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Solid state characterization of an neuromuscular blocking agent—GW280430A

Haijian (Jim) Zhu *, Mark Sacchetti [1]

GlaxoSmithKline Inc., Five Moore Drive, Research Triangle Park, NC 27709, USA

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Abstract

GW280430A is an ultrashort-acting neuromuscular blocking agent and is targeted for muscle relaxation as part of the intubation surgical procedure. The objective of this work was to perform solid state characterization on GW280430A and to evaluate the relationship between water content and glass transition temperature (T_g). GW280430A was characterized by differential scanning calorimetry, thermogravimetric analysis, powder X-ray diffraction (PXRD), microscopy and moisture sorption. The effect of water content on the T_g of GW280430A was evaluated by equilibrating the material over saturated salt solutions at a range of relative humidities (6.4–72.6%) and determining the T_g by DSC using hermetically sealed aluminum pans. GW280430A undergoes dehydration at 40 °C, glass transition at 130 °C and decomposition at 190 °C by differential scanning calorimetry. By PXRD and moisture sorption, GW280430A is an amorphous material and deliquesces at about 70% RH at room temperature. Water acts as a potent plasticizer for GW280430A and the T_g decreases significantly as the water content increases. No measurable decomposition of GW280430A was observed after 4 weeks at 40 °C/75% RH. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: GW280430A; Neuromuscular blocking agent; Solid state characterization; Glass transition temperature; Differential scanning calorimetry; Thermogravimetric analysis; Powder X-ray diffraction; Moisture sorption; Solid state stability

1. Introduction

GW280430A is an ultrashort-acting nondepolarizing neuromuscular blocking agent and is targeted for muscle relaxation as part of the intubation surgical procedure (Mook, 1996). The structure, chemical formula and molecular weight of GW280430A are shown in Scheme 1.

The most important physicochemical property of GW280430A is the hydrolytic instability of the ester group in aqueous solution. This solution instability negatively impacts the ability to formulate the compound in water for injection and presents many technical challenges to development of a stable dosage form for GW280430A (Zhu et al., 2001). Due to its instability in solution, GW280430A is formulated as a lyophilized powder. GW280430A itself has only been isolated

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* Corresponding author. Tel.: + 1-919-483-7927; fax: + 1-919-483-5652.

E-mail address: hjz40534@gsk.com (H.J. Zhu).

[1] Present address: Genentech Inc., South San Francisco, CA 94080, USA.

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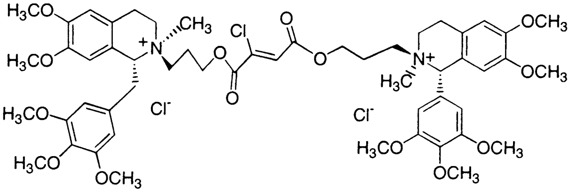

Chemical formula: $C_{53}H_{69}Cl_3N_2O_{14}$

Molecular weight: 1064.49

Scheme 1. Molecular structure of GW280430A.

in an amorphous state. Since, the glass transition temperature (T_g), of an amorphous solid affects its chemical stability, physical stability, and viscoelastic properties, and that water in an amorphous material may act as a plasticizer to decrease T_g and thus increase molecular mobility (Oksanen and Zografi, 1993), the information related to T_g and water content of GW280430A is useful.

In this study, solid state characterization was performed on GW280430A using differential scanning calorimetry, thermogravimetric analysis, powder X-ray diffraction (PXRD), microscopy and moisture sorption. A relationship between T_g and water content of GW280430A was also evaluated.

2. Materials and methods

2.1. Materials

GW280430A was manufactured at GlaxoSmithKline Inc. (Research Triangle Park, NC) with chemical purity 96.8%. All other materials were of reagent grade or better and used without purification.

2.2. Differential scanning calorimetry (DSC)

A model 910S differential scanning calorimeter (TA Instruments, New Castle, DE) equipped with a data station (Thermal Analyst 2200, TA Instruments) was used to determine the DSC curves. GW280430A samples were each placed in an open aluminum pan and heated at 10 °C/min under nitrogen purge at 40 ml/min. To determine the effect of water content on the T_g of GW280430A, 200 mg of GW280430A was each weighed into vials and the samples were kept open to saturated salt solutions at a range of relative humidities (6.4% RH, LiBr; 20% RH, K acetate; 32.3% RH, $CaCl_2$; 42% RH, $Zn(NO_3)_2$; 58%, NaBr; 72.6% RH, $NH_4Cl + KNO_3$) for 4 weeks (Nyquist, 1983). The criteria for equilibrium at a given relative humidity was less than 0.1% weight change in 24 h.

A heating rate of 20 °C/min was found to be adequate for observing and recording the T_g in GW280430A. Sample weights ranged from 1 to 3 mg. The samples were hermetically sealed in aluminum pans to prevent moisture loss during measurements.

2.3. Thermogravimetric analysis (TGA)

A Hi-Res 2950 Thermogravimetric analyzer (TA Instruments) linked to a data station (Thermal Analyst 2200, TA Instruments) was used. The weight loss was determined by placing GW280430A sample in an open aluminum pan and heating at 10 °C/min under nitrogen purge at 60 ml/min.

2.4. Powder X-ray diffraction (PXRD)

The PXRD patterns were determined on a Scintag XDS 2000 theta–theta diffractometer using − 1500 V on the detector, 45 kV and 40 mA on the X-ray tube, and slit widths of 2, 4, 0.5 and 0.3 mm. Samples were mounted on quartz zero background plates using petroleum jelly as the mounting agent and scanned from 4 to 50°, 2 theta, at a rate of 1° per min using Cu–Kα radiation.

2.5. Microscopy

GW280430A samples were examined on a Nikon Optiphot-Pol polarized light microscope. The thermal events were observed in both air and mineral oil on a hot stage (Mettler FP82HT/FP80) under a Nikon microscope using a heating rate of 10 °C/min. Photomicrographs were taken on a Sony CCD/RGB color video camera connected to a Sony UP-5200MD video printer.

2.6. Moisture sorption isotherm

The water uptake study was conducted on a vacuum microbalance (VTI Corp., Hialeah, FL) at 25 °C after drying the GW280430A samples at 60 °C under vacuum. Moisture sorption/desorption was monitored from 0 to 95% relative humidity. The criterion for equilibrium at a given relative humidity was less than 3 μg of weight change in 6 min.

2.7. Solid sate stability

To assess thermal/moisture solid state stability of GW280430A, 1 g of the drug substance was each placed in 40 °C/75% RH and 25 °C/50% RH humidity chambers for up to 4 weeks. The samples were kept open to allow continuous exposure of the samples to the controlled environment. The samples were inspected visually prior to analysis by HPLC. The peak area of non-degraded GW280430A relative to control as a percent was reported.

2.7.1. HPLC method

Instrument, Waters™ 717 Plus Autosampler; column, Supercosil ABZ, 150 × 4.6 mm, 5 μm, at ambient temperature; gradient, 15–55% B in 20 min; A = 15 mM ammonium trifluroacetate + 0.2% trifluroacetic acid; B = acetonitrile containing 0.1% TFA; flow rate, 1 ml/min; wavelength, 240 nm.

3. Results and discussion

GW280430A was identified as an amorphous material by PXRD (Fig. 1). Its amorphous nature is further confirmed by the absence of birefringence observed under polarized light microscopy. To examine whether GW280430A crystallizes during storage at different relative humidities, it was stored at 58 and 79.5% RH in desiccators containing saturated salt solutions. The sample at 58% RH was amorphous as identified by PXRD (Fig. 1). At 79.5% RH, GW280430A deliquesces. The deliquescent sample at 79.5% RH was then stored at 6.4% RH to solidify. The solidified sample remains amorphous (Fig. 1).

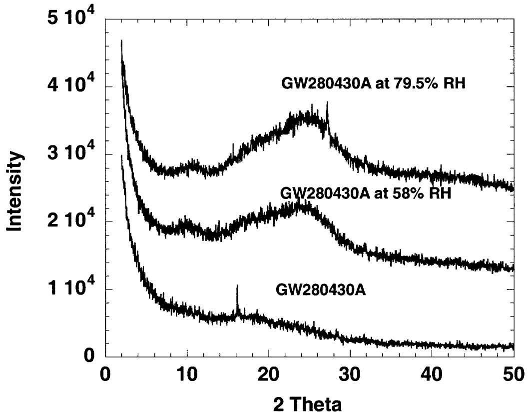

Fig. 1. Powder X-ray patterns of GW280430A and solid phases remaining after storage at various relative humidities.

When GW280430A was subjected to DSC in an open pan (Fig. 2), three endotherms were observed, with peak maximum at 40, 130 and 190 °C. The first and third endotherms corresponded to the dehydration and decomposition of GW280430A as confirmed by TGA and hot stage microscopy. The second endotherm is probably associated with glass transition and endothermic relaxation of GW280430A as it formed a liquid-like material at 130 °C when heated on hot stage. When the sample is immersed in mineral oil and heated on hot stage, the glass transition occurs at a much lower temperature (72 °C). A possible explanation for this drop in T_g is that there is minimal water loss from the solid in the mineral oil dispersion, which reduces T_g.

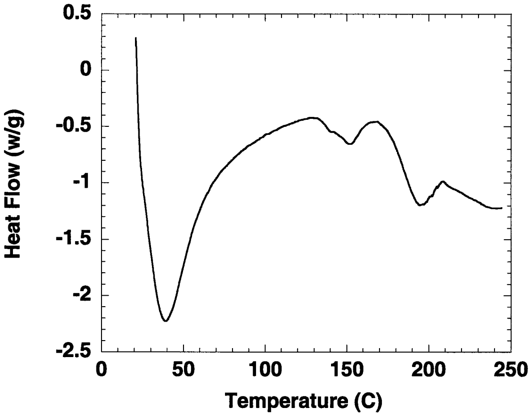

Fig. 2. DSC curve of GW280430A.

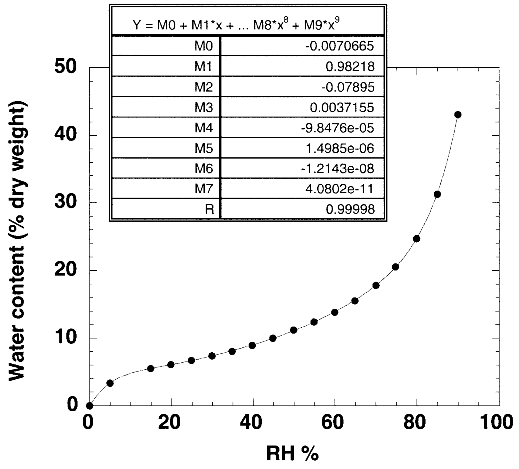

Fig. 3. Typical moisture isotherm of GW280430A.

The water uptake study indicates that GW280430A tends to sorb a significant amount of water from its surroundings, typically with 6–7% water uptake at 25% RH and 10–11% at 50% RH (Fig. 3). TGA of GW280430A shows about 8% weight loss up to 140 °C, followed by a rapid weight loss above 160 °C (not shown). Hysteresis was observed in the moisture sorption isotherm, which is common for amorphous materials. The water content values of GW280430A during sorption were fitted to polynomials of seventh degree in RH, and the equation was then used to estimate the equilibrated water content in GW280430A at any value of RH.

Fig. 4 shows the DSC curves for dry GW280430A and GW280430A equilibrated at 58% RH. The T_g is about 130 °C for dry GW280430A and 51.7 °C for samples equilibrated at 58% RH. The result suggests that water acts as a potent plasticizer for amorphous GW280430A by decreasing T_g.

Fig. 5 depicts a plot of T_g as a function of RH. T_g decreases significantly as RH increases. From Fig. 5, it is evident that T_g decreases to 70 °C after GW280430A is exposed to 10% RH at 25 °C. Fig. 6 is a plot of T_g as a function of weight fraction of water based on theoretical values calculated from the Fox equation (Eq. (1)), a simple mixing rule based on polymer free volume theory (Fox, 1950; Gordon and Taylor, 1952; Pochan et al., 1979).

$$\frac{1}{T_{\mathrm{g\,mix}}} = \left(\frac{w_1}{T_{\mathrm{g1}}}\right) + \left(\frac{w_2}{T_{\mathrm{g2}}}\right) \tag{1}$$

where $T_{\mathrm{g\,mix}}$ is the T_g of GW280430A amorphous system, T_{g1} and T_{g2} are the T_g of water and GW280430A, w_1 and w_2 are weight fraction of water and GW280430A. The Fox equation is derived assuming perfect volume additivity at T_g and no specific interaction between the two components.

The T_g of water was taken to be 135 K. The observed T_g versus weight fraction of water for GW280430A (Fig. 6) shows a negative deviation from ideality, suggesting that specific interactions between the water and GW280430A (e.g. hydrolysis) are significantly affecting the overall free volume of the system and hence, the T_g of the mixture.

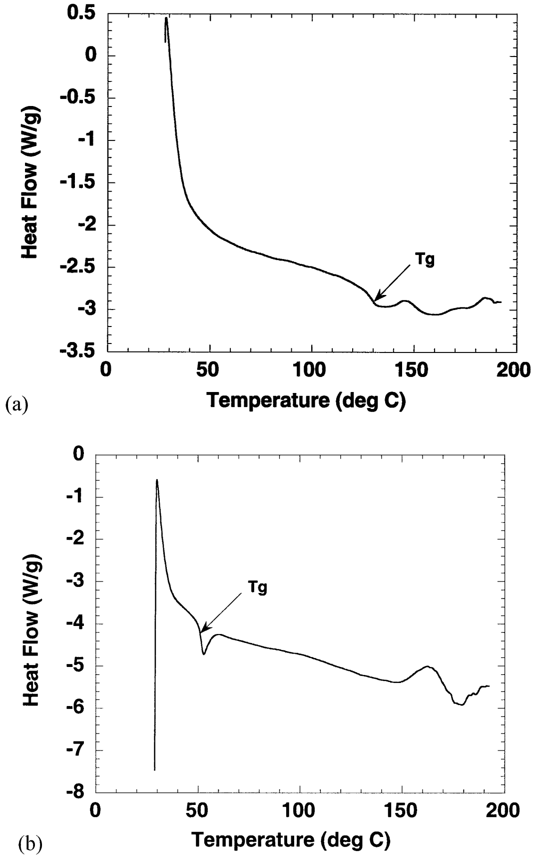

Fig. 4. DSC curves using hermetically sealed pan (a) Dry GW280430A; (b) GW280430A stored at 58% RH.

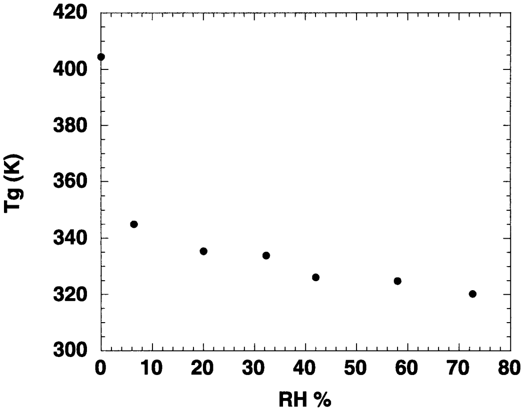

Fig. 5. T_g vs. RH for GW280430A.

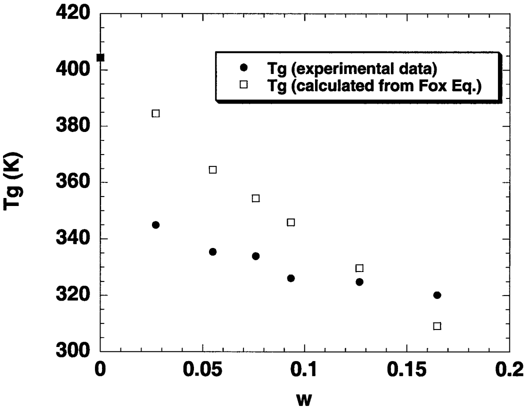

Fig. 6. T_g vs. weight fraction of water (w) for GW280430A.

The effect of temperature/humidity on the chemical stability of lyophilized GW280430A was studied under two conditions (40 °C/75% RH, 25 °C/50% RH). No significant loss in parent drug was found after storage at 40 °C/75% RH and 25 °C/50% RH up to 4 weeks. Although, no significant degradation was observed, the powder converted to a viscous liquid at 40 °C/75% RH.

4. Conclusions

GW280430A is an amorphous material that has not crystallized to date. The drug is hygroscopic and deliquesces at about 70% RH at room temperature. Thermal analysis indicates that GW280430A undergoes dehydration, glass transition and decomposition upon heating. Water acts as a potent plasticizer for amorphous GW280430A. The relationship between the water content and T_g for GW280430A evaluated in this study shows deviation from the simple mixing Fox equation assuming perfect volume additivity at T_g. Though, solution instability of GW280430A negatively impacts the ability to formulate the compound in water for injection, GW280430A is chemically stable when exists as a solid state form and can be formulated as a lyophilized powder. The physical instability (deliquesce at $> 70\%$RH) presents technical challenges in the development of a stable dosage form.

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