

Supplementary Information

Inhibition of Growth of L-Cystine Crystals by N-acety-L-Cysteine

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1. Experimental Section

Materials. L-cystine (>99.7%), L-cysteine (>98.5%), N-acetyl-L-cysteine (>99%), L-cystine dimethyl ester dihydrochloride (>95%), D-penicillamine(>98%), α -mercaptopropionylglycine and captopril(>99%) were obtained from Sigma Aldrich and used without purification. N-acetyl-D,L-serine (>98%), N,N'- diacetyl-L-cystine(>90%) were obtained from Shanghai yuanye Bio-Tecnology Co. Ltd and used without purification. Aqueous solutions were prepared using deionized water (18.2 M Ω) purified with a Nanjing Yipuyida purification system.

L-cystine crystallization. The hexagonal L-cystine crystals were grown in vitro according to method of refs1.^[1] Typical procedure includes adding 70mg of L-cystine to 100mL of deionized water (3mM), after heating under reflux at 100 °C for 20 min, the solution was then allowed to cool slowly with stirring for 70 min to room temperature. 30 mL aliquots were transferred to separate glass containers, where the desired concentration of additives were added (the additive was pre-dissolved by deionized water with concentration of 1mg/mL), the containers were then sealed to prevent evaporation and exposure to airborne particulates and stored for 72 hours at room temperature without stirring. The precipitate was collected by vacuum filtration (Whatman Grade 1 filters, >11 μ m pores) and were air dried prior to analysis. The mass yields of L-cystine crystals were obtained by dividing the mass of L-cystine crystals (collected from growth solution by filtration with additives) by the mass of L-cystine crystals collected from growth solution without additives. The solubility of L-cystine in water is 0.4-0.7 mM (pH 7, 25 °C).^[2] The mass yields in our experiments are slightly lower than the theoretical yield because the mass losses during operation.

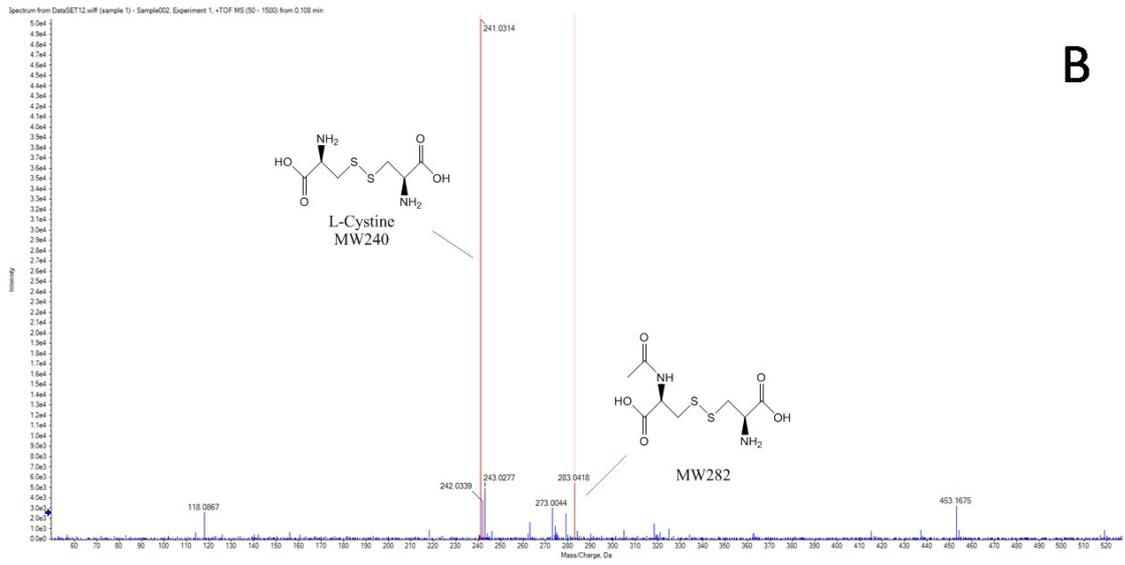
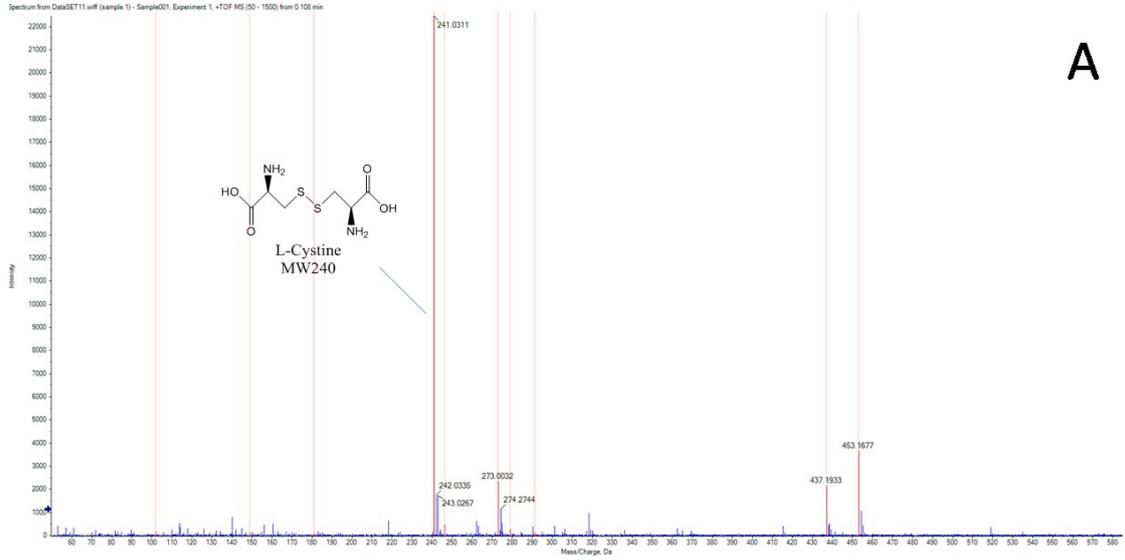
Characterization method. Powder X-ray diffraction (PXRD) was conducted on a Rigaku Ultima IV diffractometer (Japan) with Cu K α target at scanning step of 10 degrees/min. All the samples were grained for one minute in an agate mortar prior the examination. Powder size distribution was

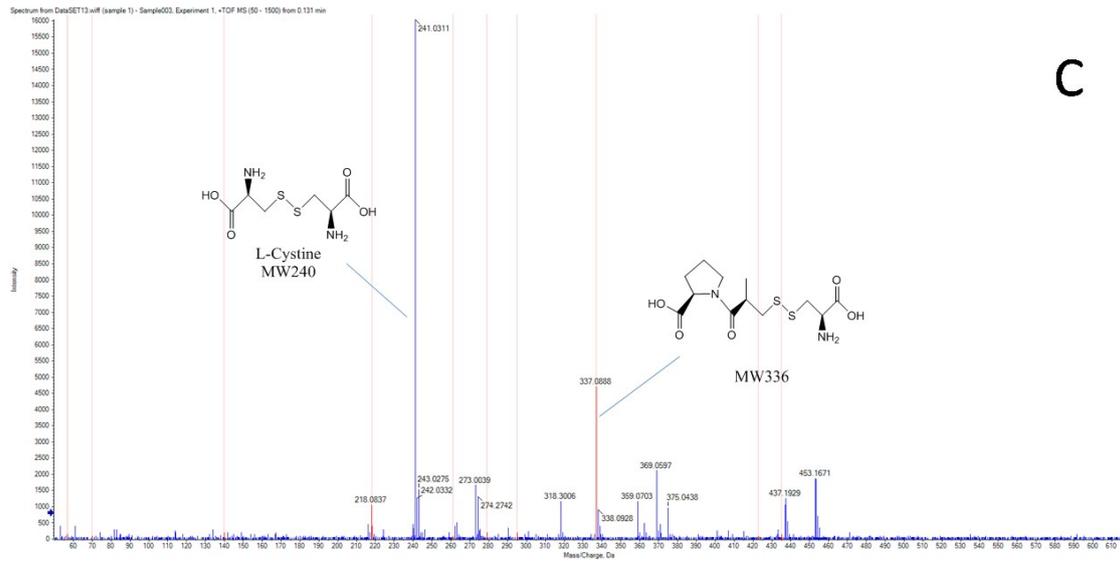
measured by a Beckman-coulter 13320 laser light scattering particle size analyzer (USA), the samples were dispersed in water and optical model was choose as rf780d. The morphology of the crystals was observed by both scanning electron microscope (SEM) and optical microscope. The SEM pictures were taken on a Hitachi S-4800 II field emission scanning electron microscope, the sample was fixed on the pillars and conductive coated by gold before measurement. The optical images were collected on Olympus DP73 microscope (Japan) with appropriate magnification. Mass spectrometry data was collected on an AB Sciex Triple TOF5600 (USA) high resolution mass spectrometry equipped with an electrospray injection system. After 72 hours of precipitation, the filtrate of Whatman Grade 1 filters was collected and filtered through 0.2 micron filtration membrane, and then diluted ten times for mass spectrometric analysis. Mass spectrometric analysis was carried out in positive ion mode, sample volume of 2 UL, atomization gas temperature of 550°C and spray voltage 5500v. Atomic force microscopy (AFM) was performed with a Bruker Instruments Dimension Icon. All measurements were performed in a cell designed to contain liquids for in situ imaging. All measurements were performed in PeakForce QNM in Fluid mode using Veeco SCANASYST-FLUID probe with a spring constant of 0.7 N/m (triangular, 70 μm length, 10 μm width). L-cystine crystals (hexagonal form) prepared by the procedure described above were transferred onto an AFM specimen disk coated with partially cured (1 hr) UV-curable optical cement by gently pressing the disk against hexagonal platelets. The (001) faces of the hexagonal plates naturally aligned parallel with the specimen disk such that the velocity of the equivalent {100} steps could be measured readily by AFM, The partially cured polymer with the adhered crystals was cured completely by additional UV radiation (2 hrs) prior to analysis. The mounted L-cystine crystals were etched slightly by immersion in deionized water for 30 sec at 60 °C to remove amorphous deposits or impurities that may be present on the

surface.

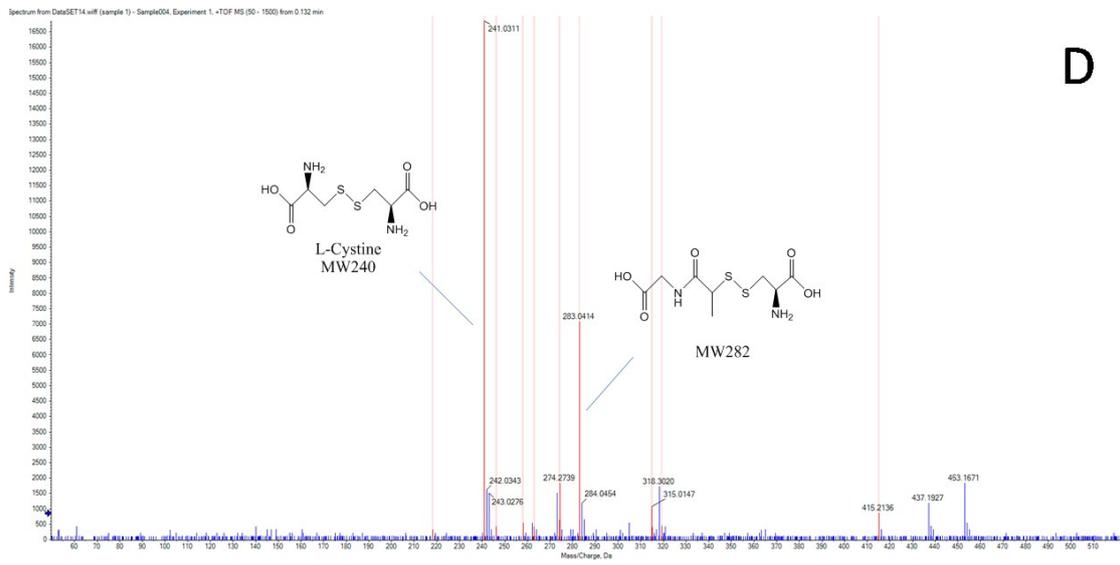
2. Results

Mass spectrometry

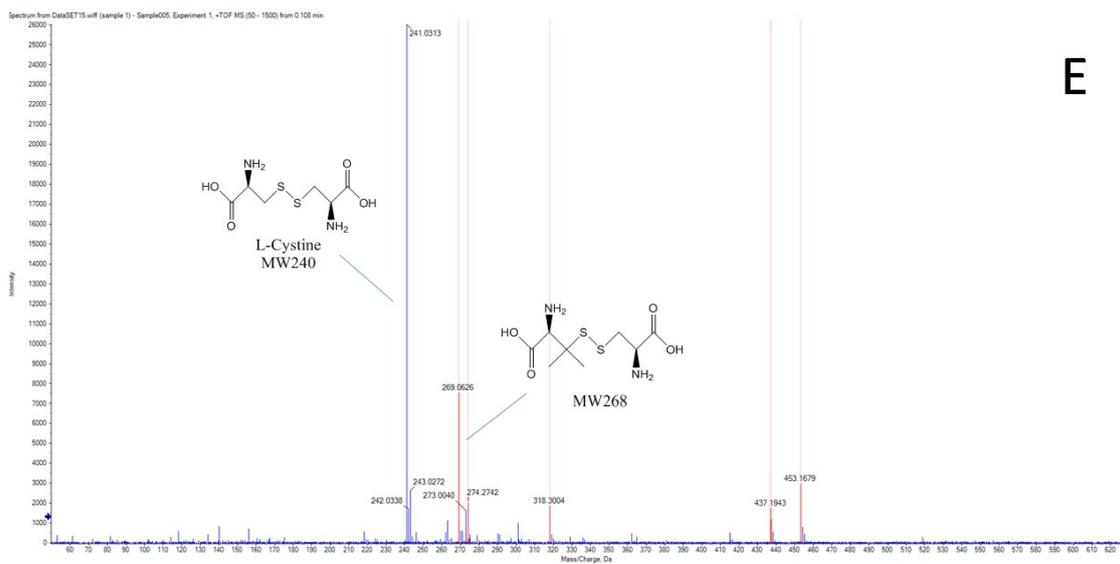




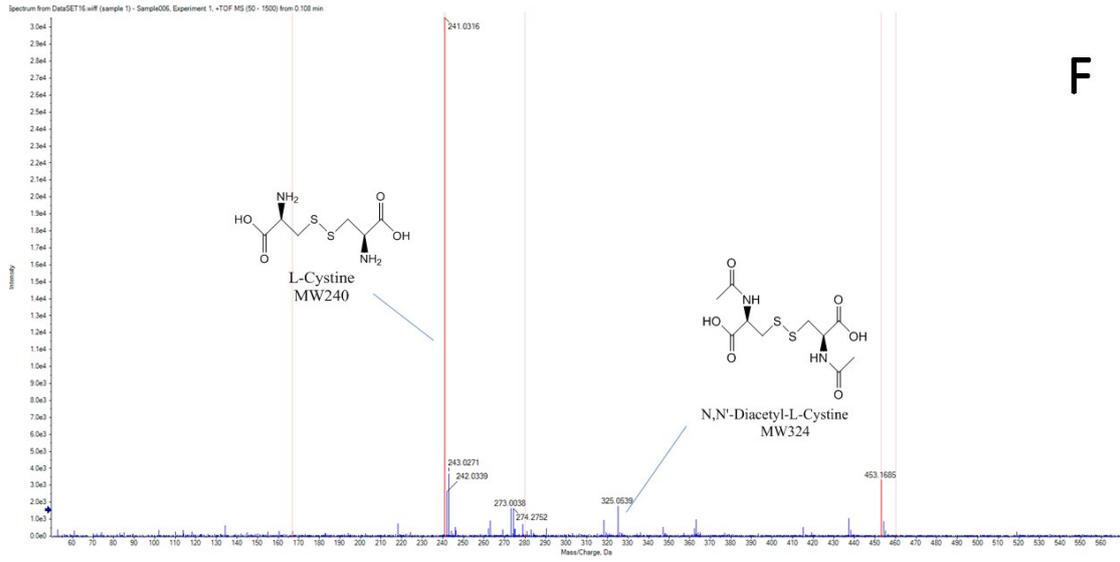
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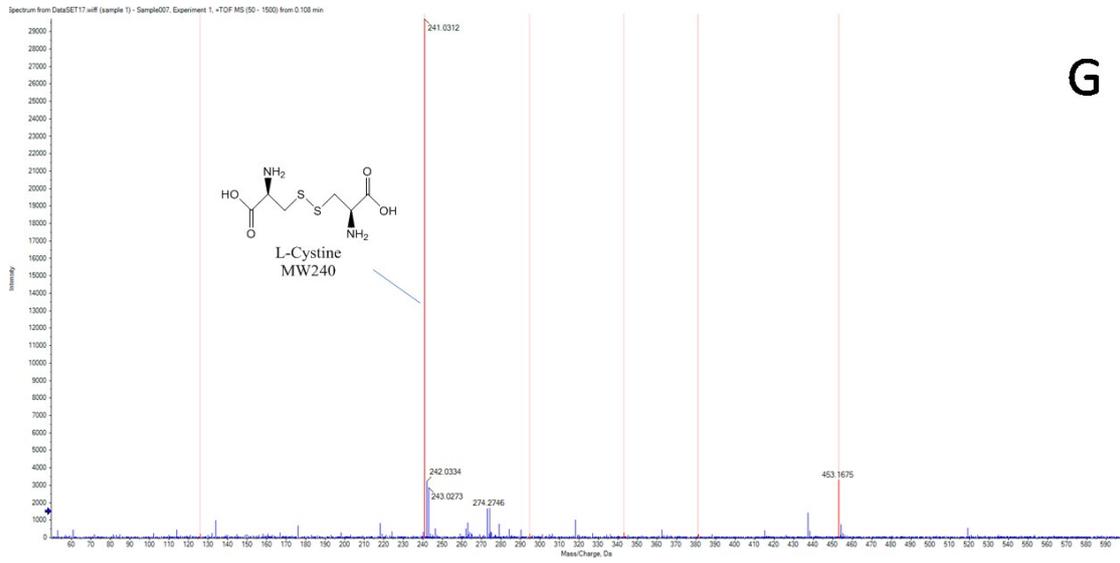
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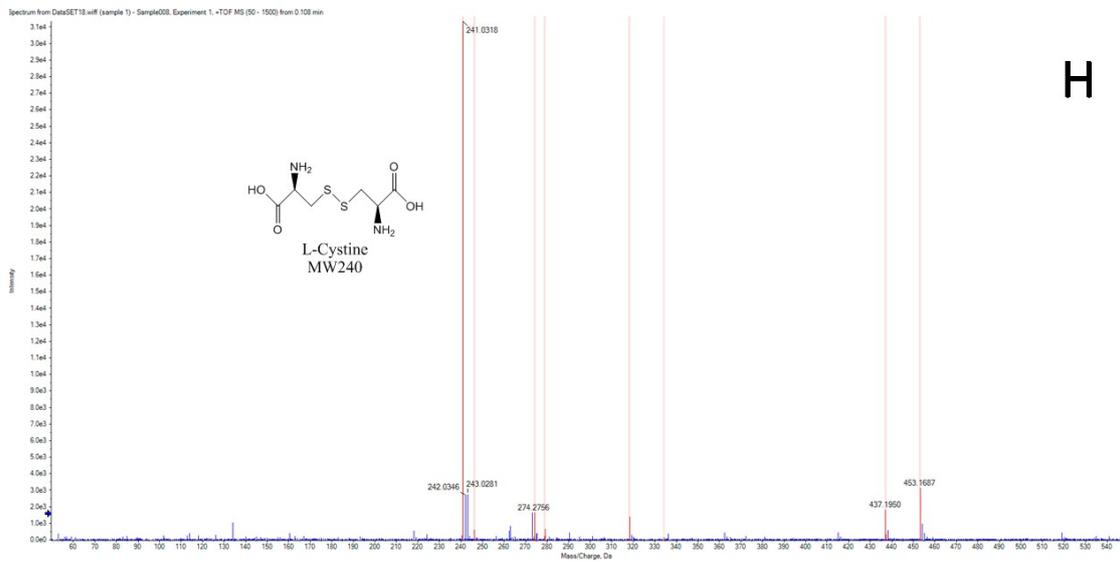
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G



H

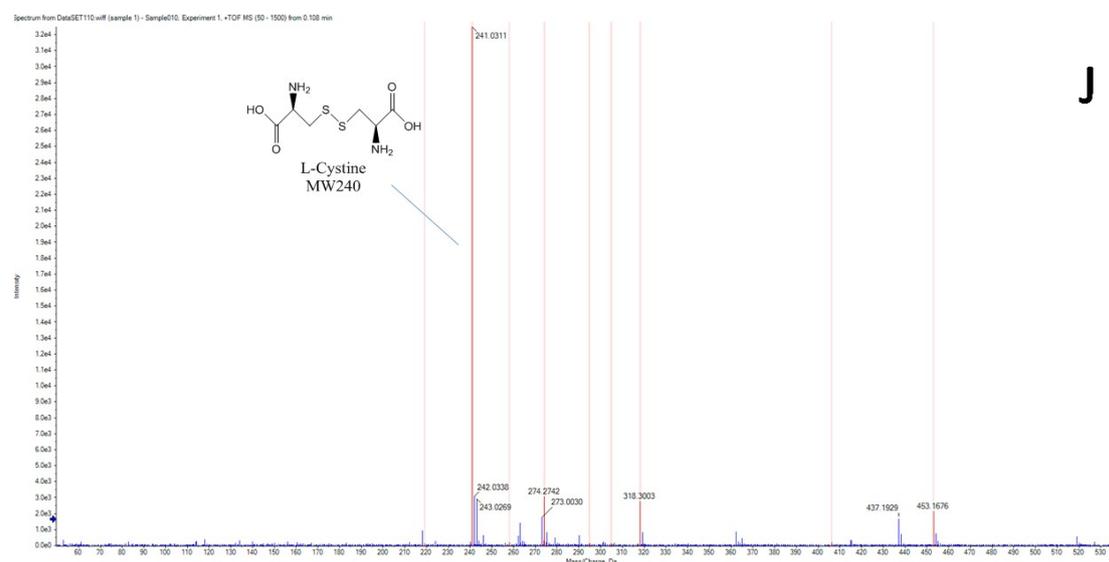
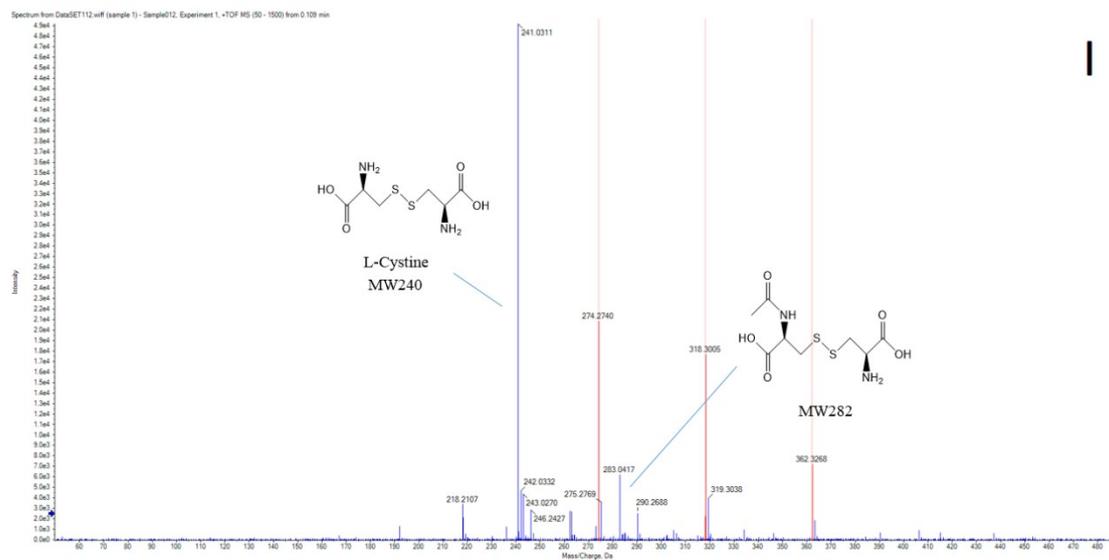


Figure S1 The positive TOF mass spectra of solutions after crystallization finished

Table 1 Peak area and height of L-cystine in the mass spectra

	Additive	Peak area of L-cystine (Mw=240)	Peak height of L-cystine (Mw=240)
A	L-cystine	230.6190	22450
B	N-acety-L-cysteine	517.9283	50423
C	Captopril	303.2126	32060
D	Tiopronin	304.9676	29483

E	D-penicillamine	276.1853	26016
F	N,N'- diacetyl-L-cystine	326.0479	30564
G	N-acetyl-L-serine	308.0193	29711
H	L-cysteine	296.2137	
I	Supernatant of NACe and L-cystine mixture	510.7256	49177
J	Citric acid	340.0041	32482

Power X-ray diffraction

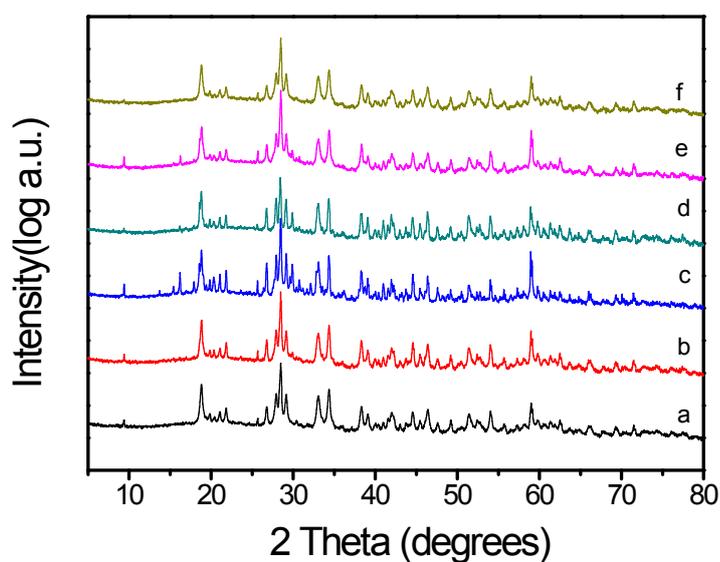


Figure S2 Powder X-ray diffraction patterns of L-cystine crystals obtained after crystallization for 72 h in the presence of 20 mg·L⁻¹ of different additives: a) no additive, b) NACe, c) N,N'- diacetyl-L-cystine, d) mixture of NACe and L-cysteine, e) NAS, f) D-penicillamine.

Particle size analysis

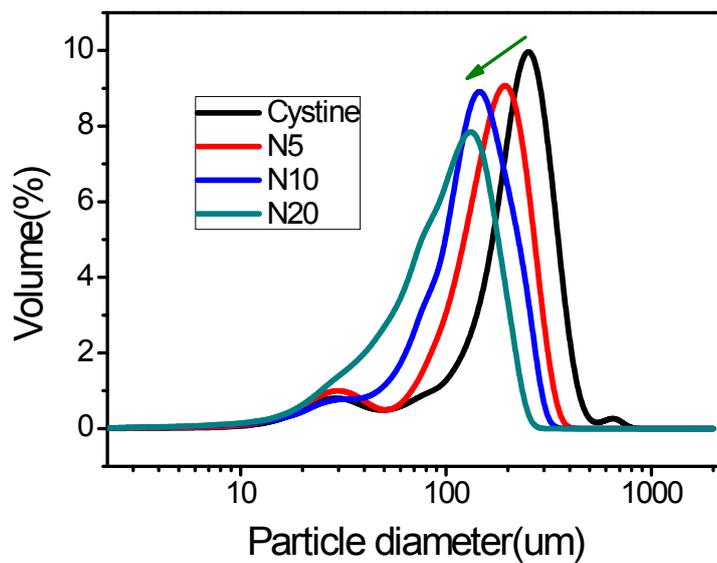


Figure S3 Distributions of particle diameter of L-cystine crystals obtained after crystallization for 72 h in the presence of various concentrations of NACe (N5, 10, 20 = 5, 10, 20 mg·L⁻¹, respectively).

- [1] J. D. Rimer, Z. H. An, Z. N. Zhu, M. H. Lee, D. S. Goldfarb, J. A. Wesson, M. D. Ward, *Science* **2010**, *330*, 337.
- [2] R. Carta, G. Tola, *J. Chem. Eng. Data* 1996, *41*, 414.