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Two-fraction and three-fraction continuous simulated moving bed separation of nucleosides

Stefanie Abel, Matthäus U. Bäbler[1], Cordin Arpagaus, Marco Mazzotti*, Joachim Stadler[2]

ETH Swiss Federal Institute of Technology Zurich, Institute of Process Engineering, Sonneggstrasse 3, CH-8092 Zurich, Switzerland

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Abstract

A new experimental set-up and a new simulated moving bed (SMB) operation are presented in this work. A desktop SMB unit developed as a modification of the commercial ÄKTA™ explorer working platform has been utilized for the separation of different mixtures of nucleosides. Both two fraction and three fraction SMB separations have been carried out, the latter made possible by the adoption of a new SMB configuration and operating mode (three fraction SMB, 3F-SMB, operation). Experiments demonstrate the feasibility of the 3F-SMB operation, and confirm the trends predicted based on considerations about retention of the components to be separated along the unit.

Keywords: Simulated moving bed; Preparative chromatography; Nucleosides

1. Introduction

The simulated moving bed (SMB) is a continuous chromatographic multi-column separation process, which has become an established technology in the pharmaceutical industry over the last 10 years, especially for the separation of enantiomers, which have identical physical properties, making it impossible to separate them by any of the classical separation processes [1–3]. Chromatography in general is a very flexible and mild separation technique, well suited especially for sensitive products. The advantages of SMB technology are the highly efficient continuous operating mode, with a higher productivity and lower solvent consumption than in batch column chromatography [2]. Moreover, SMB can be easily scaled up [2,4]. Nowadays, the separation of bio-molecules is largely performed using the less efficient batch chromatography, due to the complexity of the mixtures involved in the downstream processing of bio-products. These mixtures usually contain a variety of large molecules and a number of impurities coming from the cell broth. Some of these substances might clog the column due to their size, others might bind very tightly to the stationary phase, precipitate, or denature during the separation process. The target molecules are usually rather dilute. This makes a cleaning in place (CIP) of the column necessary after several uses. CIP is often performed in the reverse flow mode to destroy and remove molecules irreversibly bound to the stationary phase. Applying the classical SMB set-up as shown in Fig. 1, the columns are used continuously and the scheme does not allow to take out columns for cleaning without shutting down the plant.

The conventional SMB process realizes the separation of a feed mixture into two fractions by exploiting a simulated countercurrent contact of the solid and the fluid phase [5,6]. The separation principle is the different adsorption affinity of the components on the solid phase. The flow rate ratio between the fluid flow rate and the equivalent solid flow rate has to be chosen in a way that the more adsorbed compounds move in the direction of the solid flow, whereas the other compounds move with the fluid. This makes it possible to collect the two purified fractions continuously. In the simplest implementation a binary mixture is separated, and the two components are collected separately in the two product fractions. This is in particular the case of enantiomers.

* Corresponding author. Tel.: +41 1 6322456; fax: +41 1 6321141.
E-mail address: mazzotti@ivuk.mavt.ethz.ch (M. Mazzotti).
[1] Present address: Swiss Federal Institute of Technology Zurich, Institut für Chemie- und Bioingenieurwissenschaften, ETH Hönggerberg/HCI, CH-8093 Zurich, Switzerland.
[2] Present address: Amersham Biosciences Europe GmbH, Munzinger Strasse 9, 79111 Freiburg, Germany

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Nomenclature

c_i	concentration of species i (g/l)
H_i	Henry's constant of species i
m_j	flow rate ratio in section j
n_j^{col}	number of columns in section j
P_K	purity of product stream K
q_i	adsorbed phase concentration of species i (g/l)
Q_j	volumetric fluid flow rate in section j (ml/min)
t^*	switch time (s)
V	column volume (ml)
V_j^{D}	dead volume per column in section j (ml)
$V_{\mathrm{in}}^{\mathrm{D}}$	dead volume of the tubing measured from inlet port to column inlet (ml)
$V_{\mathrm{out}}^{\mathrm{D}}$	dead volume of the tubing measured from column outlet to outlet port (ml)

Greek letters

ε	bed void fraction

Subscripts and superscripts

A, B, C	components
D	desorbent
dA	2′-deoxyadenosine
dC	2′-deoxycytidine
dG	2′-deoxyguanosine
dT	thymidine
E	extract
F	feed
i	component index
j	section index ($j = 1, \ldots, 4$)
L	lower bound
R	raffinate
T	third fraction
U	upper bound

The unit is divided into four sections by the inlet and outlet streams, where the feed enters between Sections 2 and 3. The products are withdrawn in the extract stream between Sections 1 and 2 and in the raffinate stream between Sections 3 and 4, whereas the direction of the fluid flow is defined going from Sections 1–4. Sections 1 and 4 regenerate the solid phase and the solvent, respectively, which is necessary for their continuous recycle. In case of a true countercurrent configuration, this is called the true moving bed (TMB), which is a conceptual process, since the significant technical problems due to the solid phase movement cannot be solved in practice. The SMB technology overcomes these problems by using fixed-bed columns, and practically simulating the solid flow by switching all the inlet and outlet ports periodically in the direction of the fluid flow. This simulates a solid flow equivalent to $Q_{\mathrm{s}} = V(1-\varepsilon)/t^*$, where V is the column volume, ε is its void fraction and t^* is the port switching period. To calculate the net fluid flow in an SMB, the fluid amount in the column that is moved backwards in the direction of the solid flow at each port switch ($V\varepsilon/t^*$ in the case of nonporous particles) has to be averaged over the time t^* and subtracted from the fluid flow rate Q_j. Using these quantities one can define the flow rate ratio m_j for each section of the unit, which is the key parameter used to design the SMB separation:

$$m_j = \frac{\text{net fluid flow rate}}{\text{solid flow rate}} = \frac{Q_j t^* - V\varepsilon}{V(1-\varepsilon)}, \quad j = 1, \ldots, 4. \tag{1}$$

The SMB can operate in a closed loop configuration, where the regenerated solvent coming from Section 4 is directly recycled to Section 1, or in an open loop configuration, where the fluid coming from Section 4 is withdrawn and recycled indirectly (see Fig. 1).

In order to design the operating conditions of an SMB, it is necessary to know the adsorption behavior of the system under consideration. For low feed concentrations, such as those used in this work, a linear adsorption isotherm can be used:

$$q_i = H_i c_i, \tag{2}$$

where q_i is the amount adsorbed on the solid phase, H_i the Henry's constant, c_i the concentration in the liquid phase, and i is the component index. For this simple case of a linear isotherm and in the frame of the simplified local equilibrium model the necessary and sufficient conditions for complete separation of two components B and C with $H_{\mathrm{B}} > H_{\mathrm{C}}$

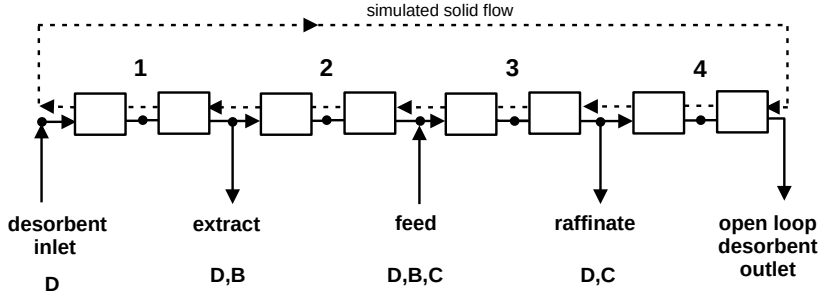

Fig. 1. Scheme of an open loop four-section SMB unit.

consist of the following inequalities (see [6] for details):

$$H_B \leq m_1 \quad (3)$$

$$H_C \leq m_2 \leq H_B \quad (4)$$

$$H_C \leq m_3 \leq H_B \quad (5)$$

$$0 \leq m_4 \leq H_C \quad (6)$$

These conditions define a region in the four-dimensional operating parameter space yielding complete separation of B and C in the frame of the ideal equilibrium model. Usually only the triangular projection of this region onto the (m_2, m_3) plane is used for graphical representation, but it has to be kept in mind that this applies only provided also the constraints on m_1 and m_4 are fulfilled.

In this work the use of SMB in the field of bio-separations is considered. The separation of nucleosides, the building blocks of DNA, using different standard and non-standard SMB set-ups, is investigated. In this context a new SMB set-up that allows for continuous CIP is introduced. Also a SMB separation into three fractions is shown, where the non-standard set-up benefits from the special dynamics of the SMB and exploits the fact that the SMB is a simulated countercurrent process and not a true countercurrent unit (see [7] for details). This type of separation can no longer be regarded as a technical implementation of the TMB process, as it is the case for the classical binary SMB separation, but it has to be viewed as a new independent multi-column chromatographic process.

2. Chromatography of nucleosides

The separation of the nucleosides thymidine, 2′-deoxyguanosine, 2′-deoxyadenosine and 2′-deoxycytidine hydrochloride (in the following referred to as dT, dG, dA and dC, respectively), all purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany), on the reversed phase SOURCE^{TM} 30RPC (trademark of Amersham Biosciences AB, Uppsala, Sweden), was considered. This stationary phase is designed for fast, high performance preparative separations of bio-molecules such as proteins, peptides and oligonucleotides and has a matrix based on rigid, polystyrene/divinyl benzene 30 μm mono-sized beads. This material was packed into two sets of SMB columns: six columns of 10 mm diameter and 10.32 ± 0.3 cm length (column set α) and seven columns of 4.6 mm diameter and 15 cm length (column set β). For column set α, columns of the type with column plungers for length adjustment and elimination of column dead volume were used, which made it difficult to pack them with identical packing length and/or identical bed porosity. For column set β, standard PEEK columns were used where the bed length is defined by the column length. The porosity was measured by injecting a potassium chloride solution as a non-adsorbing tracer,

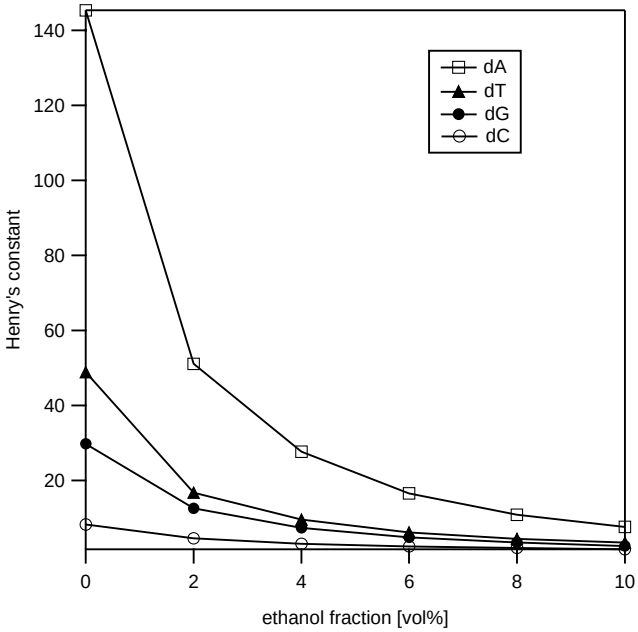

Fig. 2. Henry's constants of the four nucleosides at 30 °C as a function of the ethanol volume fraction in an aqueous mobile phase.

which is detected at the column outlet by measuring conductivity. The average porosity of both column sets is $\varepsilon = 0.78 \pm 0.02$. As a mobile phase, mixtures of water–ethanol with ethanol fractions between 0 and 12 vol.% were used.

The retention behavior of the nucleosides under investigation depends as expected on the composition of the mobile phase, e.g. changing the ratio between a polar and a less polar solvent changes the affinity of the solutes for the solid or the liquid phase. The effect of increasing the ethanol content in the water-ethanol mixture on the Henry's constants of the nucleosides dC, dG, dT and dA at 30 °C is illustrated in Fig. 2. Nucleosides are detected at the column outlet using a UV detector. Henry's constants are calculated from the retention time in the bed $t_{R,i}$, from which the residence time in the extra column dead volume is subtracted beforehand, using the standard relationship:

$$H_i = \frac{Qt_{R,i} - \varepsilon V}{V(1-\varepsilon)}, \qquad i = \text{dT, dG, dA, dC.} \quad (7)$$

3. Two fraction SMB separation

To perform a standard binary two fraction SMB separation, the two nucleosides dG and dT have been selected. From Fig. 2 it is obvious, that for any ethanol content this pair is the most difficult to separate. In order to choose a reasonable range in Henry's constants for plant operation, the ethanol fraction of 6 vol.% has been chosen, corresponding to $H_{dT} = 4.54$ and H_{dG}=6.13. For the experiments described in this chapter column set α (see Chapter 2) was used.

3.1. Experimental set-up

A modified ÄKTA™ explorer 100 (trademark of Amersham Biosciences AB, Uppsala, Sweden) was used (Fig. 3). The ÄKTAexplorer is a multi column batch chromatography system for fast method and process development, suitable for scale-up of chromatographic separations of biomolecules. To upgrade this system to an SMB, it was necessary to add a number of extra features, which could be achieved by the use of standard components, namely pumps and valves. A custom designed 'SMB strategy', based on standard UNICORN™ control software (trademark of Amersham Biosciences AB, Uppsala, Sweden) allows to control all the extra devices. The column loop of the SMB was built using identical subunits repeating for every column, as shown in Fig. 4. For the standard SMB set-up four HPLC pumps are needed, one of each to control the individual feed and desorbent inlet flows as well as the raffinate and extract outlet flows. In the configuration described here, pump 1 and 2 are provided by the standard P900 gradient pump of the ÄKTAexplorer system. The functionality of two separately operating pumps is achieved by disconnecting the outlets A and B of such a gradient pump from the normally used mixer. In order to control the third and fourth flow of the SMB unit, the system was upgraded by a second P901 module. Each pump has a flow rate range of 0.01 to 100 ml/min. For higher accuracy at low flow rates, pump type P903 (0.001–10 ml/min) would be even more ideal. In order to maintain a standardized working pressure in the pumps controlling the raffinate and extract flows, two flow restrictors (4 bar) have been installed at the corresponding pump outlets. Each of these four pumps is connected to a multi-position valve of the type PV-908 (one inlet open to one of eight outlets, or one outlet open to one of eight inlets), whereas four PV-908 valves are built-in, and an additional PV-908 valve needed for the solvent outlet used in the open loop configuration was added (see Fig. 4). Each of the eight positions of a multi-position valve is assigned to a specific physical column and connected according to the set-up shown in Fig. 4. The experiments in this chapter have been performed using only six columns, so two positions of the valves remained unused. The column switching is realized by turning the multi-position valves after each switching period to the next position in use. Check valves (CV 3301, Upchurch Scientific, Oak Harbor, USA) between each pair of columns are installed to ensure the correct flow direction. The plant has no temperature control and was operated at $30 \pm 1.5\,^{\circ}C$. Temperature variations of a few degrees, which lead to slight isotherm inaccuracies, had to be accepted.

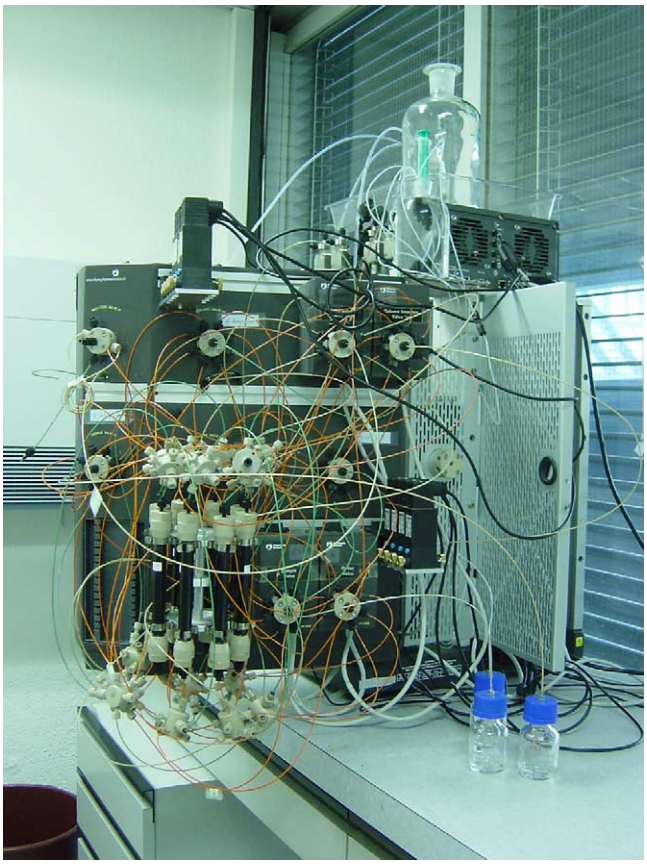

Fig. 3. Picture of the modified batch chromatography system ÄKTAexplorer used as a SMB.

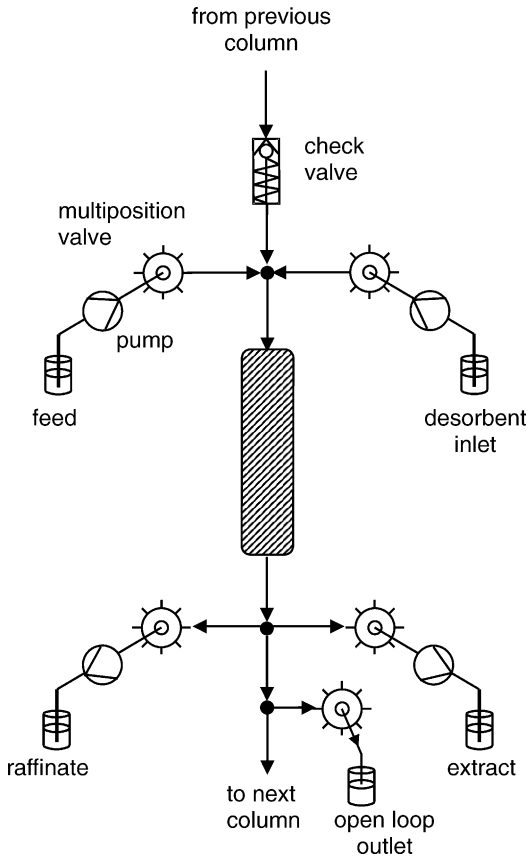

Fig. 4. Plant set-up: single column building block for a conventional SMB as shown in Fig. 1.

3.2. 2-1-2-1 configuration

First, a 2-1-2-1 configuration for an open loop SMB was adopted. The dead volume of the plant was measured to be $V_1^D = 0.598$ ml, $V_2^D = 1.066$ ml, $V_3^D = 0.598$ ml and $V_4^D = 0.754$ ml. It is worth noting that, even in the case of identical assembly for all columns, the dead volume varies from section to section due to the different tubing parts assigned to each individual section: e.g. Section 2 goes from the extract outlet to the feed inlet one column further, including the tubing with two check valves and one column, while Section 3 goes from the feed inlet to the raffinate outlet two columns downstream including tubing with only one check valve and two columns. The effective liquid flow rate in a SMB is reduced by the extra-column dead volume, which has to be accounted for when calculating the flow rate ratios m_j [8,9]:

$$m_j = \frac{Q_j t^* - V\varepsilon - V_j^D}{V(1-\varepsilon)}, \qquad j = 1, \ldots, 4 \tag{8}$$

For all experiments the external flow rates were kept constant at $Q_D = 2.5$ ml/min, $Q_E = 0.564$ ml/min, $Q_F = 0.08$ ml/min and $Q_R = 0.498$ ml/min. A feed concentration of $c_i^F = 0.5$ g/l for each nucleoside was adopted. Six SMB experiments were carried out with a switch time t^* varying between 7.7 min and 9.5 min, and the outlet concentrations and purities were measured at cyclic steady state. This corresponds to a series of six operating points crossing the region of complete separation in the (m_2, m_3) plane, going from the pure raffinate region, through the complete separation region, to the pure extract region [6]. The results are illustrated in Fig. 5 and 6, where the extract and raffinate purity, and the dG and dT concentrations in the product outlets, respectively, are plotted as a function of the switch time. When looking at Fig. 6, it is rather evident that as expected both species essentially increase their concentration in the raffinate and decrease it in the extract as the switch time increases. This leads to an increase in extract purity and decrease in raffinate purity as t^* increases. Such trends are confirmed by the purities plotted in Fig. 5, but with exceptions at small and large values of the switch time. In fact, the extract purity decreases for $t^* > 9$ min, whereas the raffinate purity decreases (for decreasing t^*) for $t^* < 8$ min. In both cases, the concentration of the main product in the relevant product stream is very low (see Fig. 6), and this makes the effect of the presence of the slightest impurity of the other species rather detrimental in terms of purity values. It is also worth noting that very high values of P_R are not accompanied by similarly high values of P_E, which is always below 98%. We attribute such behavior to the unit configuration, where only one column is used in Section 2. This can be viewed as an insufficient way of simulating a countercurrent contact between fluid and solid in Section 2. This conjecture has been confirmed by simulation results not reported here, and has led to the series of experiments reported in the next section.

3.3. 2-2-2-0 configuration

A second series of experiments has been carried out using the same column set α as before, but adopting a 2-2-2-0 configuration. This means that there are two columns in each section from Section 1–3. There is no Section 4 and the raffinate is constituted of the whole stream leaving

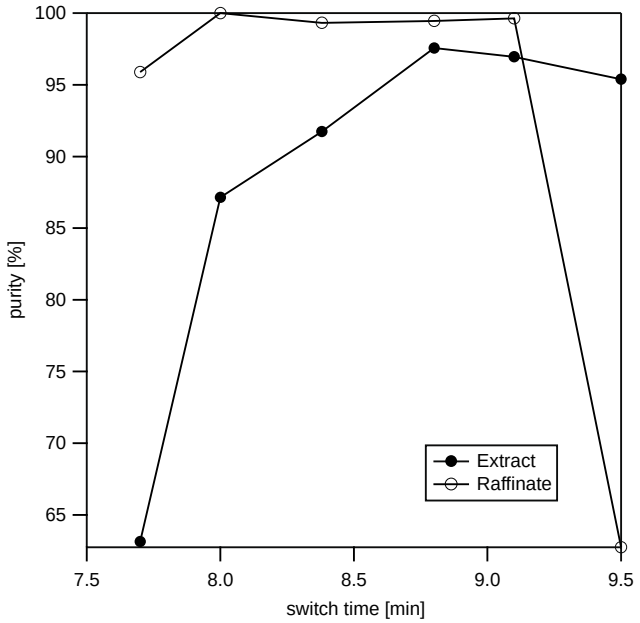

Fig. 5. Two fraction separation of dG and dT. Purity of extract and raffinate as a function of the switch time, with all flow rates kept constant. SMB configuration: 2-1-2-1.

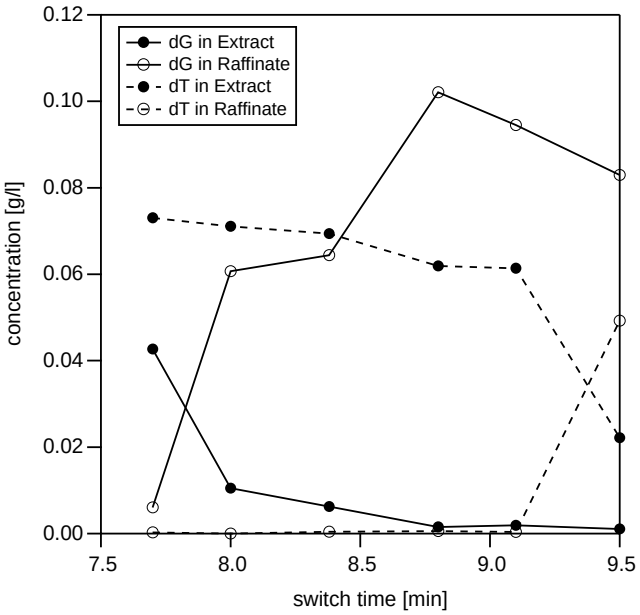

Fig. 6. Two fraction separation of dG and dT. Outlet concentrations of the nucleosides dG and dT vs. switch time, for the same experiments shown in Fig. 5.

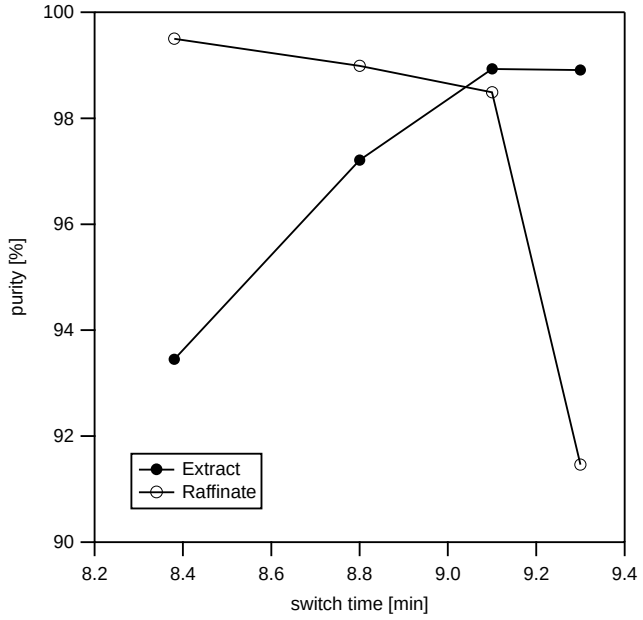

Fig. 7. Two fraction separation of dG and dT. Purity of extract and raffinate as a function of the switch time, with all flow rates kept constant. SMB configuration: 2-2-2-0.

Section 3. In this case, the extra-column dead volume is $V_1^D = 0.598$ ml, $V_2^D = 0.91$ ml, and $V_3^D = 0.598$ ml. Four SMB experiments have been performed using constant external flow rates ($Q_D = 2.5$ ml/min, $Q_E = 0.583$ ml/min, $Q_F = 0.098$ ml/min), leading to similar operating parameters m_j, as in Chapter 3.2. The switch time t^* was varied between 8.4 and 9.3 min, and the outlet concentrations and purities were measured at cyclic steady state. The results are illustrated in Fig. 7, that should be compared with Fig. 5. It can be observed that indeed the extract purity has achieved about 99%, which is a value much closer to the maximum raffinate purity, i.e. 99.5%. It is worth noting that the raffinate product is significantly more diluted due to the lack of Section 4, and this leads also to slightly lower P_R values than in the case of the 2-1-2-1 configuration.

4. Three fraction SMB separation

In the following a new SMB concept is introduced, which can be especially useful for the separation and the purification of bio-molecules. Such new concept exploits the dynamical characteristics of SMBs, without necessarily being equivalent to a TMB unit. The scheme of the new set-up is shown in Fig. 8. In the central part of the unit there is the main SMB, here shown as a 2-2-2-0 configuration as used in Section 3.3. This can be replaced by any other column configuration used in a standard SMB unit. The scheme has a Section 0, which can, if necessary, be operated using a different solvent (Des. 2) from that in the main SMB, and a new stream, called the third fraction, can be collected at the end of this section. After passing through Section 0, each column is switched to the 'equilibrate' section, which is necessary only if two different solvents are used, in order to re-equilibrate the column using the same solvent (Des. 1) as in the main SMB before the column is again included in the main SMB cycle. A separation into three pure fractions is possible for a ternary mixture consisting of three compounds A, B and C with Henry's constants in the sequence $H_A > H_B > H_C$. Under proper operating conditions, the most retained species A will be trapped by the solid phase, eluted in Section 0, and collected in the third fraction. The key to obtain both the extract and the third fraction pure is to control the dynamics of the SMB, and how component A propagates. Fig. 9 shows the position of the band of component A at different time points during one switching period after the unit has reached cyclic steady state. Component A enters the plant with the feed. It advances a bit in the first column of Section 3, but is still far away from the column outlet by the time of the switch, when this column is switched to Section 2. While the column moves through all positions of Section 2, the band advances more, but does not reach yet the end of the column. Then the column is switched to Section 1 where compound B to be collected in the extract is eluted. This position is critical for this kind of separation, since the extract must not be polluted by compound A. This is the case, if compound A does not reach the

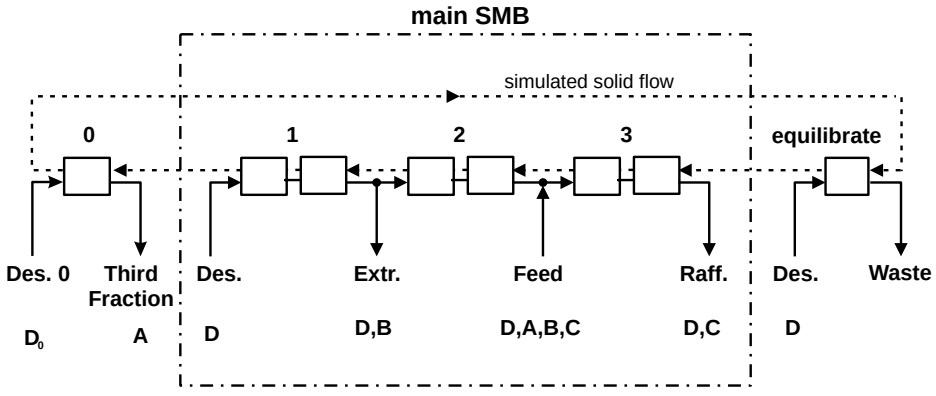

Fig. 8. Scheme of a 3F-SMB unit, that can be used for a three fraction separation, or to perform CIP continuously.

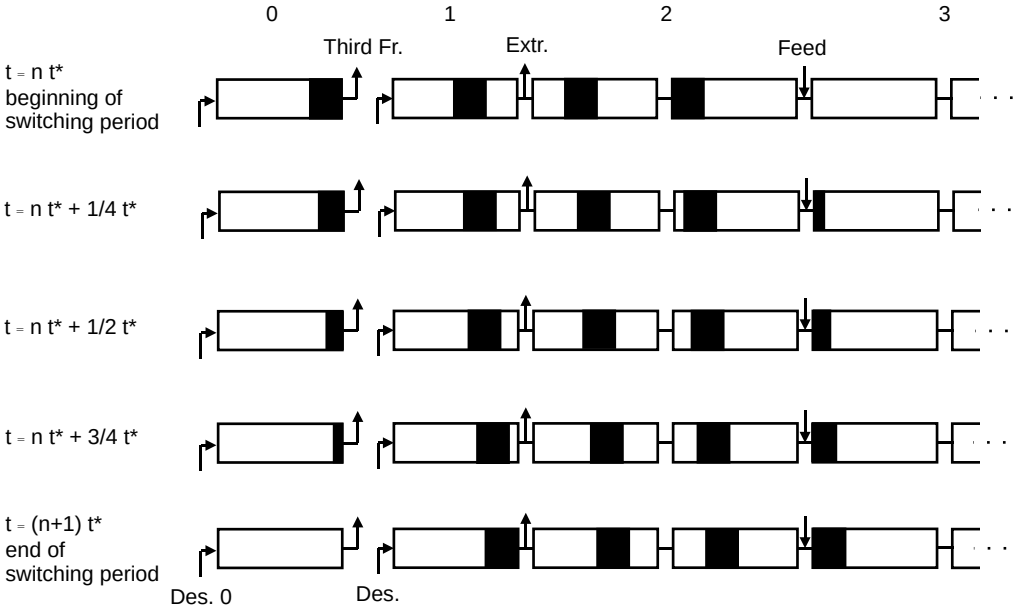

Fig. 9. Position of successive bands of the most retained component at different times during a switch period. Time increases from the top down.

end of the column at this position before the switch. After the switch the column reaches Section 0 and all of component A can be eluted, if necessary by using a different solvent, and collected in the third fraction. The key feature of this approach is that the band of component A can be switched past the extract outlet without being ever in the position to leave the unit with the extract. This is possible in the SMB, due to the simulated nature of the countercurrent solid movement, but would not be possible in a TMB unit. This new operation mode is called three-fraction SMB separation, i.e. 3F-SMB in short and it was first proposed in the patent literature [10,11]. As reported in more detail elsewhere [7], in the case of linear isotherms complete separation conditions for the 3F-SMB separation are given by the following relationships:

$$m_1^{\mathrm{L}} = H_{\mathrm{B}} \le m_1 \le m_1^{\mathrm{U}} = H_A - \frac{\varepsilon}{1-\varepsilon}(1+n_2^{\mathrm{col}}) - \phi - n_2^{\mathrm{col}} m_2 - m_3 \tag{9}$$

$$H_{\mathrm{C}} \le m_2 \le H_{\mathrm{B}} \tag{10}$$

$$H_{\mathrm{C}} \le m_3 \le H_{\mathrm{B}}, \tag{11}$$

where n_2^{col} is the number of columns in Section 2, and the parameter ϕ accounts for extra column dead volume:

$$\phi = \frac{V_1^{\mathrm{D}} + n_2^{\mathrm{col}} V_2^{\mathrm{D}} + V_3^{\mathrm{D}}}{V(1-\varepsilon)} - \frac{V_{\mathrm{in}}^{\mathrm{D}} + V_{\mathrm{out}}^{\mathrm{D}}}{V(1-\varepsilon)} \tag{12}$$

These refer to the sections of the main SMB unit in the central part of the 3F-SMB configuration in Fig. 8. It can be observed from Eq. (9) and (12) that the number of columns in Section 2, n_2^{col}, can influence the separation performance. This is because the band of component A moves through the plant as in a batch column, which means that the residence time of an individual band of component A in Section 2 is proportional to n_2^{col}, as long as it does not reach the column end. Usually this time should be kept short by choosing n_2^{col} as low as possible in order to make the separation principle described above work better (see Fig. 9). It has to be noted that conditions for complete separation also apply for m_4, in case Section 4 of the main SMB unit is not dropped, as it is done here. Proper conditions to operate the other two sections are easy to derive. It is worth noting that the inequality (9) on m_1, depends on m_2 and m_3. As a matter of fact, this enforces a constraint on the values of m_2 and m_3 that allow for the existence of a finite range of feasible m_1 values. This can be obtained by imposing the conditions that the upper bound is larger than the lower one in Eq. (9), and leads to a critical line in the (m_2, m_3) plane, below which only operating points that achieve complete separation can lie [7]. For the 3F-SMB three fraction separation complete separation is regarded as the operation regime where all outlet streams exhibit a purity of 100% with the definition of the purity as:

$$P_{\mathrm{T}} = \frac{c_A^{\mathrm{T}}}{c_A^{\mathrm{T}} + c_{\mathrm{B}}^{\mathrm{T}} + c_{\mathrm{C}}^{\mathrm{T}}} \tag{13}$$

$$P_{\mathrm{E}} = \frac{c_{\mathrm{B}}^{\mathrm{E}}}{c_A^{\mathrm{E}} + c_{\mathrm{B}}^{\mathrm{E}} + c_{\mathrm{C}}^{\mathrm{E}}} \tag{14}$$

$$P_{\mathrm{R}} = \frac{c_{\mathrm{C}}^{\mathrm{R}}}{c_A^{\mathrm{R}} + c_{\mathrm{B}}^{\mathrm{R}} + c_{\mathrm{C}}^{\mathrm{R}}} \tag{15}$$

4.1. Experimental set-up

In order to implement the 3F-SMB scheme described above, it is necessary to modify the standard column assembly (Fig. 4) to that illustrated in Fig. 10, which has to accommodate more inlet- and outlet connections to realize the scheme of Fig. 8. An additional pump and the corresponding multi-position valve PV-908 was installed to control Q_{D1} in the 'equilibrate' section. For this purpose the P950 'sample pump' of the ÄKTAexplorer system has been used, which is not a piston pump and not as accurate as the P-900 used at the other pump positions of the SMB. For the flow rate the accuracy is less critical. In the following experiments, a 2-1-2-0 configuration in the main SMB part is adopted. This means that since there is no Section 4, the raffinate pump is not needed and can be used to pump Q_{D2} to Section 0. At the D2 inlet another multi-position valve P-908 is used. In order to segregate properly the different parts of the SMB which use different solvents, the functioning of the check valve between the last column of Section 0 and Section 1 is very important. To allow for a clean partition, the pressure at the inlet of the first column in Section 1 has to be higher than the pressure at the third fraction outlet 'T' at any time, which is usually the case when using reasonable flow rates in the main SMB.

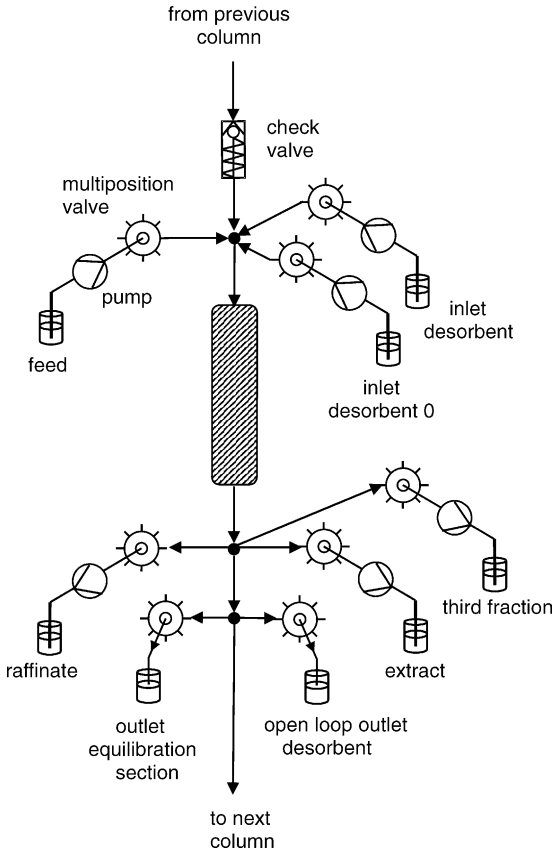

Fig. 10. Plant set-up: single column building block for a 3F-SMB, e.g. as shown in Fig. 8.

4.2. Results

The new SMB concept presented above has been investigated experimentally in a series of preliminary tests. Four 3F-SMB runs have been carried out adopting the operation mode described above. The nucleosides dA, dG and dT have been selected for this separation, where dA is to be collected in the third fraction, dT in the extract, and dG in the raffinate. Column set β (see Chapter 2) was used in an open loop 2-1-2-0 configuration in the main SMB part with one column in Section 0 and one column in the equilibration section. The dead volume per column was determined as $V_1^D = 0.245$ ml, $V_2^D = 0.5$ ml and $V_3^D = 0.245$ ml. The operating window of m_1 values according to Eq. (9) is a function of the Henry's constants which depend on the ethanol content in the solvent and on the operating point in the (m_2, m_3) plane. It can be readily demonstrated that the ethanol fraction in the solvent is restricted to values below 2.1 vol.% for this kind of separation. For the experiments presented in this chapter an ethanol fraction of 1.5 vol.%, a bit smaller than the limit, has been adopted in order to work under more robust conditions. In Section 0 an ethanol fraction of 12 vol.% has been chosen to ensure complete desorption in a reasonable time. All flow rates were kept constant ($Q_{D_0} = 1.000$ ml/min, $Q_D = 1.042$ ml/min, $Q_E = 0.258$ ml/min, $Q_F = 0.013$ ml/min and $Q_R = 0.797$ ml/min) and the switch time was varied from $t^* = 14$ min 15 s to $t^* = 16$ min 24 s. A feed concentration of $c_i^F = 0.5$ g/l for each nucleoside was adopted.

In Fig. 11 the experimental points are plotted in the (m_2, m_3) plane, together with the triangular region of complete separation for dG and dT, and the critical line below which 3F-SMB operating points should lie [7]. The grey

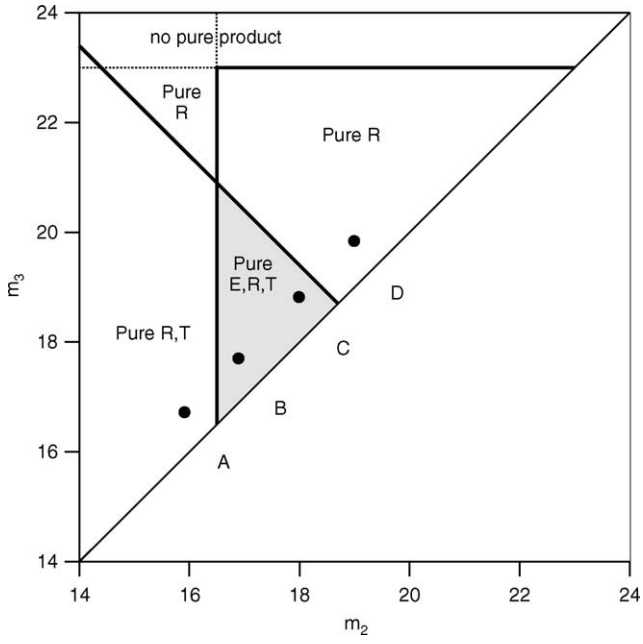

Fig. 11. Separation regions in the (m_2, m_3) plane for the three fraction separation of dA, dT and dG including experimental operating points. The grey area consists of points achieving complete three fraction separation.

Table 1
3F-SMB separation of dA, dT and dG. Operating conditions of the experimental runs

	t^* (s)	m_0	m_1	m_2	m_3	m_1^L	m_1^U	Q_0 (ml/min)	Q_1 (ml/min)	Q_2 (ml/min)	Q_3 (ml/min)
A	855	22.15	23.08	15.91	16.72	23.00	27.77	1.000	1.042	0.784	0.797
B	896	23.39	24.37	16.89	17.70	23.00	26.79	1.000	1.042	0.784	0.797
C	942	24.79	25.84	17.99	18.82	23.00	23.59	1.000	1.042	0.784	0.797
D	984	26.07	27.17	18.99	19.84	23.00	21.57	1.000	1.042	0.784	0.797

Ethanol in Section 0: 12 vol.% ($H_{dA} = 1.26$); ethanol in Sections 1–3: 1.5 vol.% ($H_{dA} = 69.0$, $H_{dT} = 23.0$, $H_{dG} = 16.5$); column set β ($\varepsilon = 0.78$, ID = 0.46 cm, length = 15 cm); dead volume per column $V_0^D = 0.160$ ml, $V_1^D = 0.245$ ml, $V_2^D = 0.5$ ml and $V_3^D = 0.245$ ml; column configuration in main SMB: 2-1-2-0, 1 column in Sections 0 and 1 column in the equilibration section; $c_i^F = 0.5$ g/l for each nucleoside.

area in Fig. 11 defines the only operating points in the (m_2, m_3) plane leading to a complete three fraction separation, provided m_1 is chosen to fulfill Eq. (9). All operating parameters of the experiments together with the corresponding upper and lower bounds on m_1 (m_1^U and m_1^L) according to Eq. (9) are reported in Table 1. It can be observed that point C and D do not fulfill Eq. (9), whereas for point D no good value for m_1 can be found, since $m_1^L > m_1^U$. The purities of the three product streams are plotted versus the switch time in Fig. 12. It can be observed that the raffinate purity is always 100%, which is consistent with the theoretical prediction (see Fig. 11). The purity of the third fraction increases from A to B, and remains constant in runs C and D at a value slightly below 95%. It has to be kept in mind that with the adopted experimental strategy of keeping the flow rates constant and just changing the switch time, not only the values of m_2 and m_3, but also that of m_1 changes according to Eq. (8). It can be observed that point A is very close to the lower bound for m_1, hence the low purity of the third fraction in this case can be explained by its pollution with dT due to a too low m_1 value. The purity of the third fraction increases from point A to B, which is consistent with the increased m_1 value that allows desorbing dT before it reaches Section 0. The extract purity increases from A to C, where the maximum purity of about 95% is reached. It can be concluded that this is the maximum possible value reachable with this configuration where only one column in Section 2 is used (see Chapters 3.2 and 3.3). The extract purity decreases again from point C to D. Also this behavior is in accordance with our expectations, since m_1 is larger than its upper boundthus leading to dA leaking out of Section 1 and polluting the extract.

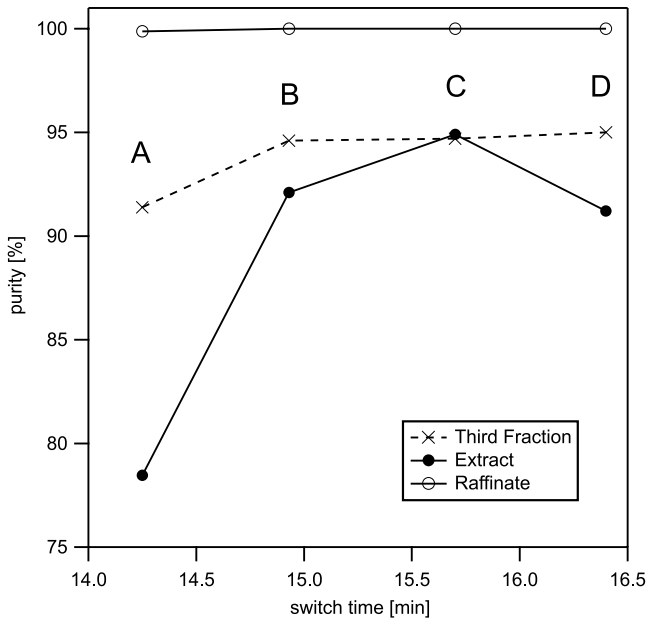

Fig. 12. Three fraction separation of dA, dT and dG. Product purity vs. switch time, with all flow rates kept constant. SMB configuration as shown in Fig. 8.

As a result it can be stated that it was possible to find an experimental point for a three fraction separation with purities for all three products above 94%. The theoretical approach used to find the region of complete separation could be used to explain the trends observed in the experiments. We believe that by using more columns in Section 2 the purity of the extract with respect to the impurity dG could be improved, as this was the case for the binary separation described in Chapter 3.2. On the other hand using two columns at this point narrows the possible operating window for m_1, due to its upper bound in Eq. (9). This implies that the application of the 3F-SMB operation mode is limited to cases where the selectivity between the two more retained components is large enough.

5. Concluding remarks

In this paper, we report about a SMB separation carried out in a desk-top SMB unit, obtained by modifying a standard ÄKTAexplorer workstation. This modification has been made possible by the rather flexible, modular hardware configuration of the standard ÄKTA system that has allowed for changes and extensions, and by the powerful UNICORN software, for which a non-standard extension could be developed to control a much larger number of devices than the commercial configuration. Two rather significant new results have been achieved.

First, on this machine we have studied experimentally the separation of different mixtures of nucleosides, and we have shown how critical the number of columns used in Section 2 of a conventional SMB is for the extract purity.

Secondly, we have introduced and experimentally tested a new operation mode, i.e. the 3F-SMB, that allows for the separation with high purity of three fractions in a single

unit and in a single process step. Such a unit has a minimum of four sections when a single mobile phase composition is used, and a minimum of five sections if two different mobile phases are used. In the latter case the 3F-SMB process allows for continuous cleaning in place in Section 0 to eliminate impurities that have been irreversibly bound to the stationary phase. Criteria to design such a new SMB operation have been identified [7], that allow to evaluate a priori whether the new operation can be applied, and which operating conditions should be selected to achieve complete three fraction separation.

References

[1] M. Juza, M. Mazzotti, M. Morbidelli, Trends Biotechnol. 18 (2000) 108.
[2] R.-M. Nicoud, Pharm. Technol. Eur. 11 (1999) 28.
[3] M. Schulte, J. Strube, J. Chromatogr. A 906 (2001) 399.
[4] S. Nagamatsu, K. Murazumi, S. Makino, J. Chromatogr. A 832 (1999) 55.
[5] D.M. Ruthven, C.B. Ching, Chem. Eng. Sci. 44 (1989) 1011.
[6] M. Mazzotti, G. Storti, M. Morbidelli, J. Chromatogr. A 769 (1997) 3.
[7] G. Paredes, S. Abel, M. Mazzotti, M. Morbidelli, J. Stadler, Ind. Eng. Chem. Res. (2004), submitted.
[8] C. Migliorini, M. Mazzotti, M. Morbidelli, AIChE J. 45 (1999) 1411.
[9] M.P. Pedeferri, G. Zenoni, M. Mazzotti, M. Morbidelli, Chem. Eng. Sci. 54 (1999) 3735.
[10] G. Hotier, J.-M. Toussaint, G. Terneuil, D. Lonchamp, Continuous process and apparatus for chromatographic separation of a mixture of at least three constituents into three purified effluents using two solvents. United States Patent No. US005093004A (1992).
[11] U. Voigt, J. Kinkel, R. Hempel, R.-M. Nicoud, Chromatographic process for obtaining highly purified cyclosporin A and related cyclosporins. United States Patent No. US006306306B1 (2001).