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Monolithic silica sorbents for the separation of diastereomers by means of simulated moving bed chromatography ☆

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Abstract

Monolithic silica sorbents with a dual pore system can be used in preparative chromatography for the separation of diastereomers. They exhibit some special features, which allows them to be operated at high linear velocities due to their reduced pressure drop and fast diffusion kinetics. Especially in the continuous set-up of simulated moving bed chromatography monolithic sorbents show high productivities, which make them well suited in pharmaceutical drug development for the production of pure isomers.

Keywords: Simulated moving bed chromatography; Monolithic silica sorbents; Diastereomer separation

1. Introduction

Since the first simulated moving bed (SMB) system has been made available for the separation of fine chemicals and pharmaceuticals by NOVASEP in the early 1990s, most separations have been performed in the field of enantiomer and diastereomer separations. This observation is driven by the fact that in modern drug development the chemical structure of new drug molecules is getting more and more complex. New drug entities with four, five or even more stereocentres are synthesized via routes of sometimes 10 to 20 different steps. During that procedure the isolation of pure diastereomers or enantiomers is of tremendous importance. For this need SMB was established over the last few years as a viable tool to obtain pure compounds. The average productivity of SMB separations over that period of time could be increased from around 300 g product day^{-1} kg^{-1} chiral stationary phase (CSP) up to over 1500 or even 2000 g product day^{-1} kg^{-1} CSP [1,2].

To achieve maximum productivity the separation is depending on three main parameters: the saturation capacity of the stationary phase, the peak resolution (combining efficiency and selectivity) and the system pressure drop (depending on the mobile phase flow-rate). A given separation on a certain stationary phase with an optimised saturation capacity can be enhanced in terms of mobile phase velocity until the maximum system pressure drop is reached or the efficiency of the sorbent decreases at higher flow-rates due to the increasing influence of the intraparti-

☆ Dedicated to Professor Klaus K. Unger on the occasion of his 65th birthday.

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cle diffusion. With standard particulate silica materials in SMB systems the maximum linear velocity is in the range of 900 cm h^{-1}. If the pressure drop and efficiency problem could be overcome the SMB flow-rates could be linearly increased and a much higher productivity would be achieved.

To increase the efficiency of sorbents at higher flow-rates several attempts have been made to develop materials with fast convective mass transfer instead of long diffusive pore transport. Those attempts include so-called perfusion adsorbents with large pores [3] and polymeric monolithic materials [4,5], which are mostly used for the isolation of large bioactive molecules.

A fascinating new possibility for preparative chromatography of small molecules arises from monolithic silica sorbents, which exhibit a double pore structure. Those monolithic silica sorbents are prepared via a sol–gel process from alkoxy-silanes [6]. The resulting silica rods have interconnecting throughpores, which can be tailored in the range from 1 to 6 μm and a silica backbone, which exhibits mesopores with a pore size of 100–200 Å [7,8]. The chemical nature of the sorbent is identical to standard silica sorbents, which makes those monolithic sorbents very well suited for diastereomer separations in the normal-phase mode. The interconnected throughpore system assures a convective transport of the analytes to the silica backbone at high mobile phase linear velocities. Even at more than 4500 cm h^{-1} preparative separations can be performed. Hence, this sorbents offer the possibility to increase the linear velocity of an SMB separation and therefore the productivity.

The astonishing properties of this new type of sorbent were examined in detail for the first time for the separation of a diastereomeric drug intermediate.

2. Experimental

2.1. Sample and analytical chromatography

Analysis of the feed stream, the products and the samples from the internal concentration profile were carried out on a 250×4 mm Hibar stainless steel column packed with LiChrospher CN, 5 μm (Merck, Darmstadt, Germany) at 25°C and a flow-rate of 1.0 ml min^{-1}. Methylcyclohexane was used as the mobile phase (HPLC grade, Merck). All chromatograms were measured on a LaChrom-HPLC system consisting of an L-7110 HPLC pump, an L-7400 UV detector, an L-7300 column oven and a D-7500 Chromato-Integrator (Merck). The detection wavelength was 254 nm.

The preparative high-performance liquid chromatography (HPLC) system for the efficiency test of the monolithic sorbent consisted of a Shimadzu LC-8A pump (Shimadzu, Japan), a Merck–Hitachi L-4200 UV detector and a Merck–Hitachi D-2500 Chromato-Integrator (Merck).

The sample was an intermediate of a commercialised drug substance from Janssen Pharmaceutica (Beerse, Belgium). The tested diastereomeric mixture has a ratio of 66% isomer A and 33% isomer B.

General column characterisation was performed with a mixture of toluene, dimethyl- and dibutylphthalate (all reagent grade, Merck).

2.2. Monolithic stationary phases

The monolithic stationary phases were synthesized according to the procedure described in Ref. [5]. They had a dimension of 100×25 mm. The pore sizes were measured with an ASAP 2400 (Micromeritics, USA) and were determined to be 2.8 μm (macropores) and 130 Å (mesopores), respectively The specific surface area of the monolithic sorbent was 260 m^2 g^{-1}. The monolithic silica rod was equipped with stainless steel end fittings on both sides and surrounded with a polymeric tube. The whole assembly was placed into a stainless steel tube of 50 mm I.D. (Fig. 1). Upfront of the tube inlet a t-piece was integrated. From this t-piece the mobile phase was directed, both into the monolith and into the stainless steel tube. The outlet of the stainless steel tube was closed, therefore the surrounding mobile phase on the outside of the monolith created a radial pressure drop which was equal to the pressure drop at the inlet of the monolith.

2.3. Determination of isotherms and calculation of the SMB starting parameters

The determination of the adsorption isotherms was achieved by the elution at characteristic point method

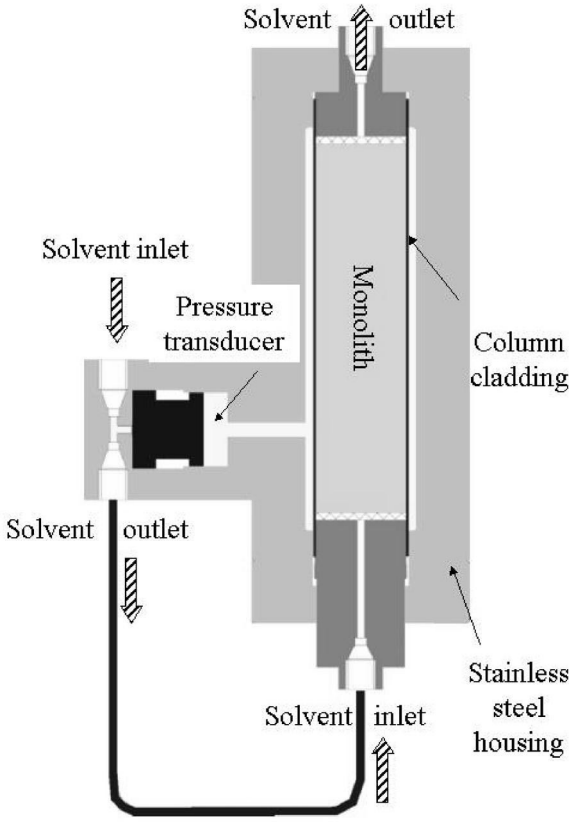


Fig. 1. Schematic drawing of the monolithic column.

[9], which has been proven to be a quick and reliable method [10]. The chromatographic data were fitted into the software HELP from NOVASEP (Nancy, France), which calculated the appropriate SMB flow-rates (basic model for the calculation is described in Ref. [11]). For the adsorption isotherm determination one monolithic column was used with a mobile phase composition of *n*-heptane–ethyl acetate (90:10, v/v) at a flow-rate of 30 ml min^{-1}.

2.4. SMB system

A Licosep Lab system from NOVASEP was used for the separation. The system is controlled by a process control system Siemens S7-300 (Stuttgart, Germany) and a personal computer (P200, Siemens Nixdorf, Stuttgart, Germany). By means of the system software all system parameters (pump flow-rates, valve switching time) are controlled and all relevant data (flow-rates, pressure drop, temperature) are continuously stored into data files. The SMB system can be equipped with up to 12 columns. For this study a six-column set-up was chosen.

3. Results and discussion

Monolithic silica sorbents are a new type of chromatographic support, which is synthesised by a sol–gel process [6]. The special feature of this sorbent is the bimodal pore structure of interconnective macropores and mesopores inside the silica backbone. Those two pore systems can be independently controlled to certain extents. A variation for the macropore size between 2 and 6 μm is possible as well as a variation of the mesopore size between 100 and 400 Å. With increasing macropore size the pressure drop of the operated column is decreasing as is shown in Fig. 2. On the other hand the column efficiency drops only slightly with increasing macropore size (Fig. 3). A good compromise between pressure drop and efficiency is found at a macropore size of around 3 μm. Monoliths with a mesopore size of 2.8 μm have been used in this study.

The most impressing feature of monolithic columns is the high efficiency at high linear flow-rates, which can also be seen in Fig. 3. As it was shown by Rodrigues for polymeric perfusion sorbents [12] the efficiency of sorbents with large pores is constant at high flow-rates due to the increasing influence of forced convective mass transfer. The diffusion length in the monolithic silica backbone is very short in comparison to an irregular or spherical shaped silica particle. Therefore the C term of the Van Deemter equation, indicating the diffusion term, is small and the slope of the function $H=f(u)$ at higher flow-rates is not as steep as it is for particulate silica sorbents. In Fig. 4 two chromatograms of a test mixture are shown, the first one at a flow-rate of 500 cm h^{-1}, which is in the flow-rate range columns with particulate sorbents are normally operated. The second chromatogram shows the same mixture at a flow-rate of 4800 cm h^{-1}. The efficiency in terms of plate height is dropping only from 29 to 50 μm.

Although the flow characteristics of the new monolithic sorbent differ from particulate silica the chemical nature of the sorbent is comparable to silica

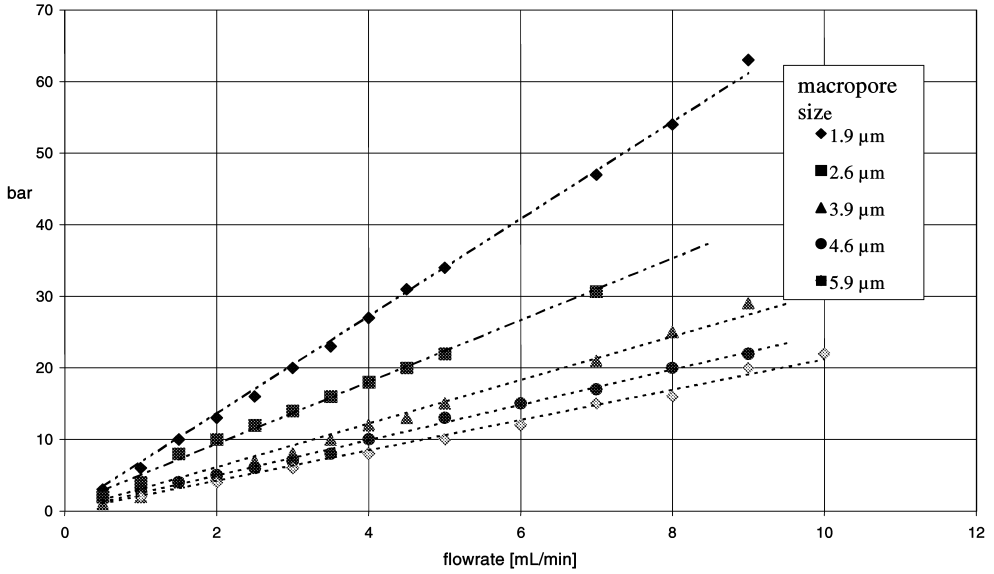


Fig. 2. Pressure drop vs. macropore size.

which is synthesised from alkoxy-silanes. Therefore the selectivity of the native silica monolith is the same as for particulate sorbents, with good separation characteristics for diastereomers and isomers.

The goal of this work was the efficient and productive separation of the diastereomeric mixture in its two individual isomers. The crude feedstock had a composition of 66% isomer A and 33% isomer B. The solubility of the sample in alkane solvents was sufficient for a preparative separation. Therefore, normal-phase chromatography is an efficient and productive method to achieve the pure diastereomers

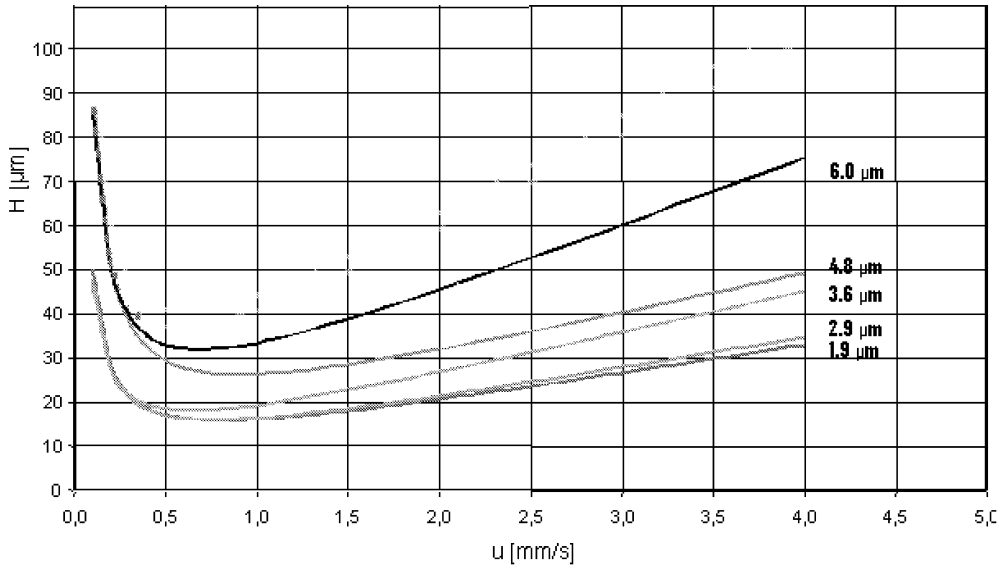


Fig. 3. Efficiency vs. macropore size.

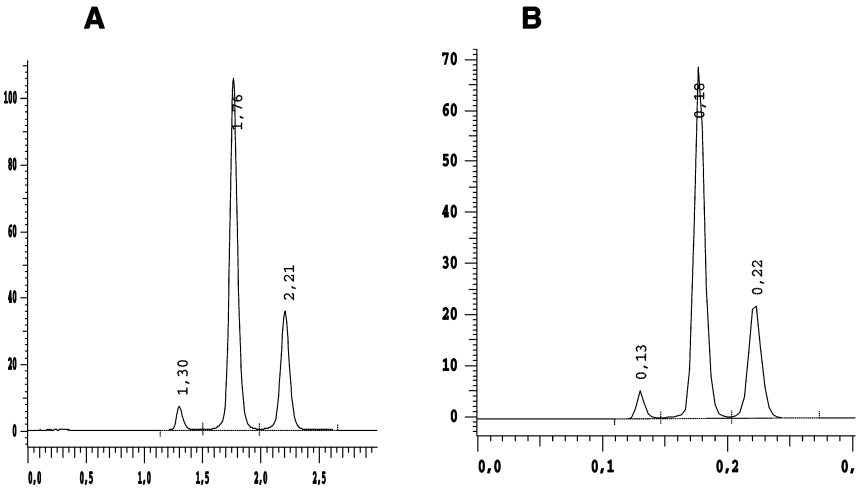


Fig. 4. Chromatograms of phthalates at (A) 500 cm h^{-1} and (B) 4800 cm h^{-1}.

which are both required for the further synthetic route.

In Fig. 5 the analytical separation of the crude mixture on LiChrospher Si60, 10 μm with a mobile phase composition of *n*-heptane–ethyl acetate (90:10, v/v) is shown. The chromatogram exhibits two chemical impurities at 4.42 and 7.23 min. As the impurities can be removed in the further synthetic route towards the final drug substance the SMB parameters were chosen in that way, that the impurities were eluted together with the raffinate diastereomer.

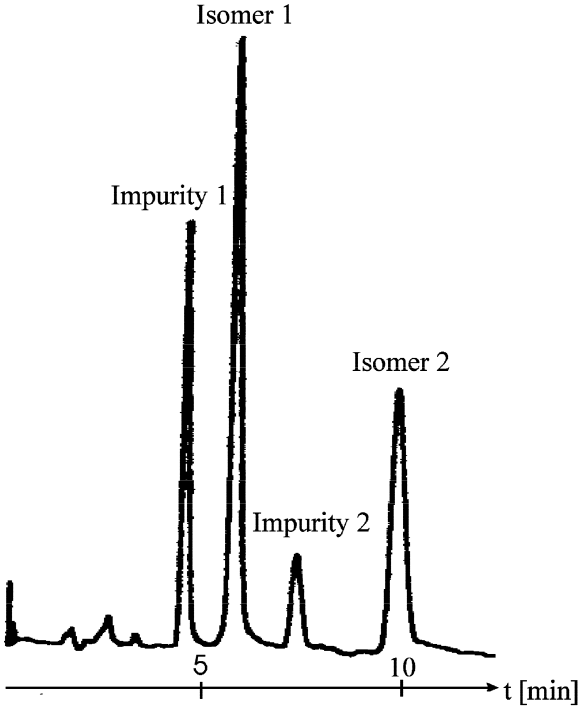


Fig. 5. Analytical injection of crude product on LiChrospher.

To determine the adsorption isotherms of the diastereomers different amounts of the feed mixture were injected onto the column. Injections of 1 ml each were performed with concentrations varying from 1 g feed l^{-1} up to 133 g feed l^{-1}. The corresponding chromatographic data are summarised in Table 1.

The isotherm parameters were calculated using the SMB calculation software HELP. The adsorption characteristics could be fitted to a modified Langmuir isotherm. For the two diastereomers the following isotherm equations have been calculated:

Diastereomer 1:

$$\bar{C}_1 = 1.40C_1 + \frac{0.388C_1}{1 + 0.00776C_1 + 0.022C_2}$$

Diastereomer 2:

$$\bar{C}_2 = 1.40C_2 + \frac{1.101C_2}{1 + 0.00776C_1 + 0.022C_2}$$

The six monolithic columns, which were used for the SMB separation were tested for their uniformity with a mixture of toluene (as t_0 marker), di-

Table 1
Injection of increasing amounts

Injection amount (mg)	Retention time (diastereomer 1) (min)	Retention time (diastereomer 2) (min)
1	2.41	3.11
2	2.40	3.10
20	2.36	2.98
67	2.30	2.85
133	2.28	2.73

Column: silica monolith, 100×25 mm, flow-rate: 30 ml min^{-1}, mobile phase: *n*-heptane–ethyl acetate (90:10, v/v), pressure drop: 3.3 bar, temperature: 24°C, injection volume: 1 ml.

butylphthalate and dimethylphthalate. The resulting k' values are summarised in Table 2.

The separation performance of the total chromatographic bed was checked, by injecting the test mixture into the coupled columns within the Licosep Lab system (pulse injection). Fig. 6 shows the chromatogram of the test mixture. The k' values of 0.31 and 0.62, respectively, correspond very well to the mean of the single columns (0.33 and 0.63). The crude feed mixture was also injected and gave k' values for the two diastereomers of 0.51 and 0.96, respectively, resulting in a selectivity of $\alpha = 1.88$ (Fig. 7).

By using the retention times from the pulse injection the following SMB starting parameters have been calculated:

Column size	6×(100×25 mm)
Zone configuration	1–2–2–1
Flow-rate recycling	95.88 ml min^{-1}
Flow-rate feed	4.76 ml min^{-1}
Feed concentration	121.5 g l^{-1}
Flow-rate eluent	30.67 ml min^{-1}
Flow-rate raffinate	9.62 ml min^{-1}
Flow-rate extract	25.81 ml min^{-1}
Period time	1.01 min

The SMB system was started with the flow parameters above and an internal concentration profile was determined in the seventh cycle. The profile is shown in Fig. 8, the resulting purities were measured using the analytical HPLC system to be 98.7% for the extract diastereomer and 98.4% for the raffinate diastereomer (Fig. 9). To achieve higher

Table 2
Regularity of columns

Column No.	k' dimethylphthalate	k' dibutylphthalate
1	0.35	0.67
2	0.34	0.65
3	0.26	0.50
4	0.33	0.65
5	0.34	0.65
6	0.33	0.65
∅	0.33	0.63

Column: silica monolith, 100×25 mm, flow-rate: 30 ml min^{-1}, mobile phase: *n*-heptane–ethyl acetate (90:10, v/v), pressure drop: 3.3 bar, temperature: 24°C, injection volume: 1 ml.

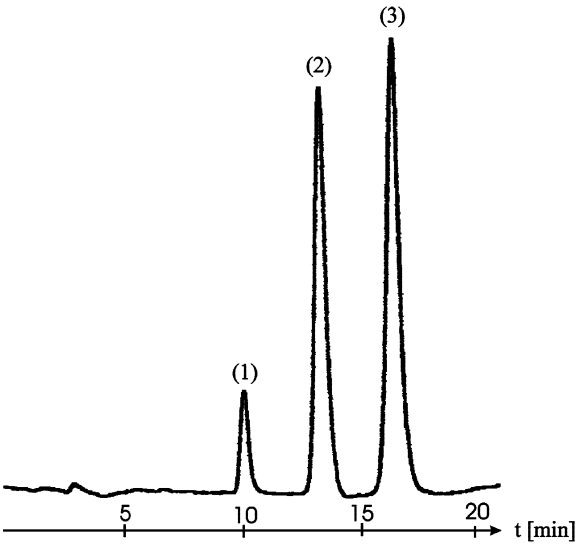


Fig. 6. Pulse injected phthalates.

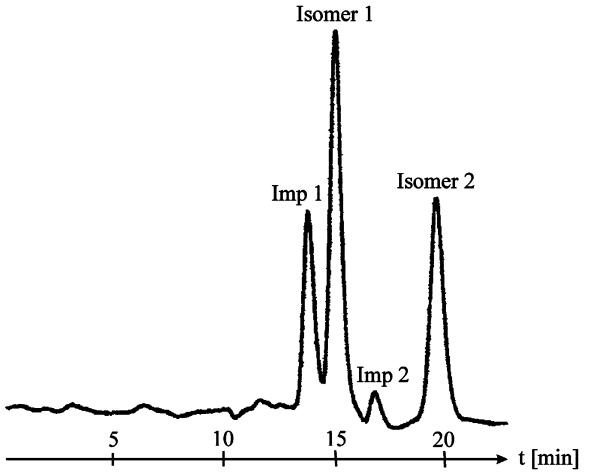


Fig. 7. Injection of diastereomer mixture onto the SMB system (pulse injection).

purities the flow-rates in zones 2 and 3 were adjusted. Table 3 illustrates the changed parameters and the resulting flow-rates.

With the adjusted flow-rates the purities for raffinate and extract increased to 99.8% and 98.8%, respectively. This SMB system was able to purify 778 g of the diastereomeric mixture per day. With a total column volume of 294 ml this corresponds to a productivity of 2642 g feed day^{-1} l^{-1} V_{col}. The resulting internal concentration profile of the optimised parameter set is shown in Fig. 10.

The total system pressure drop at a recycling

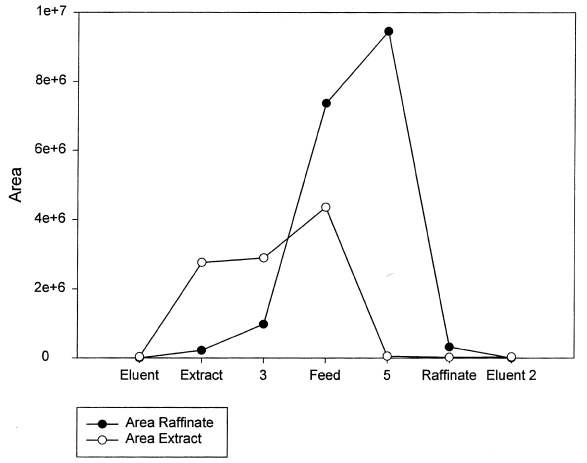
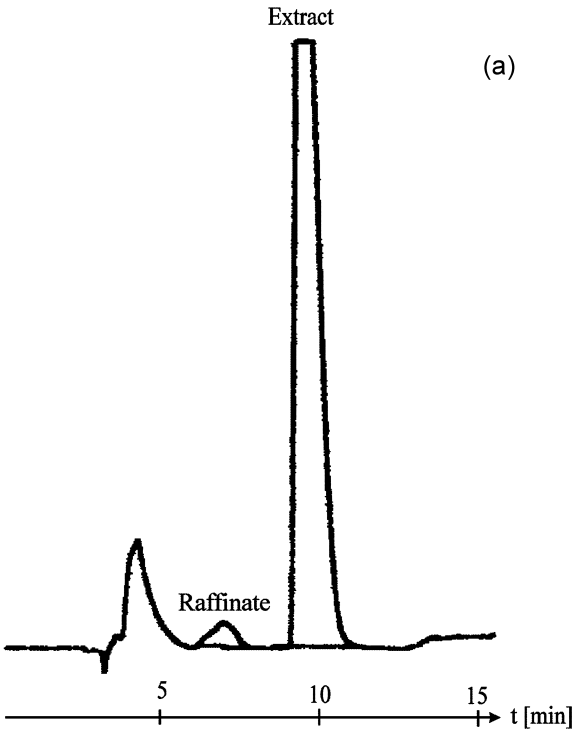


Fig. 8. Profile parameter set 1.



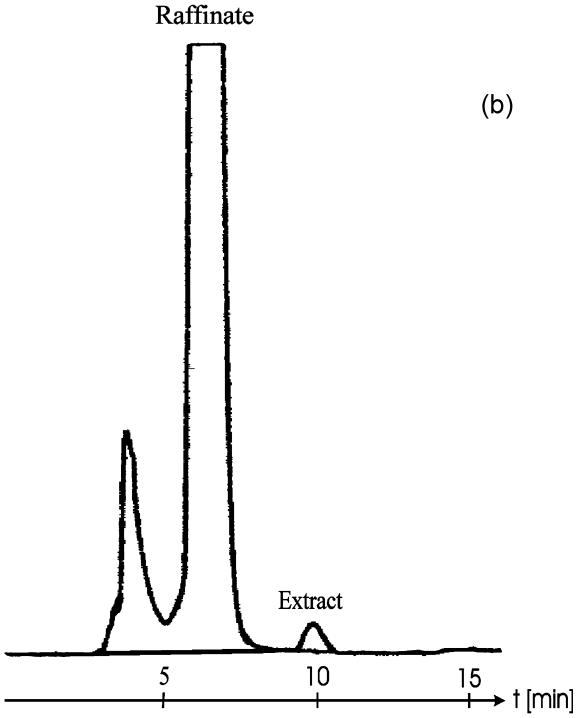


Fig. 9. Purity of (a) extract and (b) raffinate.

Table 3
Flow-rate changes for parameter optimization

Flow-rate	Parameter set 1 (ml min^{-1})	Change (%)	Parameter set 2 (ml min^{-1})
Recycling	95.88	Unchanged	95.88
Eluent	30.67	Unchanged	30.67
Extract	25.81	−5.5	24.41
Feed	4.76	−7.4	4.41
Raffinate	9.62	+10.9	10.67
Period time	1.01 min	+1	1.02 min

flow-rate of 95.88 ml min^{-1} was 50 bar. While the maximum system pressure drop for the columns and the system is 100 bar, all flow-rates could be linearly scaled up for 80% to operate the system at 90 bar. The pump flow-rates were set to:

Flow-rate recycling	172.58 ml min^{-1}
Flow-rate feed	7.94 ml min^{-1}
Feed concentration	121.5 g l^{-1}
Flow-rate eluent	55.21 ml min^{-1}
Flow-rate raffinate	19.21 ml min^{-1}
Flow-rate extract	43.94 ml min^{-1}
Period time	1.02 min

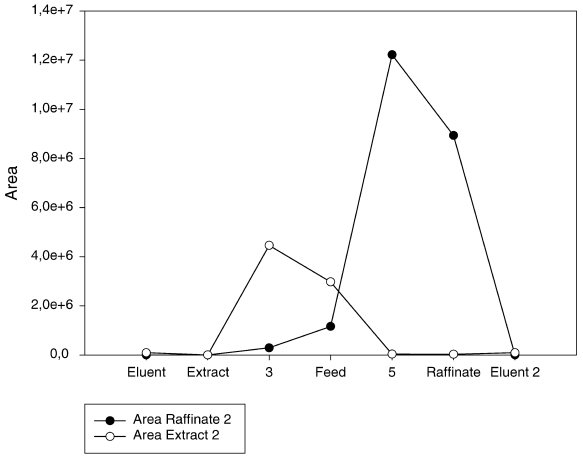


Fig. 10. Profile parameter set 2.

The daily production capacity increased with this set of parameters to 1382 g feed day^{-1}, with the productivity increasing to 4696 g feed day^{-1} l^{-1} V_{col}.

As the selectivities of the diastereomers on the particulate silica and on the monolithic column are comparable and as the saturation capacity for a given column volume is comparable too [13], the productivity of an SMB system is only depending on the linear velocity of the mobile phase. While the separation on the monolithic phases could be operated up to a linear velocity of 2000 cm h^{-1}, the maximum velocity for an SMB system operated with particulate sorbents would be in the range of 700 to 1000 cm h^{-1}. Therefore the use of monolithic stationary phases would increase the productivity of a given SMB system by a factor of 2.

4. Conclusions

The low pressure drop of the monolithic silica sorbent in combination with its high efficiency even at high flow-rates allows the operation of SMB systems at linear velocities of up to 2400 cm h^{-1}. Due to the short diffusion length inside the pore system the adsorption at high flow-rates is not affected and therefore a linear scale-up of all flow-rates up to the maximum system pressure drop is possible. On the other hand are selectivity and saturation capacity comparable to particulate silica. The combination of the good properties of selectivity and high flow-rate results in a very good productivity. Laboratory-scale SMB systems equipped with monolithic columns are therefore a powerful tool for the production of kg amounts of drug substances, which are needed for clinical trials in early phases.

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