DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 21(5), 523-536 (1995)

RESEARCH PAPERS

ISOENERGETIC POLYMORPHS

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ABSTRACT

Polymorphs are attracting a great deal of attention in present-day pharmaceutical literature. Most often the polymorphic forms are distinguished by way of solubility (or dissolution rates), DSC and X-ray diffraction. The writing to follow deals with a polymorphic pair which is virtually indistinguishable except by means of X-ray diffraction, and the essence of the report is that failing to show significant differences in solubility, and observing no, or only slight differences in melting point (which could be due to impurities) is not sufficient to rule out polymorphism[1-6]. The compound N-[2-(((-5-((Dimethylamino)methyl]-2-furanyl]thio]ethyl-N'-methyl-2-nitro-1,1-ethenediamine hydrochloride, present in two polymorphic forms, I and II, is used as a model compound.

INTRODUCTION

Although somewhat of a text book nature, the general vapor pressure diagrams of two polymorphs are shown in Fig. 1 The melting points of forms i to iv are denoted by A,C,D and E respectively, and the transition point between i and ii is denoted B. If the melt curve is removed, the y-axis could be represented a solubility.

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[a]Submitted for publication 25 August, 1994

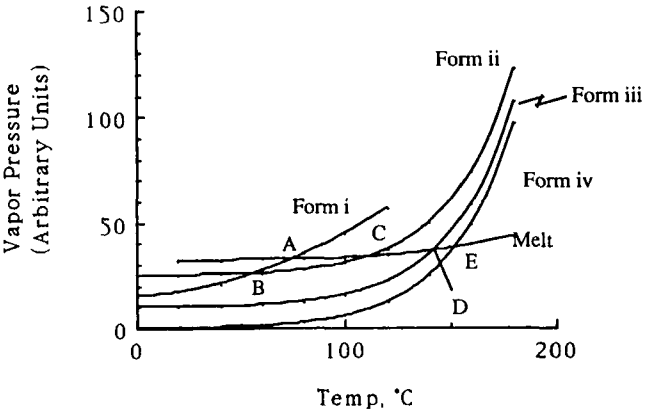

Figure 1 Schematic of polymorphism. The melting points of forms i to iv are denoted by A,C,D and E respectively, and the transition point between i and ii is denoted B. If the melt curve is removed, the y-axis could be represented a solubility. i and ii form an enantiotropic pair, (ii), iii and iv form monotropic pairs.

The general descriptive terms for the pairs would be that i and ii would form an enantiotropic pair and that iii and iv would form a monotropic pair. The latter is characterized by the vapor pressure curves not intersecting. For this to be true, either their slopes must be identical, i.e. their heats of solution must be identical, or if that is not the case, then the intersect must be below absolute zero or above the melting point.

If the curves are very close together, they can be difficult to distinguish. For instance in the case of iii and iv, if the solubilities were close throughout the temperature range, then the melting points would be close as well, to such a degree that the differences that might be noticed might be attributed to impurities.

It would also be difficult, if the heats of solution were close, so close that they were within experimental error, to determine whether one were dealing with a monotropic pair or an enantiotropic pair. Only by X-ray diffraction would it be possible to establish that there was indeed two different polymorphs, and only by having one polymorphic form, at

some temperature, convert to the other, would it be possible to state that the pair were enantiotropic not monotropic. Failing to observe such a conversion results in lack of conclusion, because the potential for conversion could be there even if the event did not occur.

The propensity for conversion is, for ideal concentration ranges, the Gibbs energy change, ΔG, in such a transformation and is given by:

$$\Delta G = -RT\ln[S_{metastable}/S_{stable}] \quad (1)$$

where S denotes solubility, R the gas constant, and T absolute temperature[1]. It is the purpose of this note to report on a case where this situation exists, viz. two polymorphic forms, I and II of the compound N-[2-(((-5-((Dimethylamino)methyl]-2-furanyl]thio]ethyl-N'-methyl-2-nitro-1,1-ethenediamine hydrochloride.

MATERIALS AND METHODS

The two polymorphic forms of forms N-[2-(((-5-((di-methylamino)methyl]-2-furanyl]thio]ethyl-N'-methyl-2-nitro-1,1-ethehediamine hydrochloride were obtained from Geneva Laboratories, Broomfield, Co. Both are off-white powders. All other chemicals used were reagent grade, except water (Milli^{Q} Water Apparatus) and methanol (HPLC grade).

Type I Flint Glass Vials (Wheaton) with Teflon-Faced Grey Butyl Stoppers (Wheaton) and 13 mm Aluminum Crimp Seals were used for storing slurries of excess drug with H_20 or 0.1 N HCl. Autosampler vials with Teflon septa were from Sunbrokers, Inc., Wilmington, NC.

The DSC instrumentation used was a SEIKO (Elk Grove Village, Illinois) Differential Scanning Calorimeter (DSC): DSC 220C Automatic Cooling (liquid N_2) with Model SSC 5200H Disk Station and Software Series v. 1.0; aluminum sample pans; were used and the instrument calibrated with Indium at 10°C/min

The X-ray diffraction pattern determinations were carried out on a Siemens Analytical X-Ray Instruments, Inc., Nicolet I_2/V Diffractometer, Cu K α radiation, 40 KV, 35 mA,

Step: 0.04° 2 theta; Incident Beam Slit, 3 mm; Diffracted beam slit, 1 mm.

Assays were carried out using HPLC manufactured by Waters consisting of 484 Tunable Absorbance Detector, set at 322 nm using a Waters WISP 712 25 mcL autosampler and a Waters 501 HPLC 1.5 mL/minsolvent delivery system; a FIAtron CH30 column heater with TC-50 controller, 30°C was used; the column: used was a Beckman Ultrasphere Octyl 5 micron, 4.6 x. 25 cm column. With this system N-[2-(((-5-((Dimethylamino)methyl]-2-furanyl]thio]ethyl-N'-methyl-2-nitro-1,1-ethenediamine hydrochloride has a retention time of 3.2 min. Data acquisition and processing was accomplished via a Rainin Dynamax Method Manager Software system using a Macintosh SE Computer

Heats of solutions were determined experimentally using a Parr 1451 Solution Calorimeter, Moline, Ill.

Microscopy was done using a Nikon Polarizing Microscope Model POH-II

Samples when tested isothermally were stored in a thermostatically-controlled Circulating Water Bath. pH was determined by a pH-meter made by Orion (Research Digital Ionalyzer/501 pH meter).

Dilutions and samplings were made with manual pipets from ICN Biomedicals, Inc. (Digiflex Dilutor and Pipetman manual pipets).

The centrifuges used were Eppendorf 5414 and 5415C Centrifuges.

RESULTS AND DISCUSSION

X-ray diffraction was carried out and showed Form I to be a different crystal modification from Form II. The powder X-ray diffraction patterns of Forms I and II are shown in Figures 2 and 3; it is seen that they are different, the peak at 2 Theta = 20.2 is absent in Form I and the peak 2 Theta = 21.8 is absent in Form II. More discussion of the X-ray diffraction will be stayed until a later point in the discussion, the point being made here being that Forms I and II were indeed of different crystal modification.

The question then arises which is the more stable form at what temperature and is the pair a monotropic pair or an enantiotropic pair. To attempt to answer this, attention is first

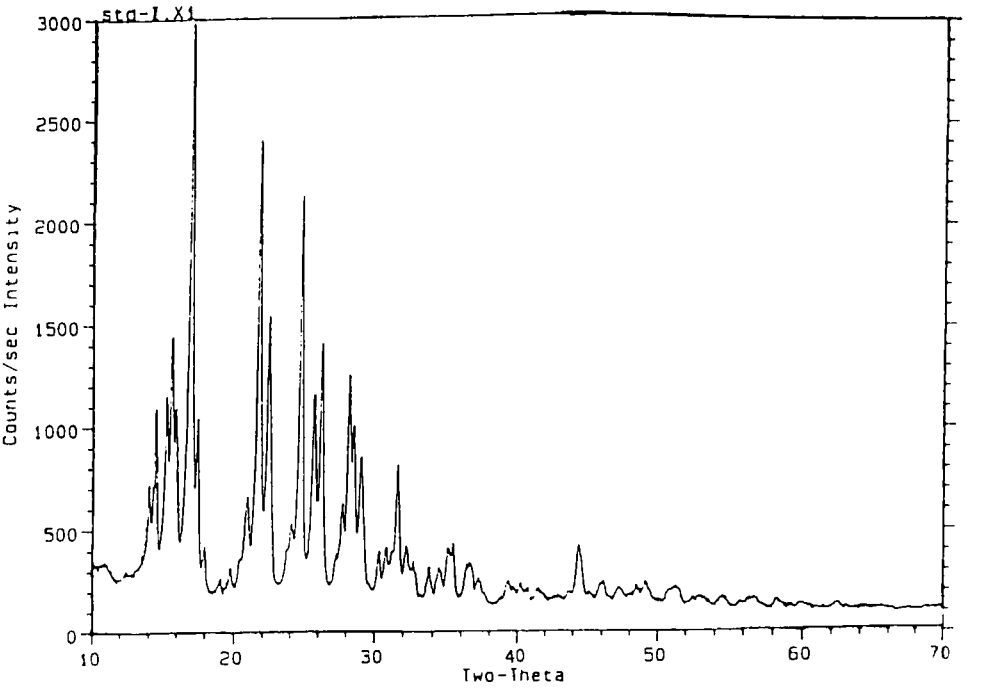

Figure 2 Powder X-ray diffraction pattern for Form I

focused on the results from DSC work. The traces were neat, without signs of transition points at all the heating rates used as shown in Table 1.

The melting points are shown as a function of heating rate in Fig 4.

The fact that form I always has a lower melting point at higher temperatures implies that it is the form which is metastable (more energetic) at these higher temperatures. For instance forms iii and iv in Fig. 1 constitute such a situation if the pair is monotropic. In that case Form I of N-[2-(((-5-((Dimethylamino)methyl]-2-furanyl]thio]ethyl-N'-methyl-2-nitro-1,1-ethenediamine hydrochloride would be equivalent to form iii with melting point D<E. If one were dealing with enantiotropism, the situation would be as shown for forms i and ii in Fig. 1. In that case Form I of N-[2-(((-5-((Dimethylamino)methyl]-2-furanyl]thio]ethyl-N'-methyl-2-

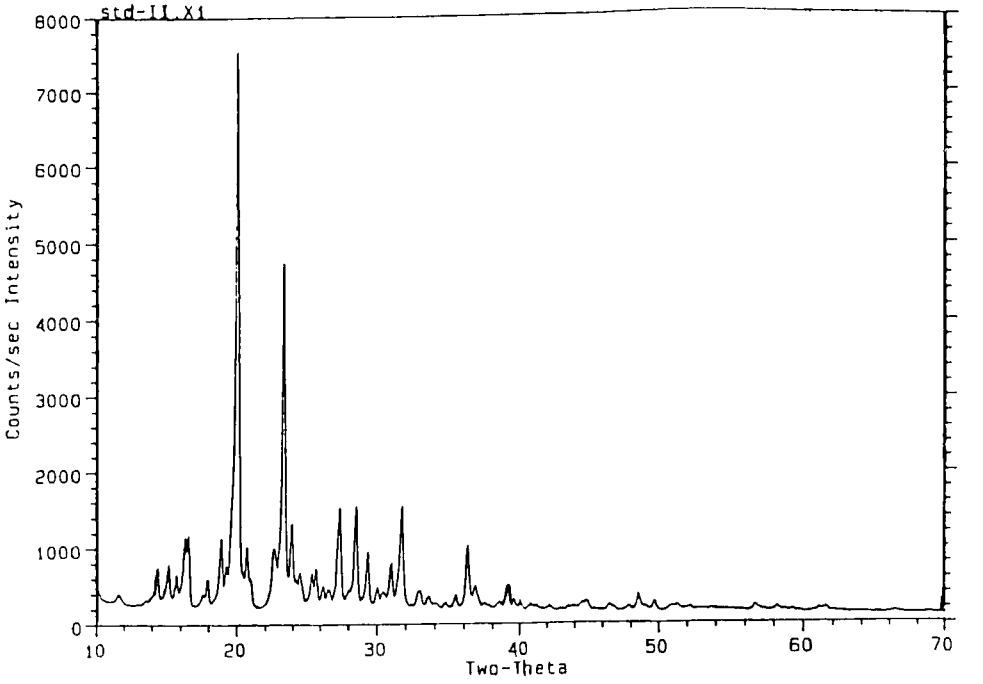

Figure 3 Powder X-ray diffraction pattern for Form II

nitro-1,1-ethenediamine hydrochloride would be equivalent to form i, with melting point A< B.

If solubilities could be carried out at this high tempeature, form I would have a higher solubility than Form II. The fact that no transition occurs during the DSC experiment in itself does, however, not prove that no transition could occur. Experiments, even at the lowest heating rates, would *imply* that this is a monotropic pair, but it is not conclusive, because the energy differences are very small if not zero.

One way of attempting to make the distinction in tropism would be by the following argument. If I is more energetic (soluble) than II at high temperature (T_{high}), and if it can be determined experimentally that I has a higher solubility at a lower temperature (e.g. 25°C), then the pair is monotropic, because in that case Form I would have a higher solubility and vapor pressure than Form II over the temperature range 25°-T_{high}.

Table 1 Unstressed Forms I and II DSC Endotherm Onset Temperatures and Melting Ranges

Heating Rate (°C/min)	Form I Onset (°C)	Melting Range	N	Form II Onset (°C)	Melting Range	N
10 (S,op)	139.5±0.6	3.2±0.5	4	144.2±0.6	2.7±0.4	4
5 (S,op)	139.1±0.4	2.0±0.1	2	143.6±0.1	1.6±0.1	2
2 (S,op)	136.6±0.5	1.8±0.0	2	141.6±0.1	1.0±0.1	2
1 (S,op)	136.7	1.1	1	140.3	0.9	1
0.5 (S,op)	133.5	1.8	1	n.d.	--	
10 (MP)	134.4±0.1	3.2±0.4	2	138.6±0.4	4.0±0.9	2
2 (MP)	124.3±1.8	2.9±0.2	5	127.5±0.9	4.3±2.2	3
5 (D,sp)	142.3	1.2		144.5	1.9	
5 (D,sp, p)	142.6	1.1		n.d.	--	
2 (D,sp)	141.0	0.5		n.d.	--	

S, op = Seiko open pan with lid, crimped
MP = Mettler sealed pan
D, sp = DuPont (TA Instruments) sealed pan
p = pierced (pinhole)
N = Number of replicates

This was partially the reason for carrying out the equilibrium solubilities at 25°C. The solubilities of Forms I and II were assessed by assaying supernatants of equilibrated samples. The equilibrium solubilities of the two forms at 25°C are listed in Table II, and are obviously within experimental error.

No significant degradation peaks were noted in the supernatants by HPLC, although two extraneous peaks were present in all chromatograms; their peak areas in the stressed samples were higher than in the unstressed sample, however, in no case did any of the peaks exceed 0.7% of the area of the parent peak.

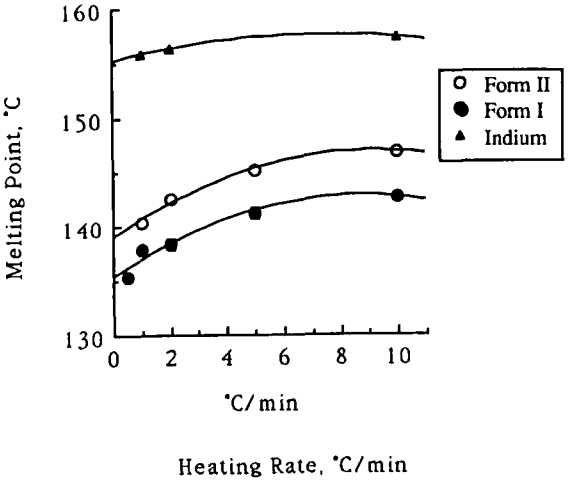

Figure 4 Melting point as a function of Heating Rate; Seiko open sample pans with lid, crimped (SP)

Table 2 Solubility of Two Polymorphic Forms of N-[2-(((-5-((Dimethylamino)methyl]-2-furanyl]thio]ethyl-N'-methyl-2-nitro-1,1-ethenediamine hydrochloride at 25°C

Polymorphic Modification	Storage	Solubility (g/g)	pH
I	5 d in H_2O	0.76*	5.6
II	5 d in H_2O	0.76	5.6
I	3 d in 0.1 N HCl	0.74	5.4
II	3 d in 0.1 N HCl	0.75**	5.4

*The filtrate from Form I, after 24 hours in H_2O, also assayed at 0.76 g/g

**The filtrate from Form II, after 2 hours in 0.1 N HCl also assayed at 0.75 g/g

The experiments were carried out both in water and N/10 HCl, the latter out of curiosity, in an attempt to answer the question, would, possibly, form I convert more readily at lower pH (and would it, for instance convert in the gastric fluid[b]). This then presumes that Form I is the metastable (high energy) state.

It is seen from the equilibrium experiments that a difference in solubility at 25°C is not established, and hence the original question of which form is the more stable at 25°C, and which type of tropism exists, is not answered by this route.

Equilibrium solubility experiments are conventionally carried out for several days, and the possibility of conversion does exist in such an experiment, but it will be seen, by further discussion of the X-ray diffraction data, that such a conversion did not take place.

Speaking generally, polymorphic conversions *can* take place (mostly in time consuming fashion) in anhydrous samples, but presence of moisture is a potent catalyst for such conversions. Moisture will, in such cases, sorb onto the surface of the solid[3] and if this is the metastable form of the compound will dissolve in the sorbed moisture layer and form a solution which is supersaturated solution with respect to the other (more stable) form. It may then recrystallize as the more stable form. It is noted that the form with the lower solubility is the more stable form at the given temperature. As mentioned (Eq. 1) if the solubilities are close, the propensity for such precipitation is small.

If one could cause a conversion to happen (e.g. through moisture exposure) at a given (lower) temperature, then the question of monotropism versus enantiotropism would be answered. To evaluate whether, in such experiments, conversion has taken place, X-ray diffraction is obviously the evaluation instrument of choice.

Prior to delving into the findings from X-ray diffraction, experiments were carried out to determine the limits of

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[b] This is essentially a moot point, because the solubilities of the compound is so high that it would take enormous doses for the entire does not to dissolve completely in the gastric fluid volume.

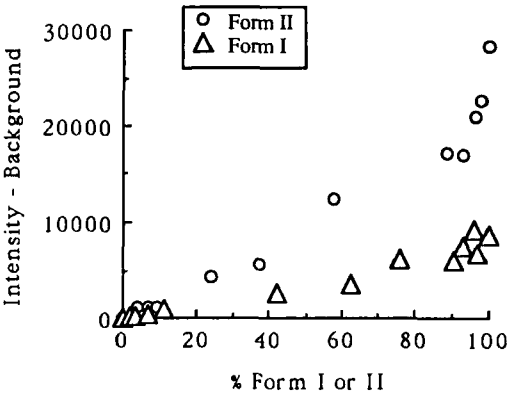

Figure 5 N-[2-(((-5-((Dimethylamino)methyl]-2-furanyl]thio] ethyl-N'-methyl-2-nitro-1,1-ethenediamine hydrochloride powder X-ray diffraction intensities at 2 Theta = 21.8 (Form I) and 2 Theta = 20.2 (Form II)

detection of Form I in Form II and of Form II in Form I. Weighed mixtures were subjected to X-ray analysis, and the limit of detection noted (as the point where in the case of Form II in Form I, the peak at 2 theta = 20.2° became detectable, and conversely in the case of Form II where Form I in Form II, the peak at 2 theta = 21.8° became detectable).

By powder X-ray diffraction, the intensities at 2 theta = 20.2 and 21.8 were found to be the two most sensitive peaks in spiked samples, i.e. Form I spiked with Form II and vice versa. When samples of known composition were analyzed by powder X-ray diffraction, it was found that 3.5 % Form I in Form II could be distinguished, and 2.8% Form II in Form I could be distinguished, and there was a positive correlation between intensity and composition, as shown in Fig. 5.

To evaluate whether moisture stress would cause conversion, the centrifuged residues from samples from the solubility experiments were now air dried, and subjected to X-ray analysis and such samples, harvested at different times, are shown in Fig. 6. Judging from X-ray diffraction patterns there was no conversion from form I to form II, or from form II to form I as noted by the absence of an intensity signal at 2 theta = 21.8° in Form I samples and an absence of an intensity

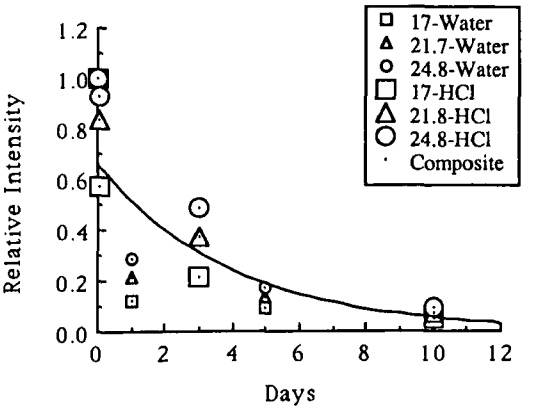

Figure 6 Relative Intensities (RI) for Form I at the exposure and at the indicated 2-Theta values as a function of time. The least squares fit is $RI = 0.66*10^{-0.11x}$. Fits, other than the exponential were not tried. In essence, if the fit were correct and the scatter small, the pre-exponential factor should be 1.0. Interpretation of intensities under the present experimental conditions is only semi-quantitative at best.

signal at 2 theta = 20.2° in Form II samples. The drop in the intensity at three 2 Theta values for each are shown in Fig. 6 both for water and hydrochloric acid exposure.

The melting points of the recovered solids were checked and Form II exhibited a higher melting point than form I in all cases.

So degree of crystallinity is lost to some degree by samples recovered from exposure to either HCl or water, in the sense that the intensity values decreased for all the samples. The relative intensities, i.e. the intensity at time t, divided by the intensity prior to stress, is plotted versus storage time in Fig. 6 for form I.

A similar picture emerges for form II (evaluated at 2 Theta values of 20.1, 23.4 and 31.8). *The important point is that neither stress at 25°C nor at 37°C make signals at 2-theta values of 20.1 appear in the stressed Form I samples nor did signals at 2-theta values of 21.8 appear in stressed Form II samples.*

Optical microscopy of these samples supports the quantitative findings in Fig. 6. The intensity changes with time at 37°C (not shown) are slower than at 25°C.

Up to this point it has been demonstrated that Form I is metastable at higher temperatures, but it has not been demonstrated which form is the more stable at lower temperatures (25°C or 37°C).

One more avenue exists to possibly show such a distinction: Solubilities mostly follow a Van't Hoff equation:

$$\ln S = -\Delta H/R((1/T_1)-(1/T_2)) \qquad (2)$$

If it can be shown, e.g., that if the heat of solution (ΔH) is lower for Form I that for Form II, or if they are equal, then the difference in solubilities would decrease with increasing temperature (or stay the same). Since Form I is more energetic (soluble) at 140°C then it would also be more soluble at 25°C if its heat of solution were equal to or lower than that of Form II. And so, in such a case, the forms would constitute a monotropic pair.

Heats of solution are shown in Table 3 below:

Table 3. Heats of Solution of Two Forms of N-[2-(((-5-((Dimethylamino)methyl]-2-furanyl]thio]ethyl-N'-methyl-2-nitro-1,1-ethenediamine hydrochloride

	Form I		Form II	
Sample	Cal/g	kCal/mole	Cal/g	kCal/mole
a	21.17	7.43	20.18	7.08
b	19.15	6.72	19.98	7.01
c	19.93	6.99	21.39	7.50
Avg	20.08	7.05	20.52	7.20
SD	1.0	0.4	0.8	0.3

It is obvious that a difference in heats of solution at 25°C has not been established.

It should finally be mentioned that transformations can, at times, be induced by pressure, e.g. such pressure as supplied in a tablet press. Such pressure induced transformations in the solid state have been reported in past literature (Matsuda and Kawaguchi, 1986, Wu et al, 1993, Matsumoto et al. 1991)[7-9]. 150 mg (round) and 300 mg (oblong) film-coated tablets produced from Forms I and II were ground (separately) in a mortar and pestle and examined (separately) by powder X-ray diffraction. The age of the tablets was at least 7 months, and samples were stored at 25°C in separate bottles.

The powder X-ray diffraction patterns of the ground tablets were superimposable on those from the corresponding standards, with no appearance of an intensity signal at 2 theta = 21.8 in Form I samples and no appearance of an intensity signal at 2 theta = 20.2 in Form II samples, indicating that the tableting process used did not result in pressure-induced polymorphic transformation, nor did storage of tablets at room temperature cause a polymorphic transformation.

CONCLUSIONS

Under the conditions tested, unseeded Forms I and II of N-[2-{{{-5-{(Dimethylamino)methyl]-2-furanyl]thio]ethyl-N'-methyl-2-nitro-1,1-ethenediamine hydrochloride were not found to interconvert in the solid state neither in dry storage nor from a slurry of excess drug in H_20 or 0.1 N HCl held at 25 and 37°C. DSC studies suggest these forms *may possibly* be a monotropic pair as no conversion was noted even at low heating rates. If so, Form II having a higher melting point, is the more stable of the two. However, until conditions are found under which one form converts to the other, it cannot be definitely distinguished whether they are monotropes or enantiotropes because their solubilities at 25°C are within experimental error, the heats of solutions are virtually identical and their melting points are only slightly different. Both forms were found to be deliquescent.

ACKNOWLEDGMENT

The authors thank Dr. Doug Powell (UW Chemistry Department) for technical assistance.

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