Supplementary Information

The Culprit of Gout: Triggering Factors and Formation of Monosodium Urate Monohydrate

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This Supplementary Information contained Instrumentations, Scheme S1, Figures S1 to S7, and Tables S1 to S3.

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INSTRUMENTATIONS

Polarizing Optical Microscopy (POM)

Crystal habit and solution morphology were examined and imaged by a polarized optical microscope (BX51; Olympus, Tokyo, Japan) equipped with a digital camera (Moticam 2000 2.0MPixel USB2.0; Motic, Inc., Xiamen, China) to take images of samples. Data were visualized by use of Motic Images Plus 2.0 ML (Motic, Inc., Xiamen, China) at the time points before harvest. Crystals were identified by their birefringence.

Scanning Electron Microscopy (SEM)

A scanning electron microscope (Hitachi S-3500N, Tokyo, Japan) was used to observe the morphology of tiny crystals. Both secondary electron imaging (SEI) and backscattered electron imaging (BEI) were used for the SEM detector and the magnification was 15- to 300 000-fold. The operating pressure was 10^{-5} Pa vacuum and the voltage was 15.0 kV. All samples were mounted on a carbon conductive tape (product no. 16073, TED Pella Inc.) and then sputter-coated with gold (Hitachi E-1010 Ion Spotter, Tokyo, Japan) with a thickness of about 6 nm. The discharge current used was about 0-30 mA and the vacuum was around 10 Pa.

Powder X-ray Diffraction (PXRD)

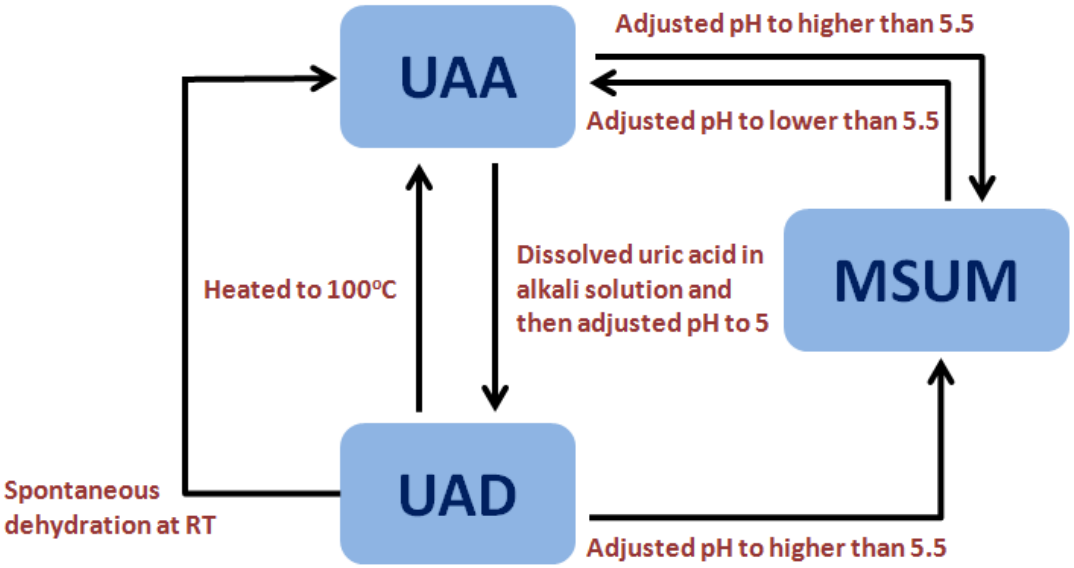
PXRD patterns were obtained from samples on a wide-angle powder X-ray

diffractometer (Bruker D8 Advance, Karlsruhe, Germany) to identify the phases if they could not be identified by polarizing optical microscopy or scanning electron microscopy simply based on their crystal habits. X-ray radiation Cu Kα1 (λ = 1.5405 Å) was set at 40 kV and 40 mA passing through a nickel filter with divergence slit (0.5^o), scattering slit (0.5^o), and receiving slit (1 mm). Samples were subjected to X-ray powder diffraction analysis with a sampling width of 0.05^o in a continuous mode with a scanning rate of 1^o min over an angular range of $2\theta = 5\text{-}60^o$.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra were recorded on a Perkin-Elmer Spectrum One spectrometer (Perkin-Elmer Instruments LLC, Shelton, CT). The KBr sample disk was scanned with a scan number of 8 from 400 to 4000 cm^{-1} and had a resolution of 2 cm^{-1}.

SCHEME



Scheme S1. The relationship among UAA, UAD and MSUM.

FIGURES

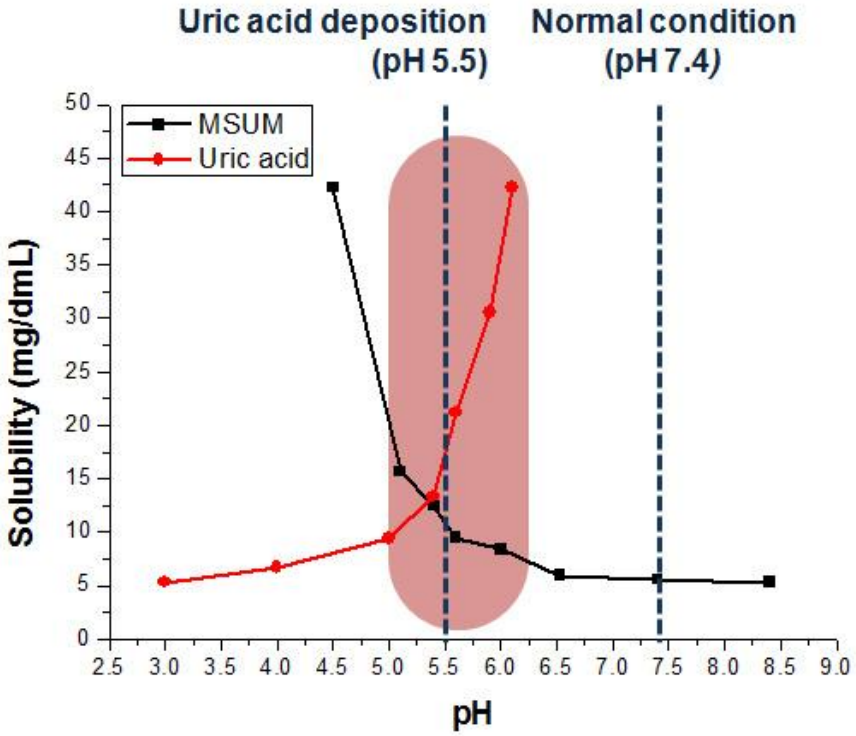


Figure S1. The pH-solubility relationships of uric acid and MSUM.

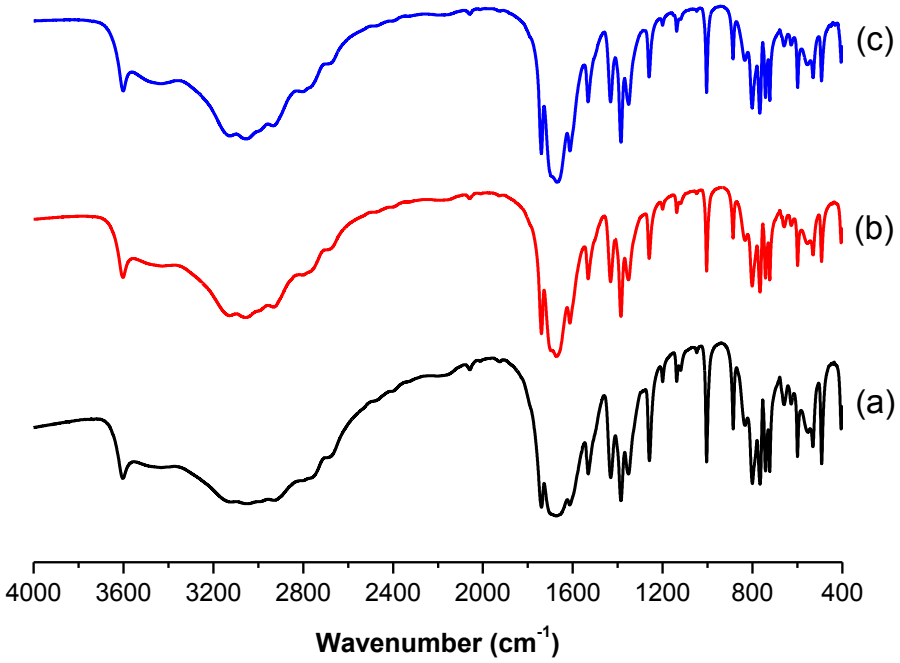


Figure S2. FTIR spectra of MSUM crystals grown under various Na^+ ion levels: (a) 100 mM, (b) 600 mM, and (c) 1500 mM.

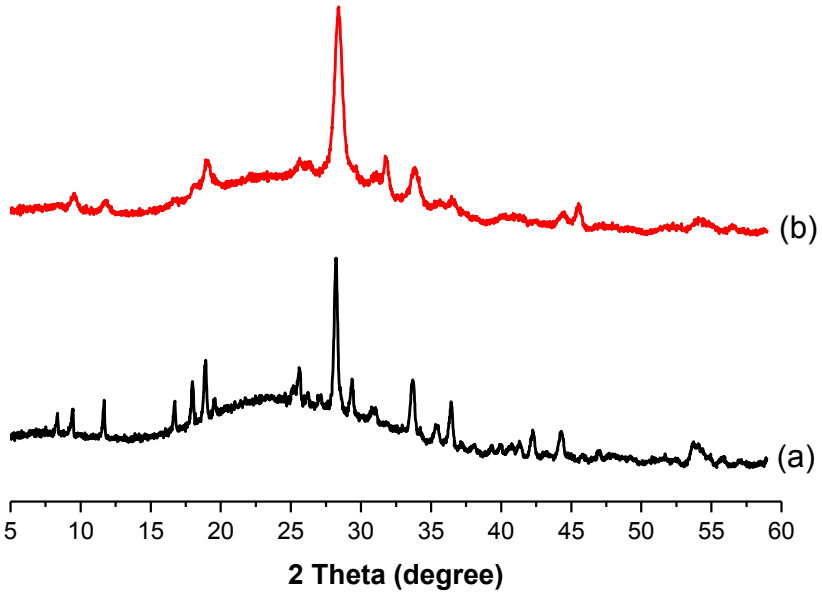


Figure S3. PXRD patterns of (a) MSUM needles and (b) MSUM beachballs.

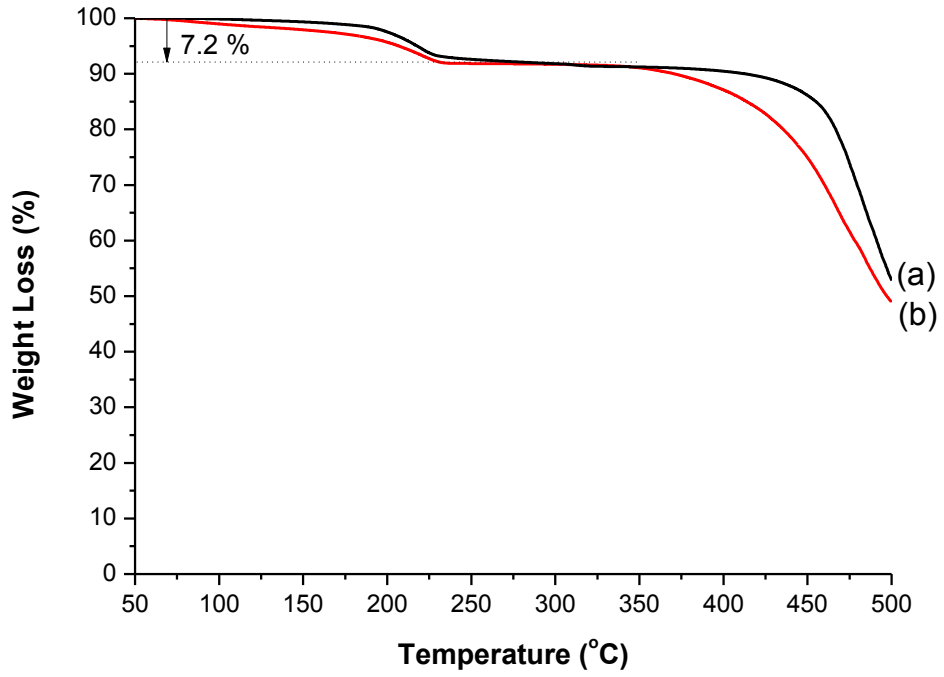


Figure S4. TGA scans of (a) MSUM needles and (b) MSUM beachballs.

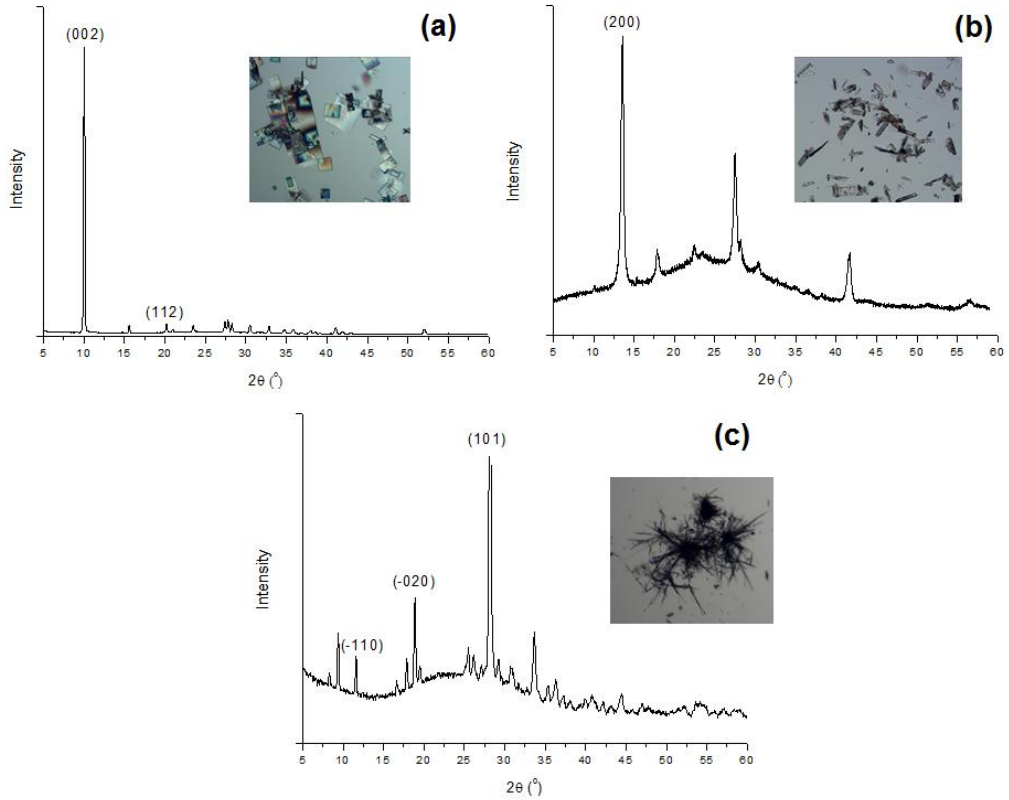
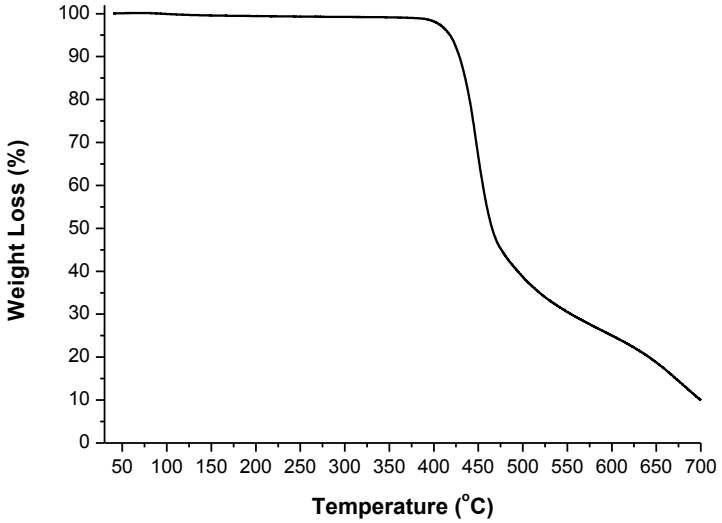
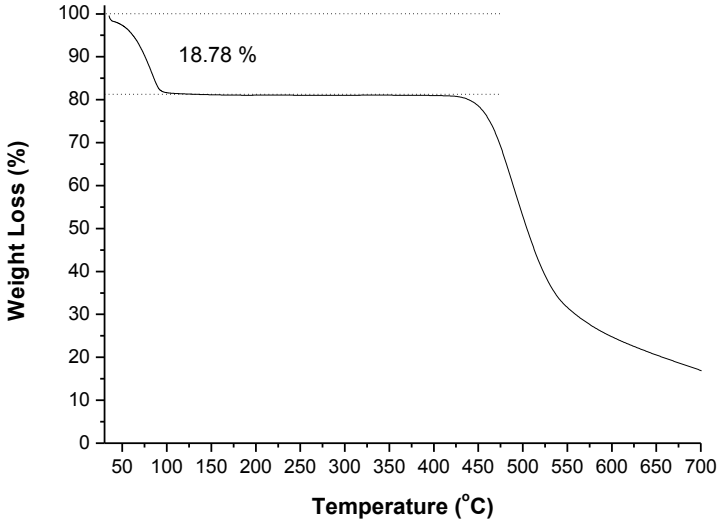


Figure S5. PXRD patterns and OM images of (a) UAD, (b) UAA and (c) MSUM.



(a)



(b)

Figure S6. TGA scans of (a) UAA and (b) UAD.

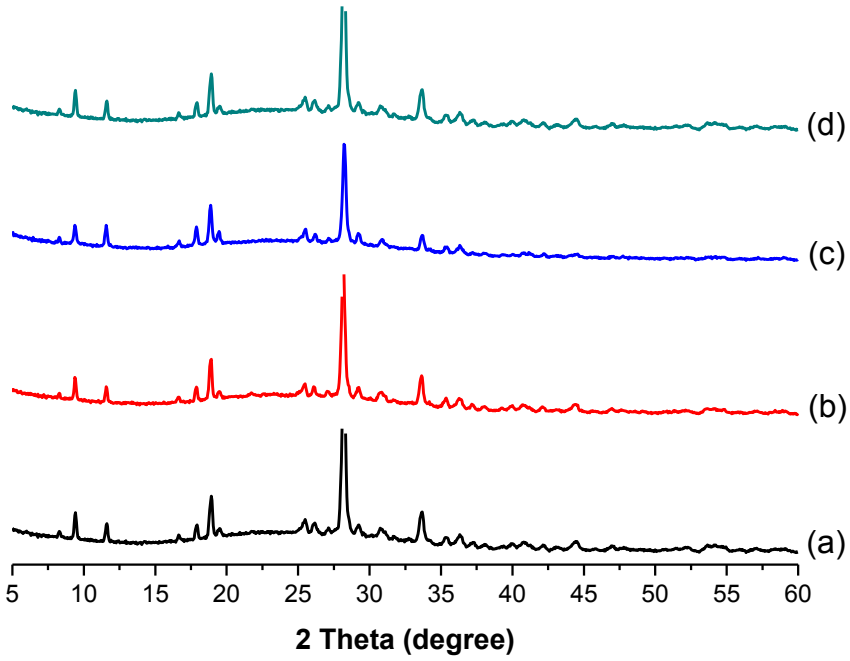


Figure S7. PXRD patterns of MSUM grown in solutions: (a) E1: 2 mg/mL of sodium hyaluronate and 140 mM of Na^+ ion, (b) E2: 2 mg/mL of sodium hyaluronate, 140 mM of Na^+ ion and 5 mM of K^+ ion, (c) E3: 2 mg/mL of sodium hyaluronate, 140 mM of Na^+ ion and 2 mM of Ca^{2+} ion, and (d) E4: 2 mg/mL of sodium hyaluronate, 140 mM of Na^+ ion, 5 mM of K^+ ion and 2 mM of Ca^{2+} ion.

TABLES

Table S1. Chemical reagents used in this study.

Chemical	Formula	Assay	M.W.	Brand
Calcium chloride dihydrate	$CaCl_2 \cdot 2H_2O$	≥ 99%	147.01	Showa
HEPES	$C_8H_{18}N_2O_4S$	≥ 99.5%	238.31	Sigma
Lactic acid	$C_3H_6O_3$	98%	90.08	Alfa Aesar
Magnesium chloride	$MgCl_2$	≥ 98%	95.22	Sigma
Potassium chloride	KCl	99.0 - 100.5%	74.55	Sigma
Potassium phosphate dibasic trihydrate	$K_2PO_4 \cdot 3H_2O$	≥ 99.0%	228.23	Sigma
Sodium bicarbonate	$NaHCO_3$	99.7 - 100.3%	84.01	J. T. Baker
Sodium carbonate	Na_2CO_3	≥ 99.5	105.99	Showa
Sodium chloride	NaCl	≥ 99%	58.44	J. T. Baker
Sodium hyaluronate	$(C_{14}H_{20}NO_{11}Na)_n$	≥ 94%	1,500,000 ~2,200,000	Echo Chemical
Sodium sulfate	Na_2SO_4,	≥ 99.0%	142.04	Riedel-deHaën
Uric acid	$C_5H_4N_4O_3$	≥ 99%	168.11	Sigma

Table S2. IR assignments for MSUM.

Assignments	**Wavenumber (cm^{-1})**
C=O	1737.7
C=C	1531.4, 1500.5
C-N	1259.4, 1351.0, 1384.8
N-H stretching	2924.8
N-H rocking	722.3, 741.6, 766.7, 800.4, 866.0, 842.0
O-H stretching	3598.0
Oxygen-metallic bond	400-600

Table S3. The crystal yields and the saturation values of MSUM in solutions.

Solution	**(a) E1**	**(b) E2**	**(c) E3**	**(d) E4**
Composition	**Hyaluronate Na^+**	**Hyaluronate Na^+, K^+**	**Hyaluronate Na^+, Ca^{2+}**	**Hyaluronate Na^+, K^+, Ca^{2+}**
MSUM Yield	47.3 wt% (19.7 mg)	39.8 wt% (16.6 mg)	26.4 wt% (11.0 mg)	7.2 wt% (3.0 mg)
MSUM Saturation	0.46 mM	0.59 mM	0.88 mM	0.69 mM