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Separation of a racemic pharmaceutical intermediate using closed-loop steady state recycling

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Abstract

Closed-loop steady state recycling (formerly called closed-loop recycling with periodic intra-profile injection or CLRPIPI) is similar to simulated moving bed (SMB) chromatography. Both steady state recycling (SSR) and SMB are steady state, binary chromatographic techniques in which fresh sample is injected into the interior of the circulating chromatographic profile and two fractions or product streams are collected from either end of the profile. However, SMB is a continuous process, whereas SSR is a repetitive, though discontinuous, process. Underlying mechanisms of closed-loop SSR were studied using the separation of a racemic pharmaceutical intermediate. We have found that creation of a stable steady state chromatographic profile is crucial to obtaining high purity fractions. The structure of the steady state profile, also called the steady state inventory, is controlled by the size of the fractions collected and by the location of the injection points. These SSR parameters have corresponding SMB parameters. For example, increasing the size of Fraction 1 in SSR is equivalent to increasing the raffinate flow-rate in SMB; increasing the distance from the injection point to fraction 1 in SSR is equivalent to increasing the size of zone III in SMB; etc. Finally, the SSR results were compared to those of an SMB separation of the same racemic pharmaceutical intermediate. Using the same chiral stationary phase (CSP) and mobile phase, the production rates (SSR, 255 g racemate/kg CSP/day; SMB, 240 g racemate/kg CSP/day), purities (SSR, 98% e.e. for both enantiomers; SMB, 98% e.e. for both enantiomers), and recoveries (SSR, 99% for both enantiomers; SMB, 99% for both enantiomers) for the two techniques were similar, but SSR used more mobile phase per gram of racemate than SMB. SSR, however, used less mobile phase than batch HPLC.

Keywords: Enantiomer separations; Closed-loop steady state recycling; Preparative chromatography; Pharmaceutical analysis

1. Introduction

The need for chirally pure drugs and drug intermediates in the pharmaceutical industry is well known [1,2]. Preparative chromatography, including recycling techniques, has been shown to be an effective means of separating and purifying enantiomers [3,4]. However, these batch modes of chromatography, while sometimes producing high production rates, require large quantities of solvent. As a result, the operating costs of large scale batch chromatography are often found to be prohibitive [5].

In recent years, simulated moving bed (SMB) chromatography has moved to the fore in large scale chromatographic separations of enantiomers [6]. The main reason for this is that SMB chromatography almost always requires less solvent to separate a given quantity of racemate, and the operating costs

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are therefore significantly lower than with batch chromatographic methods [5,7]. In addition, in those cases where the best available chiral stationary phase (CSP) gives a low resolution of the enantiomers, the production rate (throughput of racemate per kg of CSP), will usually be greater with SMB chromatography [8].

The major disadvantage of SMB is capital cost. SMB systems are significantly more expensive than batch LC systems of comparable production capacity. At very large commercial scales, the lower operating costs of an SMB system dedicated to a specific separation justify the large capital investment. At small-to-moderate scales, this might not be the case [9]. An added complication is that at these smaller scales, SMB systems are often not dedicated to single separation process, but are used to purify several different mixtures over the course of a year [10]. Therefore, a significant portion of the operating life of smaller scale SMB systems will be spent in process development, where the savings in solvent usage may not be apparent until the separation is optimized. The break even point, the scale at which SMB makes economic sense, must be determined on a case-by-case basis.

Recently, one of us (C.M.G.) reported the development of a steady state, binary chromatographic process that in several ways is similar to SMB [11]. In that paper this new process was called closed-loop recycling with periodic intra-profile injection (CLRPIPI). While CLRPIPI is an accurate name for the process, it has been determined that closed-loop steady state recycling (SSR) is a more descriptive and convenient term. Therefore, in the current paper and in future publications, we will refer to this process as closed-loop SSR.

As with SMB, in closed-loop SSR fresh sample is injected into the interior of the circulating chromatographic profile, and two fractions are collected from either end of the profile. However, because only one column is used, closed-loop SSR is not a continuous process. Rather the events (collection of fraction 1 from the front edge of the profile, injection of fresh sample, and collection of fraction 2 from the back edge of the profile) occur in sequence each cycle as the profile elutes from the column. Fig. 1 shows a schematic diagram of a closed-loop SSR system. The typical closed-loop SSR system is basically a preparative- or process-scale high-performance liquid chromatography (HPLC) chromatograph that has been modified with a recycle valve (to recirculate the unresolved portion of the chromatographic profile) and an injection valve (used to inject fresh sample at the appropriate point on the profile as it elutes from the column). Operational details of the closed-loop SSR process are given elsewhere [11].

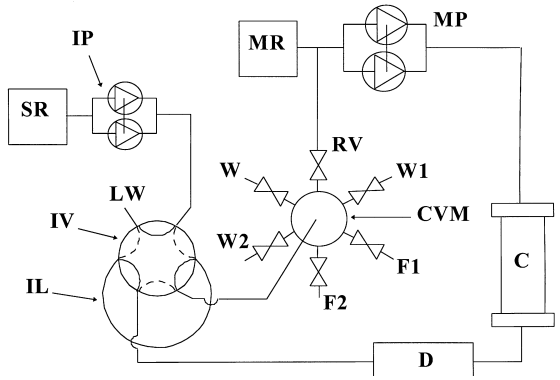

Fig. 1. Schematic diagram of a closed-loop SSR system. MR, mobile phase reservoir; MP, mobile phase pump; C, column; D, detector; CVM, collection valve manifold; W1, waste 1 fraction collection valve; F1, fraction 1 collection valve; F2, fraction 2 collection valve; W2, waste 2 collection valve; W, waste valve; RV, recycle valve; SR, sample reservoir; IP, injection pump; IV, injection valve; IL, injection loop; LW, injection valve waste port.

The capital costs of a closed-loop SSR system are marginally higher than those of the underlying HPLC system, but they are substantially lower than those of an SMB system of comparable production capacity. If closed-loop SSR exhibits some of the advantages of SMB such as higher production rates and lower solvent usage, it should fill a niche at the lower-to-moderate scales where the savings in operational costs do not yet justify the capital investment in an SMB system.

Our goals in performing the work reported here were: (1) to gain basic knowledge of the underlying mechanisms operating during the closed-loop SSR process; and (2) to compare (in a general way) SMB and closed-loop SSR as to enantiomeric purity, production rate, recovery, and solvent usage. To accomplish the first goal, we looked in detail at the SSR separation of a racemic pharmaceutical intermediate using two different mobile phases and CSPs. For the second goal, our approach was to attempt to

achieve the production rate and purities of the SMB method using the same mobile phase and CSP, and to compare recoveries and solvent usage. In the pursuit of both goals, our purity specifications were to equal or exceed enantiomeric excesses (e.e.) of 98% for both enantiomers.

2. Experimental

2.1. SSR experiments

Most of the closed-loop SSR experiments were performed at the Searle laboratories in Skokie, IL, USA using a NovaPrep 200 preparative chromatograph (manufactured by R&S Technology, Wakefield, RI, USA) modified to perform the closed-loop SSR process. This instrument had a single mobile phase pump whose maximum flow-rate was 200 ml/min. The fraction collection valves (Parker, Tucson, AZ, USA) were air-actuated. The variable-wavelength UV detector was an Hitachi Model L-7400 (Hitachi, Japan) and was equipped with a high-pressure flow cell. For these experiments the wavelength was set at 235 nm. The injection pump was an Eldex Model BBB-4-2 metering pump (Eldex Labs., San Carlos, CA, USA). The flow-rate for this pump was set manually with a micrometer. The six-port injection valve and its air-powered actuator were obtained from Rheodyne (Cotati, CA, USA). The injection loop was made of 1/16 in. O.D. stainless steel tubing and had a volume of 5.0 ml (1 in.=2.54 cm).

The remaining closed-loop SSR experiments were performed at R&S Technology using a prototype SteadyCycle™ system. This instrument's single mobile phase pump had a maximum flow-rate of 140 ml/min. The air-actuated fraction collection valves were obtained from Mace (Upland, CA, USA), and the variable-wavelength UV detector, equipped with a high-pressure flow cell, was obtained from Knauer (Berlin, Germany). For these experiments the wavelength was set at 235 nm. The injection pump was an Eldex Model B-100-S-4 metering pump (Eldex Labs.), the flow-rate of which was set manually with a micrometer. The six-port injection valve and its air-powered actuator were obtained from Rheodyne. The injection loop was made of 1/16 in. O.D. polyether ether ketone (PEEK) tubing and had a volume of 5.0 ml.

TurboPrep® software (Merck, Darmstadt, Germany) was used to control the pumps and valves of both instruments. In the experiments reported here, the relative time method [11] was used to determine the initiation times of collection and injection events. In this method, the leading edge of the profile was detected by setting a time window in which the control software looked for a detector signal (''ascending height'') of 5–10% of full scale. When this condition was met, the collection valve for fraction 1 was opened and a timer was started: the times of all other collection and injection events were then defined relative to the opening of the fraction 1 collection valve (hence the term ''relative time'').

All preparative columns were obtained prepacked from Chiral Technologies (Exton, PA, USA) and had dimensions 250×20 mm I.D. In all cases the CSP was ChiralPak AS (Daicel, Tokyo, Japan). In some experiments, the 10 μm particle size was used; in other experiments, 20 μm.

The mobile phase was either 100% acetonitrile or acetonitrile–methanol (90:10). The acetonitrile and methanol were HPLC grade and were obtained from various sources. The racemate was a proprietary pharmaceutical intermediate belonging to and synthesized at Searle (Skokie, IL, USA). For those runs in which the mobile phase was 100% acetonitrile, the sample solution was made by dissolving a sufficient quantity of racemate in acetonitrile to give a final concentration of 10 mg/ml. For those experiments using acetonitrile–methanol (90:10), the sample solution had a concentration of 12 mg racemate/ml; the sample solution's solvent had the same composition as the mobile phase.

All fractions were analyzed by analytical HPLC. The mobile phase was HPLC-grade acetonitrile, and the flow-rate was 1.0 ml/min. The column, having dimensions 250×4.6 mm I.D., was packed with ChiralPak AS (10 μm particle size) and was obtained from Chiral Technologies. The injection volume was 20 μl, and the detector wavelength was 220 nm. For the experiments performed at Searle, the analytical chromatograph consisted of a Hewlett-Packard 1050 pump (San Fernando, CA, USA), a Waters Intelligent Sample Processor (Milford, MA, USA), a Kratos Model 757 variable-wavelength detector, (Ramsey,

NJ, USA), a Kipp and Zonen BD41 two-channel recorder (Delft, The Netherlands), and Digital Equipment Corporation VAX 11/785 computer with Searle chromatography data system. At R&S Technology, a Varian (Walnut Creek, CA, USA) Vista 550 liquid chromatograph equipped with a 9090 autosampler, Knauer variable-wavelength UV detector, and Spectra-Physics SP4290 integrator was used.

2.2. SMB experiments

In previous work performed for Searle at Chiral Technologies in Strasbourg, France, SMB was used to separate the racemic pharmaceutical intermediate. The SMB system was a Licosep 12-26 (NovaSep, Nancy, France). The CSP was 20 μm ChiralPak AS (Daicel) and was packed into 12 Superformance glass columns (Merck). The I.D. of the columns was 26 mm and the bed length of each column was 9.5 cm. The mobile phase was HPLC-grade acetonitrile. The following flow-rates were used: feed, 4.5 ml/min; eluent, 17.9 ml/min; extract, 14 ml/min; raffinate, 8.4 ml/min; and recycle, 54.0 ml/min. The concentration of racemate in the feed was 1.3 mg/ml. The resulting production rate and solvent usage (see Table 3) were not optimized, but will serve as useful benchmarks for the current study.

2.3. Preparative HPLC experiments

Two sets of preparative HPLC experiments were performed at Searle. For the first set of experiments, the preparative chromatograph consisted of two Rainin (Woburn, MA, USA) SD-1 pumps, a Model UV-M variable-wavelength detector (set at 225 nm), and a Kipp and Zonen BD41 two-channel recorder. A Rheodyne Model 7125 syringe loading sample injector equipped with a 10-ml loop (Valco, Houston, TX, USA) or a Gilson Model 401 Dilutor (Middleton, WI, USA) in combination with a Rheodyne electrically actuated Model 7010 injector was used for sample injection. The column effluent was fractionated using a Gilson Model 02 fraction collector. The column was obtained from Chiral Technologies and was prepacked with 10 μm ChiralPak AS (Daicel). The column dimensions were 250×20 mm I.D. The mobile phase was HPLC-grade acetonitrile, and the flow-rate was 20 ml/min. The sample load was 40 mg of racemate per injection.

For the second set of preparative HPLC experiments, the preparative chromatograph consisted of two Rainin SD-1 pumps, a Model UV-1 variable-wavelength detector (set at 225 nm), and a Kipp and Zonen BD41 two-channel recorder. A separate Rainin SD-1 pump was used for sample injection. The CSP was 20 μm ChiralPak AS (Daicel) and was obtained in bulk form from Chiral Technologies. The CSP (1.5 kg) was packed into an 8 cm I.D. Prochrom (Indianapolis, IN, USA) dynamic axial compression column, and the final bed length was 53 cm. The mobile phase was HPLC-grade acetonitrile, and the flow-rate was 300 ml/min. The sample load was 2.0 g of racemate per injection.

3. Results and discussion

In our study of the closed-loop SSR separation of the racemic pharmaceutical intermediate, we used two CSP/solvent systems: (1) 10 μm ChiralPak AS, acetonitrile–methanol (90:10); and (2) 20 μm ChiralPak AS, acetonitrile. The capacity factors (k', using tri-*tert*.-butylbenzene as the void volume marker) and separation factors (α) on 10 μm ChiralPak AS were determined for the two solvents (column dimensions, 250×0.46 mm), and the results are shown in Table 1. Acetonitrile–methanol (90:10) is clearly the stronger solvent, a fact which will have significant influence on the closed-loop SSR production rates. In our SSR experiments, our goal was

Table 1
Separation factors measured on 10 μm ChiralPak AS

Solvent	Enantiomer 1 k'	Enantiomer 2 k'	α
Acetonitrile	0.56	0.94	1.7
Acetonitrile–methanol (90:10)	0.24	0.48	2.0

to equal or exceed the production rate previously obtained with SMB (see Table 3) while achieving a purity ≥98% e.e. for both enantiomers.

3.1. Closed-loop SSR Separation on 10 μm ChiralPak AS

For these experiments, the CSP was 10 μm ChiralPak AS, the column dimensions were 250×20 mm I.D., and the mobile phase was acetonitrile–methanol (90:10). The sample solution concentration was 12 mg of racemate/ml, and a sample volume of 5.0 ml, i.e., 60 mg of racemate, was injected through the injection valve each cycle.

Using the methods development technique described previously [11], we obtained the SSR conditions shown in Table 2 used in the initial run. Fig. 2 shows a steady state UV chromatogram (cycle 20) of this initial run. The injection point and the cut points for the waste 1, fraction 1, fraction 2 and waste 2 fractions occurred at the indicated times. For all SSR runs discussed in this paper, waste 1 and waste 2 were collected separately and were treated as very dilute fractions of enantiomer 1 and enantiomer 2, respectively. Superimposed on the UV chromatogram in Fig. 2 is a graph we shall call the profile analysis. The profile analysis was produced by slicing cycle 21 into equal fractions taken every 10 s. These fractions were analyzed by HPLC as described in Section 2.3, and the integrated absorbances were measured for each enantiomer. Note that the profile

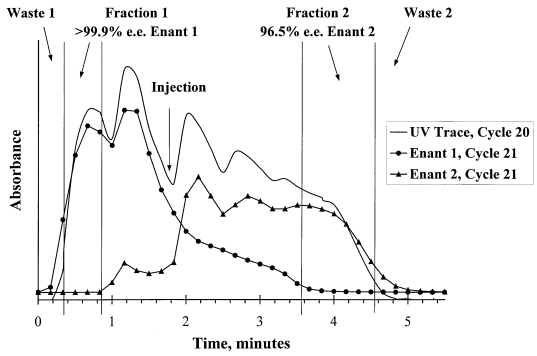

Fig. 2. Steady state chromatogram and profile analysis for first SSR experiment on 10 μm ChiralPak AS. Column dimensions, 250×20 mm I.D.; mobile phase, acetonitrile–MeOH (90:10); flow-rate, 16.9 ml/min; cycle time, 5.5 min; injection, 60 mg racemate/cycle. See Table 2 for further operating parameters.

Table 2
Conditions and event times for the closed-loop SSR processes shown in Figs. 2, 3, 5, 7 and 9

		CSP: 10 μm ChiralPak AS Mobile phase: acetonitrile–methanol (90:10) Flow-rate: 16.9 ml/min Cycle time: 5.5 min						CSP: 20 μm ChiralPak AS Mobile phase: acetonitrile F.R.[a]: 21.2 ml/min C.T.[b]: 6.0 min		CSP: 20 μm ChiralPak AS Mobile phase: acetonitrile F.R.[a]: 21.4 ml/min C.T.[b]: 5.8 min	
		Initial run, Fig. 2 Time (min)		Second run, Fig. 3 Time (min)		Final run, Fig. 5 Time (min)		Initial run, Fig. 7 Time (min)		Final run, Fig. 9 Time (min)	
Event	Description	Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative
1	Inject valve to load position, valve W opened	0.0	–	0.0	–	0.0	–	0.0	–	0.0	–
2	Load injection loop	0.0	–	0.0	–	0.0	–	0.0	–	0.0	–
3	Collection of fraction 1, valve F1 opened	0.35	0.0	0.35	0.0	0.43	0.0	0.23	0.0	0.27	0.0
4	End of fraction 1	0.85	0.5	0.85	0.5	1.03	0.6	0.73	0.5	0.87	0.6
5	Injection of sample	1.75	1.4	1.45	1.1	1.53	1.1	1.33	1.1	1.87	1.6
6	Collection of fraction 2, valve F2 opened	3.85[c]	3.5[c]	3.85[c]	3.5[c]	4.03[c]	3.6[c]	4.43[c]	4.2[c]	4.27[c]	4.0[c]
7	Collection of waste 2, valve W2 opened	4.85[c]	4.5[c]	4.85[c]	4.5[c]	5.03[c]	4.6[c]	5.63[c]	5.4[c]	5.47[c]	5.2[c]
8	Mobile phase pump switched off	5.35	5.0	5.35	5.0	5.43	5.0	5.83	5.6	5.67	5.4

[a] F.R. means flow-rate.

[b] C.T. means cycle time.

[c] These are the values entered into the TurboPrep control program. To determine the actual cut points of fraction 2 and waste 2 on the chromatograms in Figs. 2, 3 and 5, subtract 0.3 min. This correction is necessary because for these events, the volume of the injection loop (5.0 ml) separates the detector and the collection valves. At a flow-rate of 16.9 ml/min, this represents a lag time of 0.30 min between passage of the cut point through the detector and its arrival at the collection valve manifold. Similarly, for Fig. 7 (flow-rate, 21.2 ml/min) subtract 0.24 min, and for Fig. 9 (21.4 ml/min) subtract 0.23 min.

analysis plot is slightly broader than the UV chromatogram. This is because the UV chromatogram (cycle 20) measures essentially the instantaneous concentrations as the profile elutes from the column, whereas the profile analysis averages the concentrations over 10 s. Nevertheless, superimposing the two plots gives us much qualitative insight into the SSR separation at steady state.

The concentration of enantiomer 2 goes to zero at the boundary of fraction 1 near 0.85 min; thus, the purity of enantiomer 1 is very high (>99.9% e.e.). The concentration of enantiomer 1 does not go to zero before it reaches the boundary of fraction 2 (ca. 3.55 min); therefore, the purity of enantiomer 2 is only 96.5% e.e.

Note also in Fig. 2 the ledge of enantiomer 2 concentration that extends from about 1.0 to 1.8 min. The concentration of enantiomer 1 is high in this region, and the ledge appears to be due to the ''tag along effect'', first described by Guiochon et. al., for overloaded preparative chromatography [12]. In this region of high enantiomer 1 concentration, almost all of the adsorption sites are occupied by enantiomer 1 molecules. The molecules of enantiomer 2, not finding many adsorption sites, are forced to remain in the mobile phase and to ''tag along'' to lower retention times with the enantiomer 1 profile.

In SMB, the distribution of components and the structure of the chromatographic profile has been called the steady state inventory [13], and we shall follow that convention in discussing the SSR process. As will be shown later, ''inventory control'' is key to obtaining the desired purities in each fraction, and the ''tag along'' ledge has a role to play in the structure of the steady state inventory.

In an attempt to improve the purity of enantiomer 2, the injection point was moved 0.3 min to the left (toward fraction 1) to 1.1 min after the start of fraction 1. In SMB this would be equivalent to increasing the size of zone II and decreasing the size of zone III. (In SMB the zone between the extract and feed ports is usually designated zone II, and the zone between the feed and the raffinate ports is zone III [14]. In SSR fraction 1 is equivalent to the raffinate; fraction 2, the extract). All other parameters remained unchanged (see Table 2, 10 μm, second run). We hypothesized that by providing more sorbent between the injection point and fraction 2,

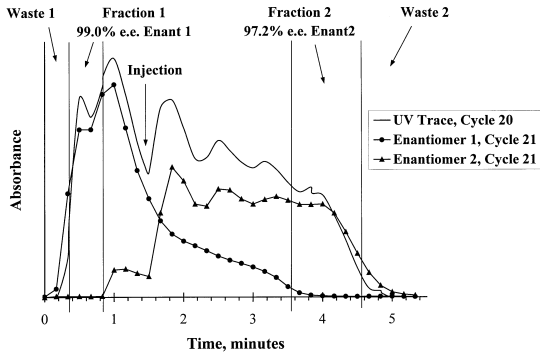

Fig. 3. Steady state chromatogram and profile analysis for second SSR experiment on 10 μm ChiralPak AS. Column dimensions, 250×20 mm I.D.; mobile phase, acetonitrile–MeOH (90:10); flow-rate, 16.9 ml/min; cycle time, 5.5 min; injection, 60 mg racemate/cycle. See Table 2 for further operating parameters.

resolution would increase in this region, thus decreasing the amount of enantiomer 1 bleeding into fraction 2.

The resulting steady state UV chromatogram (cycle 20) and profile analysis (cycle 21) are shown in Fig. 3. The purity of enantiomer 1 has decreased somewhat, but at 99.0% e.e. it still exceeds our minimum requirement of 98% e.e. The purity of enantiomer 2 has increased somewhat to 97.2% e.e. but is still below our specified purity limit.

Fig. 4 compares the profile analysis data for the initial and second experiments. For the second run the profile of enantiomer 1 appears to be sharper, and in the region of fraction 1 it appears to be more concentrated. However, the transition of the enantiomer 2 concentration from the tag along ledge to zero concentration at the boundary of fraction 1 appears

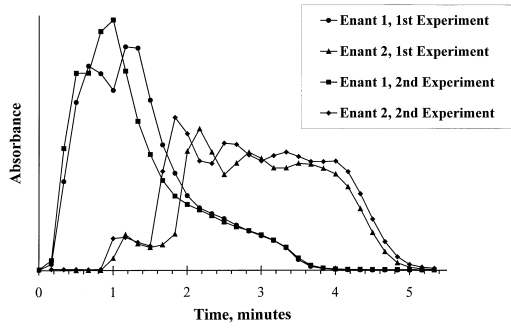

Fig. 4. Comparison of steady state profile analyses for first and second SSR experiments on 10 μm ChiralPak AS.

to be more abrupt in the second experiment. Thus, the concentration of enantiomer 2 is higher at the fraction 1 boundary, and this is probably the cause of the slight decrease in purity of enantiomer 1.

The enantiomer 1 profiles for the two experiments converge at about 2.8 min, and are virtually identical in the vicinity of fraction 2. Therefore, the attempt to increase resolution in this area was not effective and cannot explain the increase in purity of enantiomer 2. Rather, the concentration of enantiomer 2 has increased in the region of fraction 2, and this has caused the increase in purity. To explain this increase in enantiomer 2 concentration, note that the tag along ledge is shorter in the second experiment, extending only from about 0.8 to 1.5 min. Therefore, because less enantiomer 2 was diverted to the tag along ledge, more was available to increase the concentration at later times (including fraction 2). In essence, the enantiomer 2 inventory was redistributed in the second experiment, from the tag along ledge to regions of higher retention times (ca. 3.0–5.5 min).

It now appeared that the major cause of enantiomer 1 contamination in fraction 2 was that the steady state inventory of enantiomer 1 was too large. In both previous experiments an appreciable tail of enantiomer 1 extended into fraction 2. Therefore, the logical next step was to decrease the steady state inventory of enantiomer 1. Accordingly, the size of fraction 1 was increased to 0.6 min from 0.5 min (Table 2, 10 μm, final run), which decreased the build-up of enantiomer 1 in the early cycles as the separation was evolving toward steady state. In SMB, this would be equivalent to increasing the raffinate flow-rate.

Fig. 5 shows the resulting steady state chromatogram (cycle 20) and profile analysis (cycle 21). The strategy appears to have been successful: the purities of both enantiomers (enantiomer 1, 99.8% e.e.; and enantiomer 2, 99.5% e.e.) now exceed our minimum requirements of 98% e.e. The reasons for this improvement in purities are explained in Fig. 6, the profile analysis plots of the second and final experiments. The steady state inventory of enantiomer 1 has been substantially reduced in the final experiment, which has resulted in less enantiomer 1 bleeding into fraction 2. Also the concentration of enantiomer 2 at later times (including in the region

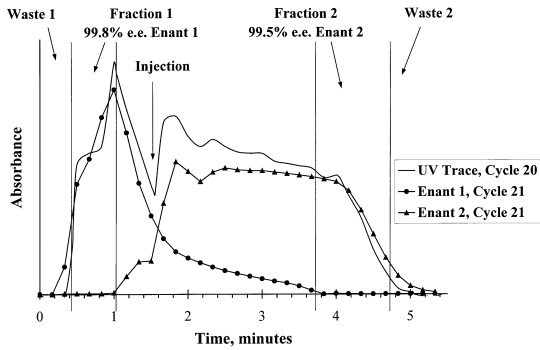

Fig. 5. Steady state chromatogram and profile analysis for final SSR experiment on 10 μm ChiralPak AS. Column dimensions, 250×20 mm I.D.; mobile phase, acetonitrile–MeOH (90:10); flow-rate, 16.9 ml/min; cycle time, 5.5 min; injection, 60 mg racemate/cycle. See Table 2 for further operating parameters.

of fraction 2) has increased significantly in the final experiment. As with the second experiment, this increase at later retention times of enantiomer 2 appears to be due to the decrease in size of the tag along ledge, which resulted in a redistribution of the enantiomer 2 inventory. However, in the final experiment the smaller tag along ledge was caused by the increase in size of fraction 1 and not by a shift in the injection point. These two factors, less enantiomer 1 contamination and higher concentration of enantiomer 2, has resulted in the substantial increase in enantiomer 2 purity observed in fraction 2.

Note also that the purity of enantiomer 1 has increased in the final experiment relative to the second experiment, even though the steady state concentration of enantiomer 1 has decreased. This could happen only if the contamination of enantio-

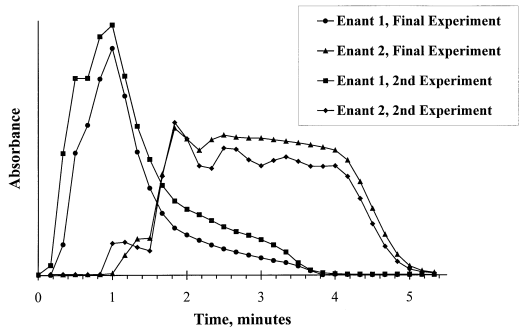

Fig. 6. Comparison of steady state profile analyses for second and final SSR experiments on 10 μm ChiralPak AS.

mer 2 in fraction 1 had decreased significantly. It appears that in the final experiment the region between the injection point and fraction 1 is much less overloaded in enantiomer 1. This has resulted in a shorter tag along ledge and a less abrupt transition in enantiomer 2 concentration between the ledge and the boundary of fraction 1. Thus the concentration of enantiomer 2 is lower at the boundary of fraction 1, and this has resulted in less enantiomer 2 contamination of fraction 1.

The production rate, solvent usage, purity, and recovery results for the final experiment (Fig. 5) are shown in Table 3. Discussion of these results will be deferred to a later section.

3.2. Closed-loop SSR separation on 20 μm ChiralPak AS

The next set of experiments was undertaken primarily to compare the performance of the closed-loop SSR process to that of SMB. However, as we shall see, in the process of developing the SSR method, we also gained some fundamental knowledge about the SSR process under overloaded conditions.

Our approach here was to approximate with SSR the production rate and purities obtained earlier with SMB and to compare the solvent usage and recoveries for the two techniques. The racemic pharmaceutical intermediate had previously been separated by SMB using 20 μm ChiralPak AS as the CSP and acetonitrile as the mobile phase. Accordingly, the SSR experiments discussed in this section used the same CSP and mobile phase. The SSR column, received prepacked from Chiral Technologies, had dimensions 250×20 mm I.D. The sample solution concentration was 10 mg of racemate/ml in acetonitrile. Once each cycle, 5.0 ml of the sample solution (50 mg of racemate) was injected through the injection valve. The mobile phase flow-rate was 21.2 ml/min, and the cycle time was 5.8 min. As with the previous series of experiments, the purity specifications called for each enantiomer to have an enantiomeric excess greater than 98%.

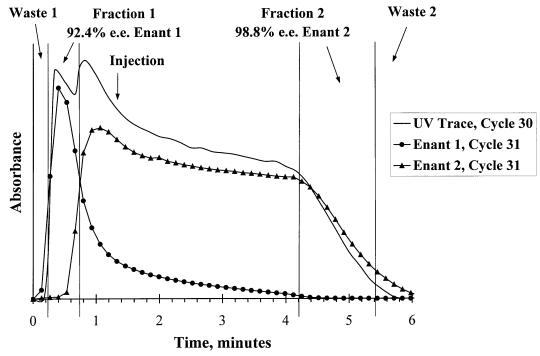

Fig. 7. Steady state chromatogram and profile analysis for first SSR experiment on 20 μm ChiralPak AS. Column dimensions, 250×20 mm I.D.; mobile phase, acetonitrile; flow-rate, 21.2 ml/min; cycle time, 6.0 min; injection, 50 mg racemate/cycle. See Table 2 for further operating parameters.

The initial event times are shown in Table 2 (20 μm, initial run) and were again determined using the methods development technique reported previously [11]. Fig. 7 shows a steady state UV chromatogram (cycle 30) and profile analysis (cycle 31) for the initial run. The purity of enantiomer 1 (92.4% e.e.) is significantly lower than our specification; however,

Table 3
Summary of SSR, SMB and HPLC results

Technique	Solvent	Particle size (μm)	Figure	Production rate (g racemate/kg CSP/day)	Solvent usage (l solvent/g racemate)	Purities (% e.e)		Recoveries (%)	
						Enantiomer 1	Enantiomer 2	Enantiomer 1	Enantiomer 2
SSR	Acetonitrile–MeOH (90:10)	10	Fig. 5	334	0.77	>99	>99	99	99
SSR	Acetonitrile	20	Fig. 9	255	1.07	>98	>98	99	99
SMB	Acetonitrile	20	–	240	0.85	>98	>98	99	99
HPLC	Acetonitrile	10	–	327	2.0	>98	>98	93	93
HPLC	Acetonitrile	20	–	192	1.5	> 98	>98	98	93

the purity of enantiomer 2 (98.8% e.e.) has exceeded our requirement. As seen in Fig. 7, the inventory of enantiomer 2 has built up to a high level near 1.1 min, and a significant amount of enantiomer 2 has spilled over into fraction 1.

The reason for this build-up in enantiomer 2 inventory is shown in Fig. 8, where the chromatograms of cycles 2–7 are plotted together. The second maximum in the chromatograms is due to enantiomer 2 (as can be verified by comparison to the profile analysis shown in Fig. 7). The second maximum increases in height and moves to shorter retention times with each succeeding cycle. This behavior is indicative of enantiomer 2 overloading in this region of the profile. (This region, between the injection point and the fraction 1 boundary, is equivalent to Zone III in SMB.) With each succeeding cycle, more and more adsorption sites are occupied by enantiomer 2 molecules, forcing an increasing proportion of the enantiomer 2 molecules to remain in the mobile phase. This causes the second maximum in Fig. 8 to shift to lower retention times as the enantiomer 2 molecules seek adsorption sites, and eventually a significant quantity of enantiomer 2 spills over into fraction 1.

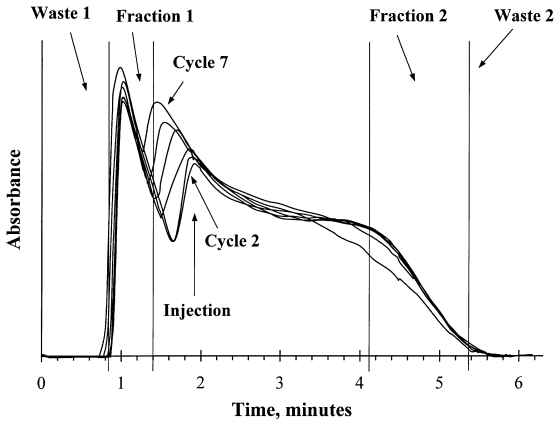

Fig. 8. Chromatograms of cycles 2–7 for the first SSR experiment on 20 μm ChiralPak AS, showing the evolution toward steady state in which the enantiomer 2 inventory is increasing and shifting to lower retention times. Note that these are early cycles in the method development process, and that the waste 1 fraction is larger than in Fig. 7. In later cycles, the distance between the injection point and fraction 2 (SMB zone II) was increased, which expanded the length of the chromatographic profile and decreased the size of the waste 1 fraction.

A means must be found, therefore, to prevent enantiomer 2 overloading in the region between the injection point and fraction 1. One way would be to decrease the quantity of racemate injected, but this would significantly lower the production rate. Another way would be to increase the number of adsorption sites in this region by moving the injection point to the right to a higher retention time. This would be equivalent in SMB to increasing the size of zone III and decreasing the size of zone II.

The injection point was therefore moved 0.5 min to the right to 1.6 min. In addition, the size of fraction 1 was increased to 0.6 min from 0.5 min; this is equivalent to increasing the raffinate flow-rate in SMB and was done to ensure that the inventory of enantiomer 1 would not build up and bleed into fraction 2 (see Table 2, 20 μm, final run). Fig. 9 shows the steady state UV chromatogram (cycle 20) and the profile analysis (cycle 21). The steady state purities of both enantiomers (enantiomer 1, 99.2% e.e.; and enantiomer 2, 99.6% e.e.) now exceed our minimum requirements of 98% e.e. Most of the enantiomer 2 profile is well away from fraction 1. There is a small tag along ledge of enantiomer 2 extending from about 1.0 to 1.7 min, indicating some enantiomer 1 overloading in this region. The concentration of enantiomer 2, however, goes to zero at the boundary of fraction 1, which results in the high purity of enantiomer 1. Also, the concentration of the enantiomer 1 profile reaches zero at the boundary of fraction 2, giving the high purity of enantiomer 2.

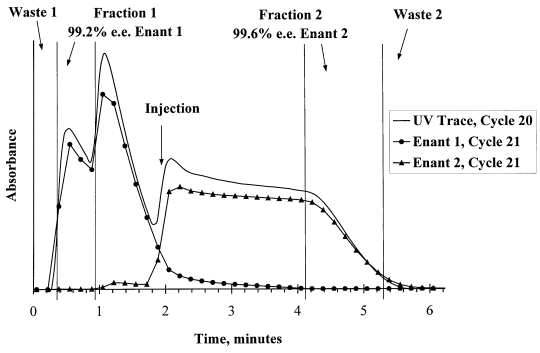

Fig. 9. Steady state chromatogram and profile analysis for final SSR experiment on 20 μm ChiralPak AS. Column dimensions, 250×20 mm I.D.; mobile phase, acetonitrile; flow-rate, 21.4 ml/min; cycle time, 5.8 min; injection, 50 mg racemate/cycle. See Table 2 for further operating parameters.

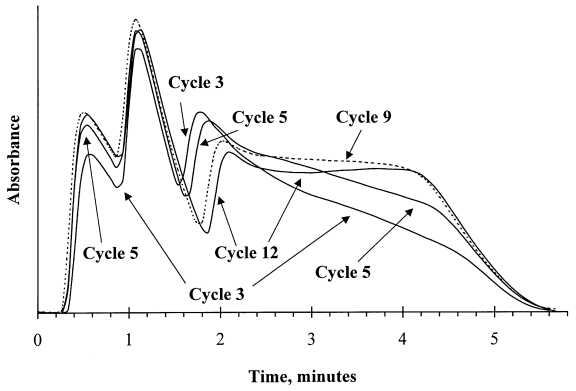

Fig. 10. Chromatograms of cycles 3, 5, 9 and 12 for final SSR experiment on 20 μm ChiralPak AS, evolution toward steady state. Conditions same as in Fig. 9.

Fig. 10 shows the chromatograms of cycles 3, 5, 9 and 12 plotted together. Note that in the range of 0.3 to 1.4 min, the concentration of enantiomer 1 increases each cycle, and in the range of 4.0 to 4.4 min, the concentration of enantiomer 2 increases each cycle. This is a common observation: as the separation evolves toward steady state, the concentration of each fraction increases until the quantity of each component collected is equal to the quantity injected. However, observe the behavior of the evolving chromatogram in the region between 1.5 and 2.0 min, a region that the profile analysis in Fig. 9 suggests should be enriched in enantiomer 2. The concentration of enantiomer 2 appears to be decreasing in this region. A possible explanation for this observation is the following. As the enantiomer 1 concentration in the range of 1.0 to 1.8 min increases, this region becomes overloaded in enantiomer 1 and a small enantiomer 2 tag along ledge is induced. The size of the tag along ledge grows each cycle, which causes the concentration of enantiomer 2 at later retention times to decrease. In other words, the enantiomer 2 inventory is redistributed to enhance the tag along ledge.

At first glance, it appears that the height of the tag along ledge at steady state (Fig. 9) is too low to account for the rather larger changes in concentration of the enantiomer 2 profile in the range of 1.5 to 2.0 min seen during the evolution to steady state (Fig. 10). In order for such an effect to occur, a small change in the size of the tag along ledge should have a large effect on the concentration of the enantiomer 2 profile at higher retention times. To test this hypothesis, the size of fraction 1 was increased to 0.7 min in order to decrease the length of the tag along ledge and also to decrease the steady state inventory of enantiomer 1, thus decreasing the overloading of enantiomer 1. Fig. 11 shows the chromatograms of some of the early cycles that resulted from these changes. The similarity to Fig. 8 is striking. Clearly, the region between the injection point and fraction 1 is becoming overloaded in enantiomer 2, and the eventual contamination of fraction 1 seems inevitable (unfortunately, the run was aborted when the overloading of enantiomer 2 became apparent; neither the profile analysis nor the purity of fraction 1 was measured). It appears that in Figs. 9 and 10, the overloading of enantiomer 1 in the region from 1.0 to 1.4 min acts a barrier to enantiomer 2. The overloading of enantiomer 1 in this region induces the tag along ledge to form, which ''siphons off'' enantiomer 2 from the highly concentrated front edge of the enantiomer 2 profile. This in turn prevents the overloading of enantiomer 2 and its subsequent contamination of fraction 1. This effect, which could be called the ''tag along siphon'', is still an hypothesis at this point. We will investigate it further experimentally in future work, but much insight could be gained into this effect (if it exists) through computer modeling.

3.3. The importance of inventory control

We have seen that ''inventory control'' is crucial

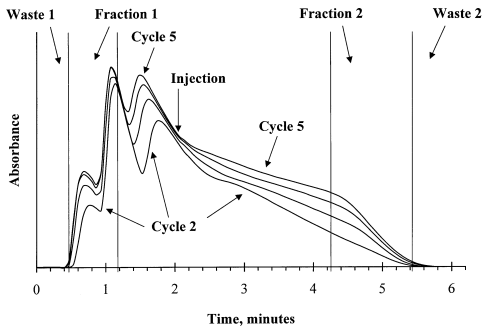

Fig. 11. Effect of increasing size of fraction 1. Conditions as in Figs. 9 and 10 except size of fraction 1 was increased to 0.7 min from 0.6 min.

to maximizing purities in SSR. If the region of the chromatographic profile between the injection point and fraction 1 (equivalent to zone III in SMB) becomes overloaded in the more retained component (enantiomer 2), the paucity of adsorption sites will cause a large proportion of all species to remain in the mobile phase and to ''rush forward'' to lower retention times. If enough adsorption sites are found downstream, the chromatographic profile will stabilize and a steady state will develop in which enantiomer 2 has not contaminated fraction 1. If enough adsorption sites are not found, the steady state which develops will result in a significant portion of enantiomer 2 being collected in fraction 1.

If the region between the injection point and fraction 1 becomes overloaded in the less retained component (enantiomer 1), a tag along ledge of enantiomer 2 concentration will form, provided that enantiomer 2 itself is not overloaded in this region. If the tag along ledge is reduced in size, either by moving the injection point to the left (equivalent in SMB to decreasing the size of zone III and increasing the size of zone II) or by increasing the size of fraction 1 (equivalent in SMB to increasing the raffinate flow-rate), the concentration of enantiomer 2 will increase at later retention times in the profile through a redistribution mechanism. Conversely, if the tag along ledge is increased in size, the concentration of enantiomer 2 at later retention times in the profile will decrease. In some cases, this ''tag along siphon'' effect appears to be able to prevent the overloading of enantiomer 2 in the crucial region between the injection point and fraction 1, and thus to create a steady state in which fraction 1 is not contaminated with enantiomer 2.

In addition to the tag along ledge, another effect will undoubtedly occur if the region between the injection point and fraction 1 becomes overloaded in enantiomer 1: a large portion of the enantiomer 1 molecules, not finding sufficient adsorption sites, will rush forward to lower retention times and will eventually be collected in fraction 1. However, this is not a problem because this is the correct fraction for enantiomer 1.

A third effect can develop if the steady state inventory of enantiomer 1 is allowed to become too large. The enantiomer 1 profile tails to higher retention times through a process that appears to be related to peak broadening in batch mode chromatography. If the inventory of enantiomer 1 is too large, the concentration of enantiomer 1 in the tail in the region of fraction 2 can be appreciable, resulting in contamination of fraction 2. This effect is not a result of overloading per se: overloading projects higher concentrations to lower retention times by forcing large amounts of sample into the mobile phase. The only way to project concentration to higher retention times is through the ordinary stochastic processes that cause band broadening. The higher the enantiomer 1 inventory at steady state, the more concentrated will be its tail at higher retention times and the more likely it will contaminate fraction 2. Of course, if enantiomer 1 is the desired product, some contamination of fraction 2 can be tolerated if a higher load of enantiomer 1 results in a higher production rate with acceptable purity and recovery.

In general, then, it appears that overloading of enantiomer 1 at steady state is not a problem, unless the steady state inventory of enantiomer 1 is so large that tailing into fraction 2 becomes unacceptable. In fact, overloading of enantiomer 1 can be advantageous if, through the ''tag along siphon'' effect, enantiomer 2 is kept from being overloaded. On the other hand, steady state overloading of enantiomer 2 will always cause contamination of fraction 1. If the desired product is enantiomer 2, this might not be a problem. Overloading of enantiomer 2 could result in a high production rate of highly pure enantiomer 2, albeit at the expense of a lower recovery of enantiomer 2 due to its contamination of fraction 1. As with other forms of chromatography, including SMB, the trade off in this case would be between throughput and recovery.

3.4. Comparison of SMB and SSR results

The production rate, solvent usage, purity and recovery results for both SSR methods (Figs. 5 and 9) are shown in Table 3. Also shown are the results of the SMB and preparative HPLC experiments that were obtained previously and whose operating conditions are given in the Section 2.3.

The SSR results compare quite well with those of the other techniques shown in Table 3. The production rate for the first SSR method was 334 g racemate/kg CSP/day, and is higher than the pro-

duction rates of any of the other procedures reported in Table 3. It should be emphasized that neither the SSR nor the SMB nor the HPLC procedures were fully optimized as to production rate. Nevertheless, we are confident that an optimized SSR method using acetonitrile–methanol (90:10) would have a higher production rate than one using 100% acetonitrile as the mobile phase. The acetonitrile–methanol mixture is a stronger solvent for this separation (see Table 1), which implies that a higher inventory of each enantiomer can be tolerated before the column becomes overloaded. This in turn implies that more racemate can be injected each cycle which should result in a higher production rate.

However, because none of the production rates were optimized and because all the purities obtained with each method were greater than our target of 98% e.e., the most meaningful results to compare for the various techniques are solvent usage and recoveries.

Qualitatively, we see (Table 3) that the SSR and SMB procedures had lower solvent usages than the HPLC methods. This is probably due to the large amount of solvent sent to waste in the HPLC procedures. In SMB, there are no waste streams per se; there are only the two product streams (the raffinate and extract). Thus the only solvent that leaves the SMB system is that needed to solvate the purified products in the raffinate and extract. In addition, the SMB product streams are often more concentrated than the HPLC fractions for a given separation [15]. Taken together (concentrated product streams and no waste streams), SMB will usually have better solvent usage performance than batch chromatographic techniques.

We note, however, that the first SSR method listed in Table 3 had the lowest solvent usage, 0.77 l solvent/g racemate. The main reason for this is that this method used a stronger solvent, as evidenced by the lower capacity factors (k') shown in Table 1. Note in Table 2 that although the first SSR method (10 μm, final run) had a lower flow-rate (16.9 ml/min) than the second SSR method (20 μm, final run, 21.4 ml/min), the first SSR method had a lower cycle time (5.5 min) than the second SSR method (5.8 min). This is because with the lower k' values, a higher proportion of the sample molecules are in the mobile phase at equilibrium, which results in a lower cycle time for a given flow-rate. It turns out that the total amount of time the collection valves (waste 1+fraction 1+fraction 2+waste 2) were open was approximately equal for the two SSR methods listed in Table 3 (2.4 min for the first SSR method; 2.3 min for the second SSR method). Therefore, with its higher flow-rate, the second SSR method used more solvent.

The most interesting comparison of solvent usage is between the second SSR method listed in Table 3 and the SMB method, both of which used acetonitrile as the mobile phase and 20 μm ChiralPak AS as the CSP. As stated earlier, an objective in developing this SSR method was to duplicate as nearly as possible the SMB production rate and enantiomeric purities. As seen in Table 3, this goal was achieved, and we can therefore make a valid comparison of solvent usage between the two techniques. At 0.85 l solvent/g racemate, SMB had a lower solvent usage than SSR (1.07 l solvent/g racemate). The reason for this appears to be the two waste fractions used with the SSR method (see Fig. 9). As mentioned before, SMB has no waste streams. The size of the SSR waste fractions could be minimized by expanding the length of the chromatographic profile and by recycling pure mobile phase instead of sending it to waste. For example, a detailed examination of the SSR data suggests that about 5.0 ml of the mobile phase could have been recycled before opening the waste 1 collection valve. If this had been done, the SSR solvent usage would have dropped to 0.97 l solvent/g racemate – better, but still higher than the SMB solvent usage. From our results here, therefore, it appears that for a given solvent/sorbent combination and a given production rate, SMB will have the lowest solvent usage followed by SSR followed by HPLC.

The recoveries of both enantiomers were 99% for the SMB method and for both SSR methods. The recoveries are high because SMB and SSR are steady state processes: for each cycle, everything that is injected is collected. In a two-component sample, if each fraction is highly pure, then the recovery of each component will be close to 100%. It should be pointed out that the SSR recoveries reported in Table 3 include the amounts of enantiomer 1 and enantiomer 2 collected in waste 1 and waste 2, respectively, as well as the amounts collected in fraction 1 and

fraction 2. This is justified, because as explained earlier, waste 1 and waste 2 were collected as separate very dilute fractions, and were not discarded.

4. Conclusions

Inventory control, i.e., maintenance of the desired chromatographic profile at steady state, is crucial to obtaining the desired purities in SSR. Because of the similarities between the two techniques, we may infer that inventory control is also crucial to SMB purities. In SSR we control the steady state inventory by adjusting the following parameters: the size and location of fractions, the location of the injection point, and the amount of sample injected (by adjusting the sample concentration and/or the size of the injection loop). In SMB, analogous parameters are adjusted: the raffinate and extract flow-rates, the sizes of zones II and III, and the amount of feed injected (by adjusting the feed concentration and/or the feed flow-rate).

For a given separation, SSR and SMB will have similar production rates, purities and recoveries. The solvent usage appears to be greater for SSR than for SMB. However, by optimizing all fraction sizes (including the waste fractions) and by recycling pure solvent where possible, the SSR solvent usage can be improved. It also appears from this study that the SSR process has lower solvent usage than preparative and process-scale HPLC. Thus in general, it appears that the three techniques can be arranged in the following order of solvent usage: HPLC>SSR> SMB.

Given the lower capital costs of SSR compared to SMB, and given the similarities of the two processes, SSR should find applications in those chromatography laboratories performing small-to-moderate scale binary (e.g., chiral) separations. Also, because an SSR system can be operated in ordinary HPLC mode, SSR equipment should find applications in general purpose laboratories that are called upon to perform many different types of separations each year – binary as well as non-binary separations.

Future work, both experimental and theoretical, will be directed toward identifying and clarifying subtle mechanistic aspects of SSR (such as the importance of the ''tag along ledge'') and in ascertaining the scales of operation appropriate to SSR from an economic perspective.

Acknowledgements

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