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Phase Solubility Studies of Poorly Soluble Drug Molecules by Using *O*-Phosphorylated Calixarenes as Drug-Solubilizing Agents

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ABSTRACT: This study is the first report on the solubilizing effect of *O*-phosphorylated calix[*n*]arenes that form complexes with neutral molecules such as nifedipine, niclosamide, and furosemide by host−guest complexation. These complexation studies were carried out by using the phase solubility technique. From the obtained results, it was observed that the solubility of guest molecules such as nifedipine, niclosamide, and furosemide was significantly increased in the presence of host molecules tetrakis-*O*-(diethoxyphosphoryl)-*p*-*tert*-butylcalix[4]arene (**1**), tetrakis-*O*-(diethoxyphosphoryl)-calix[4]arene (**2**), bis-*O*-(diethoxyphosphoryl)-*p*-*tert*-butylcalix[4]arene (**3**), bis-*O*-(diethoxyphosphoryl)-calix[4]arene (**4**), and octakis-*O*-(diethoxyphosphoryl)-*p*-*tert*-butylcalix[8]arene (**5**). The increase in solubility of drugs by the calixarene host **1** to **5** was most probably due to inclusion complexation between drug molecules and cavities of the calixarene skeleton similar to drug−cyclodextrin complexes.

INTRODUCTION

The molecular design of host systems based on calixarenes which can form molecular complexations is a focus of interest and research activity within supramolecular chemistry.[1−3]

The 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. Calixarenes are macrocyclic molecules that are generally derived from cheap starting materials as formaldehyde and phenol and can easily be used as building blocks for the design and synthesis of more sophisticated supramolecular structures.[6] Such macrocycles possess a well-defined hydrophobic upper rim and hydrophilic lower rim surrounding a hollow cavity with varied dimensions that depends on the number of the phenolic units incorporated.[7] A majority of the studies on calixarenes have focused on the calix[4]arenes, primarily because they possess open and rigid structures that are desirable for molecular recognition.[8] It is well-known that calixarenes are used for the molecular recognition of ions, peptides, amino acids, sugars, hormones, proteins, and nucleic acids which are basic substrates in biological and artificial processes.[8−12]

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 diuretics, anthelmintics, and calcium channel blockers, respectively.[13−15] The main problem of these molecules is poor aqueous solubility. Supramolecular complexation is a commonly used technique to increase the solubility of poorly water-soluble drugs.[16,17] Among the macromolecules used to solubilize drugs, 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.[18] Calixarenes may selectively include various guests according to their size and hydrophobicity in a manner similar to cyclodextrins.[19,20] Although the Food and Drug Administration (FDA) has currently not approved the use of calixarenes in medicines to date, calixarenes have showed neither toxicity nor immune responses.[21] Studies of calixarenes on human fibroblast cells using inhibition of cell growth have been showed that calixarene derivatives have the same level toxicity as glucose.[20] Furthermore, it has been observed that the calix[4]arene phosphonic acid derivative showed no effects on the cell growth of human fibroblasts.[20] On the other hand, more recently it has been observed that solid lipid nanoparticles based on amphipihilic calix[4]arenes show zero hemolytic effects at concentrations ranging up to 300 mg·L^{-1}.[22] This situation is in contrast to the behavior of the solid lipid nanoparticles based on amphipihilic cyclodextrins which show significant hemolytic effects.[23] Therefore, this situation increases interest in their use instead of cyclodextrins in the biopharmaceutical applications beyond their current use for the complex forming agents to remove molecules from the environment.[24−26] To date several works about the effect of the water-soluble *p*-sulfonic calix[*n*]arenes on the solubility of drugs has been reported.[27−29] There is no other published solubility study between poorly soluble drug molecules and lower rim functionalized phosphonate calix[*n*]arene receptors. The inclusion behaviors of water-soluble *o*-phosphonate calix[*n*]arene receptors toward nifedipine, niclosamide, and furosemide have not been explored so far by means of phase solubility process. Therefore, the aim of the present study was to explore the use of water-soluble *o*-phosphonate calix[*n*]arene as drug solubilizing agents toward nifedipine, niclosamide, and furosemide.

MATERIALS AND METHODS

Materials. All starting materials and reagents used were of standard analytical grade from Alfa Aesar, Merck, and/or Aldrich,

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Table 1. Characteristics of Reagents

chemical name	source	initial mole fraction purity	purification method	final mole fraction purity	analysis method
acetonitrile	Sigma-Aldrich	0.999			
methanol	Merck	0.999			
diethyl chlorophosphate	Alfa Aesar	0.970	distillation	0.980	GC[a]^{1}H NMR[b]
bromotrimethylsilane	Merck	0.980			
triethylamine	Merck	0.990			
acetic acid	Merck	0.998			
niclosamide	Sigma-Aldrich	0.980			
furosemide	Sigma-Aldrich	0.980			
nifedipine	Sigma-Aldrich	0.980			

[a] Gas chromatography. [b] Nuclear magnetic resonance.

and some of them were used without further purification (Table 1). 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.

Apparatus. ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on a Varian 400 MHz spectrometer in $CDCl_3$ or D_2O. 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.

Synthesis. Tetrakis-*O*-(diethoxyphosphoryl)-*p*-*tert*-butylcalix[4]arene (**1**), tetrakis-*O*-(diethoxyphosphoryl)-calix[4]arene (**2**), bis-*O*-(diethoxyphosphoryl)-*p*-*tert*-butylcalix[4]arene (**3**), bis-*O*-(diethoxyphosphoryl)-calix[4]arene (**4**), and octakis-*O*-(diethoxyphosphoryl)-*p*-*tert*-butylcalix[8]arene (**5**) were prepared according to the modified literature methods.[30−32]

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 Connors was used.[33] An excess amount of drug powders was added into the screw capped amber vials containing 3 cm^3 of water solution and the complexing agents at increasing concentrations ($(1.0 \text{ to } 7.0)\cdot 10^{-3}$ mol·kg^{-1}). The vials were rotated at 60 rpm while being kept at 303 K. After equilibrium was reached (24 h), the solutions was filtered through 0.45 μm cellulose acetate filters and analyzed for drug content by high-performance liquid chromatography (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 molal concentration of drugs dissolved versus the molal 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 thermostatted 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 0.75:0.25 mole fraction (methanol/$NH_4H_2PO_4$ (0.05 mol·kg^{-1} in water) for niclosamide, 0.60:0.40:0.01 mole fraction (water/acetonitrile/acetic acid) for furosemide and nifedipine, flow rate of 1 $cm^3\cdot min^{-1}$, and injection volume of 20 μL. Each determination was conducted in triplicate.

RESULTS AND DISCUSSION

Calixarenes **1** to **5**, containing dihydroxyphosphoryl groups at the lower rim, have been synthesized by reactions with diethyl chlorophosphate and triethylamine or sodium hydride in chloroform at reflux for 20 h (Scheme 1).[32] Obtained phospho-ethyl ester groups on lower rim of calix skeleton have been easily converted the corresponding phosphoric acid moieties by using bromotrimethylsilane in chloroform for 24 h at room temperature. Then treatment of the trimethylsilyl esters $P(O)(OSiMe_3)_2$ with absolute methanol results in cleavage of the P−O−Si bonds and formation of the corresponding acids derivatives **1** to **5** in high yields.[3,31] Related NMR data and some physical properties of compounds **1** to **5** have been given in Tables 2 and 3.

PHASE SOLUBILITY STUDIES

Furosemide. Furosemide is a derivative from the anthranilic acid, whose structure is presented in Figure 1, representing 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 oral 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.[29] 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** to **5** toward guest molecule furosemide by a phase solubility process. From the solubility studies, calixarene phosphonates **1** to **5** were found to be an effective host molecule for practically water-insoluble furosemide (38 $\mu g\cdot cm^{-3}$ at 303 K in water).

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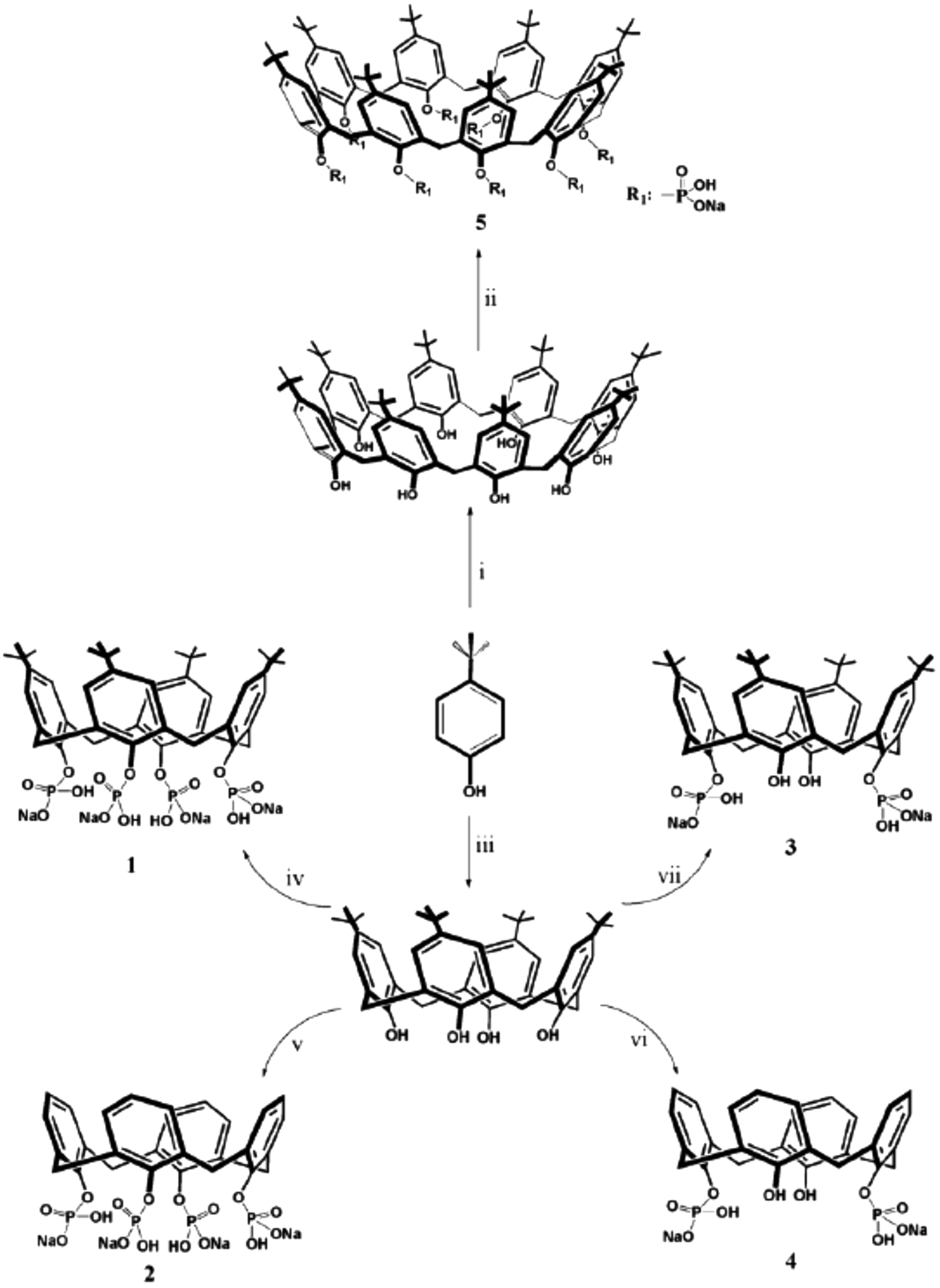
Scheme 1. Synthetic Pathway and Structures of the Studied *O*-Phosphorylated Calixarene Receptors 1 to 5. i: C_6H_6O, Paraformaldehyde, NaOH, Xylene, ii: Diethylchlorophosphate, NaH, THF, iii: CH_2O, NaOH, $C_6H_5OC_6H_5$, iv: Diethylchlorophosphate, NaH, THF, v: $AlCl_3$, C_6H_6O, Toluene, Diethylchlorophosphate, NaH, THF, vi: Diethylchlorophosphate, K_2CO_3, vii: Diethylchlorophosphate, K_2CO_3



Obtained results showed that both the molecular size and the concentration of the calixarenes significantly influenced the increase in the solubility of furosemide. The largest increase in solubility of furosemide in water from $(2.78 \pm 0.04)\cdot10^{-3}$ mol·kg^{-1} to $(10.11 \pm 0.03)\cdot10^{-3}$ mol·kg^{-1} for compound **5** and $(2.44 \pm 0.04)\cdot10^{-3}$ mol·kg^{-1} to $(9.11 \pm 0.03)\cdot10^{-3}$ mol·kg^{-1} for compound **1** was observed at a 0.007 mol·kg^{-1} concentration of calixarene compounds **1** and **5** in water (Figure 2 and Table 4). Also, the largest increase in solubility of furosemide in water for calix[4]arene compounds **2**, **3**, and **4** was seen around 7.30 ± 0.03, 7.20 ± 0.03, and $6.20 \pm 0.04\cdot10^{-3}$ mol·kg^{-1} at a 0.007 mol·kg^{-1} concentration of calixarene compounds, respectively.

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Table 2. NMR Spectra of the Receptors (1 to 5)

compounds	chemical shifts in ^{1}H and ^{31}P NMR spectra
1	^{1}H NMR: δ 6.85 (s, 8H, ArH), 4.70 (bs, 4H, $ArCH_2Ar$), 3.25 (bs, 4H, $ArCH_2Ar$), 1.13 (s, 36H, Bu^t)
	^{31}P NMR: 20.15
2	^{1}H NMR: δ 6.83 (s, 8H, ArH), 4.40 (bs, 4H, $ArCH_2Ar$), 3.25 (bs, 4H, $ArCH_2Ar$)
	^{31}P NMR: 20.00
3	^{1}H NMR: δ 7.05 (s, 4H, ArH), 6.70 (s, 4H, ArH), 4.40 (bs, 4H, $ArCH_2Ar$), 3.40 (bs, 4H, $ArCH_2Ar$), 1.15 (s, 18H, Bu^t), 0.85 (s, 18H, Bu^t)
	^{31}P NMR: 21.10
4	^{1}H NMR: δ 7.20 (d, 4H, ArH), 6.80−6.70 (m, 8H, ArH), 4.45 (bs, 4H, $ArCH_2Ar$), 3.45 (bs, 4H, $ArCH_2Ar$)
	^{31}P NMR: 20.10
5	^{1}H NMR: δ 7.07 (s, 16H, ArH), 4.30 (bs, 16H, $ArCH_2Ar$), 1.11 (s, 72H, Bu^t)
	^{31}P NMR: 20.05

Table 3. Some Physical Properties of the Receptors (1 to 5)

compound	empirical formula	color	T^a/K	yield
1	$C_{44}H_{56}O_{15}P_4Na_2 \cdot 6H_2O$	pale brown	>573	90
2	$C_{28}H_{24}O_{15}P_4Na_4 \cdot 4H_2O$	white	>573	90
3	$C_{44}H_{56}O_{10}P_2Na_2 \cdot 3H_2O$	white	>573	90
4	$C_{28}H_{24}O_{10}P_2Na_2 \cdot 2H_2O$	pale brown	>573	90
5	$C_{88}H_{112}O_{32}P_8Na_8 \cdot 6H_2O$	pale brown	>573	90

[a] T: melting point.

Comparing calixarenes **1** to **5**, the calixarene derivatives, especially having *tert*-butyl groups, gave better results for furosemide drug molecules. The cavity of the calixarene 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 like 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 behavior of calixarene skeleton.[37] 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 the solubility diagram of furosemide as shown in Figure 2 represents Type A_L phase solubility profiles, indicating the formation of 1:1 furosemide/calixarene complexes.[33]

Higuchi and Connors[33] have classified complexes based on their effect on the solubility of the substrate as shown in Figure 3. "A type" phase-solubility profiles are obtained when the solubility of the drug increases with increasing ligand concentration. The 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. The 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 the A_N type profile, which is the least encountered system, shows a negative deviation which indicates a decrease with increasing ligand concentrations.[38] 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 the formation of complexes with limited solubility in the aqueous complexation medium. This interaction is attributed to the weak interaction forces including hydrogen bonding, $\pi-\pi$ 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 noncovalent inclusion complexes with the calixarene receptors **1** to **5** similar to the complexes it forms with 4-sulfonic calix[n]arenes.[27] Comparing *para*-sulfonate calix[n]arene[27] and *ortho*-phosphonate calix[n]arene, phosphorylated calixarene compounds having both *tert*-butyl groups and phosphoryl (P═O) groups was seen to be an effective drug-solubilizing agent by supramolecular complexation for practically water-insoluble furosemide.

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.[15] A major pharmaceutical problem associated with nifedipine is its poor aqueous solubility, (5 to 6) $\mu g \cdot cm^{-3}$ over a pH range of 2 to 10, which may account for its highly variable bioavailability in humans.[39] Obtained solubility results showed that the nifedipine drug molecule could be dissolved by calixarene receptors **1** to **5** in water. The solubility of nifedipine in water was changed the some extent with calixarene receptors **2** to **5** but did significantly increase with calixarene receptor **1**. In contrast to obtained literature results,[27] it was observed that the *O*-phosphorylated water-soluble calix[4]arene derivative **1** was an effective host molecule for nifedipine in water (Table 5). This is probably due to rigid structure of calix receptor **1** having *tert*-butyl groups as explained for furosemide. Although both receptors **3** and **5** have similar *tert*-butyl groups, calix[8]arenes are more flexible than the calix[4]arenes. This situation may affected the dissolution of nifedipine by calix[8]arene receptor **5**. 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.[40] From the phase solubility profiles of the nifedipine, a linear increase in solubility of nifedipine as shown in Figure 4 represents type A_L phase solubility profiles attributable to the formation of 1:1 nifedipine−calixarene complexes. In the same time, weak interaction forces such as $\pi-\pi$ interactions, dipole−dipole bonding, and/or electrostatic attraction as mentioned above for furosemide may be another important contributions to the interaction between receptors **1** to **5** and nifedipine.

Niclosamide. Niclosamide is active against most tapeworms, including the beef tapeworm, the dwarf tapeworm, and the dog

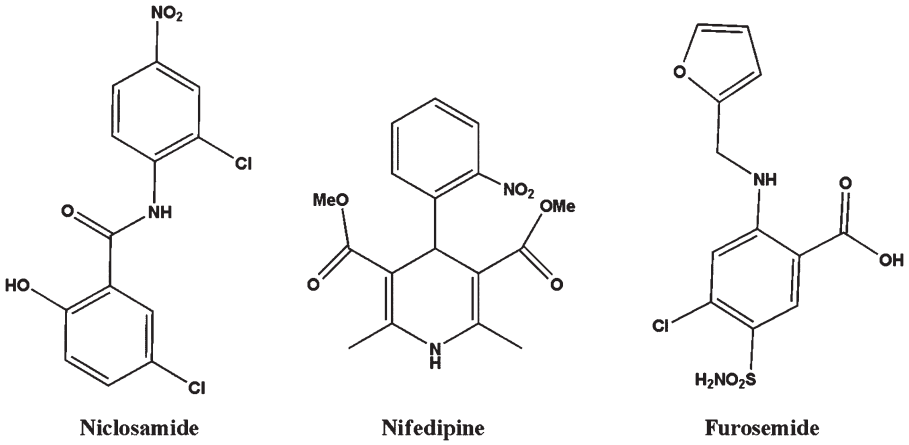

Figure 1. Structures of the drug molecules.

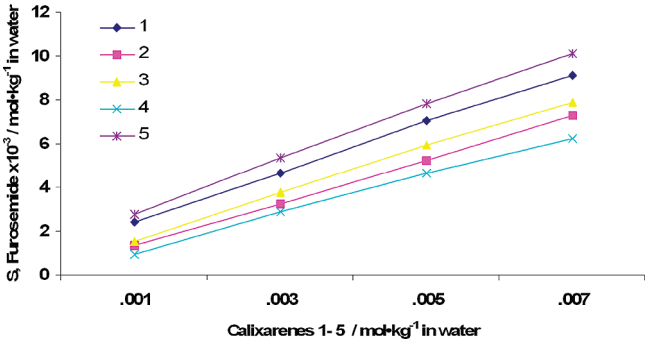

Figure 2. Phase solubility diagrams of furosemide by host receptors **1** to **5** in water at 303 K.

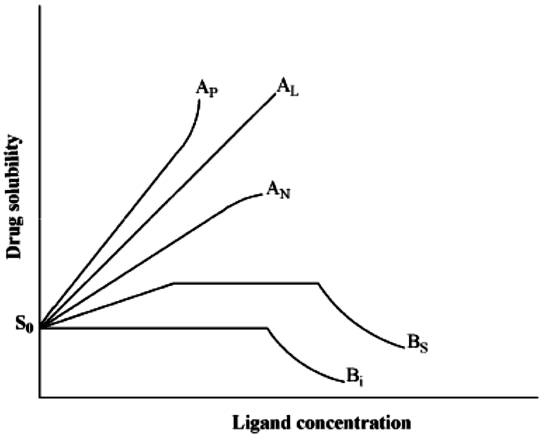

Figure 3. General phase solubility profiles of drugs.

Table 4. Concentration Values of Furosemide with Increasing Calixarene Concentrations (mol·kg^{-1} in Water)

	concentration values			
compounds	0.001[a]	0.003[a]	0.005[a]	0.007[a]
1	2.44 ± 0.03[b]	4.66 ± 0.04	7.04 ± 0.04	9.11 ± 0.04
2	1.34 ± 0.03	3.21 ± 0.04	5.22 ± 0.04	7.32 ± 0.03
3	1.52 ± 0.03	3.74 ± 0.04	5.93 ± 0.04	7.91 ± 0.03
4	0.92 ± 0.03	2.89 ± 0.04	4.64 ± 0.04	6.22 ± 0.04
5	2.78 ± 0.04	5.38 ± 0.04	7.81 ± 0.04	10.11 ± 0.03

[a] Concentration values of calixarenes. [b] Averages and standard deviations calculated for data obtained from three or four independent solubility experiments.

tapeworm.[41] This drug is also used as a molluscicide for the treatment of water in schistosomiasis control programs.[28] Niclosamide is practically insoluble (230 ng·cm^{-3}), which may severely limit its efficacy.[42] From the extraction results, it was observed that the niclosamide drug molecule could be extracted from the organic phase into the aqueous phase. Comparing the solubilizing efficiency of calix[*n*]arenes **1** to **5**, it was seen that *O*-phosphorylated water-soluble calix[4]arene derivative **1** was an effective moleculer receptor for niclosamide. From the phase solubility experiments, the increase in solubility of niclosamide from $(2.21 \pm 0.03) \cdot 10^{-6}$ mol·kg^{-1} to $(4.37 \pm 0.03) \cdot 10^{-6}$ mol·kg^{-1}

Table 5. Concentration Values of Nifedipine with Increasing Calixarene Concentrations (mol·kg^{-1} in Water)

	concentration values			
compounds	0.001[a]	0.003[a]	0.005[a]	0.007[a]
1	5.92 ± 0.03[b]	7.87 ± 0.04	9.52 ± 0.04	11.20 ± 0.04
2	3.84 ± 0.04	4.51 ± 0.03	5.22 ± 0.04	6.32 ± 0.04
3	3.52 ± 0.03	4.34 ± 0.03	4.93 ± 0.04	5.11 ± 0.04
4	3.79 ± 0.03	4.11 ± 0.03	4.34 ± 0.04	4.72 ± 0.04
5	4.64 ± 0.04	5.94 ± 0.04	6.98 ± 0.04	8.11 ± 0.04

[a] Concentration values of calixarenes. [b] Averages and standard deviations calculated for data obtained from three or four independent solubility experiments.

was observed at a 0.005 mol·kg^{-1} concentration of calixarene compound **1** in water (Figure 5 and Table 6). However, as the concentration of calixarene receptors **1** to **5** increased, the solubility of the drug is exceeded, indicating a decrease in the solubility of drug. This situation is probably due to insoluble complexes formed at higher host concentrations, or the drug was removed from the inclusion complexes.[28] Niclosamide is a highly hydrophobic molecule as nifedipine and furosemide.[43] Therefore, similar interactions to dissolve the drug molecule between

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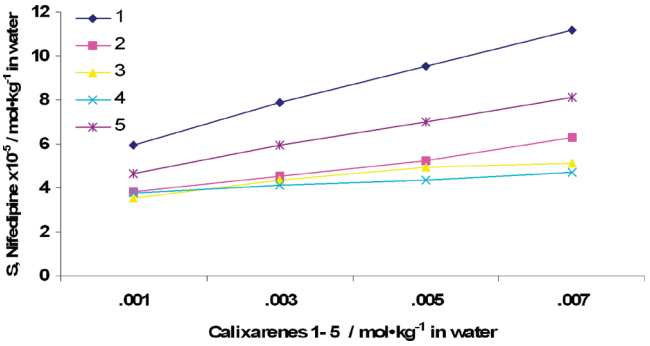

Figure 4. Phase solubility diagrams of nifedipine by host receptors **1** to **5** in water at 303 K.

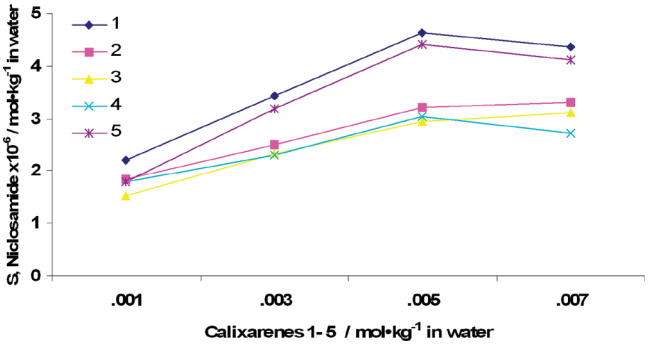

Figure 5. Phase solubility diagrams of niclosamide by host receptors **1** to **5** in water at 303 K.

Table 6. Concentration Values of Niclosamide with Increasing Calixarene Concentrations (mol·kg^{-1} in Water)

	concentration values			
compounds	0.001[a]	0.003[a]	0.005[a]	0.007[a]
1	2.21 ± 0.04[b]	3.44 ± 0.04	2.21 ± 0.04[b]	4.37 ± 0.04
2	1.84 ± 0.04	2.51 ± 0.04	1.84 ± 0.04	3.32 ± 0.04
3	1.52 ± 0.04	2.34 ± 0.04	1.52 ± 0.04	3.11 ± 0.04
4	1.79 ± 0.04	2.31 ± 0.04	1.79 ± 0.04	2.72 ± 0.04
5	1.78 ± 0.04	3.18 ± 0.04	1.78 ± 0.04	4.11 ± 0.04

[a] Concentration values of calixarenes. [b] Averages and standard deviations calculated for data obtained from three or four independent solubility experiments.

calixarene receptors **1** to **5** and niclosamide drug molecule probably occurred as mentioned for both nifedipine and furosemide. It is expected that the larger cavities would geometrically be more suited for a stronger interaction with niclosamide.[28] However, calix[8]arenes are less effective than calix[4]arenes. This situation might be attributable to the both rigid dimensions of calix[4]arene and flexible structure of calix[8]arenes with a large cavity, which are optimal for the niclosamide molecule, because "host-size selectivity" does exist in host−guest-type complexation with calixarenes.[20] Hydrogen bonding and weak interaction forces as $\pi-\pi$ interactions and dipole−dipole bonding are the most important forces responsible for the possible interaction of niclosamide with calixarene receptors **1** to **5**. Furthermore, *O*-phosphorylated calixarenes **1** to **5** contains two, four, or eight (P═O) groups, suggesting that the complexation mechanism could involve hydrogen bonding between the calixarenes and the substitute group of niclosamide.

CONCLUSIONS

In the course of this study, several *O*-phosphorylated calixarene receptors were easily synthesized. Also, aqueous solubility studies of niclosamide, furosemide, and nifedipine drug molecules were performed in water, and obtained results showed that the molecular size or the concentration of the calixarenes significantly influenced the increase in the solubility of drug molecules. Furthermore, phase solubility profiles of drugs revealed that *O*-phosphorylated calixarene receptors **1** to **5** might be useful host molecules as drug-solubilizing agents toward niclosamide, furosemide, and nifedipine. Especially comparing the drug molecules, it was observed that these receptors **1** to **5** improved the solubility of furosemide the most. It could be concluded that the solubility properties of drug molecules by host−guest complexation depend on the structural properties of the water-soluble *O*-phosphorylated calixarene such as hydrophobic cavity diameters, hydrogen binding ability, stability, or rigidity and also depend on ion−dipole attraction or electrostatic interaction between calixarenes and drug molecules.

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REFERENCES

(1) Gutsche, C. D. *Calixarenes Revisited in Monograph in Supramolecular Chemistry*; Stoddart, J. F., Ed.; The Royal Society of Chemistry: Cambridge, UK, 1998.

(2) Böhmer, V. Calixarenes, Macrocycles with (Almost) Unlimited Possibilities. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 713–745.

(3) Tairov, M. A.; Vysotsky, M. O.; Kalchenko, O. I.; Pirozhenko, V. V.; Kalchenko, V. I. Symmetrical and Inherently Chiral Water-Soluble Calix[4]arenes Bearing Dihydroxyphosphoryl Groups. *J. Chem. Soc., Perkin Trans. 1* **2002**, 1405–1411.

(4) Gokel, G. W. *Crown Ethers and Cryptands: Monographs in Supramolecular Chemistry*; The Royal Society of Chemistry: Cambridge, UK, 1991.

(5) Szetjli, J. *Cyclodextrin Technology*; Kluwer Academic: Dordrecht, 1988.

(6) Gutsche, C. D.; Dhawan, B.; Levine, J. A.; No, K. H.; Bauer, L. J. Calixarenes 9: Conformational Isomers of The Ethers and Esters of Calix[4]arenes. *Tetrahedron* **1983**, *39*, 409–426.

(7) Lo, P. K.; Wong, M. S. Extended Calix[4]arene-based Receptors for Molecular Recognition and Sensing. *Sensors* **2008**, *8*, 5313–5335.

(8) Ikeda, A.; Shinkai, S. Novel Cavity Design using Calix[n]arene Skeletons: Towards Molecular Recognition and Metal Binding. *Chem. Rev.* **1997**, *97*, 1713–1734.

(9) Koh, K.; Araki, K.; Shinkai, S.; Asfari, Z.; Vicens, J. Cation Binding Properties of A Novel 1,3-alternate Calix[4]biscrown. Formation of 1:1 and 1:2 Complexes and Unique Cation Tunneling Across a Calix[4]arene Cavity. *Tetrahedron Lett.* **1995**, *36*, 6095–6098.

(10) Mutihac, L.; Buschmann, H. J.; Mutihac, R. C.; Schollmeyer, E. Complexation and Separation Of Amines, Amino Acids, and Peptides by Functionalized Calix[n]arenes. *J. Inclusion Phenom. Macrocyclic Chem.* **2005**, *51*, 1–10.

(11) Stone, M. M.; Franz, A. H.; Lebrilla, C. B. Non-covalent Calixarene Amino Acid Complexes Formed by MALDI-MS. *J. Am. Soc. Mass Spectrom.* **2002**, *13*, 964–974.

(12) Chen, H.; Gal, Y. S.; Kim, S. H.; Choi, H. J.; Oh, M. C.; Lee, J.; Koh, K. Potassium Ion Sensing Using A Self-Assembled Calix[4]Crown Monolayer by Surface Plasmon Resonance. *Sens. Actuators, B* **2008**, *133*, 577–581.

(13) Ai, H.; Jones, S. A.; de Villiers, M. M.; Lvov, Y. M. Nano-Encapsulation of Furosemide Microcrystals for Controlled Drug Release. *J. Controlled Release* **2003**, *86*, 59–68.

(14) Devarakonda, B.; Hill, R. A.; Liebenberg, W.; Brits, M.; de Villiers, M. M. Comparison of the Aqueous Solubilization of Practically Insoluble Niclosamide by Polyamidoamine (PAMAM) Dendrimers and Cyclodextrins. *Int. J. Pharm.* **2005**, *304*, 193–209.

(15) Boje, K. M.; Sak, M.; Fung, H. L. Complexation of Nifedipine with Substituted Phenolic Ligands. *Pharm. Res.* **1998**, *5*, 655–659.

(16) Al-Omar, A.; Abdou, S.; De Robertis, L.; Marsura, A.; Finance, C. Complexation Study and Anticellular Activity Enhancement by Doxorubicin−Cyclodextrin Complexes on A Multidrugresistant Adenocarcinoma Cell Line. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1115–1120.

(17) Garcia-Rodriguez, J. J.; Torrado, J.; Bolas, F. Improving Bioavailability and Anthelmintic Activity of Albendazole by Preparing Albendazole-Cyclodextrin Complexes. *Parasite* **2001**, *8*, 188–190.

(18) Frömming, K. H.; Szejtli, J. *Cyclodextrin in Pharmacy*; Kluwer Academic: Dordrecht, 1994.

(19) Zhang, Y.; Agbaria, R. A.; Mukundan, N. E.; Warner, I. M. Spectroscopic Studies of Water-Soluble Sulfonated Calix[6]arene. *J. Inclusion Phenom. Mol. Recognit. Chem.* **1996**, *24*, 353–365.

(20) Shinkai, S.; Araki, K.; Manabe, O. Does The Calixarene Cavity Recognize the Size of Guest Molecules? On the Hole-Size Selectivity in Watersoluble Calixarenes. *J. Chem. Soc., Chem. Commun.* **1988**, *3*, 187–189.

(21) Rodik, R. V.; Boyko, V. I.; Kalchenko, V. I. Calixarenes in Bio-Medical Researches. *Curr. Med. Chem.* **2009**, *16*, 1630–1655.

(22) Shahgaldian, P.; Da Silva, E.; Coleman, A. W. A First Approach to The Study of Calixarene Solid Lipid Nanoparticle (SLN) Toxicity. *J. Inclusion Phenom.* **2003**, *46*, 175–177.

(23) Memişoglu, E.; Bochot, A.; Özalp, M.; Şen, M.; Duchene, D.; Hincal, A. A. Direct Formation of Nanospheres from Amphiphilic β-Cyclodextrin Inclusion Complexes. *Pharm. Res.* **2003**, *20*, 117–125.

(24) Bayrakci, M.; Ertul, S.; Sahin, O.; Yilmaz, M. Synthesis of Two New *p*-Tert-Butylcalix[4]arene β-Ketoimin Derivatives for Extraction of Dichromate Anion. *J. Inclusion Phenom. Macrocyclic Chem.* **2009**, *63*, 241–247.

(25) Sayin, S.; Yilmaz, M. Synthesis of A New Calixarene Derivative and Its Immobilization onto Magnetic Nanoparticle Surfaces for Excellent Extractants Toward Cr(VI), As(V), and U(VI). *J. Chem. Eng. Data* **2011**, *56*, 2020–2029.

(26) Bayrakci, M.; Ertul, S.; Yilmaz, M. Synthesis of Di-Substituted Calix[4]Arene-Based Receptors for Extraction of Chromate and Arsenate Anions. *Tetrahedron* **2009**, *65*, 7963–7968.

(27) Yang, W.; de Villiers, M. M. The Solubilization of the Poorly Water Soluble Drug Nifedipine by Water Soluble 4-Sulphonic Calix[n]arenes. *Eur. J. Pharm. Biopharm.* **2004**, *58*, 629–636.

(28) Yang, W.; de Villiers, M. M. Effect of 4-Sulphonato-Calix[n]arenes and Cyclodextrins on the Solubilization of Niclosamide, a Poorly Water Soluble Anthelmintic. *AAPS J.* **2005**, *7*, 241–248.

(29) Yang, W.; de Villiers, M. M. Aqueous Solubilization of Furosemide by Supramolecular Complexation with 4-Sulphonic Calix[n]arenes. *J. Pharm. Pharmacol.* **2004**, *56*, 703–708.

(30) Cobben, P. L. H. M.; Egberink, R. J. M.; Bomer, J. G.; Bergvelt, P.; Verboom, W.; Reinhoudt, D. N. Transduction of Selective Recognition of Heavy Metal Ions by Chemically Modified Field Effect Transistors (CHEMFETs). *J. Am. Chem. Soc.* **1992**, *114*, 10573–10582.

(31) Markovsky, L. N.; Visotsky, M. A.; Pirozhenko, V. V.; Kalchenko, V. I.; Lipkowskib, J.; Simonov, Y. A. Phosphorotropic Rearrangement in Synthesis of Asymmetrically-Substituted Calix[4]arenes. *Chem. Commun.* **1996**, 69–71.

(32) Gloede, J. Calixarene Phosphates. *Phosphorus, Sulfur Silicon Relat. Elem.* **1997**, *127*, 97–111.

(33) Higuchi, T.; Connors, K. A. Phase-Solubility Techniques. In *Advances in Analytical Chemistry and Instrumentation*; Reilley, C. N., Ed.; John Wiley and Sons: New York, 1965.

(34) Özdemir, N.; Ordu, S. Improvement of Dissolution Properties of Furosemide by Complexation with β-Cyclodextrin. *Drug Dev. Ind. Pharm.* **1998**, *24*, 19–25.

(35) Ammar, H. O.; Ghorab, M.; El-Nahhas, S. A.; Emara, L. H.; Makram, T. S. Inclusion Complexation of Furosemide in Cyclodextrins. *Die Pharm.* **1999**, *54*, 142–144.

(36) Talanova, G. G.; Talanov, V. S.; Gorbunova, M. G.; Hwang, H.-S.; Bartsch, R. A. Effect of Upper Rim *Para*-Alkyl Substituents on Extraction of Alkali and Alkaline Earth Metal Cations by Di-Ionizable Calix[4]arenes. *J. Chem. Soc., Perkin Trans. 2* **2002**, 2072–2077.

(37) Zhou, H.; Liu, D.; Gega, J.; Surowiec, K.; Purkiss, D. W.; Bartsch, R. A. Effect of *Para*-Substituents on Alkaline Earth Metal Ion Extraction by Proton Di-Ionizable Calix[4]arene-Crown-6 Ligands in Cone, Partial-Cone and 1,3-Alternate Conformations. *Org. Biomol. Chem.* **2007**, *5*, 324–332.

(38) Arun, R.; Ashok Kumar, C. K.; Sravanthi, V. V. N. S. S. Cyclodextrins as Drug Carrier Molecule: A Review. *Sci. Pharm.* **2008**, *76*, 567–598.

(39) Ali, S. L. *Nifedipine, Analytical Profiles of Drug Substances*, Vol. 18; Florey, K., Ed.; Academic Press, New York, 1989; pp 223−288.

(40) Lukin, O.; Vysotsky, M. O.; Kalchenko, V. I. O-Phosphorylated Calix[4]arenes as Li^+-Selective Receptors. *J. Phys. Org. Chem.* **2001**, *14*, 468–473.

(41) Reynolds, J. E. F., Ed. *Martindale: The Extra Pharmacopoeia*, 29th ed.; The Pharmaceutical Press: London, 1989; pp 987−991.

(42) *The Merck Index*, 13th ed.; Merck: Rahway, NY, 2001.

(43) Bayrakci, M.; Ertul, S.; Yilmaz, M. Transportation of Poorly Soluble Drug Molecules from The Organic Phase to The Aqueous Phase by Using Phosphorylated Calixarenes. *J. Chem. Eng. Data*, DOI: 10.1021/je2004319.