SUPPORTING INFORMATION

Image-based Investigation: Biorelevant Solubility of *α* and *γ* Indomethacin

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Supplementary Text

Particle morphology

Effect of particle morphology on the solubility values measured by the SPA method was assessed by the MatLab® regionprops toolbox. Three morphology-describing parameters were extracted; namely: eccentricity, major axis length and minor axis length. The morphology parameters were then plotted against solubility values of individual particles (Figure S1 and Figure S2). No specific effect of particle morphology on solubility, in the size range studied (5-25 μm), was found.

Shake-flask solubility measurements

Indomethacin α and γ samples were placed in excess amount into vials. The vials were filled with HCl, FaSSGFblk and FeSSIFblk media and left to stir on a rotating machine. This procedure was repeated in triplicate for both solid-state forms and the three media. After 24h, 48h and 72h samples of 2 mL were extracted and filtered (Whatman™ PVDF 13 mm with 0.45 μm pore size). After filtration, the samples of α indomethacin were diluted four times with the corresponding medium in order to avoid possible precipitation.

The drug concentration in the samples was determined using an Agilent High Performance Liquid Chromatography (HPLC) 1260 system (Agilent Technologies, Germany). The Phenomenex Gemini NX-C18, 3 μm, 100 x 4.6 mm column (Phenomenex, Torrance, CA) was used in the assays. The temperature of the column was set to 30°C. Mobile phase used was 0.2% H_3PO_4:Acetonitrile (40:60, v:v) with flow rate of 1.5 mL/min. Injection volume was 20 μL and total run time 5 min. Detection was preformed at 270 nm. Standard solutions of indomethacin in MilliQ:Ethanol (50:50, v:v) were prepared and analyzed with HPLC to construct the calibration curve. All samples were analyzed in triplicate: 0.1, 0.5, 1, 10, 50 and 100 μg/mL ($R^2 > 0.999$).

After 72h, the remaining excess solids, from vials were initially α form of indomethacin was placed, were filtered and dried overnight in a vacuum oven. The next day, the excess solids were analyzed by XRPD and DSC.

Shake-flaks equilibrium solubility values obtained are listed in the Table S1. XRPD diffractograms of the remaining solids are presented in the Figure S3 and DSC thermograms in the Figure S4.

When comparing the shake-flask solubility values to the SPA solubility values, they are in the same rank order and within the generally accepted interlaboratory variance, 0.6 log units.[32] Moreover, a slight shift of XRPD peaks to the left and appearance of a small melting peak at 159.3°C ± 0.1°C in the DSC thermograms of the remaining excess solid samples indicate a presence of γ form (Figure S3 and S4). The presence of γ form, in vials were initially pure α form was placed, would result in lower solubility ratio of α to γ solubility measured by shake-flask method (1.7 ± 0.4) when compared to the ratio obtained by the SPA method (3.3 ± 0.5). With the SPA method, the actual solubility of the metastable α form was measured before any possible solid-state transformation occurred that could affect the measurement. This was achieved by starting the measurement as soon as the solvent came in contact with the sample and also by maintaining the sink conditions in the flow-through cell.

Table S1. Solubility values obtained for α and γ indomethacin by the conventional Shake-flask and the SPA method. $FaSSGF_{blk}$ = FaSSGF blank, $FeSSIF_{blk}$ = FeSSIF blank.

	Shake-flask (mg/L)	**SPA method (mg/L)**
Indomethacin α in HCl pH 1.6	0.6 ± 0.1	0.34 ± 0.20
Indomethacin α in $FaSSGF_{blk}$	0.5 ± 0.2	0.14 ± 0.09
Indomethacin α in $FeSSIF_{blk}$	9.0 ± 0.2	2.79 ± 1.67
Indomethacin γ in HCl pH 1.6	0.3 ± 0.1	0.10 ± 0.05
Indomethacin γ in $FaSSGF_{blk}$	0.3 ± 0.1	0.04 ± 0.03
Indomethacin γ in $FeSSIF_{blk}$	6.4 ± 0.1	0.94 ± 0.58

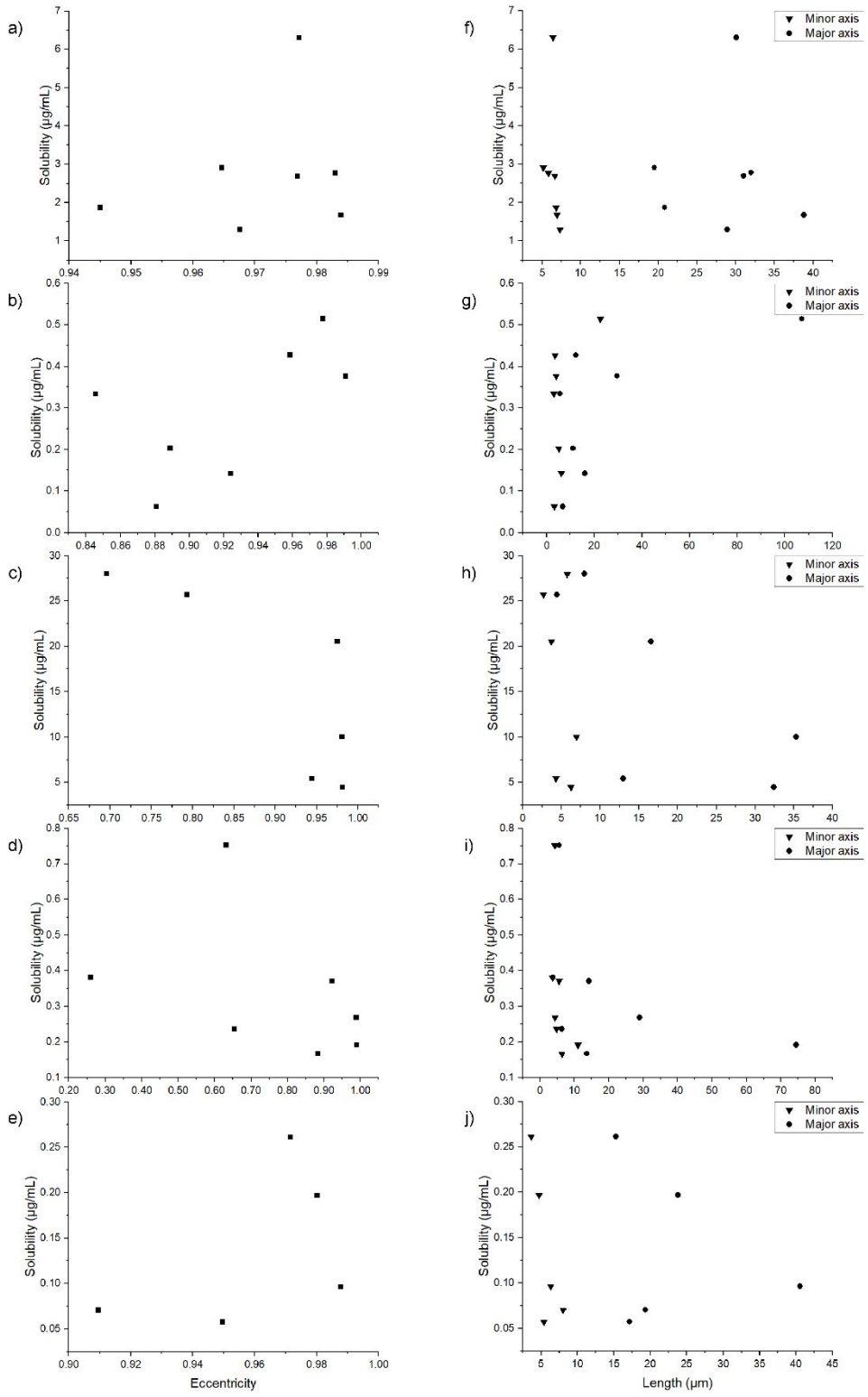

Figure S1. Solubility of individual particles plotted vs particle shape descriptors (eccentricity, minor axis and major axis) for α indomethacin. a) and f) in $FeSSIF_{blk}$, b) and g) in FaSSGF, c) and h) in FeSSIF, d) and i) in HCl, e) and j) in $FaSSGF_{blk}$ medium.

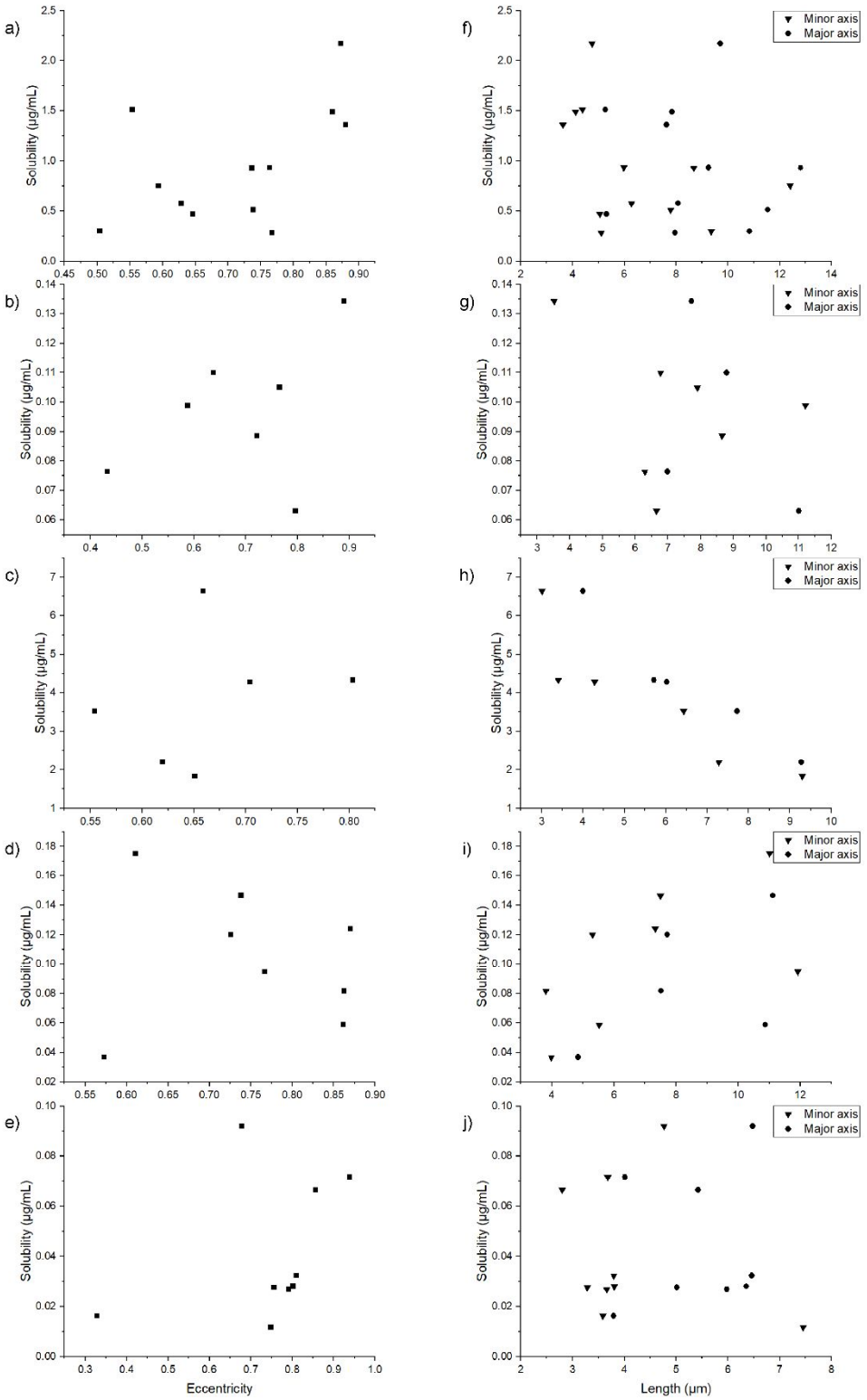

Figure S2. Solubility of individual particles plotted vs particle shape descriptors (eccentricity, minor axis and major axis) for γ indomethacin. a) and f) in $FeSSIF_{blk}$, b) and g) in FaSSGF, c) and h) in FeSSIF, d) and i) in HCl, e) and j) in $FaSSGF_{blk}$ medium.

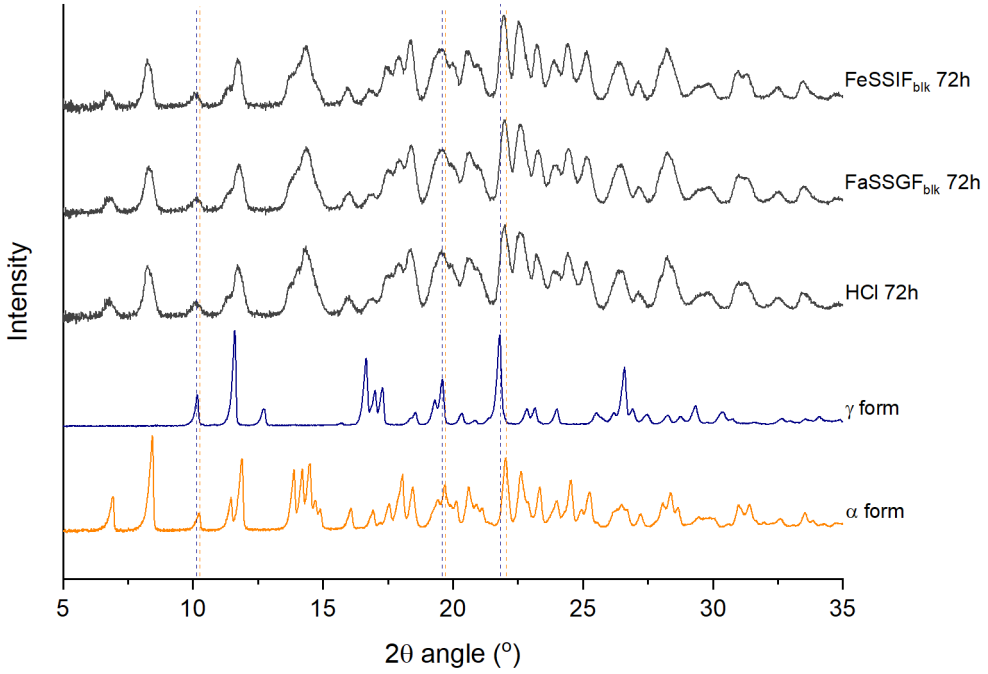

Figure S3. XRPD diffractograms of the remaining solid after 72h of shake-flask experiment started with pure α form of indomethacin. Diffractograms of γ and α form are plotted for comparison. A slight shift to the left of some peaks, indicated by dashed lines, can be noted for α samples after being exposed to dissolution media for 72h. The shift could indicate a presence of γ form in the sample.

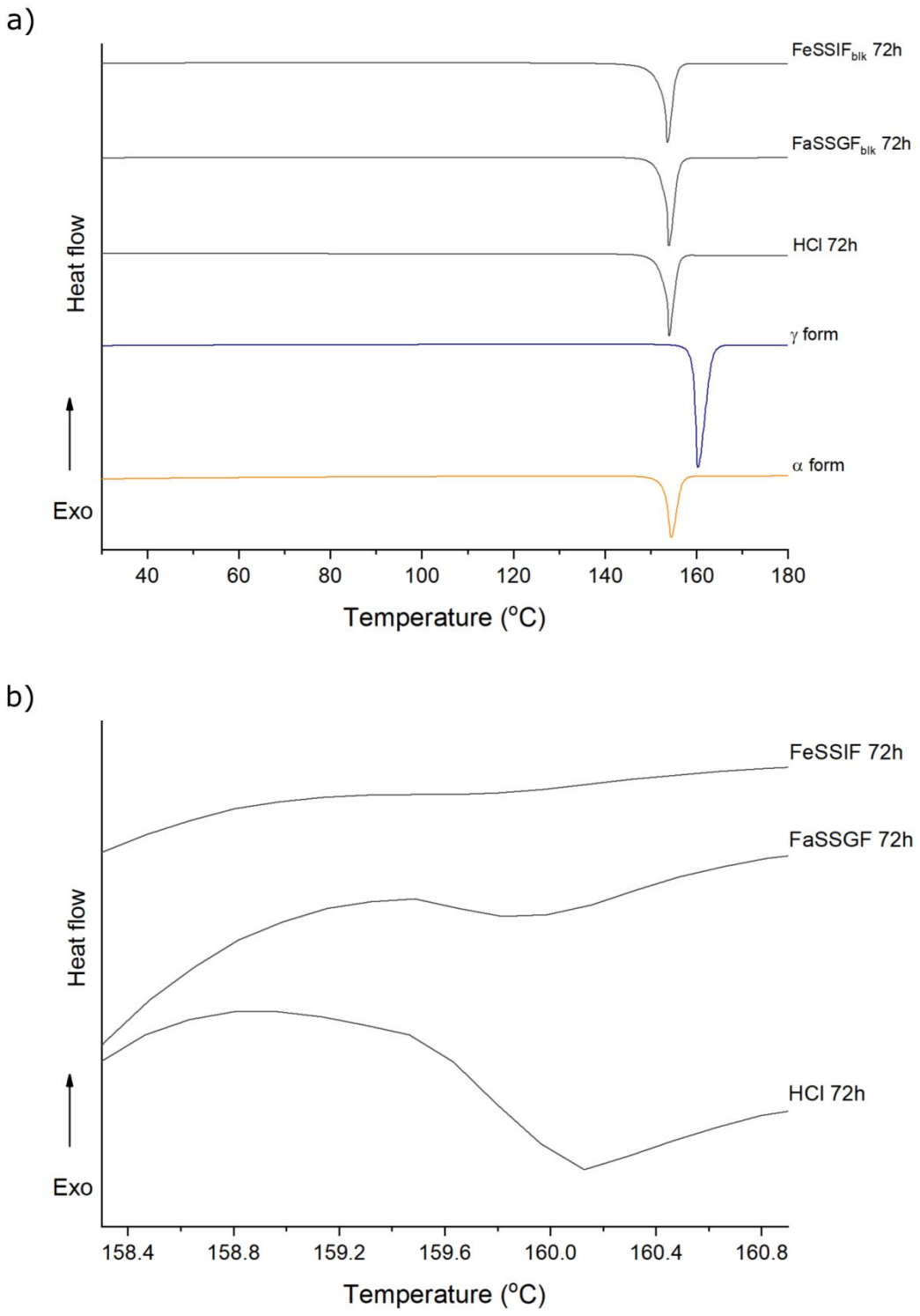

Figure S4. a) DSC thermograms of the remaining solid after 72h of shake-flask experiment started with pure α form of indomethacin. Melting peak at 152.4°C ± 0.2°C indicating the solid is the α form of indomethacin. Thermograms of γ and α form are plotted for comparison. b) A small melting peak around 159.3°C ± 0.1°C, indicating a presence of γ form, can be noted upon close up of the section from 158.4 to 160.8°C.