AAPS PharmSciTech **2004; 5 (1) Article 12 (http://www.aapspharmscitech.org).**

Preparation and Physicochemical Characterization of 5 Niclosamide Solvates and 1 Hemisolvate

Submitted: October 14, 2003; Accepted: January 15, 2004

Elsa C. van Tonder,[1] Mabatane D. Mahlatji,[2] Sarel F. Malan,[3] Wilna Liebenberg,[1] Mino R. Caira,[4] Mingna Song,[5] and Melgardt M. de Villiers[5]

[1]Research Institute for Industrial Pharmacy, Potchefstroom University for CHE, Potchefstroom 2520, South Africa
[2]Medicines Control Council, Private Bag X828, Pretoria 0001, South Africa
[3]School of Pharmacy, Potchefstroom University for CHE, Potchefstroom 2520, South Africa
[4]Department of Chemistry, University of Cape Town, Rondebosch 7701, South Africa
[5]Department of Basic Pharmaceutical Sciences, School of Pharmacy, University of Louisiana at Monroe, Monroe, LA 71209

ABSTRACT

The purpose of the study was to characterize the physicochemical, structural, and spectral properties of the 1:1 niclosamide and methanol, diethyl ether, dimethyl sulfoxide, N,N' dimethylformamide, and tetrahydrofuran solvates and the 2:1 niclosamide and tetraethylene glycol hemisolvate prepared by recrystallization from these organic solvents. Structural, spectral, and thermal analysis results confirmed the presence of the solvents and differences in the structural properties of these solvates. In addition, differences in the activation energy of desolvation, batch solution calorimetry, and the aqueous solubility at 25°C, 24 hours, showed the stability of the solvates to be in the order: anhydrate > diethyl ether solvate > tetraethylene glycol hemisolvate > methanol solvate > dimethyl sulfoxide solvate > N,N' dimethylformamide solvate. The intrinsic and powder dissolution rates of the solvates were in the order: anhydrate > diethyl ether solvate > tetraethylene glycol hemisolvate > N,N' dimethylformamide solvate > methanol solvate > dimethyl sulfoxide solvate. Although these nonaqueous solvates had higher solubility and dissolution rates than the monohydrous forms, they were unstable in aqueous media and rapidly transformed to one of the monohydrous forms.

KEYWORDS: niclosamide, solvates, solubility, physical stability

Corresponding Author: Melgardt M. de Villiers, Department of Basic Pharmaceutical Sciences, School of Pharmacy, University of Louisiana at Monroe, Monroe, LA 71209; Tel: (318) 342-1727; Fax: (318) 342-1737; Email: devilliers@ulm.edu

INTRODUCTION

Niclosamide is the generic name for 5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide, or 2',5-dichloro-4'-nitrosalicylanilide. It is an anthelmintic that is active against most tapeworms, including the beef tapeworm, the pork tapeworm, the fish tapeworm, the dwarf tapeworm, and the dog tapeworm.[1] Niclosamide is also used as a molluscicide for the treatment of water in schistosomiasis control programs.[2] The drug works by killing tapeworms, molluscs, or mussels on contact. However, niclosamide is practically insoluble in water and sparingly soluble in acetone, chloroform, and ether,[1,3] and the anhydrous form of the drug is fairly hygroscopic,[4] which complicates formulation development and reduces the effectiveness of the drug. Both the solubility and the hygroscopicity of crystalline drugs can be improved by changing the crystal form because different habits, structures,[5] surface modifications,[6] and, in some instances, solvents included into the crystal structures of solvates or clathrates can change the surface properties and the reactivity of the drug particles.[7]

Although for niclosamide, the differences in the physicochemical properties of an anhydrous form and 2 monohydrates and the single crystal structures of a monohydrate and 2 solvates crystallized from tetrahydrofuran and tetraethylene glycol have been reported earlier, little is known about the physicochemical properties of these and other niclosamide solvates.[8,9] The objective of this study was to determine structural, physicochemical, and spectral properties of several niclosamide solvates crystallized from the following solvents: methanol, dimethyl sulfoxide, N,N'-dimethylformamide, diethyl ether, and tetrahydrofuran; and a tetraethylene glycol hemisolvate. The solvates were characterized using thermal and spectral methods, including differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), hot stage microscopy (HSM), infrared spectroscopy (IR), and x-ray powder diffractometry (XRPD).

Differences in the powder dissolution, intrinsic dissolution, and the solubilities of the crystal forms are also reported.

MATERIALS AND METHODS

Materials

Niclosamide (Sigma Chemical, St Louis, MO) and the following analytical grade solvents obtained from Saarchem (Krugersdorp, South Africa) were used; namely, diethyl ether (DE), dimethyl sulfoxide (DMSO), N,N'-dimethylformamide (DMF), and tetrahydrofuran (THF). Methanol (MeOH) (BDH, Poole, England), and tetraethylene glycol (TEG) (Merck, Darmstadt, Germany) were also used.

Preparation of Solvates

The following general crystallization method was used to prepare the solvates. Saturated solutions of niclosamide anhydrate in the different solvents at room temperature were prepared by heating the solutions (not exceeding 60°C) under constant stirring until most of the niclosamide dissolved, filtering the solutions, covering them, and then leaving them to stand at room temperature until crystallization was complete. The crystals were stored in these solutions to prevent desolvation.

Morphology of the Solvates

A scanning electron microscope (SEM) (Stereoscan 250, Cambridge Scientific Instruments, Cambridge, UK) was used to obtain photomicrographs of the niclosamide solvates. Samples were mounted on a metal stub with an adhesive and coated under vacuum with carbon (Emscope TB500 sputter-coater, Emscope Laboratories, Ashford, UK) before being coated with a thin gold-palladium film (Eiko Engineering Ion Coater IB-2, EIKO Engineering, Ibaraki, Japan).

Thermal Methods of Analysis

A Leitz (Laborlux K, Leica Microsystems Inc. IL, USA) hot stage microscope was used to visually identify crystals of the different forms. Samples were placed on an object glass, covered with a coverslip, and heated to 240°C at a heating rate of 10 K per minute. Desolvation was determined using silicone oil.

DSC traces were recorded with a Shimadzu DSC-50 instrument (Shimadzu, Kyoto, Japan) or 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 instruments. A mass, not exceeding 3 mg, was measured into aluminum pans with or without a small pinhole in the lid. DSC curves were obtained under a nitrogen purge of 20 mL per minute at a heating rate of 5 to 10 K per minute. Melting temperatures were determined as extrapolated onset temperatures, defined as the point of transition, being the point of intersection between the base line and the DSC endothermal melting effect, which gives the most reproducible value, experimentally independent of the operator.[10]

Loss of solvent from the crystals was characterized by TGA obtained with either a Shimadzu TGA-50 (Shimadzu) or Hi-Res Modulated TGA 2950 (TA Instruments). TGA traces were recorded at heating rates of 2 to 10 K per minute under a nitrogen purge of 50 mL per minute. Samples with masses between 1 and 10 mg were analyzed using a platinum pan. Mass loss (%) was calculated from TG curves, based on the mass of the original sample. In addition, TG analysis was used to determine the activation energy for desolvation by measuring weight loss at 5 different heating rates.[10] The negative logarithm of the heating rates was plotted against the inverse of absolute temperature at constant predetermined weight losses. The slopes of the curves were calculated and substituted into the equation, $E \cong -4.35 d\log ß / d1/T$, to obtain the activation energy for the desolvation of the solvates. β is the heating rate in K/second and T is the absolute temperature in Kelvin.

X-Ray Powder Diffraction Analysis

The XRPD profiles were obtained at room temperature with a Philips PM9901/00 diffractometer (Philips, Eindhoven, The Netherlands). The measurement conditions were the following: target, CuKα; filter, Ni; voltage, 40 kV; current, 20 mA; slit, 0.1 mm; scanning speed, 2°/min. Crystals of the different crystal forms were ground into a fine powder with average particle size of ± 60 μm. Care was taken to avoid crystal changes during sample preparation. Approximately 200 mg samples were loaded into aluminum sample holders, taking care not to introduce preferred orientation of the crystals.

Infrared Spectroscopy

IR spectra of powdered samples were recorded on a Shimadzu FTIR-4200 spectrophotometer (Shimadzu) over a range of 4000 to 400 cm^{-1} using the KBr disc technique. For the compressed disc technique, 2 mg samples were mixed with 200 mg KBr (Merck) by means of an agate mortar and pestle. Discs were pressed using a Beckman 00-25 press (Beckman, Fullerton, CA) at a pressure of 15×10^3 kg/cm^2.

Aqueous Solubility, Intrinsic and Powder Dissolution Measurements

Before measuring the aqueous solubility at 25°C, 24 hours, and powder dissolution, the different crystals were screened using an 88-µm sieve. The particle size distributions of the samples were measured with a Galai-Cis-1 (Ashkelon, Israel) particle size analyzer. For the solubility determinations, amounts of the drugs—enough to ensure supersaturation (55 ± 5 mg)—were measured into test tubes that had screw caps. To each test tube, 10 mL distilled water was added and the caps were screwed on tightly. The test tubes were rotated at 70 rpm (Heidolph RZR-2000 rotator, Heidolph Instruments, Schwabach, Germany) in a thermostatically controlled water bath at 25°C for 24 hours. The concentration of niclosamide in the filtered samples was determined spectrophotometrically at 330 nm. The crystal forms of the undissolved powder were determined by XRPD and DSC analysis.

The intrinsic dissolution rate (IDR) was determined by the propeller-driven method as described by Singh et al.[11] Powdered samples of the solvates were slowly compressed into 13-mm tablets in a die, so that the tablet surface was flush with the die surface (Beckmann Type 00-25 IR-press, 3.8×10^5 kg/cm^2), with a dwell time of 1 minute to ensure compaction. A compression force of 2.3×10^5 kg/cm^2 could be used without an appreciable change in the apparent surface area of the disk. The back of the die was sealed and mounted directly in a holder in a water-jacketed beaker containing 400 mL of an isopropanol:water (40:60 v/v) dissolution medium, kept at 25°C ± 1°C. Stirring was achieved by a Teflon-coated stirrer rotating at 150 rpm attached to a Hurst motor (Hurst, Princeton, IN) mounted on the beaker cover. The amount of drug dissolved as a function of time was determined spectrophotometrically. DSC and TG analysis were used to determine if changes had occurred in the crystal form during compression. At the end of the dissolution, the top layer of the tablet was removed and analyzed to determine if the crystal form changed during dissolution testing.

The powder dissolutions of the screened crystal forms were performed according to the method described by Lötter et al[12] using apparatus 2 of the *United States Pharmacopeia*[13] with a paddle speed of 100 rpm (Erweka DT6R, Erweka, Heusenstamm, Germany). The dissolution medium was 900 mL of 40% isopropanol:water mixture thermostatically controlled at 37°C ± 1°C. Fifty milligrams, accurately weighed on a Sartorius analytical balance (Goettingen, Germany), of the samples were mixed with 25-mg glass beads (0.1-0.11 mm in diameter) and vortexed for 1 minute in 2 mL dissolution medium. This was introduced into the dissolution medium, and the amount of dissolved niclosamide as a function of time was determined spectrophotometrically. The dissolutions were performed in triplicate and were corrected for dilution.

Determining the Heats of Solution by Isoperibol Solution Calorimetry

Solution calorimetric measurements were performed using a Tronac model 450 isoperibol calorimeter (Tronac, Denver, CO).[14,15] The solvent employed for analysis consisted of 50 g of 90% wt/wt N,N'-dimethylformamide:water, and measurements were performed at 25°C. The size of samples used in the analysis ranged from 20 to 40 mg and were weighed (Sartorius model 1712, semi-micro balnce) into cylindrical ampoules, the top and bottom faces of which consisted of 12-mm microscope coverslips. The ampoule was submerged into the weighed solvent contained in a Dewar flask, which served as the reaction vessel. The vessel was clamped into position and lowered into the constant-temperature water bath maintained at 25°C with a deviation of ± 0.0004°C. A stirring rate of 400 rpm was employed. An operational heat-capacity calibration with heater of known resistance (101.1 Ohm) to determine the energy equivalent of the system was performed when the reaction vessel temperature reached the set point. Once the heat capacity calibration was completed, the actual analysis was initiated by mechanically breaking the glass coverslips of the ampoule, and measuring the heat produced when the sample dissolved. In this study, no correction for the heat of mixing of water in the N,N'-dimethylformamide:water mixture was made for the enthalpy of hydration, since no significant reaction heat between water and the solvent was involved when mixed.

RESULTS AND DISCUSSION

Morphology and Microscopic Properties of the Solvates

SEM photomicrographs of the crystal forms are shown in Figure 1. The crystals of the anhydrate were small rectangles and squares. Niclosamide anhydrate, the commercially available material, consists of needlelike crystals. This powder is hygroscopic and absorbs moisture. Crystals of the MeOH solvate were also needles, but the needles differed in dimensions. The surfaces of the needles are uneven with sharp edges. The THF solvate crystallized as long yellow flat tabular crystals with a layered structure. The smooth, thin needles of the DMSO-solvate also have a layered structure. The crystals of the DMF solvate were angular platelike needles with uneven surfaces. The crystals of the DE solvate were bent and tangled with uneven surfaces. The TEG hemisolvate crystallized as yellow prismatic crystals.

During heating on a hot-stage microscope, desolvation and melting occurred at different temperatures for each of the solvates. These changes are listed in Table 1. Upon desolvation, all the crystals became opaque with a simultaneous change in the crystals to small rectangles and squares that

Table 1. Transformations Observed in Crystals of the Solvates During Heating in Silicone Oil on a Hot Stage Microscope*

Solvate Form	Turns Opaque (°C)	Darkening (°C)	Desolvation (°C)	Melting (°C)
Anhydrate	-	-	-	220-230
MeOH	60	60-110	60	220-232
THF	35	35-105	35	219-232
DMSO	90	90-175	90	218-229
DMF	70	70-155	70	218-229
DE	65	65-150	65	220-230
TEG	90	95	90	195-215

*THF indicates tetrahydrofuran; DMSO, dimethyl sulfoxide; DMF, N,N'-dimethylformamide; DE, diethyl ether; and TEG, tetraethylene glycol.

started melting at 220°C. The melting was completed at 233°C, which showed that the desolvated crystals melted at the same temperature as the anhydrate. This finding was true for all the solvates except the TEG hemisolvate, where the released TEG acted as a solvent in which the smaller crystals dissolved slowly from ± 110°C to 215°C.

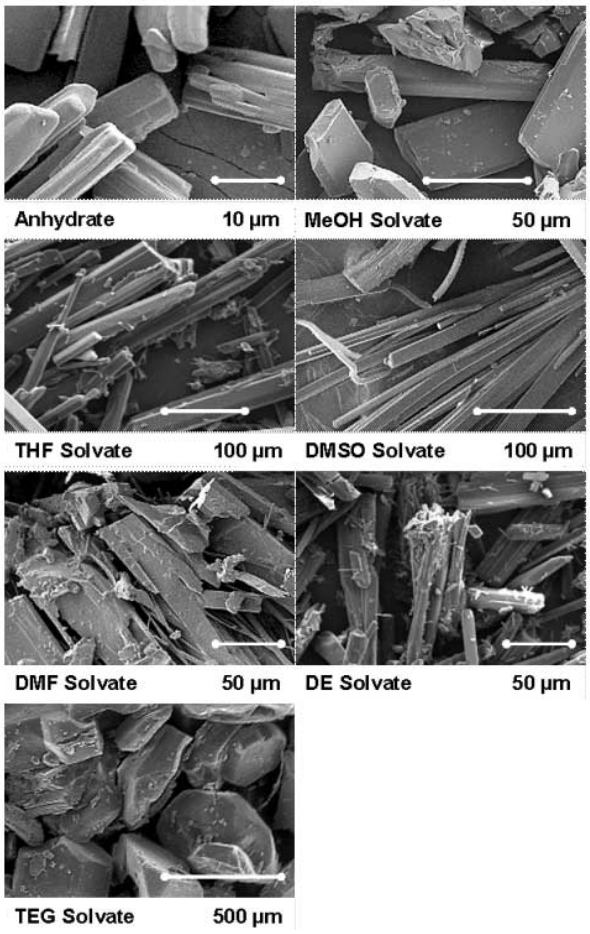

Figure 1. SEM photomicrographs of niclosamide crystal forms.

X-Ray Powder Diffraction and Spectral Properties of the Solvates

XRPD patterns of the anhydrate and solvates are given in Figure 2. There are clear differences in the XRPD patterns of the solvates compared with the anhydrate and compared with each other. Because the DMF and DE solvates have several major peaks at very similar angular locations, it was concluded that these 2 solvates were isostructural. Because of rapid loss of solvent from the THF solvate on exposure to air, it was not possible to obtain an XRPD pattern of this solvate. However, single-crystal structure determination was successfully completed by sealing the crystals in capillaries filled with mother liquor.[9]

According to Moffat et al,[16] principal peaks of niclosamide analyzed in a KBr disc appear at wave numbers 1572, 1515, 1613, 1285, 1650, and 1218 cm^{-1}. In the IR spectra (KBr disc) of the anhydrate and solvates, some of the bands were shifted. Table 2 contains the assignments and wave numbers of these different major bands. The solvates of niclosamide can also be distinguished from the anhydrate by the appearance of new stretching or bending frequencies resulting from solvation. In addition, according to Kosheleva and Bekhli[17] and Kosheleva et al,[18] niclosamide can exist in a trans (β-) and cis (α-) form, referring to 2 different conformations and intramolecular hydrogen bonding arrangements. The β-form predominates in 1,4-dioxane solutions.[18] Single-crystal x-ray analysis confirmed that the conformation of niclosamide in the THF solvate and the TEG hemisolvate is the β-form.[9] IR spectra of the pseudopolymorphs in Nujol suspension and 1,4-dioxane solution confirm the presence of the β-form.

Thermal Properties of the Solvates

DSC curves of all the solvates showed 2 endotherms, one for desolvation and the other for melting. Table 3 summa

Table 2. Wave Numbers, Assignments, and Shifts of the Major Absorption Bands in the Infrared Spectra (KBr Disc) of Niclosamide Anhydrate and Pseudopolymorphs*

Assignment	C=C Stretch	C=O Stretch	N-H Bend	C-N Stretch	NO_2 Stretch	C-O Stretch
Wave number (cm^{-1})†	1613	1650	1572	1285	1515	1218
Anhydrate	1603	1651	1572	1285	1515	1218
MeOH solvate	very weak	1686	1568	1287	1509	1223
THF solvate	very weak	1682	1570	1287	1509	1223
DMSO solvate	very weak	1680	1553	1285	1501	1223
DMF solvate	very weak	1649	1557	1283	1505	1223
DE solvate	very weak	1651	1557	1285	1505	1223
TEG hemisolvate	1597	1680	1553	1281	1511	1221

*Abbreviations are explained in the footnote to Table 1.
†Data from Singh P et al.[11]

Table 3. Melting Points, Desolvation Temperatures, and Enthalpies of Desolvation and Melting for the Solvates of Niclosamide*

Crystal Form	Melting Point (°C)	Enthalpy of Melting (J/g)	Desolvation Temperature (°C)	Enthalpy of Desolvation (J/g)
Anhydrate	229 ± 0.7	110 ± 10	-	-
MeOH solvate	228 ± 0.6	105 ± 5	90 ± 3	140 ± 10
THF solvate	230 ± 1.0	105 ± 15	75 ± 10	85 ± 35
DMSO solvate	229 ± 0.5	105 ± 15	169 ± 1	140 ± 10
DMF solvate	229 ± 0.1	105 ± 15	152 ± 5	130 ± 5
DE solvate	229 ± 0.4	105 ± 15	146 ± 4	120 ± 10
TEG hemisolvate	205 ± 10	50 ± 10	103 ± 5	54 ± 10

*Abbreviations are explained in the footnote to Table 1.

rizes the desolvation temperatures, enthalpies of desolvation, melting points, and enthalpies of melting. DSC traces of the TEG hemisolvate display only endotherms at 103°C and 205°C. The normal endotherm at 229°C was shifted to 205°C because TEG acts as a solvent after being released from the crystals. Therefore, the crystals partially dissolved in the TEG and the rest melted at 195°C to 205°C. In DSC traces of the MeOH and THF solvates, desolvation endotherms are at temperatures below 100°C, while desolvation endotherms of the DMSO, DMF, and DE solvates are at temperatures between 140°C and 170°C. Examples of the DSC and TG thermograms are shown in Figure 3 and Figure 4A.

TG analysis was used to determine the loss of solvent from the crystals, as well as the stoichiometry of the solvates. These results are listed in Table 4. The THF solvate was the least stable. X-ray crystal-structure analysis of the THF solvate revealed that the solvent molecules are located in continuous channels.[9] Due to this structure, the crystals desolvated very quickly and were taken out of the crystallizing solvent just before analysis. The first TGA trace after removal from mother liquor showed a solvent loss of 17.48%, corresponding to a drug:THF ratio of 1:0.96. A second TGA trace after 1 hour showed a 12.22% loss, and the third run after 2.5 hours showed a solvent loss of 8.82%. The TEG solvate was the most stable.[4] Single-crystal x-ray analysis confirms the existence of a solvate with a 2:1 (niclosamide:TEG) ratio.[9] It was decided to name this solvate a TEG hemisolvate. The TEG hemisolvate desolvated over a wide range, consistent with single-crystal x-ray analysis. Each TEG molecule is bound to 2 host drug molecules by very strong host-guest hydrogen bonds. Thus, because of the mode of inclusion and low volatility of TEG, a gradual desolvation was observed. Previous studies have shown that the TEG hemisolvates are stable and remain intact when suspended in organic water-free solvents.[4]

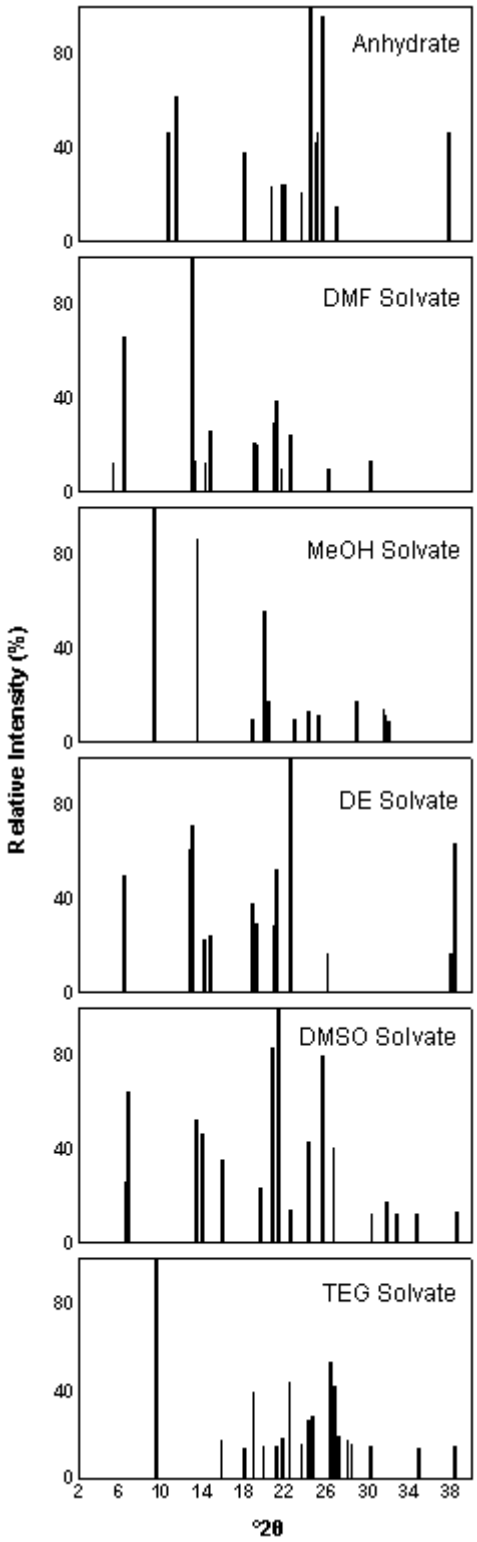

Figure 2. XRPD patterns of the niclosamide anhydrate and solvates.

To estimate the activation energy necessary for the desolvation of the solvates, a method described by Wall and Flynn[19] was used. This method involved measuring TGA runs at 5 different heating rates; namely, 2°C/min, 4°C/min, 6°C/min, 8°C/min, and 10°C/min as shown in Figure 4A. Then, the negative logarithm of the heating rates was plotted against the inverse of absolute temperature at constant predetermined weight losses (see Figure 4B). The slopes of the curves were calculated and substituted into the equation, $E \cong -4.35 d\log\beta/d1/T$, to obtain the activation energies for the desolvation of the 2 monohydrates. β is the heating rate in °C/s, and T is the absolute temperature in Kelvin. The estimated activation energies necessary for desolvation are listed in Table 5. Because of small yield and instability, it was not possible to do these measurements accurately for the DE solvate and the THF solvate, respectively. For the other 5 solvates, the activation energy necessary for desolvation was not significantly different (see Table 5).

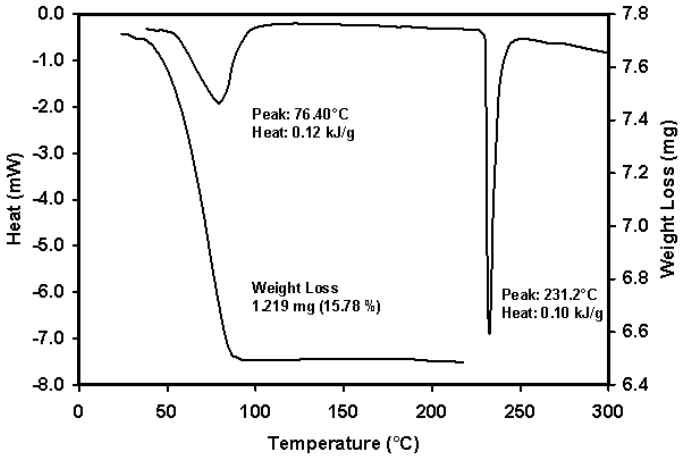

Figure 3. DSC and TG thermograms of the THF solvate.

Solution calorimetry, another thermal analysis technique, was used to measure the heats of solution of solvates.[10,14,15] Molar heats of solution and heats of transition are summarized in Table 5. For different crystal forms, the difference in heats of solution is equal to the difference in lattice energy of the solids, providing that the solids are chemically identical and differ only in morphology. In this study, the compositions of the crystal forms studied were different; therefore the transition enthalpies for the transition of the anhydrate and solvates to the more stable monohydrates (see Table 5) represent estimated and not absolute differences in thermodynamic stability.[10] A negative value indicates a metastable form converting to a stable form. The stability order according to the partial molar heats of solution was in the order anhydrate > > TEG hemisolvate > MeOH solvate > DMSO solvate >> DMF solvate > monohydrate H_A[8] > monohydrate H_B.[8] However, although these values are different it should be emphasized that the heat of solution difference between polymorphs or solvates will be maintained only when the less stable form cannot convert to the most stable form. Since, in this study, such a conversion to

Table 4. Solvent Loss From Niclosamide Solvates During Thermogravimetric Analysis*

Solvate	Temperature Range (°C) for Solvent Loss	Experimental % Mass Loss	Calculated Stoichiometric Ratio of Drug:Solvent
MeOH	64-101	8.67 ± 0.30	1:0.97 ± 0.03
DMSO	85-175	19.00 ± 0.20	1:0.98 ± 0.01
DMF	70-160	18.00 ± 0.20	1:0.98 ± 0.01
THF	30-105	14.00 ± 4.00	1:0.74 ± 0.22
TEG hemisolvate	65-230	22.00 ± 4.00	1:0.48 ± 0.11
DE	65-150	16.00 ± 2.50	1:0.84 ± 0.14

*Abbreviations are explained in the footnote to Table 1.

the more stable monohydrated form does takes place, the equilibrium heat of solution of all the forms seems to approach a common value (mean for the solvates 6.13 ± 0.41 kJ/mol); namely, that of the more stable monohydrate H_A (6.51 kJ/mol).

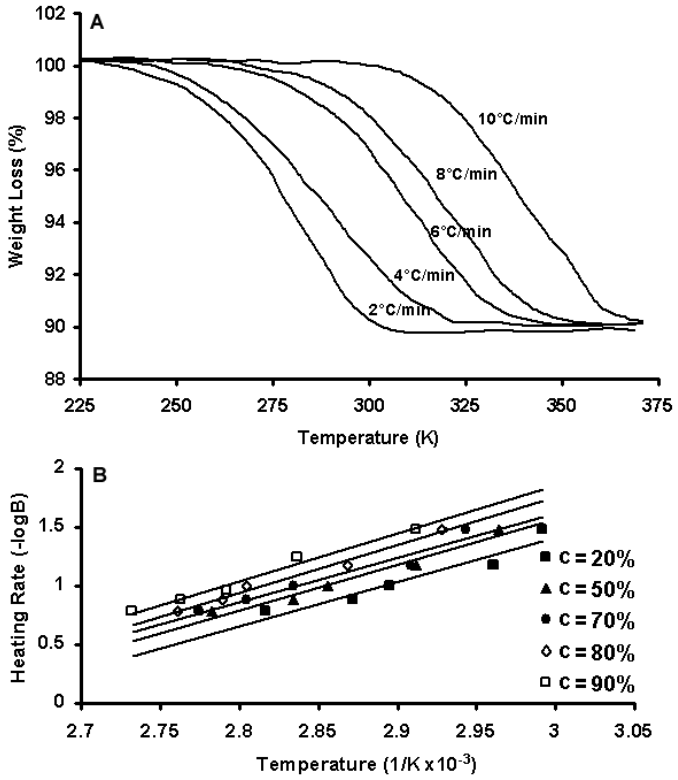

Figure 4. Example of data used for calculating the activation energy of solvates: (A) TG thermograms of the MeOH solvate at different heating rates and (B) logarithm of heating rate vs reciprocal of absolute temperature at increasing percentages of weight loss.

The differences in the energy for desolvation and heat of solution between the crystal forms are a measure of the physical stability of the crystal forms. In Table 5, these differences are summarized. The results show that in the solid state the estimated energy requirement for the transition to the anhydrate is very high, but reasonably low for the transition to the monohydrates. Therefore, in solution far less energy is required, which means that from solution (or in the presence of water) all the crystal forms would eventually transform to one of the monohydrates.

Aqueous Solubility of the Solvates

Since different crystal structures are characterized by different lattice energies (and enthalpies), it follows that the solubility of different crystal polymorphs (or solvated species) must differ as well. In Table 6, the solubilities of the solvates of niclosamide in water at 25°C after 24 hours are listed. The unsolvated equivalent solubilities of the pseudopolymorphs were also compared with that of the anhydrated form. The theoretical free energy changes (Δ G) necessary for the spontaneous change of the anhydrate and pseudopolymorphs to monohydrate H_A or H_B were also calculated using the equation, $\Delta G\ (II \rightarrow I) = -RT \ln(S_{II}/S_I)$, where S_{II} and S_I are the solubilities of the crystal forms II and I, respectively. There is a natural tendency for the free energy of a solid system to decrease so that Δ G is negative for a spontaneous process. This calculation was done to see if it were theoretically possible for the anhydrate and the solvates to convert to monohydrate H_A and H_B as was observed from DSC studies of the powders before and after intrinsic dissolution studies.

The solubility at 25°C, 24 hours, of all the crystal forms was very low in water—all below 1 mg/mL. The results listed in Table 6 show that the solubilities of all the solvated forms were significantly less than that of the anhydrate ($P < .05$). Among the solvates, the DE solvate was the most soluble, ± 6.16 µg/mL. Gibbs free energy values (see Table 6) show that the anhydrate has the highest free energy. The solubilities of the crystal forms in water at 25°C, 24 hours, decrease in the order anhydrate > DE solvate > MeOH solvate > TEG hemisolvate > DMSO solvate > DMF solvate. It should be emphasized that the solubility difference between poly

Table 5. Activation Energy (E_a), Heat of Solution ($\overline{\Delta H_S}$), and Estimated Heat of Transition ($\overline{\Delta H_T}$) to Monohydrated Form H_A and H_B Calculated From Thermogravimetric Analysis and Heat of Solution Data Obtained in 90% wt/wt N,N'

Crystal form	E_a (kJ/mol)	$\overline{\Delta H_S}$ (kJ/mol)	$\overline{\Delta H_T} \rightarrow H_A$ (kJ/mol)	$\overline{\Delta H_T} \rightarrow H_B$ (kJ/mol)
Anhydrate	-	1.10	–5.41	–6.28
Monohydrate H_A†	-	6.51	+0.00	–0.86
Monohydrate H_B†	-	7.37	+0.86	+0.00
DMF solvate	73.49	6.60	+0.09	–0.77
DMSO solvate	72.41	6.32	–0.19	–1.05
MeOH solvate	72.52	5.88	–0.63	–1.49
TEG solvate	69.17	5.69	–0.82	–1.68

*Abbreviations are explained in the footnote to Table 1.
†Data from van Tonder et al.[8]

Table 6. Intrinsic Dissolution Rates and Solubility of the Solvates in Water at 25°C After 24 Hours Compared With the Solubility of Anhydrated Niclosamide. The Change in ΔG Necessary to Convert the Solvates to Monohydrate H_A or H_B Is Also Listed*

Crystal Form	IDR (μg/cm²/min)	Solubility of Solvated form (μg/mL)	Solubility Equivalent (μg/mL)†	Change to H_A (kJ/mol)	Change to H_B (kJ/mol)
Anhydrate	2.70 ± 0.03	13.32 ± 3.18	13.32 ± 3.18	–6.69	–7.80
MeOH solvate	2.61 ± 0.36	3.26 ± 0.66	2.97 ± 0.61	–2.97	–4.07
DMSO solvate	3.49 ± 0.34	2.53 ± 0.43	2.04 ± 0.35	–2.04	–3.15
TEG hemi-solvate	3.65 ± 0.22	4.40 ± 0.55	3.39 ± 0.42	–3.30	–4.40
DMF solvate	2.69 ± 0.83	1.75 ± 0.10	1.43 ± 0.08	–1.15	–2.26
DE solvate	2.62 ± 0.67	7.56 ± 1.76	6.16 ± 0.08	–4.78	–5.88
THF-solvate	2.70 ± 0.01	-	-	-	-

*Abbreviations are explained in the footnote to Table 1.
†Solubility converted to equivalent of unsolvated form.

morphs and between solvates will be maintained only when the less stable form cannot convert to the most stable form. When such conversion to the most stable form takes place, the equilibrium solubility of all the forms will approach a common value; namely, that of the most stable forms, monohydrates H_A and H_B.

Dissolution Properties of the Solvates

In Table 6, the IDR of an anhydrate and the solvates in a 40% isopropanol:water mixture at 25°C are listed. The rates were calculated from the slopes of linear plots (mean $R^2 = 0.93$). The lines also had intercepts very close to zero (mean y-intercept = 0.017 mg). The TEG hemisolvate had the fastest dissolution rate, 3.65 μg/cm²/min. In alcoholic mixture, the dissolution rates of the other forms were the same as that of monohydrate H_B. This finding meant that, with the dissolution conditions used, all the solvated forms were converted to a monohydrated form, monohydrate H_A. Although H_B is the most stable form,[8] the transformation to H_B is a time-consuming process, and the time it took to complete an intrinsic dissolution test was insufficient for complete conversion. Previous studies also showed that monohydrate H_A could act as an intermediate phase in the formation of H_A.[4] All dissolution experiments were performed in a 40% isopropanol:water mixture because a combination of the low solubility of the drug in water and the experimental setup used for determining the IDR made it impossible to measure the IDR in water. It should be noted that the increased solubility of the solvates in this medium might also have increased the tendency for transformation to occur.

The powder dissolution curves of the different forms also showed major differences in the dissolution rates of the solvates and were in agreement with IDR results. The anhydrate was the most soluble and the DMSO solvate the least soluble. The DE solvate was also the most soluble of the solvates (43 µg/mL). There was no significant difference between the dissolved amounts after 60 minutes of the TEG hemisolvate and DMF solvate (± 39 µg/mL). The MeOH solvate was again more soluble than the DMSO solvate. The dissolved amounts of the different forms at 60 minutes decreased in the order anhydrate > DE solvate > TEG hemisolvate > DMF solvate > MeOH solvate > DMSO solvate.

The fact that the order of the amounts of solvates dissolved in 60 minutes and the IDRs are not identical to those obtained during solubility determinations may be ascribed to the differences in experimental conditions. During powder dissolutions, the media were 40% isopropanol:water mixtures at 37°C, while water at 25°C was used for the solubility determinations. The results suggest that the addition of the organic solvent to the dissolution medium not only increased the solubility but also increased the rate of change of the crystal forms to the monohydrate.

Affinity of the Solvates for Water

All the different crystal forms of niclosamide have a high affinity for water. The anhydrate sorbed moisture even when stored at room temperature in closed containers that were opened irregularly. Various stages of hydration of different raw materials were found during TGA determinations. The anhydrate stored at 37°C + 75% relative humidity (RH) transformed into monohydrate H_A within 2 weeks. During storage at 37°C + 75% RH, the MeOH and DMF solvates, and DE solvates transformed into monohydrate H_A within 1 and 2 weeks, respectively. The THF solvate stored at 37°C + 75% RH transformed into monohydrate H_A within 24 hours. The DMSO solvate stored as an aqueous suspension for 4 weeks showed a dehydration and desolvation endotherm during a DSC run. DSC studies showed that during solubility determinations and intrinsic dissolution testing, the anhydrate, MeOH, DE, THF solvates, and TEG hemisolvate transformed into monohydrate H_A. In the DMSO and DMF solvates, the crystallizing solvents were partially replaced by water. The THF solvate stored at room temperature in closed containers, desolvated and transformed into monohydrate H_A.

CONCLUSION

It was determined that the 6 solvates of niclosamide have a high affinity for water because all the forms transformed into a monohydrate (H_A) during dissolution and solubility testing. Therefore, the preparation of nonaqueous niclosamide solvates would not substantially increase the aqueous solubility or solve the problems associated with the hydration of niclosamide powder in suspensions.

ACKNOWLEDGEMENTS

This work was supported by grants from the National Research Foundation (Pretoria, South Africa), the Louisiana Board of Regents Enhancement Program (LEQSF(2001–02)-ENH-TR-82), and the National Science Foundation (BES-0210298).

REFERENCES

1. Reynolds JEF, ed. *Martindale the Extra Pharmacopoeia*. 30th ed. London, UK: The Pharmaceutical Press; 1993.

2. World Health Organization. *Niclosamide Technical*. Full specification WHO/SMT/6.R1. Geneva, Switzerland: World Health Organization; Revised 10 December 1999.

3. Budavari S, ed. *The Merck Index*. 11th ed. Rahway, NY: Merck; 1989.

4. van Tonder EC, de Villiers MM, Lötter AP, Caira MR, Liebenberg W. Correlation between hydrate formation and the physical instability of suspensions with different niclosamide crystal forms. *Pharm Ind*. 1998;60:722-725.

5. Otsuka M, Kato F, Matsuda Y. Physicochemical stability of cimetidine amorphous forms estimated by isothermal microcalorimetry. *AAPS PharmSciTech*. 2002;3(4):Article 30. Available at: http://www.pharmscitech.org.

6. Otsuka M, Ishii M, Matsuda Y. Effect of surface modification on hydration kinetics of carbamazepine anhydrate using isothermal microcalorimetry. *AAPS PharmSciTech*. 2003;4(1):Article 5. Available at: http://www.pharmscitech.org.

7. Caira MR, Nassimbeni LR. Phase transformations in inclusion compounds, kinetics and thermodynamics of enclathration. In: Attwood JL, Davies JED, Macnicol DD, Vögtle F, eds. *Comprehensive Supramolecular Chemistry*. Vol 6. New York, NY: Pergamon; 1996:132-145.

8. van Tonder EC, Maleka TSP, Liebenberg W, Song M, Wurster DE, de Villiers MM. Preparation and physicochemical properties of niclosamide anhydrate and two monohydrates. *Int J Pharm*. 2004;269:417-432.

9. Caira MR, van Tonder EC, de Villiers MM, Lötter AP. Diverse modes of solvent inclusion in crystalline pseudopolymorphs of the anthelmintic drug niclosamide. *J Inc Phenom Mol Recog Chem*. 1998;31:1-16.

10. Ford JL, Timmins P. *Thermal Analysis in the Characterization of Pharmaceutical Solids*. Chichester, UK: Ellis Horwood; 1989.

11. Singh P, Desai SJ, Flanagan DR, Simonelli AP, Higuchi WI. Mechanistic study of the influence of micelle solubilization and hydrodynamic factors on the dissolution rate of solid drugs. *J Pharm Sci*. 1968;57:959-965.

12. Lötter AP, Flanagan DR, Palepu NR, Guillory JK. A simple reproducible method for determining dissolution rates of hydrophobic powders. *Pharm Technol*. 1983;7:55-66.

13. United States Pharmacopoeial Convention. *United States Pharmacopoeia. USP* 25: NF 20. Rockville, MD: United States Pharmacopoeial Convention; 2002.

14. Guillory JK, Erb DM. Using solution calorimetry to quantitate binary mixtures of the three crystalline forms of sulfamethoxazole. *Pharm Manuf*. 1985;2:29-33Sept.

15. Botha SA, Caira MR, Guillory JK, Lötter AP. Physical characterization of the methanol solvate of urapidil. *J Pharm Sci*. 1992;78:28-34.

16. Moffat MA, Jackson JV, Moss MS, Widdop B, eds. *Clarke's Isolation and Identification of Drugs in Pharmaceuticals, Body Fluids, and Post Mortem Materials*. 2nd ed. London, UK: Pharmaceutical Press; 1986.

17. Kosheleva LI, Bekhli AF. Polymorphic modifications of phenasal and their IR spectra. *Khimiko-Farmatsevticheskii Zhurnal*. 1974;8:57-60.

18. Kosheleva LI, Shumakovish IE, Bekhli AF. IR spectra and structure of substituted salicylanilides. *Zhurnal Obshchei Khimii*. 1975;45:407-411.

19. Wall LA, Flynn JH. Quick, direct method for determination of activation energy from thermogravimetric data. *Pol Lett*. 1966;4:323-328.