Chemical Engineering Science 64 (2009) 2473 – 2482

Contents lists available at ScienceDirect

Chemical Engineering Science

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

Enzyme-assisted physicochemical enantioseparation processes—Part II: Solid–liquid equilibria, preferential crystallization, chromatography and racemization reaction

Katerina Petruševska-Seebach[a], Kerstin Würges[b], Andreas Seidel-Morgenstern[a], Stephan Lütz[b], Martin P. Elsner[a,*]

[a]Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany
[b]Institute of Biotechnology 2, Research Centre Jülich, Jülich, Germany

ARTICLE INFO

Article history:
Received 28 October 2008
Received in revised form 2 February 2009
Accepted 11 February 2009
Available online 27 February 2009

Keywords:
Amino acid racemase
Asparagine
Methionine
Integrated enantioseparation processes
Hybrid processes
Preferential crystallization
Chiral chromatography
Enzymatic reaction
Michaelis–Menten kinetics

ABSTRACT

This contribution addresses the design and investigation of two hybrid enantioseparation processes including an enzymatic racemization step in order to enhance the overall performance. Complementary to part I where the manufacturing and the characterization of an amino acid racemase (EC 5.1.1.10) was emphasized [Würges, K., Petrusevska, K., Serci, S., Wilhelm, S., Wandrey, C., Seidel-Morgenstern, A., Elsner, M.P., Lütz, S., 2009. Enzyme-assisted physicochemical enantioseparation processes—part I: production and characterization of a recombinant amino acid racemase. J. Mol. Cat. B (in print), online available: doi:10.1016/j.molcatb.2008.10.006.], the work presented in this paper tends more towards developing a data base for potential process schemes for the manufacture of selected amino acids.

The first proposed process concept (P-I) couples preferential crystallization (PC) and racemization for the production of l-asparagine (l-Asn) using racemic mixture of dl-asparagine (conglomerate-forming system) as a starting material, while the second concept (P-II) integrates chromatography and racemization for the preparation of l-methionine (l-Met) starting with racemic mixture of dl-methionine (compound-forming system). As mentioned in part I, a racemization unit, where the unwanted enantiomer will be converted into racemate, is incorporated into the hybrid processes for the sake of 100% yield, theoretically. Besides the basic investigation according to the solid–liquid equilibria, PC and chromatography, the focus of this paper is mainly on the kinetic studies of the racemization reaction. Initially, the solubility ternary phase diagrams of both examined systems were determined, leading into the idea of combination of the proposed process schemes. For P-I the concept of PC of l-Asn was experimentally proven and the kinetics of the racemization was examined for d- and l-Asn in water using purified lyophilizate (PL). Concerning P-II, for the chromatographic unit the impact on the separation of dl-Met on eremomycin based stationary phase using KPi buffer and MeOH as mobile phase was evaluated in terms of resolution and selectivity at three different temperatures by varying the content of methanol (MeOH) in the mobile phase and the pH. The experiments for determination of the racemization kinetics were done for a compromised parameter set using crude lyophilizate (CL). In both cases a Michaelis–Menten three-step model was used to describe the enzymatic reaction.

1. Introduction

Driven by the policy of regulatory authorities such as US Food and Drug Administration and the EU Committee for Proprietary Medical Products the manufacturing of pure enantiomers is distinguishably increasing in several industrial branches: pharmaceuticals, alimentary, etc. (Caner et al., 2004). As constituents of larger biomolecules with essential importance for the nutrition and health subsistence, optically pure amino acids follow the same trend. The amino acid market and the perspectives, with special accent on their biotechnological production, are reviewed by Leuchtenberger et al. (2005). So far, most of the L-amino acids are produced using microbial methods. However, the way how and in which form an amino acid is going to be produced depends mainly on the process economics, available raw material and the market, e.g. methionine is usually produced as a racemic mixture (50/50 mixture of both enantiomers) (Scheper, 2003).

Regarding the basic approaches for the production of pure enantiomers, the resolution of a racemic mixture is considered as an attractive method since numerous preparative separation techniques

* Corresponding author. Tel.: +49 391 6110 438; fax: +49 391 6110 626.
E-mail address: mpelsner@mpi-magdeburg.mpg.de (M.P. Elsner).

0009-2509/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ces.2009.02.025

can accompany the conventional organic synthesis as well as enzyme catalysis methods (Ahuja, 1996). The most often utilized methods and several novel resolution technologies are summarized in Sakai et al. (2007) and Fogassy et al. (2006), respectively.

Amino acids differ in terms of their phase diagram (Jacques et al., 1994) and that can predetermine the choice of a suitable separation technique. In case of conglomerate-forming systems (5–10% of all chiral substances belong to this group; Collet, 1999) the cost-effective and quite simple preferential crystallization (PC) holds great potential. Recently, PC in a cyclic operation mode for water–threonine system (conglomerate) was studied in detail by Elsner et al. (2005). Later on, an innovative configuration of coupled crystallizers was proposed by the same author outperforming the one-crystallizer mode by means of productivity and purity (Elsner et al., 2009). For compound-forming systems (> 90% of the chiral substances are racemic compounds) the concept of PC is rather limited. Shiraiwa et al. (1997) have resolved DL-methionine (DL-Met; racemic compound) by converting DL-Met into DL-Met · HCl (conglomerate) and then performing PC. In fact, the first approval of applying PC for optical resolution of racemic compound in a frame of a hybrid process was done recently by Lorenz et al. (2006). Subsequently, they have shown that the enantiomers of methionine can also be preferably crystallized (Polenske and Lorenz, 2008; Kaemmerer et al., 2008). Concerning their study, before racemic compound solution undergoes PC, preliminary enantiomeric enrichment is required and usually, but not necessarily, the degree of enrichment can be correlated with the eutectic composition of the system.

The enrichment can be accomplished utilizing other methods for amino acid enantioseparation, e.g. chromatography-based techniques (HPLC, SMB) since among other conveniences, the development of new chiral stationary phases broadens their range of application (Petrusevska et al., 2006; Zhang et al., 2007). On the other hand, those techniques are considered as quite expensive and their use has to be justified.

The issue of using PC, when highly enriched solutions are required, implies several questions like for instance if it is really necessary to combine sophisticated chromatography technique with PC or enantioseparation only by chromatography might be more reasonable? Is it more beneficial to combine less expensive techniques for enrichment and PC, etc.? However, a lot of physicochemical and economical parameters have to be taken into consideration before a decision of using a single or an integrated process is made. The potential of hybrid enantioseparation processes has been the subject of considerable scientific activities in the recent years, with an emphasis on the theoretical development of general design methods (Ströhlein et al., 2003) as well as on the experimental challenges emerging from the different operation windows of the single separation methods being combined (Fung and Ng, 2006). For the evaluation and design of a hybrid enantioseparation process using SMB and PC recently a shortcut method was proposed by Kaspereit et al. (2005).

Unfortunately, the separation techniques suffer with a general drawback of maximum 50% yield. Nevertheless, it is likely possible to overcome those limitations by performing racemization of the unwanted enantiomer, feed the racemate to the separation unit, and thus achieving 100% yield (Collet et al., 1980; May et al., 2002). Comprehensive overview of racemization methods is given by Ebbers et al. (1997). Due to the convenient reaction conditions, the development of biocatalysts and their rising industrial application (Bornscheuer and Buchholz, 2005), the use of an enzyme for racemization (racemase) is a challenging task for the development of efficient combined processes. So far, the functions on a molecular level and the applications of several amino acid racemases have been given by Yoshimura and Esaki (2003) and Schnell et al. (2003), respectively.

Regarding integrated processes including racemization, recently Bechtold et al. (2006a) introduced a novel process concept of coupling continuous chromatography and enzyme reactor. The general idea was analyzed in comprehensive studies with respect to the chromatographic part (Bechtold et al., 2006b) and the racemization (Bechtold et al., 2007).

Supplementing part I of this paper (Würges et al., 2009), based on an evaluation of the ternary phase diagrams of two systems (DL-Asn/water and DL-Met/solvent), two different process concepts for the production of pure enantiomers are proposed. Particularly with regard to manufacturing pure enantiomer of asparagine the integrated process consists of *crystallization* and *racemization*, whereas for methionine *chromatography* and *racemization* are coupled. Since the separation units are not the main subject of this work, we are going to present in this paper a preliminary assessment of the concept of PC for P-I and the chromatographic separation of DL-Met for P-II accompanied with a more detailed investigation about the influence of certain parameters (pH and MeOH amount in the solvent at different T) on the separation.

Distinct attention in this paper is paid on the reaction of racemization. In that manner, the impact of several parameters (the same as for the chromatographic part) was examined for the racemization of D-Met. For the racemization of D-Asn only the temperature was varied. Furthermore, for both systems the reaction kinetics was studied in detail. For that purpose the racemization was performed for different concentrations of the enantiomers and different enzyme concentrations. The experimental results were fitted into the Michaelis–Menten three-step mechanism model in order to determine the constants of the reaction.

The goal of this work is to offer a data base allowing evaluating and confirming the feasibility of combined processes for enantioseparation. The hybrid process concepts presented are not limited to amino acids. In principle, they could be extended to other chiral systems showing similar equilibrium characteristics.

2. Theory

2.1. Ternary phase diagram as a criterion for the choice of a separation technique

The general form of the ternary phase diagram for conglomerate-forming system is shown in Fig. 1a. As it was discussed before for this kind of systems the concept of PC is rather convenient for enantioseparation. Fig. 2 reveals a novel, promising and cost-effective process scheme for the manufacture of pure enantiomers by combining PC and racemization (P-I). The addition of a racemization unit results in an increase of the concentration of the wanted enantiomer and a decrease of the unwanted one in the liquid phase at the same time. Therefore, as long as the racemization is faster than the crystallization the composition moves directly towards the eutectic point belonging to the crystallization temperature T_{cryst} according to the straight trajectory in the ternary phase diagram (assigned by thick black arrow in the right-hand figure) increasing constantly the driving force for crystallization of the desired enantiomer and suppressing the occurrence of the counter enantiomer in comparison to the conventional PC process.

The choice of a separation technique is slightly more difficult for compound-forming systems. In this case the position of the eutectic points in the ternary diagram can predetermine the choice of a particular separation method or combination of few. When combined processes are used, special accent falls on the costs and the feasibility of the whole scheme. If the points tend to be more in the inner part of the triangle, i.e. not far away from the 50/50 mixture (Fig. 1b), poor enrichment is necessary in order to apply PC. In