Chemical Engineering Science 122 (2015) 416–425

Contents lists available at ScienceDirect

Chemical Engineering Science

journal homepage: www.elsevier.com/locate/ces

Reactive crystallization of selected enantiomers: Chemo-enzymatic stereoinversion of amino acids at supersaturated conditions

Luis G. Encarnación-Gómez, Andreas S. Bommarius, Ronald W. Rousseau *

School of Chemical and Biomolecular Engineering Georgia Institute of Technology, Atlanta, GA 30332-0100, USA

HIGHLIGHTS

- Racemic mixtures of amino acids were resolved through chemo-enzymatic reactions.
- Enantiomerically pure crystals were recovered from the reactive medium.
- Reaction and crystallization kinetics were estimated from independent experiments.
- A reaction-crystallization model was developed.
- Model shows the potential benefits of parallel reaction and crystallization.

ARTICLE INFO

Article history:
Received 21 January 2014
Received in revised form
26 September 2014
Accepted 30 September 2014
Available online 13 October 2014

Keywords:
Crystallization
Chiral resolution
Enantiomeric purity
Reactive crystallization

ABSTRACT

A new route for resolution of amino acids that form racemic compounds was developed using chemo-enzymatic stereoinversion reactions in saturated or supersaturated solutions: i.e. at high solute concentrations. The reactions enrich the solution in the desired enantiomer and move the solution composition to a thermodynamic region where the pure desired enantiomer can be crystallized. The reaction sequence first oxidizes the undesired D-enantiomer with D-amino acid oxidase, and the resulting intermediate imino acid is reduced into a racemic mixture of the enantiomers by ammonia borane. The result of this reaction network is completely enantioselective because the first step is enantioselective. The concept has been demonstrated by resolving racemic mixtures of phenylalanine and methionine, which allowed recovery of L-phenylalanine and L-methionine crystals with chemical and enantiomeric purities greater than 99%. Finally, reaction and crystallization kinetics were determined and combined to model this reactive-crystallization system. These results were used to demonstrate the potential of combining chemo-enzymatic reactions and crystallization to enhance the productivity of the process.

1. Introduction

Chirality refers to the difference in spatial orientation of identically bonded chemical species. It was discovered by Louis Pasteur in 1850 when he identified the enantiomers of tartaric acid and its salts (Jacques et al., 1981). Amino acids play an important role in the synthesis of enantiomerically pure compounds (EPCs), either as end products (e.g. in the food and agro industries) or building blocks in the manufacture of drugs. Implementation of efficient processes for the manufacture of EPCs in the pharmaceutical industry has become increasingly important because of either the inactivity or the harmfulness of undesired enantiomers. Furthermore, regulatory agencies such as the Food and Drug Administration (FDA) are implementing standards for high enantiomeric purity (typically enantiomeric excess, ee, greater than 99%) for marketable chiral compounds.

High enantiomeric purity may be obtained either by reaction or separation. In the former, the desired enantiomer is synthesized selectively (e.g., asymmetric synthesis, dynamic resolution, and stereo-inversion) or, alternatively, the undesired enantiomer is selectively consumed through kinetic resolution (Wolf, 2007; Bommarius et al., 2001; Gruber et al., 2006), which enriches the solution in the counter-enantiomer. In the absence of racemization, kinetic resolution is limited to a 50% yield since one of the enantiomers is consumed, but it is an attractive alternative when the production of a racemate is economical (Keith et al., 2000).

Separation of enantiomers can be achieved by chromatography, membranes, or crystallization (Francotte et al., 2002; Svang-Ariyaskul and Rousseau, 2012; Gou et al., 2011; Lorenz and Seidel-Morgenstern, 2014). Chromatography and membranes rely

* Corresponding author.

http://dx.doi.org/10.1016/j.ces.2014.09.055
0009-2509/

on chiral binders that selectively attach to one of the enantiomers, but crystallization is a thermodynamically controlled process in which the difference between the chemical potential of a solute in solution and in the solid state drives the process. Moreover, the integration of crystallization into biochemical processes can reduce toxic or inhibitory impurities in the final product (Urbanus et al., 2012). As a result, crystallization-based chiral separations are an attractive alternative for manufacture of enantiomerically pure compounds.

The vast majority of enantiomeric compounds are categorized into systems that form either conglomerates or racemic compounds. Ternary phase diagrams of such systems are illustrated in Fig. 1 with superimposed qualitative solubilities at two different temperatures, T_1 and T_2, with $T_1 > T_2$.

Vertices of the triangles represent pure components with solvent at the top and the enantiomers at the bottom. The sides of the triangle represent the solvent–enantiomer or enantiomer–enantiomer systems. In both diagrams, Zone 1 represents undersaturated solutions; Zones 2 and 3 correspond to saturated solutions in equilibrium with enantiomerically pure crystals; Zone 4 represents saturated solutions in equilibrium with a physical mixture of crystals of the two enantiomers in Fig. 1(a) and between pure enantiomer and racemic compound in Fig. 1(b). The distinguishing characteristic of a racemic-compound-forming system is Zone 5, which has saturated solutions coexisting with crystals of a racemate compound. Such crystals incorporate both enantiomers in a single crystal lattice (Jacques et al., 1981).

The thermodynamics just outlined mean that crystallization of enantiomerically pure compounds can be assured only if the solution from which the crystals are formed has a solution concentration within the boundaries of either Zone 2 or Zone 3. Accordingly, solutions of racemic mixtures must be enriched in the desired enantiomer beyond the eutectic point in a supersaturated solution; in other words, the enantiomeric excess (ee) must exceed that at the eutectic (ee_{Eu}), where $ee = 100\% \times (x_L - x_D)/(x_L + x_D)$ and x_D and x_L are mole fractions of the D and L enantiomers. In recognition of these requirements the present work has the following objectives:

1. Formulate a strategy for chemo-enzymatically enriching an initially racemic mixture in one enantiomer at the expense of the other. The enantiomeric enrichment must be accomplished at solution compositions appropriate for subsequent crystallization of the desired enantiomer.
2. Demonstrate the feasibility of the strategy on two amino-acid systems: methionine and phenylalanine. This will require several intermediate steps:
   (a) Appropriate equilibrium data and models for the two systems must be obtained so that we can determine the regions in which crystallization is feasible and set targets for enantiomeric enrichment.
   (b) Identify an appropriate enzyme and test its effectiveness at the temperatures and high solute concentrations essential to the success of the strategy. Use the enzyme with the two model systems to obtain data sufficient for development of a model for the reaction kinetics.
   (c) Perform cooling crystallization experiments to verify that crystals of high enantiomeric purity can be obtained. Use the data from *in situ* measurements of solute concentrations during crystallizations to obtain rudimentary models of nucleation and growth kinetics.
   (d) Combine the models of reaction and crystallization kinetics to demonstrate how these two steps can be sequenced to produce the desired isomer.
   (e) Use the two-step model to show how the productivity of the process can be enhanced through initiating crystallization while enzymatic enrichment is occurring.

Fig. 2 is a visual representation of the objectives of the present work. Obtaining appropriate equilibrium data (Objective 2a) is essential in devising a process that will meet our objectives. Such data include (a) solubilities of enantiopure and racemate compounds as functions of temperature and enantiomeric excess, and (b) dependence of the eutectic point and corresponding enantiomeric excess, on temperature. Such data are illustrated qualitatively in Figs. 1 and 2 for two different temperatures, T_1 and T_2. Step 1 shows how the system composition is to be adjusted by enantiomeric enrichment (Objective 2b) using specific chemo-enzymatic

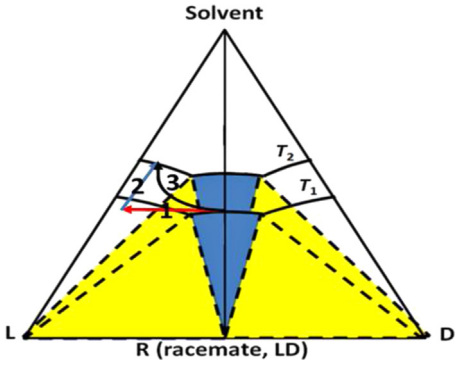

Fig. 2. Reactive crystallization process.

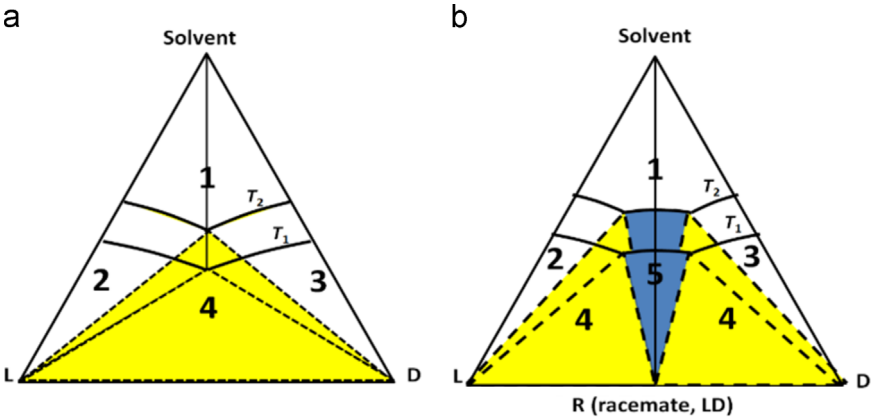

Fig. 1. Conglomerate-forming (a) and racemic-compound-forming (b) systems.