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Performance optimization of ultra high-resolution recycling liquid chromatography

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ABSTRACT

The optimization of a twin-column recycling separation process (TCRSP) for maximum resolution or maximum speed-resolution was investigated. The general optimization method was based on the construction of kinetic plots by assuming an ideal TCRSP (no efficiency loss upon recycling). For the optimization, we examined three chromatographic parameters: operation pressure (3000, 6000, 9000, and 12,000 psi), column length (10, 15, and 25 cm), and column inner diameter (i.d.) (2.1, 3.0, and 4.6 mm). Accordingly, the highest TCRSP resolution level is expected for 25 cm long columns packed with 2.5, 2.0, 1.7, and 1.6 μm particles at pressures of 3000, 6000, 9000, and 12,000 psi, respectively. The maximum speed-resolution performance is expected for 10 cm columns packed with 3.7, 3.0, 2.6, and 2.4 μm particles. 3.0 mm i.d. columns are best to minimize the negative impacts of thermal and inter-column dispersion effects on the TCRSP performance. The method was illustrated for the challenging separation (selectivity factor $\alpha < 1.02$) of small molecules in RPLC at a maximum pressure of 6000 psi using commercially available columns. Accordingly, 3.0 × 150 mm columns packed with 2.5 μm cellulose-1 Trefoil particles (chiral separation, γ-phenylbutyrolactone, $\alpha = 1.01$, efficiency $N = 4500$) and 2.7 μm Cortecs-C_{18} particles (isotope separation, $\alpha = 1.02$, $N = 14,500$) particles were found to be the most suitable columns to maximize speed-resolution performance. Further optimization of the TCRSP performance was required by reducing the inter-column sample dispersion that could cause undesirable peak tailing. A standard 2.4 μL Rheodyne valve and 100 μm i.d. tubes were replaced with a home-made 0.5 μL low-dispersion prototype valve and 75 μm i.d. perfect connection tubes. As a result, the experimental resolution factors were increased by +60% (γ-phenylbutyrolactone, 25 cycles, $R_s = 0.7 \rightarrow 1.1$) and +80% (deuterated benzenes, 22 cycles, $R_s = 1.1 \rightarrow 2.0$). Direct comparison between the experimental and the predicted TCRSP performance unambiguously demonstrated that the resolution gain was explained by the significant reduction of the peak tailing after a large number of cycles ($n > 20$).

1. Introduction

Modern HPLC/UHPLC development methods may still fail to deliver satisfactory resolution levels at either conventional (<6000 psi) or very high pressures (15,000 psi) regardless of the optimization approach used to select the best isocratic experimental conditions (eluent composition, stationary phase nature, temperature, flow rate, pH, etc.). The twin-column recycling separation process (TCRSP) could resolve such challenging separation problems, which are caused by too small selectivity factors and/or too poor column efficiencies [1]. After application of LC optimization methods that maximize selectivity, the remaining adjustable experimental parameter left for the improvement of the separation power is the column length in order to maximize column efficiency. The column length can always be increased but at the expense of longer analysis times. Two options are then possible: (1) physically increase the column length by connecting in series a large number of commercial columns [2]; (2) virtually increase the column length by recycling the sample zone back to the inlet of the same column (direct-pumping recycling chromatography or DPRC) or to the inlet of a second twin column (alternate-pumping recycling chromatography or APRC) [1,3,4]. Note that APRC is preferred to DPRC because, in APRC, the analyte is not required to pass through the internal volume of the pump, which causes additional band broadening. In both scenarios, the column efficiency inevitably increases but each option has its own limitation. In the first option, the resolution power is limited by the available pressure drop of the HPLC/UHPLC system used. The column efficiency is eventually bounded above

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by the fact that the linear velocity decreases and tends towards zero as the column length is indefinitely increased [2,5–7], which is an impractical situation. In the second option, the separation performance of both DPRC and APRC is limited by the available separation space, e.g., the length of each one of the twin columns selected. The highest resolution power is then fixed by the maximum allowable number of cycles [8]. TCRSP has been applied in the past for the challenging separation of isomers [9,10], isotopes [11], and optically active compounds [12–15] by adsorption chromatography and for the fractionation of polymers/biopolymers by size-exclusion chromatography (SEC) [16–19].

The potential of the TCRSP was recently investigated regarding its range of practical applications at moderate pressure drops (<6000 psi) for the separation of small and large molecules by adsorption and size exclusion chromatography, respectively [19]. In particular, TCRSP could potentially be advantageous for the isolation of trace compounds present under the main peak of active pharmaceutical ingredients (unknown impurities) or under that of monoclonal antibodies (unknown degradation products such as aggregated and fragmented forms of the monomeric antibody). At any arbitrary pressure drop along the two twin columns, the inherent advantages of the TCRSP over the non-recycling process have been fully demonstrated in terms of analysis time (at a given resolution level), resolution power (at a given analysis time), sensitivity (at a fixed number of column lengths involved during the separation process), and cost (only two columns are required in the TCRSP) [19].

Nevertheless, some practical limitations of the TCRSP have also been identified because the observed resolution power of the actual TCRSP was often found smaller than that of the ideal recycling process (for which the elution times and efficiencies are simply additive when accumulating a large number of cycles) [8]. Three sources of non-ideality were experimentally recognized: (1) column aging as the bed structure could be altered by the many successive pressure transients caused by the actuation of the recycling valve, (2) pressure-dependence of the local retention factor along the column (this becomes problematic at very large numbers of cycles) [8], and (3) inter-column sample dispersion occurring during the sample transfer from one to the second twin column causing undesirable peak tailing [19]. While the negative impacts of the phenomena (1) and (2) on TCRSP performance can be minimized by operating at relatively low pressures (<6000 psi), the third cause of non-ideality would require minimizing the lengths/inner diameters of the different transfer lines of the TCRSP, reducing the void volume of the recycling valve, and using perfect connections between the different parts of the equipment [20]. Finally, once the operating pressure has been selected during the TCRSP, three important experimental parameters have yet to be unambiguously chosen by the user: which are the optimum particle diameter, column length, and column i.d. (among those commercially available) that will maximize either resolution power or speed-resolution? This fundamental question has yet to be answered from the optimization of the kinetic performance of the TCRSP. This is the main incentive of this work. The ultimate goal is to provide the users with a simple tool that will allow them to complete the set of experimental conditions in the TCRSP besides the operating pressure, the nature of the eluent/stationary phase, and the other usual chromatographic parameters (temperature, flow rate, pH, etc.) that maximize column selectivity.

In this work, the performance optimization of the TCRSP based on two twin columns (10, 15, and 25 cm long; 2.1, 3.0, and 4.6 mm i.d.) packed with particle diameters ranging from 1.0 μm to 7.5 μm and used on HPLC/UHPLC systems operating at pressures from 3000 to 12,000 psi is theoretically investigated in order to anticipate the optimum experimental conditions for the pressure, particle diameter, and column dimensions that will maximize either resolution or speed-resolution of small molecules in RPLC. This simple theoretical tool is based on the construction of kinetic plots [21,7,2,6] for the ideal TCRSP (no efficiency loss upon the accumulation of cycles). Besides the aforementioned theoretical works, it is assessed experimentally how the TCRSP performance can be further improved by minimizing the inter-column dispersion that could cause nefarious peak tailing. Optimized experimental conditions are based on the combination of a home-made low-dispersion recycling valve (0.5 μL) and connection tubes (75 μm i.d.) with zero sample dispersion due to the perfect face-to-face sealing between these tubes and the different parts of the TCRSP (injection valve, recycling valve, twin columns, and in-line detection flow cell). The expected benefits are tested and discussed for the challenging and incomplete TCRSP separations of one racemic mixture (γ-butyrolactone, $R_s = 0.7$) and of three deuterated isotopes (benzene/benzene-1,3,5-d_3/benzene-d_6, $R_s = 1.1$) initially observed with a 2.4 μL recycling valve and 100 μm i.d. pre-cut fused-silica glass capillaries.

2. Theory

For more details regarding the general theory of the TCRSP, all the results are available in the theory sections of references [8] and [19]. Only the relevant results used in this work are listed below without demonstration.

2.1. Kinetic plot of the TCRSP

Kinetic plots permit the evaluation of the performance of a chromatographic system by simultaneously considering its speed and resolution charateristics. The kinetic plot representations such as $\frac{t_0}{N}$ (hold-up time per unit of plate number) *versus* N or $\frac{t_0}{R_s}$ (hold-up time per unit of resolution factor) *versus* R_s permit the discrimination between different separation technologies [21,7,2,6]. Its construction enables the user to select the most suitable separation system that maximizes both speed and performance. In this work, we arbitrarily choose the $\frac{t_0}{R_s}$ *versus* R_s kinetic plot representation (note that both representations are strictly equivalent) because it directly informs on the success of a separation process when $R_s > 1.5$.

Let us consider two compounds A and B to be separated, symmetrical Gaussian peaks, and a $2m\sigma$ baseline peak width. m is an arbitrary integer that will be fixed at $m = 3$ and σ is the peak standard deviation. A 6σ baseline peak width will then be considered in this work. The maximum allowable number, n_{max}, of TCRSP cycles or the largest apparent column length, $n_{max}L$, is given by (for the detailed information, cf. Ref. [8]):

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

where the discriminant Δ in Eq. (1) is given by (for the detailed information, cf. Ref. [8]):

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

In Eqs. (1) and (2), the coefficient $f < 0.5$ is the ratio of the migration velocity of the most retained compound B to the sum of the migration velocities of the two compounds (so, $f = 0.5$ if the two peaks strictly co-elute). H and L are the column plate height and the column length, respectively.

The plate height H is a function of the selected particle size, d_p, and of the applied interstitial linear velocity, u, which depends on the selected pressure drop ΔP along the two columns. ΔP itself depends on the column length L. In this work, we consider small molecules (diffusion coefficient $D_m = 10^{-5}$ cm^2/s) and a RPLC-like retention mode. Based on the classical Knox plate height model,