Intensified deracemization via rapid microwave-assisted temperature cycling

Supporting Information

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1. Solubility of glutamic acid conglomerates in acetic acid

In order to assess the solubility of the model compound in the given solution, gravimetric analysis were conducted at different temperatures: 60, 70, 80 and 90 °C. In all the experiments 0.2 g of racemic conglomerates crystals of glutamic acid was added to 3.5 mL acetic acid solution. The suspension was placed in the microwave reactor and kept at constant temperature for 23h while stirring at 600 rpm with a magnetic stirring bar. The solid obtained after this time was collected by filtering the suspension on a P4 glass filter and the solid was weighed after drying overnight in an oven set at 40 °C. The same procedure was used for calculating the solubility of glutamic acid in acetic acid when salicylaldehyde was added as catalyst agent (13.1 mg/mL).

The curves obtained from these experiments show only a slight difference in the solubility trend between the conditions with and without the catalyst, thus it can be concluded that the catalyst does not affect significantly the solubility of the crystals in this particular system at the concentration of the deracemization experiments. On the other hand, it is clearly visible that the solubility limits are rather close between 60 and 80 °C whereas the curve gets steeper above 80 °C.

The temperature cycles chosen for this compound act between 60 and 80 °C, as at 60 °C the racemization reaction is still fast and the solubility is low enough to promote crystallization. The upper limit is set at 80 °C, because above this value the decomposition reactions can be favored thus jeopardizing the yield of the process[1].

However, only 5 mg/mL difference is observed between 60 and 80 °C (1.69 mg/mL and 6.69 mg/mL respectively), hence the solid involved in each temperature sweep is rather modest. By applying this swing in temperature in the microwave configuration, the supersaturation ratio created when cooling down rapidly is almost 4 (S=6.69/1.69=3.97).

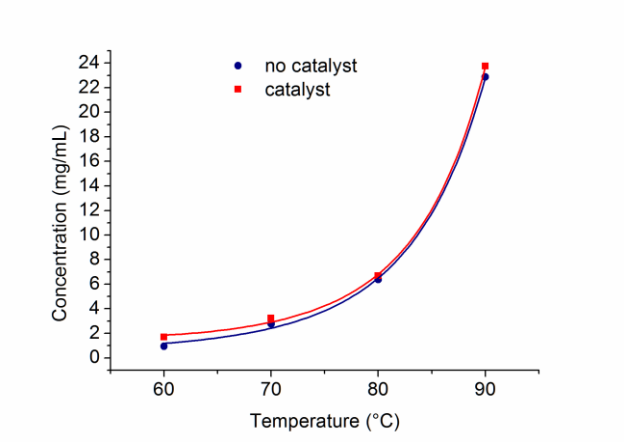

Figure 1. Solubility of glutamic acid in acetic acid at different temperatures with and without salicylaldehyde.

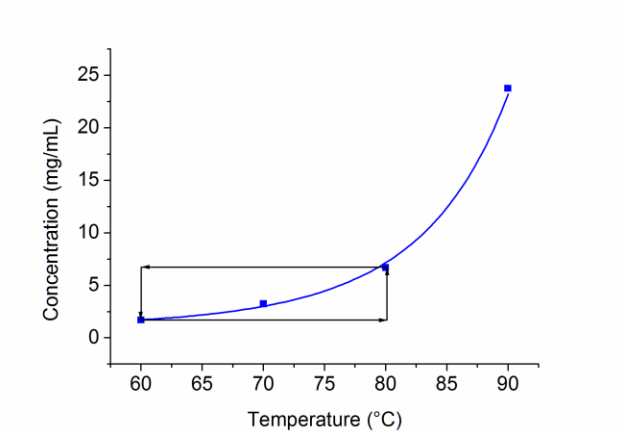

Figure 2. Temperature limits imposed in the cycles with the corresponding solubility values.

2. Experimental procedure

2.1. Preparation

All deracemization experiments were conducted by weighing L- and D-glutamic acid conglomerates separately (Sigma Aldrich), with a total solid amount of 546 mg. Prior to using in the deracemization experiments, every set of crystals was manually ground and sieved so as to have an initial particle distribution between 25 and 63 µm.

In the case of experiments with an initial enantiomeric excess of 22%, the amount of conglomerate crystals was:

- 333 mg L-glutamic acid
- 213 mg D-glutamic acid

In the experiments conducted at 60% initial enrichment, the proportion of the reagents was:

- 436 mg L-glutamic acid
- 110 mg D-glutamic acid

Both in the jacketed reactor configuration and in the microwave one, the solid mass was added into the reactor with 10.5 mL of acetic acid and 120 µL of salicylaldehyde. In both cases, the suspension was magnetically stirred at 600 rpm by an oval PTFE magnetic stirring bar (L 12 mm, $\emptyset$ 4.5 mm).

2.2. Analysis of the solid phase

For sampling, 100 µL of the suspension were withdrawn with a pipette and filtered on a P4 glass filter. After completely drying overnight in an oven set at 40 °C, the cystals were dissolved in water (1 mg/mL) and the enantiomeric excess was evaluated via an HPLC apparatus (column: Phenomenex Chirex 3126, stationary phase: (D) penicillamine, eluent $CuSO_4$, flow 1 mL min^{-1}, injection volume: 20 µL, retention times: L-glutamic acid 46 min, D-glutamic acid 55 min). The relative concentration of the two species was calculated by the chromatogram peak areas (A_L for the L species and A_D for the D glutamic acid). The formula used to estimate the enantiomeric excess is:

$$e.e.(\%) = \frac{A_L - A_D}{A_L + A_D} * 100$$

2.3. Analysis of the liquid phase

The liquid recovered by filtering the suspension on a P4 glass filter was utilized to investigate the composition of the liquid phase. The different solutes present in the suspension were recovered by complete evaporation of the solvent at room temperature in a vacuum centrifuge. Thus, the solid amount obtained from this procedure was dissolved in water (1 mg/mL) and analyzed in a HPLC column. The same chiral column used for analyzing the solid phase was used with the same procedure described above. The column utilized is not suitable for separating the two chiral forms of pyroglutamic acid, hence only one peak of the chromatogram corresponding to pyroglutamic acid is detected at 12 min retention time. The peaks referring to glutamic acid were detected again at 46 min (L-glutamic acid) and 55 min (D-glutamic acid), hence a relative composition of the solutes in the liquid was obtained from the peak areas in the chromatogram.

2.4. Estimation of solid yield

The total amount of solid recovered after a complete set of cycles (*i.e.* 110 in the experiment with an initial 60% enantiomeric excess) was calculated by subtracting the weight of the empty filter (P4) on which the whole suspension was filtered, to the final mass weight of the filter containing the solid mass of crystals after one day in the oven at 40 °C. Therefore, the percentage value of the solid yield was calculated as:

$$Y\ (\%) = \frac{m_{f+s} - m_f}{m_i} * 100$$

Where m_{f+s} is the mass of the filter plus the crystals at the end of the process, m_f is the mass of the empty filter and m_i is the initial mass of solid.

2.5. Conversion of D-glutamic acid into L-glutamic acid

The conversion of D-glutamic acid into the L species is calculated through the following formula:

$$D_c(\%) = \frac{D_i - D_f - D_d}{D_i} * 100$$

Where D_c is the amount of D species converted, D_i is the initial mass of D-glutamic acid, D_f is the final mass and D_d is the mass of D-glutamic acid that is lost (by dissolution and conversion to pyroglutamic acid).

2.6. Productivity

The value of productivity of the two processes compared in this study is defined as the mass enrichment of the major enantiomer obtained within the process time per unit volume of solvent[2]. Therefore the formula for the productivity (P [g L^{-1} h^{-1}]) is:

$$P = \frac{1}{Vt}\frac{\left(m_L^f - m_D^f\right) - \left(m_L^i - m_D^i\right)}{2}$$

Where V is the solvent volume, t is the total process time, m_L^f and m_D^f are respectively the final mass of L and D species and m_L^i and m_D^i are the initial masses of the two chiral crystals.

3. Effect of decomposition on deracemization

As mentioned elsewhere[1], glutamic acid can undergo a condensation reaction at high temperature in acetic acid solution, leading to the formation of pyroglutamic acid. To assess the influence of the decomposition reaction on the deracemization process, an isothermal experiment at 80 °C was conducted at the same conditions as the deracemization experiments reported in the main text. From Fig. 3, it is apparent that enantiomeric excess does not increase substantially up to about 234 h. However, after 234 h, a sudden rise in the enantiomeric excess is achieved, due to the dissolution of solid glutamic acid and its subsequent conversion to the more soluble pyroglutamic acid that drives further dissolution of glutamic acid. Eventually, the system reaches enantiopurity by the complete dissolution of the racemic solid part and the survival of only a few of the L-glutamic acid crystals, right before complete dissolution occurs. Analysis of the liquid phase revealed that the final liquid phase consisted of pyroglutamic acid (54 mg/mL), with no trace of glutamic acid.

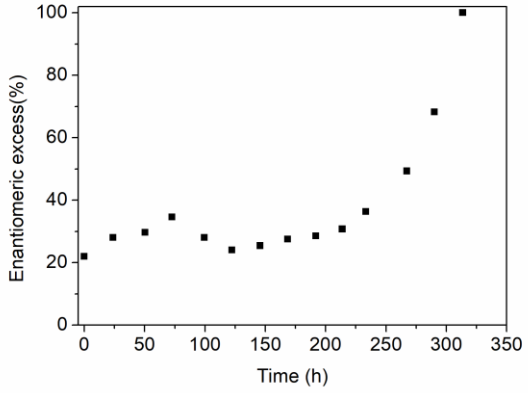

Figure 3. Enantiomeric enrichment due to gradual dissolution and subsequent decomposition of the suspension kept at constant temperature (80 °C).

4. Powder X-ray diffraction

Since for glutamic acid, the conglomerate crystals are metastable and can slowly convert to racemic crystals, at the end of every deracemization experiment the resulting crystals were subjected to XRPD in order to determine the crystal phase present. In all experiments, the final crystals were these of the conglomerate crystal phase. PXRD analysis was done using a Philips PW1830 diffractometer with Bragg/Brentano theta-2theta setup, CuKα radiation, 45 kV, 30 mA and a graphite monochromator. The scan range was between 10 and 40°, using a step size of 0.01, and the time/step set at 2 s.

5. Particle size distribution

The crystals collected after the yield experiments, both from the microwave and the jacketed reactor were analyzed in a laser diffractometer (Malvern Mastersizer 3000) in order to determine the particle size distribution. The same procedure was applied for the initial particles so that a comparison between final and initial conditions for both cases can be obtained.

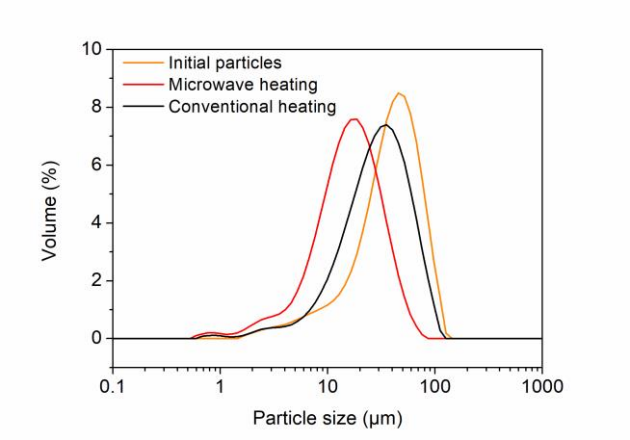

Figure 4. Particle size distribution of initial crystals and crystals obtained from the deracemization experiments conducted under microwave and conventional heating.

To calculate the number ratio between final and initial crystals, the values of solid yield and mean particle size ($D_{4,3}$) were used so to estimate the number of crystals by:

$$N = \frac{m}{\bar{V}\rho}$$

Were m is the mass, $\bar{V}$ the mean particle volume and ρ is the crystal density. Thus the ratio between the final and initial number of crystals is defined as:

$$N_R = \frac{N_f}{N_i} = \frac{M_f \bar{D_i}^3}{M_i \bar{D_f}^3}$$

References

1. L. Spix, H. Meekes, R. H. Blaauw, W. J. P. Van Enckevort and E. Vlieg, *Cryst. Growth Des.*, 2012, **12**, 5796–5799.

2. F. Breveglieri, G. M. Maggioni and M. Mazzotti, Cryst. *Growth Des.*, 2018, **18**, 1873–1881.