Characterization of the Salts of a Cyclic RGD Peptide

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INTRODUCTION

Cyclic[D-2-aminobutyryl-N^2-methyl-L-arginylglycyl-L-aspartyl-3-(aminomethyl)-benzoic acid (XL118) is the zwitterion of a cyclic RGD peptide that is a potent antagonist of the glycoprotein IIb/IIIa receptor (Fig. 1) (1,2). Irrespective of the stimulus of platelet aggregation, fibrinogen binding to the glycoprotein IIb/IIIa receptor is the common convergent pathway prior to aggregation (3,4). By acting as an antagonist to the glycoprotein IIb/IIIa receptor, XL118 blocks the binding of fibrinogen to the receptor thereby inhibiting platelet aggregation and providing a mechanism for antithrombotic therapy (5).

A significant body of literature has existed on the optimal characteristics of salts of non-peptide organic molecules (6). However, little information is available on the preparation or chemical and physical characterization of salts of peptides. The ability of salts of zwitterions to improve solubility has been presented (7). The authors also describe the general tendency of salts of zwitterions to be quite hygroscopic and the ability to overcome the behavior by forming hydrates (7). As part of the preclinical development of XL118, a series of salts were prepared with a goal of identifying a salt that afforded facile and reproducible conversion to a single stable crystalline form that would be amenable to processing into a dosage form.

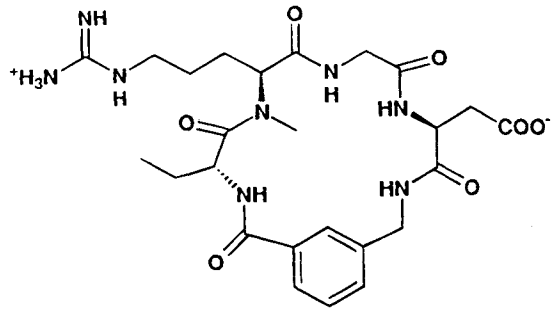

Fig. 1. Chemical structure of cyclic[D-2-amino-butyryl-N^2-methyl-L-arginyl-glycyl-L-aspartyl-3-(aminomethyl)-benzoic acid (XL118).

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EXPERIMENTAL SECTION

Materials

XL118 (lot 4) was prepared by Chemical Sciences at the Kilo Lab, Experimental Station, Wilmington, Delaware and was used as received. The amorphous acetate salt of XL118 was prepared by lyophilization from a 5 mg/ml aqueous solution of XL118 containing two equivalents of acetic acid. The sulfate salt was prepared by dissolving the acetate salt at 10 mg/ml in 1 N sulfuric acid, pH adjusted to 2.5. The resulting sulfate crystals were filtered and dried under vacuum. The besylate salt was prepared by adding 1.2 equivalents benzenesulfonic acid to a 10 mg/ml aqueous solution of the acetate salt. The resulting crystals were filtered, rinsed with 2-propanol and dried under vacuum. The mesylate salt was prepared by the Pharmacy R&D Group, Experimental Station, Wilmington, Delaware and was described previously (8). Elemental analyses were consistent with the appropriate structure following correction for solvent content. The purity of all crystalline compounds was greater than 98% by HPLC.

The water was house-deionized water that was passed through a Nanopure II (Barnstead) ion-exchange cartridge system and had a specific resistance of greater than 17 MΩ-cm. All solvents were HPLC grade. All other reagents were of analytical grade.

Thermal Analysis

The thermal properties were characterized with hot stage microscopy (Hot Stage FP82 and Central Processor FP80, Mettler), differential scanning calorimetry (DSC 910, TA Instruments) and thermogravimetric analysis (TGA 2950, TA Instruments) with data analysis via a thermal analyzer (Analyzer 2100, TA Instruments). Heating rates of 5°C/min or 10°C/min were employed for the techniques over a temperature range of 25–300°C for hot stage microscopy and DSC and 25–200°C for TGA.

Polarized Light Microscopy

Microscopic observations were performed by suspending the sample in silicone immersion oil and examining with a polarized light microscope equipped with cross polars (Aristomet Microscope, Wild Leitz).

X-ray Powder Diffraction

Powder x-ray diffraction data were obtained with an automated powder diffractometer (Model 3720, Phillips). The diffractometer was equipped with a variable slit, a scintillation counter and a graphite monochromator. The radiation was CuK_α (40 kV, 30 mA). Data were collected at room temperature from 2–60 degrees 2θ; step size was 0.02 degrees; count time was 0.5 sec. Powder samples were prepared as thin layers on glass or quartz specimen holders. The powder diffraction data have been deposited with the International Centre for Diffraction Data (Newtown Square, PA, 19073-3273, USA).

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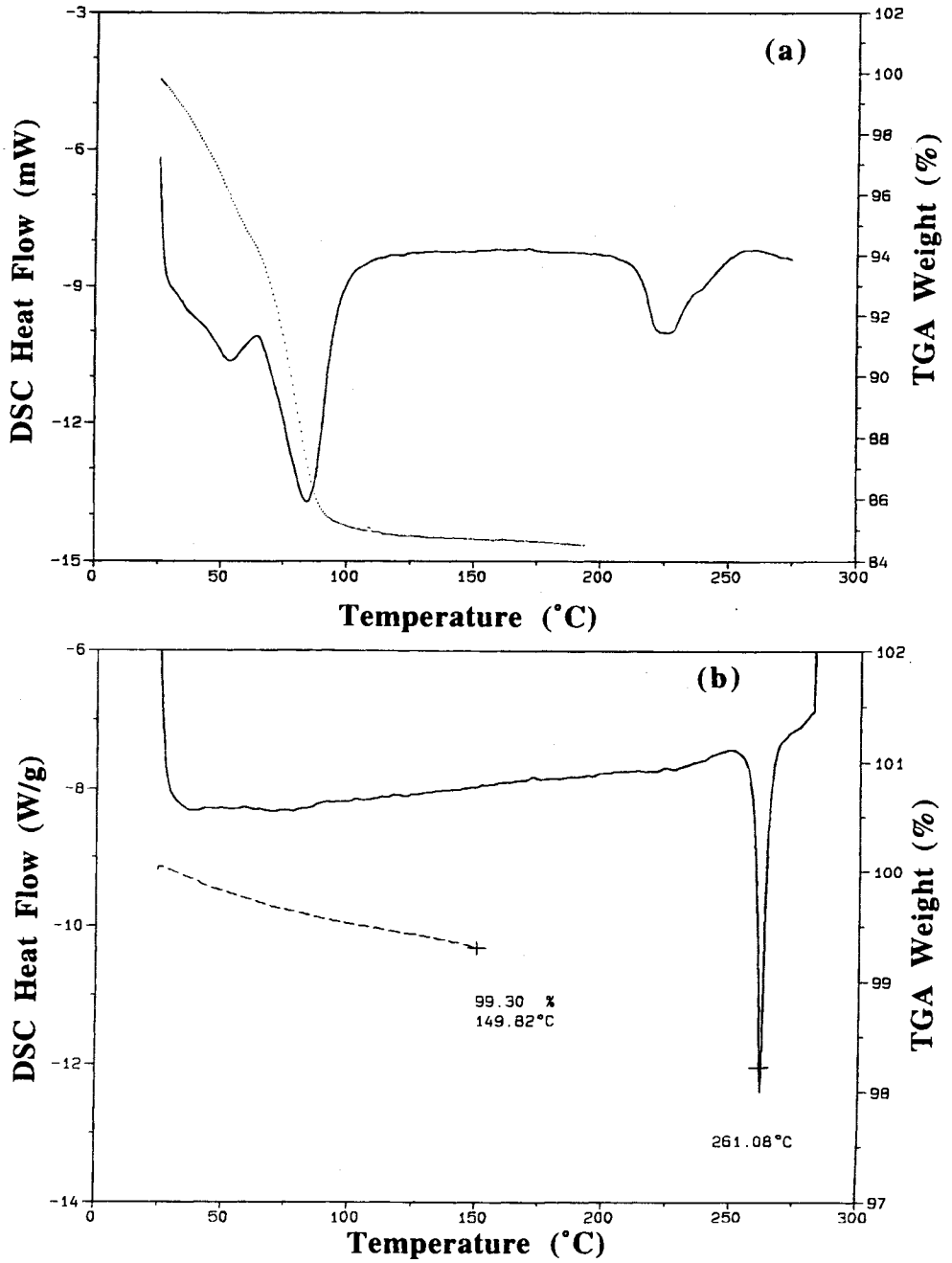

Fig. 2. DSC thermograms (solid lines) and TGA profiles (dashed lines) of XL118 (a) and the mesylate salt (b) at 10°C/min.

Hygroscopicity

The water content of the salts was measured with direct coulometric analysis (Coulometer 684KF, Metrohm). Due to insolubility in a variety of coulometric reagents, the water content of XL118 was measured indirectly with coulometric analysis (Coulometer 684KF, Metrohm) of the water that evolved into the stream of an ultra high purity nitrogen flush (40 cc/min, MG Industries) while heating at 180°C (688KF Oven, Metrohm). The 85% RH was maintained in a sealed chamber (Dry Keeper, Samplatec Corporation) with a layer of a saturated aqueous potassium chloride solution in contact with excess potassium chloride. For the sorption/desorption profile of XL118, an isothermal dynamic water uptake instrument with humidity control provided by mass flow controllers that regulated the blending of dry and humidified house nitrogen (MB-300W, VTI Corporation). The relative humidity increased from 40% RH to 95% RH for sorption and decreased from 95% RH to 5% RH for desorption in 5% RH increments. The initial sample weight was 54.7 mg and the equilibrium criteria was less than 0.005 mg weight gain per 5 min with three consecutive full intervals required prior to proceeding to the next humidity.

Aqueous Solubility

Solubility studies were carried out by placing excess drug substance into a suitable container with the appropriate solvent.

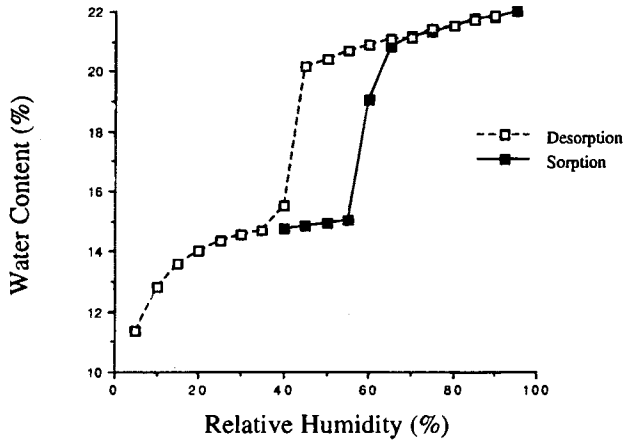

Fig. 3. Water sorption/desorption of XL118 as a function of relative humidity at 25°C.

The suspensions were rotated end-to-end for at least forty-eight hours at room temperature (≈24°C). Preliminary experiments indicated that three hours provided sufficient time to reach equilibrium. The suspension was passed through a 0.45-μm filter (Acrodisc® LC13 PVDF, Gelman Sciences) with the first portion discarded to ensure saturation of the filter. An aliquot of the filtrate was diluted and analyzed by HPLC and the remainder of the filtrate was employed for pH determination.

Chromatographic Method

Concentrations were measured with a gradient HPLC method. Separation was performed on a 15 cm reverse phase Novapak® C_{18} column (Waters Chromatography) with the temperature maintained at 30°C (Column Heater Module and Temperature Control Module, Waters Chromatography). The mobile phase was composed of methanol: water with 0.1% trifluoroacetic acid from 10:90 to 20:80 programmed linearly over 25 minutes. A flow rate of 1.0 ml/min was employed (Automated Gradient Controller, Model 680 and 2 HPLC Pumps, Model 510, Waters Chromatography). Ultraviolet detection was employed at 220 nm (Tunable Absorbance Detector 486, Waters Chromatography). Data acquisition was completed with a VAX-based program that calculated the sample concentrations from a standard curve using peak area with gradient background subtraction (Multichrom® software, VG Instruments). The standards were prepared freshly before each analysis.

RESULTS AND DISCUSSION

The mesylate salt underwent a rapid phase change via a hot crystallization in isopropanol. As soon as the XL118 was

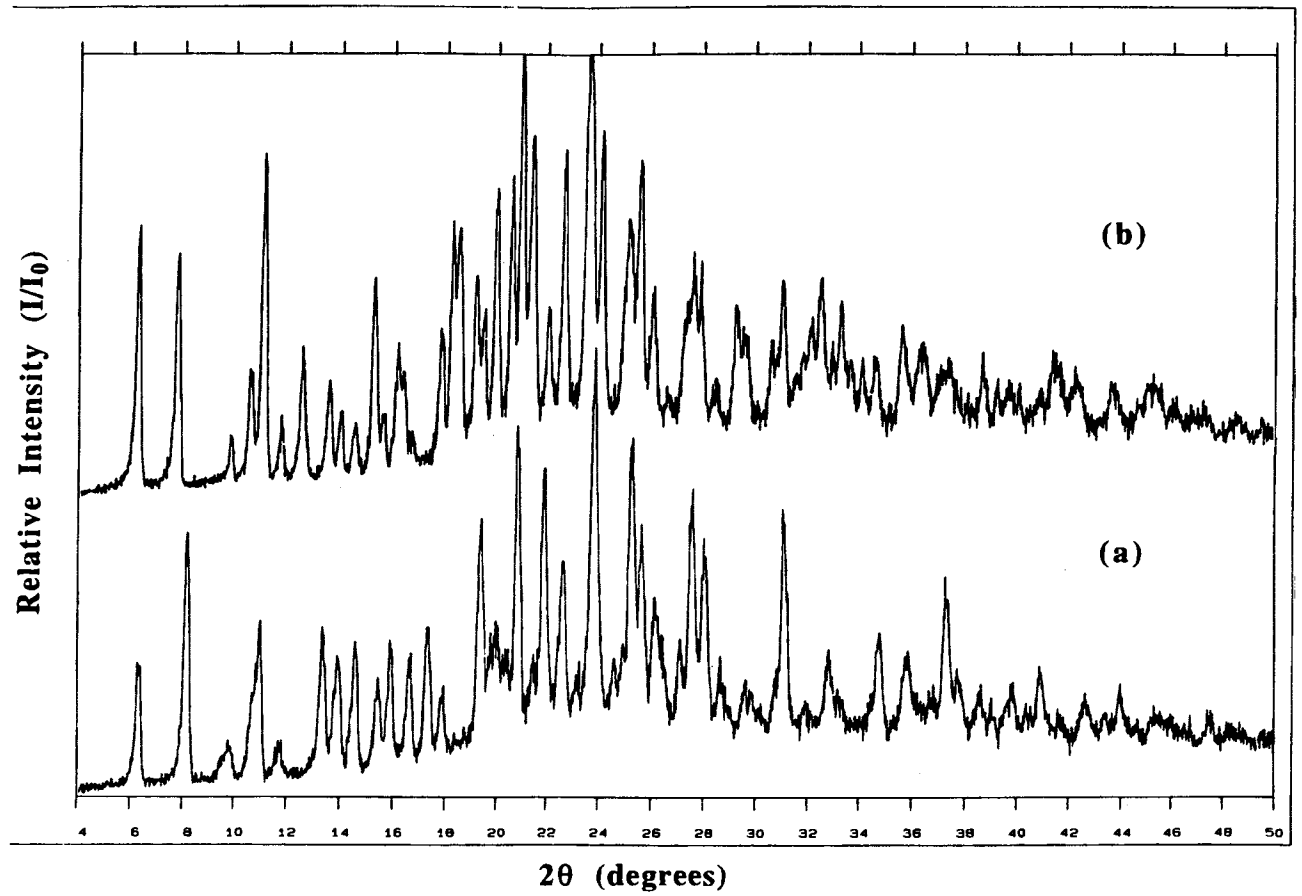

Fig. 4. Powder x-ray diffraction patterns for XL118 as received with a water content of 14.9% (a) and XL118 incubated at 85% RH with a water content of 18.5% (b).

solubilized in the boiling isopropanol following the addition of the methanesulfonic acid, a white crystalline salt nucleated instantaneously while the isopropanol continued to boil. Elemental analysis of the mesylate salt was in agreement with theoretical values and HPLC found no degradation or esterification. The mesylate salt was insoluble in its recrystallization solvent, isopropanol.

The acetate salt was amorphous. Polarized light microscopy found XL118 and the mesylate, sulfate, and besylate salts to be birefringent with distinct extinctions when rotated under cross polarization consistent with a crystalline material. Powder x-ray diffraction pattern indicated that XL118 and the mesylate, sulfate, and besylate salts were crystalline. DSC revealed a broad water release between 30°C and 100°C followed by melting at 226.5°C, 227.4°C, and 232.0°C for XL118 and the sulfate and besylate salts, respectively. A representative thermogram of XL118 is presented in Fig. 2a. DSC of the mesylate produced a single melt transition at 261.5°C (Fig. 2b). TGA to 150°C found weight losses of 0.6%, 11.8%, 3.8%, and 15.2% for the mesylate, sulfate, besylate, and XL118, respectively. Weight loss profiles for XL118 and the mesylate salt are provided in Fig. 2a–b.

The mesylate, sulfate, and besylate salts were nonhygroscopic with water contents remaining unchanged at 1%, 17%, and 6%, respectively, on storage at 85% RH. XL118 was hygroscopic with the water content increasing from 14.7% to 21.7% upon reaching 85% RH. Desorption of XL118 to 70% RH was superimposed on the water sorption profile (Fig. 3). However, below 70% RH, a hysteresis with greater than 20% water retention was maintained until the humidity became less than 45% RH at which point the desorption profile returned to initial water concentration levels (Fig. 3).

Powder x-ray diffraction patterns were obtained for XL118 before and after storage at 85% RH. Comparison of the powder diffraction patterns (Fig. 4) indicated that different crystalline phases were present when the water content increased from 15% to 19%. After storage at 85% RH, two prominent additional signals appeared in the diffraction pattern at 12.6 degrees and 18.4 degrees. In addition, a significant peak shift occurred from 8.1 degrees to 7.7 degrees following incubation at 85% RH. The water content increase was consistent with changes in stoichiometry from a pentahydrate to an octahydrate.

The aqueous solubility of XL118 and the mesylate, sulfate, and besylate salts were 5.37 mg/ml, 73.5 mg/ml, 50.0 mg/ml, and 5.0 mg/ml, respectively.

In conclusion, XL118 was hygroscopic and a different crystalline phase was observed as the water content increased. The sulfate and besylate salts were nonhygroscopic but contained high water contents and provided less water solubility than the mesylate. The mesylate was crystalline, nonhygroscopic, and water soluble and was the preferred crystalline form that entered clinical development.

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