

## Article

# Mapping the solid-state properties of crystalline lysozyme during pharmaceutical unit-operations

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## Tables

Table 1. Apparent denaturation temperature ( $T_m$ ), calorimetric enthalpy ( $\Delta H_{cal}$ ), Van't Hoff enthalpy ( $\Delta H_{vH}$ ), and cooperative unit ( $n'$ ) of lysozyme samples in the solid state; determined by differential scanning calorimetry (DSC). Conditions: samples heated from 30 to 210 °C; heating rate: 10 °C/min.

Table 2. Apparent denaturation temperature ( $T_m$ ), calorimetric enthalpy ( $\Delta H_{cal}$ ) of the second and third batches of the milled lysozyme samples (3M, 10M, 20M and 60M); determined by differential scanning calorimetry (DSC). Conditions: samples heated from 30 to 210 °C; heating rate: 10 °C/min.

Table 3. Apparent denaturation temperature ( $T_m$ ), calorimetric enthalpy ( $\Delta H_{cal}$ ), and the percentages of crystalline (Cr%), amorphous (Am%), and denatured (De%) in the mixtures of amorphous lysozyme (unprocessed) and crystalline lysozyme (3M) samples at different ratios; determined by differential scanning calorimetry (DSC). Conditions: samples heated from 30 to 210 °C; heating rate: 10 °C/min.

Table 4. The effect of pharmaceutical operations including milling time on the enzymatic activity of lysozyme powders.

Table 1. Apparent denaturation temperature ( $T_m$ ), calorimetric enthalpy ( $\Delta H_{cal}$ ), Van't Hoff enthalpy ( $\Delta H_{vH}$ ), and cooperative unit ( $n'$ ) of lysozyme samples in the solid state; determined by differential scanning calorimetry (DSC). Conditions: samples heated from 30 to 210 °C; heating rate: 10 °C/min.

Lysozyme samples	$T_m$ °C	$\Delta H_{cal}$ kJ.mol <sup>-1</sup>	$\Delta H_{vH}$ kJ.mol <sup>-1</sup>	$n'$
Unprocessed	202.3 (0.5)	134.1 (4.6)	1400.9 (35.6)	10.4 (0.6)
Crystals	187.3 (0.3)	125.3 (7.2)	1045.9 (82.6)	8.3 (0.7)
Dried crystals	187.8 (0.7)	127.0 (4.2)	1016.1 (39.7)	8.0 (0.5)
Milled dried crystal for 3 minute (3M)	187.9 (0.6)	114.4 (1.2)	940.2 (79.1)	8.2 (0.8)
Milled dried crystal for 10 minutes (10M)	187.5 (0.4)	38.0 (11.3)	307.9 (71.2)	8.1 (0.5)
	201.1 (0.2)	69.9 (8.5)	762.6 (61.3)	10.9 (0.4)
Milled dried crystals for 20 minutes (20M)	201.0 (0.4)	105.9 (3.6)	1180.1 (60.7)	11.1 (0.6)
Milled dried crystals for 30 minutes (30M)	200.8 (0.6)	52.8 (7.5)	-	-
Milled dried crystals for 45 minutes (45M)	201.1 (0.5)	19.6 (2.9)	-	-
Milled dried crystals for 60 minutes (60M)	No peak	-	-	-

Values within parenthesis are standard deviation, n = 3.

Table 2. Apparent denaturation temperature ( $T_m$ ), calorimetric enthalpy ( $\Delta H_{cal}$ ) of the second and third batches of the milled lysozyme samples (3M, 10M, 20M and 60M); determined by differential scanning calorimetry (DSC). Conditions: samples heated from 30 to 210 °C; heating rate: 10 °C/min.

Lysozyme samples	Second batch		Third batch	
	$T_m$ °C	$\Delta H_{cal}$ kJ.mol <sup>-1</sup>	$T_m$ °C	$\Delta H_{cal}$ kJ.mol <sup>-1</sup>
Milled dried crystal for 3 minute (3M)	187.4 (1.1)	122.3 (3.7)	188.4 (0.7)	119.7 (8.9)
Milled dried crystal for 10 minutes (10M)	188.2 (0.7)	48.9 (6.2)	187.1 (0.9)	37.4 (3.6)
	200.9 (0.5)	64.8 (5.9)	200.1 (1.2)	74.9 (7.1)
Milled dried crystals for 20 minutes (20 M)	200.6 (0.9)	112.8 (4.6)	200.9 (0.8)	109.2 (5.2)
Milled dried crystals for 60 minutes (60M)	No peak	-	No peak	-

Values within parenthesis are standard deviation, n = 3.

Table 3. Apparent denaturation temperature ( $T_m$ ), calorimetric enthalpy ( $\Delta H_{cal}$ ), and the percentages of crystalline (Cr%), amorphous (Am%), and denatured (De%) in the mixtures of amorphous lysozyme (unprocessed) and crystalline lysozyme (3M) samples at different ratios; determined by differential scanning calorimetry (DSC). Conditions: samples heated from 30 to 210 °C; heating rate: 10 °C/min.

Mixtures	Crystalline Peak		Amorphous peak		Cr%	Am%	De%
	$T_m$ °C	$\Delta H_{cal}$ kJ.mol <sup>-1</sup>	$T_m$ °C	$\Delta H_{cal}$ kJ.mol <sup>-1</sup>			
Unprocessed/3M (3:7)	187.1 (0.7)	79.1 (8.0)	201.8 (0.4)	41.8 (6.0)	63.3 (6.4)	31.2 (4.5)	5.5 (1.6)
Unprocessed/3M (5:5)	188.1 (0.5)	58.6 (4.9)	202.1 (0.3)	63.4 (7.0)	46.9 (3.9)	47.3 (5.2)	5.8 (2.0)
Unprocessed/3M (7:3)	187.6 (0.3)	43.5 (6.9)	202.2 (0.8)	82.5 (8.7)	34.8 (5.5)	61.6 (6.5)	3.6 (1.4)

Values within parenthesis are standard deviation, n = 3.

Table 4. The effect of pharmaceutical operations including milling time on the enzymatic activity of lysozyme powders.

Lysozyme samples	% Activity
Unprocessed	100.0 (1.5)
Crystals	102.3 (2.0)
Dried crystals	100.1 (2.9)
Milled dried crystal for 3 minutes (3M)	100.4 (1.8)
Milled dried crystal for 20 minutes (20M)	101.3 (2.7)
Milled dried crystals for 60 minutes (60M)	99.9 (1.6)

Values within parenthesis are standard deviation, n = 3.