Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1075 (2005) 77–86
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Separation of amino acids with simulated moving bed chromatography☆

Zoltán Molnár[a,*], Melinda Nagy[a], Antal Aranyi[b], László Hanák[a], János Argyelán[a], István Pencz[a], Tibor Szánya[a]

[a] *University of Veszprém, Department of Chemical Engineering, P.O. Box 158, 8201 Veszprém, Hungary*
[b] *Gedeon Richter Pharmaceutical Works, P.O. Box 27, 1475 Budapest, Hungary*

Received 28 May 2004; received in revised form 10 March 2005; accepted 18 March 2005
Available online 19 April 2005

Abstract

Authors have constructed an automatized four-column large laboratory scale (I.D. = 50 mm, L = 500 mm) simulated moving bed (SMB) equipment. The applied model system for separation of biomolecules is glycine, L-phenylalanine, water and Sepabeads SP825 adsorbent. The authors determined the adsorption equilibrium data and the packing characteristics. The operating conditions of SMB equipment were calculated with the help of the Morbidelli variables. During the SMB experiments, glycine and L-phenylalanine were separated in water on Sepabeads SP825 with an average particle size 0.3 mm at temperatures 20 °C and 60 °C. The measurement series were carried out on a four-column three-zone open loop SMB. Both L-phenylalanine and glycine were produced with more than 99.9% (m/m) purity and 99% yield at productivity 1.7–3.7, with productivity 3.7–8.1 mg/(g adsorbent h) in case of 2-1-1-0 column configuration. The measured and the calculated data agreed well.

Keywords: Column liquid chromatography; Three-zone simulated moving bed; Reversed phase polymeric resin; Effect of temperature

1. Introduction

It is typical in biotechnology and pharmaceutical industry that water-phase mixture for processing contains an end-product or an active ingredient in a small concentration besides the contaminant or polluting components. SMB preparative chromatography can be advantageous among the end-product recovery methods for continuous processing of high purity products. The SMB applicability in the separation of diluted aqueous amino acid solution was investigated and presented in this publication. The diluted solution of amino acids can arise, e.g. from protein hydrolysis.

SMB liquid chromatography (hereafter-abbreviated SMB-LC) (Fig. 1) is a multi-column system with two inputs (fresh eluent and feed to be separated) and outputs (products: extract and raffinate), in which liquid phase moves in counter-current of adsorbent phase. Above all, in the basic case regenerated eluent of recirculation stream is added to the fresh eluent. The counter-current stream is not real, but simulated, since the packed chromatographic stationary phase moves periodically after each switching time.

The inlet and the outlet fluid flow streams divide the column system into four zones (I–II–III–IV). The sections I–II of the SMB are rich in the more binding component and sections III–IV contain the less binding component.

Three-zone open loop SMB (Fig. 2) is preferred in systems with a high selectivity coefficient, when the less binding component has a low capacity factor (k) almost running together with the mobile phase [1,2].

Summarizing the column liquid chromatographic methods, which are feasible for SMB chromatographic separation of amino acids, the following were applied in practice:

(1) Ion-exchange column liquid chromatography.
The continuous version is the SMB packed with ion-exchange resin chromatographic quality [3].
(2) Size exclusion chromatography.

☆ Presented at the 17th International Symposium on Preparative and Process Chromatography, Baltimore, MD, 23–26 May 2004.

* Corresponding author. Fax: +36 88 421 905.
E-mail address: hmolnar78@freemail.hu (Z. Molnár).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.03.083

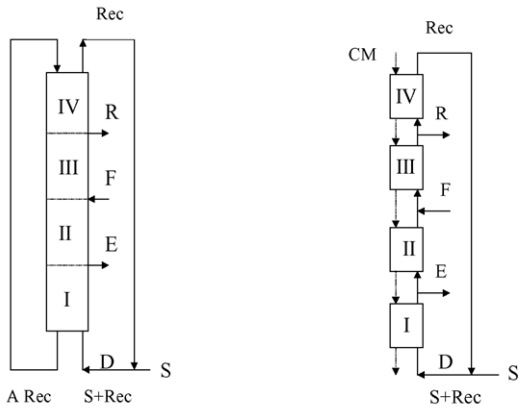

Fig. 1. (A) True moving bed (TMB) adsorber: I, II, III, IV—zones; S—desorbent (solvent, eluent); Rec—recirculated eluent; A Rec—adsorbent recirculation; E—extract stream with the better adsorbed component A; F—feed stream with the components A and B; R—raffinate (liquid outlet) stream with the less adsorbed component B. (B) Simulated moving bed (SMB) adsorber: I, II, III, IV—zones, respectively HPLC columns; CM—direction of simulated (relative) moving of HPLC columns.

Protein hydrolizate separation and fractioning on chromatographic gel column [4].

(3) Reversed-phase adsorption chromatography.

The styrene–divynilbenzene copolymers with nonpolar surface are favorable for the separation of amino acids dissolved in water, electrolyte solutions or any polar solvent. In such systems, the adsorption equilibrium depends on the temperature, the solvent strength, the pH value [5–7] and also the electrolyte concentration in aqueous solution.

The examined model system during research is the diluted aqueous solution of glycine and L-phenylalanine, which must continuously be recovered. For this purpose, the reversed-phase adsorption chromatography is probably the most favorable solution, as the ion-exchange characteristics of both

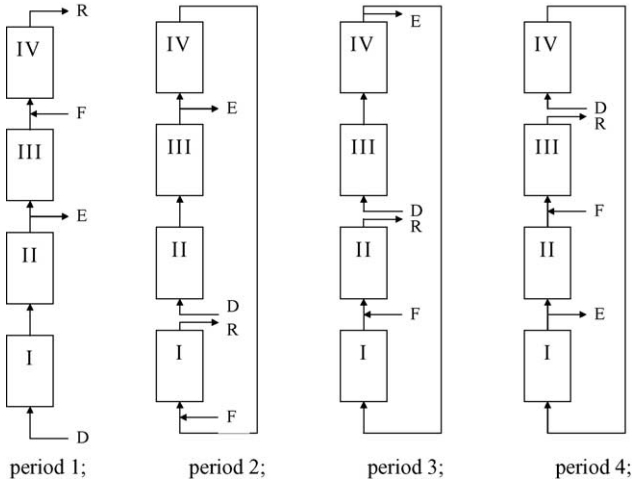

Fig. 2. Fluid streams in the four period (total cycle) of a four columns three zones open loop SMB with 2-1-1-0 column/zone configuration.

the L-phenylalanine and glycine are similar. The size exclusion is not applicable because of the small molecule size of both the examined amino acids.

The initial step of designing an SMB-LC operation is to choose the most advantageous mobile phase–stationary phase combination namely selectivity and capacity factors. For this purpose, the Diaion HP20 and Sepabeads SP825 reversed-phase adsorbent resins were compared with frontal chromatography. Both the examined resins are styrene–divynilbenzene copolymers with large specific surface area (600–1000 m^2/g). On the bases of selectivity factors, application of both adsorbents' seems to be advantageous for chromatographic separations of the better-adsorbed L-phenylalanine and less-adsorbed glycine. In this publication, experiments carried out with SP825 resin are described, however the results of HP20 resins used successfully were published elsewhere [8]. The first experiments with SP825 verified whether the high capacity factor of the phenylalanine is not advantageous for the SMB separation, namely the regeneration of the adsorbent in the SMB zone I. Therefore, capacity-reducing investigations were carried out, e.g. increasing temperature. Further, higher temperature can be advantageous to fluid dynamics, because of smaller viscosity and faster adsorption–desorption kinetics.

After determining the equilibrium data of the selected systems and the column packing characteristics, the initial operating parameters of the SMB can be calculated. Through the small capacity factor of glycine, a three-zone open loop SMB is advisable for the separation. The initial operating parameters for a three-zone open loop SMB were calculated by the triangle method of Morbidelli and co-workers [9].

The equation for the total regeneration of the first column of the first zone is:

$$K_A < m_I = \frac{(D/A)T - L\varepsilon}{L(1-\varepsilon)} \tag{1}$$

where K_A = equilibrium distribution coefficient of better adsorbed component, m_I = Morbidelli's velocity ratio for the first SMB-zone, D = eluent flow rate (mobile phase volumetric velocity in the first zone (cm^3/min)), T = switching time (min), A = cross-section of one SMB-column (cm^2), L = SMB column length (cm), and ε = total porosity of the SMB column.

In the second and third zone, the components must be separated by their distribution coefficient. The less-binding component must be removed from the second zone till the end of the switching time. The function of the third zone is the retardation of the better-adsorbed component, that is, this component must not break through the third zone. Obviously, the Morbidelli conditions for the second and third SMB-zones are:

$$K_B < m_{II} = \frac{((D-E)/A)T - L\varepsilon}{L(1-\varepsilon)} < K_A \tag{2}$$

$$K_B < m_{III} = \frac{((D-E+F)/A)T - L\varepsilon}{L(1-\varepsilon)} < K_A \tag{3}$$