KINEMATIC STABILIZATION OF CONTINUOUS-FLOW ELECTROPHORESIS AGAINST THERMAL CONVECTION*

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The Problem of Thermal Convection.—Electrophoresis presupposes the main tenance of an electric field in a conductive fluid (usually a buffer solution). The electric field \( \frac{dV}{dx} \) cannot be maintained without a concomitant current density \( J_z = \sigma \frac{dV}{dx} \). In an electrophoretic column of uniform cross section, the heat generated in a unit volume per unit time will thus be: \( \frac{dQ}{dt} = J_z^2 / \sigma = (\frac{dV}{dx})^2 \sigma \). In the absence of turbulence, this heat would escape by heat conduction through the walls of the electrophoretic channel at a rate determined by the temperature gradients in the fluid and in the walls confining it. Actually, however, the transfer of heat from the fluid to its surroundings is greatly accelerated by the phenomenon of thermal convection which is normally engendered in the presence of a temperature gradient transverse to the walls. There are two special cases in which thermal convection is avoided: (1) when the density of the fluid is independent of its temperature (this condition is approached in the vicinity of 4°C near the density maximum of water and is utilized for stabilization in the Tiselius electrophoresis apparatus); (2) when the direction of a temperature gradient which is parallel to the gravitational field is such that the fluid density increases in the gravitational field direction. (Lord Rayleigh showed that under certain special conditions a horizontal fluid sheath may remain undisturbed by thermal convection even if the temperature gradient is inverted.)

The steady-state convection pattern depends on the direction of the temperature gradient relative to the gravitational field axis and on the geometry as well as orientation of the fluid container relative to the field of gravity. The mathematical treatment of thermal convection is extremely difficult and has been successful only in a few special cases under simplifying conditions.

Generation of Thermal Convection in Configurations of Practical Interest.—Several configurations, which are relevant to the present considerations, will be discussed qualitatively with the aid of photographs and schematic diagrams based on observations. Figures 1–3 illustrate convection patterns which will be considered below in connection with methods of inhibition of thermal convection.

(a) Convection between two vertical walls: In Figure 1a, the fluid of initial temperature \( T_0 \) is introduced into a vertical container whose opposite walls \( W_a \) and \( W_b \) are maintained at the constant temperatures \( T_a > T_b \) and \( T_b = T_0 \). \( g \) represents the direction of the gravitational field. Under usual circumstances when the density of the fluid decreases with temperature, the buoyant force upon a heated fluid element adjacent to wall \( W_a \) will exceed its weight, and a circulation of the pattern indicated in the figure will result, as can be easily ascertained by observing streak patterns of injected dye.

In Figure 1b, the temperature of the fluid introduced between the walls \( W_a \) and \( W_b \) exceeds the temperature of both walls \( T_a < T_0 > T_b; T_a = T_b \). The liquid in the central plane rises for the reasons given in the previous case, while the denser
fluid at the walls descends, engendering the convection pattern shown schematically in the diagram.

(b) *Convection between two horizontal plates:* The convection pattern between two horizontal plates (Fig. 2a) is much more complex than between vertical plates. For a fluid whose density diminishes with temperature, a stable turbulent flow pattern of striking appearance can be obtained when the temperature $T_b$ of the bottom plate ($W_b$) is higher than the temperature $T_a$ of the top plate ($W_a$) (the case of $T_b > T_a$ being stable for a fluid whose density decreases with the temperature). Bénard\(^4\) has shown that under these conditions numerous adjacent convection cells are formed in the fluid layer after an initial transient period during which the convection pattern changes as it approaches a steady-state configuration. The convection cells in the initial transient convection pattern are polygonal in shape including irregular polygons of 4–7 sides. The network of polygons rapidly becomes more regular with hexagons gaining predominance, as shown in Figure 2b. This pho-

![Diagram](image-url)

**Fig. 1.** (a) Type of pattern engendered by heating wall $W_a$ to a temperature $T_a > T_b$. $g$: gravitational field vector. $T_a$, $T_b$: temperatures of the walls $W_a$, $W_b$. (b) Type of convection engendered when fluid of temperature $T_b$ is introduced between walls $W_a$, $W_b$ maintained at temperatures $T_a > T_b$. $W_a$, $W_b$, $T_a$, $T_b$, $g$. (c) Arrangement for study of heat transfer through a layer of fluid between two horizontal surfaces $W_a$ and $W_b$ maintained at temperatures $T_a$ and $T_b$. (b) Photograph of convection cells (natural size) visualized by suspended graphite particles in a 0.81-mm layer of Spermaceti oil at $T_a > T_b$. (c) Stable steady-state convection cell pattern in a 0.64-mm thick horizontal layer of liquid (natural size). (d) Convection pattern in cross-sectional view. The vortices illustrate circulation in Bénard’s hexagonal convection cells (redrawn after Bénard\(^4\)). $A$ represents the axis of symmetry of the convection cell. Three adjacent convection cells are shown in sectional view. The section is taken through the plane bisecting the hexagons by passing through the centers of their sides (compare 2c). The warm fluid rises around the axis of symmetry $A$ (center of the regular hexagon), moves centrifugally along the upper surface where it loses heat, turns abruptly downward at the edges of the convection cell, and moves centripetally along the bottom wall $W_b$, which heats the fluid.
Fig. 3.—(a) Convection scheme in the annular space between two long horizontal cylinders, the outer cylinder being maintained at a lower temperature than the inner one (after Jacob). (b) Convection pattern for centrifugal heat flow between a large horizontal cylinder and a small one passing through its center (redrawn after Liu). (c) Convection pattern under conditions similar to those in (b) for a 2:3 diameter ratio of the cylinders (redrawn after Liu). (d) Convection pattern for centrifugal heat flow through a narrow annulus between horizontal cylinders (redrawn after Liu). Individual convection cells form in the upper part of the annulus similar to those encountered between horizontal plates, except that in this case the convection cells are nearly cylindrical filaments. In the lower portion the convection pattern resembles the pattern between vertical walls shown in Fig. 1a. (e) Drawing of convection pattern observed by means of dye streaks when water at room temperature is introduced between two horizontal cylinders kept at 0°C. The convection pattern is analogous to the pattern between walls shown in Fig. 1b.

tograph has been obtained by Bénard by suspending fine graphite particles in a 0.81-mm layer of Spermaceti oil and heating the bottom plate to 100°C while maintaining the top surface at room temperature. The graphite particles disappear by sedimentation from zones where circulation is absent. These zones appear dark in reflected light. The bright zones of Figure 2b depict regions where circulation of fluid maintains the graphite particles in suspension. Figure 2c, obtained by a different method described by Bénard, shows the extraordinary regularity of the hexagonal cell network established eventually in the stabilized convection pattern. By careful microscopic observation of motions of suspended particles, Bénard established the convection pattern shown in cross section in Figure 2d. The central axes A of the hexagons are lines of zero turbulence. Turbulence is also zero at the boundaries between two adjacent cells (i.e., along the sides of the polygons). The fluid is rising along the axis A, streams centrifugally along the top boundary while losing heat, and dips downward abruptly at the periphery of the convection cell turning toward the axis A in a centripetal flow during which heat is absorbed from the bottom plate. In any of the vertical planes passing through the axis A, the closed convection paths enclose a point of zero linear velocity. The locus of such points forms a hexagonal belt (with rounded corners) about which the convection
revolves. Heat is thus transported in a mosaic pattern from the bottom plate to the top surface.

(c) Convection between two concentric horizontal cylinders: Another configuration which is of interest to us is a pair of long horizontal concentric cylinders enclosing a fluid in the annular gap between them. A series of convection patterns is shown in cross section in Figure 3a–d under the assumption that the wall \( W_a \) of the inner cylinder is maintained at a higher temperature \( T_a \) than the wall \( W_b \) of the outer cylinder \( (T_b) \).\textsuperscript{2} As the difference between the cylinder diameters decreases, the convection pattern becomes more complex exhibiting formation of multiple convection cells.\textsuperscript{5} Figure 3b–d shows convection patterns for different diameter ratios determined experimentally by Liu.\textsuperscript{5} Figure 3b, obtained with a very small warm inner cylinder, approximates roughly the case of a cylindrical volume of fluid heated by an axial electric current and losing heat uniformly through the outer cylinder. This condition is obtained in Hjertén's zone electrophoresis apparatus.\textsuperscript{6} In Figure 3c the diameter of the inner cylinder is about two thirds of the outer diameter, and in Figure 3d we reach the other extreme of two nearly equal cylinders i.e., of a very narrow annular gap, which is utilized in the author's magnetically stabilized electrophoresis.\textsuperscript{7} Here, the convection pattern is more complex, exhibiting convection cells of dimensions diminishing in the upward direction. Figure 3e shows a convection pattern under conditions approximating those in the author's electrophoretic column stabilized by electromagnetic rotation.\textsuperscript{8} The annular fluid column is heated by a uniform electric current, and the two cylinder walls are cooled by a liquid coolant. The temperature reaches a maximum in the annular gap between the two cylinders. The convection pattern shown in Figure 3e is obtained by filling the annular space between the two cylinders with water at room temperature while cooling the cylinder surfaces with an ice-water mixture. The dye streak tracing technique is utilized to visualize the convection pattern.

Inhibition of Thermal Convection.—A complete elimination of thermal convection in our environment is possible only in a freely falling system, such as an artificial earth satellite, where gravitational manifestations are completely abolished. In the presence of gravitational influence, we can only inhibit thermal convection. We can use static methods of suppression (such as establishing a density gradient\textsuperscript{8} or a conductivity gradient in crossed electric and magnetic fields,\textsuperscript{9} or kinematic methods, such as rotation of the cell\textsuperscript{5,10} or fluid circulation in a narrow stationary channel\textsuperscript{7} shown in Figure 4a. Instead of referring to this fluid motion as rotation of the fluid in the annulus, we shall consider it as flow of an endless belt of fluid through a channel whose origin and end are united to form a closed loop as shown in Figures 4a and 4b for a circular and a noncircular channel. We shall consider below the basis for stabilization against thermal convection in this type of fluid motion and will generalize the conditions under which thermal convection will be inhibited in a fluid flowing through a nonunidirectional narrow channel.

(a) Circulation through an annular channel: It is possible to maintain a circulation of fluid indicated in Figure 4a by placing the pole of a bar magnet into the inner cylinder and sending an axial electric current through the fluid in the annulus between the cylinders in Figure 4a, as is shown in detail in a previous paper.\textsuperscript{7} Let us now assume that a radial temperature gradient is maintained in the fluid between the two cylinders as has been assumed in the cases shown in Figure 3a–e. What
effect will establishment of the circulation shown in Figure 4a have upon convective motion shown in Figure 3a–e?

The downward direction of the gravitational field vector accounts for the fact that the fluid moves upward adjacent to the warmer inner cylinder in the thermal circulation pattern shown in Figure 3a and on the right-hand side in a solid loop in Figure 4a. If we rotate the fluid ring with its streaming pattern 180°, the warm fluid near the inner cylinder will now be moving downward, as shown by the dotted loop in Figure 4a. This motion will, however, be opposed by the force of buoyancy so that the inverted convection will be retarded and eventually reversed if the fluid is left long enough in the new position. Let us now assume that the fluid is circulating counterclockwise through the annular channel at a uniform rate, as indicated in Figure 4a. It is clear that the convection pattern shown in the right half of the annular channel section of Figure 4a, where warm (less dense) fluid moves opposite to the direction of the gravitational field, will be subjected to retarding forces as the fluid rotation carries it into the left channel section in which the previously ascending warm fluid near the inner cylinder becomes a descending stream. The retardation will apply in similar fashion to all parts of the convection pattern shown in Figures 3a and 4a when it is inverted. It can be shown by the same argument that the analogous and in some cases more complex convection patterns shown in Figure 3b–e will be similarly inhibited by inversion in the course of the circulation of fluid through the ring-shaped channel. It is obvious that the extent to which the convection will be inhibited depends on the rate of circulation of the fluid in the annulus (Fig. 4a). The shorter the time between two successive inversions of the convection pattern, the less kinetic energy will be accumulated in the developing convection. Experiments have shown that evidence of thermal convection is nearly completely absent at moderate circulation rates corresponding to periods of revolution in the vicinity of 40 sec in annuli of about 3-cm diameter at current densities of 20 m A/cm² in buffers of 1,500 ohm-cm resistivity. A thin stream of fluid containing ions or charged microscopic particles to be subjected to electrophoretic fractionation can thus be injected into the circulating buffer at the center of the annulus of Figure 4a and be maintained in circulation without objec-
tionable deterioration by thermal convection up to 22 revolutions in favorable cases. This corresponds to a linear particle path of about 2 meters. The circulation channel does not have to be circular, as shown in Figure 4a. Other channel shapes, such as "squares" with rounded corners or cross sections approximating elliptical shapes, have been used successfully. Figure 4b shows, merely as an illustration of a general case, a channel of arbitrary noncircular cross section which would operate in a fashion similar to the circular cross section.

(b) **Horizontal transfer of fluid through a serpentine channel:** The essential condition for stabilization against thermal convection is the alternating inversions of the convection pattern described above. It can also be obtained in a noncirculating mode of fluid motion as shown in Figure 5a. The vertical sections of the

![Diagram](image)

Fig. 5.—(a) "Horizontal" serpentine channel. The buffer is transferred from left to right as it meanders through the channel. The pattern of convection engendered in vertical sections is inverted relative to the gravitational field vector \( g \) as it is transferred from one vertical channel to the adjacent vertical channel. (b) "Vertical" serpentine channel. The buffer is transferred upward in a meandering flow pattern. The pattern of convection engendered in the horizontal sections is periodically inverted relative to the \( g \) vector.

The vertical sections of the serpentine channel are labeled \( A B C D \). Assume the convection pattern shown in Figure 1b to have formed in section \( A \). The fluid flow will transfer it into section \( B \) (indicated in dashed lines) where it will be suppressed by inversion. We obtain in the meandering flow of Figure 5a a repeated inversion of the convection pattern...
analogous to conditions prevailing in the circulating flow illustrated in Figure 4a and 4b. This results in equally effective stabilization against thermal convection. The horizontal sections joining the vertical channel sections A, B, C . . . are kept short so as not to leave enough time for the complex cellular thermal convection pattern described above for horizontal fluid sheaths to develop during the transit of fluid through them. Continuous electrophoretic separation can thus be conducted successfully by injecting (by means of injector IN) at the center of the channel A a thin stream of a mixture to be fractionated which is carried to the end of the channel without objectionable deterioration through thermal convection.\textsuperscript{11}

(c) \textit{Vertical transfer of fluid through a serpentine channel:} An established convection pattern represents storage of kinetic energy in the fluid. A certain amount of time is required to establish a given convection pattern as energy for its development is supplied at a constant rate. Conversely, when gravitational forces which establish a given convection pattern cease to act, the fluid motion decays through damping action of viscosity. The narrower the confines through which the fluid flows, the greater will be the velocity gradients at a given average speed, and the greater will be the rate of dissipation of the kinetic energy of the fluid by internal friction. Let us assume that a convection pattern shown in the short ascending stream section a of Figure 5a has been engendered by gravitational forces as described above. As soon as this convection pattern enters the horizontal channel section B, it is reoriented relative to the gravitational field so that this type of convection pattern can no longer be maintained. It will thus decay due to viscosity as it is transported through the horizontal section B. Similarly, the remaining horizontal sections (C, D . . . ) will attenuate the convection patterns engendered in the vertical sections which precede them. The effectiveness of this attenuation will increase as the channel width W is diminished.

As has been stated in a preceding section, complex convection patterns can also be engendered between horizontal plates in the presence of vertical temperature gradients creating an unstable density distribution in the fluid. Figure 5b shows that the type of convection pattern described above for the case of a thin fluid layer between horizontal plates will also tend to be inhibited by successive inversions of the convection pattern as it moves consecutively through the sections A, B, C . . . G. This is illustrated by the vortices in channels D and E. Experiment\textsuperscript{11} shows very satisfactory stabilization against thermal convection in this mode of operation, resulting in sharp separation patterns.

Increase in viscous damping of thermal convection by utilizing narrower channels has an important limitation. The streak, injected at the center of the buffer stream, containing the particles to be separated by electrophoresis tends to sediment and may be deposited on the bottom walls of the horizontal channel sections. This would, for instance, happen in a serpentine cell of the type illustrated in Figure 5a if it were made very long by adding many more sections to A B C D. In the rotating column of Figure 4a and in the vertical serpentine cell of Figure 5b the particles oscillate about the channel midline instead of settling continuously downward. For instance, in Figure 5b, a particle issuing from injector IN at the channel center will drop below the midline as it enters the vertical section a. It will be to the right of the midline in a and will enter the horizontal section B above the midline. As it moves through B, it drops below the midline, and is to the left of the midline.
as it rises in b to enter c above the midline, etc. It can be easily seen that a similar oscillation about the midline takes place in the rotating fluid annulus of Figure 4a and in the more general situation of Figure 4b. These oscillations can be made quite small by adjusting the density of the buffer to match the particle density and by speeding up the rate of buffer flow to shorten the period of settling.

The principle of eliminating thermal convection in continuous flow electrophoresis presented here can be implemented experimentally in a variety of ways. The various methods of approach will be published in this journal.

Summary.—The process of generation of thermal convection is considered with an emphasis on two special cases: (1) a thin fluid sheath between two parallel vertical walls with a horizontal temperature gradient maintained in the fluid; (2) a thin fluid sheath between two parallel horizontal plates with a vertical temperature gradient maintained in the fluid to obtain a density decrease with depth. The patterns of thermal convection are described, and it is shown how both types of convection can be inhibited by allowing the fluid sheath to flow through a closed or a meandering channel in such a fashion that the relative orientation between the gravitational field and the convection pattern is periodically reversed.

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STUDIES ON THE ACTIVE SITE OF CHYMOTRYPSIN*

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Studies were undertaken to investigate the nature of the amino acids, especially of the serine residue, associated with the phosphopeptides of DIP-chymotrypsin,1 DIP-trypsin,2 and DIP-subtilisin (Novo).4  

α-Chymotrypsin was first investigated. In a typical experiment, 2.5 gm of 3 X recrystallized α-chymotrypsin was converted to its DIP-derivative by treatment with DFP at pH 8.0. After the removal of excess DFP by extensive dialysis against 0.001 N HCl, the DIP-protein was subjected to enzymatic degradation at 37°C in the following order: the pepsin at pH 2.10 (8 hr); chymotrypsin and trypsin at pH 8.0 (8 hr); carboxypeptidases A and B at pH 7.80 (8 hr); and finally with