(hydroxyphosphonoyl)- methyl-11,23-di-tert-butyl-25,26,27,28-tetrahydroxycalix[4]arene **2**, 5,11,17,23,29,35-hexakis(hydroxy phosphonoyl)methyl-37,38,39,40,41,42-hexamethoxycalix[6]arene **3** and 5,11,17,23,29,35,41,47-octakis(hydroxyphosphonoyl)methyl- 49,50,51,52,53,54,55,56-octamethoxycalix[8]arene **4** were prepared according to the literature methods [10, 33] (Scheme 1).

Solubility measurements

The aqueous solubility of niclosamide, furosemide and nifedipine in water was determined at increasing concentrations of the *p*-phosphonate calixarenes. The solubility method of Higuchi and Conners was used [36]. An excess amount of drug powders was added into the screw capped amber vials containing 3 mL of water solution and the complexing agents at increasing concentrations (1.0–7.0 × 10^{-3} M). The vials were rotated at 60 rpm while being kept at 30 °C. After equilibrium was reached (24 h), the solutions was filtered through 0.45 μm cellulose acetate filters and analyzed for drug content by HPLC. All of the solubility experiments and HPLC analysis were carried out in the dark to prevent photodegradation of the drug molecules. Phase solubility

diagrams were constructed by plotting the molar concentration of drugs dissolved versus the molar concentration of complexing agents.

HPLC analysis of drugs

Drug content was analyzed by an HPLC Agilent 1200 Series were carried out using a 1200 model quaternary pump, a G1315B model diode array and multiple wavelength UV–Vis detector, a 1200 model standard and preparative autosampler, a G1316A model thermostated column compartment, a 1200 model vacuum degasser and an Agilent Chemstation B.02.01-SR2 Tatch data processor at 254, 342, and 338 nm for niclosamide, furosemide and nifedipine, respectively. Niclosamide, furosemide and nifedipine eluted on a Supelco Discovery RP Amide C16 column (25 cm × 4.6 mm, 5 μm, Bellefonate, PA) after 14, 8, and 13 min, respectively. The mobile phase was 75:25 (methanol:0.05 M $NH_4H_2PO_4$ v/v) for niclosamide, 60:40:1 (water:acetonitrile:acetic acide v/v) for furosemide and nifedipine, flow rate of 1 mL/min, and injection volume of 20 μL. Each determination was conducted in triplicate.

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