DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY®
Vol. 30, No. 6, pp. 581–592, 2004

RESEARCH PAPER

Comparison of the Physical and Chemical Stability of Niclosamide Crystal Forms in Aqueous Versus Nonaqueous Suspensions

Melgardt M. de Villiers,[1,*] Mabatane D. Mahlatji,[2] Elsa C. van Tonder,[3] Sarel F. Malan,[4] Antonie P. Lötter,[3] and Wilna Liebenberg[3]

[1]Department of Basic Pharmaceutical Sciences, School of Pharmacy, University of Louisiana at Monroe, Monroe, Louisiana, USA
[2]Medicines Control Council, Pretoria, South Africa
[3]Research Institute for Industrial Pharmacy and
[4]Pharmaceutical Chemistry, School of Pharmacy, Potchefstroom University for CHE, Potchefstroom, South Africa

ABSTRACT

In an effort to produce physically stable and pharmaceutically acceptable suspensions of niclosamide, this study reports the differences in physical and chemical stability of aqueous vs. nonaqueous suspensions of a niclosamide anhydrate, two monohydrates H_A and H_B, a 1:1 niclosamide N,N-dimethylformamide solvate, a 1:1 niclosamide dimethyl sulfoxide solvate, a 1:1 niclosamide methanol solvate, and a 2:1 niclosamide tetraethylene glycol hemisolvate. Evaluation of aqueous and nonaqueous suspensions showed that in aqueous suspensions anhydrous, and solvated niclosamide crystal forms were transformed to a monohydrate, H_A, which was reasonably stable but which did eventually transform to the most stable monohydrate H_B. The order in which these crystal forms transformed to monohydrate H_B were: Anhydrate > N,N-dimethylformamide > dimethyl sulfoxide > methanol > tetraethylene glycol > monohydrate H_A. In a nonaqueous propylene glycol vehicle, the transformation to the monohydrous forms was not observed and on desolvation the solvated crystals transformed to the anhydrous form. In all cases, immediately upon desolvation or dehydration, the crystal structures of the desolvated materials were similar to that of the solvated materials. However, the isomorphic structures, formed after desolvation, were unstable and rehydrated or resolvated when exposed to the solvent or converted to the anhydrous

*Correspondence: Melgardt M. de Villiers, B.Pharm., M.Sc., Ph.D., Department of Basic Pharmaceutical Sciences, School of Pharmacy, University of Louisiana at Monroe, 700 University Ave., Monroe, LA 71209, USA; Fax: (318) 342-1737; E-mail: devilliers@ulm.edu.

DOI: 10.1081/DDC-120037489
0363-9045 (Print); 1520-5762 (Online)

form in a dry environment. The crystal forms remained chemically stable in both aqueous and nonaqueous suspensions for the length of the study.

Key Words: Niclosamide; Polymorphs; Suspensions; Aqueous vehicle; Nonaqueous vehicle.

INTRODUCTION

Niclosamide, Fig. 1, is an anthelmintic drug that is used in the treatment of worm infestations in humans and animals.[1] It is available mainly as two types of dosage forms, tablets and suspensions. Suspensions are mainly used for animals, especially cattle and sheep. The biggest problem with the formulation of niclosamide suspensions is that the anhydrous crystal form has an affinity for water and as such, on hydration, the suspension forms a cementlike sediment during storage or even during formulation.[2] These sediments are difficult to resuspend. In suspensions, such caking can be overcome by the addition of organic solvents or coating of the drug with water insoluble materials such as polyvinyl pyrrolidone (PVP) or encapsulated with polyethylene glycol (PEG). Niclosamide also crystallizes, as do a number of solvates that also turn into a stable monohydrate when suspended in water.[3–5] These solvates were obtained from methanol, dimethyl sulfoxide, N,N′-dimethylformamide, and tetraethylene glycol. Three of the solvates were 1:1 stoichiometric compounds, while the tetraethylene glycol solvate was a hemisolvate with a 2:1 niclosamide:solvent ratio.[6]

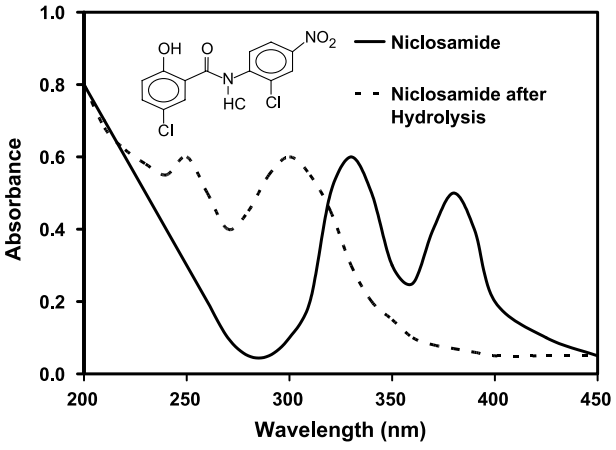

Figure 1. Molecular structure and UV spectrum of niclosamide before and after hydrolysis (MW = 327.13). (From Refs. [15,16].)

The interconversion of crystal forms can cause the physical instability of suspensions. Normally, crystal forms can be stabilized in suspensions in a variety of ways. The use of stable crystal forms is one the methods used to keep the suspension stable because the stable form does not undergo further transformation in suspension and consequently does not change in shape and size distribution.[7,8] When the metastable crystal form is used, it is advisable to add formulation additives that include a structurally related compound, viscosity imparting materials, or surfactants to stabilize the crystal form.[9] This, combined with the elimination of seeds of the stable—and in the case of water—the water-stable seeds normally would improve the stability of the metastable crystal form in suspension.[10] The use of additives and the elimination of seed crystals is also outlined in a description of the stabilization of a sulfamethaxazole suspension.[11]

Another important factor that determines the usability of the suspension, especially in view of the crystal transformations discovered in previous studies, is the chemical stability of niclosamide in the vehicles, because the nature of crystals also affects the chemical stability of the drug substance.[12] In addition, particles that are insoluble in liquid vehicles are unlikely to undergo chemical degradation, but most drugs in suspension have a finite solubility even though this may be in the order of a fraction of micrograms per mL; as a result, the material in solution may be susceptible to degradation.[13] Differences in the aqueous solubility of the niclosamide crystal forms could therefore lead to differences in the chemical stability in solution. Schultz and Harman[14] studied the hydrolysis and photolysis of niclosamide and found that no hydrolysis occurred in pond water or distilled water buffered at pH 5.0, 6.9, or 8.7 after 56 days. Wollmann and Zimmerman[15] confirmed these results and also found that the crystallization of water has no effect on the stability of niclosamide. Based on these studies it is claimed that the shelf-life of niclosamide can be extended to 10 years. However, these studies found that the drug is light sensitive, because when radio-labeled niclosamide solutions are exposed to long-wave ultraviolet (UV) for 24 and 168 hours less

than 50% and 5%, respectively, of the radioactivity of the parent compound was still present.[14]

Based on the results of studies that showed the difficulty in preparing physically stable and pharmaceutically acceptable niclosamide suspensions[2,5] and with the expectation that the physical and chemical stability of suspensions prepared with the niclosamide solvates will be different from the anhydrate and monohydrates, this study was undertaken to determine the effect of the crystal form on the properties of aqueous and nonaqueous suspensions of niclosamide. To assess the tendency towards caking, the sedimentation volume and resuspendability of suspensions prepared with seven niclosamide crystal forms, stored at elevated temperatures for up to 3 months, were measured. The chemical stability and crystal form changes during storage were also determined.

MATERIALS AND METHODS

Materials

Niclosamide was obtained from Sigma Chemical Company (St. Louis, MO). The following analytical grade solvents were obtained from Saarchem (Krugersdorp, South Africa), namely dimethyl sulfoxide, N,N-dimethylformamide, and tetraethylene glycol. Methanol (BDH, Poole, England) and ethanol (Merck, Darmstadt, Germany) were also used. Xanthan gum (high molecular weight polysaccharide gum, Spectrum Chemical Company, Gardena, CA), polyvinyl pyrrolidone (PVP K25, BASF, Ludwigshafen, Germany), sodium benzoate (Saarchem, Krugersdorp, South Africa), potassium sorbate (Saarchem, South Africa), and propylene glycol (Crodamol PC, Croda Chemicals, South Africa) were used to prepare the suspensions.

Preparation of Crystal Forms

Generally, niclosamide raw materials were found to be the anhydrate, but this form was also prepared by crystallization from absolute (water free) ethanol in a 5% w/v concentration. Raw material composed of a monohydrate, H_A, was obtained from Bayer (Midrand, South Africa) and Centrachem (Pretoria, South Africa). The second monohydrate, H_B, was formed through the transformation of the anhydrate into transparent, yellow, needlelike crystals when suspended in ethanol, ethyl acetate, benzene, or acetone and stored in open containers. The solvates were prepared as described by Van Tonder et al.[5] and Caira et al.[6] by crystallization from saturated solutions in dry methanol, N,N-dimethylformamide, dimethyl sulfoxide, and tetraethylene glycol. Solutions were covered and left at room temperature to crystallize. Crystals were stored in the solutions to prevent desolvation or hydration. Prior to making the suspensions, the crystal forms were removed from the mother liquors and allowed to dry on filter paper to remove the excess solvent, taking care that the solvates did not desolvate or were exposed to water, which would facilitate change to one of the monohydrated forms.

Characterization of the Crystal Forms

The crystal forms were characterized by their x-ray powder diffraction (XRPD) and thermal, DSC and TG analysis, properties. X-ray powder diffraction profiles of the solvates, air-dried and lightly screened through a 250 μm sieve, were obtained at room temperature with a Philips PM9901/00 diffractometer. The measurement conditions were: target, CuKα; filter, Ni; voltage, 40 kV; current, 20 mA; slit, 0.1 mm; and scanning speed, 2°/min.

Differential scanning calorimetry thermograms 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. The DSC curves were obtained under a nitrogen purge of 20–30 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. Thermogravimetric (TG) traces were obtained with either a Shimadzu TGA-50 (Shimadzu, Kyoto, Japan) or Hi-Res Modulated TGA 2950 (TA Instruments, New Castle, DE). The 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.

A scanning electron microscope (Philips, XL 30, Eindhoven, Netherlands) was used to obtain photomicrographs of the anhydrate and monohydrates H_A and H_B. Samples were mounted on a metal stub with an adhesive and coated under vacuum with carbon (Emscope TB500 sputter-coater, Emitech, Ashford,

England) before being coated with a thin gold-palladium film (Eiko Engineering Ion Coater IB-2, Hitachinaka, Japan).

Preparation of Aqueous Suspensions

A 0.1% xanthan gum suspension medium was prepared by introducing 1 g of xanthan gum into vigorously stirred 900 mL distilled water and filling it to 1000 mL with the rest of the water. Before suspension formulation, the niclosamide crystals were screened through a 85-mesh sieve to ensure the particle size of the different suspensions were approximately the same. One gram of the crystals, accurately weighed, was then suspended in 15 mL of the suspension vehicle with mild stirring. The suspension was then accurately filled up to 20 mL with the vehicle, producing a 5 % suspension of the solvate in the 0.1% xanthan solution. The second aqueous vehicle contained PVP, xanthan gum, potassium sorbate, and sodium benzoate. To prepare this vehicle, 1 g PVP K25, 0.1 g sodium benzoate, and 0.02 g potassium sorbate accurately weighted were dissolved in 40 mL of distilled water, and while stirring, the xanthan gum was introduce into the solution and the volume was made to 50 mL with distilled water. The niclosamide crystal forms were sieved and 1 g was introduced into about 15 mL of the well-stirred suspension vehicle. After all the particles were suspended, the final mixture was filled to 20 mL with the vehicle.

Preparation of Nonaqueous Suspensions

The nonaqueous suspensions contained only 5% w/v niclosamide in propylene glycol. The method of preparing this suspension involved the inclusion 1 g of the screened crystal forms into 15 mL of continuously stirred propylene glycol, and then filled to 20 mL with more of the excipient.

Stability Testing of Suspensions

The suspensions were prepared and then stored at room temperature for 3 months. The sedimentation volume and resuspendability were determined periodically. Most commonly, sedimentation volume, V_s, is defined as $V_s = H/H_0 \times 100$. Where H is the ultimate settling height and H_0 is the original height of the suspension before settling. 100 % is equal to no sedimentation and as the value decreases, the sedimentation increases. The chemical stability of the suspensions stored at 30°, 40°, and 50°C were determined using a calibrated UV-spectrophotometric method with measurements at 330 nm.[15,16] This method was applicable to all the solvates. However, an increase in the slopes of the lines with an increase of the molecular weight of the niclosamide solvates was found. The increase in the slope might be the effect of the solvent of crystallization liberated in solution, which affects the absorptivity of the drug molecule. However, the UV absorption of the main hydrolysis products, 5-chlorosalicylic acid and 2-chloro-4-nitroaniline, did not interfere with the UV absorption at 330 nm (Fig. 1).[15,16] Crystal transformations in the suspensions were followed by TG and DSC analysis. Results are the mean of three determinations.

RESULTS AND DISCUSSION

Previous studies have shown that niclosamide crystallizes in a number of solvates, two monohydrates, and the anhydrous form.[3,4] Additional studies also showed that several of these crystal forms were transformed to a monohydrous crystal form when suspended in water, and water:polyethylene glycol or

Table 1. Main XRPD diffraction peaks and desolvation and melting temperatures and weight loss upon heating of the niclosamide crystal forms.

Crystal form	Main XRPD peaks d/Å(I/I_0 %)	DSC peaks		TG weight loss (%)
		Desolvation (°C)	Melting (°C)	
Anhydrate	3.47(100); 3.33(96.2); 6.44(62.4); 6.81(46.0)	–	229.1	–
H_A	3.36(100); 3.28(79.6); 3.48(58.0); 7.76(34.6)	100.5	228.2	5.1
H_B	3.27(100); 2.82(60.7); 2.81(43.7); 3.43(36.3)	173.2	230.4	4.6
DMF	5.86(100); 9.76(66.1); 3.91(39.1); 3.95(28.6)	144.8	220.6	18.5
DMSO	3.90(100); 3.98(83.4); 3.32(79.7); 9.31(64.4)	158.3	217.4	19.1
MeOH	8.18(100); 5.67(85.7); 4.10(56.4); 4.06(16.8)	49.8	215.5	8.6
TEG	9.36(100); 3.38(52.24); 3.97(43.93); 3.33(42.44)	97.9	181.9	28.9

water:isopropanol mixtures.[5] To further characterize suspensions prepared with different niclosamide crystal forms, this study reports the differences in physical and chemical stability of aqueous vs. nonaqueous suspensions of a niclosamide anhydrate, two monohydrates H_A and H_B, a 1:1 niclosamide N,N-dimethylformamide solvate, a 1:1 niclosamide dimethyl sulfoxide solvate, a 1:1 niclosamide methanol solvate, and a 2:1 niclosamide tetraethylene glycol hemisolvate.

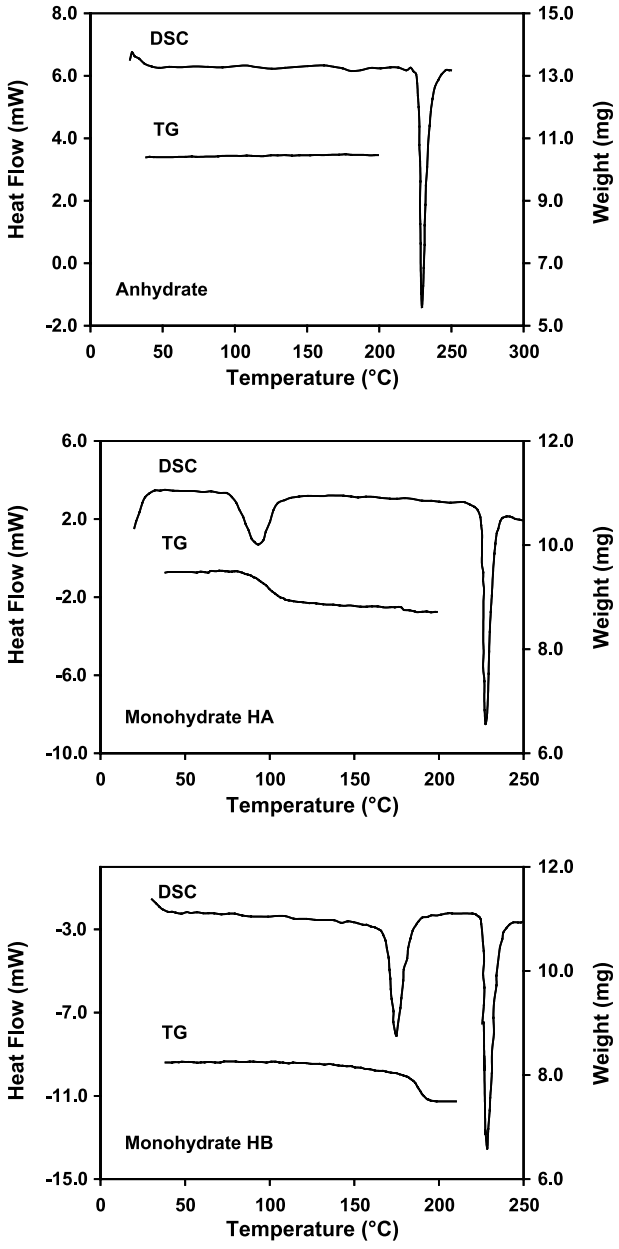

Figure 2. DSC and TG thermograms of the niclosamide anhydrate and two monohydrates.

Characterization of the Crystal Forms

The crystal forms were characterized by their XRPD patterns, melting points, and thermogravimetric weight loss upon heating. These results are summarized in Table 1 and DSC and TG analysis results for the crystal forms are given in Figs. 2 and 3. X-ray and thermal analysis were the same as reported earlier.[3,4] Each solvate has a distinct desolvation temperature, and the weight loss upon desolvation corresponded with the molecular ratio of solvent to drug molecules in the crystal structures, Table 1. Upon desolvation, the solvates transformed to the stable anhydrous polymorph, which melted between 215–230°C. However, the final melting point of the desolvated TEG solvate was lower, 181°C, because this solvent does not evaporate upon heating and the niclosamide dissolves in the TEG with an increase in temperature. This is indicated by the broad melting endotherm, Fig. 3, which ranged from about 175–220°C.

Effect of Moisture and Temperature on Crystal Transformations

Before suspensions were prepared, the physical stability of the crystal forms were studied by comparing changes in XRPD, DSC, and TG analysis results to the data listed in Table 1. Firstly, it was confirmed that niclosamide anhydrate is hygroscopic, because several raw material samples already contained variable amounts of moisture. Crystals of the raw materials containing small amounts of water are needles, while crystals of the anhydrate consist of small rectangles and squares. This hinted at the transformation of the anhydrous form to a monohydrous form on exposure to moisture. This was confirmed when dried raw material samples were stored in water, Fig. 4, and the following moisture absorptions (% by weight) were determined by TG analyses: 0.5% after 1 week and 1.2% after 4 weeks at 25°C+30% relative humidity (RH) and 3.4% after 1 week, 4.5% after 2 weeks, 4.6% after 3 weeks, and 4.9% after 4 weeks at 37°C+75% RH. Complete dehydration of monohydrate H_A was achieved by heating crystals at 100°C for only 15 minutes.

X-ray powder diffraction analysis showed that the crystal structure of H_A immediately after dehydration did not change, Fig. 5; however, after continued drying the anhydrate was formed because the XRPD patterns of the dried dehydrated H_A and anhydrous raw material were the same. It also remained stable when stored at room temperature in open containers, and a sample stored at 37°C in an open container for 1 month also remained stable. Monohydrate H_A remained stable for

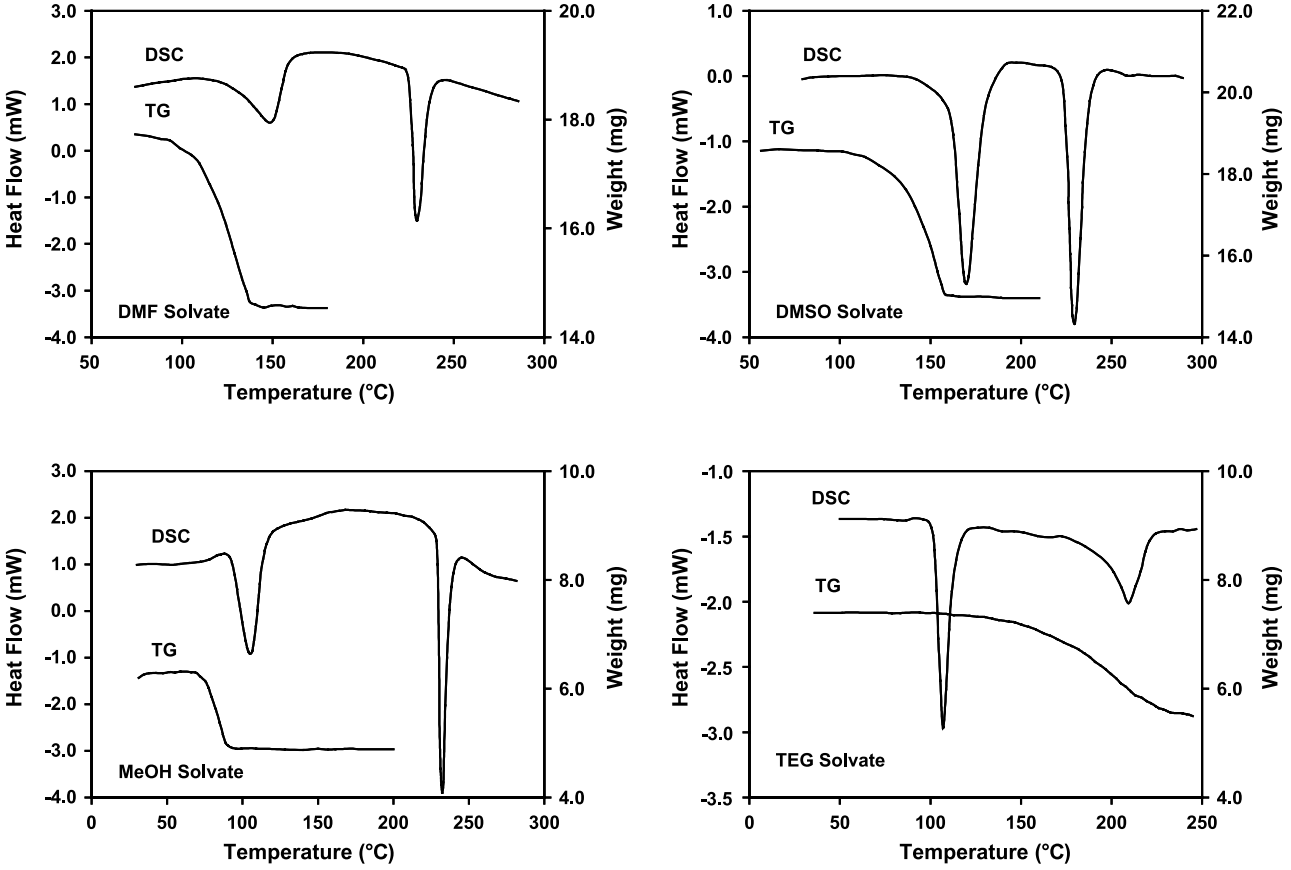

Figure 3. DSC and TG thermograms of the niclosamide solvates.

2 months when stored at 25°C+30% RH and 37°C+75% RH. Monohydrate H_B stored at 37°C for 1 month and at room temperature remained stable, and heating at 100°C for 15 minutes did not dehydrate monohydrate H_B. However, heating crystals at 200°C for 15 minutes resulted in dehydration. Crystals of monohydrate H_B, when stored in open containers in desiccators at 25°C+30% RH and 37°C+75% RH, remained stable for 2 months. X-ray powder diffraction and thermal analysis showed that the crystal structures of H_A and H_B changed with dehydration relative to those of the anhydrate.[4]

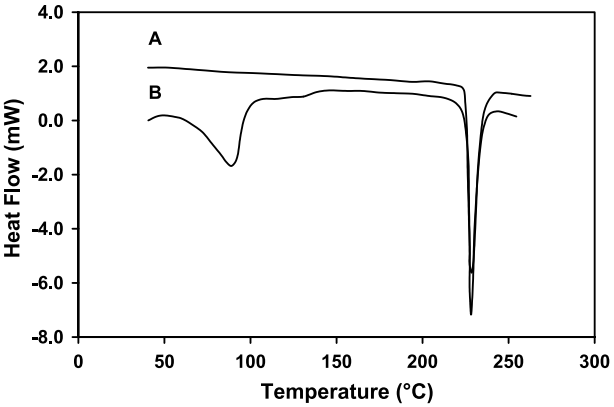

Figure 4. DSC thermograms showing the change of niclosamide anhydrate (A) to monohydrate H_A (B) in an aqueous suspension stored at 25°C for 3 months.

Crystals of the MeOH solvate remained stable and did not desolvate when removed from the mother liquor. Heating of the crystals to 50°C for 24 hours only partially desolvated the crystals, but heating to 100°C for 15 minutes removed the MeOH completely. X-ray powder diffraction patterns before and after desolvation showed that MeOH solvate desolvated to form the anhydrate. When the desolvated crystals were stored in a desiccator containing MeOH for 2 to 7 days at 25°C, TG analysis showed that the crystals were resolvated. However, when MeOH-solvate crystals were stored in desiccators, the solvate was transformed into monohydrate H_A within 1 week at 37°C+75% RH and within 3 weeks at 25°C+30% RH for 3 weeks. The transformation to monohydrate H_A was confirmed by DSC and TG traces and SEM microscopy, Fig. 6. This

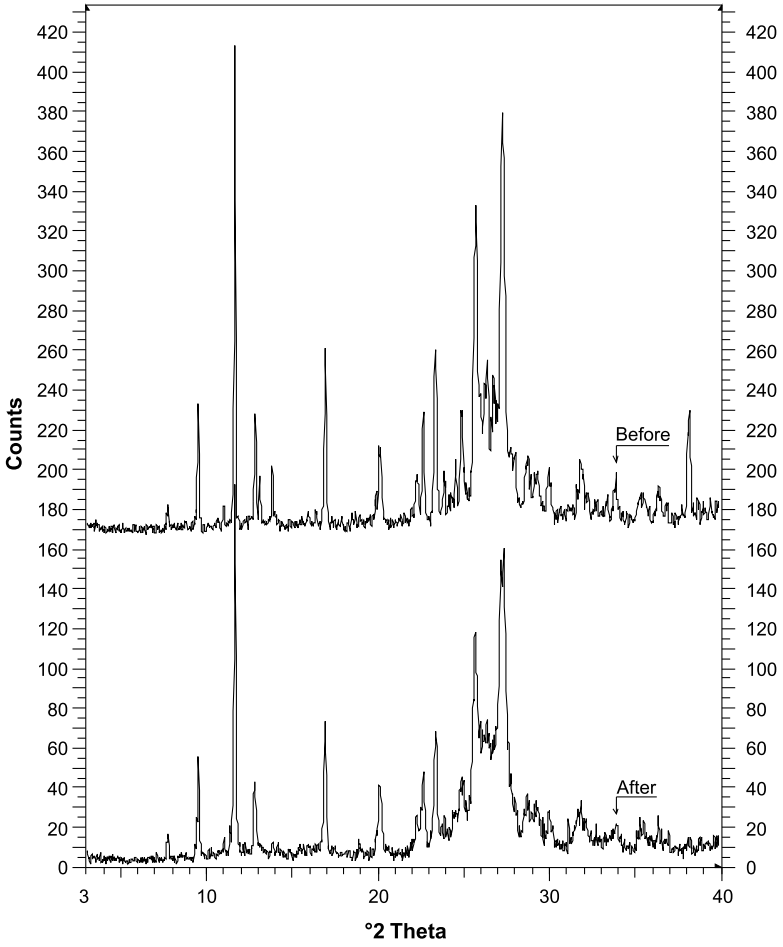

Figure 5. Identical XRPD patterns of niclosamide monohydrate H_A immediately before and after dehydration.

micrograph clearly shows that these desolvated crystals maintain the basic structure of the MeOH solvate, but channels are formed due to desolvation. The monohydrous H_A crystals then grow from the surface of these desolvated crystals, as indicated by the needles protruding from the surface of the desolvated crystals.

Crystals of the DMSO solvate remained stable after removal from the crystallizing solvent and storage in closed containers at room temperature. Heating of the crystals at 170°C for 15 minutes removed the DMSO and XRPD patterns of the desolvated crystals, and anhydrate were again the same. When desolvated crystals were stored in DMSO for 1, 2, and 7 days at 25°C, TG traces showed that 8.3%, 16.1%, and 18.9% DMSO were absorbed, respectively. This means that DMSO solvates with stoichiometric ratios of 1:0.38, 1:0.80, and 1:0.98 of niclosamide:DMSO were formed, respectively. When DMSO-solvate crystals were stored in desiccators at 25°C+30% RH and 37°C+75% RH; DSC curves showed that some of the DMSO was

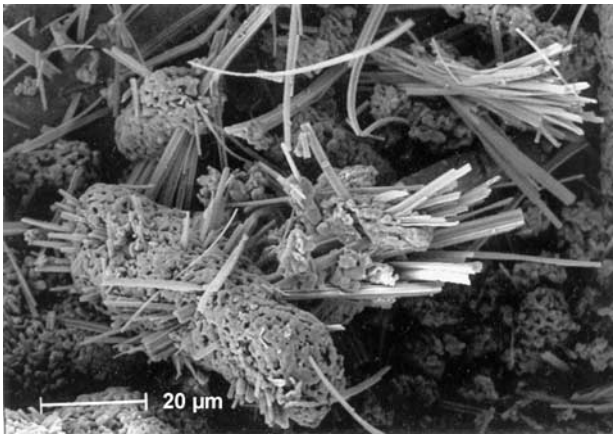

Figure 6. SEM photomicrograph of desolvated MeOH solvate crystals, showing desolvation as channels on the surface of the crystals. The protruding needles show the growth of monohydrous crystals from the surface of the desolvated crystals. (*View this art in color at www.dekker.com.*)

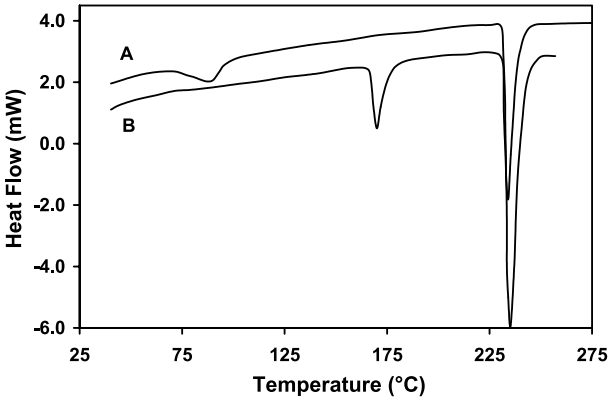

Figure 7. DSC thermograms showing the change of monohydrate H_A (A) to monohydrate H_B (B) in an aqueous suspension stored at 25°C for 6 months.

replaced by water, because dehydration and desolvation peaks corresponding to those of monohydrate H_A were observed. After an initial replacement of the DMSO with water, the system stabilized and less DMSO was replaced.

N,N-Dimethylformamide solvate crystals removed from the crystallizing solvent and stored in closed containers at room temperature remained stable, but heating of the crystals to 150°C for 15 minutes removed the DMF. Again, XRPD patterns of the solvated and desolvated crystals immediately after desolvation were the same, but on storage the desolvate crystals changed to the anhydrate. When desolvated DMF-solvate crystals were stored in DMF for 1 and 7 days at 25°C, TG curves showed that 17.7% and 18.1 % DMF were absorbed, corresponding to DMF solvates with stoichiometric ratios of 1:0.96 and 1:0.99 of niclosamide:DMF, respectively. Differential scanning calorimetry analysis confirmed that the DMF solvate transformed into monohydrate H_A during storage for 1 week at 37°C+75% RH and within 3 weeks at 25°C+30% RH, because the desolvation endotherms was lowered from±150°C to±100°C.

The tetraethylene glycol (TEG) hemisolvate remained stable when stored at room temperature in closed containers. Crystals of the TEG hemisolvate only desolvated when washed with diethyl ether and heated at 200°C for 30 minutes. It was difficult to remove TEG from the solvated crystals because TEG is a highly viscous, nonvolatile liquid with a boiling point of 328°C. A DSC trace of the desolvated crystals showed the formation of the anhydrate, because only one endotherm at±230°C was observed. In warm water TEG-hemisolvate crystals were transformed into monohydrate H_A, because DSC traces obtained afterwards showed peaks corresponding to monohydrate H_A and a TG analysis showed a mass loss of±5% at a temperature range corresponding to that of monohydrate H_A (Fig. 2).

In this study the desolvates, produced by desolvation of hydrated or solvated crystalline niclosamide, retained most of the three-dimensional order of the solvated lattice. This was demonstrated by similarities in their diffraction patterns immediately before and after desolvation, Fig. 5. Also, when the samples were re-exposed to atmospheric moisture or the solvents, it was extremely hygroscopic, rapidly gaining several percent of water or solvent. However, the desolvated lattices were physically unstable relative to the solvated lattice, because while studying these ''isomorphic desolvates,'' we found that when the desolvates were kept in a dry environment (<20% RH), the crystal lattices relaxed over time to a more efficiently packed structure with XRPD patterns similar to those of the anhydrate.[17] However, in a moisture rich environment the crystals convert to the monohydrous form, Fig. 6. Processing properties, chemical stability, and the ability to develop stable suspensions

Table 2. Sedimentation volumes and resuspendability of the xanthan gum aqueous suspensions containing the different niclosamide crystal forms.

	Sedimentation volume %				Resuspendability			
Crystal form	1 Day	2 Day	3 Day	4 Day	1 Day	2 Day	3 Day	4 Day
Anhydrate	80±2	80±3	80±5	S	Easy	Easy	Difficult	Caked
H_A	100±3	100±2	100±2	100±1	Easy	Easy	Easy	Easy
H_B	100±2	100±3	100±1	100±1	Easy	Easy	Easy	Easy
DMF	100±2	90±2	70±3	S	Easy	Easy	Difficult	Caked
DMSO	90±3	90±3	80±4	S	Easy	Easy	Easy	Caked
MeOH	100±3	90±4	70±5	100±1	Easy	Easy	Easy	Difficult
TEG	100±2	100±2	90±3	80±4	Easy	Easy	Easy	Easy

Note: S = separated into three layers.

Table 3. Sedimentation volume and resuspendability of PVP aqueous suspensions of the niclosamide crystal forms.

	Sedimentation volume %			Resuspendability		
Crystal	1 Month	2 Month	3 Month	1 Month	2 Month	3 Month
Anhydrate	100±1	50±4	25±4	Easy	Easy	Easy
H_A	100±0	95±4	90±3	Easy	Easy	Easy
H_B	100±1	100±1	100±1	Easy	Easy	Easy
DMF	100±1	95±2	40±4	Easy	Easy	Easy
DMSO	100±1	90±2	50±5	Easy	Easy	Easy
MeOH	100±1	98±1	70±2	Easy	Easy	Easy
TEG	100±1	100±2	98±3	Easy	Easy	Easy

may be impacted by these properties if controls on drying and storage processes are not maintained to ensure that the optimal crystalline form is produced and maintained. Monohydrate H_A was reasonably stable, but did change to H_B, Fig. 7, when suspended in water for 6 months.

Physical Stability of the Suspensions

Except for the suspension of the TEG solvate and the monhydrates, suspensions prepared in 0.1% xanthan gum in water were resuspendable for a period of only 4 days, Table 2, without caking, but caked so much within 6 days that it was not resuspendable. Within this time the sedimentation volumes fluctuated between 70% and 100%. Some suspensions even separated into three layers. In the xanthan gum vehicle, the TEG-solvate suspension remained resuspendable for at least 2 weeks. The suspensions that were prepared in the PVP vehicle, Table 3, all remained resuspendable for the 3 months they were tested. The sedimentation volume for all the suspensions stored at room temperature remained 100% for up to 1 month, but after 3 months the sedimentation volumes ranged from 25–98%. However, the suspensions were easily resuspended. When suspended in propylene glycol, which is more viscous than water, the suspensions settled rapidly with a clear supernatant forming within a couple of hours. However, the suspensions were easily resuspended, Table 4, showing that these suspensions exhibited the behavior of flocculated systems. The suspensions remained resuspendable for the 3 months they were tested.

The physical stability of the propylene glycol suspension together with the stability evaluation of the aqueous suspension indicated that water and the additives added during the preparation of aqueous suspensions had a significant effect on the suspension stability of suspensions prepared with niclosamide crystal forms. Nonaqueous vehicles seemed to be best for preparing niclosamide suspensions, but aqueous suspensions can be improved when potassium sorbate and PVP were added to a xanthan gum suspension. These additives kept the suspensions uniform for at least 1 month.

Crystal Transformations in the Suspensions

Based on the results of the studies characterizing the crystal transformations of the polymorphs, DSC and TG analysis was used to quantify changes in the crystal forms in the suspensions stored at 30° and 40°C. In the xanthan gum vehicle all the crystal forms

Table 4. Sedimentation volume and resuspendability of nonaqueous propylene glycol suspensions of the niclosamide crystal forms.

	Sedimentation volume %			Resuspendability		
Crystal	1 Month	2 Month	3 Month	1 Month	2 Month	3 Month
Anhydrate	20±4	20±6	20±7	Easy	Easy	Easy
H_A	100±1	100±0	100±1	Easy	Easy	Easy
H_B	100±2	100±1	100±1	Easy	Easy	Easy
DMF	50±4	40±3	40±6	Easy	Easy	Easy
DMSO	25±6	20±6	20±5	Easy	Easy	Easy
MeOH	55±4	50±5	50±4	Easy	Easy	Easy
TEG	20±5	20±5	20±6	Easy	Easy	Easy

Table 5. Changes in desolvation and melting temperatures, and TG weight loss of niclosamide crystal forms suspended in the xanthan gum vehicle after storage for 3 months at 30° and 40°C.

	Stored at 30°C		Stored at 40°C			
Crystal form	Desolvation (°C)	Melting (°C)	Desolvation (°C)	Melting (°C)	Weight loss 30°C (%)	Weight loss 40°C (%)
Anhydrate	98.5	227.1	101.3	228.6	5.2	4.6
H_A	100.2	226.3	100.6	229.0	4.8	5.0
H_B	93	229.7	173.2	230.1	4.7	4.8
DMF	102.3	228.5	101.8	230.3	18.5	18.5
DMSO	98.7	229.8	100.3	227.9	19.1	19.1
MeOH	99.8	230.1	99.7	225.9	8.6	8.6
TEG	100.1	191.2	96.5	187.4	26.3	25.3

except the TEG hemisolvate and monohydrate H_B changed to monohydrate H_A (Fig. 4). This change was confirmed by DSC and TG analysis, Table 5, because in each case the desolvation temperature was lowered to around 100°C with a weight loss of about 5%. In the PVP vehicle, the transformations were similar and DSC and TG results closely matched those of the crystals recovered from the xanthan gum vehicle. After being suspended for 3 months in propylene glycol, none of the crystal forms changed to monohydrate H_A, but small decreases in weight loss were observed. This suggests that when changes in the crystal form occurred, the crystals were desolvated to the anhydrate, rather than hydrated to monohydrate H_A, since the vehicle was nonaqueous. However, when the propylene glycol suspensions were diluted with water, transformation to monohydrate H_A started almost immediately and rapidly progressed until DSC and TG did not detect any of the original solvates in the suspensions prepared with the DMF, DMSO, anhydrate, and MeOH solvates, Table 5.

Chemical Stability of the Suspensions

Another important factor that determines the usability of the suspension, especially in view of the crystal transformations discovered in this study, is the chemical stability of niclosamide in the vehicles. In this study, a UV spectrophotometric method was used to estimate the chemical stability of niclosamide crystal forms in the nonaqueous and aqueous suspensions.[15,16] Ultraviolet analysis obeyed the Lambert-Beer's law, and the alkaline hydrolysis products 5-chlorosalicylic acid and 2-chloro-4-nitroaniline did not interfere with the UV-absorption at λ_{max}, 330–380 nm (Fig. 1).[16] Due to the high sedimentation rate of some of the suspensions, it was very difficult to draw a uniformly distributed sample, and that allowed some variation in the assay results, Fig. 8. Assay results for

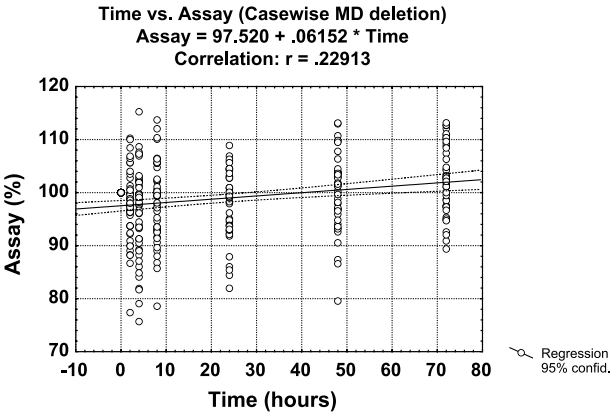

Figure 8. Correlation matrix showing the change in assay results (quality) of the suspensions with an increase in time in the xanthan, PVP, and propylene glycol vehicles stored at 30°, 40°, and 50°C (values shown are that of three suspensions at each temperature).

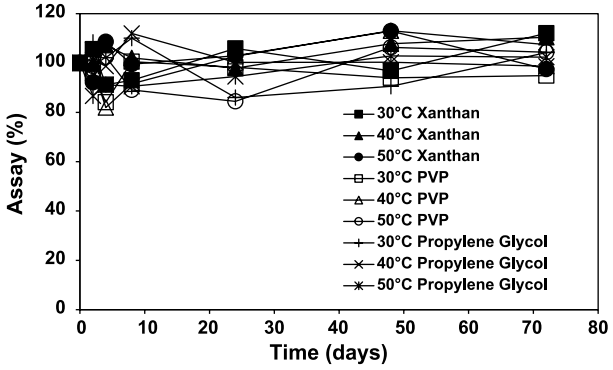

Figure 9. Assay results for the anhydrate suspended in different vehicles and stored at different temperatures.

niclosamide, even the least physically stable anhydrate, Fig. 9, in the suspensions indicated that the crystal forms were chemically stable in the aqueous and nonaqueous suspensions, because there was no significant decrease in the concentration of the drug. The mean assay results for all the suspensions tested at all the temperatures were 98.9±2.7%, well within limits set for suspension quality in major pharmacopoeia.[18] These results showed that the chemical stability of the niclosamide crystal forms was not adversely affected by the suspension vehicle composition or the presence of the solvent in the crystal lattice.

CONCLUSIONS

Evaluation of aqueous and nonaqueous suspensions prepared with anhydrous, monohydrous, and solvated niclosamide crystal forms showed that in aqueous suspensions these crystal forms were transformed to a monohydrate, H_A, which was reasonably stable but which did eventually transform to the most stable monohydrate H_B. The order in which these crystal forms transformed to monohydrate H_B: Anhydrate > DMF > DMSO > MeOH > TEG > H_A. In a nonaqueous vehicle comprised of propylene glycol, the transformation to the monohydrous forms was not observed and on desolvation the solvated crystals transformed to the anhydrous form. In all cases, immediately upon desolvation or dehydration, the crystal structures of the desolvated materials were similar to that of the solvated materials. However, these isomorphic structures, formed after desolvation, were unstable and rehydrated or resolvated when exposed to the solvent or converted to the anhydrous form in a dry environment. The crystal forms remained chemically stable in both aqueous and nonaqueous suspensions within the time the study was conducted.

ACKNOWLEDGMENTS

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. Niclosamide Technical. *Full Specification WHO/SMT/6.R1*; World Health Organization Revised: Geneva, 10 December 1999; 13 pp.
2. Van Tonder, E.C. *Preparation and Characterisation of Niclosamide Crystal Modifications*, Ph.D. Thesis; Potchefstroom University for CHE: South Africa, 1996; 261 pp.
3. Van Tonder, E.C.; Lötter, A.P.; De Villiers, M.M.; Caira, M.R.; Liebenberg, W. Correlation between hydrate formation and the physical instability of suspensions prepared with different niclosamide crystal forms. Pharm. Ind. **1998**, *60*, 722–725.
4. Van Tonder, E.C.; Maleka, T.S.P.; Liebenberg, W.; Song, M.; Wurster, D.-E.; De Villiers, M.M. Preparation and physicochemical properties of niclosamide anhydrate and two monohydrates. Int. J. Pharm. **2004**, *269*, 417–432.
5. VanTonder, E.C.; Mahlatji, M.D.; Malan, S.F.; Liebenberg, W.; Caira, M.R.; Song, M.; DeVilliers, M.M. Preparation and physicochemical characterization of five niclosamide solvates and one hemisolvate. AAPS PharmSciTech **2004**, *5* (1), *in press.* http://www.pharmscitech.org.
6. Caira, M.R.; Van Tonder, E.C.; De Villiers, M.M.; Lötter, A.P. Diverse modes of solvent inclusion in crystalline pseudopolymorphs of the anthelmintic drug niclosamide. J. Incl. Phenom. Mol. Rec. Chem. **1998**, *31*, 1–16.
7. Khalil, S.A.; Motawi, M.M.; Ebian, A.R.; Moustafa, M.A. Effect of additives on the kinetics of interconversion of sulphamethoxydiazine crystal forms. J. Pharm. Pharmacol. **1973**, *25*, 13–20.
8. Khalil, S.A.; Motawi, M.M.; Ebian, A.R.; Moustafa, M.A. Succinylsulfathiazole crystal forms: crystal growth studies. J. Pharm. Sci. **1975**, *64*, 1481–1489.
9. Martin, A. Coarse dispersion. In *Physical Pharmacy*; Lea & Febiger: Philadelphia, 1993; 477–511.
10. Pearson, J.T.; Varney, G. Crystal studies involving phase transitions in aqueous drug suspensions. J. Pharm. Pharmacol. **1969**, *21*, 60s–90s.
11. Abdallah, O.; Graf, E.; Beyer, C. The physical instability of sulfamethoxazole suspensions. Pharm. Ind. **1982**, *44*, 1071–1074.
12. Haleblian, J.K.; Mccrone, W. Pharmaceutical applications of polymorphism. J. Pharm. Sci. **1969**, *58*, 911–929.
13. Zografi, G.; Schott, H.; Swarbrick, J. Dispersed systems. In *Remington's Pharmaceutical Sciences*, 18th Ed.; Genarro, A.R., Ed.; Mack: Pennsylvania, 1990; 257–309.
14. Schultz, D.P.; Hardman, P.D. Hydrolysis and photolysis of the lampricide 2′,5-dichlorosalicylanilide (Bayer 73). Invest. Fish Control **1978**, *3*, 83–85.
15. Wollmann, H.; Zimmermann, M. Stability testing of niclosamide and vanillin. Part 20. Stability of

pharmaceuticals. Zentralblat Fuer Pharmazie. Pharmakotherapie und Laboratoriumsdiagnostik **1983**, *126*, 571–579.
16. Bergisadi, N.; Sarigul, D. Determination of niclosamide and its stability in simulated gastric and intestinal media. Acta Pharm. Turc. **1986**, *28* (2), 51–54.
17. Stephenson, G.A.; Groleau, E.G.; Kleeman, R.L.; Xu, W.; Rigsbee, R.D. Formation of isomorphic desolvates: creating a molecular vacuum. J. Pharm. Sci. **1998**, *87*, 536–542.
18. United States Pharmacopoeia. *USP 25: NF 20*; United States Pharmacopoeial Convention: Rockville, MD, 2002; 2569 pp.

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