EFFECTS OF NONUNIFORM ELECTRIC FIELDS ON REAL DIELECTRICS IN WATER

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CHAPTER I

INTRODUCTION

Electrical force effects in liquid dielectrics have been the objects of research as early as the eighteenth century when it was found that a wet sponge could be wrung of its water by hooking it to a static generator (1). It is the object of this paper to present a study of one of these electrical force effects and an application of this force.

This particular effect, dielectrophoresis, is the motion of an electrically neutral body in a liquid dielectric caused by polarization effects of the body in the presence of a nonuniform electric field. It is to be distinguished from motion due to a charged body in an electric field, termed electrophoresis. Four aspects of the dielectrophoresis effect are investigated. First, the dielectrophoretic theory obtained by using the assumption that the dielectrics are perfect insulators is tested experimentally for a variety of real materials. Second, the effect is experimentally examined for a dependence on the conductivity of the particle and medium. Third, it is studied experimentally for a dependence on the frequency of the applied potential. And last, the feasibility of separating biological cells using the dielectrophoresis effect is examined.

If a uniform electric field is applied to a system containing a liquid dielectric and suspended dielectric particles, the particles will polarize, but the net force on the particles will be zero. However, if the particles are charged, there will be a net force on the particles and they will tend to migrate towards the oppositely charged terminal. A different situation exists in a nonuniform field: here, the charged particles will again migrate in a direction depending on the particular charge of the particle, but this time the neutral particle will also experience a force. If the body is more polar than the medium, it will migrate towards the higher electric field intensity region. If the body is less polar than the medium, it will migrate to the lower field region, other forces being neglected. Another interesting aspect of the force is that the sign of the field is unimportant, and, as can be seen from the force equation for ideal dielectrics, the field need not be stationary. This can be attirbuted to the dependence of the force on the square of the impressed field. The equation can be shown to be:

$$\bar{F} = 2\pi R^3 K_1 \epsilon_o \frac{K_2 - K_1}{K_2 + 2K_1} \bar{\nabla} |\bar{E}_o|^2$$

where R is the radius of the particle, K_2 is the dielectric constant of the particle, K_1 is the dielectric constant of the medium, and $\bar{E}_o$ is the applied electric field (2). As can be seen from the equation, the direction of the force can be influenced only by the effective dielectric constants of the two media. This is an important point because if we have a charged particle immersed in a dielectric liquid and apply an alternating field the net electrostatic force over a cycle due to the particle being charged is zero. Thus, if the electric field alternates fast enough so that the particle is not moved an appreciable distance on one half of the cycle, the electrophoretic force effect can be eliminated. This frequency does not need to be of some great order of magnitude since the particles we are concerned with are macroscopic particles (that is: they are large when compared with atoms and molecules). As an order of magnitude, one thousand cycles per second will suffice. For these reasons, alternating current sources were used to observe the dielectrophoretic phenomena in the present work.

The force equation given is that for ideal dielectrics. From this equation, the behavior of the system can be predicted correctly, providing the materials have properties approaching those of ideal dielectrics; that is to say, their dielectric loss components are negligible compared to the real component. If the real component of the dielectric is not negligible, then the dielectric is called a lossy dielectric. The first part in the investigation was to test the validity of the simple equation for several types of materials of varied lossiness.

Since the force equation for ideal dielectrics (i.e. perfect insulators) does not include any frequency dependent terms it might then be assumed that the frequency is of no consequence. This assumption is valid for ideal dielectrics, but since conduction is a frequency dependent process, it is not valid for lossy materials. Accordingly, the frequency dependence of their behavior in dielectrophoresis was studied.

The third aspect of the investigation was to study experimentally the effect of the resistivity of the particle and the medium and the importance of the role played by each. Finally, a more complete theory of the dielectrophoretic behaviors of lossy dielectrics in lossy media was sought.

One exciting possible application of this force is the separation of living organisms from dead ones based on the difference in their polarizability. As reported by Schwan (3) the dielectric constants of living tissues are on the order of 10^2 or greater, while the dielectric constant of water at room temperature is approximately 80. In a divergent electric field, the cells in a water suspension would be expected to migrate towards the region of highest field intensity. If for some reason, dead cells have a lesser dielectric constant than live ones, then their separation in a nonuniform field is possible. But before this can be done, the behavior, the optimum ranges of frequency and resistivity, and reasons for neutral particles exhibiting these effects in water must be known if this phenomena is to be used on living organisms.

To sum up, this thesis describes a study on inanimate systems to understand the dielectrophoretic behavior of lossy particles in lossy media, and concludes with an account of our successful application of it to the separation of living and dead organisms.

CHAPTER II

PERTINENT PAST WORK

To our best present knowledge, no one has ever devised a separator for biological cells using dielectrophoretic forces. However, separations of biological cells have been made using uniform fields, and separations of inanimate particles have been accomplished using non-uniform fields in insulating liquids.

Fulwyler (4) uses a method that counts cells on the basis of electronically measured interference with conduction in the medium. Electrical conduction was used to count particles, both biological cells and inanimate, on the basis of their effect in partially blocking conduction in a fine capillary filled with conductive liquid. The suspension then emerges drop-wise into a uniform charging field which separates indicated droplets by charging them and then causing them to be deflected to collection vessels at different deflection distances. That separator is reported as using an electric field of 7,000 volts per centimeter, but it is important to note that the survival rate of the cells used (Chinese hampster ovary cells) was reported as 96 percent.

Pohl and Schwar (5) used the dielectrophoretic force to separate particles such as solid ammonium chloride, polyvinyl chloride and sulfur in a liquid dielectric of carbon tetrachloride-benzene mixture. Later Pohl (6) used it to separate diamond and carborundum particles, which are difficult to separate by gravity or magnetic methods because of their nearly identical properties.

The latter separations were performed in liquid dielectrics of extremely low conductivity. In contrast, this thesis is concerned with the dielectrophoretic force on particles in water, a substance with an appreciable conductance depending on the amount and type of impurities it contains. This is a very important difference, for, as we show later the conductivity can be as important a factor as the polarization.

CHAPTER III

THEORY

The dielectrophoretic force equation for ideal dielectrics is well known. It is obtained by starting with the general force equation for a dipole in an inhomogeneous electric field and solving for the two variables ($\bar{\mu}$ and $\bar{E}_o$) in this general force equation (7). The equation is:

$$\bar{F} = (\bar{\mu} \cdot \bar{\nabla}) \bar{E}_o$$

where $\bar{\mu}$ = dipole moment

$\bar{\nabla}$ = del operator

$\bar{E}_o$ = applied electric field

By noting that the dipole moment is the product of the polarization and the volume

$$\bar{\mu} = V_1\bar{P}$$

where V_1 = volume

$\bar{P}$ = polarization vector

and then noting that the excess polarization on the particle is given by

$$\bar{P} = (\varepsilon_2 - \varepsilon_1)\bar{E}_i$$

where ϵ_2 = permittivity of particle

ϵ_1 = permittivity of medium

$\overline{E}_i$ = internal electric field vector

the force equation can be rewritten as

$$\overline{F} = \left\{ V_1 (\epsilon_2 - \epsilon_1) \overline{E}_i \cdot \overline{\nabla} \right\} \overline{E}_o$$

where V_1 = volume of the particle

One next solves Laplace's equation (using the appropriate boundary conditions) for the internal electric field inside a spherical particle in terms of the applied field at some distance from the particle. The internal field is given by

$$\overline{E}_i = \left(\frac{3\epsilon_1}{2\epsilon_1 + \epsilon_2} \right) \overline{E}_o$$

On combining the last two equations and simplifying one obtains

$$\overline{F} = \frac{3}{2} V_1 \; \epsilon_1 \left(\frac{\epsilon_2 - \epsilon_1}{2\epsilon_1 + \epsilon_2} \right) \nabla |\overline{E}_o|^2$$

For a spherical particle of radius R this reduces to

$$\overline{F} = 2\pi R^3 \epsilon_1 \left(\frac{\epsilon_2 - \epsilon_1}{2\epsilon_1 + \epsilon_2} \right) \nabla |\overline{E}_o|^2$$

For a field produced by two concentric spheres, the force equation becomes

$$\overline{F} = -\overline{r}_o \; 8\pi R^3 \; \epsilon_1 \frac{r_1 r_2}{r^5 (r_2 - r_1)^2} V^2 \left(\frac{\epsilon_2 - \epsilon_1}{\epsilon_2 + 2\epsilon_1} \right)$$

where r_1 and r_2 are the inner and outer radii of the two concentric spheres respectively, r is the distance from the center of the spheres to any point between them, V is the applied potential, and $\bar{r}_o$ is the unit vector in the radial direction (2). For a system of two concentric cylinders, the force equation is (2)

$$\bar{F} = +\bar{r}_o \; 2\pi R^3 \; \epsilon_1 \frac{-2V^2}{\left\{\ln(r_1/r_2)\right\}^2} \left(\frac{1}{r^3}\right) \left(\frac{\epsilon_2 - \epsilon_1}{2\epsilon_1 + \epsilon_2}\right)$$

So, for either situation, the magnitude of the force on a spherical dielectric particle embedded in another dielectric is dependent on the permittivity of the material in which the spherical particle is embedded, the difference in permittivities of the particle and medium, the voltage squared, the volume of the particle, and on its position in the field.

The dielectrophoretic force equation for lossy dielectrics is more difficult to obtain, but has an identical form to that for ideal dielectrics. It is given by Sher (8) as

$$\bar{F} = \frac{3}{2} V_1 \text{ Real } \left\{\epsilon_1^* \left(\frac{\epsilon_2^* - \epsilon_1^*}{2\epsilon_1^* + \epsilon_2^*}\right) \nabla |\bar{E}_o|^2\right\}$$

where V_1 is the volume of the particle, ϵ_1^* and ϵ_2^* the complex permittivities of the particle and medium respectively, and $\bar{E}_o$ is the applied electric field strength.

In a lossy dielectric, the properties of the materials reflect two processes. One is an in-phase conduction and the other is the polarization mechanism. They can be lumped together by the introduction of complex notation; namely by the complex dielectric constant K^* which is equal to

$$K^* = K' - iK''$$

where K' is the dielectric constant of the material, K'' is the term containing the conduction, and i is the $\sqrt{-1}$. K'' can be written as

$$K'' = \frac{\sigma}{\epsilon_o \omega}$$

where σ is the specific conductivity and ω is the angular frequency of the electric field.

To evaluate the force, the polarization effect must be examined. First, consider a small volume of the liquid dielectric, V, with dielectric constant K^* and susceptibility, X^*. The polarization vector, $\overline{P}$, is given by

$$\overline{P} = X^* \overline{E}$$

or since

$$X^* = (K^* - 1)\epsilon_o$$

then P may be written

$$\overline{P} = (K_1{}^* - 1)\epsilon_o \overline{E}$$

for the water.

Similarly for the particle of dielectric constant $K_2{}^*$, the polarization is given by

$$\overline{P_2} = (K_2{}^* - 1)\epsilon_o \overline{E}$$

Looking at the magnitudes of these polarizations and assuming that the particle does not affect the applied field in the first approximation, then when the magnitudes are equal, the force on the particle will be zero. That is

$$\overline{F} = 0 \quad \text{when} \quad |\overline{P}_1^*| = |\overline{P}_2^*|$$

or when

$$|K_1^* - 1| = |K_2^* - 1|$$

which is approximately equivalent to saying

$$\overline{F} = 0 \quad \text{when} \quad |K_1^*| = |K_2^*| \quad K_1', K_2' \gg |$$

Rewriting these magnitudes as

$$\left|K_1' - \frac{i\sigma}{\omega\varepsilon_o}\right| = \left|K_2' - \frac{i\sigma}{\omega\varepsilon_o}\right|$$

it is apparent that the value of resistivity of water can be calculated for which the force on the particle is equal to zero. For a given frequency, one point is calculated for each particle. In Chapter V, the calculated values and experimental values of resistivity for zero force will be compared and discussed.

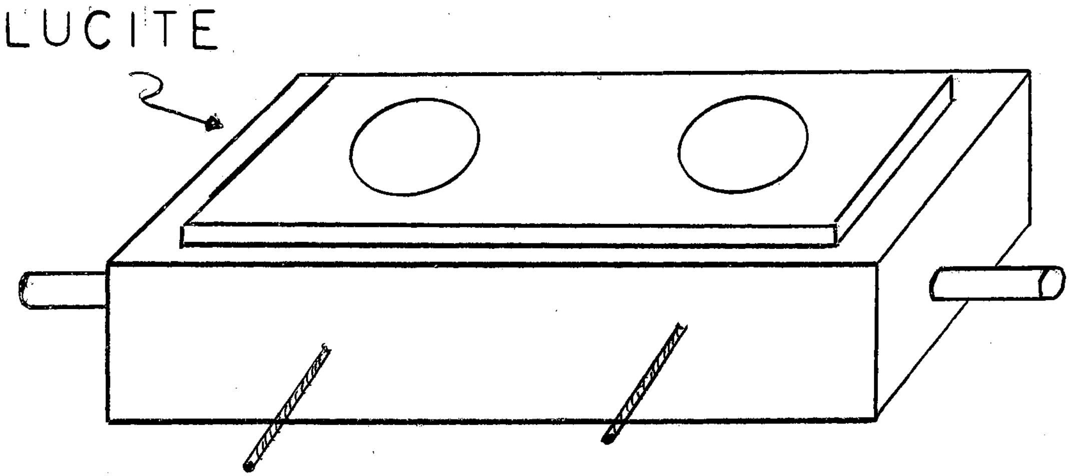
CHAPTER IV

APPARATUS AND EXPERIMENTAL PROCEDURE

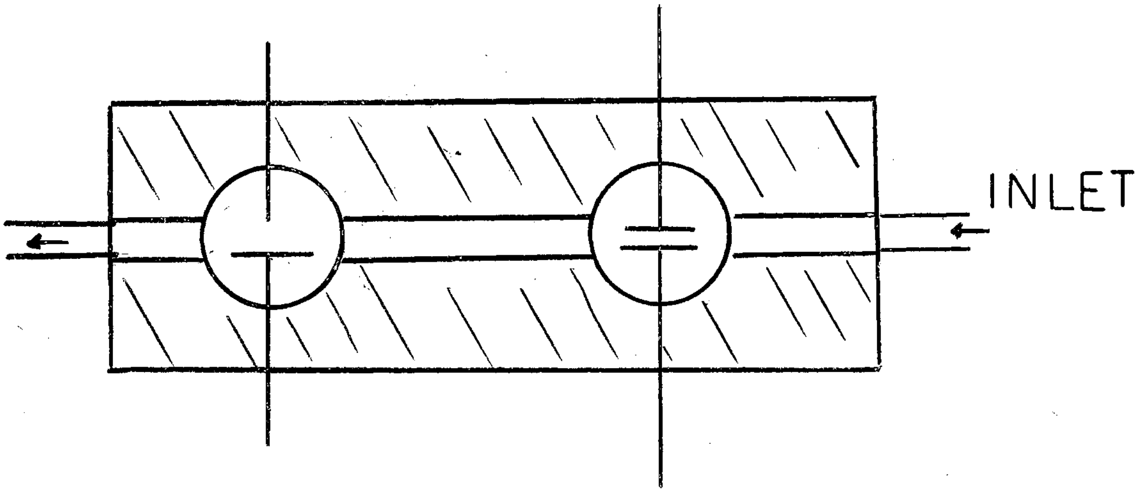
Two pieces of apparatus were constructed for the research problem. The first was constructed to study the dielectrophoretic force effects of hard insoluble particles in water, and the second was constructed to study the dielectrophoretic force effects on biological cells.

The first piece of apparatus (the "test" cell) was constructed of lucite, or polymethylmethacrylate (See Figure 1). The cell contained two sections--one for measuring the resistivity of the water and the other for measuring the movement of a particle in a nonuniform electric field. The section for measuring the resistivity of the water was a cylindrical well drilled in the lucite provided with inlet and outlet ports. It contained two circular stainless steel plates one centimeter in diameter and separated by a distance of 0.178 centimeters. These plates were fastened to stainless steel screws which were in turn threaded in the lucite to permit the separation to be adjusted if necessary. The resistance, area, and separation of a parallel plate capacitor are related to the resistivity of the substance between the plates by the equation

$$\rho = \frac{A}{d} R$$



TEST CELL W/O COVER



SCHEMATIC TOP VIEW

Figure 1. Inanimant Test Cell

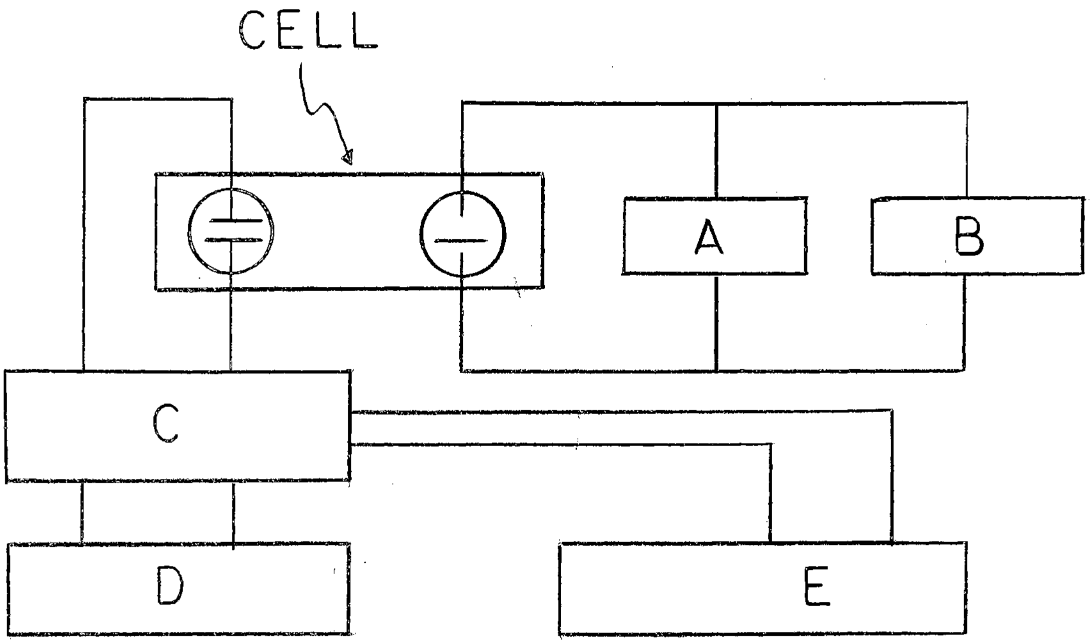
where ρ = resistivity

A = area of one plate

d = separation of plates

The resistance across the parallel plates was determined by using a Wheatstone bridge and a one-kilocycle alternating current source. The two equal legs of the Wheatstone bridge were made with one percent precision resistors (500 kilo-ohms), and the balancing arm consisted of two stepping switches and a potentiometer. The stepping switches had nine equal steps of 0.1 meg-ohms and one meg-ohms respectively while the potentiometer was a linear taper type calibrated in divisions of one thousand ohms. The capacitance of the parallel plates was not completely negligible, so it was necessary to add an adjustable capacitor across the balancing arm to obtain a good null. This was especially necessary for higher resistivity water, since the impedance due to conduction approaches that of the capacitive part. The one kilocycle source for the bridge was a Heath audio oscillator in series with a large blocking capacitor (a very low loss mylar capacitor) to inhibit any direct current flow. For a null detector, a Hewlett Packard 1404 oscilloscope with a Hewlett Packard 1402 dual-trace amplifier was used with one channel across the balancing leg and the other across the cell leg. The function switch on the oscilloscope was set at -A +B such that the scope read a null when the voltage drops across the two legs were equal. See Figure 2 for the block diagram of electrical circuit.

The second part of the lucite block (See Figure 1) contained two electrodes (a circular disk electrode and a pin electrode) in a cylindrical well with inlet and outlet ports. Both electrodes were made of



A – Voltmeter
B – AC Source
C – Wheatstone Bridge
D – Null Detector
E – IKC Generator

Figure 2. Block Diagram of Electrical Circuit

stainless steel. The circular electrode and pin electrode were 0.5 centimeters and 0.05 centimeters in diameter respectively, and were separated a distance of 0.21 centimeters. The pin electrode had a tip that was carefully rounded such that its appearance in the direction of the plate electrode was one of a sphere. Again, both electrodes were mounted on stainless steel screws to permit the separation to be varied. The pin-plate form was picked because it gives a highly divergent field, yet it can be approximated by assuming spherical geometry.

The field across these electrodes was produced with high voltage alternating current power supplies. For the high frequency, a 2.55 mega-cycle source capable of delivering 200 volts rms was used, and for the low frequencies the power supply consisted of a Hewlett Packard 200 CD audio oscillator and a Heath A9 audio amplifier. The voltmeter was a Hewlett Packard 410 A radio frequency voltmeter which had a frequency range capability from 20 cycles per second to 200 mega-cycles per second.

As can be seen from Figure 1, the cell contains two stainless steel tubes--one an outlet port, the other an inlet port. These ports allow the water to be run gently through the cell so the resistivity of the water can be varied without removing the cover.

To obtain high resistivity water (specific resistivity 10^6 ohm-cm or more) distilled water must be passed through an ion exchange bed. To obtain the lower resistivity water, small amounts of tap water were added to the high resistivity water. This gives any desired value of resistivity between the high level (10^6 ohm-cm) to that of tap water (10^2 ohm-cm). A schematic diagram of the water circuit is shown in Figure 3.

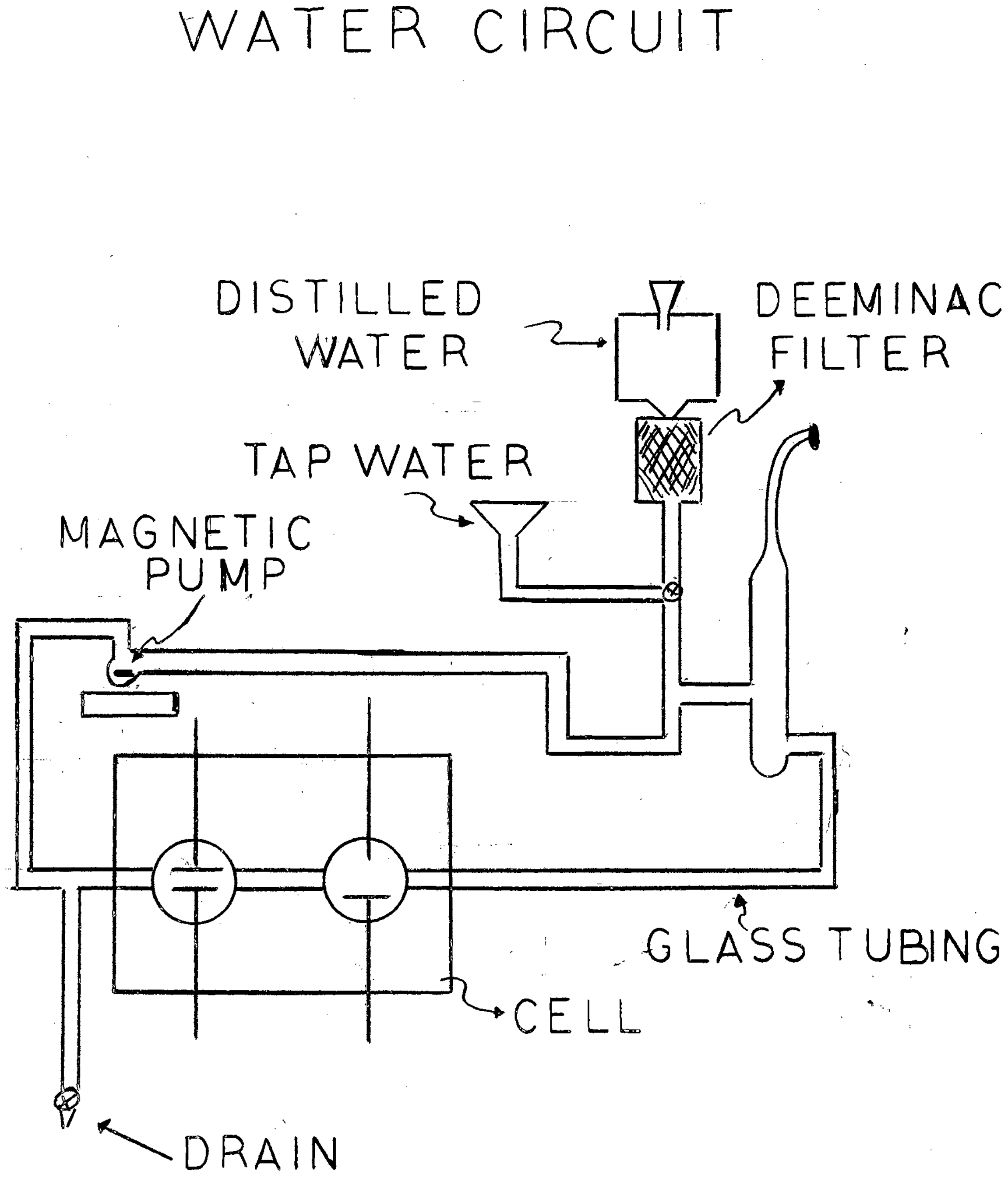
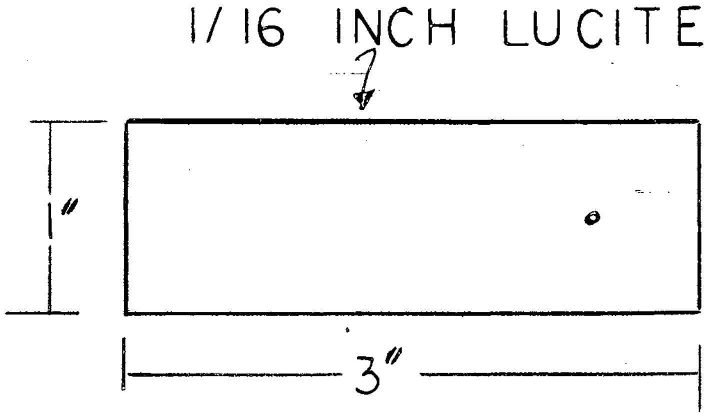


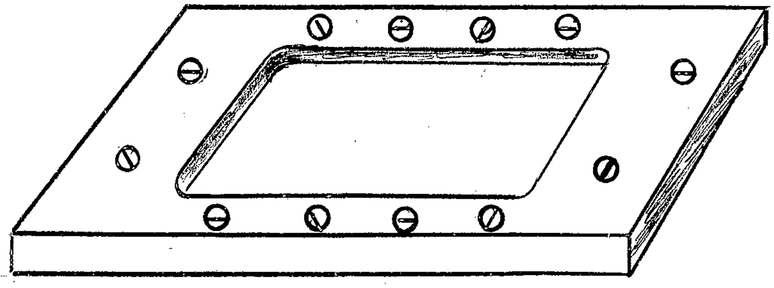
Figure 3. Water Circuit

Several problems arose when starting the investigation. Air bubbles were quite a problem for when they appeared on the electrodes in the cell they were very difficult to remove in the closed system. The only practical solution found was prior deaereation of the water. The water could then absorb the air, dissolving the bubbles. Another problem encountered in early work was that the data taken in these early runs gave inconsistent results--the curves from two values of resistivity of water apparently crossed. This led us to recognize that the resistivity measured on one side of the cell was not necessarily that where the data was being recorded. This led to the introduction of a magnetic circulating pump. The pump consisted of a magnetic stirrer encased in a glass tube built so that the rotary motion would induce a small circulating action in the liquid. The pump removed the inconsistency and allowed us to take trustworthy data. A third problem was a mechanical one. For the cover of the cell, a glass slide was used. It had a lucite lip which screwed down to make a water tight bond. Upon standing, the glass slide would break and vitiate the readings. This problem was remedied by substituting a slide made of optical quality lucite.

The final cell had a cover slide (See Figure 4) made of 1/16 inch thick lucite. It was held in place by a rim which had several small screws to bolt it snugly to the main part of the cell, although some lateral movement was possible to align the cover on the cell. In the lucite cover was a tiny hole in which was placed a pyrex fiber with hard wax as the adhesive. To afix a particle to the fiber, a glue was needed which was not soluble in water, but was only temporary. Common



COVER



RIM

Figure 4. Cover and Rim on Test Cell

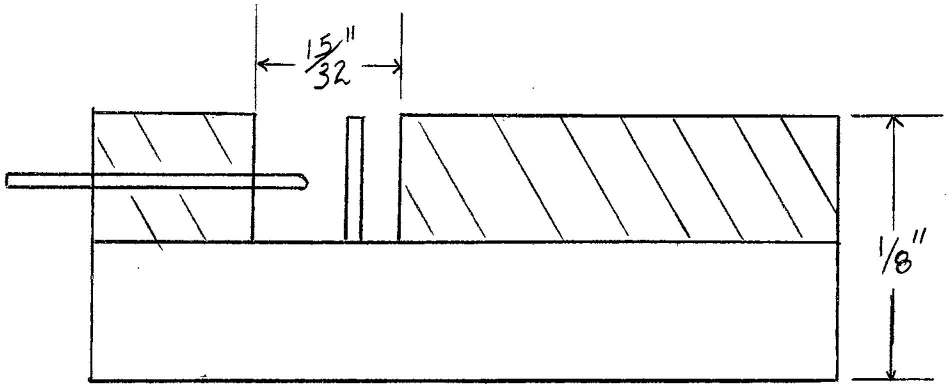
fingernail polish was found acceptable for this duty. Fingernail polish can be easily removed with acetone without damage to the fiber or the particles.

The test cell had a base which made attachment to a microscope possible. Particle deflection measurements were taken using a Monolux measuring microscope.

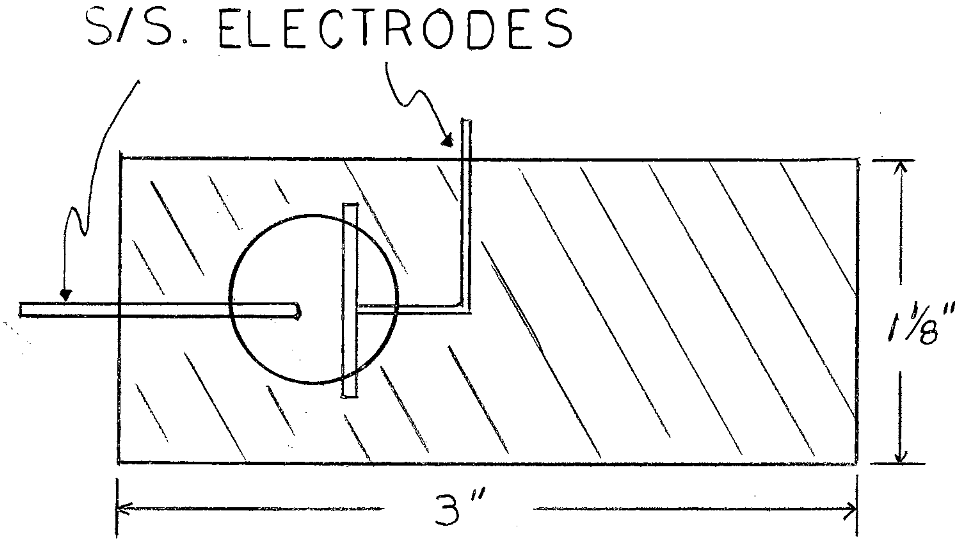
The test cell described was used for making studies with inanimate hard particles in water. The data taken would hopefully lead to the rules needed to be obeyed if this effect is to be used to separate living cells from dead ones. The next cell constructed was a simple one. It was to be used to test the force on living cells. It consisted of two pieces of 1/16 inch thick lucite. The top piece had two holes drilled in it as shown in Figure 5. One was a large hole, 15/32 inches in diameter, which forms the well while the other was a small hole laterally drilled into the slide slightly larger than the wire, and was used to support a rounded tip-pin electrode. In addition to these two holes, a slot was made so the rectangular plate electrode would fit snugly in place at the center of the well. This top lucite plate was bonded to the lower plate with rubber cement. The dimensions were approximately those of a microscope slide so that observations could be easily made with a microscope.

Methods

The crystals, or particles, that were used in the first cell needed to be fairly spherical, and could not contain sharp edges. Therefore, before any runs were made the particles were polished. The polisher



SIDE VIEW



TOP VIEW

Figure 5. Slide for Viewing Motion of Biological Cells

consisted of a small air centrifuge lined with fine grit paper. The centrifuge consisted of a brass cylinder one inch in outside diameter with a small pipe entering tangentially on the side. Its base was a solid rubber stopper, and its top was a rubber stopper with a small hole to serve as an air outlet. Air entering through the jet and hitting the side of the cylinder created a stirring action which hurled particles inside the cylinder against the abrasive wall and against each other.

The particles were placed in this centrifuge and periodic checks were made until at least some of the particles were noticed to be quite round. Some materials proved especially difficult to polish by this method, e.g. the abrasion time required for spinning varied depending on the type of particle. Specific examples: Lead hafnate; two hours using approximately 100 ml/sec flow rate of air, rutile; 48 hours at the same flow rate, and silicon; 10 days but with poor results.

The deflection of the particles in the field does not only depend on the polarization effect, but also depends on the stiffness of the pyrex fiber, and the density of the particles. By tilting the microscope and recording the angle of tilt versus deflection, a stiffness constant or deflection per degree of tilt could be found. This constant can be used to eliminate the stiffness peculiar to a given fiber and the mass peculiar to a given particle. Thus, a relative deflection, or deflection per stiffness constant, can be obtained which is a function only of polarization forces.

The general method if investigation was: first, select the frequency wanted and attach the corresponding power supply to the electrodes of the "inanimate test cell". Starting at high water resistivity and going down, tables of deflection versus voltage for each value of resistivity were recorded. From this data, a family of deflection versus voltage-squared curves were obtained. The deflection may or may not be normalized for the stiffness constant of the system. Next, by changing the frequency, a different set of curves which would show the effect of frequency on the force were obtained. But, it was found that a much better way to compare the frequency effects was to plot the initial slope of these deflection versus voltage squared curves for a given frequency against the resistivity for each curve in the family. This gives a family of curves for each particle. The importance of frequency is at once visible upon inspection of these graphs. These figures are given in Chapter V.

CHAPTER V

RESULTS

As was explained earlier, the data taken was for the specific purpose of obtaining deflection versus voltage squared curves. Graphs for lead hafnate, silicon, silicon dioxide, rutile, and lead particles are to be found on the following pages. See Figures 6 - 17. A table of the properties of these materials is given for quick reference in Table 1.

All of these graphs show that the force depends on the voltage squared for small deflections except in the case of lead. This is easily explained by looking at the force equation. As the change in r, denoted by Δr, approaches zero, the deflection in this limit will depend only on the voltage squared. But, this is the case only if the movement of the particle is very small. Such is the case for the first two or three divisions of motion measured with a calibrated recticle on the Monolux microscope because the total distance to the pin electrode is about 17 divisions. Therefore, the great rate of change in the deflection curves for deflections of several divisions can be attributed to the dependence of the energy on a high power of the radial distance out from the pin electrode. Furthermore, at high field intensities turbulence is created in the liquid making simple correlations difficult. The latter effect is thought to account for the anomalous curve

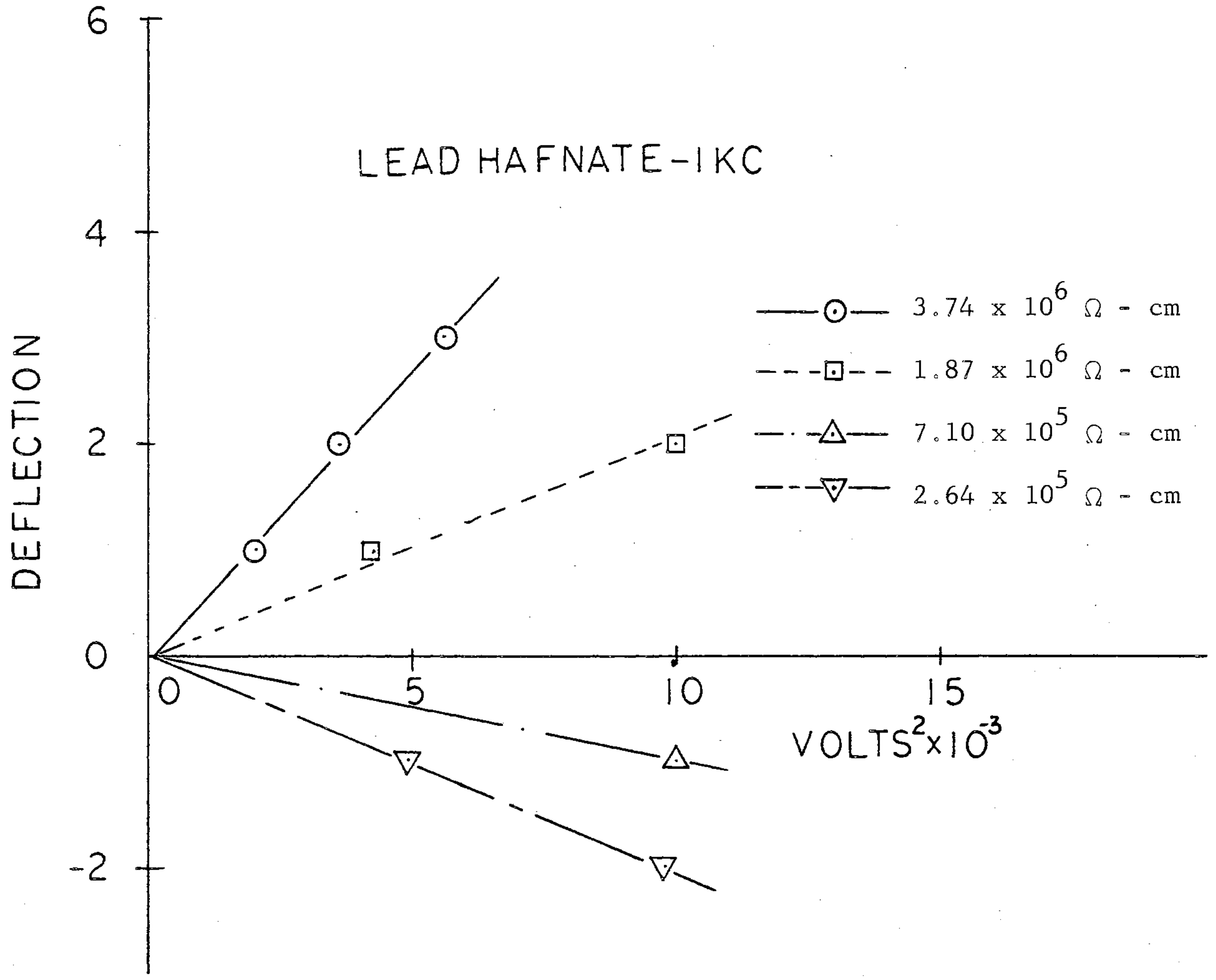


Figure 6. Deflection versus Voltage Squared for Lead Hafnate at 1 KC

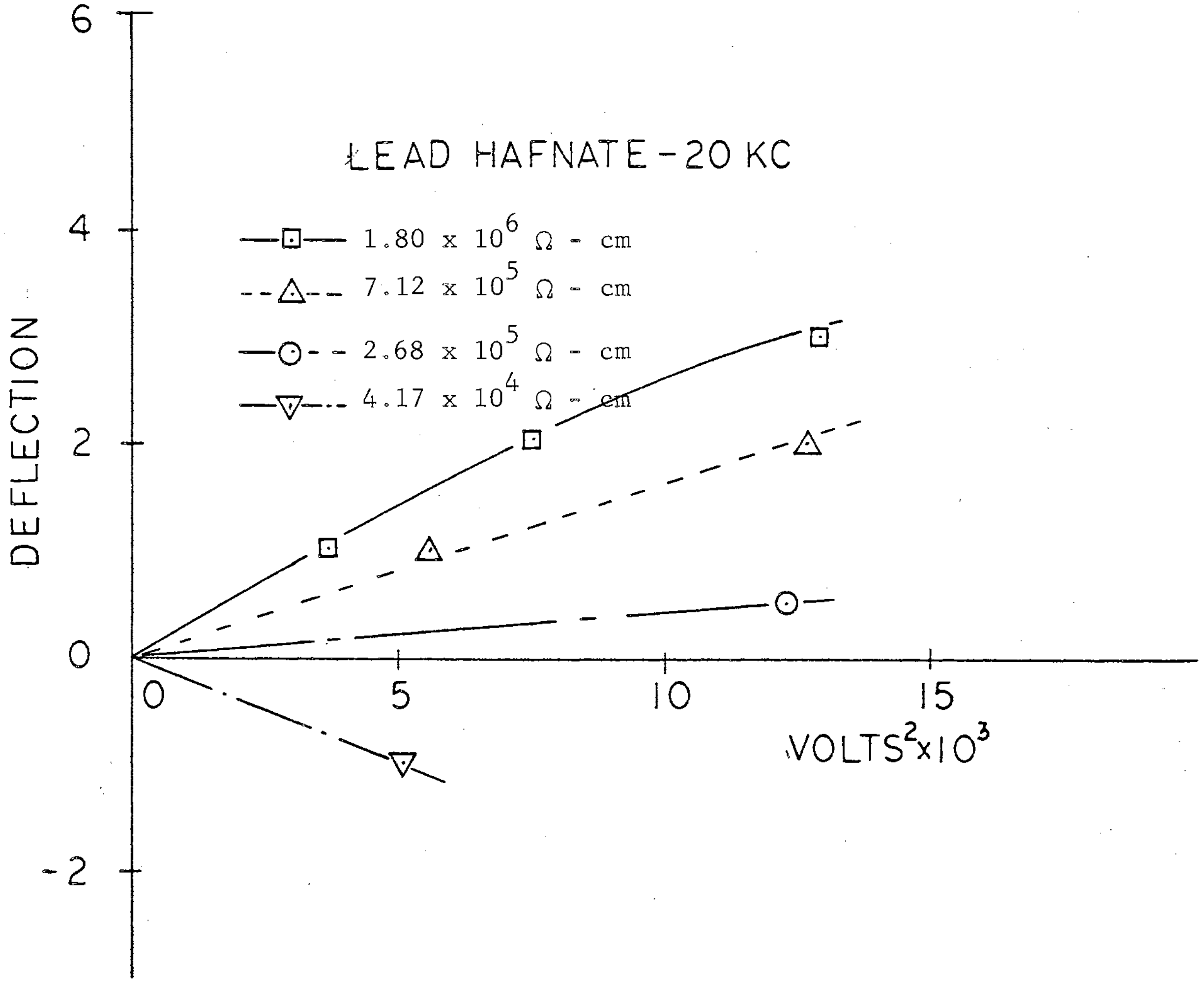


Figure 7. Deflection versus Voltage Squared for Lead Hafnate at 20 KC

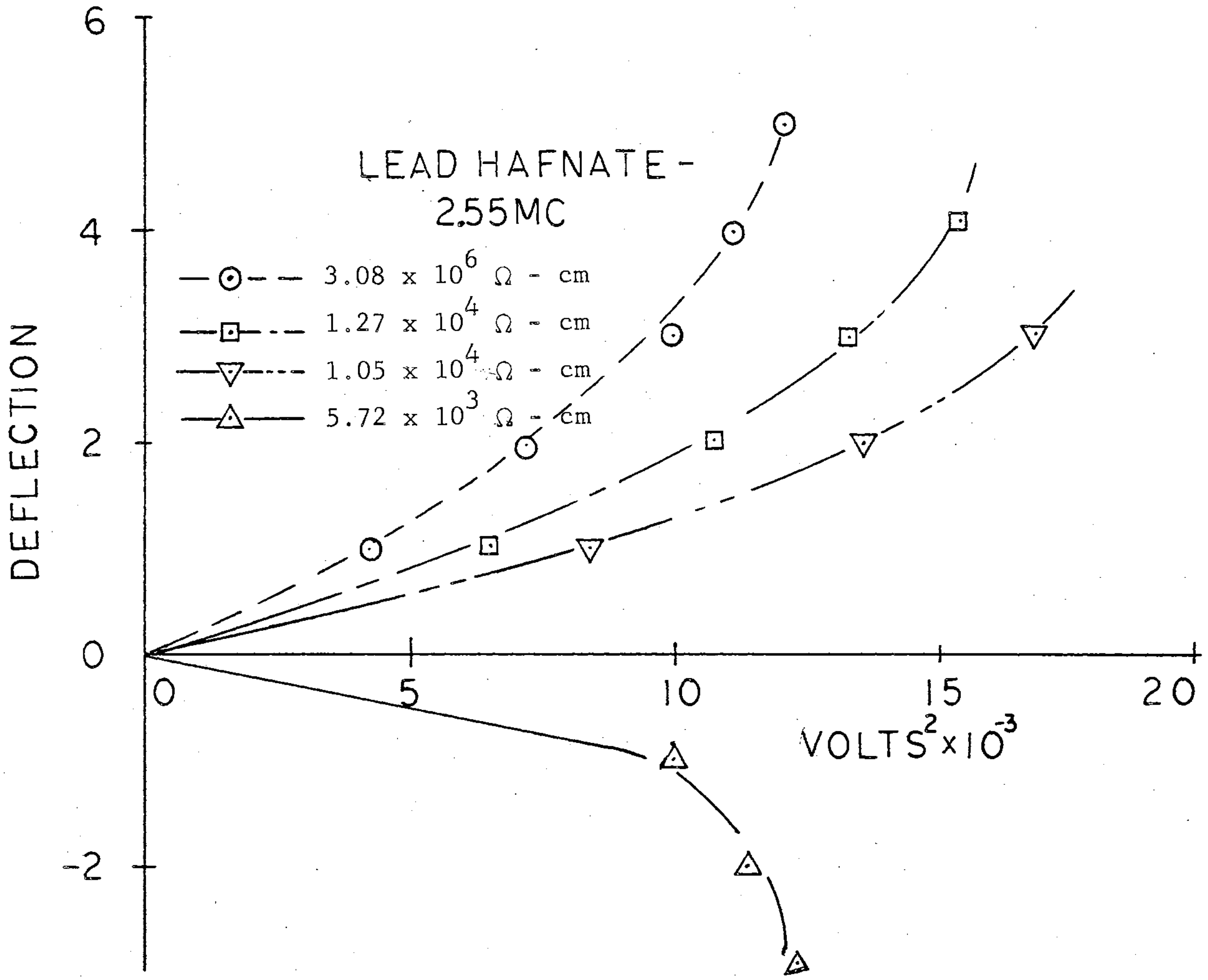


Figure 8. Deflection versus Voltage Squared for Lead Hafnate at 2.55 MC

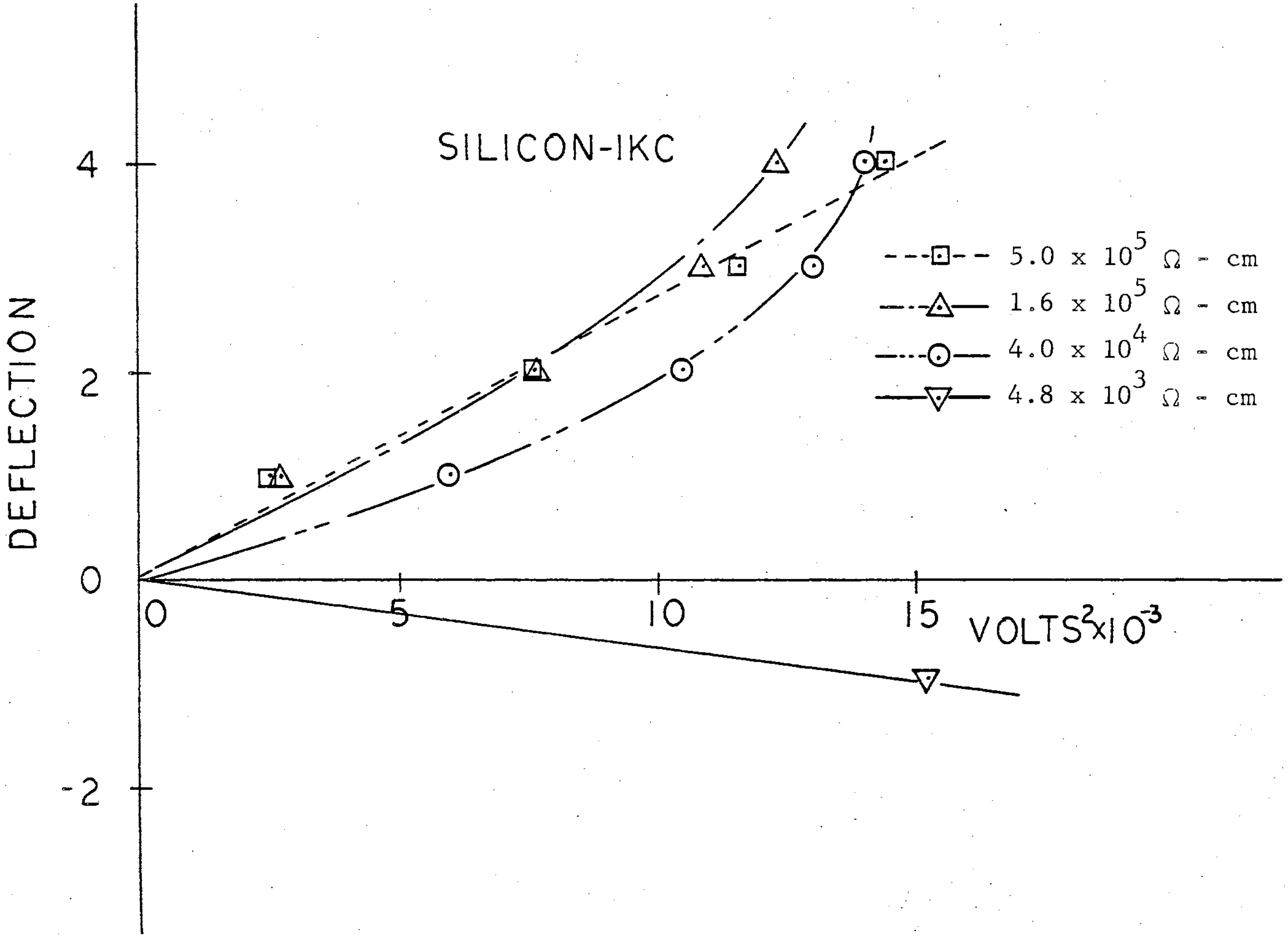


Figure 9. Deflection versus Voltage Squared for Silicon at 1 KC

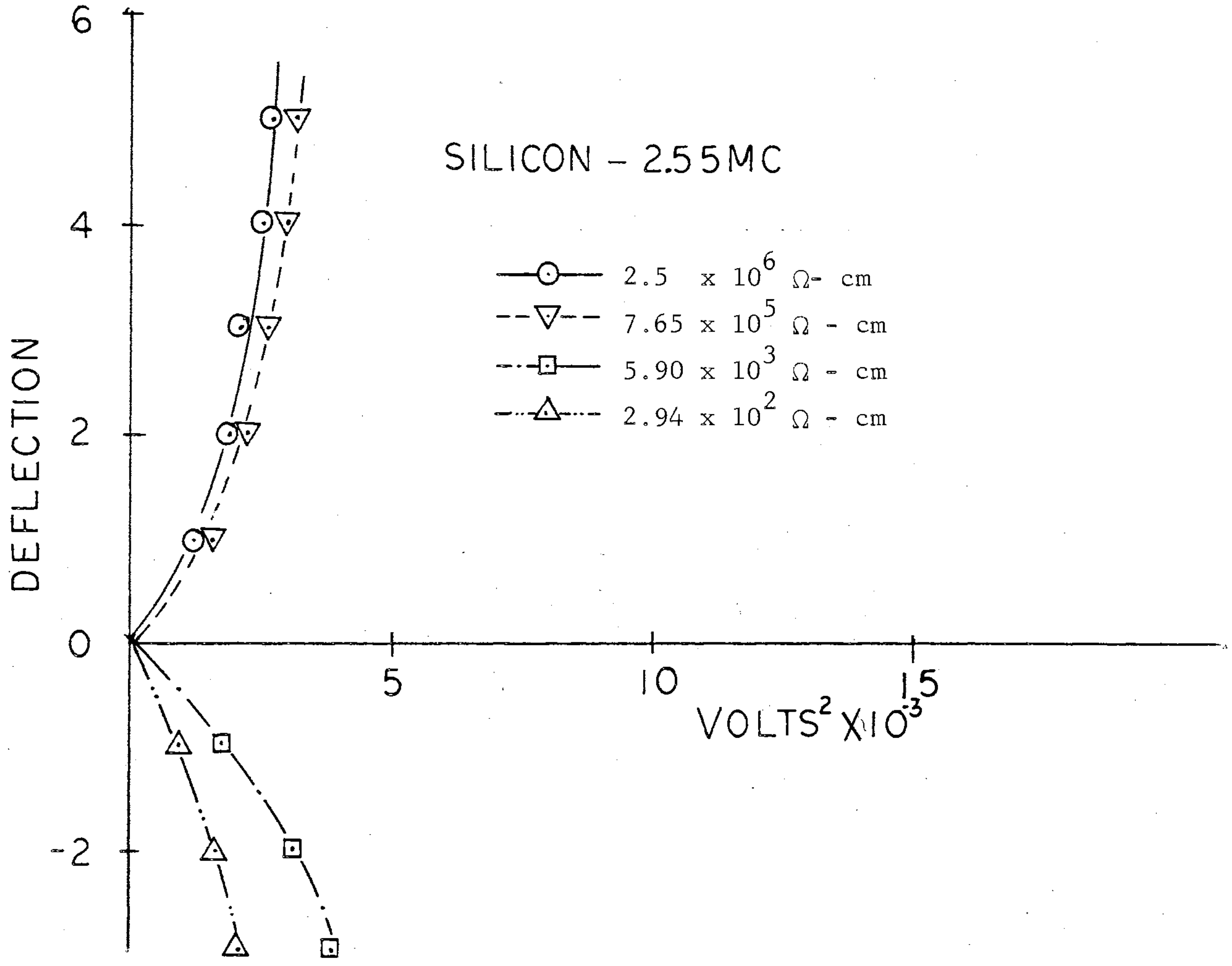


Figure 10. Deflection versus Voltage Squared for Silicon at 2.55 MC

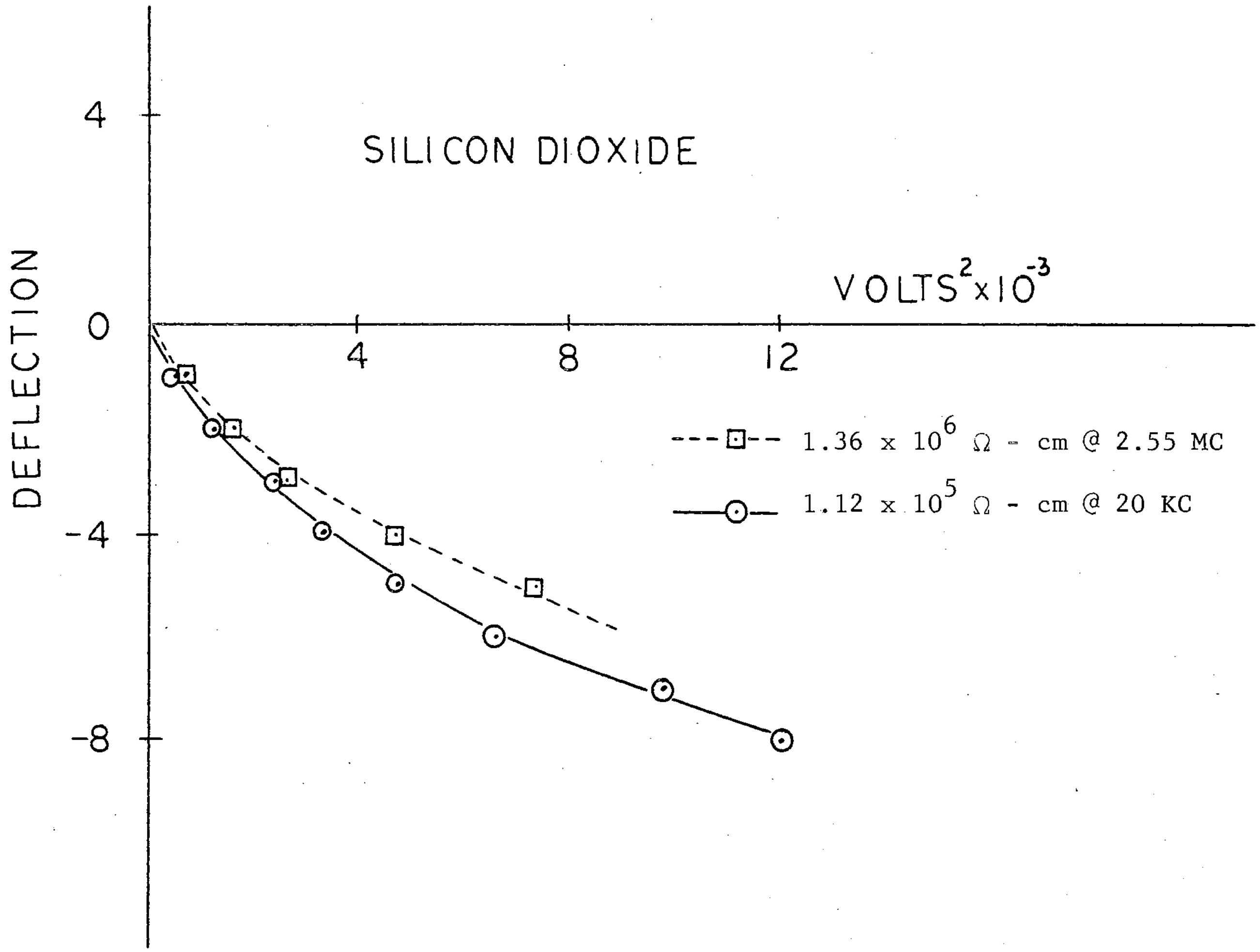


Figure 11. Deflection versus Voltage Squared for Silicon Dioxide at 20 KC and 2.55 MC

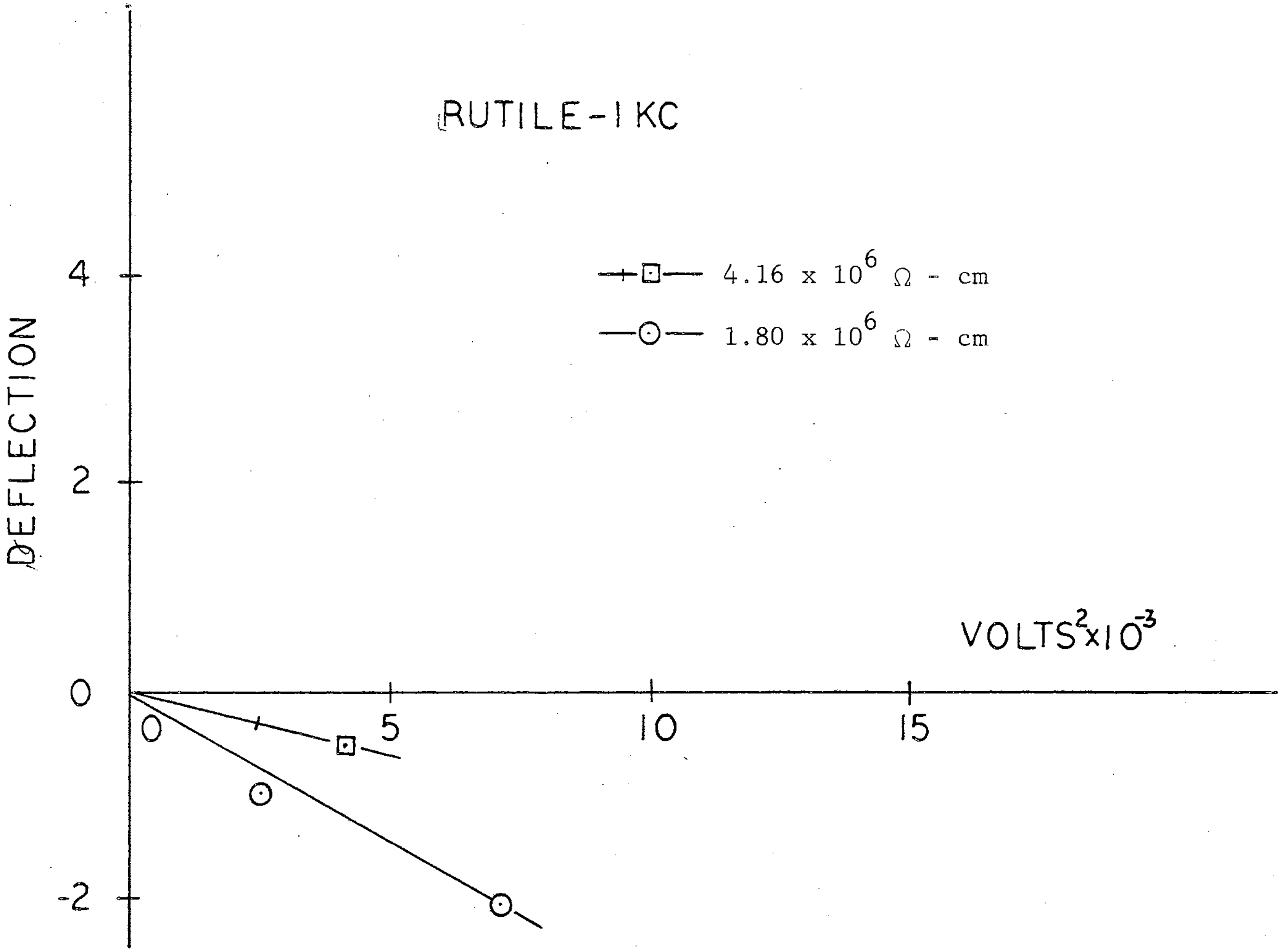


Figure 12. Deflection versus Voltage Squared for Rutile at 1 KC

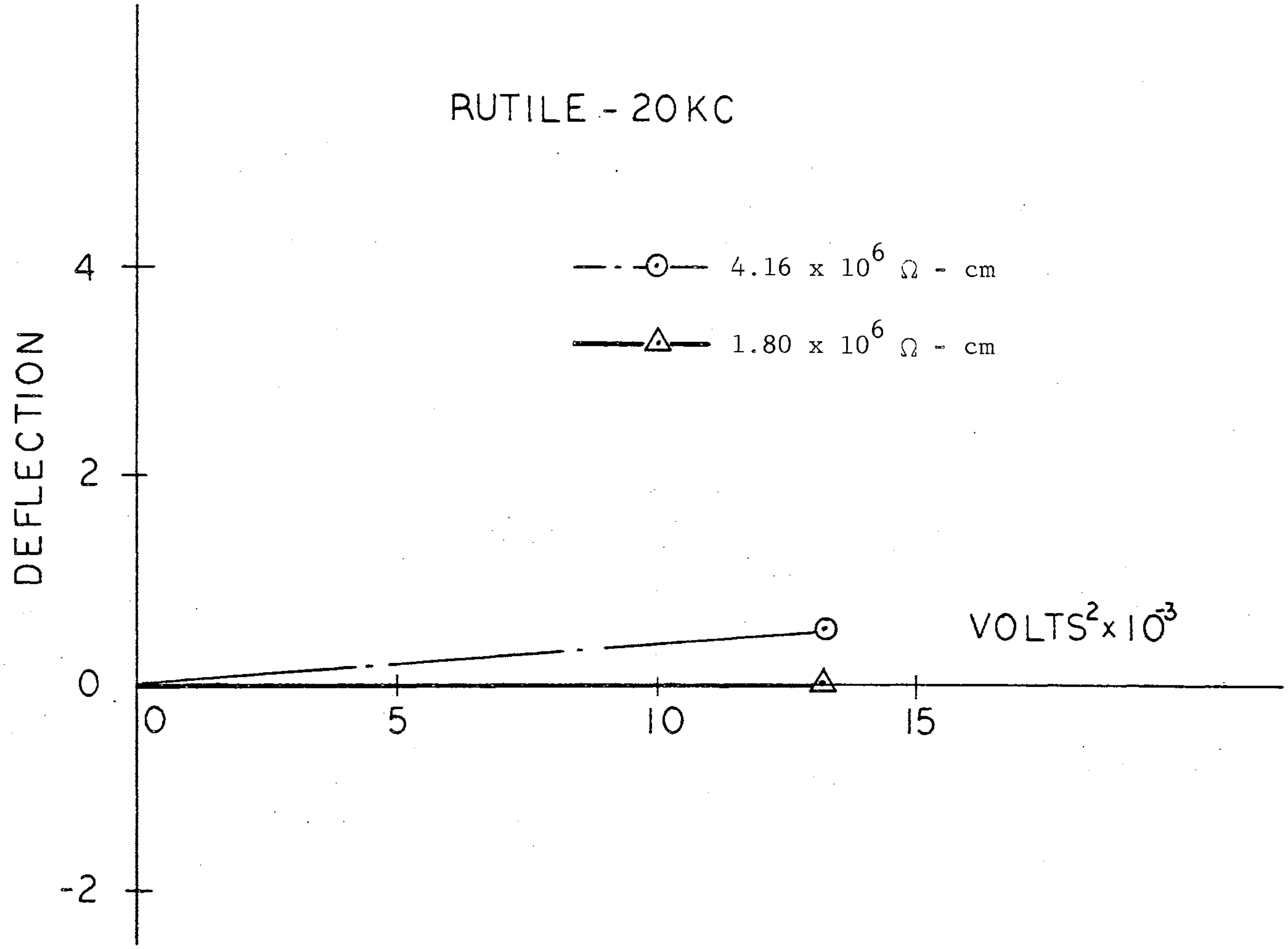


Figure 13. Deflection versus Voltage Squared for Rutile at 20 KC

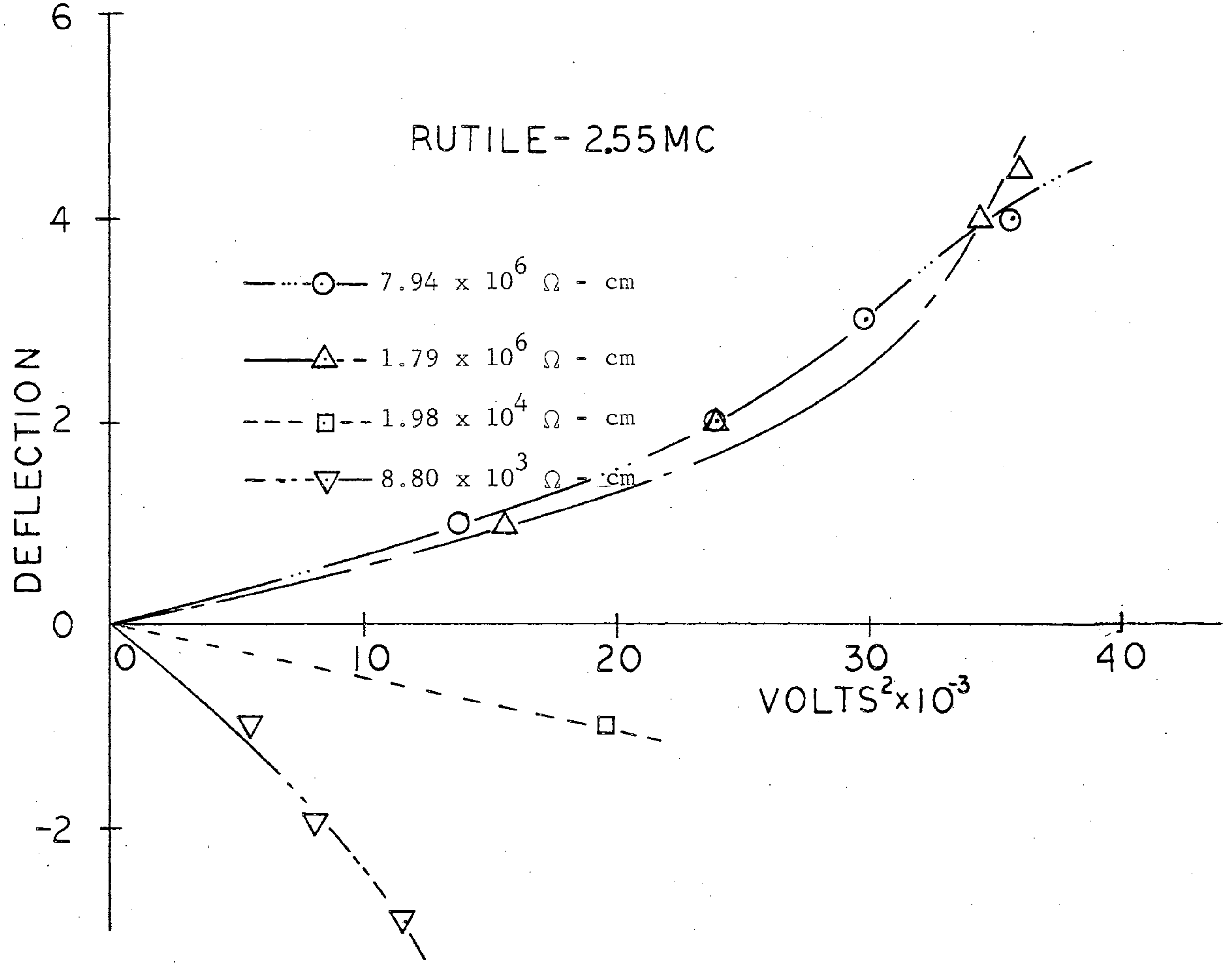


Figure 14.— Deflection versus Voltage Squared for Rutile at 2.55 MC

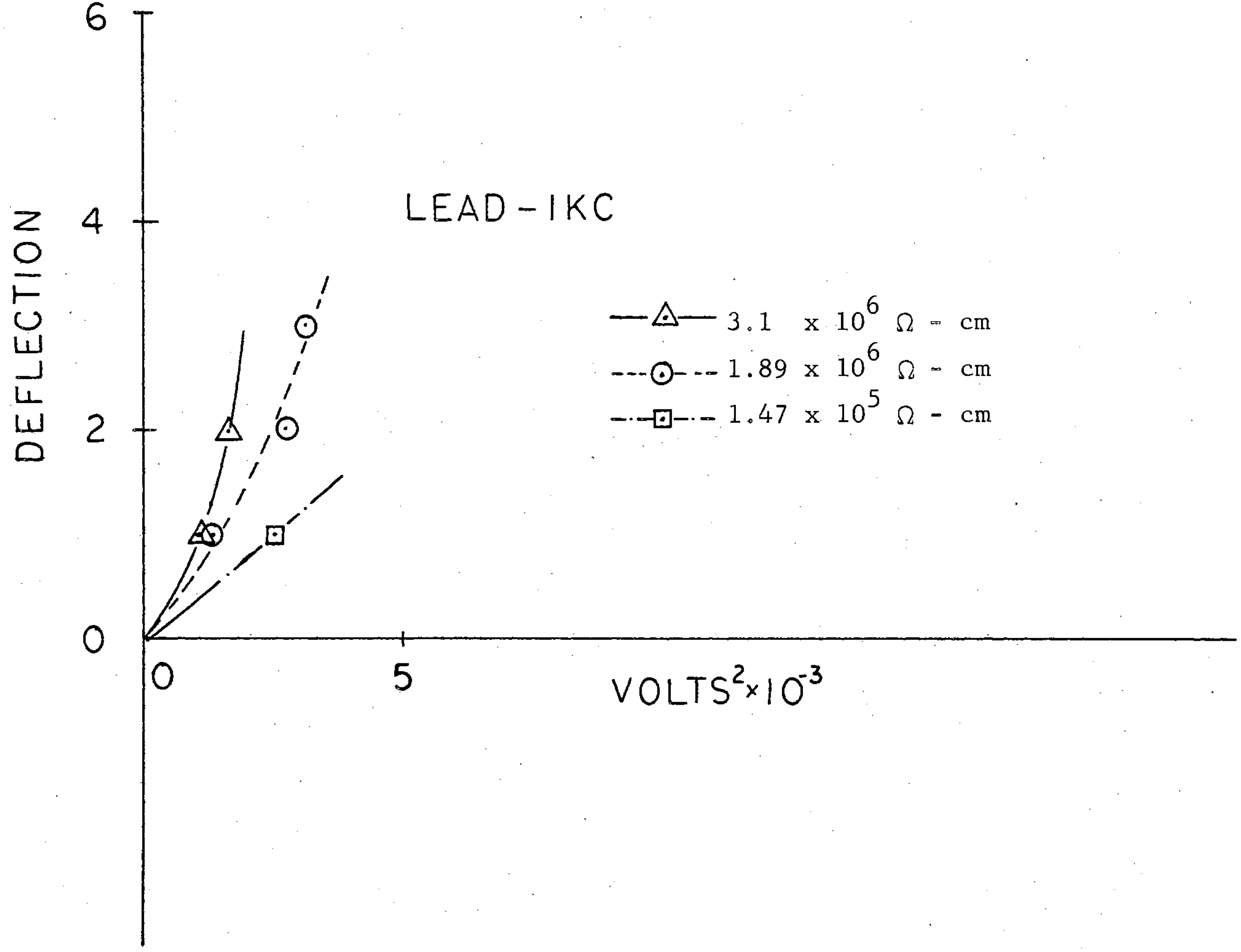


Figure 15. Deflection versus Voltage Squared for Lead at 1KC

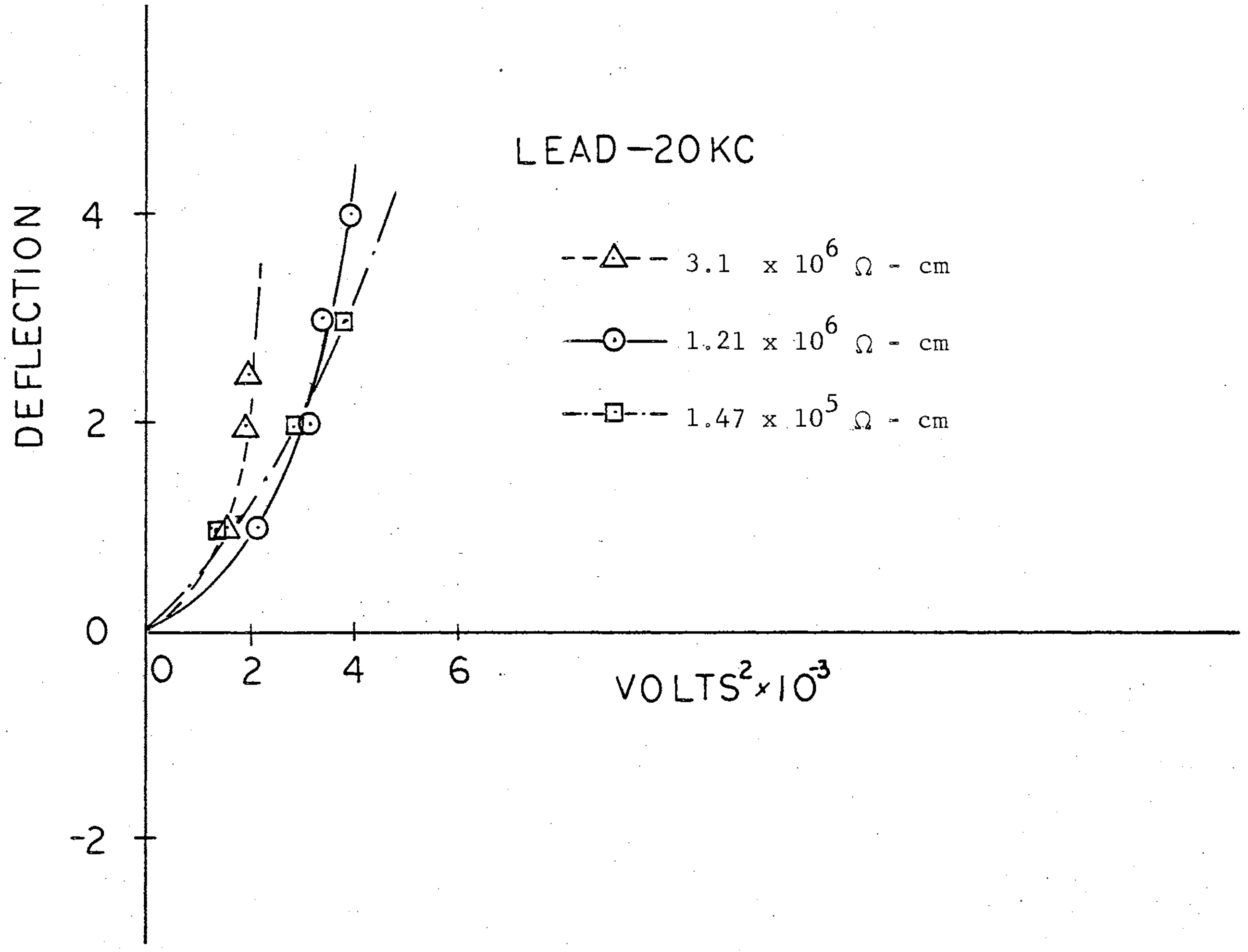


Figure 16. Deflection versus Voltage squared for Lead at 20 KC

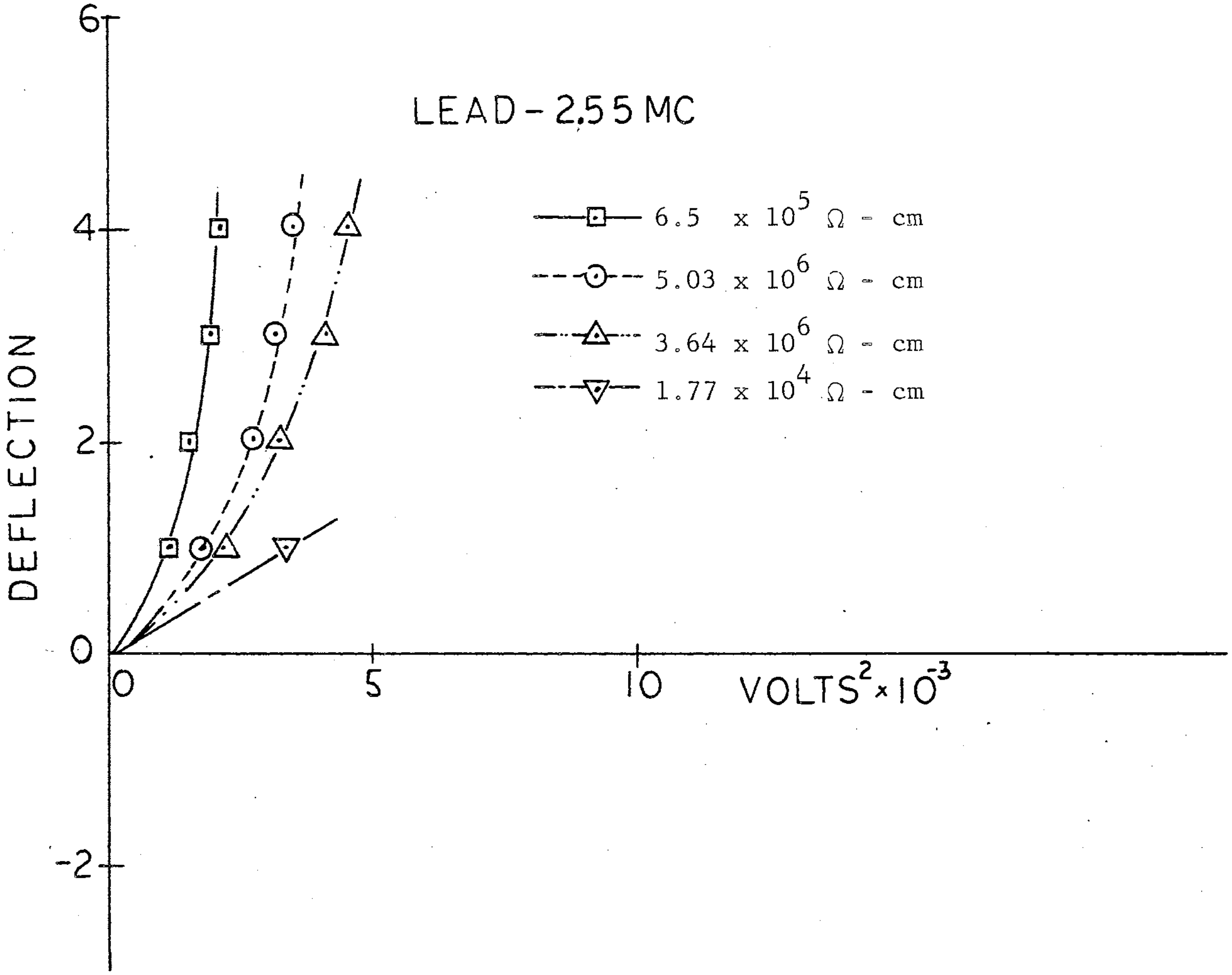


Figure 17. Deflection versus Voltage Squared for Lead at 2.55 MC

TABLE I

VALUES OF DIELECTRIC CONSTANTS AND SPECIFIC RESISTIVITIES USED

Material	Dielectric Constant	Frequency	Reference Source	Resistivity	Reference
lead hafnate	350	1MC	A	10^{16}	D
silicon dioxide	3.78	1MC	B	10^{14}	E
lead	------	---	---	10^{-5}	E
silicon	11.9	13GC	C	5×10^{4}	D
rutile					
(‖ optic axis)	170	1MC	B	--------	---
(⊥ optic axis)	86	1MC	B	--------	---

A -- Dr. E. Kohnke, Okla. State University, private communication.

B -- A. Von Hippel, <u>Dielectric Materials and Applications</u>, pub. jointly by the Tech. Press of M.I.T. and John Wiley and Sons, Inc., New York (1954).

C -- H. B. Briggs, Phys. Rev., <u>77</u>, 287 (1950).

D -- Measured in our laboratory.

E -- <u>Handbook of Chemistry and Physics</u>, pub. by Chemical Rubber Co., 44th ed.

crossings to be noted in Figures 9, 14 and 16. The turbulence effect becomes especially marked in the more conductive media at high fields.

It is easy to see that the force equation predicts accurately the direction of motion for the particles and water where both have high resistivity. For instance, see Figures 6, 7 and 8 for lead hafnate. The resistivity of lead hafnate is very high (see Table 1), and it can be noted that the force equation would predict its motion toward the higher field intensity region. This is exactly what happens for high resistivity water--regardless of frequency. But, at low resistivity water, something strange occurs--the particle goes the other direction. It is in this region that the assumptions for the ideal force equation are no longer sufficient. No longer is the resistivity a negligible quantity when compared with the permittivity. A little later in this section, a method will be discussed to calculate a point in the water's resistivity range where the crossover from the important term being the imaginary permittivity lies. Next, looking at the curves for silicon dioxide, it is seen that this particle always goes to the plate electrode which is evident from the force equation since its dielectric constant is much less than water.

Having the particles with dielectric constant greater than that of water going in the wrong direction was at first a puzzle, but by looking at particles like silicon and lead (see Figures 9, 10, 15, 16 and 17) the anomoly was quickly resolved. The explanation for the particle going "backwards" was due entirely to the resistivities of the materials.

It is important at this point to list the results obtained from using the relations in Chapter III and specifically the relation

$$|K_1' - i/\rho_1\omega\epsilon_o| = |K_2' - i/\rho_2\omega\epsilon_o|$$

From this equation, the value of the resistivity of water can be calculated where the force will equal zero. Using the values listed in Table 1, the values of ρ_1 are calculated and listed in Table 2.

This table shows that the lead particle will always go forward because water could never reach a resistivity of 10^{-5} ohm-cm., and it shows that the silicon particle will cross over at nearly its own resistivity since this term is much greater in magnitude than is its real permittivity at low frequencies.

The initial slope of the deflection versus voltage squared curves is important for it represents the proportionality constant between the force and the voltage squared in the limit as Δr approaches zero. This slope constant shall be denoted as c which has units of "deflection per volts squared". Plotting c versus the resistivity of the particular curve from which c was taken, gives a family of curves for a single particle, one curve for each frequency. See Figures 18-22. These graphs are particularly interesting because they instantly show the effect of the frequency on the dielectrophoretic force. In addition, the points at which the curves intercept the axis are the points of the water's resistivity at which the force is equal to zero. Table 2 contains a comparison between these crossover points taken from the graph and calculated from the equation listed previously. The agreement is satisfying.

TABLE II

CALCULATED AND EXPERIMENTAL CROSSOVER RESISTIVITIES

Particle	Calculated Crossover Resistivity (Ω - cm)	Frequency	Estimated Crossover From Graphs (Ω - cm)
lead hafnate	2.06 x 10^{3}	2.55 MC	1.5 x 10^{4}
	2.63 x 10^{5}	20 KC	4.5 x 10^{5}
	1.05 x 10^{6}	5 KC	1.5 x 10^{6}
	5.30 x 10^{6}	1 KC	2.0 x 10^{6}
rutile	1.19 x 10^{4}	2.55 MC	6 x 10^{4}
	1.52 x 10^{6}	20 KC	1.7 x 10^{6}
	6.0 x 10^{6}	5 KC	---------
	3.0 x 10^{7}	1 KC	None obtained
silicon dioxide	None		None obtained
lead	10^{-5}		Always Forward
silicon	6 x 10^{3}	2.55 MC	1.3 x 10^{4}
	5 x 10^{4}	20 KC	8 x 10^{3}
	5 x 10^{4}	5 KC	---------
	5 x 10^{4}	1 KC	9 x 10^{3}

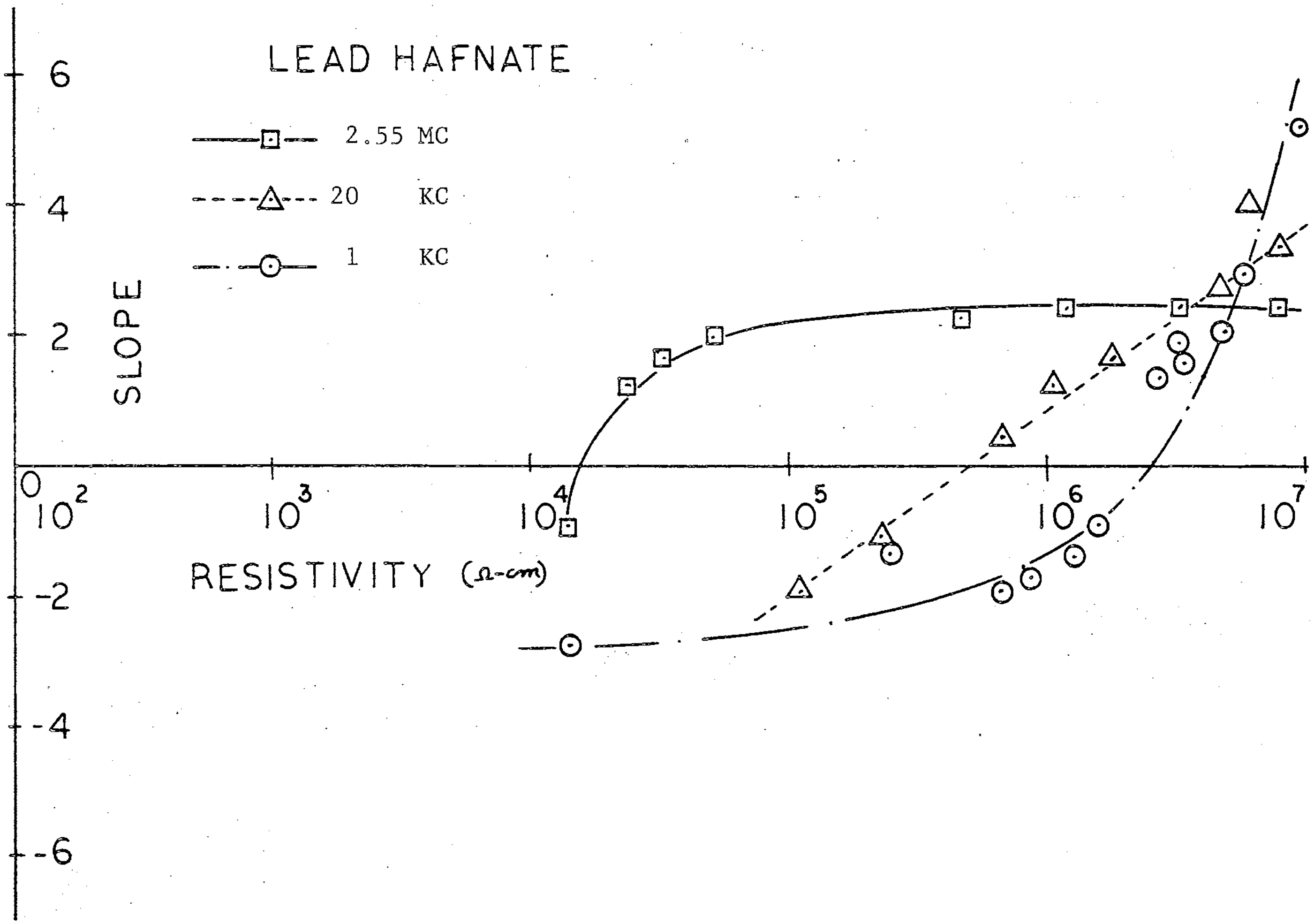


Figure 18. Slope versus Resistivity of Water for Lead Hafnate

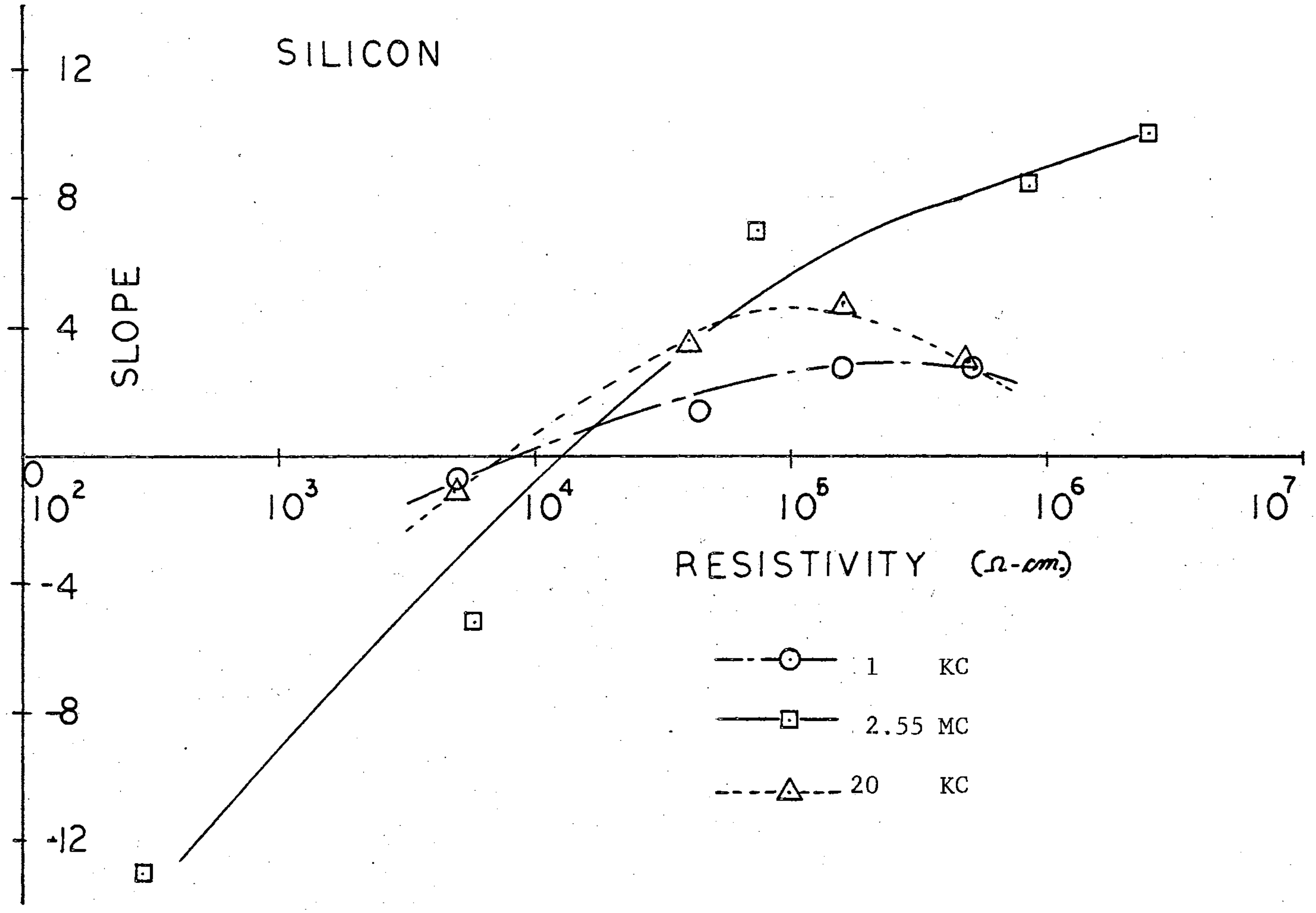


Figure 19. Slope versus Resistivity of Water for Silicon

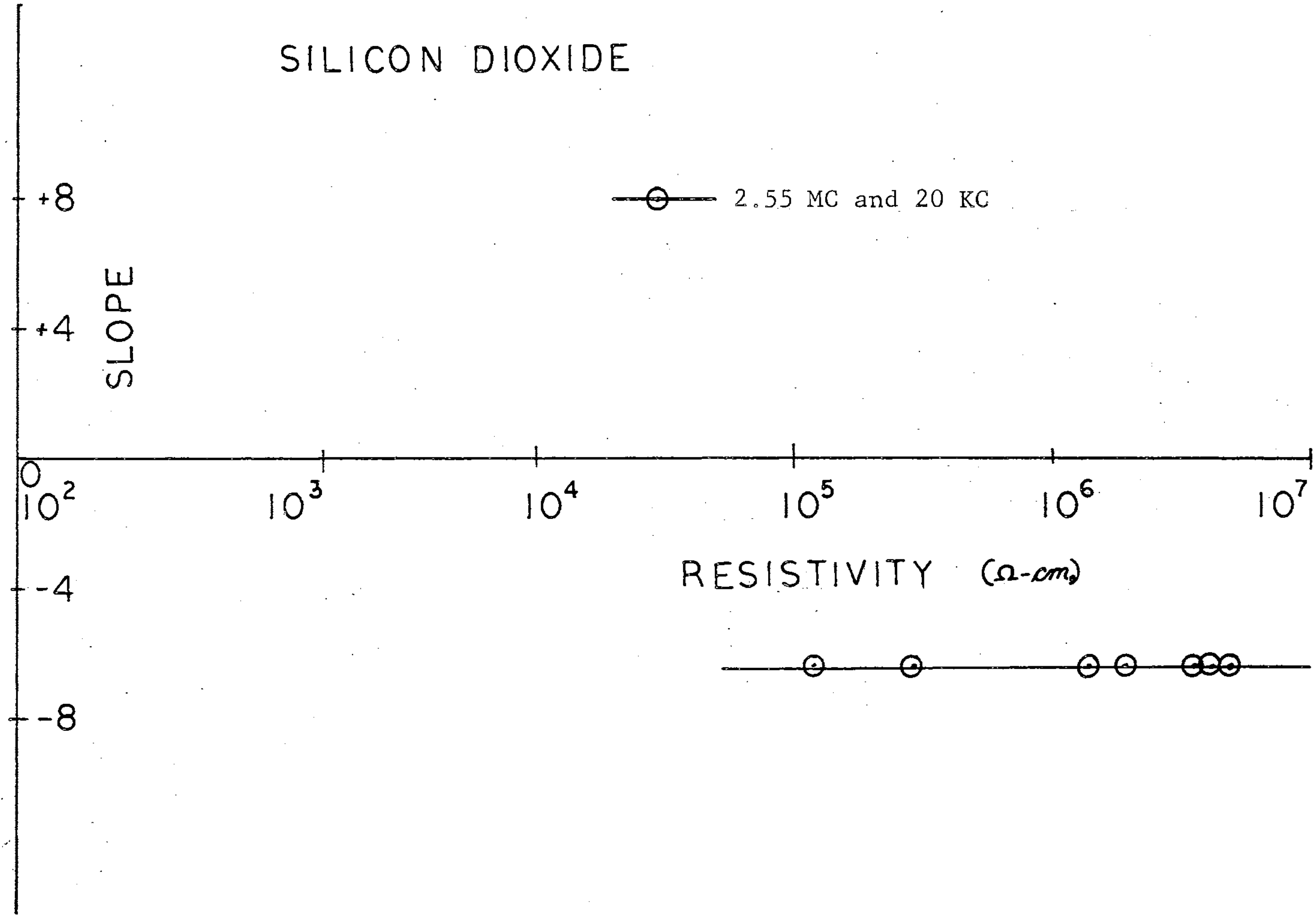


Figure 20. Slope versus Resistivity of Water for Silicon Dioxide

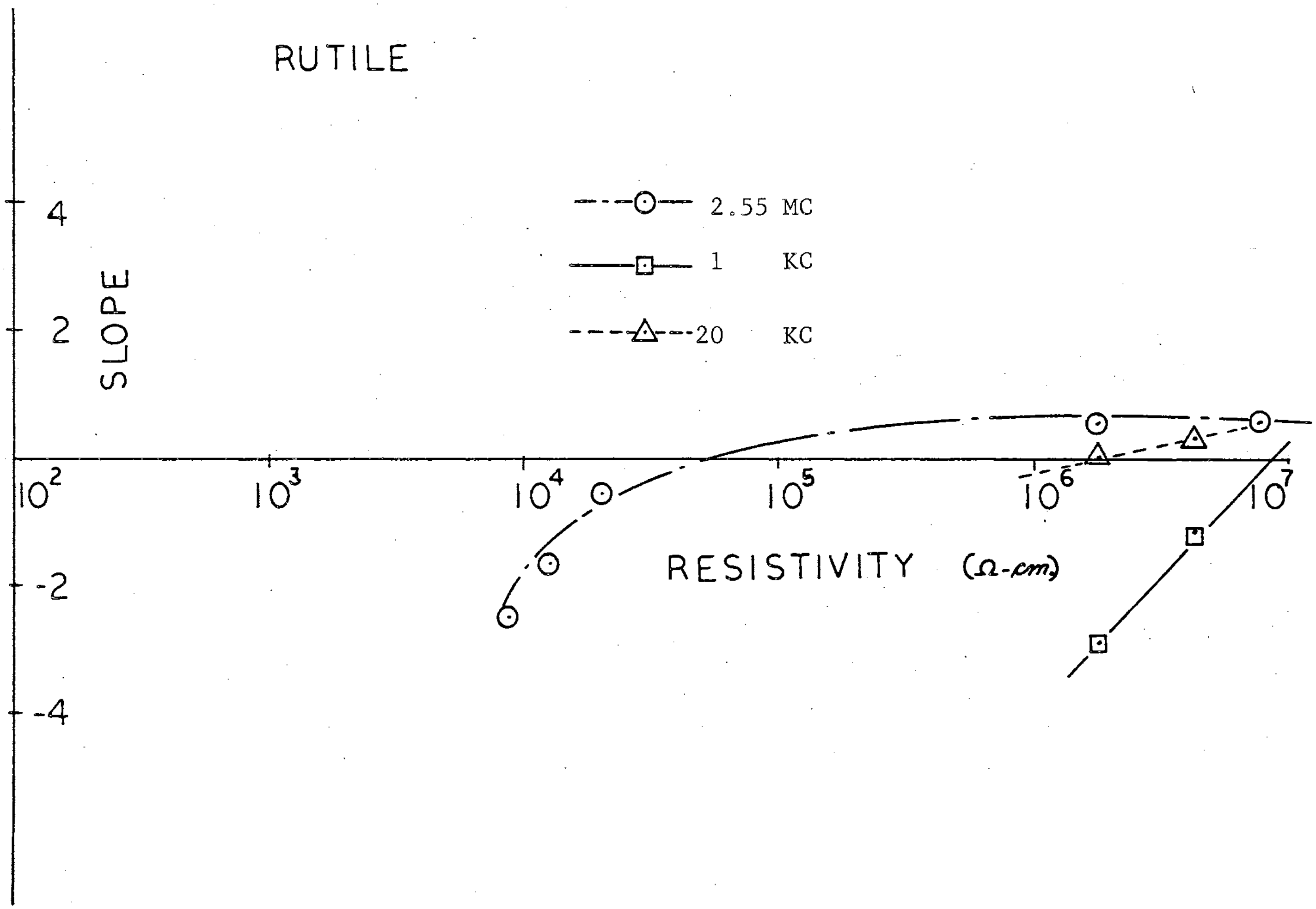


Figure 21. Slope versus Resistivity of Water for Rutile

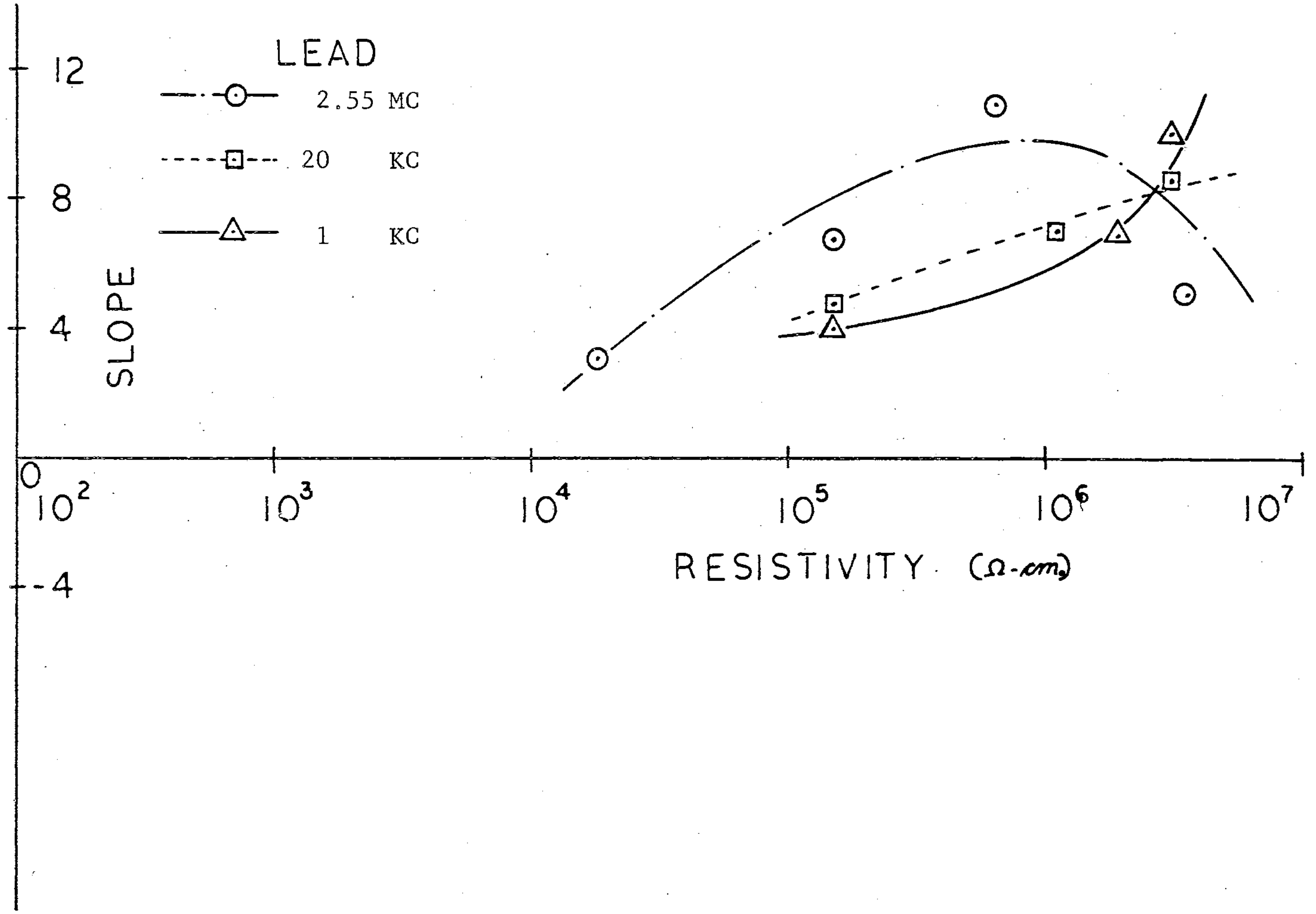


Figure 22. Slope versus Resistivity of Water for Lead

From these curves, the general result is: the higher the frequency, the more effective the force unless the purity of the water is extremely high, in which case, the lower frequency becomes more effective.

CHAPTER VI

USE OF FORCE ON ORGANISMS

After ascertaining some of the basic rules governing the dielectrophoretic force, the logical step was to experiment with living cells. As was mentioned in an earlier chapter, the apparatus used to investigate the force effect was like that of a microscope slide. The experiment was a simple one: place some cells into the well with pin and plate electrodes and view their motion with a microscope.

The first experiment was with yeast. There are several reasons for choosing yeast for the biological cells to study. First, the organism must be capable of survival in highly purified, distilled water; second, the organism should preferably be present as individual, spherically shaped particles; and last, the organism should be of easy access and have rapid reproduction: all of these criteria are met by yeast cells.

The yeast cells studied either collected at the pin electrode or remained in the suspension, depending on the resistivity of the suspension. When the purity was high enough that the cells collected with no turbulence, the yeast cells formed chains or lines which looked like tiny magnets aligned in a magnetic field. When the water suspension was in the low resistivity range, stirring was observed. An explanation of the cause of stirring is discussed by Pohl (9).

On two occasions yeast cells, subjected to an electric field great enough to collect them, were tested for viability. In both cases the yeast survived.

Another test with yeast cells was to "kill" an amount of cells, stain them, and add them to an approximately equal density of living cells. The suspension was then placed in the well of the slide, and the motion of the cells was observed with the electric field on. The cells were killed by heating a suspension of yeast cells in water to 60° C, cooling, and adding 10% by volume of 0.3% crystal violet solution. Next, they were allowed to stand for three minutes for the dye to fix and then centrifuged to wash free the suspension of dissolved dye. This gave a preparation of yeast cells, which under inspection at a magnification of 600X, showed the fixing to be strong and uniform. In addition, the cells appeared to retain their spherical shape, which is a necessity when comparing with normal cells. Next, the cells were mixed with the ones containing washed living cells and placed into the slide's well for observation. The number of white cells collected at the pin electrode with the field applied was much greater than the number of stained cells.

Other trials were conducted with 1:1, 1:3, and 3:1 volume ratios of living cells to dead ones, and each trial showed the collection at the pin electrode contained a predominant amount of living cells with a trace of dead ones. Test conducted on each group of cells separately (with approximately the same cell concentration and volume resistivity) showed the live cells to be attracted strongly and the dead cells weakly.

These tests indicate that living and dead cells do differ in polarizability, and that the difference is large enough to permit dielectrophoretic separation.

The highly divergent field that was used is strongly dependent on the distance from the pin electrode, thus materials only slightly more polar than water would be attracted to the pin if they were in close proximity to it. This is one part of the explanation why the dead cells partially collected at the pin electrode. Another reason for dead cells to collect at the pin is that materials of high dielectric constant, even in uniform fields, condense the lines of force and create a divergent field in the region near them. So in the case of a suspension of yeast cells in water, the live ones could exert a large force on the dead ones near by as they migrate toward the pin electrode.

In building a practical separator, a better geometry than this pin-plate electrode system would be one in which the force whose magnitude is a constant at any point in the field, and whose direction is radially outwards. This isomotive field would eliminate the strong attraction by the dead cells near the electrodes, but would not affect the attraction of dead cells by live ones near to or in contact with the dead cells.

The experiments that followed were done with dog erythrocytes and thrombocytes in suspension in aqueous 5% glucose. These dog cell preparations were made by Dr. Ralph Buckner of the Pathology Department of Oklahoma State University. The photographs were made with his considerable assistance. Those cells in the divergent electric field aligned in chains as can be seen in the photographs taken with a

Poloroid camera atop an American Optical microscope (see Figure 23). That there is a difference between the cells taken from three normal dogs and two hemophilic transmitter dogs, is evident from the photographs since the cell concentration resistivity of the solution, applied potential, and collection time were carefully controlled.

These experiments with simple cells showed that since the force on the particles is in the direction of highest field intensity, the cells must necessarily have a higher effective dielectric constant than water, and the idea of using this force to differentiate between cells on the basis of their polarization properties is thereby confirmed.

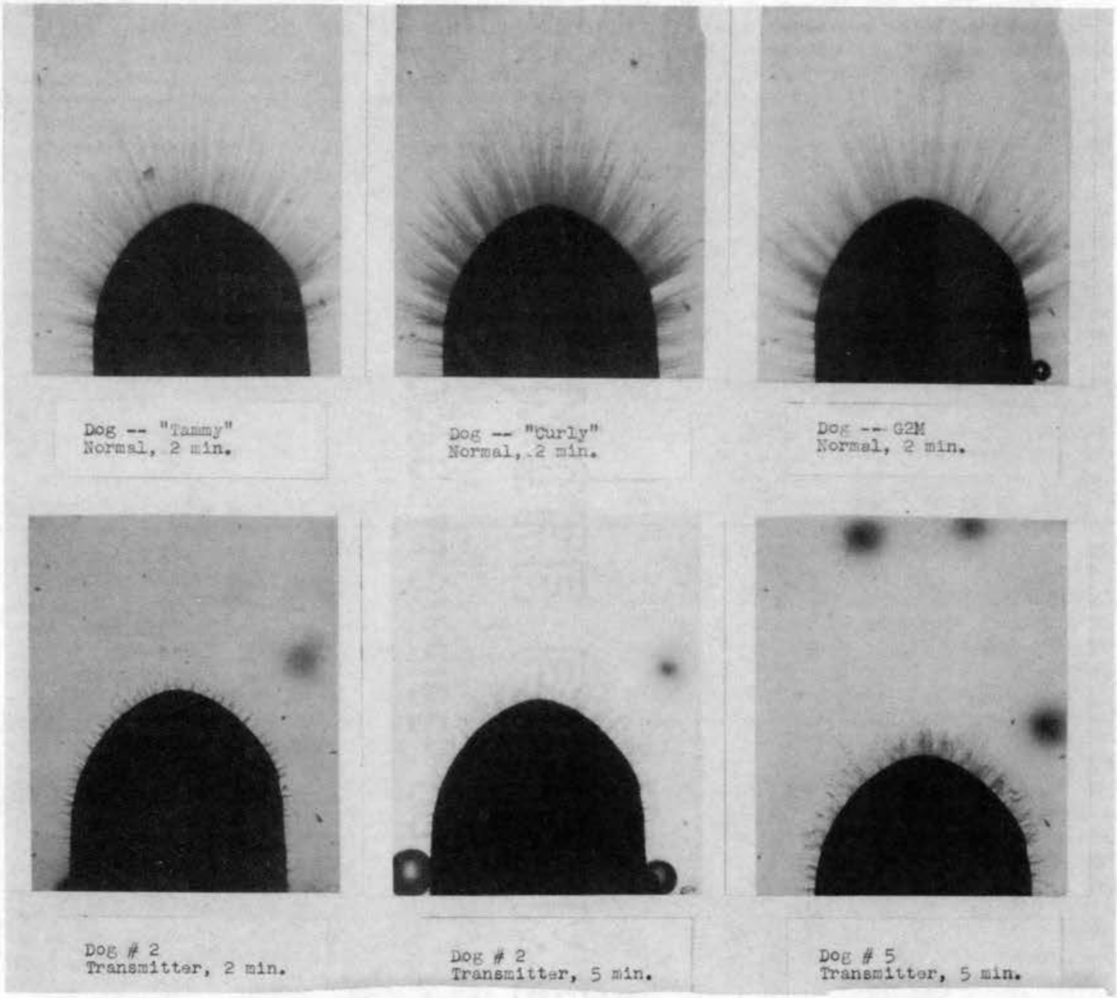


Figure 23. Comparative Dielectrophoretic Collection in Pin-Plate Cell of Dog Thrombocytes (mag. - 50X).

CHAPTER VII

SUMMARY

The behavior of real particles in a conducting dielectric has been studied at various frequencies. This was investigated partly for the purpose of extending the use of the dielectrophoretic force to biological cells and organisms.

We observe that the force not only depends on the real dielectric constant, but on the imaginary part as well, and this in turn causes the frequency to be an important factor. At low values of resistivity of the water, the higher the frequency, the more effective the force. This should have an important consequence in the study of living organisms and cells because of their tendency to give up ions in dilute solutions. In this case, low conductivity is virtually impossible to obtain, and if the force is to be used, then the applied field must alternate at a moderately high frequency.

In conclusion, much work is needed before use of the force as a differentiator between cells is possible, but this background research allows us to believe that this technique can be developed to become a valuable aid in biological studies of organisms and their parts.

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