Another means of minimizing the required precision would be to make the ratio $W/d$ as small as possible.

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# SUPPRESSION OF THERMAL CONVECTION AND OF SIMILAR TYPES OF FLUID STREAMING BY AN ELECTROMAGNETIC FORCE FIELD

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SUMMARY

A method is described for increasing the stability of a fluid column against convection (such as thermal or electromagnetic convection) by superimposing upon a stabilizing vertical density gradient a parallel gradient of electromagnetic force density. The latter gradient is generated by creating a gradient of electrical conductivity concomitant with the vertical density gradient and maintaining at right angles to this gradient electric current traversed by a perpendicular homogeneous magnetic field. To obtain a stable column, one must choose the directions of the current and the magnetic field such as to make the electromagnetic force vector point in the direction of increasing electrical conductivity. Conditions under which the stability of a density gradient is reinforced, diminished or neutralized are considered as well as the engendering of instability by electromagnetic forces in a column stabilized by a density gradient.

## INTRODUCTION

Thermal convection is one of the main experimental difficulties in electrophoresis in liquid columns[1] and in ultracentrifugation[2]. It is equally disturbing in electromagneto-

*References p. 543.*

phoresis[3,4,10]. In the latter processes, in addition to thermal convection, an electromagnetic convection can be observed which is due to non-uniformities in the magnetic and the electric current fields[5]. For successful uses of fractionation methods based on these processes, it is essential to suppress fluid convection as completely as possible.

An effective method of suppressing thermal convection has been used in connection with rapid electrophoretic separations in gradient columns[6,7]. This method consists of stabilizing the liquid column against thermal convection by establishing in it a steep density gradient concomitant with a chemical concentration gradient in which the density increases in the downward direction. This gradient is generated by dissolving a non-ionizing substance, such as sucrose or glycerol, in the fluid column with an appropriate concentration gradient. Density gradient electrophoresis has also been used by SVENSSON AND VALMET[8] in longer electrophoretic separation columns.

An alternative general method for the suppression of thermal convection[9] is based on rotation of the migration cell about an axis perpendicular to the force of gravity. Thus, the volume elements in the rotating fluid "see" a rotating gravitational field and thermal convection, which depends on the presence of a unidirectional field of gravity, cannot develop.

The present paper suggests an electromagnetic method of stabilization of fluid columns which may be used instead of the density gradient method or, preferably, in conjuction with it to augment the stability of the fluid against convection. The method is based upon the fact that a force field, similar to a gravitational field, can be generated in a conductive fluid traversed by an electric current of density $\boldsymbol{J}$ at right angles to a magnetic field of flux density $\boldsymbol{B}$[3]. The force density $f = [\boldsymbol{J} \times \boldsymbol{B}]$ corresponds to $\delta g$, the weight of a unit volume, in the analogous gravitational case ($\delta$ being the mass per unit volume and $g$ the acceleration of gravity). If $\boldsymbol{B}$ and $\boldsymbol{J}$ are uniform, the case is analogous to a fluid of uniform density in an homogeneous

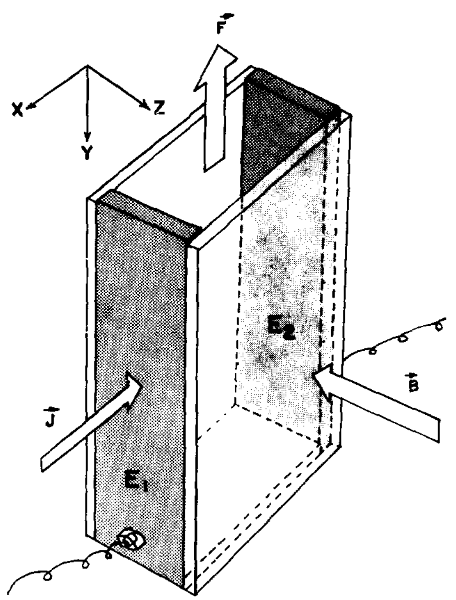


Fig. 1. Electromagnetophoresis cell. $J$: current density; $B$: magnetic flux density. $F$: electromagnetically engendered force. $E_1$, $E_2$: electrodes.

References p. 543.

gravitational field. If $J$ in the product $|J|\,|B|$ varies along the $f$ axis due to a variation in the electrical conductivity $\sigma$ of the solution, we have a case analogous to a fluid column with an axial density gradient. If the magnitude $|f|$ increases in the direction of the force vector, $f$, we have a stable condition which corresponds to a density gradient in which the density increases in the downward direction. In the converse case, the liquid column is as unstable as a fluid column with an upward increase in density in a gravitational field.

A gradient of the $[\boldsymbol{J} \times \mathbf{B}]$ force magnitude can be created by establishing a conductivity gradient in a cell which is provided with electrodes and is exposed to a magnetic field $\mathbf{B}$ as illustrated in Fig. 1. While the potential difference V between the parallel electrodes $E_1$ and $E_2$ is constant, the current density $\boldsymbol{J}$ between them varies in accordance with

$$\boldsymbol{J} = \sigma \frac{\partial \mathbf{V}}{\partial x}, \tag{1}$$

where $\sigma$ is the conductivity which varies along the $Y$ axis and $\partial \mathbf{V}/\partial \boldsymbol{x}$ the constant potential gradient. For the magnetic field vector $\mathbf{B}$ shown in Fig. 1, the force density $\boldsymbol{f}$ is parallel to the $Y$ axis. A stable conductivity gradient can be established easily by coupling it with a density gradient. For instance, one can dissolve sodium chloride in a highly concentrated sucrose or urea solution and fill the lower half of the cell shown in Fig. 1 with this dense liquid of high conductivity. Over this solution, saline of much lower conductivity and density is then poured. After stirring of the boundary of this solution a conductivity gradient with a concomitant density gradient is spread over the entire length of the cell. For simplicity, we assume these gradients to be uniform. If the directions of $\mathbf{B}$ and $\boldsymbol{J}$ are chosen so that $\boldsymbol{f}$ has the same direction as the gradients of conductivity and density, the gradient of the electromagnetic force density reinforces the gravitational stabilizing action of the density gradient. If, on the other hand, the direction of $\boldsymbol{f}$ is reversed by reversing $\mathbf{B}$ or $\boldsymbol{J}$, the stabilizing action of the density gradient is counteracted by the gradient of the electromagnetic force density and the fluid column becomes unstable when the product $[\boldsymbol{J} \times \mathbf{B}]$ exceeds a critical value. To determine this critical condition, we consider the following equation for the resultant force density $\boldsymbol{F} = F\,\mathbf{y}$:

$$F\,\mathbf{y} = (\delta\, g)\,\mathbf{y} + [J\,B]\,\mathbf{y} \tag{2}$$

where $\mathbf{y}$ is the vertical unit vector, $\delta$ the mass per unit volume in g/ml, $g$ the acceleration of gravity in cm/sec$^2$, $B$ the magnetic flux density in gauss and $J$ the current density in abamperes/cm$^2$. If $J^*$ designates the current density in amp/cm$^2$, we can express $J = 1/10 \cdot J^*$ in terms of the potential gradient $\delta V/\delta x$ (volts/cm) and the conductivity $\sigma$ ((ohm–cm)$^{-1}$) as follows:

$$\boldsymbol{J}^* = \sigma \frac{\partial V}{\partial x} = 10\,\boldsymbol{J}\,. \tag{3}$$

The following relation between the gradients can be obtained from (2) and (3):

$$\frac{\partial F}{\partial y} = g \frac{\partial \delta}{\partial y} + \frac{1}{10} B \frac{\partial V}{\partial x} \frac{\partial \sigma}{\delta y} = g \frac{\partial \delta}{\partial y} + \frac{\partial f}{\partial y}\,. \tag{4}$$

Assuming the downward direction of the density gradient $\partial\delta/\partial y \cdot \mathbf{y}$ as the positive

*References p. 543.*

(1) The conductivity increases, as does the density, in the downward direction and $\partial V/\partial x$ is positive, *i.e.*, force $f$ points in the direction of increasing $\sigma$. The second term in equation (4) is positive and the electromagnetic force density gradient reinforces the gradient of weight density so that $\partial F/\partial y > g \cdot \partial \delta/\partial y$. The stability of the solution against thermal convection is increased by the electromagnetic field combination.

(2) $\partial \sigma/\partial y < 0$ and $\partial V/\partial x < 0$. The conductivity decreases in the downward direction and $\partial V/\partial x$ is negative. As in (1), $\partial F/\partial y > g \cdot \partial \delta/\partial y$ and the stabilizing action of the density gradient is again increased by the electromagnetic effect.

(3) $\partial \sigma/\partial y < 0$ and $\partial V/\partial x > 0$. The conductivity distribution is as in (2) and the electromagnetically engendered force points in the direction of decreasing $\sigma$. The second term in equation (4) is negative and $\partial F/\partial y < g \cdot \partial \delta/\partial y$. The fluid column is reference direction, we distinguish the following cases which are of practical interest*: rendered less stable against convection. We can distinguish three cases under these conditions:

a) $|g \cdot \partial \delta/\partial y| = |1/10 \cdot B \cdot \partial V/\partial x \cdot \partial \sigma/\partial y|$: There is no stabilizing gradient.

b) $|g \cdot \partial \delta/\partial y| > |1/10 \cdot B \cdot \partial V/\partial x \cdot \partial \sigma/\partial y|$: The stability of the fluid column is diminished by the electromagnetic force.

c) $|g \cdot \partial \delta/\partial y| < |1/10 \cdot B \cdot \partial V/\partial x \cdot \partial \sigma/\partial y|$: The fluid column is unstable.

Equation (a) is the critical condition under which the fluid column becomes unstable.

(4) $\partial \sigma/\partial y > 0$ and $\partial V/\partial x < 0$. The conductivity distribution is as in (1) and the electromagnetically engendered force points upward in the direction of decreasing $\sigma$. The second term in equation (4) is negative and $\partial F/\partial y < g \cdot \partial \delta/\partial y$. We obtain again the same three special conditions of stability as in (3).

Thus, only cases (1) and (2) have practical value for stabilization against thermal and other types of convection.

A practical example, using rough round figures will provide an idea of the order of magnitude of the effect to be expected. Let us suppose that we establish by stirring a concentration gradient between a dense, good electrolytic conductor prepared from 5 % NaCl solution and sucrose (58 g sucrose per 100 ml solution) having the approximate conductivity of $\sigma \simeq 1.5 \cdot 10^{-2}$ $(\Omega\ \text{cm})^{-1}$ and density of $\delta \simeq 1.16$ g/ml and a less dense inferior electrolytic conductor (0.1 % NaCl) of conductivity $\sigma \simeq 3.3 \cdot 10^{-3}$ $(\Omega\ \text{cm})^{-1}$, and density $\delta \simeq 1.00$ g/ml. Assume a uniform gradient extending over a length of 1 cm, the density gradient, conductivity gradient, the force of gravity and the electromagnetic force having the same direction. We shall now compare the magnitude of the electromagnetically engendered gradient of force density to the gravitational weight density gradeint:

1. Electromagnetically engendered gradient: $\partial f/\partial y = 1/10 \cdot B \cdot \partial V/\partial x \cdot \partial \sigma/\partial y$. Assuming $B = 10^4$ gauss; $\partial V/\partial x = 25$ volts/cm and $\partial \sigma/\partial y$ $(1.5 \cdot 10^{-2} - 3.3 \cdot 10^{-3}) \simeq 1.2 \cdot 10^{-2}$ $(\Omega\ \text{cm})^{-1}$/cm, we get $\partial f/\partial y \simeq 3.10^2$ dynes/ml/cm.

By comparison, the weight density gradient $g \cdot \partial \delta/\partial y \simeq 10^3$ $(1.16 - 1.00) = 1.6 \cdot 10^2$ dynes/ml/cm is considerably smaller.

The effect of a conductivity gradient upon stability of fluid columns exposed to the combination of magnetic and electric current fields described above is illustrated by the following sequence of photographs, Fig. 2. They depict an aqueous solution

* In all of these cases $\partial \delta/\partial y$ is assumed to be $> 0$; the case of $\partial \delta/\partial y < 0$ is unstable (density increases upward) and, hence, of no interest.

*References p. 543.*

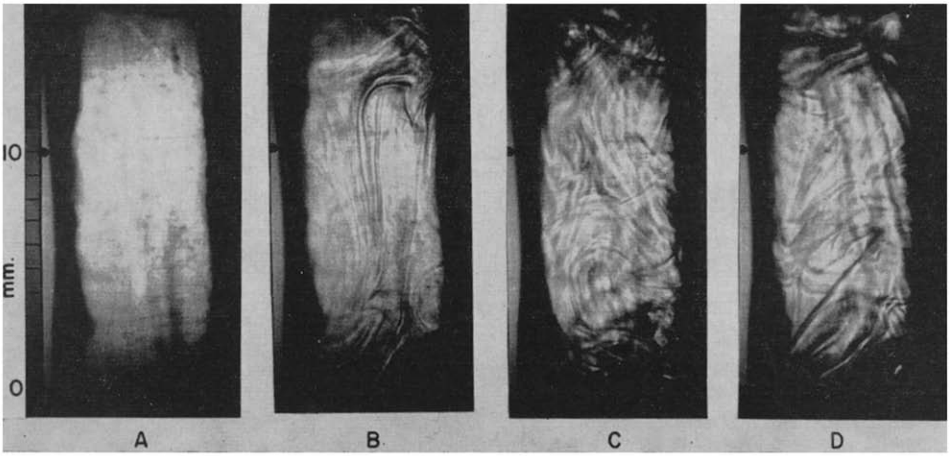


Fig. 2. Effect of phase relation between the magnetic field and current upon stability of the fluid. A: The electromagnetic force points in the direction of the density and conductivity gradients. The fluid column is stable. B, C, D: The electromagnetic force points in the direction of diminishing density and conductivity. Instability of the column is evidenced by turbulence.

of NaCl and urea with a density gradient and conductivity gradient both directed downward. The cell is of the type shown in Fig. 1. It is traversed by a 60 cps alternating current (of density $J \simeq 500$ mA/cm$^2$ in Figs. *A* and *B* and $J \simeq 250$ mA/cm$^2$ in Figs. C and D). The transverse alternating magnetic field of 6,000 gauss is in phase with the cell current. The electromagnetic force exerted upon the fluid does not vary in direction under these conditions. Fig. 2A was taken with the electromagnetic force density $f$ pointing in the direction of the gradients of $\delta$ and $\sigma$. The gradient was prepared by mixing a 20% NaCl solution saturated with urea with distilled water in A and B. In C and D the dense solution was 1% NaCl solution saturated with urea. In A the electromagnetic and the gravitational force density gradients reinforce each other assuring stability. Figs. 2B–D show the turbulence which is engendered by instability as soon as the phase of the cell current is reversed. The direction of $f$ is now reversed and the conductivity gradient becomes "top heavy" in the same sense as an inverted density gradient with layers of high density located above less dense layers. To produce turbulence, the current $J = \sigma\, \partial V/\partial x$ must exceed a minimum value since, for instability to develop, we must have $|1/10 \cdot B \cdot \partial V/\partial x \cdot \partial\sigma/\partial y| > |g \cdot \partial\delta/\partial y|$.

This principle has been used successfully to suppress thermal and electromagnetic convections in electromagnetophoresis. The possibility of using it for elimination of thermal convection in electrophoresis will be explored.

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# ELECTROPHORETIC STUDIES ON CYTOCHROME OXIDASE

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SUMMARY

A modified preparation of cytochrome oxidase has been described. The preparation is extremely low in contaminating hemoproteins. Because it required only a low salt concentration for solubility, it was suitable for electrophoretic studies. Paper electrophoresis resulted in the separation of two fluorescent and three hemoprotein areas. Employing a spray reagent which contained a leuco dye and cytochrome *c*, it was possible to locate active cytochrome oxidase in only one area of the paper. Column electrophoresis of the preparation showed that at least partial separation of the hemoprotein having cytochrome oxidase activity from inactive hemoprotein had occurred. Both hemoproteins fractions exhibited the spectral properties of cytochromes $a$ and $a_3$, and the ratio of these cytochromes was uniform throughout.

## INTRODUCTION

The cytochrome "$a$" components of the respiratory chain are defined by two distinct properties, spectral character and enzymic activity. Employing spectral examination, most workers have concluded that cytochromes $a$ and $a_3$ are distinct entities[1-3]. Spectral studies show that cytochrome $a_3$ is autoxidizable and that it reacts with carbon monoxide and cyanide, while cytochrome $a$ is not autoxidizable and is unreactive toward these respiratory inhibitors. In their kinetic studies SMITH[4], CHANCE[5], and LUNDEGARDH[6] noted that in the steady state the absorption spectra of the two cytochromes could be altered to different extents. It was also observed[3,7,8] that the molar ratio of cytochrome $a_3$ to cytochrome $a$ varied according to the tissue studied, this ratio being 2.3 in *Bacillus subtilis* and 1.0 in yeast and heart muscle. Further evidence in support of the separate existence of cytochrome $a_3$ is noted in

*References p. 550.*