Supporting Information

Polymeric Curcumin Nanoparticle Metabolism and Pharmacokinetics in Bile Duct Cannulated Rats

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The following are supplemental tables and figures for the Molecular Pharmaceutics manuscript titled, "Polymeric Curcumin Nanoparticle Metabolism and Pharmacokinetics in Bile Duct Cannulated Rats". The tables below, tables S1-S5, contain supplemental information for the bioanalytical methods utilized in the pharmacokinetic studies. The tables displayed are the mass spectrometry parameters for analytes (Table S1), limits of quantification (LOQ) for the analytes in tissue matrices (Table S2), calibration standards for analytes in tissue matrices (Table S3), precision, accuracy and recovery of curcumin in tissue matrices (Table S4), and precision, accuracy and recovery of tetrahydrocurcumin, hexahydrocurcumin and vanillylidenacetone in tissue matrices (Table S5). The following figures display the curcumin degradation pathways and in vitro stability studies (Figure S1), nanocurcumin physicochemical characterization data (hydrodynamic size and zeta potential) (Figure S2), evaluation of curcumin aggregation in plasma (Figure S3), and validation studies for curcumin-glucuronide hydrolysis by β-glucuronidase (Figure S4).

Table S1. Mass spectrometer parameters.

Parameters	Tetrahydrocurcumin	Hexahydrocurcumin	Vanillylidenacetone
Interface Temperature (ºC)	350	350	350
DL Temperature (ºC)	300	300	300
Heat Block (ºC)	400	400	400
Nebulizing Gas Flow (L/min)	1.5	1.5	1.5
Dry Gas Flow (L/min)	15	15	15
m/z	373.4	375.4	193.2
Interface Voltage (kV)	4.5	4.5	4.5
DL Voltage (V)	0	0	0
Qarray DC (V)	0	0	0

Table *S*2. LOQs of curcumin, tetrahydrocurcumin, hexahydrocurcumin and vanillylidenacetone in biofluids and tissue homogenates (μg/mL).

Matrix	Curcumin	Tetrahydrocurcumin	Hexahydrocurcumin	Vanillylidenacetone
Plasma	0.01	0.5	0.25	0.2
Bile	0.02	2	0.5	1
Urine	0.02	2.5	1	1
Feces	0.02	2	1	0.5
Lung	0.02	2	0.4	0.4
Liver	0.02	2	0.4	0.4
Spleen	0.02	1	0.4	0.2
Heart	0.01	1	0.2	0.2
Kidneys	0.02	1	0.2	0.2

Table S3. Calibration curves of curcumin, tetrahydrocurcumin, hexahydrocurcumin and vanillylidenacetone in biofluids and tissue homogenates (µg/mL).

Matrix	Linearity range and R^2 (in brackets)			
	Curcumin	Tetrahydrocurcumin	Hexahydrocurcumin	Vanillylidenacetone
Plasma	0.01-10 (0.999)	1-200 (0.994)	0.5-200 (0.995)	0.2-200 (0.998)
Bile	0.02-50 (0.999)	2-200 (0.997)	1-200 (0.994)	1-200 (0.999)
Urine	0.02-10 (0.996)	2.5-50 (0.997)	1-50 (0.998)	1-50 (0.999)
Feces	0.02-10 (0.999)	2-50 (0.998)	1-50 (0.997)	0.5-50 (0.998)
Lung	0.1-100 (0.999)	2-200 (0.997)	1-200 (0.997)	0.5-100 (0.998)
Liver	0.05-5 (0.997)	2-50 (0.998)	0.5-50 (0.996)	0.5-50 (0.999)
Spleen	0.05-10 (0.999)	1-50 (0.995)	0.5-50 (0.997)	0.5-50 (0.998)
Heart	0.01-5 (0.999)	1-50 (0.996)	0.5-50 (0.998)	0.5-50 (0.997)
Kidneys	0.02-5 (0.999)	1-50 (0.998)	0.5-50 (0.996)	0.5-50 (0.999)

Table S4. Precision, accuracy and recovery of curcumin in biofluids and tissue homogenates.

Matrix	Spiked concentration (µg/mL)	Precision (RSD%) (n = 5)	Accuracy (RE%) (n = 3)	Recovery (%) (n = 3)
Plasma	0.02	8.7	5.2	89.3±10.1
	0.5	4.6	-2.1	91.9±5.3
	10	6.9	3.0	93.1±6.4
Bile	0.05	8.6	-6.5	92.3±7.5
	1	6.9	0.9	102.4±6.1
	50	5.3	-4.2	95.8±4.2
Urine	0.05	10.5	7.4	85.2±9.4
	0.5	4.3	5.1	91.8±7.3
	10	5.9	3.9	92.5±5.2
Feces	0.05	8.5	-5.7	87.1±9.9
	0.5	6.8	6.1	93.5±5.4
	10	9.0	-4.9	90.1±6.2
Lung	0.1	5.1	5.5	93.0±4.7
	2	2.4	7.8	89.0±7.9
	100	3.6	2.1	95.2±6.8
Liver	0.05	6.2	7.4	107.1±8.5
	0.5	3.3	-6.9	98.2±7.6
	5	4.9	-3.7	96.9±7.9
Spleen	0.05	8.9	-6.0	88.5±7.4
	0.5	3.6	-4.9	93.9±4.6
	10	5.8	5.6	99.1±6.3
Heart	0.02	11.2	7.9	107.4±8.8
	0.5	6.7	7.4	104.1±6.9
	5	4.1	-3.6	94.8±7.3
Kidneys	0.05	9.9	-7.7	90.3±12.5
	0.5	4.8	6.2	89.5±6.4
	5	7.1	3.5	94.2±6.0

Table S5. Precision, accuracy and recovery of tetrahydrocurcumin, hexahydrocurcumin and vanillylidenacetone in biofluids and tissue homogenates.

Compounds	Matrix	Spiked concentration (μg/mL)	Precision (RSD%) (n = 5)	Accuracy (RE%) (n = 3)	Recovery (%) (n = 3)
Tetrahydrocurcumin	Plasma	1	11.6	-7.5	82.1±8.6
		20	7.2	-3.9	84.2±7.1
		200	5.9	7.2	85.8±8.4
	Bile	2	11.4	10.9	80.4±9.3
		20	6.3	-5.8	86.7±7.9
		200	7.9	6.1	84.5±6.8
	Urine	2.5	9.3	-9.7	86.7±9.2
		10	8.1	7.1	89.6±10.4
		50	5.2	7.8	86.0±5.8
Hexahydrocurcumin	Plasma	1	10.3	-8.9	84.8±9.9
		20	7.8	6.8	80.4±7.3
		200	8.2	-5.5	86.6±4.9
	Bile	2	11.2	9.2	81.2±7.6
		20	7.9	-6.4	83.7±5.5
		200	6.1	-7.3	82.3±8.1
	Urine	2	9.5	-8.5	88.1±9.0
		10	8.9	7.4	84.5±7.2
		50	8.4	8.2	85.9±6.4
	Liver	1	9.3	-6.5	80.6±8.5
		10	9.7	7.9	83.6±8.9
		50	7.6	-5.7	82.5±7.1
Vanillylidenacetone	Plasma	0.5	11.4	8.4	85.1±9.2
		20	7.3	-6.7	82.6±7.9
		200	8.6	-7.2	87.2±6.8

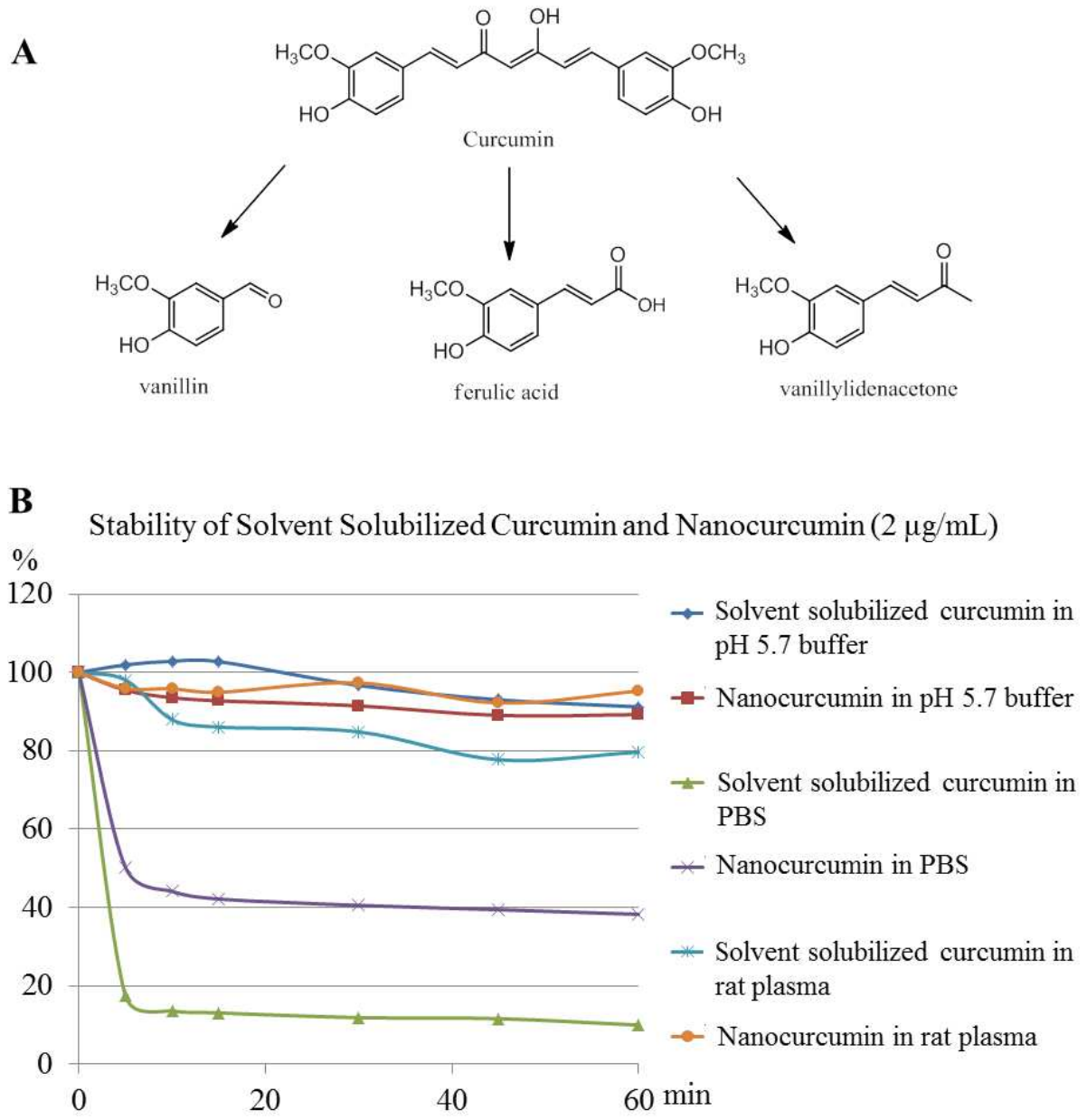

Figure S1. Degradation of solvent solubilized curcumin and nanocurcumin. (A). Degradation pathways of curcumin in PBS [1]. (B). Stability of solvent solubilized curcumin and nanocurcumin (2 µg/mL curcumin) in pH 5.7 buffer, pH 7.3 PBS buffer and rat plasma.

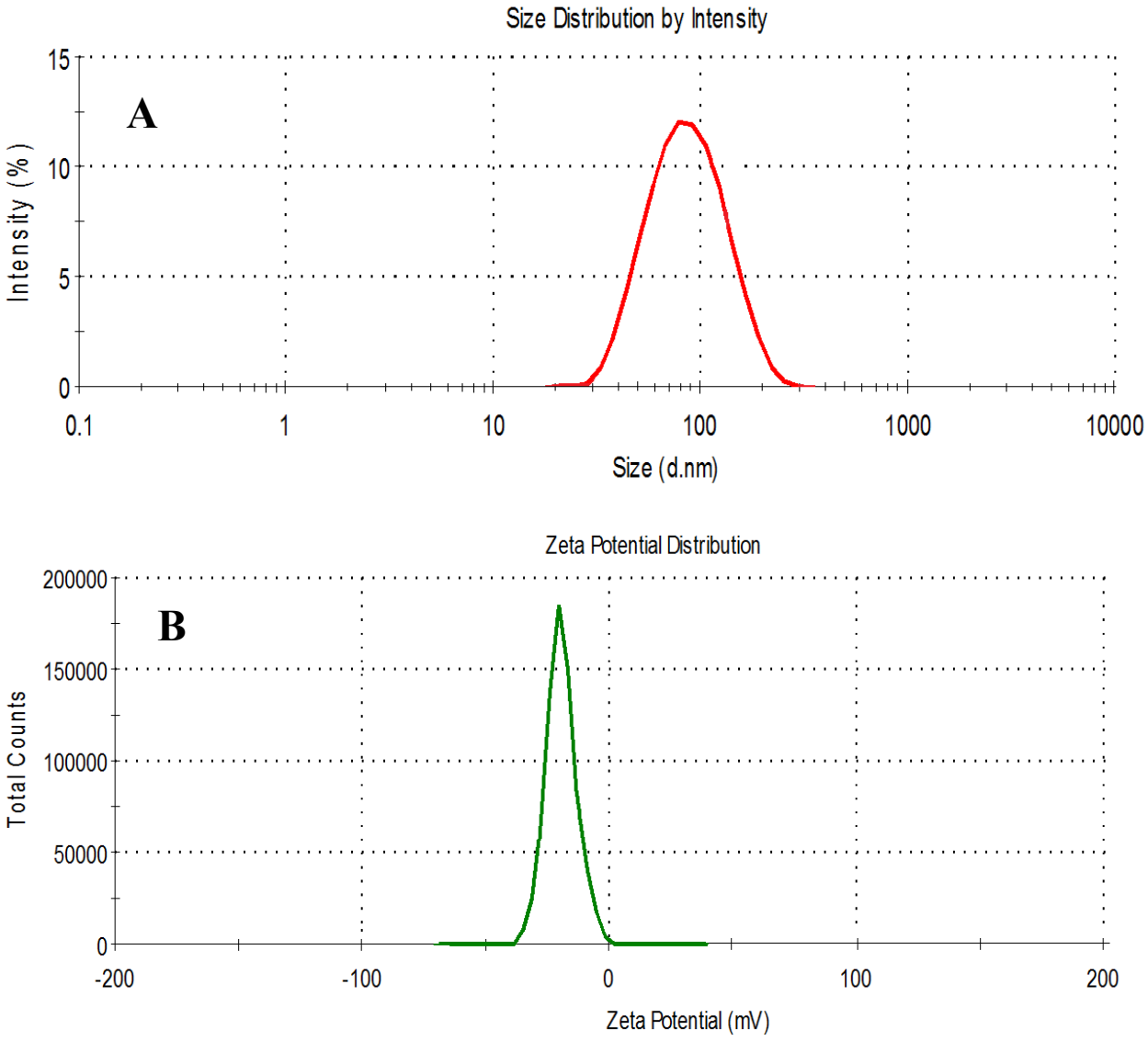

Figure S2. **(A) Hydrodynamic size of nanocurcumin in phosphate buffered saline and (B) zeta potential of nanocurcumin in 10 mM NaCl.**

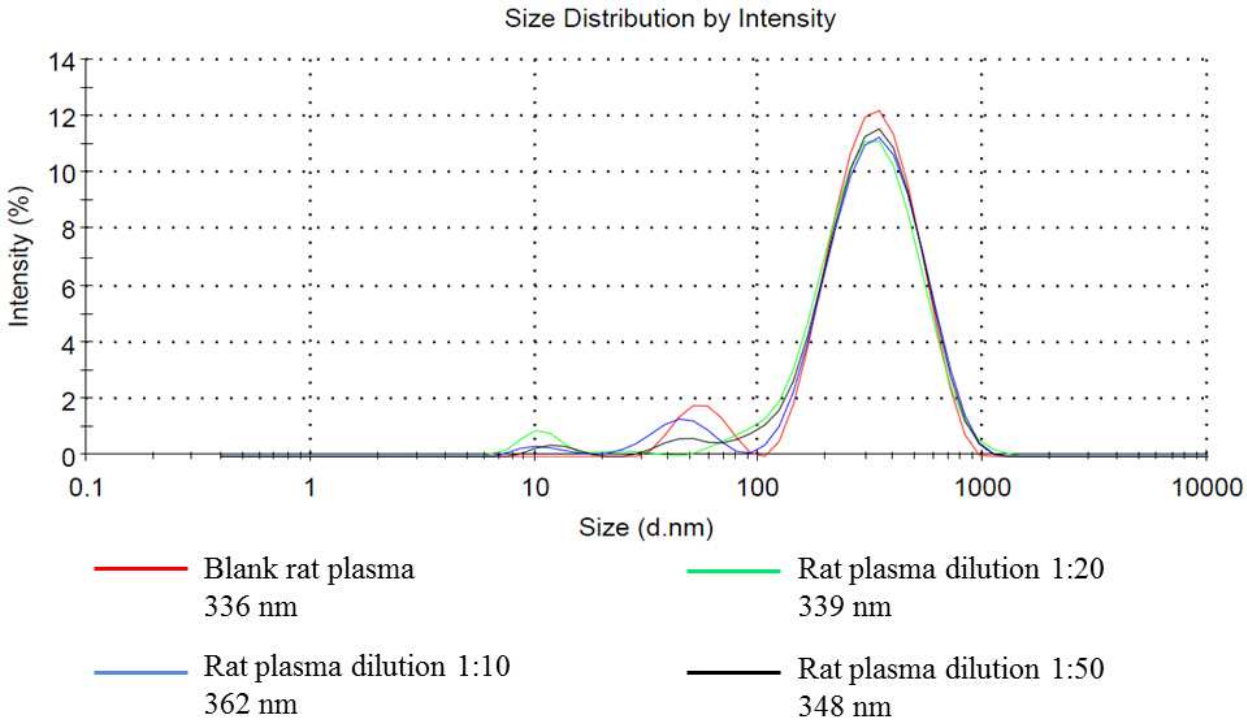

Figure S3. Effect of rat plasma dilution on the aggregation of solvent solubilized curcumin. Solvent solubilized curcumin (5 mg/mL in DMSO/PBS, 1:1, v/v) was diluted 10, 20, and 50-fold with rat plasma. The figure shows the average curve from three measurements. No aggregation of curcumin was observed. The peaks at 300-400 nm were likely due to the platelet-derived microparticles in rat plasma.

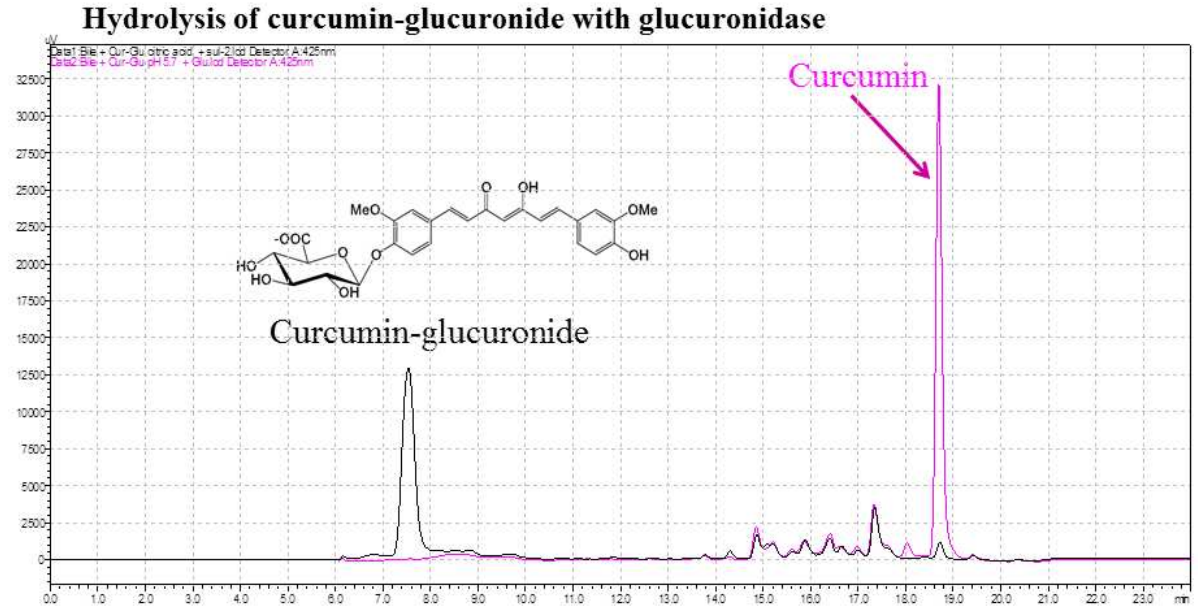

Figure S4. Complete hydrolysis of curcumin-glucuronide by β-glucuronidase. Curcumin-glucuronide (40 µg/mL) was incubated with 100 units of β-glucuronidase at 37°C for 1 h in triplicate. The average hydrolysis yield was 87.4%. The black line shows the peak of curcumin-glucuronide and the pink line shows the peak of curcumin, for a representative incubation.

References

1. Wang, Y. J.; Pan, M. H.; Cheng, A. L.; Lin, L. I.; Ho, Y. S.; Hsieh, C. Y.; Lin, J. K. *J Pharm Biomed Anal* **1997,** 15, (12), 1867-76.