Journal of Chromatography A, 1346 (2014) 34–42

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Model-based identification of optimal operating conditions for amino acid simulated moving bed enantioseparation using a macrocyclic glycopeptide stationary phase

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ARTICLE INFO

Article history:
Received 27 December 2013
Received in revised form 17 March 2014
Accepted 21 March 2014
Available online 12 April 2014

Keywords:
Simulated moving bed technology
Mobile phase optimization
Model-based SMB design
Amino acid enantioseparation
Macrocyclic glycopeptide stationary phases

ABSTRACT

Teicoplanin aglycone columns allow efficient separation of amino acid enantiomers in aqueous mobile phases and enable robust and predictable simulated moving bed (SMB) separation of racemic methionine despite a dependency of the adsorption behavior on the column history (memory effect). In this work we systematically investigated the influence of the mobile phase (methanol content) and temperature on SMB performance using a model-based optimization approach that accounts for methionine solubility, adsorption behavior and back pressure. Adsorption isotherms became more favorable with increasing methanol content but methionine solubility was decreased and back pressure increased. Numerical optimization suggested a moderate methanol content (25–35%) for most efficient operation. Higher temperature had a positive effect on specific productivity and desorbent requirement due to higher methionine solubility, lower back pressure and virtually invariant selectivity at high loadings of racemic methionine. However, process robustness (defined as a difference in flow rate ratios) decreased strongly with increasing temperature to the extent that any significant increase in temperature over 32 °C will likely result in operating points that cannot be realized technically even with the lab-scale piston pump SMB system employed in this study.

1. Introduction

Simulated moving bed (SMB) technology has matured into a standard tool in the pharmaceutical and fine chemical industry, in particular for the generation of enantiopure products from racemic mixtures [1,2]. Today, a large number of efficient chiral stationary phases (CSPs) are commercially available [3] and a number of reliable design tools [1] were established making SMB chromatography a versatile and rapid to implement technology.

In order to obtain economically viable SMB productivities the selection of stationary and mobile phase needs to be aligned with the character of the molecules to be separated, not only in terms of selectivity and capacity but also with regard to solubility. The bioavailability of a small molecule API typically requires a hydrophobic overall character ($\log P > 5$ [4]), and correspondingly a set of workhorse CSPs has emerged that provide favorable CSP-solute interactions (e.g. π–π, dipole–dipole, hydrogen bonds) in normal or polar organic phase mode which typically supports sufficient solubility for a small molecule API.

When addressing molecules with pronounced hydrophilicity, which often implicates the presence of charged functional groups, aqueous mobile phases clearly support best a high solubility of the compounds to be separated. The switch to aqueous mobile phases drastically changes the CSP-solute interaction landscape with ionic interactions becoming increasingly relevant [5]. The surface chemistries of today's workhorses of chiral chromatography (polysaccharide-based CSPs such as Chiralcel OD/OJ, Chiralpak AS/AD, Daicel, Japan [2]) are not designed for such interactions, and measures for neutralization of charges such as lowering the pH below the pK_a in case of acidic compounds or acidification and addition of an ion-pair reagent in case of zwitterions are required [6]. In particular the latter case appears rather unappealing for large scale separation due to the requirement of ion-pair reagent in at least stoichiometric amounts. In that respect other CSP types provide a better balance between solute–water and enantiodiscriminating CSP–solute interactions, as evidenced for example by the successful separation of amino acids in aqueous–organic phases using crown-ether [7], glycopeptide [8–10], cyclodextrin [11,12], or ligand-exchange CSPs [13].

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http://dx.doi.org/10.1016/j.chroma.2014.03.056
0021-9673/

Amino acids arguably constitute the most important class of hydrophilic amphoteric compounds [14] ($\log P < -1$ [15]) and are virtually insoluble in non-polar organic solvents [16]. Although a number of efficient biocatalytic process routes to enantiopure amino acids are established [14,17–20], a frequently required extension of the substrate scope usually involves lengthy strain or enzyme engineering procedures, which should serve as ample motivation to pursue direct physical enantioseparation, such as provided by SMB technology, as a process alternative, in particular in view of the well-established organic synthesis methods for racemic amino acids [21].

In the scope of this work we elucidate the potential of amino acid SMB enantioseparation with a particularly promising CSP from the class of commercialized macrocyclic glycopeptide stationary phases, teicoplanin-aglycone (TAG, Chirobiotic TAG, Sigma-Aldrich), on the basis of one specific example, the separation of methionine.

The TAG CSP is widely recognized for its ability to separate a broad set of amino acids using aqueous–organic mobile phases [22]. However, when the column was subjected to overload conditions a change in adsorption behavior was observed which manifested itself in prolonged retention times of both enantiomers in finite injection HPLC runs after overloading the column. This effect was termed memory effect and is reversible upon flushing the column with solute-free solvent [23].

Since SMB processes are characterized by cyclic ad- and desorption of compounds under overload conditions [1], the memory effect needs to be accounted for in the determination of adsorption isotherm parameters for SMB design. Recently, we demonstrated that a reliable SMB design can be obtained by using a modified perturbation method and stable SMB operation is possible for a number of days, despite the memory effect [24].

Methionine SMB separation has also been recently implemented employing a structurally related eremomycin chiral stationary phase and 80/20 100 mM NaH_2PO_4/methanol (MeOH) mobile phase at an undisclosed temperature with a reported productivity of 15 g D-Met/L_{CSP}/h and moderate purities (e.g. 91% for both ports) [25].

However, no attempts have been made so far to consequently optimize such SMB amino acid enantioseparations with respect to decision variables (m-values and switch time) and process conditions. Ribeiro et al. [26] recently introduced an efficient method for the selection of optimal SMB process conditions for a flurbiprofen separation on Chiralpak AD. For a set of potentially applicable process conditions varying by mobile phase composition and temperature adsorption isotherms over a concentration range relevant for SMB operation were measured. From the adsorption isotherm data, SMB performance parameters (throughput and desorbent requirement) were estimated by triangle theory and a flow rate constraint for zone I. Comparison of the performance parameters could then easily identify the conditions yielding the most promising SMB performance. In a subsequent study the SMB design was refined by switching to a more realistic transient SMB model that provided operating points according to the desired specification and provided reliable productivity predictions [27].

In this work we follow a similar approach to elucidate the economic potential of amino acid enantioseparation using TAG columns. Previous studies indicated that the chromatographic process is strongly influenced by the organic phase content, specifically MeOH and temperature [24]. Hence, we measured adsorption isotherms, solubility and column back pressure as a function of MeOH content and temperature and calculated the achievable productivities at feed concentrations close to the solubility limits and taking into account the pressure constraints of SMB systems. In contrast to the above mentioned study we based the estimation of the performance parameters on a TMB model that accounts for non-idealities of the chromatographic system and hence should provide a more accurate estimation of actual SMB behavior.

2. Experimental

2.1. Chemicals

Methionine, MeOH, ammonium acetate (NH_4Ac), and acetonitrile were purchased from Sigma–Aldrich (Buchs, Switzerland). All organic solvents were HPLC grade. Water was deionized using a TKA-Genpure machine. Solvent compositions are given in % (v/v) unless specified otherwise.

2.2. Analytics

All HPLC experiments were carried out on an Agilent 1100 HPLC system equipped with a diode array detector. For the quantification of L- and D-methionine concentrations a stainless steel Chirobiotic TAG column (250 mm × 4.6 mm ID, 5 μm) was used with a mobile phase of 5 mM NH_4Ac/MeOH (75/25). The flow rate of the mobile phase was set to 1 mL min^{-1} and the volume of injected samples was 10 μL.

2.3. Determination of solubility limits

Mobile phases containing a mix of 5 mM aqueous NH_4Ac and MeOH at ratios of 85/15, 75/25, 65/35, 55/45 and 45/55 were prepared and excess racemic methionine was added to a theoretical concentration of 40 g L^{-1}. The mixtures were stirred continuously in a flask and placed inside an incubator overnight at 12, 22 and 32 °C, respectively. After sterile filtration (pore diameter 0.2 μm) of the liquid fraction to remove the remaining solids, the methionine concentration was determined by HPLC.

2.4. Measurement of column back pressure

Measurements of column back pressure were performed by HPLC with the column temperature set to 12, 22 or 32 °C by the column oven and employing five different mobile phases of ratios of 85/15, 75/25, 65/35, 55/45 and 45/55 of 5 mM NH_4Ac/MeOH. The flow rate of the mobile phase was increased from 1 mL min^{-1} to 6 mL min^{-1} in steps of 1 mL min^{-1} and the column-specific pressure drop was calculated by subtracting the back pressure of the system (same procedure without column) from the recorded back pressure. The specific flow resistance φ was calculated by linear regression from the set of flow rate (expressed as superficial velocity u_s) and pressure drop data using the D'Arcy relationship (Eq. (1)) with ΔP being the pressure drop over the column length L.

$$\frac{\Delta P}{L} = \phi u_s \tag{1}$$

2.5. Determination of the adsorption isotherm

Adsorption isotherms were determined by the perturbation method (PTM) [28–30]. Specifically, a "top–down" approach was applied [24] that comprised at least 15 concentration levels. Prior to each PTM run the column was regenerated with a 50/50 mixture of 50 mM NH_4Ac/acetonitrile at 4 mL min^{-1} for a minimum of 30 min followed by flushing with the respective mobile phase for 15 min. A bi-Langmuir isotherm assuming equal saturation capacities for both enantiomers was applied (Eq. (2)),

$$q_i = q_{sI}\frac{b_{Ii}c_i}{1 + b_{I1}c_1 + b_{I2}c_2} + q_{sII}\frac{b_{IIi}c_i}{1 + b_{II1}c_1 + b_{II2}c_2} \quad i = (1, 2) \tag{2}$$

with c_i and q_i denoting the concentration of component i (1: L-methionine, 2: D-methionine) in the mobile and stationary phase,