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Comparison of the aqueous solubilization of practically insoluble niclosamide by polyamidoamine (PAMAM) dendrimers and cyclodextrins

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Abstract

This study is the first report of the solubilization of niclosamide by cyclodextrin complexation or the interaction between the drug and polyamidoamine (PAMAM) dendrimers. Half generation dendrimers with more polar carboxylate surface functional groups did not increase the solubility of niclosamide. From the phase solubility studies, when the fold enhancement in solubility of niclosamide combined with full generation amine terminated PAMAM dendrimers was compared with that obtained when the drug was combined with β- or hydroxypropyl-β-cyclodextrin, the results showed that, except for G-0 dendrimer at pH 7, the solubility of niclosamide was significantly higher in the presence of the dendrimers. In addition, higher equilibrium stability constants and complexation efficiency showed that the dendrimers formed stronger more stable complexes than the CDs. However, the strong interaction between the amine surface functional groups and the niclosamide molecule complexes caused a decrease in dissolution rate compared to the CDs because the interaction retarded the release of the drug from the dendrimers. In addition to increasing the solubility, PAMAM dendrimers therefore also offer the possible for the controlled release of the drug from solid dosage forms.

Keywords: Niclosamide; Dendrimers; Cyclodextrins; Solubilization; Dissolution

1. Introduction

Niclosamide ((5-chloro-*N*-2-chloro-4-nitrophenyl)-2-hydroxybenzamide), Fig. 1, is an anthelmintic drug that is active against most tapeworms, including the beef tapeworm, the dwarf tapeworm and the dog tape-

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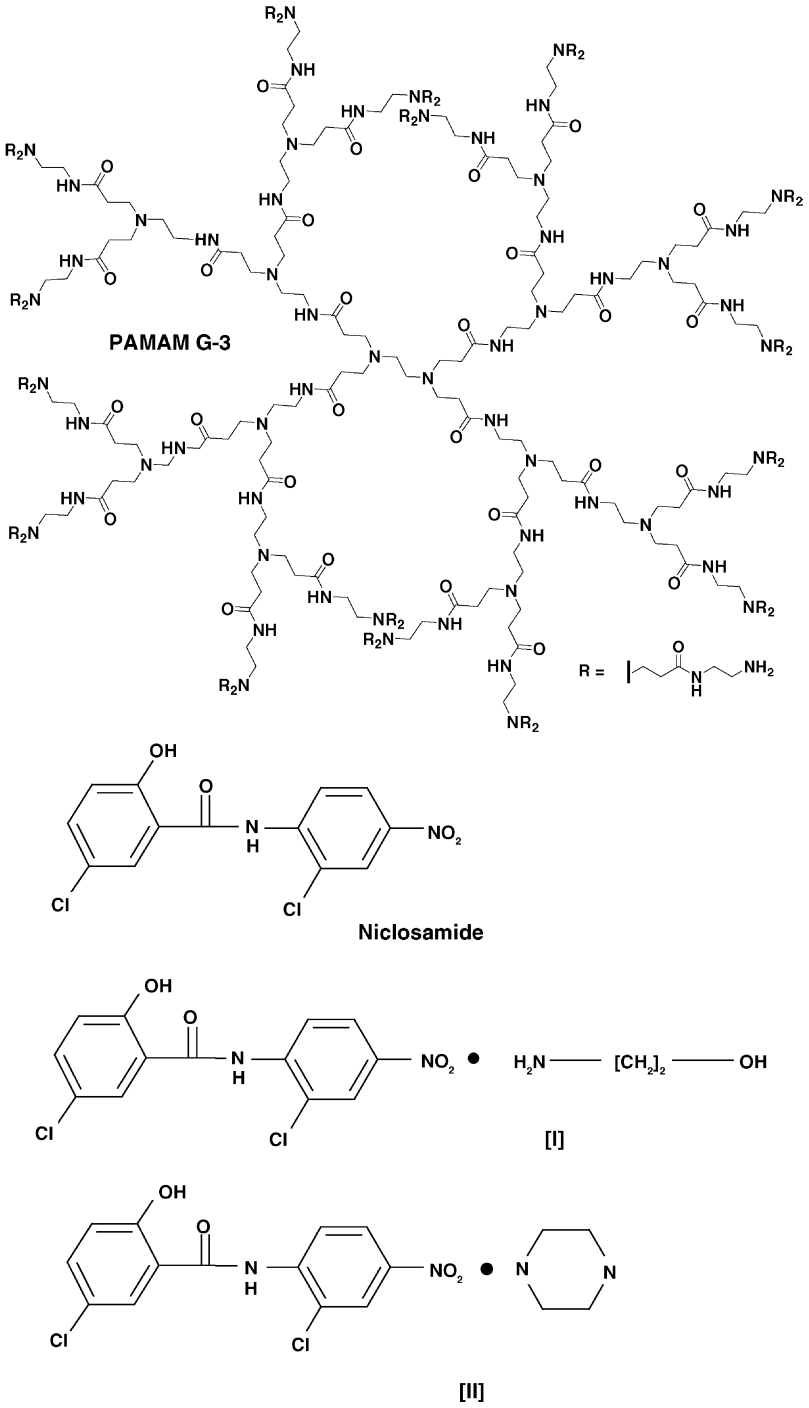

Fig. 1. Chemical structures of PAMAM G-3 dendrimer, niclosamide ($C_{13}H_8Cl_2N_2O_4$, MW = 327.1), (I) 1:1, 5-choloro-salicyl-(2-chloro-4-nitro)anilide-2-aminoethanol salt ($C_{15}H_{15}Cl_2N_3O_5$, MW = 388.2) and (II) 2:1, 5-choloro-salicyl-(2-chloro-4-nitro)anilide-piperazine salt ($C_{30}H_{26}Cl_4N_6O_8$, MW = 740.4).

worm (Reynolds, 1989). Niclosamide is also used as a molluscicide for the treatment of water in schistosomiasis control programs (Goldsmith, 1984; Reynolds, 1989). Its activity against these worms appears to be due to inhibition of mitochondrial oxidative phosphorylation and anaerobic ATP production.

However, many problems are associated with the preparation of successful niclosamide formulations, the most severe being that this drug is practically insoluble in water. The solubility does increase and the drug is sparingly soluble over the pH range of 8–10 (Merck Index, 2001). Other factors that causes problems during formulation is that the drug exist in various polymorphic, hydrated, solvated, salt and isomeric forms (Kosheleva and Bekhli, 1974; Caira et al., 1998; Van Tonder et al., 2004). Currently, niclosamide is formulated as a suspension of the active ingredient, which may be niclosamide, its ethanolamine salt, piperazine salt or niclosamide monohydrate (WHO/VBC/DS/88.63, 1988) (Fig. 1). Several other techniques, such as the preparation of solid dispersions with PEG-6000, prodrug formation and pH-control methods have been investigated for improving the solubility and in vitro dissolution properties of niclosamide (Mally et al., 1983; Laila, 1993; Klkihel et al., 1994).

Another, commonly used technique to increase the solubility of poorly water soluble drugs is by supramolecular complexation. Among the macromolecules used to solubilize drugs the cyclodextrins are the most widely used (Szejtli, 1982). Natural cyclodextrins (CDs) are cyclic oligosaccharides made up of six (α-CD), seven (β-CD) and eight (γ-CD) D-glucopyranose units linked by α-1,4-glycosidic bonds. The molecular structure of these glucose derivatives generates a hydrophilic exterior surface and a cylinder-shaped electron-rich internal hydrophobic cavity. The lipophilic cavity enables CDs to form non-covalent inclusion complexes with a wide variety of appropriately sized poorly water-soluble compounds in aqueous solutions. These complexes have been extensively studied for the solubilization of poorly soluble drugs because they offer a variety of physicochemical advantages, including the possibility for increased water solubility, chemical stability, bioavailability and clinical activity (Loftsson et al., 1991; Linares et al., 1997; Choi et al., 2003). However, due to various reasons such as their high molecular weight, relatively low water solubility and possible parenteral toxicity, cost and dosage, the amount of CD that can be used in most pharmaceutical formulations is limited (Loftsson and Brewster, 1996).

Recently dendrimers, due to their well defined structure, compact globular shape, size, monodispersity and controllable surface functional groups, have gained much attention as carriers for drug delivery. The interactions between drugs and dendrimers can also increase the solubility of poorly soluble drugs (Malik et al., 1999; Twyman et al., 1999; Milhem et al., 2000; El-Sayed et al., 2001, 2002; Beezer et al., 2003; Chauhan et al., 2003; Purohit et al., 2003; D'Emanuel et al., 2004; Devarakonda et al., 2004). The interactions that lead to an increase in solubility include physical entrapment of the drug molecules inside the dendrimer structure or the drug being attached onto the dendrimer surface to prepare dendrimer-drug conjugates (Liu and Frechet, 1999; D'Emanuel et al., 2004).

To date, the ability of CDs and dendrimers to solubilize the same drug has not been reported. In this study, we studied niclosamide as a model drug because it has not been combined with either CDs or dendrimers. Niclosamide therefore offers an opportunity to compare solubilization by cyclodextrins and polyamidoamine (PAMAM) dendrimers. The PAMAM dendrimers have primary amines on their surface with internal tertiary amines and amide linkages (Tomalia et al., 1985). Therefore, these dendrimers could interact with niclosamide by a mechanism similar to the interaction between the drug and ethanolamine or piperazine (Fig. 1). Solubility studies of niclosamide were carried out in the presence of amine terminated full generation dendrimers (G-0 to G-3) and ester terminated half generation dendrimers (G-0.5 to G-2.5) in water, at pH 4, 7, 11 and in aqueous solutions containing β- and hydroxypropyl-β-cyclodextrin.

2. Materials and methods

2.1. Materials

Niclosamide monohydrate was purchased from Sigma Chemical Company (St. Louis, MO, USA). It is a yellowish grey, odorless, crystalline solid which melts between 224 and 229 °C. Niclosamide is practi-

cally insoluble in water, 5–8 mg/L at 20 °C, sparingly soluble in ether, ethanol and chloroform, but soluble in acetone. β-CD and HP-β-CD were purchased from Sigma Chemical Company. Ethylenediamine, methylacrylate, methanol (HPLC grade), sodium dihydrogen phosphate, citric acid, potassium chloride and standard pH buffers were obtained from Spectrum Chemical Company (Gardena, CA, USA). Triethanolamine (TEA) was from J.T. Baker Chemical Co. (Phillisburg, NJ). The dendrimers were synthesized as described by Devarakonda et al. (2004) or purchased from Sigma–Aldrich.

2.2. HPLC analysis of niclosamide

Niclosamide was analyzed by high performance liquid chromatography (Spectra System, AS 1000 autosampler and P2000 pump, Thermo Separation Products, Waltham, MA) equipped with a multiple wavelength UV detector (UV 3000 detector) set at a wavelength of λ_{max} 254 nm. Chromatographic separation was performed using a C_{18} column (Econosil, 5 μm particles, 250 mm × 4.6 mm, Alltech, Deerfield II). The mobile phase was methanol: ammonium phosphate 0.05 M (9:1, v/v) adjusted to pH 3.6 using phosphoric acid; flow rate 1.0 mL/min; injection volume 50 μL; retention time 8 min and limit of detection 1.0 ng/mL. Results were the mean of three analyses. The method complied with the specifications as required by ICH Q2A (1995).

To prepare calibration curves, 10 mg of niclosamide was accurately weighed into a 50 mL volumetric flask and filled to volume with methanol. From this stock solution, graded dilutions were made with the methanol solution to obtain standard solutions of niclosamide in the concentration range of 0.5–50 μg/mL. The peak area of the solutions was measured using the HPLC method and standard curves were constructed by plotting the peak area versus concentration. These calibration curves were used to calculate the amount of niclosamide in the solutions ($y = 182432.6x + 65400$, $R^2 = 0.9996$).

2.3. Phase solubility studies

The aqueous solubility of niclosamide in water (pH 6–6.5), McIlvaine buffers at pH 4 and 7 (composition: different ratios of 0.1 M citric acid and 0.2 M sodium dihydrogen phosphate adjusted to 0.5 M ionic strength using potassium chloride), and at pH 11 in phosphate buffer (0.05 M sodium dihydrogen phosphate and 0.1 M sodium hydroxide) was determined in the presence of increasing concentrations of dendrimers (0–5%, w/v: full generation dendrimers; G-0, G-1, G-2 and G-3 and half generation dendrimers; G-0.5, G-1.5 and G-2.5). The solubility of niclosamide was also measured in aqueous solutions containing increasing concentrations of β-and HP-β-CD (0–5%, w/v).

An excess amount of niclosamide monohydrate was added to 3 mL buffer solutions in 5 mL screw-capped amber colored vials containing dendrimers or CDs with or without TEA. The vials were rotated at 60 rpm while being kept at 30 ± 1.0 °C. Preliminary experiments indicated that 24 h provided sufficient time to reach equilibrium. After 24 h, samples were filtered through a 0.45 μm cellulose acetate filters (Osmonics Inc., Minnetonka, MN), diluted appropriately with the mobile phase and analyzed by HPLC. Measurement of pH at the end of the solubility studies showed no significant change in the pH of the mediums at pH 4 and 7, but for the medium at pH 11 it decreased slightly to pH 10.5. The final pH of the aqueous solutions at the end of the study was in the range of 8–9 depending upon the concentration of dendrimer were observed.

From the results of the phase solubility studies, phase solubility diagrams were constructed by plotting the molar concentrations of niclosamide (solubility) versus molar concentration of dendrimers or CDs. From these plots, stability constants for the complexation of niclosamide with dendrimers or CDs were calculated according to Eq. (1) (Higuchi and Conners, 1965; Yalkowsky, 1999).

$$S_t = \frac{K_{1:1} S_0}{1 + K_{1:1} S_0} L_t + S_0 \quad (1)$$

where S_t is the observed molar solubility of niclosamide, $K_{1:1}$ the equilibrium stability constant for 1:1 complex, S_0 the intrinsic molar solubility of niclosamide and L_t is the total molar dendrimer concentration.

When the total solubility versus ligand concentration curve was parabolic, it was assumed that higher-order complexes were formed. The data were analyzed via non-linear regression analysis using Eq. (2), assum-

Table 1
Preparation of niclosamide–dendrimer mixtures in *N*:1 molar ratio of drug to dendrimer (*N* = 1, number of primary amines and total number of amines in each dendrimer)

	Dendrimer	Drug molar ratio = *N*		
		1	Primary amines	Total amines
Dendrimer molar ratio = 1	G-0	1	4	6
	G-1	1	8	14
	G-2	1	16	30
	G-3	1	32	62

ing the formation of niclosamide to dendrimer or CD complexes in molar ratios of 1:1 and 2:1.

$$S_t = S_0 + S_0 K_{1:1} L_t + S_0 K_{2:1} L_t^2 \quad (2)$$

where $K_{2:1}$ is the equilibrium stability constant for a 2:1 complex.

2.4. Preparation of niclosamide–dendrimer mixtures

The solid mixtures of niclosamide with the dendrimers were prepared by a co-precipitation method. Niclosamide was dissolved in acetone and the dendrimers in water. The two solutions were mixed thoroughly at 50 °C for 2 h, and the solvent was allowed to evaporate at room temperature. Since dendrimer molecules have many binding sites (primary and tertiary amines), it was assumed that one molecule of dendrimer could interact with many molecules of drug. In other words, depending on the generation size the number of drug molecules interacting with each dendrimer varies. Therefore, niclosamide–dendrimer mixtures were prepared in the drug:dendrimer molar ratio where the dendrimer is always 1 and the drug varies from *N* being equal to: *N* = 1; *N* = the number primary amines in each dendrimer and *N* = total number of amines in each dendrimer as shown in Table 1.

2.5. Preparation of niclosamide–cyclodextrin mixtures

The solid mixtures of niclosamide with β-CD, γ-CD and HP-β-CD were prepared by using the following five methods. *Kneading*: aqueous methanol (50%, v/v) was added to mixtures of niclosamide–CDs until creamy homogenous products were obtained. These mixtures were transferred to a mortar, kneaded for 15 min, and then dried in a vacuum oven at 50 °C until constant weight was obtained. *Freeze-drying*: a minimum volume of distilled water was added to physical mixtures of niclosamide and CDs and the suspensions were sonicated in an ultrasonic bath for 1 h, shaken in a constant temperature water bath at 25.0 ± 0.1 °C for 48 h and finally, filtered through a 0.45 μm membrane (Millipore, USA). Filtrates were frozen at −40 °C before freeze-drying (Freeze Dye 4.5 Labconco Corp., Kansas City, MI). *Co-precipitation*: mixtures of niclosamide–CDs and niclosamide–CD–TEA were dissolved in 50% acetone; the solvent was allowed to evaporate. The resulting precipitates were dried in a vacuum oven at 50 °C for 24 h. *Grinding*: mixtures of niclosamide–CDs were ground together in a mortar with a pestle for 15 min until a uniform mixture was obtained. *Physical mixing*: physical mixtures were prepared by simple spatulation in a mortar for 10 min.

2.6. Dissolution studies

The dissolution of pure niclosamide was determined using Apparatus II of the United States Pharmacopeia (USP XXIV, 2000) with a paddle speed of 75 rpm (Vanderkamp 600, Van-Kel Instruments Inc., USA). Niclosamide, 10 mg, were mixed with 25 mg glass beads (0.1–0.11 mm in diameter) and vortexed for 1 min in 2 mL dissolution medium before being introduced into the dissolution flask. The dissolution medium was 900 mL of a 35% (v/v) isopropanol:water mixture thermostatically controlled at 37 ± 0.1 °C. Since the niclosamide–dendrimer mixtures were sticky gels, samples containing the equivalent of 10 mg of the drug were placed in gelatin capsules (Apothecary Products, Inc., MN, USA). The powder dissolution of the niclosamide–CD mixtures were similarly measured from capsules. The amount of niclosamide dissolved as a function of time was determined spectrophotometrically at λ_{max} 331 nm. Measurements were performed in triplicate and were corrected for dilution. The dissolution profiles were compared to that of pure niclosamide using a similarity factor (Eq. (3)) (Moore and Flanner, 1996). No difference was assumed when the f_2 is greater than 50 (50–100).

$$f_2 = 50 \times \log\left\{\left[1 + \left(\frac{1}{n}\right)\sum_{t}^{n}(R_t - T_t)^2\right]^{-0.5} \times 100\right\} \quad (3)$$

where n is number of time points, R_t the dissolution value of the reference batch at time t and T_t is the dissolution value of the reference batch at time t.

2.7. Differential scanning calorimetry

DSC measurements were conducted using a DSC-2920 system (TA Instruments, DE, USA). Samples containing 0.5 mg of niclosamide, alone or in niclosamide–dendrimer mixtures, were heated at a scanning rate of 10 K/min from 25 to 275 °C in hermitically sealed aluminum pans under nitrogen purge.

2.8. X-ray powder diffraction analysis

X-ray powder diffraction (XRPD) profiles were measured with a Bruker D8 Advance diffractometer (Bruker, Karlsruhe, Germany). The measurement conditions were: target, Cu; voltage, 40 kV; current, 30 mA; divergence slit, 2 mm; anti-scatter slit, 0.6 mm; receiving slit, 0.2 mm; monochromator; detector slit, 0.1 mm; scanning speed, 2° min^{-1} (step size 0.025°, step time, 1.0 s). Approximately 300 mg samples were weighed into aluminum sample holders, taking care not to introduce a preferential orientation of crystals.

2.9. Zeta potential measurements

To measure changes in the charge of the dendrimers upon complexation, the zeta potential of dendrimers solutions were measured. The reported results represent the mean of 10 measurements determined with a Zeta-plus photon correlation spectroscopy and microelectrophoresis instrument (Brookhaven Instruments, Holtsville, NY, USA).

3. Results and discussion

3.1. DSC studies of niclosamide, dendrimer and cyclodextrin mixtures

DSC thermograms of niclosamide and niclosamide–dendrimer mixtures are shown in Fig. 2. The dendrimers displayed no peaks in the temperature range between 25 and 300 °C, while niclosamide exhibited a sharp endothermic melting peak (T_m) at 233.42 °C. Table 2 lists the T_m and heat of fusion (ΔH_f) of niclosamide and the drug in niclosamide–dendrimer mixtures prepared with different molar ratios of the drug. In the presence of all the other dendrimers, the melting temperature of the drug changed significantly with decreased heats of fusion. The niclosamide–dendrimer mixtures prepared with 1:1 molar ratio of drug to dendrimer did not show the endothermic melting peak of the drug. This peak was also not seen in drug–dendrimer mixtures prepared with G-0 and G-1 dendrimers combined with a molar ratio of drug equal to the number of primary amines, and mixtures prepared with G-0 dendrimer combined with a molar ratio of drug equal to the total number of amines in the dendrimer.

Table 3 lists the melting temperature (T_m) and heat of fusion (ΔH_f) of niclosamide alone and in the presence of CDs. DSC thermograms of niclosamide and niclosamide–CD are shown in Fig. 3. The CDs displayed no peaks between 25 and 300 °C, while niclosamide exhibited a sharp melting endotherm at

Table 2
Melting temperature and heat of fusion for niclosamide–dendrimer mixtures prepared with different generation PAMAM dendrimers

Niclosamide:dendrimer molar ratio	Dendrimer generation	T_m (°C)	ΔH_f (J/g)
Niclosamide	–	233.21	122.92
1:1	G-0	–	–
	G-1	–	–
	G-2	–	–
	G-3	–	–
N (primary amines):1	G-0	–	–
	G-1	–	–
	G-2	234.04	5.08
	G-3	229.98	45.32
N (total amines):1	G-0	–	–
	G-1	230.73	19.88
	G-2	244.84	60.90
	G-3	230.70	67.81

(–) No melting peak was observed.

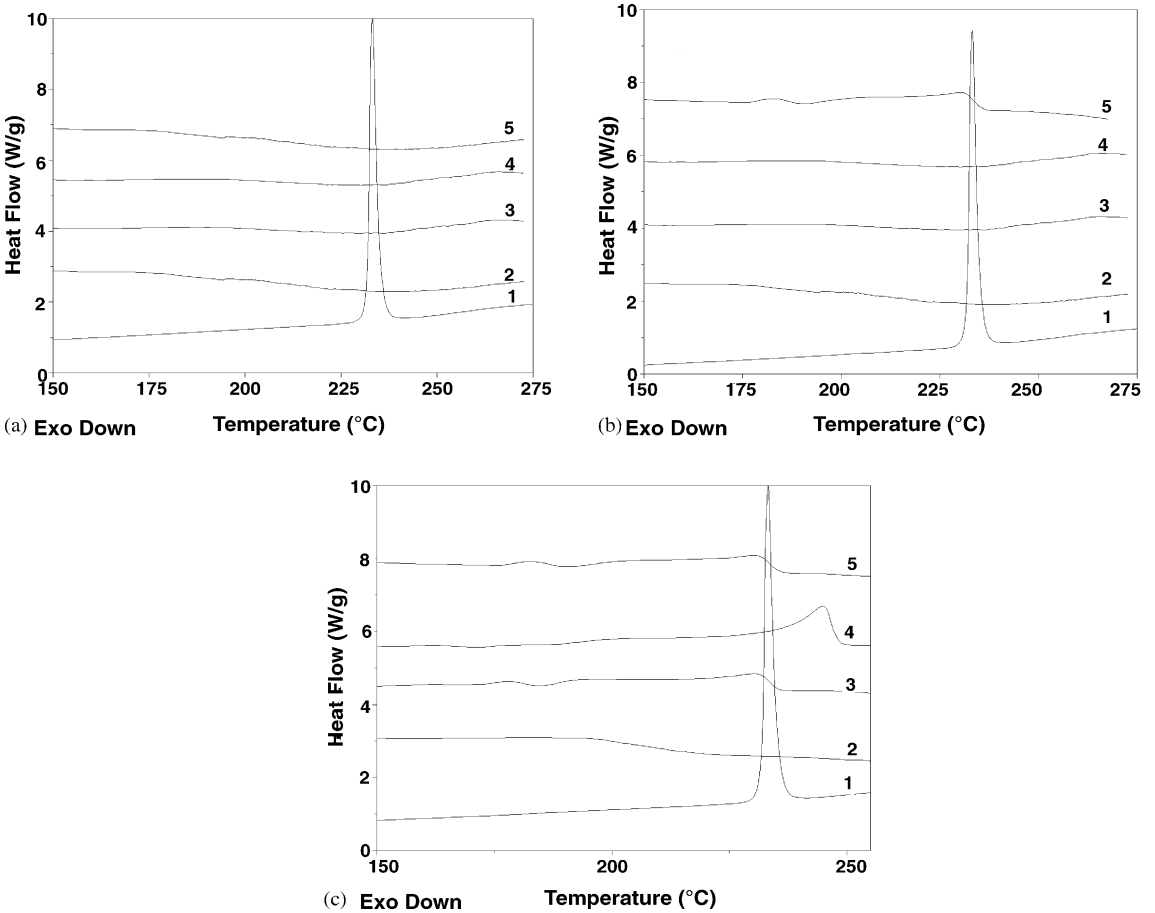

Fig. 2. DSC thermograms of niclosamide–dendrimer mixtures: (1) niclosamide; (2) niclosamide-G-0; (3) niclosamide-G-1; (4) niclosamide-G-2 and (5) niclosamide-G-3 prepared with: (a) 1:1 molar ratio of drug to dendrimer; (b) *N* (number of primary amines in each dendrimer):1 molar ratio of drug to dendrimer and (c) *N* (number of total amines in each dendrimer):1 molar ratio of drug to dendrimer (Table 2).

233.42 °C. For niclosamide–β-CD mixtures prepared by various methods, no changes in the melting point of the drug were observed but the heat of fusion required for melting was much lower than that for the pure drug. For niclosamide–HP-β-CD systems, the melting temperature of the drug was reduced with decreased heats of fusion. The products prepared by the freeze-dried method showed complete disappearance of the endothermic peak with no heats of fusion for niclosamide. The endothermic changes observed for niclosamide in the presence of CDs could be due to reduced crystallinity and/or the formation of inclusion complexes with the CDs.

3.2. X-ray powder diffraction analysis

The dendrimers are gel like in nature and mixtures with the drugs in the proportions listed in Table 1 produced amorphous semi-solids or glasslike solids with no measurable X-ray patterns. The X-ray diffraction patterns of niclosamide, the CDs and niclosamide–CD mixtures are shown in Figs. 4 and 5. Niclosamide and β-CD have well-defined crystalline X-ray patterns, whereas HP-β-CD is amorphous. The patterns of the mixtures prepared by physical mixing, grinding, kneading and co-precipitation appears to represent the superimposition of each component's spec-

Table 3
Melting temperature and heat of fusion for niclosamide in niclosamide–CD mixtures prepared by different methods

CD	Preparation method	T_m (°C)	ΔH_f (J/g)
β-CD	Physical mixture	233.63	25.39
	Ground	233.42	20.31
	Kneaded	233.37	3.66
	Co-precipitated	233.42	66.63
	Freeze-dried	–	–
Niclosamide:HP-β-CD (1:1)	Physical mixture	230.97	9.54
	Ground	229.18	3.98
	Kneaded	217.72	15.43
	Co-precipitated	219.62	9.56
	Freeze-dried	–	–

(–) No melting peak was observed.

trum although peaks attributable to niclosamide are remarkably diminished, indicating lower degree of crystallinity. In contrast, freeze-dried products showed halo-patterns, suggesting amorphous drug:CD complexes with the two CDs.

3.3. Charge changes

The zeta potential (ζ) of suspended niclosamide in water was −24(±2) mV. The charge of this suspension changed with a change in pH. At pH 4 the niclosamide suspension was positively changed +15(±3) mV and negatively charged at pH 7 −8(±3) mV and pH 11 −32(±4) mV. The amine terminated dendrimer solutions were positively charged in the pH range 4–11. However, the positive charge of the dendrimer surface increased with a decrease in pH as a result of an increasing degree of protonation of the dendrimer surface. For example, the charge of the G-3 dendrimer was +50(±4) mV at pH 4 and +32(±5) mV at pH 11. The charge also increased with an increase in generation size because at pH 4 the charge of the dendrimers were G-0 = +25(±3) mV, G-1 = +34(±2) mV, G-2 = 45(±3) mV and G-3 = +50(±4) mV.

When the dendrimer solutions were added to the niclosamide suspensions there was on average for G-0 to G-3 a reversal in charge at pH 7 (+15(±4) mV) and pH 11 (+8(±2) mV). However, at pH 4 an increase in the positive charge of the suspension to +35(±4) mV was observed. This same trend in ζ was observed for solutions recovered after solution studies. At pH 4 there was an increase in the positive charge, at pH 7 the solutions was weakly positively charged ∼+6 to 9 mV and at pH 11 the positively charge was stronger ∼+25 to 30 mV but weaker than the charge observed for the dendrimers alone.

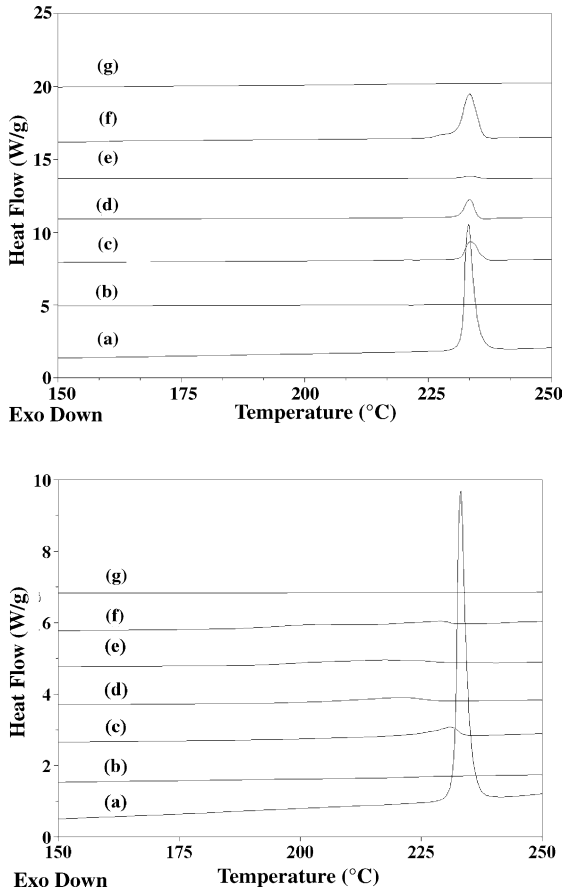

Fig. 3. DSC thermograms of: top, niclosamide–β-CD complexes. (a) Niclosamide; (b) β-CD; (c) physical mix; (d) ground; (e) kneaded; (f) co-precipitated and (g) freeze-dried. Bottom: niclosamide–HP-β-CD (1:1) complexes. (a) Niclosamide; (b) HP-β-CD; (c) physical mix; (d) ground; (e) kneaded; (f) co-precipitated and (g) freeze-dried.

3.4. Solubility of niclosamide in the presence of PAMAM dendrimers or cyclodextrins

The solubility of niclosamide ranged from 0.20 to 0.25 μg/mL between pH 2–22 and 0.5–0.6 μg/mL in water. Fig. 6 shows the phase solubility diagrams of niclosamide in the presence of dendrimers in water and at pH 7 and 11. In the presence of aqueous solutions of half generation dendrimers (G-0.5, G-1.5 and

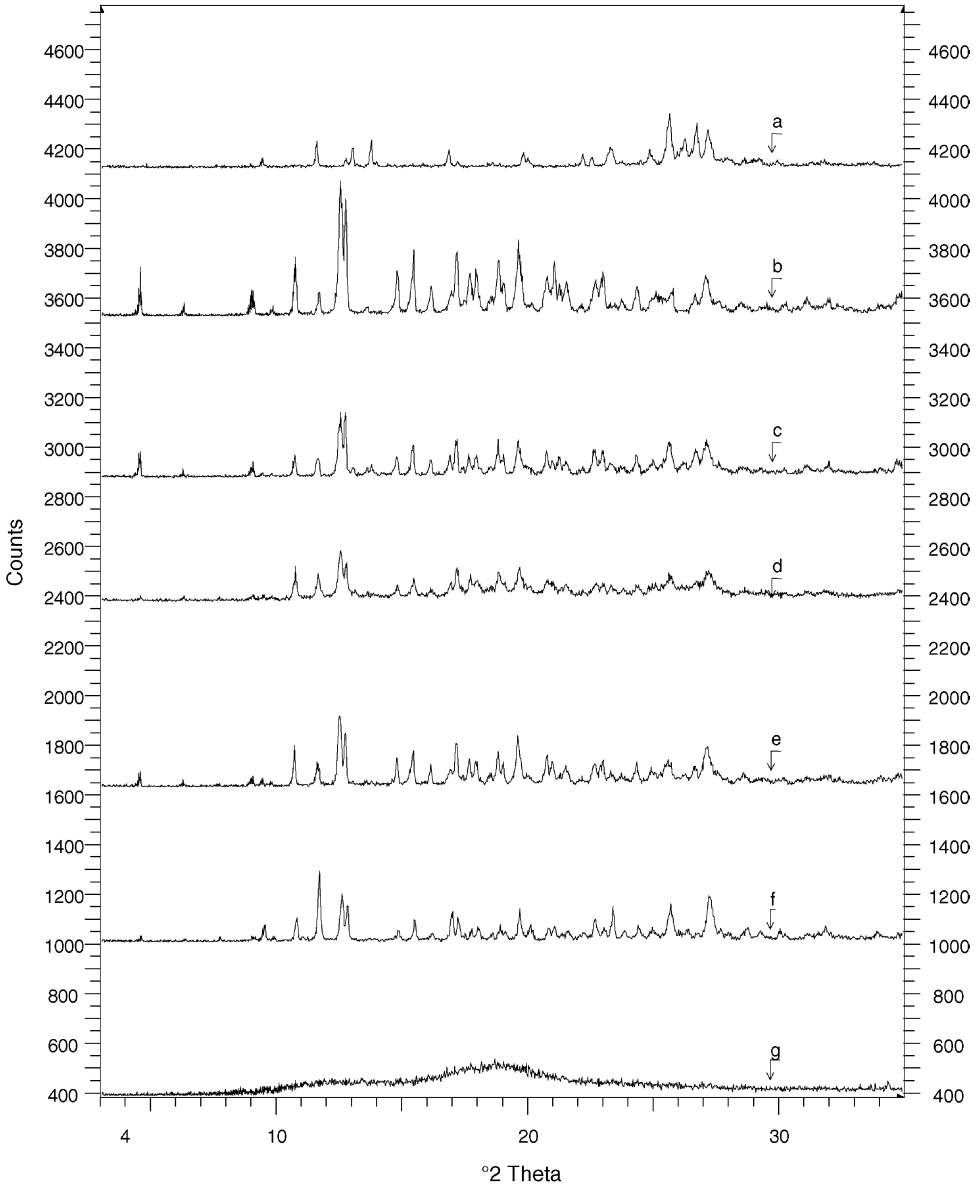

Fig. 4. X-ray powder diffraction patterns of (a) niclosamide; (b) β-CD; (c) niclosamide:β-CD 1:1 physical mixture; (d) niclosamide:β-CD 1:1 ground mixture; (e) niclosamide:β-CD 1:1 kneaded mixture; (f) niclosamide:β-CD 1:1 co-precipitated mixture and (g) niclosamide:β-CD 1:1 freeze-dried.

G-2.5) and at the three pHs, no increase in aqueous solubility of niclosamide was observed. Similarly, the full generation dendrimers did not increase the aqueous solubility of niclosamide at pH 4, but in water and at pH 7 and 11, the solubility of niclosamide was increased with increasing concentrations of these dendrimers. The highest increase in niclosamide was observed in aqueous solutions of dendrimers, followed by solutions at pH 11 and 7 (Table 4).

Analysis of the phase-solubility profiles according to Eq. (1) showed that, except for G-3 in water, the slopes of the curves was less than unity (A_L-type

diagrams) indicating the formation of drug:dendrimer complexes with a drug to dendrimer molar ratio of 1:1 (Table 4). For G-3 dendrimer in water, approximately four molecules of drug were associated with each molecule of dendrimer. Dendrimers were found to have higher complexation efficiency (product of intrinsic molar solubility of niclosamide and stability constants for niclosamide–dendrimer complexes) in water, followed by pH 11 and 7. The solubility of niclosamide also increased with an increase in the generation size

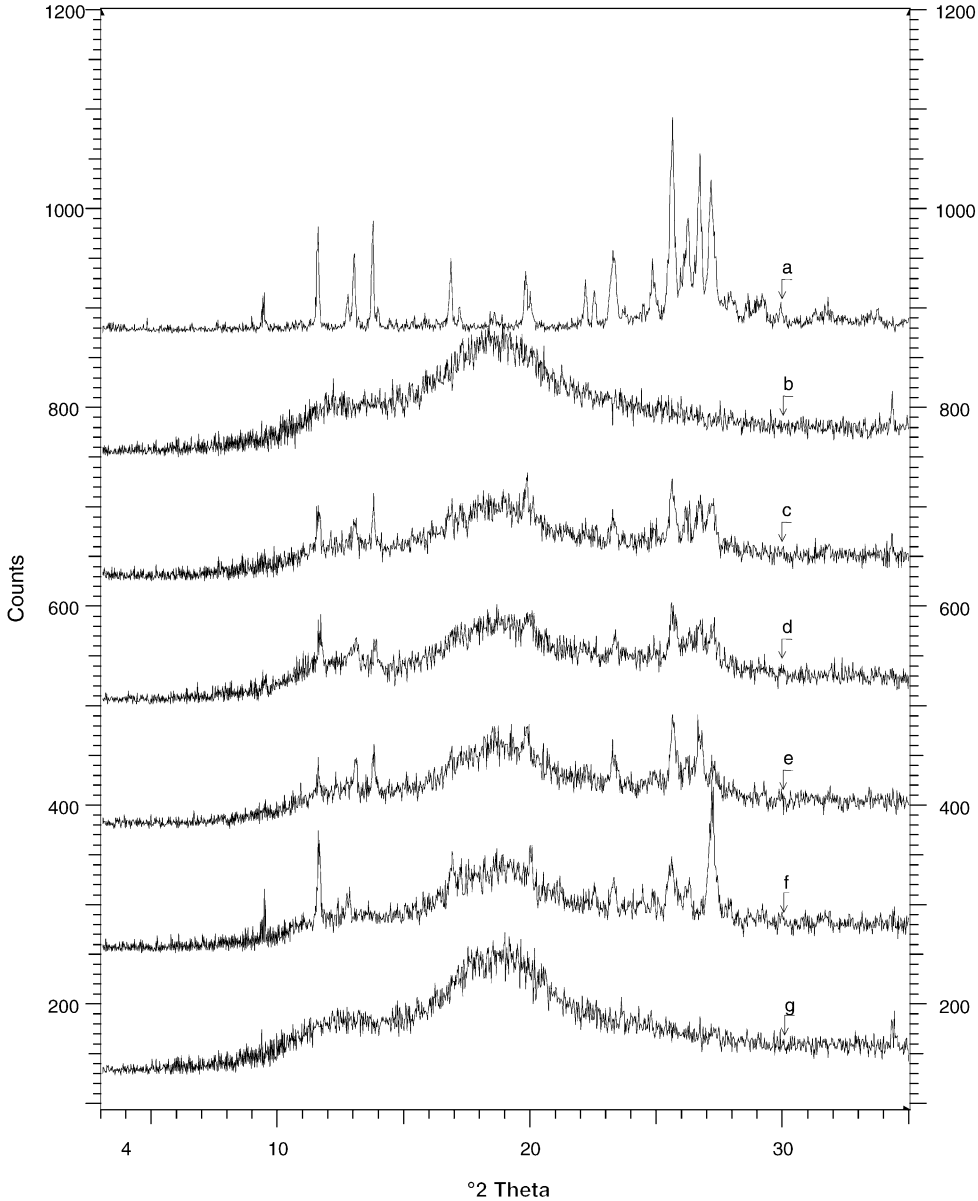

Fig. 5. X-ray powder diffraction patterns of (a) niclosamide; (b) HP-β-CD; (c) niclosamide:HP-β-CD physical mixture; (d) niclosamide:HP-β-CD ground mixture; (e) niclosamide:HP-β-CD kneaded mixture; (f) niclosamide:HP-β-CD co-precipitated mixture and (g) niclosamide:HP-β-CD freeze-dried.

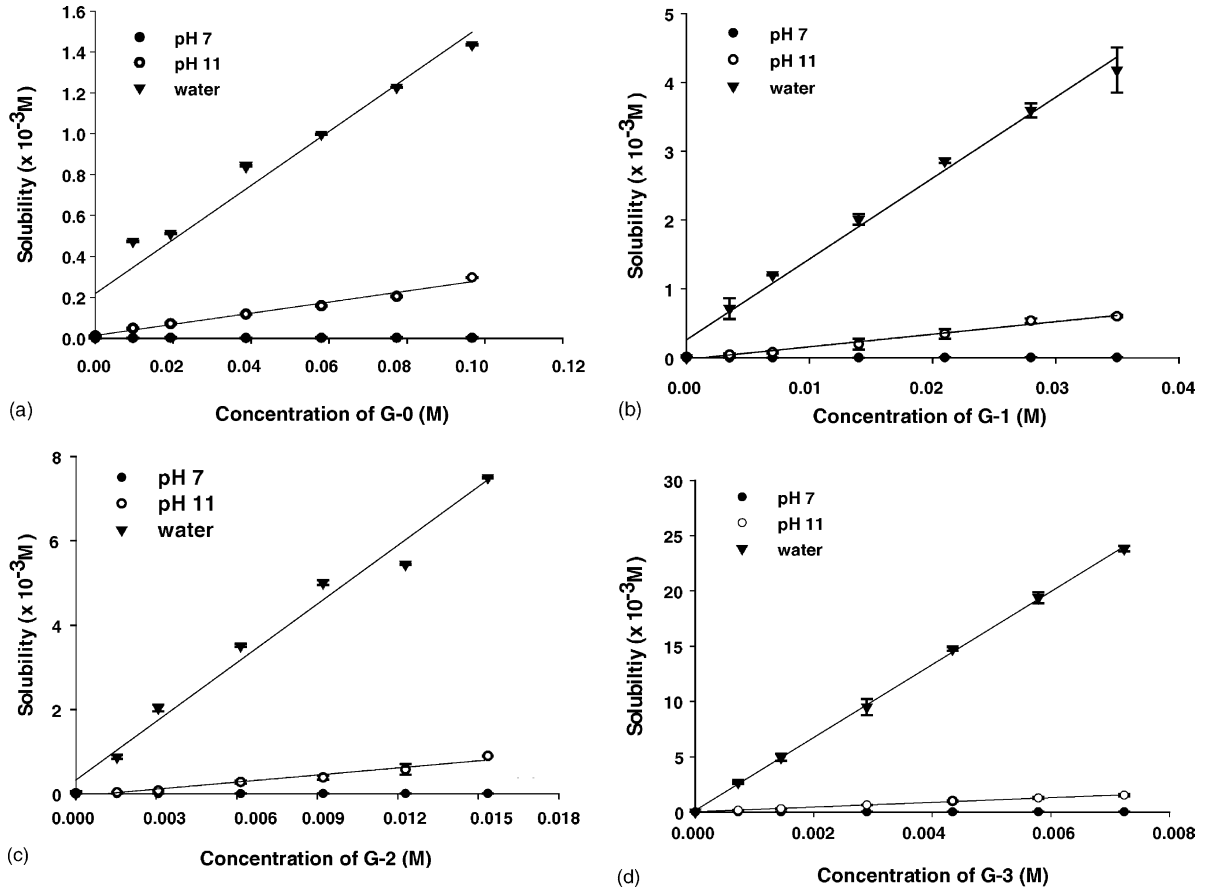

Fig. 6. Phase solubility diagram of niclosamide in the presence of full generation PAMAM dendrimers in water and at pH 7 and 11: (a) G-0; (b) G-1; (c) G-2 and (d) G-3.

(Table 4) at all the concentrations of dendrimer added with the highest increase at 0.008 M dendrimer.

The increase in aqueous solubility of niclosamide could be due to the electrostatic interactions between the positively charged primary amines of the dendrimers and negatively charged drug molecules as indicated by the ζ measurements. The extent of electrostatic interactions, however, depended on the pH of the aqueous medium. For ethylenediamine core PAMAM dendrimers, primary amines have higher pK_a (7–9) than the tertiary amines (3–6). In addition, tertiary amines closer to the core were found to have a reduced pK_a relative to identical residues closer to the surface (Tomalia et al., 1985; Ottaviani et al., 1996; Chen et al., 2000). This difference in the pK_a means that the ionization state of the full generation dendrimers will change, in the interior and at the surface, as a function of the pH of the solubility medium.

Niclosamide is a weak acid with an acidic pK_a of 7.3 (Merck Index, 2001). At pH 4, the drug would be completely in its unionized form and would probably not interact with the dendrimer molecules unless through non-ionic interactions. This was confirmed by ζ potential measurements. In water, the basic dendrimers have positively charged primary amines and it increase the pH to 8–9 where niclosamide in completely ionized leading to the largest increase in solubility. At pH 11, 100% of the drug was in its anionic form as shown by the ζ potential measurements, but PAMAM (pK_a less than 9) is almost completely unionized (weaker positive charge) leading to a decrease in interaction. At pH 7 only 50% of the drug is in its anionic form (weaker neg-

Table 4
Stoichiometry of niclosamide–dendrimer complexes, increase in solubility, equilibrium stability constants and complexation efficiency calculated from the phase solubility profiles of niclosamide in the presence of dendrimers

Dendrimer	pH	Stoichiometry (drug:dendrimer)	Solubility (fold enhanced)[a]	Stability ($K_{1:1}$) (M^{-1})	Complexation efficiency[b] ($\times 10^{-3}$)
G-0	7	1:1	6.29	22.51	0.07
	11	1:1	26.75	209.26	0.08
	Water	1:1	372.13	61.47	1.11
G-1	7	1:1	28.58	292.61	0.09
	11	1:1	54.3	808.41	0.16
	Water	1:1	1353.99	509.65	4.01
G-2	7	1:1	39.16	1620.78	0.12
	11	1:1	80.81	2196.61	0.24
	Water	1:1	1944.52	2649.71	5.81
G-3	7	1:1	110.08	1175.31	0.33
	11	1:1	137.59	1513.92	0.41
	Water	Multiple	6175.78	–	–

[a] At a concentration of 0.008 M dendrimer.
[b] Complexation efficiency = intrinsic molar solubility of niclosamide × equilibrium stability constant.

ative charge) and although PAMAM is in its cationic form the increase in the solubility of niclosamide was less than at pH 11 and in water.

The increase in solubility of niclosamide observed with an increase in generation size could be due to the increased number of available binding sites for interaction with the drug molecules (Table 1). Half generation dendrimers have only tertiary amines in their internal cavities, which are less susceptible to protonation than primary amines (Ottaviani et al., 1996). In water and at pH 4, 7 and 11, these dendrimers would be in their unionized state and could not interact with the drug molecules causing no increase in the solubility of the drug. These differences in aqueous solubility of niclosamide in the presence of full and half generation dendrimers suggest that the niclosamide seem to interact electrostatically only with the primary amines at the surface of the full generation dendrimers.

Fig. 7 shows the phase solubility diagrams of niclosamide in aqueous solutions containing CDs. The results obtained from the analysis of the solubility profiles using Eqs. (1) and (2) are shown in Table 5. In the presence of β-CD, no improvement in the solubility of niclosamide was observed. The addition of HP-β-CD improved the solubility of niclosamide by 10-fold, showing a positive deviation from linearity indicating Type A_P solubility profiles, suggesting the formation of higher order complexes. The increase in solubility of niclosamide by CDs could be due to the formation of inclusion type guest–host complexes.

In binary mixtures the addition of PAMAM G-3 increased the aqueous solubility of niclosamide the most (∼6178 times), followed by PAMAM G-2 (∼1945 times), PAMAM G-1 (∼1354 times), PAMAM G-0 (∼372 times) and HP-β-CD (∼10 times). As shown in Fig. 8, the increase in the solubility achieved with the PAMAM dendrimers followed a log–linear relationship that depended on the generation size and the number of primary amine (NH_2) groups at the

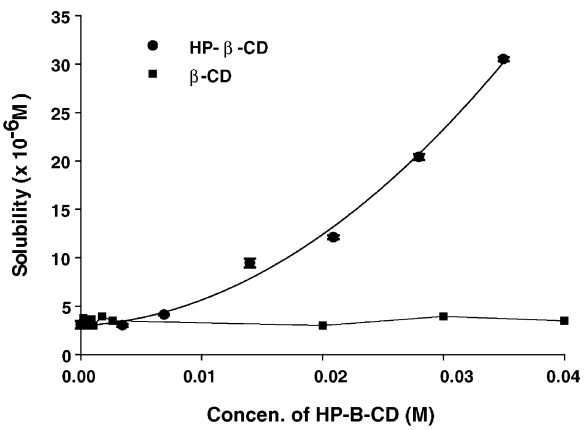

Fig. 7. Phase solubility diagram of niclosamide in the presence of β-CD and HP-β-CD.

Table 5
Stoichiometry of niclosamide:CD complexes, increase in solubility, equilibrium stability constants, and complexation efficiency calculated from the phase solubility profiles of niclosamide in the presence of CDs

CD	Stoichiometry (drug:CD)	Solubility (fold enhanced)	Stability (M^{-1})		Complexation efficiency ($\times 10^{-3}$)	
			$K_{1:1}$	$K_{1:2}$	$K_{1:1}$	$K_{1:2}$
β-CD	–	–	–	–	–	–
HP-β-CD	2:1	9.87	20.13	342.06	0.06	1.01

Complexation efficiency = intrinsic molar solubility of niclosamide × equilibrium stability constant.

Table 6
Percentage of niclosamide dissolved after 90 min and similarity factors for the dissolution of niclosamide–dendrimer complexes prepared with different generation dendrimers

Niclosamide:dendrimer molar ratio	Dendrimer	Niclosamide dissolved (%)	Similarity factor (f_2)
Niclosamide	–	13	–
1:1	G-0	66	37
	G-1	73	24
	G-2	66	21
	G-3	70	32
N (primary amines):1	G-0	70	34
	G-1	64	37
	G-2	55	40
	G-3	44	52
N (total amines):1	G-0	66	30
	G-1	57	41
	G-2	46	50
	G-3	33	58

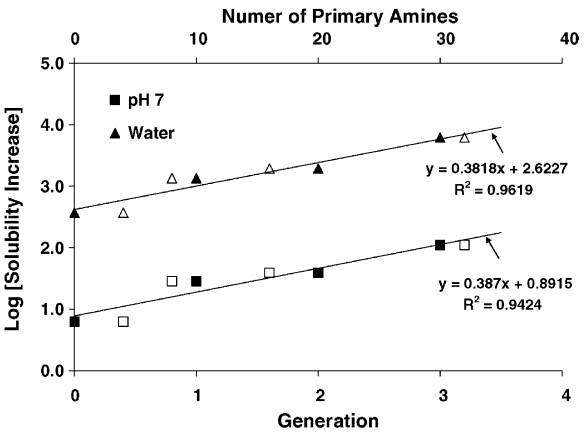

Fig. 8. Semi-logarithmic plot showing the potential increase in the aqueous solubility of niclosamide with an increase in PAMAM dendrimer generation size (closed symbols) and number of primary amines (open symbols) on the surface of the dendrimer.

surface of the dendrimer. There was an exponential increase in the magnitude of the solubility increase with an increase in the generation size from 0 to 3 and the number of primary amines from 4 to 32.

3.5. Niclosamide dissolution from PAMAM dendrimer or cyclodextrin mixtures

Table 6 shows the percent of niclosamide dissolved at the end of 90 min along with similarity factors (comparing the dissolution profiles of the niclosamide–dendrimer complexes with that of the pure drug). The dissolution profiles of niclosamide and the niclosamide–dendrimer mixtures are shown in Fig. 9. The dissolution from niclosamide–dendrimer complexes was significantly greater than that for pure niclosamide, the increase being dependent on the dendrimer generation size and amount of drug loaded in the complexes. All the complexes prepared with 1:1 molar ratio of drug to dendrimer showed a similarity factor of less than 50, indicating significant improve-

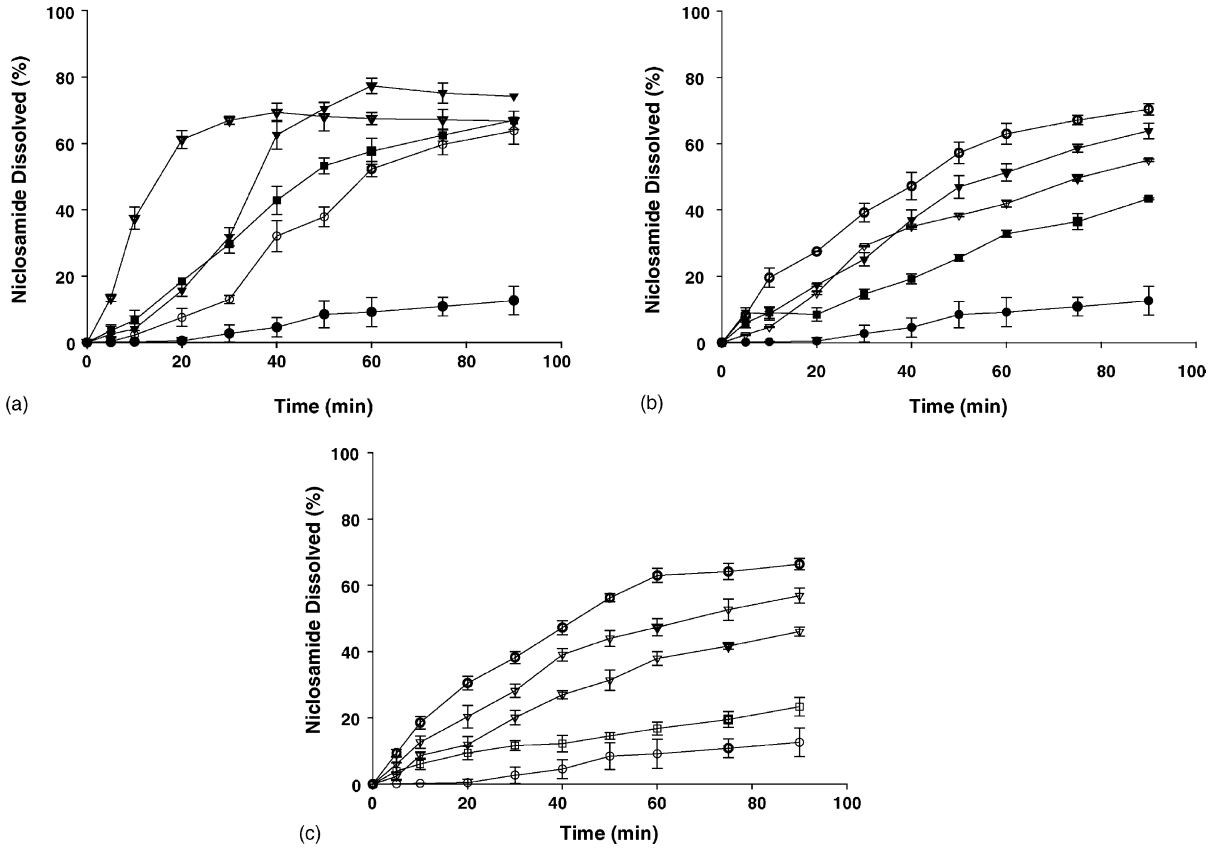

Fig. 9. Dissolution profiles of niclosamide from niclosamide–dendrimer mixtures: (●) niclosamide; (○) niclosamide-G-0; (▼) niclosamide-G-1; (▽) niclosamide-G-2; (■) niclosamide-G-3 prepared with: (a) 1:1, (b) *N* (number of primary amines):1 and (c) *N* (number of total amines):1 molar ratio of drug to dendrimer.

ment in the dissolution rate of the drug, but not the total amount dissolved, with an increase in generation size (Table 6).

The mixtures prepared with increased drug loading (1:*N* molar ratio of dendrimer to drug, *N* being equal to the number primary amines or total number of amines in each dendrimer) improved the dissolution rate of the drug but this increase was highest with low (G-0) versus higher (G-3) generations (Fig. 9). These changes in the dissolution profiles show that 1:1 molar mixtures of the drug and full generation dendrimers represented the optimum increase in dissolution and that combination of higher generation dendrimers with multiple drug molecules slowed down the release. This result suggest that the interaction between the drug and the higher generation dendrimers was stronger and that this slowed down niclosamide release compared to weaker associations between the drug and smaller dendrimers.

The dissolution profiles of both niclosamide and the niclosamide–CD mixtures are shown in Fig. 10. Table 7 lists the % of niclosamide dissolved at the end of 90 min along with the similarity factors observed when the dissolution profiles of the mixtures were compared to the pure drug. Only the drug:CD mixtures prepared by physical mixing did not show any improvement in the dissolution of drug. For the other method of preparing the mixtures the dissolution rate of drug–CD mixtures was significantly higher than that of the drug alone (Fig. 10 and Table 7). When combined with CDs the significant increase in the dissolution rate could be explained by the increase in solubility caused by complexation, a marked reduction in crystallinity as con-

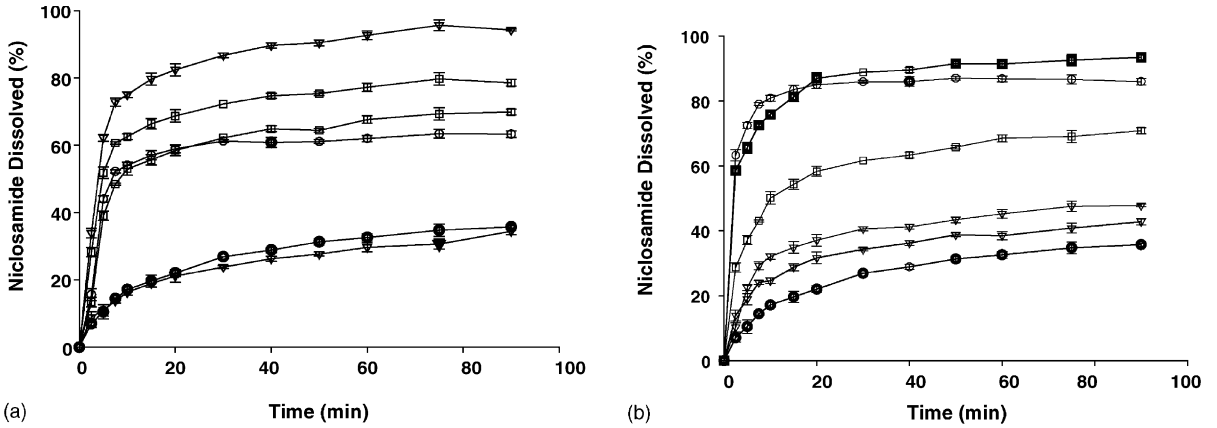

Fig. 10. Dissolution profiles of niclosamide from niclosamide–CD mixtures prepared by different methods: (a) niclosamide:β-CD complexes and (b) Niclosamide:HP-β-CD: (●) niclosamide; (▽) physical mixture; (▼) co-precipitation method; (■) kneading method; (◯) grinding method and (□) freeze-dried method.

firmed by powder X-ray diffraction and/or improved wettability of the drug when combined with the CDs. Increased dissolution rate of the ground product might also be attributable to particle size reduction of the drug along with a significant amount of drug transformed into soluble complexes.

Overall, the dissolution rate and extent of dissolution of niclosamide was significantly improved from mixtures with PAMAM dendrimers and CDs. Dissolution was fastest from niclosamide–CD mixtures prepared by co-precipitation and freeze-drying. Combination of the drug with the PAMAM dendrimers also significantly increased the dissolution compared to the pure drug and the magnitude of the increase in dissolution was the same as from the CD mixtures (Tables 6 and 7). However, the rate of dissolution was slower from dendrimer compared to CD mixtures (Figs. 9a and 10). In addition, although the dissolution of niclosamide from 1:1 molar mixtures with the dendrimer did not vary with an increase in PAMAM dendrimer generation size, the dissolution from mixtures with increased molar ratios of the drug decreased with an increase in the dendrimer generation size. This represented a decrease in the rate of dissolution and a linear decrease in the amount of drug dissolved after 90 min as shown in Fig. 11.

Table 7
Percentage of niclosamide dissolved after 90 min and similarity factors for the dissolution of niclosamide–CD mixtures prepared by different methods

CD	Preparation method	Niclosamide dissolved (%)	Similarity factor
Niclosamide	–	36	–
β-CD	Co-precipitated	94	13
	Freeze-dried	79	19
	Kneaded	70	25
	Ground	63	26
	Physical mix	35	81
Niclosamide:HP-β-CD (1:1)	Freeze-dried	93	12
	Ground	86	12
	Kneaded	71	20
	Co-precipitated	48	45
	Physical mix	43	56

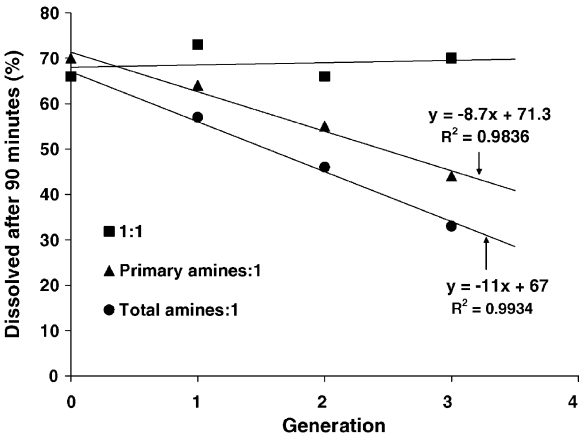

Fig. 11. Linear decrease in the amount of niclosamide dissolved after 90 min from drug-PAMAM dendrimer mixtures with an increase in generation size and the molar ratio of drug present in the mixtures.

4. Conclusions

This study is the first report of the solubilization of niclosamide by CD complexation or the interaction between the drug and PAMAM dendrimers. Half generation dendrimers with more polar carboxylate surface functional groups did not increase the solubility of niclosamide. From the phase solubility studies, it was clear that except for G-0 dendrimer at pH 7, the solubility of niclosamide was significantly higher in the presence of the dendrimers. In addition, higher equilibrium stability constants and complexation efficiency showed that the dendrimers formed stronger more stable complexes than the CDs. However, the strong interaction between the amine surface functional groups and the niclosamide molecule complexes caused a decrease in dissolution rate compared to the CDs because the interaction retarded the release of the drug from the dendrimers. In addition to increasing the solubility, PAMAM dendrimers therefore also offer the possible for the controlled release of the drug from solid dosage forms.

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