Review

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Cyclic electrophoretic and chromatographic separation methods

A review is given of the application of cyclic analytical methods in capillary electroseparation (CE) and liquid chromatography (LC) systems. Cyclic methods have been used since the early sixties in chromatographic systems to overcome pressure limitations to resolution. From the early nineties on they have also been applied in capillary electroseparation systems to overcome voltage limitations. Some basic theory is given, outlining the temporal development of resolution in cyclic CE and LC systems and calculating the maximal resolution that can be obtained as a function of the operational parameters of pressure and electrical field. Simple equations are given for the temporal change in the peak capacity and the loss of peaks from the systems as it occurs in some cyclic systems. Finally, a circular open tubular chromatographic system is proposed using integrated pumping and continuous detection. The performance of such a system is discussed using magnetohydrodynamic and alternating current electroosmotic pumping as examples of integrated pumps and Shah Convolution Fourier transform detection as an example of a continuous detection method.

Keywords: Cyclic chromatography / Electrophoretron / Fourier transform / Lab on a chip / Miniaturization / Review / Synchronized cyclic capillary electrophoresis DOI 10.1002/elps.200305750

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Abbreviations: DPRC, direct-pumping recycling chromatography; **HV**, high voltage; **OTLC**, open-tubular liquid chromatography; **SCCE**, synchronized cyclic capillary electrophoresis

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1 Introduction

In an address given in August 1957, Martin [1] suggested an intriguing way of tackling difficult separations in gas chromatography (GC). Instead of manufacturing as he put it ‘a column a quarter of a mile long’, he proposed to use two much shorter columns in an alternating fashion. The chromatographer would drive the substances consecutively through one and then the other by repeated column switching, connecting the end of column 1 to the entrance of column 2 and *vice versa.* A column of, in principle, infinite length would be simulated in this way. In the years following Martin’s address, this idea was taken up in different ways and in several separation methods. Almost certainly it was sometimes also reinvented by researchers not aware of Martin’s original address. In GC it never became very popular, but instead it was mainly employed in analytical and preparative liquid chromatography (LC) and to a lesser extent in analytical capillary electroseparation (CE) methods. Recycling chromatography, synchronized cyclic CE (SCCE), synchronous cyclic CE and electrophoretron are names that appear in literature for the different cyclic systems developed. The main aim of this paper is to review the literature that has appeared on the

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methods mentioned above, lumping them together under the name of cyclic separation methods. We will confine ourselves hereby to analytical cyclic systems, omitting the preparative (nonlinear) ones. Apart from offering a review, a second important aim of this paper will be to construct a simple theoretical framework to interpret and compare the different efforts. This theory can be used to compare the different methods, but also to describe a chip-based analytical recycling system, based on the theoretical limits derived. Such a system will finally be discussed with particular attention to the subject of detection, showing that a continuous detection method combined with simple chemometrics can significantly enhance the usefulness of cyclic separation methods.

In our discussion of the merits of the different recycling methods we will characterize them concerning analysis speed, resolving power and peak capacity. Practical circumstances generally dictate the relative importance of these three. For difficult separations, *e.g.*, of chiral compounds, resolving power will be of prime importance. In complex mixtures with many substances of interest, it will rather be a combination of peak capacity and resolving power. In routine analyses on the other hand, analysis time can be the prime parameter to optimize. In this paper, all three parameters will be evaluated for the different cyclic methods described. The parameters will be expressed as a function of time instead of separation length as customary. This choice is motivated by the fact that in cyclic methods we are free to terminate the analysis once the object has been achieved. Our theoretical analysis will, if necessary, in addition consider the peak loss (the total disappearance of analyte peaks from the system) as a function of time, which occurs in some of the methods described.

2 Capillary electroseparation methods

In the last two decades, capillary electroseparation methods have become increasingly popular due to their inherent speed of analysis, the high separation efficiencies obtainable, and the ease of quantification and automation. An additional advantage of these methods is that they are inherently orthogonal to HPLC. However, in spite of the high separation speed, some analytes with very similar electrophoretic mobilities can still remain unresolved due to limits posed by Joule heating and limits to the maximal applicable voltage (the maximum voltage applied under special precautions has been 120 kV [2]). To address this problem, a number of solutions have been offered in the course of time, always aimed at increasing the analysis time. As early as in 1937, hydrodynamic flow-counterbalanced electrophoresis was pioneered by Tiselius [3, 4]. Later, it was applied to CE by Culbertson and Jorgenson [5] and Dasgupta and Liu [6]. Another approach to increase analysis time has been to modify EOF by applying a radial electrical field over the capillary wall [7–10]. In this context it is interesting to note that it has been shown on chip that very low radial electrical fields (0–50 V) can be used to vary the EOF over a wide range [11]. Using this method to influence the EOF to a different magnitude in a number of parallel channels, it would in principle be possible to keep different analytes stationary in different parallel channels, obtaining very high resolutions for each of them simultaneously. A system for continuous detection would be needed for feedback to the radial field such that the analytes do not “escape” from the separation region.

Quite a different way to increase the analysis time is to use the continuous column extension as suggested by Martin [1] and cited in the introduction of this paper. There are two fundamentally different approaches for this, both of which are schematically illustrated in Fig. 1. The left hand side of Fig. 1 shows the “electrophoretron” as developed by Choi *et al.* [12], where two capillaries, respectively, with negative and positive surface charge are joined by porous sleeves. The sleeves offer high resistance to hydrodynamic flow but allow current to pass and are submerged into buffer solutions for high-voltage (HV) application. On application of HV the EOF in both branches will have the same direction due to the different surface charge. Though an analyte will move in opposite directions in the two branches, it still moves around the circle when its mobility is smaller than the electrophoretic mobility, thus allowing a continuous separation.

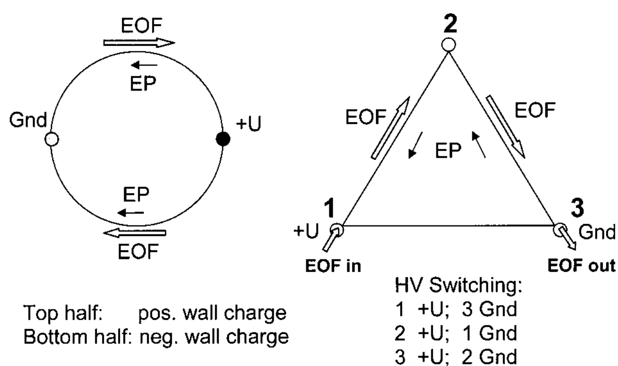

Figure 1. Schematic representation of two cyclic methods: the “electrophoretron” (left) and SCCE (right). The electrophoretron employs two capillary branches with opposite surface charge to generate circular EOF. In SCCE the voltage application points are switched in a circular fashion around a polygonal channel structure. Note that only one possible direction of electrophoretic analyte movement (EP) is indicated.

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The second method for column extension is SCCE [13], schematically shown at the right hand side of Fig. 1. In this method, the HV application points are continuously switched around a polygonal closed channel structure, at a frequency determined by the substances to be analyzed. A minimum of three application points is therefore necessary (Fig. 1), though four points is the most commonly used. This method is akin to column switching in LC [14].

2.1 Theoretical limits

2.1.1 Resolution

The following equations describe the resolution obtained in CE and SCCE [15],

$$R_S = E\sqrt{t}\frac{\mu_1 - \mu_2}{4\sqrt{2D}} \tag{1}$$

$$R_S = \sqrt{\frac{V}{\mu_2 + \mu_{eo}}}\frac{\mu_1 - \mu_2}{4\sqrt{2D}} \tag{2}$$

where E is the applied electrical field (V/m), t is the time (s), μ_i is the electrophoretic mobility of ionic species i, μ_{eo} is the electroosmotic mobility ($m^2/V \cdot s$), D is the analyte diffusion coefficient (m^2/s) (assumed equal for species 1 and 2), and V is the applied potential difference (V). Equation (2) is useful to show that conventional systems have intrinsically limited resolution because the applicable voltage is limited, and because the EOF decreases the available separation time. The time-dependent Eq. (1) is most useful for SCCE, since with this method the separation can be terminated when sufficient resolution is reached. The increase of resolution with the square root of time in Eq. (1) is a result of the proportionality of the peak separation to time and of the peak broadening to the square root of time. The conceptual advantage of SCCE can be read from this equation, since SCCE aims at applying a high field E for a long time t, by successive application of a relatively low voltage over a limited length of column. When Eq. (2) is used for SCCE, an (imaginary) cumulative voltage will result, determining the resolution.

The development of resolution in the electrophoretron (Fig. 1, left hand side) is slower than in SCCE, because the resolution obtained in one branch is diminished in the other. Analytes are only resolved because they spend unequal times in both branches. It can be calculated that for the electrophoretron (averaging the velocities and for μ_1, $\mu_2 < \mu_{eo}$)

$$R_S = E\sqrt{t}\frac{\mu_1^2 - \mu_2^2}{4\mu_{eo}\sqrt{2D}} \tag{3}$$

Interestingly, cations and anions with equal absolute mobilities will not be resolved in the electrophoretron. The reason for this is that during one complete cycle cations and anions undergo identical stages of moving against and with the EOF, albeit in opposite branches. A further limitation of the device is that analytes with mobilities higher than the electroosmotic mobility cannot be separated but end up in the cathodic well.

2.1.2 Maximum resolution

A second parameter of interest is the maximum obtainable resolution. This provides the theoretical limit of the method, even though gradual loss of analyte will almost always prevent this limit to be reached. Maximum resolution is reached when $t = L^2/(128D)$, at which moment two peaks totally fill the separation length L (m). In the electrophoretron L is the circle circumference, and in SCCE the distance between the points of HV application (*e.g.*, between points 1 and 3 in Fig. 1). Thus, the maximum obtainable resolution in SCCE will be

$$(R_S)_{max} = V\frac{\mu_1 - \mu_2}{64D} \tag{4}$$

while in the electrophoretron it will be

$$(R_S)_{max} = V\frac{\mu_1^2 - \mu_2^2}{32\mu_{eo}D} \tag{5}$$

where $EL = V$ was used for SCCE and $EL = 2V$ for the electrophoretron. The power of cyclic systems is clearly demonstrated by the dependence of the maximal resolution on the applied voltage, whilst in classical CE it depends on the square root of the voltage (*cf.* Eq. 2). Interestingly, the maximum resolution does not depend on the separation length but only on the applied voltage. This property derives from the higher field and faster separation when L is made smaller, compensating for the shorter available analysis time. The maximal resolution for SCCE and the electrophoretron in practice might be approximately equal for equal applied voltage V, since the slower generation of resolution in the electrophoretron is compensated for by the twice longer available separation length L.

2.1.3 Peak capacity

Another important parameter is the peak capacity n. We will define

$$n = \frac{L}{w} = \frac{L}{4\sqrt{2Dt}} \tag{6}$$

where w (m) is the baseline peak width. The peak capacity decreases with the square root of time, and is proportional to the separation length L. In both SCCE and the

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electrophoretron it is therefore advantageous for the peak capacity to use a large separation length. It should be noted that Eq. (6) is a best case approximation of the peak capacity in the systems mentioned since other peak broadening mechanisms than longitudinal diffusion will play a role (see Section 2.2 below).

2.1.4 Peak loss

A fundamental difference between the electrophoretron and SCCE is that all peaks are retained in the electrophoretron, while there is a continuous loss of compounds in SCCE. Neglecting the injection plug length, a length of $v_{range}t$ will be taken up by analyte peaks, where v_{range} is the range of velocities of all analytes. In SCCE, peak loss will occur as soon as $v_{range}t$ is larger than L. The fractional peak loss is described by

$$t > \frac{L}{v_{range}} : \frac{NP_0 - NP_t}{NP_0} = 1 - \frac{L}{v_{range}t} \quad (7)$$

where NP is the number of analyte peaks in the separation length L.

2.2 Examples

The feasibility of SCCE was first demonstrated by Burggraf *et al.* [13, 16] who made good use of the ease of sample manipulation on a planar glass chip device. The authors used a square channel geometry; Fig. 2 shows different steps during the separation process.

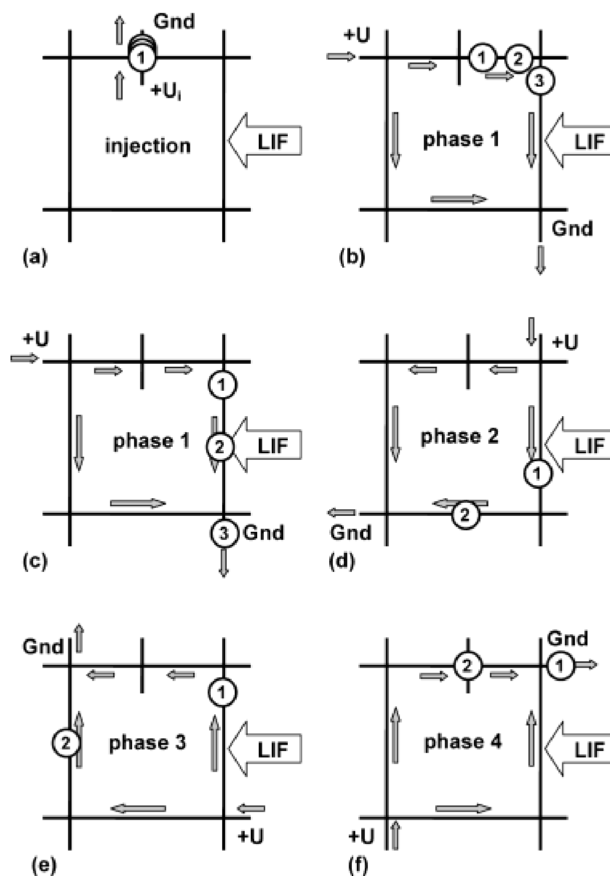

Figure 2. Principle of SCCE. The black lines represent channels of 10 μm deep and 40 μm wide. Three sample components are symbolized by the circled numbers 1, 2, and 3. The voltage-switching procedure is synchronized to component 2: (a) injection phase; (b) during phase 1; (c) at the end of phase 1; (d) phase 2; (e) phase 3; (f) end of the cycle. Reprinted from [16], with permission.

In this setup it is important to switch to a new separation channel at the moment determined by the migration rate of the compounds of interest. Other compounds, like No. 3 in Fig. 2, are allowed to leave the system (the peak loss described in the previous section). The system therefore 'zooms in' at a chosen migration window. Since the switching rate is synchronized with the compounds of interest, Burggraf called the method 'synchronized' electrophoresis. On-chip SCCE was later applied by von Heeren *et al.* [17] for on-chip separation of amino acids and components of human urine using MEKC. The authors made good use of the efficient power dissipation of the high aspect ratio chip channels to apply fields of up to 2000 V/cm, achieving sub-μm plate heights. Using an applied voltage of only 5 kV, 150 000 plates were generated in about 100 s. The same authors also demonstrated capillary gel electrophoresis in this chip [18]. Later, Manz *et al.* [19] discussed the pro's and con's of using the chip-based format for cyclic CE. Advantages are the ease of fluid handling and voltage switching, the high power dissipation possible on a chip, and the possibility of using very low applied voltages in combination with short channel lengths. Disadvantages are the diffusional loss of analyte at each intersection (about 8% per cycle in [17]), the peak dispersion caused by the many corners turned and the limited separation length L available on a chip. The last factor can be regarded as the negative side of the advantage that low voltages can be used. Manz *et al.* [19] addressed the first two disadvantages by making the channels connecting the intersections to the HV reservoirs more shallow and by narrowing the channel at the corners. The authors also demonstrated that good separation performance can be obtained using very low voltages (down to 500 V). Finally they discuss different channel geometries (triangular, square, pentagonal, . . .). In the paper, a triangular geometry is used.

A nonchip-based approach was chosen by Zhao *et al.* [15, 20] employing a setup with conventional fused-silica capillaries. Their device addresses two limitations of the

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chip-based SCCE devices. Firstly, they employ a capillary length *L* of 1 m, giving a 25-fold increase in peak capacity as compared to the *L* = 4 cm of the chip though at the cost of high applied voltages. Secondly, they employ capillary joints at the points of HV connection that can be actively opened to apply voltage and closed when the analyte peaks pass, to reduce analyte loss and peak dispersion. The authors separated amongst others L-phenylalanine and its ring-deuterated derivative with MEKC, and obtained 30–100 million plates in separations lasting about 15 h. Significant analyte loss was still observed (about 5% per cycle), possibly due to adsorption.

Regarding the electrophoretron, only the principle has been successfully demonstrated [12]. Also with this device a substantial analyte loss was observed (20% per cycle), attributed by the authors to loss through the Teflon capillary joints and analyte wall adsorption. Since an electrical connection through the joints must be present, the problem of analyte loss by migration through pores in the joints seems to be inherent to this method.

3 Liquid chromatography

HPLC has become the most popular separation method both for routine analysis and research, due to its general applicability and great versatility. In the past 30 years the state-of-the-art and especially the column technology has evolved to such an extent, that routine analyses with plate numbers of $>$ 10 000 are now performed in just a few minutes. This evolution has been mainly due to the development of ever smaller particles, improving mass transfer and decreasing plate height. Since both the plate number and the backpressure roughly increase with the square of the particle size, similar plate numbers can be obtained at the same pressure in a quarter of the time and at a quarter of the column length by halving the particle size. However, for very challenging separations, for example of racemic mixtures or close structural analogues, very high plate numbers can be necessary, requiring longer columns and creating large backpressures. Then conventional pumps can no longer be used. Though very high plate numbers can still be provided by using very long columns packed with larger particles, this will come at the enormous cost of a quadratically increasing analysis time [21, Table 1]. Alternatively, special pumps can be used capable of very high pumping pressures (up to 5000 bar [22]). This, however, comes at the price of having to construct special pumps and valves. Another approach is to use open-tubular chromatography with its more reduced pressure resistance. This method still holds a great promise for the generation of high resolution at low pressure gradients [21, 23].

Table 1. Separation times and operational parameters for a cyclic OTLC using different separation column lengths *L*

Column length Time (s)	(m)	Velocity (mm/s)	Diameter (μm)	Revolutions
6	2870	2.1	4.5	1
1	869	3.8	2.5	3.3
0.6	618	4.5	2.1	4.6
0.1	187	8.1	1.1	15.2

The indicated separation time gives a resolution of 1.0 for two analytes with $k_1' = 1$, $k_2' = 1.01$. The effects of extra-column band broadening are neglected. $D = 10^{-9}$ m^2/s; $\eta = 10^{-3}$ Pa·s; $\Delta P = 200$ atm

A fundamentally different approach to increase column efficiency and therefore resolution is the subject of this review, namely continuous column extension. Two methods have been employed for this in HPLC which, interestingly, conceptually are the same as those used in CE as discussed above. The first method was introduced in 1962 by Porath and Bennich [24] in gel filtration chromatography and is schematically depicted in Fig. 3. In this method, in the literature called closed-loop or direct-pumping recycling chromatography (DPRC), the column effluent is led back to the column *via* the pump. A detector and an injection valve are taken up in the closed loop as well. The second method was introduced by Biesenberger *et al.* [25] and is called alternate-pumping recycling chromatography (APRC; Fig. 4). In this method two columns are used. After the analyte has passed through the first column it is led to a second column, which is switched behind the first column by means of a valve. When the analyte has passed through the second column, the first column is at its turn switched behind it, and this process is repeated as many times as necessary. This method therefore follows the original suggestion of Martin for GC as cited in the introduction. Figure 4 illustrates that one or two detectors have to be employed, depending on whether a six- or an eight-port valve is used.

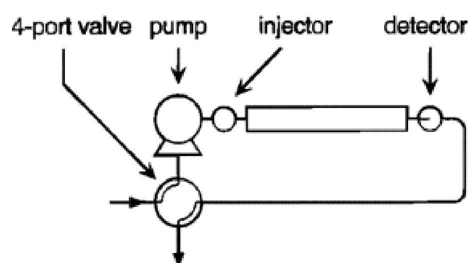

Figure 3. Direct pumping or DPRC. Reprinted from [27], with permission.

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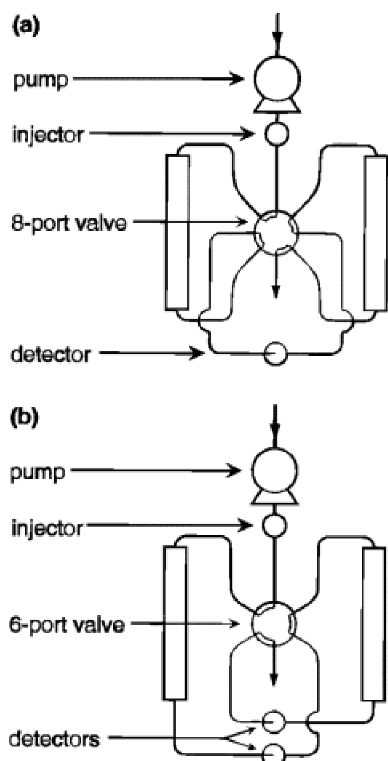

Figure 4. APRC using an eight-port (top) or six-port (bottom) valve. Reprinted from [27], with permission.

Both the direct- and the alternate-pumping method have been applied for analytical and preparative purposes. For analytical purposes, the methods provide quick results, without need for optimization, which is especially useful in the exploratory phase [26, 27]. For preparative purposes, the recycling methods provide a more cost-effective solution than, *e.g.*, simulated moving-bed methods [28] since existing columns and equipment can be used. Theoretical analyses of both analytical [27] and preparative [29, 30] recycling methods have appeared. In this paper, we will limit our theoretical treatment to analytical recycling chromatography.

3.1 Theoretical limits

The plate height in HPLC depends on a large number of parameters describing properties of the stationary phase, mobile phase, analyte, and column packing, and is generally described using variants of the van Deemter equation [21, 31]. For simplicity and to demonstrate the principle of cyclic LC, we will here use the equations for open-tubular liquid chromatography (OTLC). In cyclic chromatography we are interested in generating resolution as quickly as possible (separation speed), implying that we want to work at the mobile phase velocity where the so-called plate time is minimal [21]. The plate time hereby is defined as the time needed to generate one plate or *H/u* where *u* is the linear flow velocity and *H* is the plate height. It is the inverse of the plate generation rate *N/t*. From classical theory [23] the optimum resolution that can be obtained as a function of time can then be derived from the separation between peaks 1 and 2 and the plate height

$$(R_S)_{opt} = \frac{\sqrt{t}}{4d} \cdot \sqrt{\frac{D}{2A}} \cdot \frac{k'_2 - k'_1}{(k'_1 + 1)(k'_2 + 1)} \tag{8}$$

if the column is operated at the velocity where the plate time *H/u* is minimal:

$$u_{opt} = \frac{2D}{d\sqrt{A}} \tag{9}$$

It should be noted that the optimal velocity defined here, where *H/u* is minimal, is a factor of sqrt(2) faster than the commonly used optimal velocity where *H* is minimal [23]. In the above equations for channels of circular cross section

$$A = \frac{1 + 6k' + 11k'^2}{96(1 + k')^2} \tag{10}$$

(For OTLC in capillaries of rectangular cross-section see [32]). Resolution thus increases with the square root of time, a situation that is familiar when we consider for example replacing a short column by a longer identical one to improve resolution in a conventional case. This dependence stems from the increase of peak separation with *t* and the increase of peak broadening with sqrt(*t*). Since the resolution depends on the inverse of the column diameter, it will be favorable to use as small diameter columns as possible. When downsizing the diameter of course the limit will be posed by pressure constraints. This dependence of the maximal resolution on the column pressure gradient can be explicitly formulated by combining Eq. (8) and the Poiseuille flow equation for a circular cross-section capillary to give

$$(R_S)_{opt} = \left(\frac{\Delta P}{L}\right)^{1/3} \sqrt{t} \frac{k'_2 - k'_1}{(k'_1 + 1)(k'_2 + 1)} B \tag{11}$$

where

$$B = \frac{1}{4\sqrt{3}} \left(\frac{\sqrt{D}}{16A\eta}\right)^{1/3} \tag{12}$$

where η is the viscosity (Pa·s).

Equation (11) makes clear that it is favorable to have a system with a maximal pressure gradient $\Delta P/L$. This dependence of the optimum resolution on the cube root of the pressure gradient can be understood from the fact that u_{opt} increases when *d* is scaled down (Eq. 8). The

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pressure increase needed to increase u_{opt} is proportional to d while the pressure increase on downscaling d is proportional to d^2. Stated in a different way, when we make optimal use of the separating speed of the column by working at u_{opt}, the generation of resolution will depend on the cube root of the pressure gradient. Table 1 illustrates the impact of recycling on the basis of Equation (11). Assuming a pressure limit of 200 bar, it shows the time needed to attain $(Rs)_{opt} = 1$ for an analyte couple that is very difficult to separate ($k_1' = 1$, $k_2' = 1.01$) as a function of the column length L. It can be seen that the separation time will decrease with $L^{-0.67}$, the velocity increases with $L^{0.33}$ and the channel diameter has to decrease with $L^{-0.33}$. For a column of 6 m length the system is in fact a conventional OTLC system (No. revolutions = 1). For shorter columns an increasing amount of revolutions is necessary to obtain a resolution of 1, but the analysis time still decreases because of the larger velocity. Though useful to illustrate the principle, the practical relevance of Table 1 is limited due to the small channel diameters implied, limiting analyte detection methods to laser-induced fluorescence [21, 23]. It should further be noted that the independence of k' on column diameter d as assumed in the above analysis, implies that the stationary phase thickness decreases with decreasing d.

In conventional laboratory situations packed columns will rather be used, implying a much slower generation of resolution at a certain pressure drop (relative to an open column operated at the same pressure, the resolution drops with a factor of about 3.2 in a packed column; see [21]). Thus, packed columns were used in all the examples of recycling chromatography given below. They were, however, always of commercially available lengths. When the approach illustrated for open columns in Table 1 would be applied to packed columns, special very short columns could be constructed, and the gain in separation speed could be even greater than already demonstrated in these papers. By chopping up the column into many small lengths L with over each a pressure drop ΔP of 200 bar, very high separation speeds could be obtained. In theory there is no limit to this process. In practice, however, limits will be encountered in column construction, detectability issues and extra-column band broadening.

It is interesting to compare Eq. (1) and (11), respectively, representing the optimum resolution as a function of time in SCCE and cyclic OTLC. In both equations the same elements appear: applied force field (E or $\Delta P/L$), square root of time, difference in mobility of the two analytes and a factor related to the peak broadening. The most interesting difference between these equations is that separation speed in CE is directly proportional to the force field while in LC it is proportional to the cube-root. As we saw above, this has its origin in the requirement to work at the optimal velocity in LC, coupled to the increasing difficulty of moving liquid through tiny capillaries by pressure.

3.1.1 Maximum resolution

Just as in SCCE, the maximum resolution in cyclic LC is reached when two peaks totally fill the separation length L (m). Since the peak broadening at the u_{opt} defined above is three times as rapid as in CE, this occurs at $t = L^2/(384D)$. We then obtain from Eq. (11)

$$(R_S)_{max} = \left(\Delta P L^2\right)^{1/3} \cdot \frac{B}{8\sqrt{6D}} \cdot \frac{k_2' - k_1'}{(k_1'+1)(k_2'+1)} \tag{13}$$

In chromatography the maximum obtainable resolution thus depends both on the pressure drop applied and the separation length. Since the assumption for the derivation of Eq. (13) is that we are working at the optimal velocity, it is favorable to decrease the channel diameter so that a higher ΔP can be applied.

3.1.2 Peak capacity and peak loss

Peak capacity can be treated in a way entirely analogous to SCCE, substituting in Eq. (6) the baseline peak width w with $4\sigma = 4\text{sqrt}(Hut) = 4(\text{sqrt}(6Dt))$ at u_{opt}. The peak capacity again will decrease with the square root of time and considering peak capacity it will therefore be favorable to use long columns. Just as peak loss occurs in SCCE but not in the electrophoretron, peak loss occurs only in APRC, where analytes can be flushed off the column to the waste before the valve is switched. In APRC a long column will therefore be favorable if peak loss must be limited. In DPRC all analytes will be retained but maximal resolution is reached when analytes start overtaking since the peak identity is lost [27]. In the system proposed at the end of this paper, the latter restriction does not hold, since analytes are not identified by their location but by their speed.

3.2 Examples

All recycling separations reported in the literature have been performed using packed columns. DPRC was first demonstrated by Porath and Bennich [24], and APRC by Biesenberger *et al.* [25], in both cases applied for gel permeation chromatography. It was directly recognized that it was of great importance to limit extra-column band broadening. In DPRC, extra-column band broadening is mainly caused by the internal volume of the pump. Small volume pumps and/or large volume columns therefore have to be used. In APRC, extra-column band broadening

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is mainly caused by the connective tubing, which is more easily controlled and limited. This method seems to hold the larger promise and has indeed been more widely used. Martin *et al.* [27] were the first to give a thorough theoretical treatment of analytical recycling chromatography, in which they explicitly treated the influence of the extra-column band broadening. The authors showed that under realistic conditions, even conditions far from the optimal because of extra-column band broadening, resolution always increased in a recycling system if the cycle number becomes sufficiently large. Recycling chromatography has been used for difficult separations of a small number of analytes such as isotopic [33, 34], isomeric [35–37], oligomeric [38], and chiral [39, 40] separations. The important feature of the system was the rapid generation of resolution as described by the Eqs. (8) and (11).

An interesting phenomenon in APRC is, that some analytes will be continuously chromatographed at a higher pressure than others. A theoretical treatment on analytical APRC that includes the effect of pressure-induced retention variations was given by Lan and Jorgenson [26]. Resolution was shown not to be influenced by the pressure variations, though band broadening and retention both were affected. Lan and Jorgenson's experimental results with APRC demonstrated the expected increase of resolution with the square root of time. The authors were able to separate phenylalanine and ring-deuterated phenylalanine in 30 min, a separation that in a (linear) conventional system would have necessitated the use of four columns in series and an applied pressure of 800 bar. The authors also provide a list of practical considerations for choosing between APRC and DPRC. The greatest advantage of recycling HPLC appears to be the fact that high efficiencies can be reached in a relatively short time using conventional equipment in a nonconventional setup. This is especially of use for difficult separations in the exploratory phase, when there is no time to develop a dedicated separation procedure.

3.3 Proposed chip-based system for cyclic OTLC applying continuous pumping, detection, and chemometrics

All systems for cyclic HPLC have up till now employed conventional columns and pumps. It would, however, be of interest to investigate chip-based systems for cyclic OTLC, especially regarding the usefulness of chip-based cyclic CE as described above. Figure 5 shows a possible chip-based cyclic OTLC system. Mobile phase is pumped around a circular open column and the detection signal is sampled in a continuous fashion using multiple detection windows. The detection signal obtained in this fashion can be, *e.g.*, subjected to a Fourier transform, resulting in a frequency signal that is proportional to the analyte velocity which will enable continuous analyte tracking. No pump is envisaged in the channel in order to minimize dispersion effects. Instead a body force is exerted on the solution. This cyclic OTLC system will be preferable over a conventional linear OTLC system if the added advantage of a flexible analysis time does not come at the price of a reduced efficiency. In addition, the chip format will then add advantages such as compactness and ease of operation.

To establish the performance of a reference conventional OTLC system, we will use the analysis of Knox and Gilbert [23] as a guide. On the basis of their paper we regard an OTLC system with a channel diameter of 8 μm and a channel length of 63 m that operates at a flow velocity of 0.6 mm/s as the practical optimum in the light of detection and pressure requirements (see Table 1 in [23]). Our pumping method should therefore be able to generate a flow of 0.6 mm/s in a channel of 8 μm diameter, applying a body force on the solution. At least two types of pumping methods can be employed for this, those exerting a force on the entire solution like alternating current magnetohydrodynamic flow (AC MHD) flow [41, 42], generating a parabolic flow profile, and those exerting a force on the solution in the electrical double layer like alternating current electroosmotic flow (AC EOF) [43–46]. AC MHD has been the first pumping method investigated for chip-based cyclic systems. The maximal force experimentally demonstrated with this method has been 850 Pa/m while the estimated maximum force is about 3×10^3 Pa/m [42]. Since this is at least two orders of magnitude less than the pressure drop per unit length occurring in the optimal linear system (3×10^5 Pa/m), a MHD-based circular system is expected to be performing worse than a linear pressure-driven system [42].

In contrast, AC EOF pumping can possibly meet the requirements. This method, propelling liquid above an asymmetric interdigitated electrode array by the application of an AC actuation voltage of typically a few volts was first theoretically described by Ajdari [43], after the phenomenon had been spotted by Ramos *et al.* [47]. Soon a working device with a straight channel was demonstrated by Brown *et al.* [44]. Because the force is exerted on the ions in the electrical double layer, the method creates an approximately triangular flow profile if the electrode array covers one side of the channel, or an approximately flat profile if electrode arrays are present on both sides [45]. Typical velocities obtained are in the order of 100 μm/s, but average velocities of up to 500 μm/s have been observed, approximating the optimal system defined above (Studer, personal communication; [45]). A theoreti-

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cal investigation of AC EOF, especially considering the temporal variation of the flow, has also been presented [48]. An advantage of AC EOF is that the flow speed is independent of the channel height just as in (conventional) DC EOF. However, a limit to the channel height will still be posed by the minimal size of the interdigitated electrodes, which is determined by cleanroom production considerations. Electrode dimensions of 1 μm width and hence channel heights of 10 μm should be feasible, which are sufficient for our optimal system. Recently, Debesset *et al.* [49] demonstrated AC EOF pumping in a circular device designed especially for chromatographic purposes. Though we limited the integrated pumping principles to AC MHD and AC EOF, these are by no means the only principles suited for application in circular channels. Another principle that may for example very well be used is peristaltic pumping [50].

Continuous detection will enable chemometrics to be applied on the signal in order to identify and follow individual analyte bands around the circular channel. This principle was demonstrated for the first time by Crabtree *et al.* [51] using fluorescence detection. The authors applied SCOFT (Shah Convolution Fourier Transform detection) to extract the electrophoretic velocity of two analytes moving in a straight channel from a continuous detection signal (compare Fig. 5). In the circular OTLC system proposed here, such identification by separation speed would be favorable since it allows analyte bands to overtake each other. Crabtree *et al.* and later also Kwok and Manz [52, 53] demonstrated that by the application of SCOFT the detection sensitivity increases with the square root of the sampling time. In a follow-up study, Eijkel *et al.* [54] showed that wavelet transform coupled to continuous sampling allows the observation of small changes in analyte speed in time. SCOFT does not seem to increase the resolution, judging from measurements and system simulations. Another limitation is, that with increasing peak broadening the analyte bands become much wider than the slit width, decreasing the signal amplitude. With one exception, all setups for SCOFT have used fluorescence detection by laser illumination in a spatially periodic fashion of a separation channel and sampling of the emission by a photomultiplier tube, including one variant in which integrated waveguides are used, demonstrated by Mogensen *et al.* [55]. The exception is formed by the work of McReynolds *et al.* [56] who obtained continuous information on all analytes by sampling the entire separation channel with a charge-coupled device. The authors afterwards had the freedom to apply any form of chemometrics, and amongst others obtained the SCOFT signal by software processing. In summary, SCOFT detection and its variants allow continuous identification of individual analytes and increase detection sensitivity. Other

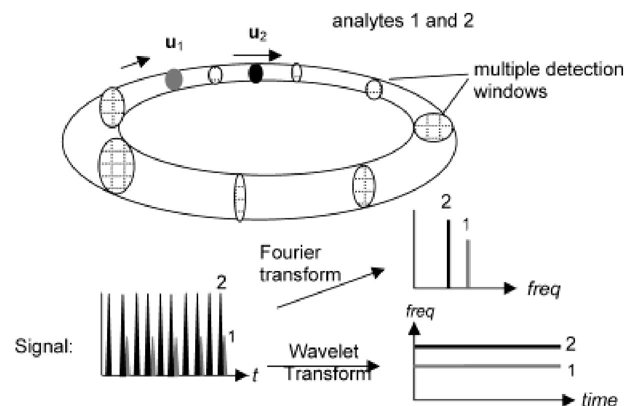

Figure 5. Proposed device for cyclic OTLC using integrated pumping and Fourier or wavelet transform detection.

more universal detection methods, like UV absorption or conductometry, will certainly also form good candidates for the application of SCOFT or similar chemometric methods.

4 Conclusions

It is shown that many similarities exist between cyclic analytical separation methods in CE and LC, both in practical setup and theoretical description. Design rules for practical systems are determined by the particular application of the system. Separation speed can be optimized by increasing the electrical field or the pressure gradient. Peak capacity can be optimized by increasing the separation length. Literature on cyclic CE methods provides examples of both approaches. Thus, the chip-based systems for SCCE can generate high resolution with low applied voltages but show considerable peak loss because of the short separation length, and a low peak capacity. These systems therefore seem most suited for difficult separations of a small number of substances using low voltages. In contrast, the SCCE systems composed from conventional columns and HV power supplies need high voltages, but offer a higher resolving power, high peak capacity, and less peak loss. Work on the electrophoretron still has to continue to determine its advantages and disadvantages in detail.

All publications on analytical cyclic LC have concerned the application of conventional columns and pumps in a cyclic fashion. In this paper, a cyclic chip-based OTLC system is proposed based on integrated pumping and a continuous detection method. It is shown that AC EOF can possibly generate a pumping velocity in such a system that will enable the circular system to compete with optimal linear OTLC systems as described by Knox *et al.*

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[23] but with the additional advantage of a flexible analysis time. As a continuous detection method in the system, continuous sampling would be most suited combined with chemometrics, *e.g.*, SCOFT detection to extract analyte velocities.

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