Journal of Chromatography A, xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Applications of high-resolution recycling liquid chromatography: From small to large molecules

Fabrice Gritti*, Sebastien Besner, Sylvain Cormier, Martin Gilar

Waters Corporation, 34 Mapple Street, Milford, MA 01757, USA

ARTICLE INFO

Article history:
Received 3 August 2017
Received in revised form
11 September 2017
Accepted 23 September 2017
Available online xxx

Keywords:
Twin-column recycling chromatography
Isomers
Enantiomers
Isotopes
Polymers
Biomolecules

ABSTRACT

A twin-column recycling separation process (TCRSP) is assembled and used to generate higher speed and/or higher resolution levels than those of the usual non-recycling process at the same back pressure. It enables the users to solve very challenging separation problems caused by too small selectivity factors and/or too low column efficiencies. The relative gain in speed-resolution performance increases with increasing the number of cycles in the TCRSP, decreasing the maximum allowable pressure imposed by the LC system, decreasing the column permeability, and with reducing the separation speed. TCRSP is then particularly attractive for conventional LC systems (5000 psi maximum) and columns packed with sub-2 μm to 3.5 μm particles. The performance of the real TCRSP was compared to that of the ideal TCRSP for which the retention factor is strictly pressure-independent. A broad range of separation problems encountered in conventional non-recycling chromatography can be easily solved by using a TCRSP assembly based on two 15 cm long columns. Under adsorption conditions, the TCRSP enables the full baseline separation of polycyclic aromatic hydrocarbon (PAH) isomers (benzo[a]anthracene and chrysene) on a 3.5 μm XSelect-HSS T_3 phase, the complete or improved resolution of racemic mixtures (4-phenylbutanol and bromacil) using the same 2.5 μm cellulose-1 chiral stationary phase, and the full resolution of isotopic compounds (benzene/1,3,5-benzene-d_3/benzene-d_6) on a 2.7 μm Cortecs-C_{18} phase. Under non-adsorption conditions or in size-exclusion chromatography (SEC), the fractionation of a polystyrene standard mixture (molecular weights of 35, 66, 130, 277, 552, 1210, and 2500 kDa) was completed after only 8 cycles on a 1.7 μm BEH 200 Åphase. Similarly, a mixture of intact proteins with molecular weights of 16.7, 66.4, 150, 660, and 1320 kDa was fully resolved on a 2.5 μm BEH 450 Åphase after only 6 cycles. Finally, TCRSP enables the complete separation of a few high-molecular-weight species (monoclonal antibody aggregates, small relative abundance of 1 for 250) from the intact monomeric monoclonal antibody (Vectibix).

1. Introduction

There are many practical cases for which a single chromatographic run is insufficient to achieve full baseline separation. They include the separation of isomers, isotopes, or enantiomers by adsorption chromatography because the compounds present in these mixtures have very similar physico-chemical properties. They also concern the fractionation of larger molecules by SEC such as polymers, bio-polymers (proteins), and aggregated/fragmented species of monoclonal antibodies (IgG) because both the separation space and the column efficiency are extremely limited in exclusion chromatography. In all cases, even after intensive optimization of column and mobile phase chemistry using any design-of-experiment or quality-by-design methods, selectivity factors and/or column efficiencies are not large enough to enable a complete separation with a single column. The first and obvious solution would consist in increasing the column length by coupling a large number of commercial columns. However, speed inevitably decreases due the limitation in the operating pressure of standard LC instruments. The plate height eventually increases and the column efficiency cannot exceed a maximum value, which is imposed by the allowable system pressure, the particle size (or the specific column permeability), the eluent viscosity, and by the effective diffusivity of the analyte along the column. The full separation is then either physically impossible or unacceptably long and poorly sensitive.

Alternatively, a second and more elaborated solution based on the known principle of recycling chromatography can alleviate this

* Corresponding author.
E-mail address: Fabrice_Gritti@waters.com (F. Gritti).

https://doi.org/10.1016/j.chroma.2017.09.054
0021-9673/

resolution barrier [1,2]. In this option, the sample zone is simply recycled back into the column to continue the separation process until the zone width becomes as large as one column length. Note that this type of recycling chromatography fundamentally differs from recycling simulated moved bed chromatography [3–5] because it is not a continuous and highly productive process. Only a small sample volume is injected and the process ends when a few compounds are fully separated. Therefore, the TCRSP cannot be as productive as a multi-column solvent gradient process (MCSGP) or any variant of the simulated moving bed (SMB) process but it has a much higher resolution power than any other processes. A direct-pumping (using only one column) or an alternate-pumping (using two twin columns and a switching valve) recycling process can be used as suggested in the 1960s and 1970s in gel permeation chromatography [1,2,6–9] for the improved fractionation of polymer mixtures. Recycling chromatography was also favorably applied for the preparation of pure isotopes and isomers by preparative gas chromatography [10,11] and for the collection of optically active compounds by preparative chiral liquid chromatography [12–16]. The maximum allowable system pressure is no longer a problem because only two columns are connected in series. The main advantage is the continuous recycling of the unresolved sample mixture back into the same column (direct pumping) or into a second twin column (alternate-pumping) at near-optimum linear velocities until the spatial width of the separation zone becomes as large as one column length [17].

In a previous report, the separation mechanism of a TCRSP was analyzed in depth [17]. It was possible to predict quantitatively the maximum allowable number of cycles given the chromatographic properties (retention, selectivity, and efficiency) of a single column. Additionally, it was shown that the performance of the real process could significantly deviate from that of the ideal process in which the overall resolution factors are directly predicted from the number of cycles and the intrinsic efficiency of a single column. These deviations originate from 1) the extra-column sample band broadening during the sample transfer (first column to valve, valve, valve to inline detector, inline detector to valve, valve, and valve to second column), 2) the potential loss in column performance due to the repetitive valve actuations (and the subsequent pressure transients) which may affect the stability and the structure of the packed bed (the two columns switch position from high to low and from low to high pressures), and 3) from the pressure-dependence of the local retention factor along the column [18] (the separation time between peaks and the overall efficiency are no longer proportional to the cycle number). For these last two reasons, it is preferable to keep the pressure drop moderate in order to reduce the risk of column failure and pressure-dependent retention behavior which both compromise the performance of the recycling process [17]. Most importantly, when using two 15 cm long twin columns packed with 2–3.5 μm particles, the TCRSP provides the highest resolution level for small molecules when the pressure drop remains around 3000 psi [19]. Therefore, the separation performance of the real TCRSP has yet to be demonstrated at moderately high pressures for a broad range of applications and validated form a theoretical viewpoint.

In this work, the intrinsic advantages (speed, resolution, cost, and sensitivity) of the TCRSP over the classical non-recycling process are demonstrated for a wide range of challenging applications covering small to very large molecules at relatively low pressures. An easy-to-use TCRSP device was built in order to follow in real time the progression of the separations. The device can be directly connected to any standard LC system through a short connecting tube enabling the experimenter to automate the recycling process with its own instrument. A small volume (≃25 nL) detection cell is placed in series between the two twin columns and in the vicinity of the recycling valve to minimize the extra-column band spreading. The UV–vis light of the LC instrument is then guided through fiber optics from the light source to the detection cell and from the detection cell to the photodiode. The speed-resolution performances of this home-made TCRSP were measured and compared to those of the ideal process for the tough separation of small molecules such as shape isomers (polycyclic aromatic hydrocarbons benzo[a]anthracene and chrysene), isotopes (benzene, 1,3,5-benzene-d_3, and benzene-d_6), and a few racemic mixtures (4-phenylbutanol and bromacil). Additionally, the TCRSP was applied for the separation of larger molecules by size-exclusion chromatography (SEC). The fractionation of a polystyrene standard mixture (molecular weights of 35, 66, 130, 277, 552, 1210, and 2500 kDa), intact protein mixtures (16.7, 66.4, 150, 660, and 1320 kDa), and of monoclonal antibodies present as both monomeric and aggregated species is tested using conventional 2.5 μm (450 Åpore size) and 1.7 μm (200 Åpore size) SEC particles.

2. Theory

Let us consider the separation of two compounds *A* and *B*. Their retention factors are k_A (least retained compound) and $k_B = \alpha k_A$ (most retained compound, α>1 is the selectivity factor). The linear migration velocities of these two compounds along the column are then:

$$u_A = \frac{u_0}{1+k_A} \text{ and } u_B = \frac{u_0}{1+k_B} \quad (1)$$

where u_0 is the chromatographic linear velocity.

Let us define the velocity fraction $f_B = u_B/(u_A + u_B)$.

2.1. Intrinsic advantages of recycling separation process

For a given pressure drop ΔP, the advantages of recycling chromatography (alternate-pump recycling with two twin columns) over conventional non-recycling chromatography for high-resolution separations are three fold:

1 The speed-resolution performance is no longer limited by the longitudinal diffusivity of the sample when using extremely long columns in absence of recycling. In the TCRSP, it is only limited by the available separation space, e.g., the length of one column. Considering a $2m\sigma$ baseline peak width (m is an arbitrary integer) and symmetric peaks, the maximum apparent column length or the maximum number, n_{max}, of TCRSP cycles can be calculated. It is given by [17]:

$$n_{\max} = \text{Int}\left(\frac{-\left[f_B(2f_B-1) + m^2(f_B-1)H/L\right] - (m^2\sqrt{\Delta}/2)}{(2f_B-1)^2} \right) \quad (2)$$

where Δ is given by:

P

$$\Delta = 4\left[\frac{f_B(2f_B-1)}{m^2} + (f_B-1)\frac{H}{L}\right]^2 - 4\frac{(2f_B-1)^2}{m^2}\left[\frac{f_B^2}{m^2} + (f_B-1)\frac{H}{L}\right] \quad (3)$$

and H and L are the column plate height and the column length, respectively.

At the same time, the interstitial linear velocity, u, at which the TCRSP is run is no longer decreasing to infinitely small values as the column length is increased since it involves only two columns connected in series (u can actually be chosen close to the optimal velocity):

$$u = \frac{k_0 \Delta P}{2\epsilon_e \eta L} \quad (4)$$

where η is the eluent viscosity and k_0 is the specific bed permeability.

Please cite this article in press as: F. Gritti, et al., Applications of high-resolution recycling liquid chromatography: From small to large molecules, J. Chromatogr. A (2017), https://doi.org/10.1016/j.chroma.2017.09.054