Effect of 4-Sulphonato-Calix[n]Arenes and Cyclodextrins on the Solubilization of Niclosamide, a Poorly Water Soluble Anthelmintic

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ABSTRACT

The present study investigated the effect of water-soluble 4-sulphonato-calix[n]arenes, cyclodextrins, and combinations of these macromolecules on the aqueous solubility of a poorly water-soluble drug, niclosamide. Complexation between the macromolecules and niclosamide was confirmed by thermal analysis and phase solubility studies in a pH 7.0 McIlvaine buffer kept at 30°C. Results show that the increase in solubility ranked as follows: 4-sulphonato-calix[6]arene + hydroxypropyl- β -cyclodextrin (HP- β -CD) > 4-sulphonato-calix[6]arene + β -cyclodextrin > 4-sulphonato-calix[6]arene + γ -cyclodextrin = HP- β -CD > 4-sulphonato-calix[6]arene > 4-sulphonato-calix[8]arene = 4-sulphonato-calix[4]arene > β -cyclodextrin. Type B phase solubility profiles were observed, indicating a decrease in solubility at concentrations > 0.004 to 0.005 mol/L of the 4-sulphonato-calix[n]arenes or combinations of 4-sulphonato-calix[6]arene and the cyclodextrins. However, below this concentration, the greatest increase in the aqueous solubility niclosamide was observed when 4-sulphonato-calix[6]arene and HP- β -CD were combined. This increase in solubility was additive.

KEYWORDS: niclosamide, solubilization, 4-sulphonato-calix[n]arenes, cyclodextrins

Niclosamide (5-chloro-*N*-2-chloro-4-nitrophenyl)-2-hydroxybenzamide; Figure 1) is an anthelmintic drug that is active against most tapeworms, including the beef tapeworm, the dwarf tapeworm, and the dog tapeworm.¹ This drug is also used as a molluscicide for the treatment of water in schistosomiasis control programs.² Niclosamide is practically insoluble in water (230 ng/mL), which may severely limit its efficacy.³

One way to increase the aqueous solubility of this drug is to use complexing agents to form host:guest complexes. Among the complexing agents available, cyclodextrins (CDs) are most widely used in drug formulations.⁴ Alterna-

tive complexing agents are calixarenes (Figure 1), a new class of cyclooligomers formed via phenol-formaldehyde reactions. Each calixarene contains a repeating phenolic unit formed into a macrocycle via methylene bridges.⁵ Among the water-soluble calixarenes, the *p*-sulphonato-calixarenes have been widely studied as complexing agents for organic molecules, because they possess the highest-known aqueous solubility, >0.1 mol/L.⁶ These calixarenes provide not only a hydrophobic environment (benzene rings), but also hydrophilic heads (SO₃⁻), that is, they possess properties of both CDs and micelles.⁷

Although little systematic research had been conducted to date on the toxicity of the calixarenes either at the cellular level or in vivo, the calixarenes showed no activity in the Ames test.⁵ Da Silva et al⁸ reported that *p*-sulphonato-calixarenes are innocuous with regard to hemolytic effects at doses of <50 mmol/L, but the hemolytic effects increase with increased ring size. They also reported no acute toxicity for the *p*-sulphonato-calix[4, 6, and 8]arenes when injected in mice.⁹ These results demonstrate the extremely low levels of toxicity of these compounds at the cellular level, but much of the behavior in animals remains to be determined.

The calixarenes are often compared with CDs, and, indeed, there are a number of structural similarities. Both are macrocyclic molecules with a repeating unit, and both are cage-like molecules with a hydrophobic cavity. However, there is a significant difference between the oligosaccharide units of CD and the phenols of calixarenes. Whereas the CDs are quite rigid molecules, the parent calixarenes are highly flexible molecules, capable of minor flexing and possessing the ability to undergo complete ring inversions.⁶ The α -, β -, and γ -CDs have inner-cavity diameters of 5.7, 7.8, and 9.5 Å, respectively.¹⁰ In contrast, the inner cavity diameters of calix[4]arene, calix[6]arene, and calix[8]arene are 3.0, 7.6, and 11.7 Å, respectively.⁶ Thus, the inner-cavity diameter of calix[6]arene is comparable with that of β -CD. The CDs provide a hydrophobic environment with hydrophilic outer surfaces, but the sulphonato-calix[n]arene cavity does not provide much of a hydrophobic environment, because there are strongly hydrophilic heads (SO₃⁻) occupying the upper rim.⁷ Nevertheless, the sulphonato calixarenes may selectively include various guest molecules according to their size and hydrophobicity in a manner similar to CDs.⁷

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4-sulphonato-calix[n]arenes

Figure 1. Molecular structures of niclosamide and the 4-sulphonato-calix[n]arenes.

Noncovalent inclusion complexes formed between *p*-sulphonato-calix[n]arenes and steroids, furosemide and nifedipine, have also been investigated.¹¹⁻¹⁴ Few studies reporting the comparative effect of CDs and calixarenes on solubilization are available. Liu et al¹⁵ reported the inclusion complexation behavior of some dye guest molecules with native α -, β -, and γ -CDs; chemically modified β -CDs; and water-soluble calix[n]arenesulphonates ($n = 4, 6$, and 8) and their lower-rim alkylated derivatives and found that the fluorescence intensity of the guest molecules was decreased by the calix[n]arene-sulphonates ($n = 4, 6$, and 8), whereas the CDs cause gradual increases in the fluorescence intensity. In a similar study, the complexation properties of two complementary hosts, α -CD and a water-soluble sulphonated calixarene, with dimeric viologen guests was investigated.¹⁶ Both hosts show high affinity for the viologen guests, but whereas the complexation behavior of the CD host was driven by hydrophobic interactions, that of the calixarene was largely electrostatic in nature.

However, the complexation and effect on the aqueous solubility of drug molecules by these calixarenes or combinations of calixarenes and CDs have not been explored. The purpose of this study, therefore, was to study the effect

of the water-soluble 4-sulphonato-calix[n]arenes, CDs, and combinations of these macromolecules on the aqueous solubility of a poorly water-soluble drug, niclosamide.

MATERIALS AND METHODS

Materials

Niclosamide monohydrate ($C_{13}H_8Cl_2N_2O_4$, MW = 327.13; Figure 1) was purchased from the Spectrum Chemical Company (Gardena, CA). 4-Sulphonato-calix[4]arene hydrate ($C_{28}H_{24}O_{16}S_4 \cdot 9 H_2O$, anhydrous MW = 744.75), 4-sulphonato-calix[6]arene hydrate ($C_{42}H_{36}O_{24}S_6 \cdot 13 H_2O$, anhydrous MW = 1117.13), and 4-sulphonato-calix[8]arene hydrate ($C_{56}H_{48}O_{32}S_8 \cdot 21 H_2O$, anhydrous MW = 1489) were purchased from Acros Organics (Geel, Belgium). Hydroxypropyl- β -CD (HP- β -CD), β -CD, and γ -CD were supplied by Roquette Corporation (Gurnee, IL). The methanol used for the chromatographic assay of niclosamide was of high-performance liquid chromatography (HPLC) grade (Spectrum Chemical Company). For both the solubility studies and HPLC analysis, deionized water was used (Nanopure, Barnstead International, Dubuque, IA). All of the other chemicals and solvents were of analytical reagent grade and were used as received.

Preparation of 4-Sulphonato-Calix[n]Arene (Cyclodextrin): Niclosamide Mixtures

Physical mixtures of niclosamide and 4-sulphonato-calix[n]arenes at 1:0.25, 1:0.5, and 1:1 molar ratios were prepared by geometric mixing. Ground mixtures of niclosamide and 4-sulphonato-calix[n]arenes at 1:0.25, 1:0.5, and 1:1 molar ratio were prepared in a mortar by grinding with a pestle for 30 min. Samples from these mixtures were analyzed by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). Ground mixtures of niclosamide and CDs at 1:1 molar ratio were made by grinding in a mortar with a pestle for 30 min. Samples from these mixtures were analyzed by DSC.

Thermal Analysis of Calixarene- and Cyclodextrin-Niclosamide Mixtures

DSC thermograms were recorded with a DSC 2920-modulated DSC (TA Instruments, New Castle, DE). Indium (melting point, 156.6°C) and tin (melting point, 231.9°C) were used to calibrate the DSC. A mass, ≤ 3 mg, was measured into aluminum pans. DSC curves were obtained under a nitrogen purge of 20 to 30 mL per minute at a heating rate of 3 K/min. DSC scans were recorded for niclosamide, the calixarenes, niclosamide:calixarenes mixtures, and niclosamide:CD mixtures. Changes in the melting temperatures, determined as the onset temperatures, were used as an indication of complex formation.

Figure 2. TGA and DTGA of niclosamide monohydrate. The release of water is seen between 50 and 100°C.

The amount of niclosamide in each of the samples analyzed by DSC was approximately the same. TGA traces were recorded with a Hi-Res Modulated TGA 2950 (TA Instruments). Samples weighing approximately 5 mg were heated at 20 K/min under nitrogen gas flow of 35 mL/min.

Solubility Studies

The aqueous solubility of niclosamide in pH 7.0 McIlvaine buffers at 0.5 mol/L ionic strength was determined at increasing concentrations of the 4-sulphonato-calix[n]arenes and CDs (at equal molar concentrations). The solubility method of Higuchi and Connors¹⁷ was used. An excess amount of niclosamide (10 mg) was added into the screw capped amber vials (three replicates) containing 3 mL of buffer solution and the complexing agents at increasing concentrations (1.0×10^{-3} – 8.0×10^{-3} M). The vials were rotated at 60 rpm while being kept at 30°C. After equilibrium was reached (48 hours), the solutions were filtered through 0.45- μ m cellulose acetate filters (Osmonics Inc, Minnetonka, MN) and analyzed for niclosamide content by HPLC. Phase solubility diagrams were constructed by plotting the molar concentration of niclosamide dissolved (solubility) versus the molar concentration of complexing agents. From these diagrams, the stability constants for the complexation of niclosamide with calix[n]arenes were calculated.¹⁷

HPLC Analysis of Niclosamide

The HPLC method used in this study complied with specifications for precision, accuracy, selectivity, linearity, and ruggedness as required by ICH Q2A.¹⁸ Analysis was conducted with an automated HPLC (Spectrum System, AS1000 autosampler and P2000 pump, Thermo Separation Products, Waltham, MA) with a UV detector (UV3000 detector) set at 254 nm. Niclosamide eluted on a Supelco Discovery RP Amide C₁₆ column (25 cm $\times$ 4.6 mm, 5 μ m, Bellefonte, PA) after 14 min, whereas 4-sulphonato-calix[n]arenes or CDs eluted at approximately 2.5 or 10 min using a mobile phase of 75:25 (methanol:0.05 mol/L NH₄H₂PO₄ v/v %), flow rate of 1 mL/min, and injection volume of 20 μ L. Each determination was conducted in triplicate.

Statistical Analysis

The enhancement in aqueous solubility niclosamide (3 replicates) in the presence of the different complexing agents were evaluated using a one-way ANOVA (SAS Institute Inc, Cary, NC). In both cases, posthoc comparisons of the means of individual groups were performed using the Duncan's test. A *P* value of <.05 denoted significance in all of the cases.

RESULTS AND DISCUSSION

Thermal Analysis of the Niclosamide:

Calix[n]Arene Mixtures

Niclosamide is a monohydrate (Figure 2) that dehydrated around 50 to 100°C and melted at $232.2 \pm .2^\circ\text{C}$ with a heat of fusion of $40.7 \pm .5$ kJ/mol, whereas the 4-sulphonato-calix[n]arenes melted above 260°C (Table 1). TGA results showed that 4-sulphonato-calix[n]arenes started to dehydrate at temperatures around 50°C, and water was released in multiple steps up to 200°C. The calixarenes melted with decomposition that started shortly after the melting was completed.

Evaluation of the possible complexation between niclosamide and the 4-sulphonato-calix[n]arenes was done by studying drug-calix[n]arene mixtures by DSC and TGA. The results of this experiment clearly showed changes in

Table 1. Melting Parameters of Niclosamide and the 4-Sulphonato-Calix[n]Arenes (n = 3)

Compounds	Melting Point (T_m °C)	Heat of Melting (ΔH_f kJ/mol)	TGA Start of Decomposition (T_D °C)
Niclosamide	232 ± 0.2	41 ± 0.5	245
Calix[4]arene	277 ± 0.2	192 ± 4.2	272
Calix[6]arene	262 ± 0.2	242 ± 5.8	264
Calix[8]arene	270 ± 0.7	351 ± 3.9	272

Figure 3. Top: DSC thermograms of the physical mixtures of 4-sulphonato-calix[4]arene and niclosamide (molar ratios: S1P: niclosamide:calixarene = 1:1; S2P: niclosamide:calixarene = 1:0.5; S3P: niclosamide:calixarene = 1:0.25). Bottom: TGA and DTGA curves of a physical mixture of 4-sulphonato-calix[4]arene and niclosamide.

the DSC and TGA thermograms of niclosamide in the presence of the calix[n]arenes (Figure 3). Both the DSC thermograms of physical and ground mixtures showed that the melting of niclosamide shifted to a lower temperature in the presence of the calix[n]arenes. The effect of the calix[n]arenes on the melting temperature of niclosamide was stronger as the molar ratio of calix[n]arenes in the mixtures increased.

The data listed in Table 2 show that the peak representing the endothermic melting of niclosamide at around 232°C was reduced in the physical mixtures by as much as 9 to 18°C. In addition, the heat of fusion was reduced from 41 to 35 kJ/mol, to 25 kJ/mol, and to 27 kJ/mol, respectively, in the case of the calix[4]arene, calix[6]arene, and calix[8]arene at a 1:1 molar ratio. TGA results of these

mixtures showed that the weight loss peak temperature of 245°C for niclosamide shifted to 225°C in all of the physical mixtures. These results indicated a strong interaction between calixarenes and niclosamide, resulting in lower melting and decomposition temperatures.

Similarly, the peak representing the endothermic melting of niclosamide at around 232°C was lowered even more in the ground mixtures (decrease between 9 and 19°C). In these mixtures, the heat of fusion was reduced from 41 to 27 kJ/mol, to 27 kJ/mol, and 26 kJ/mol, respectively, with calix[4]arene, calix[6]arene, and calix[8]arene, at a 1:1 molar ratio. TGA results showed that the weight loss peak at 245°C shifted to 220°C in all of the ground niclosamide mixtures. The larger decrease in melting and decomposition temperatures indicated stronger interactions after grinding because of more uniform molecular contact among the calixarene and niclosamide molecules.¹⁹

Thermal Analysis of the Niclosamide: Cyclodextrin Mixtures

Preliminary evaluation of the possible complexation between niclosamide and the CDs was done by studying niclosamide-CD mixtures at a 1:1 molar ratio with DSC. The results of this experiment (Figure 4) clearly showed changes in the DSC thermograms of niclosamide in the presence of the CDs. Niclosamide melted around 232°C (ΔH_f (heat of fusion) = 41 kJ/mol). The melting of niclosamide in the mixtures was lowered from 232 to 230°C, and the heat of fusion was reduced from 41 to 8 kJ/mol in the case of HP- β -CD at 1:1 molar ratio. However, the melting peak of niclosamide in the presence of β -CD and γ -CD did not change much. The heat of fusion was reduced by 2 kJ/mol in the presence of β -CD and reduced by 1 kJ/mol in the presence of γ -CD. These results indicated that HP- β -CD strongly interacted with niclosamide on heating, but β -CD and γ -CD did not.

Solubility of Niclosamide in the Presence of 4-Sulphonato-Calix[n]Arenes

Figure 5 shows the phase-solubility diagrams of niclosamide with 4-sulphonato-calix[n]arenes in an aqueous solution at pH 7.0 and 30°C. This phase-solubility diagram can be classified as type B. The water solubility of niclosamide increased with an increase in the concentration of 4-sulphonato-calix[n]arenes (<.005 mol/L for n = 4 and 6 and 0.004 mol/L for n = 8), but as the concentrations increased additionally, the solubility dropped to a lower level, indicating that insoluble complexes were formed at higher concentrations.

The increased solubility of niclosamide in the presence of 4-sulphonato-calix[n]arenes indicated that calixarenes formed complexes with niclosamide. According to Higuchi

Table 2. Heat of Fusion, ΔH_f (kJ/mol), and Melting Point, T_m (°C), of Niclosamide in the Niclosamide:4-Sulphonato-Calix[n]Arene Mixtures With Increased Drug/Calixarene Molar Ratios*

Calix[n]arene		Physical Mixture			Ground Mixture		
		1:1	1:0.5	1:0.25	1:1	1:0.5	1:0.25
4	ΔH_f	35.3 $\pm$ 5.1	39.2 $\pm$ 2.2	41.4 $\pm$ 1.9	26.5 $\pm$ 3.7	33.0 $\pm$ 0.6	37.5 $\pm$ 0.8
	T_m	218.6 $\pm$ 0.9	221.1 $\pm$ 1.6	222.4 $\pm$ 0.2	215.1 $\pm$ 1.0	218.3 $\pm$ 0.5	219.9 $\pm$ 0.3
6	ΔH_f	25.3 $\pm$ 2.0	33.0 $\pm$ 0.9	36.1 $\pm$ 3.0	27.0 $\pm$ 1.5	27.9 $\pm$ 3.8	29.0 $\pm$ 0.3
	T_m	220.5 $\pm$ 0.9	222.8 $\pm$ 0.3	223.1 $\pm$ 0.5	220.8 $\pm$ 0.5	220.5 $\pm$ 2.3	222.8 $\pm$ 0.3
8	ΔH_f	26.9 $\pm$ 5.1	36.8 $\pm$ 3.2	39.5 $\pm$ 6.3	26.2 $\pm$ 3.5	27.5 $\pm$ 2.7	32.1 $\pm$ 1.9
	T_m	214.4 $\pm$ 5.7	216.3 $\pm$ 1.0	220.7 $\pm$ 0.3	213.1 $\pm$ 0.2	214.1 $\pm$ 0.4	217.4 $\pm$ 0.4

*Values are given as mean $\pm$ SD.

and Connors,¹⁷ the linear increases in solubility seen in these phase solubility profiles could be attributable to one or more molecular interactions between the niclosamide and the calixarenes to form distinct chemical species, which may be called soluble niclosamide:4-sulphonato-calix[n]arene complexes. However, as the concentration of 4-sulphonato-calix[n]arenes increased, the solubility of the complexed drug is exceeded, causing precipitation of the complex and a reduction in the solubility.

To compare the complexing tendencies between different complexing agents, linear regression analysis was performed on the linear part of the solubility profiles shown in Figure 5. The results are listed in Table 3. All of the slopes were less than unity, thus, the stoichiometry of the complex is, at present, unknown. Stability constants (Table 3), assuming 1:1 complex formation, were calculated according to the following equation: $K_{st} = \text{slope}/S_0 (1 - \text{slope})$, where S_0 is the solubility of niclosamide in the absence of

calix[n]arenes. Based on the calculated stability constants, 4-sulphonato-calix[n]arenes formed stable complexes with niclosamide at pH 7.0.

Solubility of Niclosamide in the Presence of Cyclodextrins

Figure 6 shows the phase-solubility diagrams of niclosamide in aqueous medium containing increasing concentrations of CDs at pH 7.0 and 30°C. The solubility of niclosamide increased with an increase in the HP- β -CD concentrations with a positive deviation from linearity (type $\text{NH}_4\text{H}_2\text{PO}_4$ diagram).¹⁷ The solubility of niclosamide was increased slightly with an increase in the concentration of β -CD with a type $\text{NH}_4\text{H}_2\text{PO}_4$ phase solubility diagram. However, γ -CD did not improve the solubility of niclosamide significantly.

Type $\text{NH}_4\text{H}_2\text{PO}_4$ diagram indicated 1:2 complexes produced in the presence of β -CD and HP- β -CD. A more

Figure 4. DSC thermograms of mixtures of CDs and niclosamide (1:1 molar ratio) prepared by grinding in a mortar with a pestle. **Figure 5.** Phase-solubility profiles for niclosamide in aqueous medium at pH 7.0 at 30°C, in the presence of increasing concentrations of 4-sulphonato-calix[n]arenes.

Table 3. Apparent Stability Constants for Complexes of Niclosamide and 4-Sulphonato-Calix[n]Arenes and/or CDs, Calculated From Phase-Solubility Profiles

Calix[n]arene	CD	Type Solubility Profile	Stability Constant ($\times 10^3 \text{ M}^{-1}$)	R^2
4	—	B	1.38	0.990
6	—	B	3.92	0.997
8	—	B	1.46	0.993
—	HP- β -CD	A _p	2.17	0.999
—	β -CD	A _p	0.15	0.998
6	HP- β -CD	B	9.65	0.951
6	β -CD	B	6.95	0.887
6	γ -CD	B	5.47	0.982

quantitative description of the type A_p diagram may be achieved by assuming that it results from the formation of two complexes, SL and SL₂. The stability constants, K_{st}, can be calculated by the following equation: $(S_t - S_0)/L = K_{st1}S_0 + K_{st1}K_{st2}S_0L$, where S_t is the total solubility in the presence of complexing agents, S₀ is the solubility in the absence of complexing agents, and L is the concentration of the complexing agents.¹⁷ If $(S_t - S_0)/L$ is plotted versus L, K_{st1} and K_{st2} can be calculated from the slope and intercept. Stability constants for SL complexes are listed in Table 3. HP- β -CD showed stronger complexation than β -CD.

Solubility of Niclosamide in the Presence of 4-Sulphonato-Calix[6]Arene and Cyclodextrins

Because 4-sulphonato-calix[6]arene improved the aqueous solubility of niclosamide the most, this water-soluble calixarene was combined with each CD to see the combined solubilizing effect. Figure 7 shows the phase-solubility diagrams of niclosamide combined with increasing concen-

trations of equal molar concentrations of 4-sulphonato-calix[6]arenes and the CDs in aqueous buffer pH 7.0 and 30°C. The phase-solubility diagrams followed type B diagrams. The solubility of niclosamide first increased with an increase in the concentration of 4-sulphonato-calix[6]arene and CDs, then the solubility decreased, indicating that insoluble complexes formed at higher concentrations or that the drug was removed from the complexes.

The increased water solubility of niclosamide in the presence of combinations of 4-sulphonato-calix[6]arenes and CDs indicated that these complexing agents may form bimacromolecular complexes with niclosamide. According to Higuchi and Connors,¹⁷ the linear increases in solubility seen in these phase-solubility profiles could be attributable to one or more molecular interactions among the niclosamide and calix[6]arene and CDs to form distinct chemical species, which may be called soluble niclosamide: 4-sulphonato-calix[6]arene:CD complexes. But as the concentration of 4-sulphonato-calix[6]arene and CDs increased more, insoluble complexes formed with decreased aqueous

Figure 6. Phase-solubility profiles for niclosamide in aqueous medium at pH 7.0 at 30°C, in the presence of increasing concentrations of CDs. **Figure 7.** Phase-solubility profiles of niclosamide in the presence of equimolar concentrations of 4-sulphonato-calix[6]arene and CDs at 30°C.

Figure 8. Solubility enhancement of niclosamide by different complexing agents. (A) calix[4]arene, (B) calix[6]arene, (C) calix[8]arene, (D) HP-β-CD, (E) β-CD, (F) γ-CD, (G) calix[6]arene + HP-β-CD, (H) calix[6]arene + β-CD, (I) calix[6]arene + γ-CD.

solubility. To compare the complexing tendencies between different combinations of the complexing agents, linear regression analysis was performed on the linear part of the solubility profiles. Results are listed in Table 3. All of the slopes were less than unity, thus, it was assumed that 1:1 complexes were formed in the presence of calix[n]arene/CD mixtures at lower concentrations.

The stability constants of the complexes between niclosamide and calix[6]arene/CDs was in the following order: calix[6]arene + HP-β-CD > calix[6]arene + β-CD > calix[6]arene + γ-CD, as shown in Table 3. These stability constants were much larger than those obtained using either calix[6]arene or the CD alone and showed that the effects of the two polymers on solubility enhancement is additive.

Comparison of the Enhancement in Solubility

The solubility enhancement (percentage per millimole concentration of complexing agent) of niclosamide at the maximum solubilizing concentration of each complexing agent was calculated by the following equation: Solubility enhancement factor = $[(S - S_0)/S_0]/\text{mM}$, where S and S_0 are the solubility in the presence and absence of complexing agents, and mM is the molar concentration of the complexing agent(s). As shown in Figure 8, the increase in solubility ranked as follows: calix[6]arene + HP-β-CD > calix[6]arene + β-CD > calix[6]arene + γ-CD = HP-β-CD > calix[6]arene > calix[8]arene = calix[4]arene > β-CD. The γ-CD did not increase the solubility. The calix[6]arene had the greatest solubilizing effect among the

three calix[n]arenes tested, whereas HP-β-CD showed the greatest increase in solubility among the CDs. The combination of calix[6]arene and CDs produced an additive increase in solubility, because the increase was more than the solubility increase obtained with the 2 individual excipients.

Possible Mechanisms Involved in the Complexation Between Niclosamide and the Macromolecules

Both thermal analysis of the mixtures and solubility experiments showed strong interaction between niclosamide and calixarenes, and the complexation could be explained by several interactions. Hydrogen bonding is one of the most important forces responsible for the formation of host-guest complexed systems. 4-Sulphonato-calix[n]arenes contains free intraannular OH groups, suggesting that the complexation mechanism could involve hydrogen bonding between the phenolic hydrogen atoms of the calix[n]arenes and the oxygen atom, nitrogen atom, or chloride atom on the substituted benzene ring of niclosamide similar to the interactions between nifedipine and substituted phenolic ligands.²⁰

Hydrophobic bonding could also contribute to the increased solubility. This term describes the attractive forces between nonpolar (usually hydrocarbon) molecules or portions of molecules. These forces are small, but large solvent-solvent intermolecular-attractive forces enhance them when the molecules are in a polar medium, such as water.¹⁷ Niclosamide, a highly hydrophobic molecule, is in a sense “squeezed out” of the polar phase by its high-internal pressure, leading to an appreciable degree of interaction between niclosamide and the nonpolar hydrophobic cavity of calixarenes. It is expected that the larger cavities would geometrically be more suited for a closer and stronger interaction with niclosamide than the smaller calixarene cavity. This was confirmed by the higher solubility shown in Figure 5 afforded by 4-sulphonato-calix[6]arene compared with 4-sulphonato-calix[4]arene. However, calix[8]arene has less effect than calix[6]arene, and this might be attributable to the higher reactivity of calix[6]arene, or the dimension of calix[6]arene is most optimal for the niclosamide molecule, because “host-size selectivity” does exist in host-guest-type complexation with calixarenes.^{6,21,22} The solubility differences as a function of cavity size also suggest that, based on the molecular structure of niclosamide, the complexes between the sulphonated calixarenes and this molecule should be classified as intermolecular complexes.²³

In the case of CDs, HP-β-CD has stronger solubilizing effect than β-CD and γ-CD. HP-β-CD is a partially substituted poly(hydroxypropyl) ether of β-CD. In this macromolecule, the glycosidic oxygen forming the bond between

the adjacent glucose monomers and the hydrogen atoms lining the cavity of the CD impart an electron density and hydrophobic character to the cavity. The hydroxyl groups and the hydroxypropyl groups on the exterior of the molecule can interact with water to provide the increased aqueous solubility of HP- β -CD and complexes made with it. These groups may also account for the stronger hydrogen bonding between niclosamide and HP- β -CD than the other two CDs. On the other hand, hydrophobic bonding may be another important contribution to the interactions between niclosamide and HP- β -CD.

In the binary systems, containing equal amounts of calix[6]arene and CD, the solubility enhancement was additive. The increase in the solubility could be explained by the same interactions as discussed earlier for the calix[n]arenes or CDs alone. These interactions include hydrogen bonding, hydrophobic bonding, and electron donor-acceptor interaction.^{15,16} Because the calix[6]arenes and CDs contain free hydroxyl groups, they will also interact with each other by hydrogen bonding in the solution. This interaction depends on concentration, and at higher concentrations it might be so dominant that it will restrict the complexation of niclosamide with these molecules. That could explain the decrease in solubility of niclosamide at higher concentrations of calix[6]arenes and CDs.

CONCLUSION

Aqueous solubility studies showed that the molecular size of the 4-sulphonato-calix[n]arenes or CDs and the concentration of the calix[n]arenes or CDs significantly influenced the increase in the solubility of niclosamide achieved with niclosamide:4-sulphonato-calix[n]arene or CD complexes in aqueous solutions. Overall, HP- β -CD outperformed the calixarenes as a potential solubilization agent for niclosamide, suggesting that the drug-HP- β -CD complexes are more soluble compared with drug-calixarene complexes. However, the greatest increase in the aqueous solubility of niclosamide was observed when 4-sulphonato-calix[6]arene and HP- β -CD were combined, because the increase in solubility was additive.

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