1-1-1976

Investigation into a system for the automatic measurement of electrophoretic velocity.

Louis Filo

Follow this and additional works at: http://preserve.lehigh.edu/etd

Part of the Electrical and Computer Engineering Commons

Recommended Citation

This Thesis is brought to you for free and open access by Lehigh Preserve. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of Lehigh Preserve. For more information, please contact preserve@lehigh.edu.
INVESTIGATION INTO A SYSTEM FOR THE AUTOMATIC MEASUREMENT OF ELECTROPHORETIC VELOCITY

by

Louis Filo III

A Thesis

Presented to the Graduate Committee of Lehigh University in Candidacy for the Degree of Master of Science in Electrical Engineering

Lehigh University

1976
This thesis is accepted and approved in partial fulfillment of the requirements for the degree of Master of Science.

(date)

Professor in Charge

Chairman of Department
### Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>1</td>
</tr>
<tr>
<td>I. Theory</td>
<td>2</td>
</tr>
<tr>
<td>II. Optics</td>
<td>19</td>
</tr>
<tr>
<td>III. Electronics</td>
<td>33</td>
</tr>
<tr>
<td>IV. Conclusions and Suggestions for Further Investigation</td>
<td>57</td>
</tr>
<tr>
<td>Bibliography</td>
<td>61</td>
</tr>
<tr>
<td>Vita</td>
<td>62</td>
</tr>
</tbody>
</table>
ABSTRACT

A method of automatic measurement of drift-velocity of microscopic particles in watery suspension is proposed. It is especially conceived to measure the electrophoretic velocity of colloidal particles in order to determine the \( \zeta \) potential. The particle images pass along a Ronchi grating and create a signal in a photomultiplier. The average frequency of that signal is a measure of that velocity.

The basic optical setup is tested and circuitry developed and tested that would process the rather noisy and random primary signal and would allow digital read out. Part of the objective is also to develop means to detect the polarity of the movement (direction) in addition to the velocity magnitude. This is achieved by using a split image and two Ronchi gratings, producing two signals in phase quadrature.
I. Theory

Electrophoresis is an electrochemical process in which small charged particles suspended in a liquid are caused to migrate under the influence of an electric field. The potential difference across the interface between the two phases (solid and liquid), at which movement occurs, is referred to as the electrophoretic or zeta potential. The scope of this section is to briefly describe electrophoresis, to discuss existing electrophoretic measuring techniques and to propose a new system for electrophoretic measurement.

Colloidal particles dispersed in water are prevented from sticking together and separate out as a coagulum because of the repelling action of like electric charges. These charges are found on the particle surface and are caused either by adsorbed ions that have been taken from the surrounding water or by ions which are an integral part of the chemical structure of the particle. By virtue of these charges, a colloidal particle will move toward an electrode of opposite charge.

The theoretical treatment of electrophoresis is based on a model which assumes the existence of an electrical double layer at the solid-liquid boundary. Helmholtz\(^1\) (1879) considered this double layer to consist of two oppositely charged layers at a fixed distance apart (of the order of a molecular diameter), which could be regarded as the equivalent of a capacitor. If these layers are \(d\) cm. apart and each carrying a charge \(\sigma\) per sq. cm., according to

electrostatics:

\[ \mathcal{R} = \frac{4\pi \sigma d}{\varepsilon} \]  

(Eq. 1.1)

where \( \mathcal{R} \) is the difference of potential between the layers, the zeta potential, and \( \varepsilon \) is the dielectric constant of the medium.

Guouy\(^2\), in 1910, utilized the idea of a diffuse double layer in which the solution side of the double layer is not merely one molecule, or so, in thickness, but extends for some distance into the liquid phase. In this region, thermal agitation permits the free movement of the ions present in the solution. The distribution of positive and negative charges is not uniform, though, since the electrostatic field arising from the charge on the solid will result in preferential attraction of particles of opposite sign. This is analogous to the Debye-Hückel concept of the oppositely charged ion-atmosphere surrounding a given ion. However, with approximations, the diffuse double layer leads to a result for the zeta potential of the same form as Helmholtz's sharp double layer.

A combination of Helmholtz's double layer and Guouy's diffuse double layer resulted in a model proposed by Stern\(^3\)(1924). According to Stern, the double layer consists of two parts; one, which is approximately of a molecular diameter in thickness and is supposed to remain fixed to the surface, while the other, is a diffuse layer extending for some distance into the solution. The fall of potential in the fixed layer is sharp, as seen in

\(^3\)Ibid., p. 525-526.
Fig. 1.1, between points A, C, as compared to the gradual fall in the diffuse layer, C, B. A, is the potential at the boundary of the solid phase and C, is the potential at the limit of the fixed part of the double layer. B, represents the potential of the bulk of the liquid.

Fig. 1.1. Stern's model.

The fall in potential may occur in two general ways (I and II versus III in Fig. 1.1) depending upon the nature of the ions and molecules present in the solution. The zeta potential, $\zeta$, is the potential difference between the fixed and freely mobile parts of the double layer, in other words, between points C and B in Fig. 1.1. In spite of differences in charge density distributions between this model and Helmholtz's and Gouy's models, for dilute solutions all give correct results.

The zeta potential is influenced by the nature of the electrolyte.

Generally, it is made more positive by acids (hydrogen ions) and more negative by solutions of alkalis (hydroxyl ions). As the concentration of the electrolyte increases, the zeta potential reaches a maximum and then decreases. In Fig. 1.1, a maximum zeta potential exists at I and as the electrolyte concentration is increased, the zeta potential decreases (II) and changes sign at III.

A simple equation for electrophoretic movement in free solution was derived by Alexander and Johnson\(^5\) in 1949 for small non-conducting particles, where the diffuse double layer is larger than the radius of the particle. If a particle of radius \(r\) is immersed in a fluid of viscosity \(\eta\) (poise), and dielectric constant \(\varepsilon\), it bears a net charge \(Q\), and will attain a terminal velocity \(v\), under the influence of an electric field \(\vec{E}\), such that the frictional resistance of the medium in which it moves just balances the electrical force. For a spherical particle Stoke's law can be applied and

\[
Q\vec{E} = 6\eta rv
\]  
(Eq. 1.2)

Now, by introducing the mobility \(u\) and rearranging yields

\[
u = \frac{v}{E} = \frac{Q}{6\pi\eta r}
\]  
(Eq. 1.3)

which shows that the velocity is governed by particle size and net charge. Alexander and Johnson discovered experimentally that changing the geometry of the particle only changed the numeral 6 in the denominator (from 6 to 4 or 8). Thus, by measuring the velocity of the particle, the electrophoretic mobility can be

determined and then the zeta potential by the equation

\[ \eta = \frac{4\pi \eta u}{\varepsilon} \quad (\text{Eq. 1.4}) \]

The electrophoretic velocities of small colloidal particles, as well as larger quartz particles and oil drops are usually about 2 to \( 4 \times 10^{-4} \) cm./sec. in water. Thus, the value of the zeta potential as given by Eq. 1.4, with \( \eta \) being .01 poise and \( \varepsilon \) approximately 80, is between 0.03 and 0.06 volt in each case.\(^6\)

Various methods exist for making electrophoretic measurements and will be briefly discussed. The first is the microscopic method in which the colloidal solution or suspension of particles under examination is placed in a special micro-electrophoresis cell which is fixed on the stage of a microscope. The cell is usually in the form of a hollow microscope slide with electrodes sealed in at each side and connected to the EMF source. Velocity is measured by timing particle movement across the plane of a calibrated graticule placed in the microscope eyepiece. This method has the advantages of simplicity, time required, and the information it gives concerning the size, shape, and orientation of the particles. It has been suitable for measuring the electrophoresis of metal sols, emulsions, suspensions of bubbles, and of biological materials such as bacteria, blood cells, or fungi.

A more satisfactory measurement of electrophoresis is the moving boundary "macroscopic" method advanced by the developmental

---

\(^6\) Glasstone, op. cit., p. 522 - 533.

\( \eta \) is expressed in electrostatic (e.s.) units. Multiply Eq. 1.4 by \( 9 \times 10^4 \) when the electrophoretic mobility is for a potential gradient of 1 volt/cm. and the zeta potential is to be in volts.
work of Tiselius\(^7\)(1930). In this method, the boundary between a buffer solution and a solution of colloidal particles moves with a speed equal to that of the electrophoresis of the particles when an EMF is applied.

Fig. 1.2(a)\(^8\), shows a simple Tiselius electrophoretic cell.

Fig. 1.2. Tiselius electrophoretic cell.

The channels in sections A and B are filled with the experimental

system up to bb'. Section C is slid aside and filled with a buffer solution and then slid back to its original position, thus, forming a sharp boundary at bb'. The cell is connected through a closed system to reversible silver and silver chloride electrodes in vessels containing a reservoir of buffer. Upon application of electrical potential, the boundaries move at the electrophoretic velocity, Fig. 1.2(b). By making use of the refractive index difference between the colloidal particle solution and the buffer solution, direct optical observation and photographic recording can be made of the concentration of the particles as well as the rate of migration. This method has proved very effective in the analysis of protein components. The distinguishing features of the moving boundary method are: (1) migration of a boundary in a free solution, (2) thermostatic control to reduce temperature convection, (3) photographic recording of the pattern, and (4) quantitative evaluation of electrophoretic composition and mobility.

The method proposed by this paper is an extension of the microscopic method. Electrophoretic movement is observed through the microscope, but unlike the microscopic method, particle velocity is determined electronically. Fig. 1.3 depicts the experiment setup.
Fig. 1.3. Sketch of proposed system for automatic electrophoretic measurement.
An electrophoretic cell containing a capillary tube for supporting the suspension is mounted in front of a microscope. Illumination of the cell is provided by a helium neon laser and upon application of potential $V_{dc}$, particle motion is detected by the photomultipliers. The signals detected contain information about the directionality and velocity of the particles and are processed in the electronics section; direction of the particle flow and velocity are thus determined.

On the beamsplitter are mounted two Ronchi gratings, each with 50 lines to the inch. It is proven below that as particles are detected passing the gratings, the summation of the detected signals is approximately a sinusoidal function, the frequency being proportional to the terminal velocity of the particles. Fig. 1.4 shows the gratings mounted $90^\circ$ out of phase with one another producing a sine and a cosine function, which are required for electrical processing.

![Diagram](image)

**Fig. 1.4.** Beamsplitter and Ronchi gratings.
In Fig. 1.5 it is seen, that as the image of a particle moves across the gratings, a periodic signal with some average dc level is detected by the photomultipliers. If the image diameter is comparable to the grating spacing this signal will be approximately sinusoidal. As the number of particles, \( N \), increases, assuming they are all of the same size, the resultant signal observed is a summation of \( N \) sine waves of equal amplitudes with random phase angles, and therefore, a sine wave itself (except for the rare case when accidentally there is complete destructive interference).

The diagram of Fig. 1.6 depicts the vector summation of sine functions with random phase angles, represented by phasors, and the resultant phasor, which rotates at the frequency representing the particle velocity.
To simulate the proposed system, a model with $n$ particles is constructed. All particles are assumed to move at the same velocity and at the end of a "cycle", one second in this case, one particle is removed and replaced by another with random phase. A function of the form

$$X_k = A \sin (\omega t + \phi_k)$$  \hspace{1cm} (Eq. 1.5)

is assumed to describe a particle moving across the field, where

- $A = \text{constant}$
- $t = \text{constant}$
- $\phi_k = \text{random phase angle uniformly distributed}$
  over the interval 0 to $2\pi$.

Therefore, the total output of the system, $n$ particles, can be expressed mathematically

$$X_n(t) = A \sum_{k=1}^{n} \sin (\omega t + \phi_k)$$  \hspace{1cm} (Eq. 1.6)

and every second, out of the collective $\phi_1, \ldots, \phi_n$, one $\phi_k$ is replaced by a different random number out of the interval 0 to $2\pi$.

After the first second, $\phi_1$ is replaced, after the second, $\phi_2$, etc.

In determining the mean amplitude of Eq. 1.6, the probability
density function for $X_n(t)$ has to be found. Lathi\(^9\) describes a random function process such as Eq. 1.5, where

$$p_*(\phi) = \begin{cases} 
\frac{1}{2\pi} & 0 < \phi < 2 \\
0 & \text{otherwise} 
\end{cases} \quad (\text{Eq. 1.7})$$

for which the probability density function is

$$p(X; t) = \frac{p_*(t)}{dX_k/d\phi} \quad (\text{Eq. 1.8})$$

Since here $X_k$ is a double-valued function of $\phi$,

$$p(X; t) = \frac{p_*(\Phi_1)}{|dX_k/d\Phi_1|} + \frac{p_*(\Phi_2)}{|dX_k/d\Phi_2|} \quad (\text{Eq. 1.9})$$

and,

$$\frac{dX_k}{d\phi} = A \cos(\omega t + \phi)
= A \left[ \sqrt{1 - \sin^2(\omega t + \phi)} \right]
= A \sqrt{A^2 - X^2} \quad (\text{Eq. 1.10})$$

Upon substitution of Eq. 1.7 and Eq. 1.10 into Eq. 1.9

$$p(X; t) = \frac{1}{\pi \sqrt{A^2 - X_k^2}} \quad (\text{Eq. 1.11})$$

and this density is shown in Fig. 1.7.

![Probability density distribution of Eq. 1.11.](image)

**Fig. 1.7.** Probability density distribution of Eq. 1.11.

By analogy, the probability density for $X_n(t)$ is

$$p_n(x_k; t) = \frac{1}{\pi \sqrt{n A^2 - x_k^2}}$$

(Eq. 1.12)

Now, the mean square value of $X_n(t)$ can be computed;

$$\overline{X_n^2(t)} = \int_{-\infty}^{\infty} x_n^2 p(x_k; t) dx$$

$$= \int_{-A}^{A} x_n^2 \frac{n}{\pi \sqrt{n A^2 - x_k^2}} dx$$

$$= \frac{n A^2}{2} \left[ -\frac{X_k}{2} \sqrt{A^2 - x_k^2} + \frac{A^2}{2} \sin^{-1} \frac{x_k}{|A|} \right]_A$$

(Eq. 1.13)

Since the mean amplitude is equal to the square root of twice the mean square value,

$$\text{Mean Amplitude} = \sqrt{2 \overline{X_n^2(t)}} = \sqrt{2 \frac{n A^2}{2}}$$

$$= \sqrt{n} A$$

(Eq. 1.14)

This result implies that for a system of $n$ random functions (particles) as described by Eq. 1.5, the mean amplitude of the summed total $X_n(t)$ is nothing more than the square root of the total number of functions (particles), $n$, times the function amplitude $A$.

With the aid of a computer, a program was written to calculate and plot Eq. 1.6 for the case, $n = 100$. A program flow chart appears in Fig. 1.8.
\text{Start} \\
\begin{align*}
\text{Generate 200 } \\
\phi_k \text{'s and} \\
\text{store} \\
\text{t} = t_{\text{start}} \\
\text{t} = t + .025 \\
\text{Generate 1 sine} \\
\text{wave} \\
x = A\sin(\omega t + \phi_k) \\
\text{40 time} \\
\text{points} \\
\text{no} \\
\text{SUM}(1-40) \\
\text{no} \\
\text{100} \\
\text{x_k's} \\
\text{yes} \\
\text{Output Wave}(1-40) \\
\text{= SUM}(1-40) \\
\text{no} \\
\text{10} \\
\text{seconds} \\
\text{yes} \\
\text{Plot} \\
\text{Output Wave}(1-40)
\end{align*}

\text{Fig. 1.8. Flow chart of computer program to calculate Eq. 1.6.}
Initially, 200 $\Phi_k$'s are generated by a random number generator and stored in an array. Then $\Phi_1$ is used to compute $X_1(t)$ with 40 time points per second resolution over the first one second interval (0 to 1 second) and $X_1(t)$ is stored in the array $\text{SUM}(1-40)$. Next, $X_2(t)$ is computed, and then $X_3(t)$, and so forth until $\text{SUM}(1-40)$ contains 100 summed $X_k$'s, which is represented by Eq. 1.6 over the zero to one second interval. $\text{SUM}(1-40)$ is then transferred to Output Wave(1-40). For the next one second interval (1 second to 2 seconds) $\Phi_1$ is removed and $\Phi_{101}$ added to the collective which is again summed and stored in $\text{SUM}(1-40)$. This process is continued for ten seconds and illustrates a hypothetical electrophoretic experiment in which particles are entering and leaving a network being observed. The output, Output Wave(1-40), is plotted in Fig. 1.9 and shows an average amplitude of approximately 18 as compared to a theoretical mean amplitude (Eq. 1.14) of 10.
Thus, it has been proven that particles randomly distributed and passing by a network of gratings with spacing comparable to the particle diameters sum together and can be used for further processing. In this experiment, glass particles of approximately 20 $\mu m$ size are used and in the next section the optical system and experimental results are discussed.
II. Optics

The optical section, designed for the detection of particle motion, is to be discussed in this chapter. At first, the overall optical system will be described and then results will be presented. Shown in Fig. 2.1 is a sketch of the components of the system, which was bread-boarded on an optical table. Fig. 2.2 contains a photograph of the optical setup and Fig. 2.3, the optical schematic.

Fig. 2.1. Sketch of proposed optical system.
The need for high contrast dark field illumination dictated the use of the helium neon laser, which illuminates the capillary tube in the cell. Not only does the laser provide more brightness than some standard source (incandescent light bulb), but also reduces reflections from the capillary wall as shown in Fig. 2.4.
Fig. 2.4. Comparison of standard light source with laser for capillary illumination.

The ease of making and cleaning a capillary tube as compared to one with a rectangular cross section suggested its use. A drawback is that fluid flow in a cylinder creates a velocity gradient related to the viscosity of the suspension (fluid velocity is greatest in the center and least at the stationary walls). Experimental results of other researchers suggest taking electrophoretic measurements at one fifth the distance from one wall to the next. However, since this experiment was designed to demonstrate the feasibility only, strict quantitative results were not taken.

The cell, converging lens, and mirrors were all mounted on X - Y positioning blocks, thus providing means for making the critical optical adjustments. Fluid composed of a sodium chloride solution and glass particles was pumped through the capillary via
a syringe and collected in a flask at the other end. A scanning electron microscope (SEM) photograph of the glass particles appears in Fig. 2.5. The 5X objective lens setting was used on a standard microscope with a 10X eyepiece for direct observation. A beamsplitter with Ronchi gratings attached to its faces and two photomultipliers for detecting the particle motion complete the optical setup.

The cell was constructed using plexiglass, projector slide cover glasses, and silicone sealer (see Fig. 2.6). The insert was also made of plexiglass and contained the glass capillary and brass tubes which doubled as electrodes. To prevent conduction between the brass electrodes and the water which filled the cell, the exposed sections were painted with an enamel paint. The capillary, which was of .058" inside diameter, was bent towards the cell glass window so that it was within the focal length of the objective lens of the microscope. A microscope slide was placed on top of the cell making contact with the water to make a window for the laser beam. This is shown in Fig. 2.7. With the mirror and collimating lens mounted above the cell, the laser beam could be focused on the area to be observed.
Fig. 2.5. Scanning electron microscope photograph of glass particles.
Fig. 2.6. Electrophoretic cell construction.
Fluid was pumped into the tubing using a syringe and care had to be taken in avoiding air bubbles in the capillary. Repeated manual pumping provided means for stirring the fluid when necessary. With the application of a potential across the electrodes, an electric field was established causing particle motion towards one electrode. Reversal of the potential produced particle flow towards the other electrode. Experimentation necessitated the use of a few grains of sodium chloride to the fluid for better conduction. Through the microscope, the particles appeared as bright spots against a dark red background. (oscilloscope photograph appears in Fig. 2.8).
Fig. 2.8. Photograph of particle motion viewed through the microscope with an applied potential of 150 volts and an exposure time of approximately 1 second.
To detect the signals through the beamsplitter, two DuMont 6467 photomultiplier tubes were used. These were mounted with the beamsplitter inside a blackened box and were powered by a 1 kVdc supply. To amplify the small outputs of the photomultipliers, the amplification circuit of Fig. 2.9 was built for each tube.

Fig. 2.9. Photomultiplier amplifier.

Table 2.1 contains measured currents at various potentials and the calculated electric field strengths between the electrodes, 5.32 cm. apart.
<table>
<thead>
<tr>
<th>Potential (volts)</th>
<th>Current (mA)</th>
<th>E (v/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>0.015</td>
<td>469.9</td>
</tr>
<tr>
<td>50</td>
<td>0.032</td>
<td>939.8</td>
</tr>
<tr>
<td>75</td>
<td>0.05</td>
<td>1409.8</td>
</tr>
<tr>
<td>100</td>
<td>0.07</td>
<td>1879.7</td>
</tr>
<tr>
<td>125</td>
<td>0.092</td>
<td>2349.6</td>
</tr>
<tr>
<td>150</td>
<td>0.117</td>
<td>2819.5</td>
</tr>
<tr>
<td>175</td>
<td>0.145</td>
<td>3289.5</td>
</tr>
</tbody>
</table>

Table 2.1. Measured electrophoretic currents for different electric fields.

The photograph in Fig. 2.8 shows particle motion with an applied potential of 150 volts. This photograph was taken using a Polaroid camera with manual shutter and positioned in the optical system as shown in Fig. 2.10.

Fig. 2.10. Photographing an electrophoretic experiment.
From an exposure time of approximately 1 second and measured trace length (from photograph in Fig. 2.8 which is 5X multiplication) of $\frac{1}{16}$" (0.159 cm.) the electrophoretic velocity is:

$$v = \frac{x}{t} = \frac{0.318 \text{ cm.}}{1 \text{ sec.}} = 0.0318 \text{ cm/sec.}$$

(Eq. 2.1)

Now, the electrophoretic mobility can be calculated, knowing the electric field strength present at 150v of potential difference.

$$u (1 \text{ v/cm}) = \frac{V}{E} = \frac{0.318 \text{ cm/sec}}{28.195 \text{ v/cm}} \times 1 \text{ v/cm.}$$

$$= 1.13 \times 10^{-3} \text{ cm/sec.}$$

(Eq. 2.2)

Finally, by using Eq. 1.4, the zeta potential is calculated:

$$\zeta = \frac{4\pi \eta u}{D} \times 9 \times 10^4 \text{ v}$$

$$= \frac{4\pi (0.01 \text{ poise})(1.69 \times 10^{-3} \text{ cm/sec})}{80} \times 9 \times 10^4 \text{ v}$$

$$= 0.16 \text{ v}$$

(Eq. 2.3)

which is approximately three times higher than the predicted range .03 to .06 volts.

The photograph in Fig. 2.11 depicts the summation of the outputs of the photomultiplier tubes. One photomultiplier output was connected to the y input of the oscilloscope, the other to the x input and both x and y amplifiers were set to the .5 v/cm. sensitivity scale. A Fairchild oscilloscope camera attachment was used to take the picture at a shutter speed of 1 second. A circle whose frequency represents the electrophoretic velocity should be observed if the photomultiplier signal outputs are clean and 90° out of phase with one another, but the photograph indicates that problems exist. These problems could be attributed to noise due to background illumination as seen in the photograph in Fig. 2.8.
and inaccurate alignment of the Ronchi gratings on the beamsplitter.

These results do, however, indicate the feasibility of this optical setup for detecting electrophoretic velocity. In this study more emphasis was given to the electronic circuitry. It was not attempted to finalize the overall optical design.
Fig. 2.11. Photograph of combined output of both photomultiplier amplifiers. Exposure time is 1 second and oscilloscope horizontal and vertical sensitivity is .5 v/cm.
III. Electronics

The sine and cosine functions obtained from the outputs of the photomultipliers contain information about the particle motion. These signals are utilized by the electrical system in determining the particle velocity and also the sign of the velocity, or direction of particle flow for a given electric field. The block diagram of Fig. 3.1 describes this system. It will be mathematically analyzed and then individual electrical sections will be discussed in detail and experimental results presented.

To determine the sign of the velocity, or particle direction, the $\pm V_1 \cos(\omega t)$ signal is differentiated and multiplied with $V_1 \sin(\omega t)$, resulting in

$$
V_3 = \left[ \mp \omega V_1 \sin(\omega t) \right] \left[ V_1 \sin(\omega t) \right]
= \mp V_1^2 \omega \sin^2(\omega t)
= \mp V_1^2 \omega \left[ \frac{1}{2} - \frac{1}{2} \cos(2\omega t) \right]
$$

(Eq. 3.1)

From this, the dc component, which indicates particle direction by its sign is

$$
V_{3dc} = \mp \frac{1}{2} \omega V_1^2
$$

(Eq. 3.2)

The velocity determining portion of the circuit involves a frequency control loop in which the photomultiplier signals are processed to control a local oscillator which locks onto the signal frequency and is proportional to the particle velocity. Mathematically, from Fig. 3.1,
Fig. 3.1. Block diagram of electrical system.
\[ V_4 = V_1 V_2 \left[ \cos(\omega t) \right] \left[ \cos(\omega + \Delta \omega t) \right] + \]
\[ V_1 V_2 \left[ \sin(\omega t) \right] \left[ \sin(\omega + \Delta \omega t) \right] \]
\[ = V_1 V_2 \cos(\mp \Delta \omega t) \quad (\text{Eq. 3.3}) \]

and,
\[ V_5 = V_1 V_2 \left[ \sin(\omega t) \right] \left[ \cos(\omega + \Delta \omega t) \right] - \]
\[ V_1 V_2 \left[ \cos(\omega t) \right] \left[ \sin(\omega + \Delta \omega t) \right] \]
\[ = V_1 V_2 \sin(\mp \Delta \omega t) \quad (\text{Eq. 3.4}) \]

Now,
\[ V_6 = V_4' \times V_5 = \Delta \omega (V_1 V_2)^2 \sin^2(\mp \Delta \omega t) \]
\[ = \pm \Delta \omega (V_1 V_2)^2 \left[ \frac{1}{2} - \frac{1}{2} \cos(2\Delta \omega t) \right] \quad (\text{Eq. 3.5}) \]

and,
\[ V_7 = V_4 \times V_5' = -\Delta \omega (V_1 V_2)^2 \cos^2(\mp \Delta \omega t) \]
\[ = \mp \Delta \omega (V_1 V_2)^2 \left[ \frac{1}{2} + \frac{1}{2} \cos(2\Delta \omega t) \right] \quad (\text{Eq. 3.6}) \]

Therefore,
\[ V_8 = V_7 - V_6 \]
\[ = \pm \Delta \omega (V_1 V_2)^2 \quad (\text{Eq. 3.7}) \]

the dc error voltage to the local oscillator.

Fig. 3.2 shows the electronics used in determining particle direction and the polarity reversing switch needed to guarantee a positive \( V_1 \cos \omega t \) signal to the frequency control loop. Since low frequency operation (under 20 Hz) was anticipated, a design goal of 10 Hz was established. A linear four quadrant multiplier IC1 (MC1495) was selected to multiply the photomultiplier outputs together, \( V_1 \sin(\omega t) \) and the differentiated signal of \( \pm V_1 \cos(\omega t) \), C4, C5, R23, R24, and IC4 comprise the differentiator which produces an output of \( \mp \frac{V_1}{K_1} \sin(\omega t) \) where \( K_1 \) is the gain of this circuit as shown in Fig. 3.3.
**Fig. 7.2.** Particle direction and polarity reversing switch circuitry.

\[ \theta = \theta_{\text{input}} \]

\[ V_y = V_{y\text{output}} \]
Fig. 3.3. Graph showing gain of differentiator.

The oscilloscope photograph in Fig. 3.4 represents the input (X channel) and output (Y channel) of the differentiator summed together at 10 Hz.

Several external adjustments must be made to the multiplier. R1, R2, R3 and R4 are biasing resistors for the X offset and Y offset potentiometers, R5 and R6, respectively. With a 1 kHz, 1 v p-p signal connected to the Y input and the X input grounded, the X offset is adjusted for an output null. The same is done for the Y offset. R19 and R20 provide an output offset adjustment for zero dc output. The gain of the multiplier, $k (kV_x V_y)$, is determined by the combination of R10 and R11; with +5 vdc connected to both inputs, R11 is adjusted for $k = \frac{1}{10}$ or +2.5 vdc at the output.
Fig. 3.4. Photograph of differentiator input and output summed together. X and Y oscilloscope input sensitivity is 1 v/div.
R9 is selected for an I_{13} design current of 1 mA and is balanced by I_3 to give 1 mA operating current in each side of the differential amplifier. Errors introduced by nonlinear base-emitter voltage variations within the multiplier are avoided by placing resistors R7 and R8 (emitter degeneration resistors) across the emitters of the X and Y input diff. amps. R12 is a dropping resistor and R13 and R14 are loads for the outputs, pins 2 and 14. Supply noise rejection is accommodated by C1 and C2.

Removal of multiplier common mode output voltage is accomplished by IC2 which also provides for dc level shifting as mentioned previously. R15, R16, R17, and R18 set the amplifier for a gain of one. Fig. 3.5 is a photograph of the output of the op amp showing multiplication of two input signals, a 2 V p-p, 5 Hz signal on the X input and a 2 V p-p, 120 Hz signal on the Y input.

The scope photographs of Fig. 3.6 depict the op amp output for a 2 V p-p, 10 Hz sine and cosine function input; Fig. 3.6(a) is for a +V_1\cos(2\pi 10t) signal and Fig. 3.6(b) is for a -V_1\cos(2\pi 10t) signal.
Fig. 3.5. Photograph of multiplication of two input signals. Y oscilloscope input sensitivity is .2 V/div. and X sweep rate is 20 msec/div.
Fig. 3.6(a). Photograph of multiplier output for 2 v p-p, \( \sin(2\pi 10t) \) and \( +\cos(2\pi 10t) \) inputs. \( Y \) oscilloscope input sensitivity is .1 v/div. and \( X \) sweep rate is 20 msec/div.
Fig. 3.6(b). Photograph of multiplier output for 2 v p-p, sin(2π10t) and -cos(2π10t) inputs. Y oscilloscope input sensitivity is 0.1 v/div. and X sweep rate is 20 msec/div.
Fig. 3.7(a). Photograph of Fig. 3.6(a) filtered. Y oscilloscope input sensitivity is .05 v/div. and X sweep rate is 20 msec/div.
Fig. 3.7(b). Photograph of Fig. 3.6(b) filtered. Y oscilloscope input sensitivity is .05 v/div. and X sweep rate is 20 msec/div.
R21 and C3 form a low pass filter and the voltage across C3 is \( \pm \frac{1}{2} \frac{V_1}{A} \) with some ac ripple, where A is the filter attenuation factor. Fig. 3.7 shows the results of the wave forms of Fig. 3.6 filtered.

R22 limits the input current to IC3, whose output \( (V_o) \) switches between +15 v and -15 v because of its high gain in open loop configuration. Thus, with a + cosine input, \( V_o \) is -15 v and with a - cosine input, \( V_o \) becomes +15 v. Therefore, this circuit demonstrates a means for determining particle direction (plus represents one direction and minus the opposite) from the sign of the cosine signal.

Limitations exist with this circuit in terms of input signal amplitude and frequency response. Motorola\(^{10} \) specifications indicate that input signal amplitude must not exceed (ac or dc):

\[
-5\,\text{v} < V_x < 5\,\text{v} \\
-5\,\text{v} < V_y < 5\,\text{v}
\]

otherwise nonlinear output can be expected. Experimentally, signal amplitude and frequency response as a function of the voltage across C3 were measured with results listed in Table 3.1.

---

\(^{10}\) Linear Integrated Circuits, Semiconductor Data Library, Vol. 6, Motorola Semiconductor Products inc., 1975, p. 8-389 to 8-404.
<table>
<thead>
<tr>
<th>Frequency (Hz)</th>
<th>Amplitude (V p-p)</th>
<th>$-V_1\cos(\omega t)$</th>
<th>$+V_1\cos(\omega t)$</th>
<th>$-V_1\cos(\omega t)$</th>
<th>$+V_1\cos(\omega t)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>+0.077</td>
<td>+0.201</td>
<td>-0.195</td>
<td>(7Hz) +0.530</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>(8Hz) +0.041</td>
<td>+0.133</td>
<td>-0.128</td>
<td>+0.394</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>*</td>
<td>+0.07</td>
<td>-0.085</td>
<td>+0.278</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>*</td>
<td>+0.048</td>
<td>-0.051</td>
<td>+0.123</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>* (23Hz) +0.041</td>
<td>(23Hz) -0.039</td>
<td>+0.086</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* state uncertain

Table 3.1. Particle direction circuitry limitation as a function of VC3.

It was noted that below a C3 voltage of ±0.04 v the circuit was unable to accurately define the difference between a ± cosine function. The data in Table 3.1 indicates that an input signal between 5 Hz - 25 Hz with a 2 v - 4 v p-p amplitude will provide accurate particle direction information.

The polarity reversing switch in Fig. 3.2 is needed to provide a positive $V_1\cos(\omega t)$ signal to the frequency control loop at all times. FET, T1, is used to channel $±V_1\cos(\omega t)$ to either the positive or negative input of IC5 depending on the control signal from $V_Q$ of IC3. For a $-V_1\cos(\omega t)$ input signal to the multiplier, $V_Q$ is +15 v, T1 is off, and the gain of IC5 is

$$\frac{V_{out}}{V_{in}} = -\frac{R_{30}}{R_{26}} = -1$$

(Eq. 3.8)

For $+V_1\cos(\omega t)$, $V_Q$ is -15 v, the FET is on, and the gain becomes

$$\frac{V_{out}}{V_{in}} = \left(\frac{R_{28}}{R_{27} + R_{28}} -1\right)\left(\frac{R_{30}}{R_{26}}\right) + \left(\frac{R_{28}}{R_{27} + R_{28}}\right)\left(\frac{R_{30}}{R_{29}} +1\right)$$

$$= +1$$

(Eq. 3.9)

Thus, $+V_1\cos(\omega t)$ is guaranteed at the output of IC5.
Fig. 3.8 shows circuitry used in the frequency control loop to obtain signals $V_4$ (Eq. 3.3) and $V_5$ (Eq. 3.4). The photomultiplier outputs, $V_1 \sin(\omega t)$ and $V_1 \cos(\omega t)$, and the local oscillator outputs, $V_2 \cos(\omega \pm \Delta \omega)t$ and $V_2 \sin(\omega \pm \Delta \omega)t$, are multiplied together as shown and sum and differences taken to obtain $V_4$ and $V_5$. Again, the four quadrant multiplier is used, IC101 and IC201, and external components are identical to those used in Fig. 3.2. Components are numbered similarly to those of Fig. 3.2 and accomplish the same as previously described, but numbers have been incremented by 100 to denote new circuits. Two differences exist between the adder and subtractor circuits: for the adder, $A$ is connected to $A'$ and $B$ to $B'$; for the subtractor, $A$ is connected to $B'$ and $A'$ to $B$ and the underlined inputs are used.

In Fig. 3.9, $V_5$ is multiplied by $V_4'$ and $V_4$ by $V_5'$, again using four quadrant multipliers. Differentiation is accomplished by differentiators C304, C305, R323, R324, IC302 and C404, C405, R423, R424, IC402 which are identical in components and characteristics (Fig. 3.3) to the one used in the particle direction circuitry. Resultant signals $V_6$ and $V_7$ are added together to give

$$V_8 = \pm \Delta \omega (V_1 V_2)^2$$

(Eq. 3.7)

the error voltage to the local oscillator. C306 reduces ripple and holds the dc level when the local oscillator is locked onto the particle frequency. All multiplier and level shifting components are identical as are the circuit limitations to those described in Fig. 3.2.
Fig. 3.8. Adder and subtractor circuitry.
Fig. 3.9. Circuitry for determining error voltage to oscillator.
The local oscillator shown in Fig. 3.10 is comprised of two Intersil 8038 function generators, IC401 and IC402. By using the triangular output of IC401 along with the level detector IC403, the charging cycle of IC402 is controlled, thus slaving IC402 to IC401. This results in a sine/cosine generator whose frequency is controlled by the dc voltage at pin 8.

Waveform symmetry is determined by R401, R402, R404 and R405; a 50% duty cycle is established. Potentiometers R403 and R406 are provided to minimize sine wave distortion and capacitors C401 and C402 control the timing of each device. R407, R408, and R409 set up a reference for one side of the comparator or level detector, such that IC403 output switching occurs when the triangular input waveform goes through zero (a 90° phase shift) as shown in Fig. 3.11. R414 and R415 provide for IC403 output adjustment and the generator output signal amplitude is controlled by resistive bridges R410, R411 and R412, R413. Multiplier input impedance of approximately 20 MΩ prevents loading problems by the resistive bridges. Fig. 3.12 shows the scope photograph of the sine and cosine signals each with a 4 V p-p amplitude (V2) added together; a circle indicates two signals in phase quadrature.
Fig. 3.11. Timing signals used to slave IC402 to IC401.
Fig. 3.12. Photograph of local oscillator outputs, sine and cosine, summed together. X and Y oscilloscope input sensitivity is 1 v/div.
The frequency of the waveform generator is a direct function of the dc voltage at terminal 8 as measured from $+V_{cc}$. By altering this voltage, frequency modulation is performed and a graph of the frequency range as a function of tuning voltage of the local oscillator appears in Fig. 3.13.

![Graph showing frequency range versus tuning voltage](image)

**Fig. 3.13.** Oscillator frequency range as a function of tuning voltage to pin 8.
IC404 in Fig. 3.10 is connected as a non-inverting summing amplifier providing the sweep voltage to the oscillator. R416, R417, and R420 are selected for an amplifier gain of one and R419, R418 establish a +10 v dc level on one of the summing inputs (A); +10 v dc at pin 8 of the oscillator sets the output frequency to approximately 10 Hz. With \( V_2 \) set to 4 v p-p and two multiplier gain factors taken into consideration, Eq. 3.7 becomes

\[
V_8 = \pm \Delta \omega \left[ \frac{V_1(4)}{10} \right]^2
= \pm 0.16 \Delta \omega V_1
\]  

(Eq. 3.10)

which is applied to the B input.

Upon using the limitations established by the particle direction circuitry for the multiplier and differentiator, the locking range of the oscillator was determined. Experimental measurements were taken using an oscilloscope and appear in Table 3.2. A frequency counter can be hooked up to the sine wave output of the oscillator to obtain digital readout.

<table>
<thead>
<tr>
<th></th>
<th>( V_1 = 2 \text{ v p-p} )</th>
<th>( V_1 = 4 \text{ v p-p} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>upper limit</td>
<td>18.6 Hz</td>
<td>19.2 Hz</td>
</tr>
<tr>
<td>lower limit</td>
<td>6.25 Hz</td>
<td>6.25 Hz</td>
</tr>
</tbody>
</table>

Table 3.2. Locking range of local oscillator.

Therefore, the limitations on the input signal for determining particle velocity are a 2 v - 4 v p-p amplitude and a frequency range of 6.25 Hz - 18.6 Hz.
Thus, it has been shown in this section that electronic circuitry can process two input signals, $\pm V_1 \cos(\omega t)$ and $V_1 \sin(\omega t)$, of unknown frequency (within limitations) and determine the sign of the $\pm V_1 \cos(\omega t)$ signal as well as the unknown frequency. It is applicable to automatic electrophoretic measurements provided the input information is within the circuit limitations described.
IV. Conclusions and Suggestions for further Investigation.

The original goal of this project was to demonstrate a new method for making automatic electrophoretic measurements. Although this was not completely achieved, some new and important ideas have resulted from this experiment.

It was shown in chapter one that particle images moving past a grid could be observed as sinusoidal functions, as long as the image diameters approached the grid spacings. For a system containing \( n \) particles the resultant signal would also be a sinusoidal with some phase angle associated with it. The average amplitude of this phasor was mathematically computed to be the square root of the number of particles, \( n \), times the sinusoidal amplitude \( A \).

An electrophoretic cell was constructed using a capillary immersed in water and illuminated by a He-Ne laser. In an experimental setup, the electrophoretic velocity of small glass particles in water under a potential difference of 150 v was measured and a zeta potential of .16 v calculated. This compares to measured zeta potentials between .03 v - .06 v for small quartz particles. A beamsplitter with two Ronchi gratings mounted on its faces was then tried in an effort to derive the sine and cosine functions necessary for electrical processing. Results were poor and were attributed to the fact that background illumination was prevalent and alignment of the 90° phase difference between Ronchi gratings was difficult.

The electrical circuit designed utilized sine and ± cosine inputs (simulated particle signals) to determine the sign of the
cosine function and the frequency, which is directly related to the velocity of particles. The sign or particle direction determining circuitry used a differentiator and four quadrant multiplier to indicate a ± cosine function and had limitations of 2 v - 4 v p-p input signals between 5 Hz - 25 Hz. For determining input signal frequency, a frequency control loop comprised of six multipliers, two differentiators, and two function generator IC’s was constructed. Input signal amplitude range was also 2 v - 4 v p-p and an oscillator locking range of 6.25 Hz to 18.6 Hz was experimentally measured.

Thus, the results of both the optics and electronics suggests the feasibility of such a system for making automatic electrophoretic measurements. However, improvement is necessary and a few ideas are given below for such work.

In the optical area, the need to reduce background illumination for sharper particle contrast is important. A liquid medium whose index of refraction approaches that of glass could be used in the cell, as well as the transport medium in the capillary, to reduce reflections within the capillary due to water - glass interfaces. Table 4.1 lists various liquids whose indexes of refraction approach that of glass.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Index of Refraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>water</td>
<td>1.33</td>
</tr>
<tr>
<td>glass</td>
<td>1.50</td>
</tr>
<tr>
<td>benzyl alcohol</td>
<td>1.538</td>
</tr>
<tr>
<td>phenetole</td>
<td>1.505</td>
</tr>
<tr>
<td>tetrachloroethylene</td>
<td>1.504</td>
</tr>
<tr>
<td>1,2 dimethylbenzene</td>
<td>1.503</td>
</tr>
<tr>
<td>1,2 diethylbenzene</td>
<td>1.501</td>
</tr>
<tr>
<td>pentachloroethane</td>
<td>1.501</td>
</tr>
<tr>
<td>benzene</td>
<td>1.498</td>
</tr>
<tr>
<td>toluene</td>
<td>1.494</td>
</tr>
</tbody>
</table>

Table 4.1. Liquids which approach the index of refraction of glass.

The mechanical stability of the breadboarded system has room for improvement as well. The microscope, beamsplitter, and photomultiplier tubes should all be solidly mounted and adjustable. A small positioning block should also be used for mounting one of the Ronchi gratings on the beamsplitter to precisely adjust the 90° phase shift between gratings.

In the electronics section the circuit limitations could be greatly enhanced by designing a differentiator with some AGC. Fig. 4.1 shows an idea how to accomplish this.

![Differentiator with AGC](image)

Fig. 4.1. Differentiator with AGC.
The output of the differentiator is rectified and filtered, controlling the gate voltage of an FET, which varies the FET's forward resistance. By varying the input impedance to the amplifier, gain control is obtained. This should greatly increase the dynamic range of the electronics both in the amplitude of the signal permitted and its frequency response.

A sine/cosine generator of a different type that does not rely on locking of two separate oscillators should be used. This would also increase the dynamic range of measurable velocities.

These have been a few suggestions for future investigators to perfect this system for automatic electrophoretic measurements. This work, however, has proven some viable techniques that can be carried forth and greatly improved upon.
BIBLIOGRAPHY


"Viscosity", Medical Electronics and Data, Pittsburgh, Pa., May - June, 1975, pg. 17-32.

VITA

Louis Filo III was born the son of Louis Filo, Jr. and Mary Korpics Filo on October 16, 1950 in Bethlehem, Pennsylvania. He attended public schools in Bethlehem and entered Lehigh University in 1968. Degrees obtained by him while at Lehigh include: Bachelor of Science in Electrical Engineering (1972), Bachelor of Science in Engineering Physics (1973) and Master of Science in Electrical Engineering (1976). He is a member of the IEEE and of Eta Kappa Nu. Louis presently works as a design engineer in the consumer products electrical engineering area of Eastman Kodak Company's Apparatus Division in Rochester, New York. He is married to the former Althea Ellis of Northampton, Pennsylvania.