Polymorphism of Nicotinamide Riboside Chloride and Crystal Structure Determination of the Most Stable Crystalline Form

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Abstract

Three crystalline forms of nicotinamide riboside chloride (NR-Cl), namely Form A, Form B and Form C, were prepared and characterized. Form A and Form B are true polymorphs of anhydrous forms, while Form C is a pseudo-polymorph of a methanolate solvate form. Physical stability relationship among these three crystalline forms was established, and the crystal structure of the most stable form, Form B, was determined by single crystal X-ray diffraction analysis.

Introduction

Nicotinamide riboside (IUPAC name: 3-Carbamoyl-1-[(2R,3R,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]pyridin-1-ium) is a naturally occurring pyridine-nucleoside present in almost all living organisms and is a form of vitamin B3 [1]. Nicotinamide riboside (NR) functions as a precursor to nicotinamide adenine dinucleotide or NAD+ and has been demonstrated as a promising candidate for preventing and/or treating several conditions including metabolic and neurodegenerative disorders [2]. NR is generally recognized as safe (GRAS) by the FDA and is widely commercialized in food supplements for anti-aging and longevity [3].

Nicotinamide riboside is marketed as nicotinamide riboside chloride salt (NR-Cl) with a chemical structure shown in Figure 1. Preparation methods of various crystalline forms of NR-Cl were disclosed in a number of patents in recent years [4-9], and these crystalline forms are believed to have advantageous physicochemical properties compared to the amorphous form, e.g. higher chemical purity and better physical stability. The single crystal growth by the vapor diffusion method and crystal structure determination by single crystal X-ray crystallography of a meta-stable crystalline form of NR-Cl were also reported recently [10]. However, the preparation and polymorphism relationship among all disclosed crystalline forms were not systematically investigated. Herein, we report the preparation of three crystalline forms, the physical stability relationship among these forms, and the crystal structure determination of the most stable polymorph of nicotinamide riboside chloride (NR-Cl).

Experimental Section

Nicotinamide riboside chloride (NR-Cl) (Form B) was purchased from UltraHealth LLC (727 Central Avenue, Worthington, IN 47471, USA), and all crystallization solvents were used without further purifications.

Preparation of NR-Cl-Form A

200 mg of white solid nicotinamide riboside chloride (NR-Cl) was dissolved in 0.5 mL of water (H_2O) to achieve a clear solution. 4.0 mL of ethanol (EtOH) was added, resulted in a cloudy solution. 4.0 mL of tert-butyl methyl ether (TBME) was added slowly to the cloudy solution to precipitate white solid, and then the slurry was stirred at the room temperature for 2 hours. 170 mg of white solid NR-Cl-Form A was obtained after the homogenous slurry was filtered and dried in vacuum for 4 hours (yield: 85%).

Preparation of NR-Cl-Form B

500 mg of white solid NR-Cl was dissolved in 2.5 mL of water to achieve a clear solution. 5.0 mL of acetone and 2.5 mL of isopropanol (IPA) were added to the solution causing a white solid to precipitate. The suspension was stirred at room temperature for 1 day and a homogenous slurry was formed. 385 mg of white solid NR-Cl-Form B was obtained after the homogenous slurry was filtered and dried in vacuum for 4 hours (yield: 77%).

Single crystals of NR-Cl-Form B were grown from evaporation of a MeOH/dichloromethane (DCM) solution (or IPA/H_2O solution) of NR-Cl with NR-Cl-Form B seeds.

Preparation of NR-Cl-Form C

50 mg of white solid NR-Cl was dissolved in 1.0 mL of methanol (MeOH) to achieve a clear solution. 2.0 mL of tert-butyl methyl ether (TBME) was added to the solution causing a white solid to precipitate. The suspension was stirred at the room temperature for 1 day and a homogenous slurry was formed. 22 mg of white solid NR-Cl-Form C was obtained after the homogenous slurry was filtered and dried in vacuum for 4 hours (yield: 44%).

Competitive Slurry Study of NR-Cl-Form A and NR-Cl-Form B

20 mg of white solid NR-Cl-Form A and 20 mg of NR-Cl-Form B were suspended in 0.50 mL of ethanol. The slurry was stirred at the room temperature for 5 days. XRD showed that a pure crystalline form of NR-Cl-Form B was obtained.

Slurry Study of NR-Cl-Form C

20 mg of white solid NR-Cl-Form C was suspended in 0.50 mL of acetone. The slurry was stirred at room temperature for 1 day. XRD showed that NR-Cl-Form C was converted to NR-Cl-Form A. NR-Cl-Form A retains after the slurry was stirred at the room temperature for 3 days.

X-Ray Powder Diffraction (XRPD) Analysis

XRPD analysis was performed using a Bruker D8 diffractometer, Bruker AXS Inc™ (Madison, Wisconsin). The XRPD spectrum of each sample was obtained by mounting a sample (approximately 5–10 mg) on a single silicon crystal wafer mount (e.g., a Bruker silicon zero background X-ray diffraction sample holder) and spreading out the sample into a thin layer with the aid of a microscope slide. The sample was spun at 30 revolutions per minute (to improve counting statistics) and irradiated with X-rays generated by a copper long-fine focus tube operated at 40 kV and 40 mA with a wavelength of 1.5406 angstroms. The sample was exposed for 1 second per 0.02 degree 2-theta increment (continuous scan mode) over the range of 5 degrees to 40 degrees 2-theta in theta-theta mode.

Differential Scanning Calorimetry (DSC) Analysis

DSC analysis was performed on samples prepared according to standard methods using a Q SERIES™ Q1000 DSC calorimeter available from TA INSTRUMENTS® (New Castle, Delaware). A sample (approximately 2–5 mg) was weighed into an aluminum sample pan and transferred to the DSC. The instrument was purged with nitrogen at 50 mL/min and data were collected between 22°C and 300°C, using a dynamic heating rate of 10°C/minute. Thermal data was analyzed using Universal v.4.5A from TA INSTRUMENTS®.

Thermogravimetry Analysis (TGA)

TGA was performed on samples prepared according to standard methods using a Q SERIES™ Q5000 thermogravimetry analyzer available from TA INSTRUMENTS® (New Castle, Delaware). A sample (approximately 5–8 mg) was placed into an aluminum sample pan and transferred to the TGA furnace. The instrument was purged with nitrogen at 50 mL/min and data were collected between 25°C and 300°C, using a dynamic heating rate of 10°C/minute. Thermal data was analyzed using Universal v.4.5A from TA INSTRUMENTS®.

Single Crystal Analysis

Single crystal analysis was performed using a Bruker Apex diffractometer, Bruker AXS Inc™ (Madison, Wisconsin). The diffraction data were collected at 23°C using Mo X-ray source. The crystal structure was solved and refined with the SHELXTL package. The H atoms on the N and O atoms were located in the electronic density map while all other H atoms on the C atoms were calculated.

The simulated X-ray powder pattern of NR-Cl-Form B was calculated from the single crystal structure data with the help of the program Mercury from The Cambridge Crystallographic Data Centre (CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax:+44 1223 336033).

Results And Discussion

Patent Summary and Nomenclature of Crystalline Forms

Three crystalline forms of NR-Cl have been discovered so far and disclosed in various patents as listed in Table 1. Table 1 also designates these forms into an ambiguous nomenclature. The crystalline form nomenclature is based on observation of XRPD pattern similarity, and the figures of XRPD overlays of each crystalline form are shown in supplementary information.

Table 1. Patents for crystalline forms of nicotinamide riboside chloride (NR-Cl).

Patent number	Ref	Assignee(s)	Crystalline form and condition	Form
WO2015186068	[4]	GlaxoSmithKline	Crystalline form from H_2O/EtOH	Form A
WO2016014927	[5]	W. R. Grace & Co.	Form I from MeOH/TBME	Form A
WO2016144660	[6]	W. R. Grace & Co.	Form II from MeOH/Acetone/H_2O	Form B
WO2018089830	[7]	The Queen's University / ChromaDex Inc.	Form I from MeOH/TBME Methanolate Form II from MeOH	Form A Form C
WO2019126482	[8]	Elysium Health, Inc.	Crystalline form from H_2O/EtOH/TBME	Form B
CN111808156	[9]	Xuchang Yuanzhi Biotechnology Co ltd	Form IA from MeOH Form IB from DCM	Form C Form A

Three distinguishable crystalline forms were observed in the previous patents and crystalline forms are designated as Form A, Form B and Form C, respectively.

Preparation of Crystalline Forms

White solid NR-Cl is readily dissolved in H_2O or MeOH to obtain a clear colorless solution. NR-Cl shows a better solubility in water compared to MeOH so that water is preferred to dissolve white solid NR-Cl, except for NR-Form C, which is a methanolate solvate and requires methanol during crystallization. Interestingly, both polymorphs of NR-Cl-Form A and NR-Cl-Form B were identified by addition of different anti-solvents into the aqueous solution.

It is worth mentioning that a ternary solvent system with two mixed anti-solvents were used in preparation of both NR-Cl-Form A and NR-Cl-Form B. In preparation of NR-Cl-Form A, the single anti-solvent of ethanol only precipitated a small amount of the product. Yield was significantly improved when using the second anti-solvent of TBME. In this case, the ethanol not only serves as an anti-solvent but also overcomes miscibility issue between water and TBME. In preparation of NR-Cl-Form B, a ternary solvent system is necessary for crystallization of NR-Cl-Form B. It was observed that water/acetone binary solvents only produced a gel-like solid, while water/isopropanol binary solvents gave a low yield of the product, which was often found as a mixture of NB-Cl-Form A and NR-Cl-Form B.

Solid State Characterization of NR-Cl crystalline forms

The overlay of powder X-ray diffraction (XRD) patterns of NR-Cl-Form A, NR-Cl-Form B and NR-Cl-Form C is shown in Fig. 2. While Form A and Form B demonstrated high crystallinity, Form C had some background noise, indicating that the material contained some content of amorphous form and was likely not very stable due to de-solvation of methanol solvate. Some caution should be taken in the XRD characterization of Form C since the conversion of Form C to Form A was observed when Form C was pressured with a microscope slide while being flattered on the XRD sample holder. Such an observation of form conversion in the XRD measurement correlates with the crystalline solvate form which was disclosed in GlaxoSmithKline's patent WO2015186068 [4]. This solvate form had an XRD pattern comparable to that of Form A, even though the solvate form itself contained about 0.9 molar ratio of methanol in the material. Likely, this was due to the form conversion during XRD measurement. More studies are needed to confirm this hypothesis.

The overlay of differential scanning calorimetry (DSC) curves of NR-Cl-Form A, NR-Cl-Form B and NR-Cl-Form C is shown in Fig. 3. The first thermal event for Form A and Form B was chemical decomposition of the nicotinamide riboside into nicotinamide and sugar, confirmed by a previous study [11]. The endothermic event of Form B with an onset temperature of 123°C revealed a higher decomposing temperature compared to that of Form A with an onset temperature of 119°C, suggesting Form B is more thermally stable than Form A. The first endothermic event of Form C with an onset temperature of 79°C was assumed to be a de-solvation process of this methanolate solvate form.

The overlay of thermal gravimetric analysis (TGA) curves of NR-Cl-Form A, NR-Cl-Form B and NR-Cl-Form C is shown in Fig. 3. The TGA indicates that both Form A and Form B exhibit an insignificant weight loss (< 0.5%) in the solvent region (25–105°C), confirming that Form A and Form B are anhydrous crystalline forms, and true polymorphs. Form C exhibits a weight loss of about 7.5% upon heating from 25°C to about 105°C, close to the methanol content for a mono-methanolate form (The theoretical methanol content of mono-methanolate is 32.04/(290.70 + 32.04) = 9.9%).

Crystal structure of NR-Cl-Form B

Single crystals of NR-Cl-Form B were obtained from slow evaporation of the NR-Cl solution. It should be noted that seeding with NR-Cl-Form B in the saturated solution is critical to obtain the desired Form B crystals. Otherwise, Crystalline NR-Cl-Form A was obtained. The crystallographic data are shown in Table 2 and the content of the asymmetric unit and crystal packing is shown in Fig. 5. The absolute configuration of the enantiomerically pure molecule was confirmed by the Flack parameter (0.02(1)) and matched with the expected stereochemistry.

Table 2. Crystal data and structure refinement for NR-Cl-Form B.

Identification code	nr-cl-b	
Empirical formula	C11 H15 Cl N2 O5	
Formula weight	290.70	
Temperature	297(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	P2(1)2(1)2(1)	
Unit cell dimensions	a = 7.0008(11) Å	α= 90°.
	b = 9.6465(15) Å	β= 90°.
	c = 17.971(3) Å	γ = 90°.
Volume	1213.7(3) $Å^3$	
Z	4	
Density (calculated)	1.591 Mg/m^3	
Absorption coefficient	0.335 mm^{-1}	
F(000)	608	
Crystal size	0.28 x 0.20 x 0.04 mm^3	
Theta range for data collection	2.27 to 25.73°.	
Index ranges	-8<=h<=8, -11<=k<=11, -21<=l<=21	
Reflections collected	27748	
Independent reflections	2317 [R(int) = 0.1156]	
Completeness to theta = 25.73°	99.8 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7453 and 0.4989	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2316 / 4 / 190	
Goodness-of-fit on F^2	1.083	
Final R indices [I>2sigma(I)]	R1 = 0.0519, wR2 = 0.1294	
R indices (all data)	R1 = 0.0671, wR2 = 0.1369	
Absolute structure parameter	0.05(6)	
Extinction coefficient	N/A	
Largest diff. peak and hole	0.626 and -0.289 $e.Å^{-3}$	

NR-Cl-Form B crystallizes in an orthorhombic $P2_12_12_1$ space group, the same space group with NR-Cl-Form A, which was determined previously [10]. NR-Cl cations in Form A and Form B adopted different molecular conformations, as shown in Fig. 6. The torsional angle connecting nicotinamide and sugar shows about 180° rotation, consequentially the amide group of nicotiamide lies towards the same side of the sugar group in Form B but at the opposite side in Form A. Besides, the hydroxyl groups in the riboside group are in different directions. As a consequence, NR-Cl-Form B reveals unique hydrogen bonding and crystal packing in crystal lattices compared to those of NR-Cl-Form A

As shown in Fig. 7, the overlay of the simulated powder X-ray diffraction pattern calculated from single crystal structure data matches well with the experimental XRD pattern of NR-Cl-Form B. The XRD identicalness not only indicates that the characterized crystal structure represents NR-Cl-Form B but also confirms that the prepared bulk material is a pure crystalline form of NR-Cl-Form B.

Relative physical stability of NR-Cl crystalline forms.

Polymorph studies aim to identify as many as possible crystalline forms and establish physical stability relationship among these forms. As a result, it helps to ensure the optimal crystalline form, usually the most stable form, is selected for commercial product development. Form A and Form B are true polymorphs of anhydrous forms, while Form C is a pseudo-polymorph of the solvated form. The solvated crystalline form is usually not preferred due to its potential physical stability issue and the possible toxicity caused by the extra solvent content. Thus it becomes critical to establish the physical stability relationship between the two true polymorphs, Form A and Form B. A quick competitive slurry study demonstrated that a mixture of Form A and Form B converted to pure Form B at ambient temperature, which suggests that NR-Cl-Form B is more stable than NR-Cl-Form A, thus Form B is preferred to be used in pharmaceutical or nutraceutical development.

It is also worth pointing out that NR-Cl-Form B, as the most physically stable form, is also expected to possess a better chemical stability. Crystalline form control is crucial in order to produce the desired NR-Cl-Form B with the optimal physical and chemical stability, which would benefit the shelf life of a commercial product. It should be kept in mind that the experimental polymorph study itself does not guarantee that any identified crystalline form is the most stable. There is always an opportunity to discover a more stable form during further crystallization experiments. While ongoing crystallization study continues to identify novel crystalline form of NR-Cl, other substitute products, including new crystalline NR organic salts [12], NR prodrugs [13] and nicotinamide mononucleotide (NMN) as an alternative NAD + precursor [14], have also been under investigations in order to achieve optimal physicochemical properties.

Conclusions

In summary, three crystalline forms of NR-Cl were prepared and characterized. NR-Cl-Form A and NR-Cl-Form B are two polymorphs of anhydrous form, while NR-Cl-Form C is a methanolate solvate. Competitive slurry studies conclude that NR-Cl-Form B is more stable compared to NR-Cl-Form A at ambient temperature, suggesting that NR-Cl-Form B is preferred in pharmaceutical or nutraceutical substance development. The single crystal structure of NR-Cl-Form B revels molecular conformation and crystal packing of this most stable polymorph at the molecular level.

Declarations

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Data Availability:All data generated or analyzed for this work are included in the article and the Supplementary Information document. CCDC number 2244277 contains the crystallographic data for NR-Cl-Form B reported in this paper. These data can be obtained free of charge via

www.ccdc.cam.ac.ukldata_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax:+44 1223 336033.

Conflict of interest: The authors have no conflict of interest to declare that are relevant to the content of this paper.

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Figures

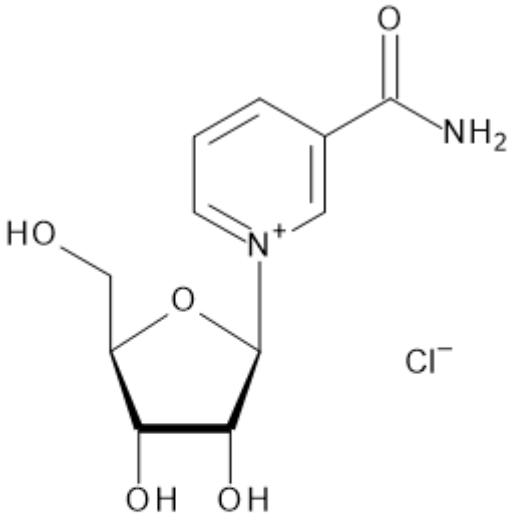

Figure 1

Chemical structure of nicotinamide riboside chloride (NR-Cl).

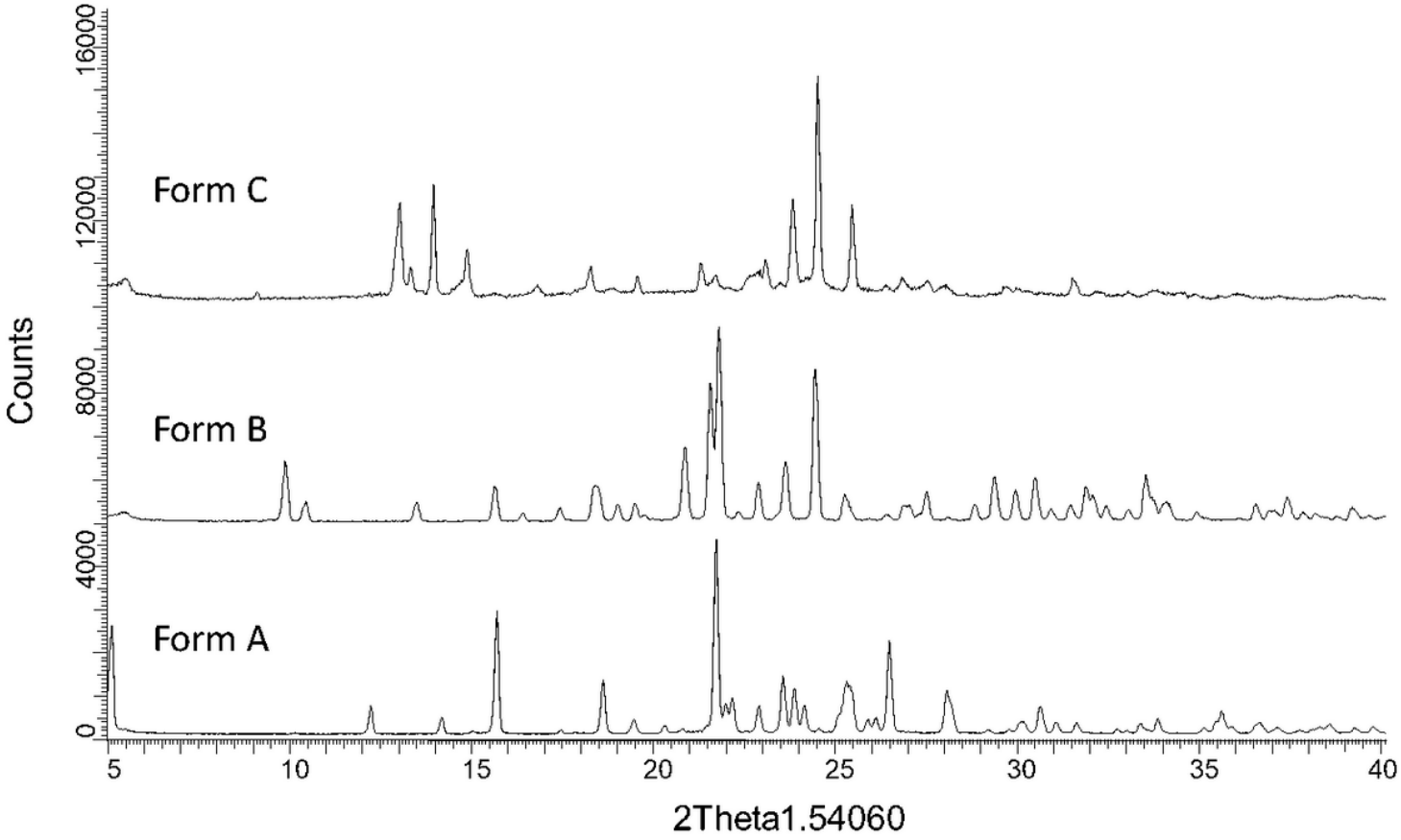

Figure 2

Overlay of X-ray powder diffraction of crystalline forms of NR-Cl.

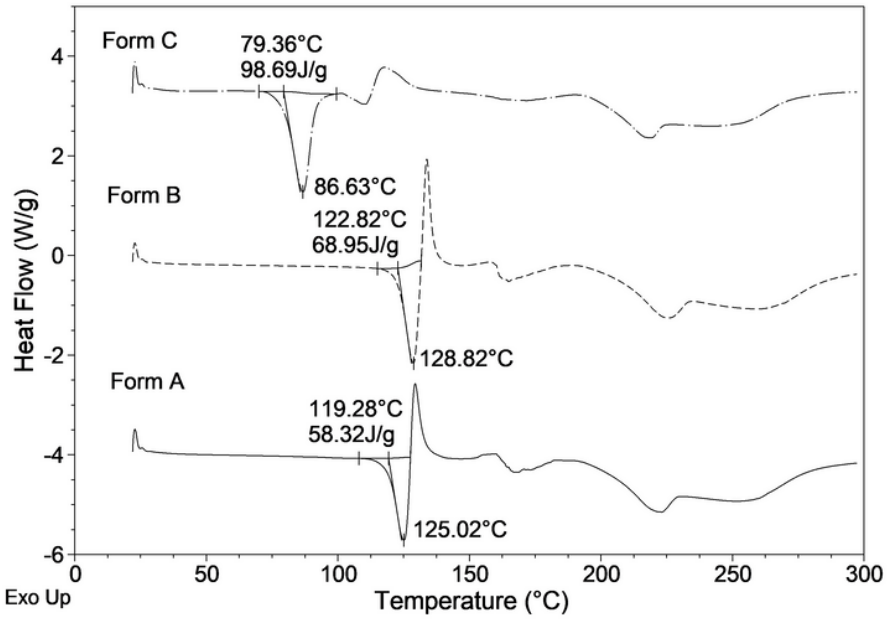

Figure 3

Overlay of DSC curves of crystalline forms of NR-Cl.

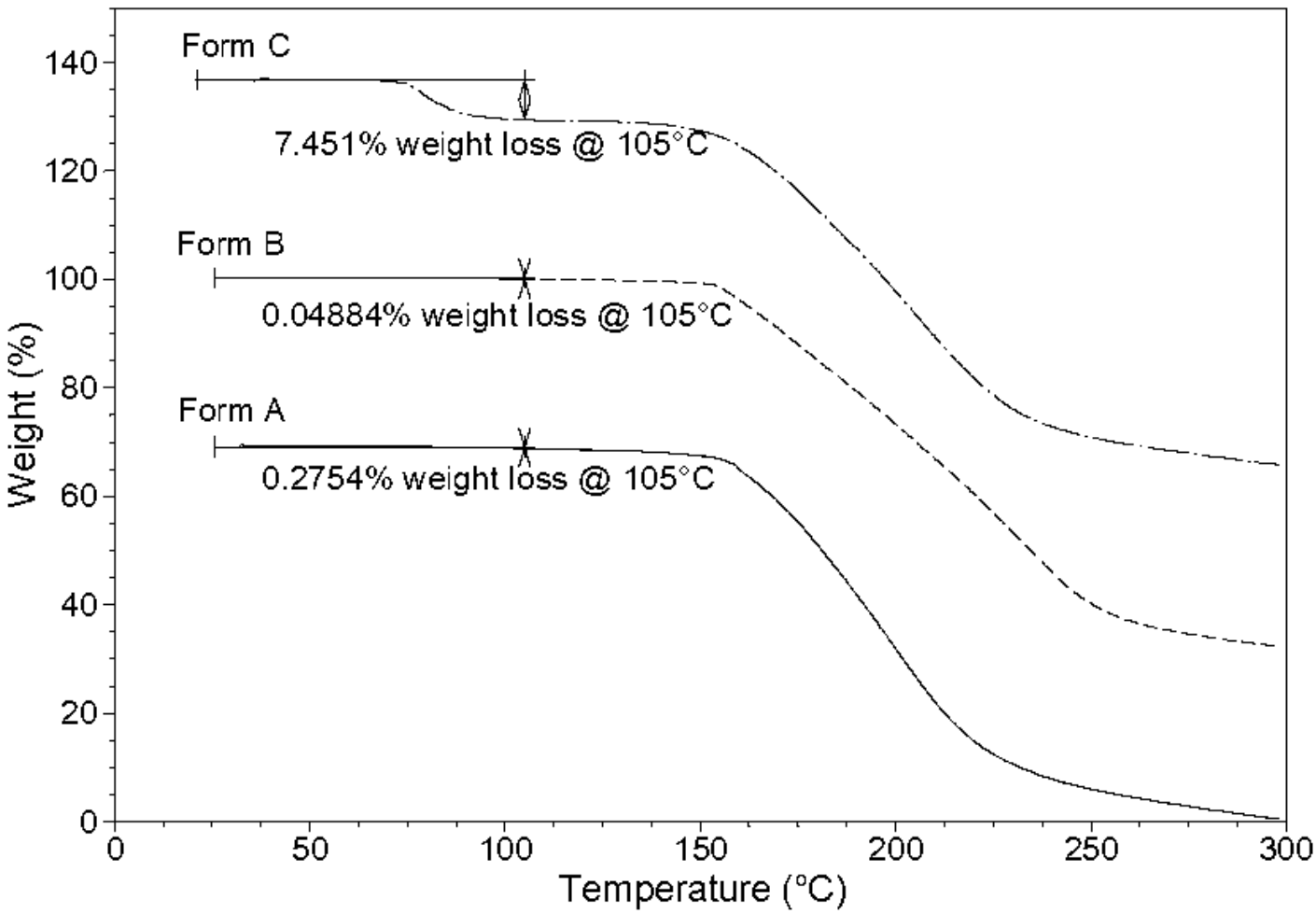

Figure 4

Overlay of TGA curves of crystalline forms of NR-Cl.

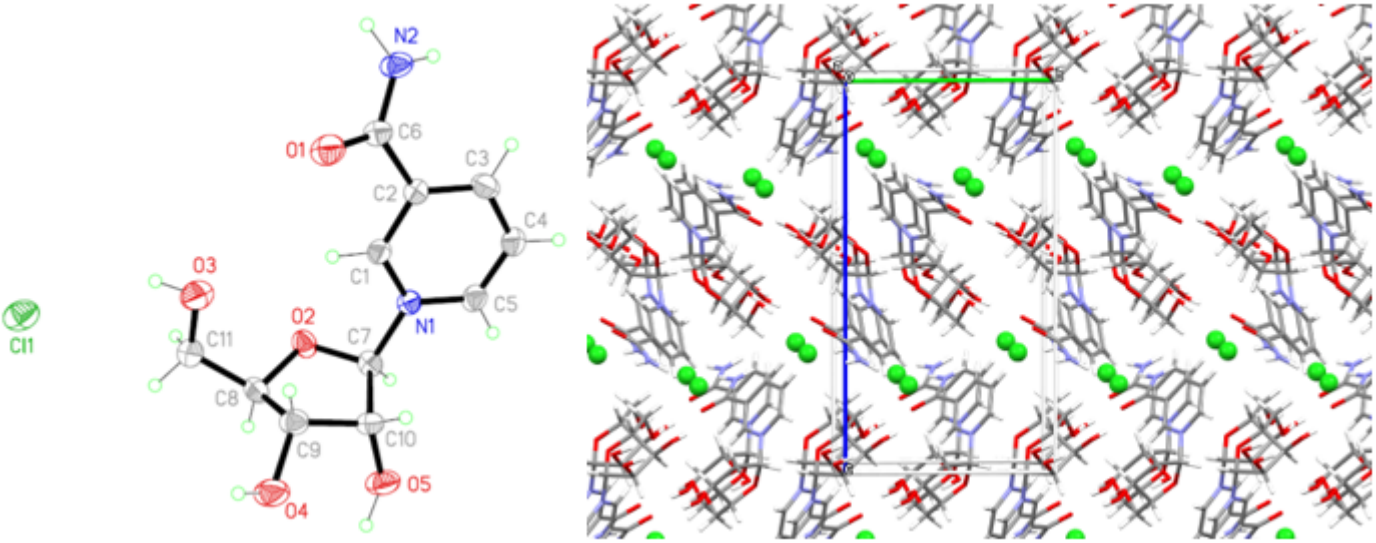

Figure 5

(a) Displacement ellipsoid diagram of the asymmetric unit of NR-Cl-Form B, ellipsoids drawn at 50% probability. (b) Crystal packing diagram of NR-Cl-Form B along the *a* axis.

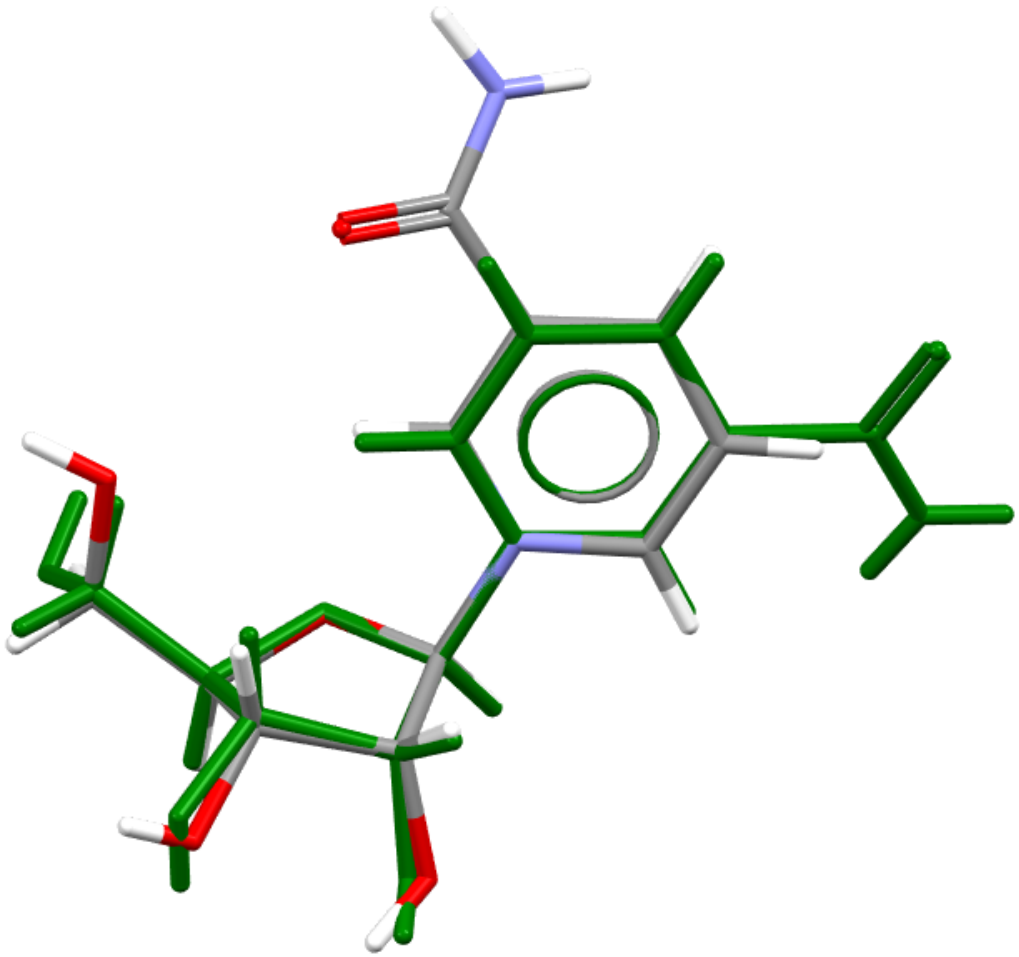

Figure 6

Overlay of NR-Cl cation in NR-Cl-Form A (green) and NR-Cl-Form B (multi-color).

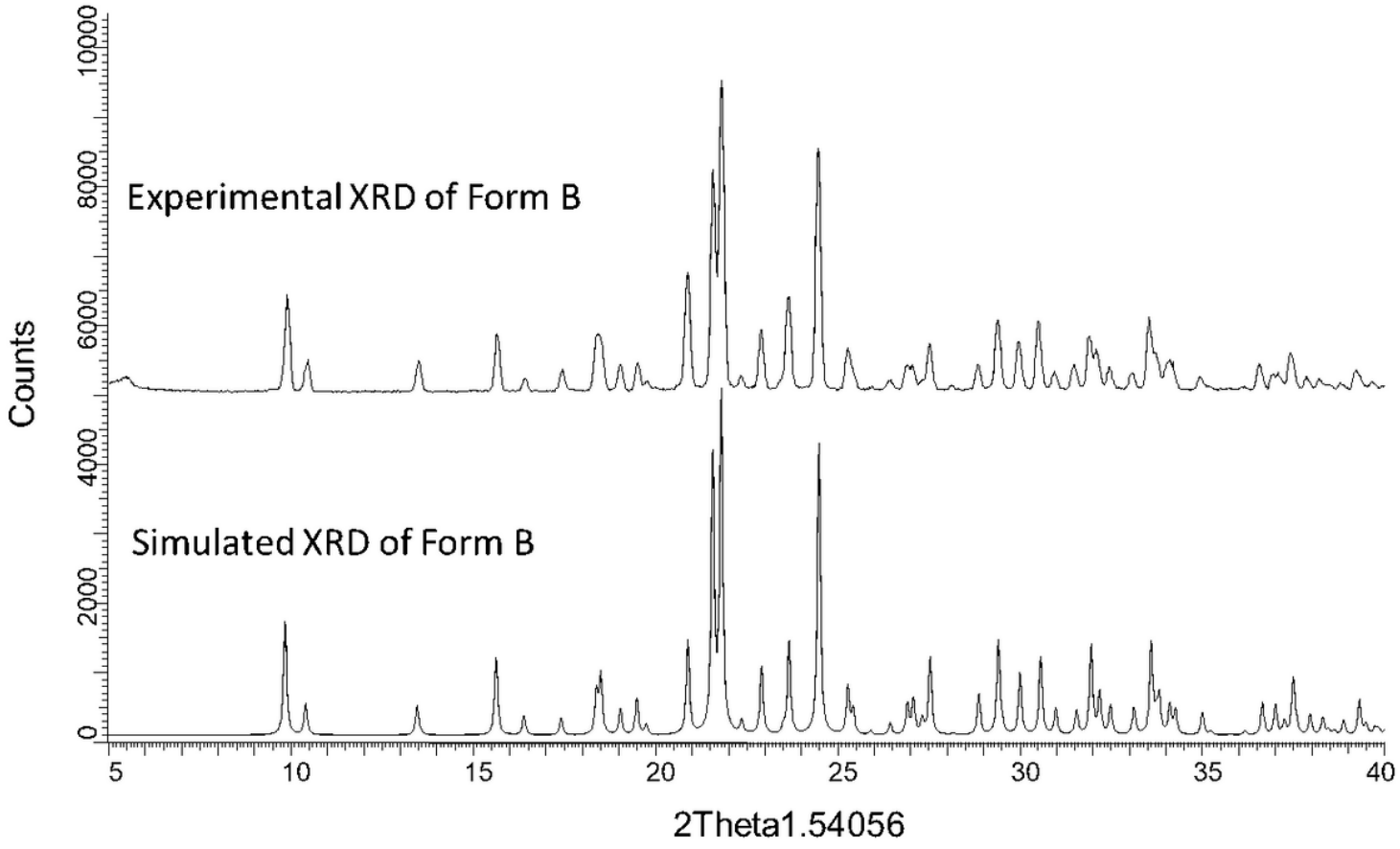

Figure 7

Overlay of the simulated XRD and the experiental XRD of NR-Cl-Form B.

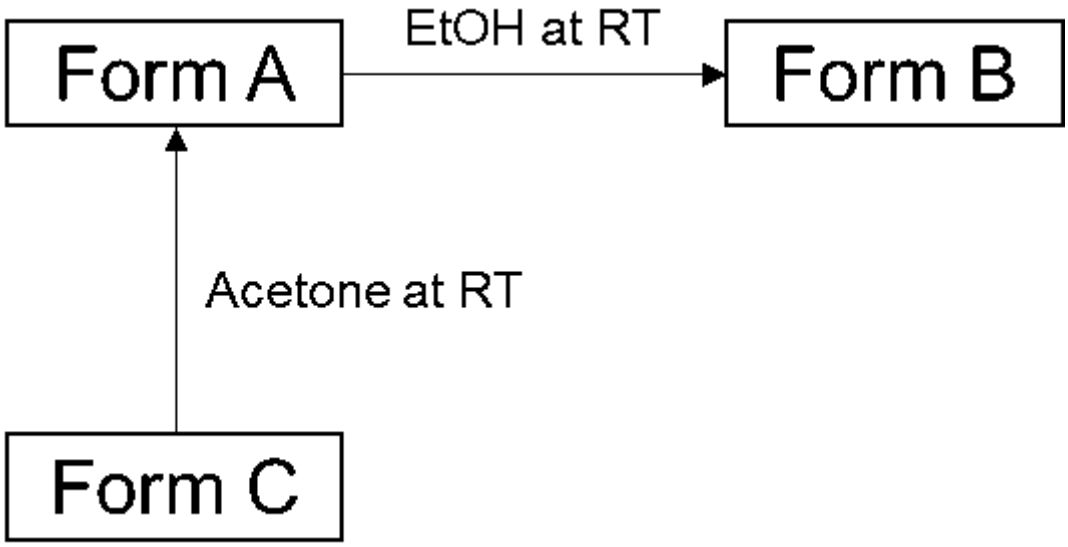

Figure 8

Crystalline form conversion diagram of NR-Cl.

Supplementary Files

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- GA.png
- NRClWu2023Supplementary.docx