Circular Geometry in Molecular Stream Separation to Facilitate Non-Orthogonal Field-to-Flow Orientation

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Abstract: Molecular stream separation (MSS) is a promising complement for continuous-flow synthesis. MSS is driven by forces exerted on molecules by a field applied at an angle to the stream-carrying flow. MSS has only been performed with a 90° field-to-flow angle because of a rectangular geometry of canonic MSS; the second-order rotational symmetry of a rectangle prevents any other angle. Here, we propose a non-canonic circular geometry for MSS, which allows changing the field-to-flow angle. We conducted in silico and experimental studies of circular geometry for continuous-flow electrophoresis (CFE, an MSS method). Counterintuitively, circular CFE was found to support better flow and electric-field uniformity than rectangular CFE. We proved that the nonorthogonal field-to-flow orientation can result in a higher stream resolution than the orthogonal one. We foresee that circular CFE will serve as a new testbed for the investigation and creation of new CFE modalities.

Continuous-flow organic synthesis has several important advantages over its batch counterpart,[1–3] and continuous separation is its logical extension.[4–6] There are several continuous-separation methods; however, the only widely used one is liquid-liquid extraction, which can hardly separate multiple species from each other.[7,8] In contrast, molecular stream separation (MSS) can continuously separate multiple species; though, its early stage of development hinders the efficient combination with continuous-flow synthesis.[9–11] MSS is driven by a force exerted discriminatively on the separated species by a force field oriented at an angle to the hydrodynamic flow carrying the streams. The interplay of the separating force and the hydrodynamic flow causes the splitting of the stream of mixed species into their individual molecular streams (Figure 1a).

Fundamentally, MSS is defined by three variables: the field-to-flow angle, the field strength, and the flow rate. The influence of the varying magnitudes of field strength and flow rate on MSS has been extensively studied, and these parameters are used to control separation.[12] On the opposite, the field-to-flow angle has always been 90° owing to the apparent obviousness that the 90°-angle is optimal as well as conceptual challenges and technical difficulties associated with the non-orthogonal field-to-flow orientation.[13] The aim of this work was to understand if the field-to-flow orientation can be included as a degree of freedom, thus, facilitating non-orthogonal MSS for exploration and potential use.

There are two major MSS methods: continuous-flow electrophoresis (CFE, Figure 1b) and continuous annual chromatography (CAC, Figure 1c).[14,15] Both CAC and CFE utilize a rectangular geometry; the rectangle is planar for CFE and folded into a cylinder for CAC. In CFE, two straight parallel electrodes constitute two sides of a thin rectangular separation zone in which the electric field is perpendicular to the hydrodynamic flow. In CAC, a thin cylindrical layer of the chromatographic stationary phase is rotating orthogonally to the hydrodynamic flow creating an infinitely long stationary phase and a continuous force field.

The rectangular geometry of canonic MSS does not allow the change of the field-to-flow angle because of the 2nd-order rotational symmetry of a rectangle (Figure 2a). A rectangle has only two equivalent rotational positions and allows only orthogonal field-to-flow orientation. To allow all field-to-flow angles, the geometry must be of the infinite-order symmetry, i.e., circular rotational symmetry (Figure 2b).

In an ideal CFE device, the electric field and the hydrodynamic flow should be uniform, i.e., field and flow lines must be straight, parallel, and uniformly spaced inside most of the separation zone. Intuitively, a classic rectangular device with straight and parallel electrodes and a rectangular separation zone between them is best to satisfy the uniformity condition. In contrast, a circular device, having arc-shaped electrodes circularly oriented around a circular separation zone, a priori appeared to be

Figure 1. Schematic depiction of conventional orthogonal MSS: a) general principle and its implementation in b) continuous-flow electrophoresis (CFE) with differential electrophoretic (EP) mobility of molecules being the driving force of MSS, and c) continuous annual chromatography (CAC) with differential affinity to the solid phase driving MSS.

Figure 2. Illustration of a) the second-order rotational symmetry for conventional rectangular-geometry MSS and b) the high-order rotational symmetry for hypothetical circular-geometry MSS. A rectangle has two mirror axes, and a circle has an infinite number of mirror axes in the x-y plane.
completely incompatible with the uniformity requirement. The major goal of our investigation was to understand if circular-geometry CFE was feasible.

Developing a circular CFE device with both a uniform field and a uniform flow requires a quantitative measure of uniformity. We used an index of uniformity \( \Gamma \) which provides the relative degree of uniformity inside the separation zone of our CFE device with a maximum value of \( \Gamma \) being unity (Note S1).

This study requires evaluating multiple hypotheses via testing multiple CFE device designs. We use a milling process to fabricate CFE devices. Making one device takes a day and incurs a non-negligible cost in materials and supplies. Accordingly, this study could become impractically long and/or cost-prohibitive if carried out experimentally only. To keep the project within reasonable time- and cost-scales, we investigated multiple virtual devices within COMSOL Multiphysics software. Modeling in COMSOL allowed us not only to assess the feasibility of circular CFE but also to optimize the design of a CFE device before fabricating it. The details of our COMSOL model can be found in Note S2.

The hydrodynamic flow and the electric field do not interfere with uniformity separately starting with the flow uniformity. As an initial point, we selected the design with a circular separation zone of uniform thickness and a single source and sink for the flow (Figure 3a). Expectedly, the flow in a virtual design with such features diverges from the source and converges to the sink. The flow lines are visibly curved resulting in the uniformity index \( \Gamma_{\text{flow}} = 0.82 \).

When developing rectangular CFE devices we identified and successfully used two device features as a means of flow uniformization: (i) deep sacrificial channels around the separation zone, which also serve for placing electrodes, and (ii) deep and large entry and exit zones. We decided to explore these two features in a circular CFE device before even looking for any other means of flow uniformization.

As in a rectangular design, adding sacrificial channels to a circular design drastically improved flow uniformity. We gradually increased the depth of the channels to reach flow uniformity of \( \Gamma_{\text{flow}} > 0.9 \) (Figure 3b). We did not proceed with further depth increase as it started causing an unjustifiable decrease of the flow velocity in the separation zone. Instead, we explored adding the entrance and exit zones to the device with the channels, which proved to further increase flow uniformity. The shape of the zones was varied to achieve the maximum value of \( \Gamma_{\text{flow}} = 0.97 \) with an eye-shaped device (Figure 3c). Notably, this flow uniformity in a circular device was higher than in the best rectangular device previously assessed \( \Gamma_{\text{flow}} = 0.95 \) and allowed us not to look for any other means of flow uniformization. While the reason for better flow uniformity in a circular device was not further investigated, we attribute this fact to the absence of flow-disturbing corners in the circular device. Solving the flow uniformity issue allowed us to concentrate on electric field uniformity in the eye-shaped device (Figure 3c).

We chose segmented arced electrodes with segments spaced by a short distance from each other and occupying the entire perimeter of the separation zone (Figure 4). Several segments would be connected to each other to form each of the two electrodes on the opposite sides of the separation zone. A voltage would be applied to these electrodes to create an electric field in the separation zone. We varied (i) the total number of segments and (ii) the length of electrodes (the number of segments per electrode) in our virtual instrument to assess their influence on field uniformity.

Three total numbers of electrode segments were considered 24, 20, and 16. The total length of the electrodes was kept approximately constant by activating approximately half of the segments. This varying segmentation had a negligible effect on the field uniformity; the index of uniformity was \( \Gamma_{\text{field}} = 0.96 \) for all three cases (Figure S2) allowing us to choose either number of segments. To decide between 24, 20, and 16, we used a practical consideration. In theory, a large number of segments provides a high degree of flexibility for setting the direction and magnitude of the electric field. However, in practice, a larger number of segments creates a greater engineering challenge. Accordingly, we chose 16 segments for our evolving a functional circular CFE device.

We then varied the length of electrodes by changing the number of segments per electrode from two to six (Figure 4). Increasing the electrode length increases the surface area of the electrode, which, in each other in the first approximation. Hence, we consider their...
turn, would increase the electric current in a real CFE device. Since high currents are undesirable in electrophoresis (due to Joule heating, bubble generation, etc.), we aimed to find the minimum length needed for field uniformity of $\Gamma_{\text{Efield}} \geq 0.95$ in the separation zone. We found that four segments per electrode (Figure 4b) resulted in $\Gamma_{\text{flow}} = 0.96$ and, thus, satisfied our criterion. Accordingly, this electrode length was used in our further in-silico and experimental studies.

The above-described COMSOL simulation proved that our circular CFE device could support both a uniform hydrodynamic flow ($\Gamma_{\text{flow}} = 0.97$) and a uniform electric field ($\Gamma_{\text{Efield}} = 0.96$). It is instructive to compare the overall field-flow uniformity of a circular CFE device to that of a classic rectangular CFE device (Figure 5). The shaded areas (16 cm$^2$) indicate parts of the separation zones in which both the flow and field are uniform with $\Gamma_{\text{Efield}} > 0.95$. Those parts have similar sizes relative to the total area of separation zones: 57% for the circular device and 64% for the rectangular device. This comparison clearly shows that the overall field-flow uniformity of a CFE device is not compromised when the device’s rotational symmetry changes from the 2-nd order to high order.

We then used the circular device optimized for flow and field uniformity to simulate the separation of three analytes: two with negative electrophoretic mobilities and one with zero mobility. The chosen electrode design with 16 segments in total and four segments per electrode allows eight different field-to-flow angles from $0^\circ$ to $157.5^\circ$ with a step of 22.5°. Each of them was used to investigate the influence of the angle on CFE separation. The streams for eight angles are shown in Figure 6a, while the corresponding angulagrams can be found in Figure S3. The angular resolution was calculated from the angulagrams using the previously reported approach. The resolution was found to depend on the field-to-flow-angle (Figure 6b). The maximum and minimum resolutions are achieved at angles different from $90^\circ$, which agrees with the theory of CFE. The improvement in resolution is obtained only for angles greater than $90^\circ$.

Overall, our COMSOL investigation of circular CFE proved its feasibility and provided a pre-optimized design for further fabrication and evaluation of the circular CFE device. No optimization of real CFE devices was necessary. The circular device was fabricated using the same Solid Edge code as the one used for creating device geometry in COMSOL. Using the same code ensured that the physical device was identical to the virtual device within the precision of our fabrication process. The details of the fabrication steps are described in Note S4 while experimental details on CFE can be found in Note S5.

Flow uniformity in the physical CFE device was tested by flowing fluorescent beads added to the background electrolyte through the device at a zero electric-field strength. Figure 7a demonstrates an image with flow lines derived from a video of fluorescence from the moving beads (Video S1). The video was used to create a vector field (Figure 7b) using Fiji/TrackMate[20–22] which, in turn, was utilized to calculate flow uniformity index $\Gamma_{\text{flow}} \approx 0.91$ (Note S6). This value is similar to that predicted in COMSOL, providing cross-validation for COMSOL modeling and experiments in a physical device.

We did not have a direct way of investigating electric field uniformity. Therefore, we assessed it via evaluating stream linearity in CFE separation; if the electric field is non-uniform, then streams are non-linear even if the flow is uniform (Note S7). We run the separation of four species and found that stream linearity was $\geq 0.95$ for all four streams (Figure 8a). The results of these experiments indirectly proved that the electric field was uniform in a circular CFE device.

Proving that the real circular CFE device supports uniform flow and field allowed us to move to the ultimate goal of this study: an experiment assessing the influence of the field-to-flow orientation on CFE. We used three analytes: negatively charged fluorescein, negatively charged α-naphtholbenzene, and Sudan Black, respectively, measured in non-aqueous capillary electrophoresis as described in Note S3. Panel a show the separated stream at eight different angles, and panel b shows the dependence of the angular resolution (calculated from the streams in panel a via intermediate angulagrams) on the field-to-flow angle. The volumetric flow rate and electric field strength were typical for real CFE experiments in a device of this scale: 2 mL/min and 25 V/cm, respectively.

![Figure 5. Comparison of field-flow uniformity of a) circular CFE to that of b) rectangular CFE. The top panels show general geometries of the respective devices, and the bottom panels show COMSOL-simulated overlaid flow lines and field vectors in these devices. The red 40 × 40 mm area is where the uniformity index for the circular device in greater than 0.95 for both field and flow. The uniformity indexes within the same square within the rectangular device were lower. The bottom graphs show flow lines and field vectors.](image)

![Figure 6. The effect of the field-to-flow angle on simulated CFE of three species with electrophoretic mobilities $\mu_1 = -1.01 \times 10^{-8}$, $\mu_2 = -5.66 \times 10^{-9}$, and $\mu_3 = 0$ m$^2$/V*s. These mobilities correspond to fluorescein, α-naphtholbenzene, and Sudan Black, respectively, measured in non-aqueous capillary electrophoresis as described in Note S3. Panel a show the separated stream at eight different angles, and panel b shows the dependence of the angular resolution (calculated from the streams in panel a via intermediate angulagrams) on the field-to-flow angle. The volumetric flow rate and electric field strength were typical for real CFE experiments in a device of this scale: 2 mL/min and 25 V/cm, respectively.](image)
naphtholbenzein, and neutral Sudan Black. Note that their mobilities were utilized in simulated CFE shown in Figure 4, and 112.5° was an angle for the best resolution of both fluorescein and α-naphtholbenzein from Sudan Black. Accordingly, in our CFE experiments separation quality was compared at two field-to-flow angles, i.e., 90° and 112.5° (Figure 9). The dependence of stream deflection on the angle was in a manner predicted in both COMSOL modeling and in the theory of CFE. Stream resolution was significantly higher for 112.5° than for 90°. These experiments provided the final proof-of-concept for circular CFE.

While the results of experiments correspond qualitatively to those of in-silica study, there is a significant quantitative disagreement. The two major potential reasons for this disagreement are: (i) experimental imperfections (e.g., inaccurately measured values of electrophoretic mobilities of the two charged species) and (ii) secondary phenomena which were present in the experiment but not included in COMSOL simulation. The second reason is more interesting as it can potentially reveal a phenomenon that has been previously disregarded by CFE practitioners. A known secondary phenomenon is the electroosmotic flow (EOF), which is very common in aqueous electrophoresis, and can also be beneficial. However, here we used non-aqueous CFE in which EOF is negligible, which was confirmed by no deflection of the stream of the electrically-neutral analyte. Therefore, we looked for another reason.

While using the field-to-flow angles different from 90°, we noticed a decrease in the flow velocity in the separation zone, which could be seen by following stream evolution for the neutral molecule. We hypothesized that this flow slowing was caused by ion concentration polarization, a well-known phenomenon in the area of ion transport, which has never been mentioned in the context of CFE. This phenomenon is caused by the ion concentration gradient and is accompanied by recirculation of the flow near the electrodes. To check if the recirculation was detectable, we followed the flow pattern near the electrodes with fluorescent beads added to the background electrolyte. We found profound flow recirculation (Video S2) which confirms that ion concentration polarization is always present in CFE, but does not have the same magnitude when electrodes aren’t segmented or in hydrodynamic flows with high Peclet numbers. This phenomenon can also explain the curving of the streams at angles other than 90°. While it would be interesting to include it in our simulation of CFE to see if this could improve quantitative agreement with the experiment, COMSOL does not have suitable capabilities. We did not find in our experimental results any signs of other phenomena that could contribute to the quantitative disagreement.

To conclude, we proposed the concept and proved the feasibility of high-order symmetry in MSS. It was implemented in a CFE device with a circular separation zone and arc-shaped electrodes positioned in a deep channel surrounding the separation zone. Circular CFE can support flow and electric-field uniformity on par with or higher than those of canonic rectangular CFE. Both simulations and experiments confirmed the theoretical prediction that the best stream resolution is achieved at a field-to-flow angle different from 90°. Some quantitative disagreements between the results of simulation and experiments prompted us to look for secondary phenomena unaccounted in simulation. We identified one such phenomenon: ion concentration polarization. This phenomenon does not clearly manifest itself unless the field-to-flow angle is varied or the flow pattern near the electrodes is analyzed in detail. Accordingly, it’s the first time when ion concentration polarization is reported in the context of CFE. We foresee that circular CFE will serve as a testbed for the investigation and creation of new CFE modalities. It is instructive to mention that the idea of circular CFE was conceived by recent advances in presenting CFE in polar rather than a Cartesian system of coordinates.

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