CELLULAR SPIN RESONANCE

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PREFACE

This study will concern itself with cellular spin resonance mainly as it applies to cells and to a lesser extent, particles. The procedure necessary for the studies as well as the apparatus necessary will also be discussed. Appendix A (a manuscript for a chapter in press) with theory and additional information will be included in this thesis.

The selection of cellular spin resonance as the topic for this thesis came about for a variety of reasons. My having a biological background, the conducting of a study on cells seemed to be a very logical and natural choice. My desire to do some work with some electrical apparatus and with computers and to learn a little applied physics also contributed to my choice. Ultimately, and most importantly, the chance to work in collaboration with Dr. Herbert Pohl who had started me off in research while I was still an undergraduate and has become a good friend made it all very appealing and worthwhile. It has been said that doing graduate work is like doing an apprenticeship in some trade. If this is so I have labored under one of the best in his trade.

I also wish to express my sincere gratitude to Dr. L. Herbert Bruneau who advised me on the academic portion of my graduate degree. My thanks also go to Kent Pollock, who will soon become Dr. Kent Pollock, for answering my many questions as they pertained to physics.

Special thanks go to the Pohl Cancer Research Laboratory for the

financial support it provided while conducting this study.

My parents Hiram and Gladys Rivera deserve my most sincere and deepest appreciation for their unending support and love. Finally a special thanks go to my wife Jean for encouraging me while I strove to finish this thesis, and for her support when it seemed it would never get done.

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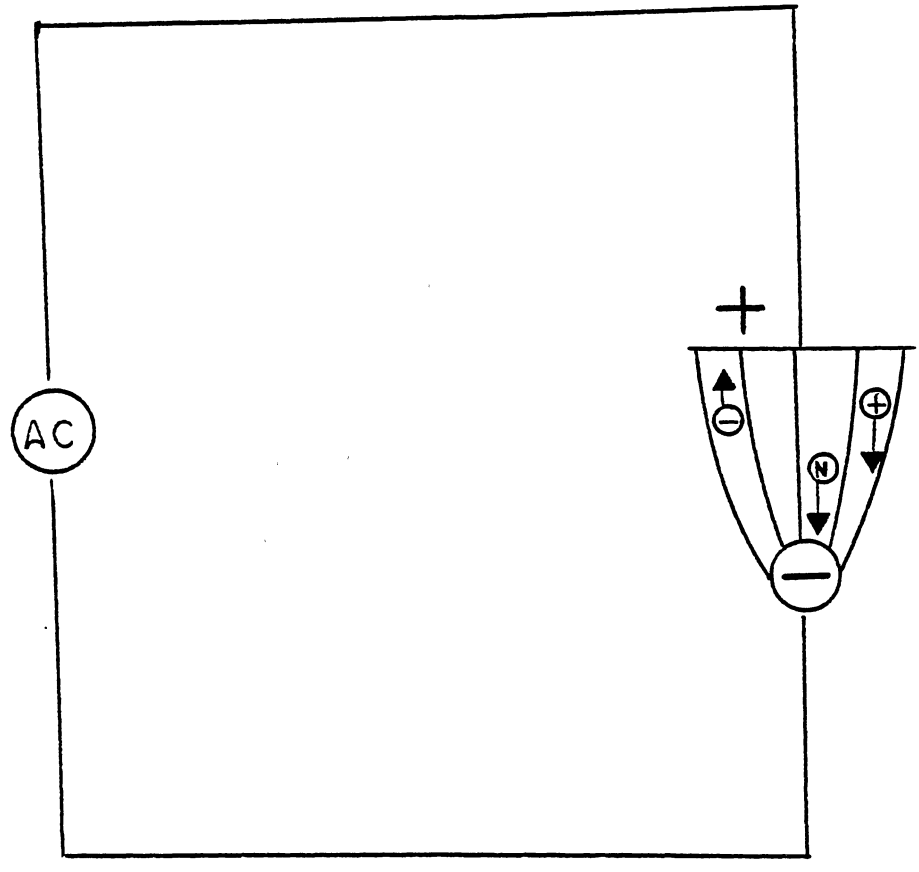
CHAPTER I

INTRODUCTION

Cellular spin resonance (CSR) is a new method that has the potential for some very exciting applications. This phenomenon of resonating spinning in response to an AC field was first observed by Pohl and Crane (1971) while they were studying the dielectrophoretic behavior of Saccharomyces cervesiae. They observed that cells were seen to spin while in the presence of an alternating current (ac) field. What is intriguing is that the cells are observed to rotate at only a few Hertz while the applied frequency of up to 1 MHz was applied. In addition, the cells responded very sharply to the frequency of the applied field. These cells can be made to spin, stop, or change direction of spinning in a resonant manner depending on what external frequency is being applied at the moment. Hence the name "cellular spin resonance".

At the heart of CSR is the phenomenon known as dielectrophoresis. Dielectrophoresis (DEP) is defined as the motion of uncharged particles or fluid induced by a non-uniform electric field due to their induced, or permanent dipoles. It should be pointed out that dielectrophoresis should be distinguished from electrophoresis. Electrophoresis is the movement of a charged particle due to a uniform or non-uniform field. Figure 1 shows the difference between the movement of a charged particle and a neutral

Figure 1
Effect of a non-uniform AC field on
a neutral and charged objects.


AC
+
−

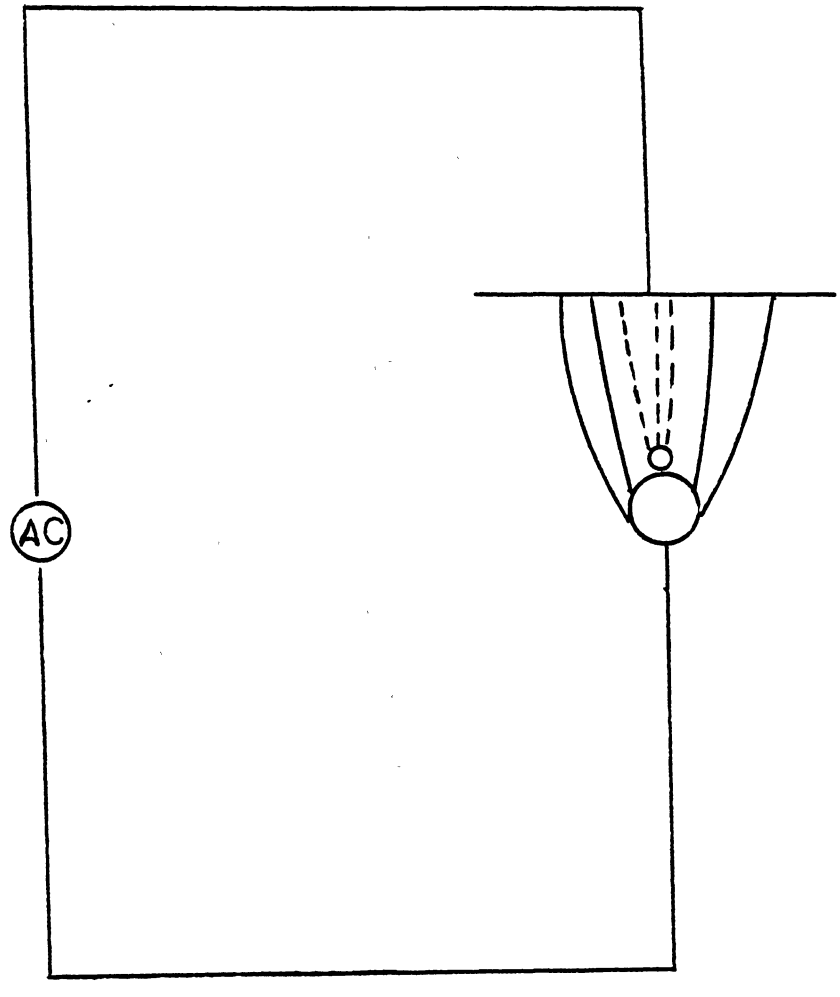
particle while under the influence of an ac field. The charged particle will be attracted by its opposite charge. Inside a rapidly changing field (ac field) it will just shudder as it tries to always follow its attracting charge. In contrast, the neutral particle (or cell) will always be attracted by the region of stronger field. An interesting sidelight of dielectrophoresis is that when a particle or cell is attracted to and finally comes in contact with the electrode it still perturbs the field creating a new region of high field strength (Fig. 2.) Now if a cell or particle comes close to it, this new region of increased field strength divergence will attract the object. This phenomenon has been dubbed "pearl-chaining" since one may end up with chains of many "links".

The response of the cells to CSR has been observed to be directly dependent on the physiological state of the cell. Culture age, cell health, and possibly even culture temperature all serve to change the CSR spectrum of that particular cell. Because of this, a standardized spectrum could be made up of a particular cell while in a healthy state. When another cell is compared to this standard spectrum, and a deviation is seen, it can be assumed that the cell is different in some way. This could be used as a diagnostic test between healthy cells and diseased cells.

Particles have also been observed to display a certain CSR spectrum. In this case the governing factor for spinning, be it with the field (+ rotation) or against the field (- rotation), is the polarizability of the particular particle under study.

Appendix A has a review on the literature as it pertains to cellular spin resonance. The reader is referred to that section for more details.

Figure 2
Particle or cell creating a new,
higher area of field strength.


AC

CHAPTER II

APPARATUS AND EXPERIMENTAL PROCEDURES

Chamber

The chamber used for the studies of CSR was that of a 4-pole configuration, (Figure 3). This chamber is connected to an electrical circuit which produces a rotating sinsoidal field (Appendix B). A "block" diagram illustration of the entire experimental set-up required is shown in Figure 4.

Cells

Various cells were used in this study, namely Saccharomyces cerevesiae (yeast), bovine kidney cells (BKC), African green monkey kidney cells (VERO), and Crandell feline kidney (CRFK) cells. The yeast cells were grown in Difco Sabouraud liquid medium at room temperature (25^{o}C) while being kept aerated by a magnetic stirrer. Mammalian cells were obtained from Mrs. Jill F. Dotson and were taken off their culture plates by the use of trypsin.

Figure 3

Glass Chamber. The platinum electrodes are 100 microns in diameter. The spheres are approximately 150 microns and the spacing between spheres is 400 microns x 450 microns. The glass slide is a standard slide, 3 inches long by 1 inch wide.

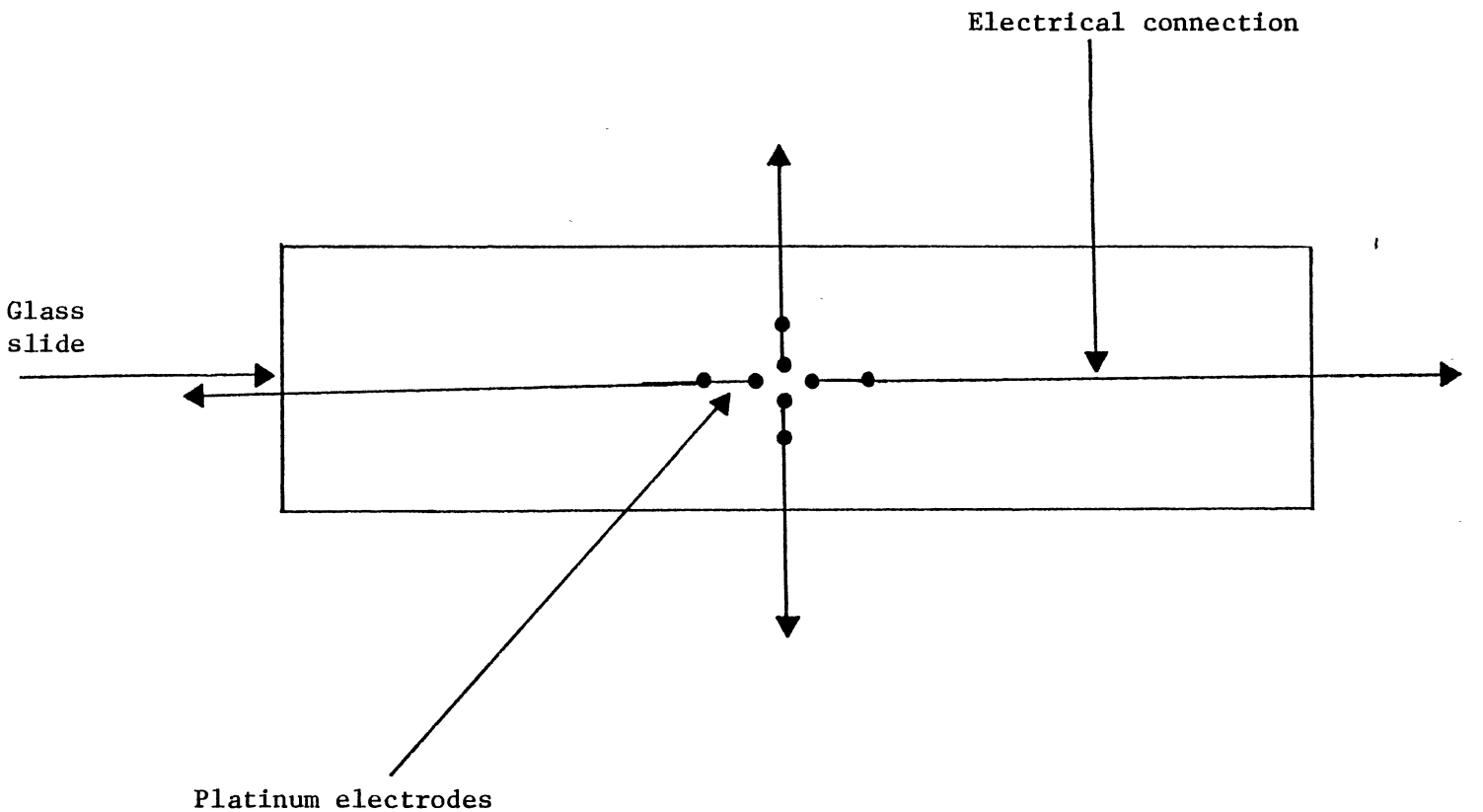
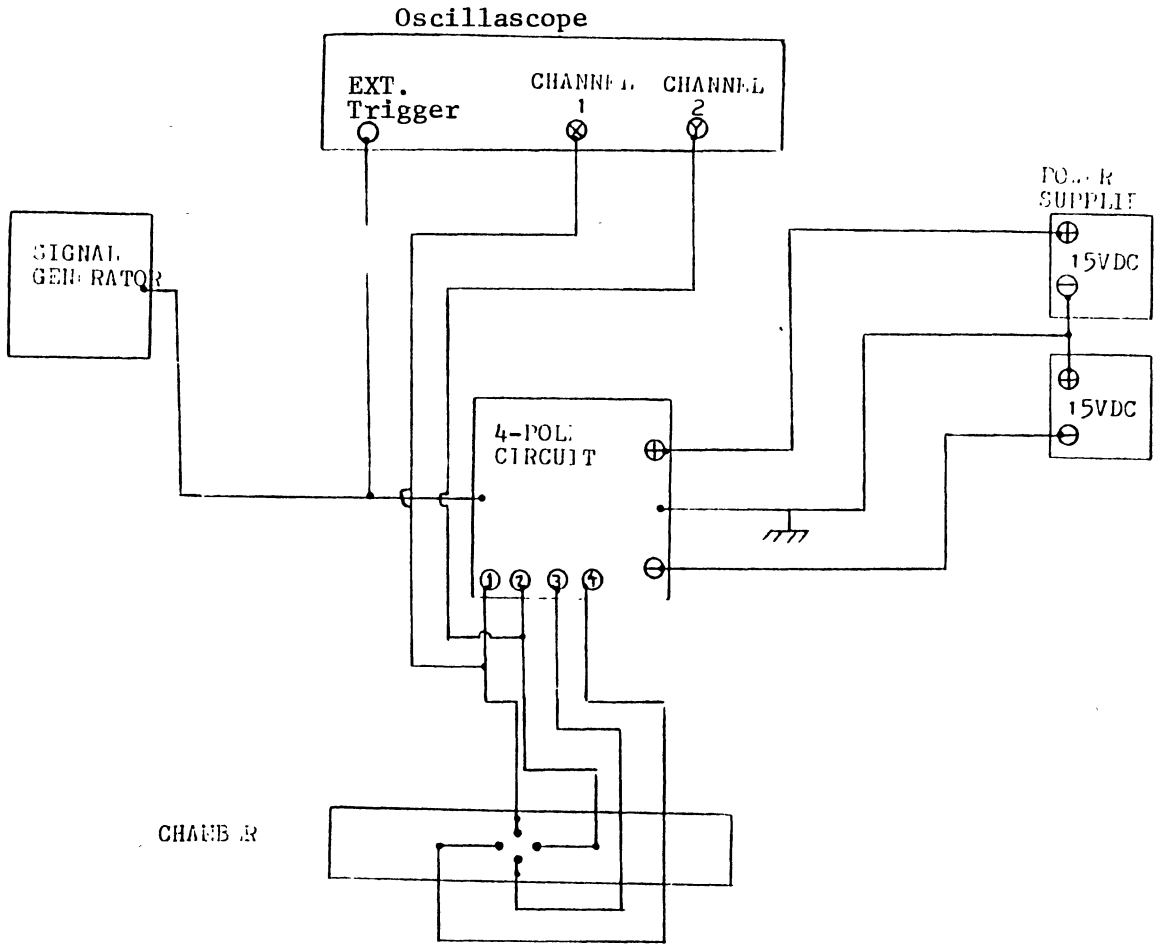

Electrical connection
Glass
slide
Platinum electrodes

Figure 4
Experimental apparatus lay out.

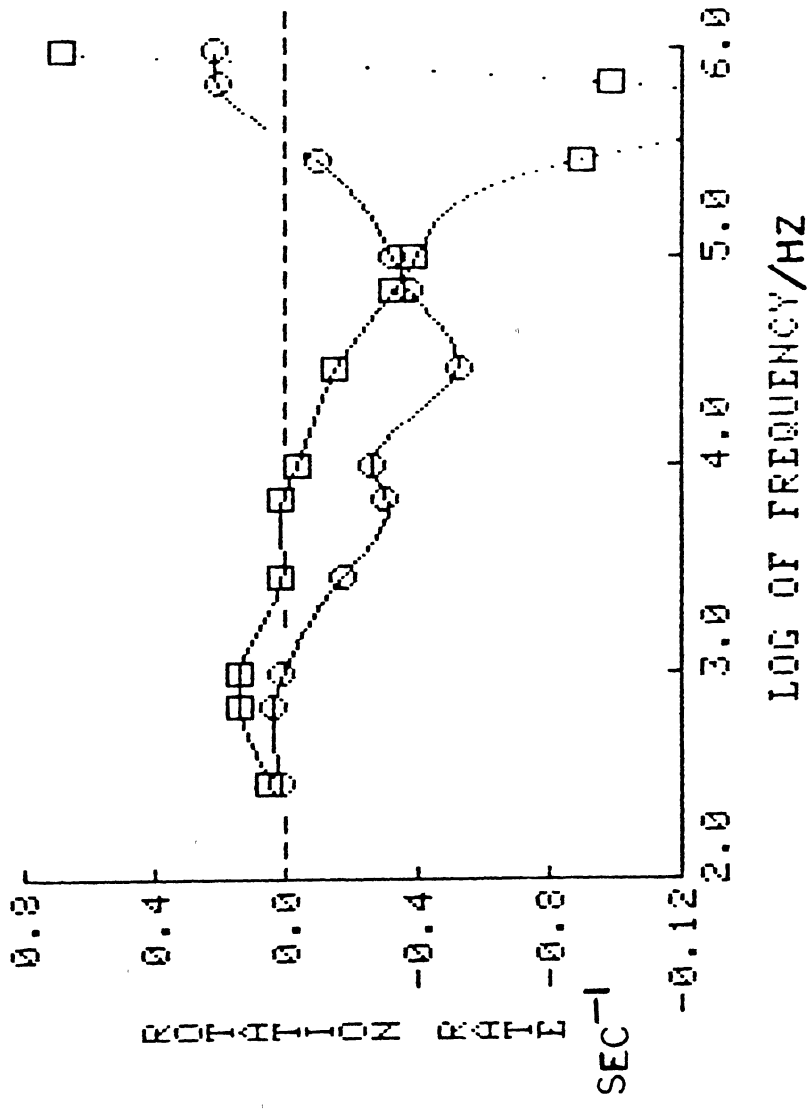

Oscillascope
EXT.
Trigger
CHANNEL
1
CHANNEL
2
SIGNAL
GENERATOR
15VDC
15VDC
4-POL
CIRCUIT
1
2
3
4
CHAMBER

Cell Preparation

Cell preparation was done in two ways. In the first, cell preparations were spun out from their original medium at 2000g's for 2 minutes. The cells were then washed in 300 Kohm/cm water three times to get a cell suspension of low conductivity. It is imperative to have a low conductivity solution since Ohmic heating must be minimized and the DEP spectrum is affected by the conductivity. The latter effect can be appreciated by Fig. 5. In the second, more rapid protocol, cells were spun down initially to achieve a high concentration per milliliter (ca. 10^9). These cells were then diluted by a factor of 250:1 into high resisitivity solution, thus diluting the salts and achieving a favorable conductivity for the experiment, while retaining at least small amounts of critically necessary ions about the cells to be examined. The advantage to the second procedure is that it greatly improves the condition of cells. This is due partly to reduction in time that the cells are exposed to the physiologically unfavorable environment in which the studies are conducted. When a mammalian cell was used which required a physiological osmotic medium, sucrose was added at 330 mM concentration to the diluting liquid.

Particle Preparation. Particle preparation was done in much the same way. In this case time was not a critical factor since there is no degradation of the particles as is encountered when working with live cells. Care must be taken though, to thoroughly wash the particles being tested to remove any extraneous material or coatings which might alter the CSR spectrum.

Figure 5
Effect of resistvity on CSR spectrum.
Circles, barium titanate in pure
water, 200Kohm. Squares, Barium
titanate in 30 Kohm solution.


0.8
0.4
0.0
-0.4
-0.8
-0.12
2.0
3.0
4.0
5.0
6.0
LOG OF FREQUENCY/HZ
ROTATION RATE
SEC⁻¹

Procedure. Measurement of the final resistivity of the solution was done with a bridge, General Radio Model 1650A.

Once the cells or particles were placed in the chamber by using a Pasteur pipette, they were then covered with a cover slip. A magnification of 400X was used to observe rotation. A long focal length objective was needed to focus in to the chamber. The concentration that was used ranged from between 1 to 60 cells or particles inside the chamber. Actual performance of the experiment can be broken down as follows:

1. Cells or particles are placed in a pre-washed chamber and covered.

2. Power is then applied to the four pole circuit and "balancing" of the circuit is done. This is done by selecting the frequency range to be used and then balancing both the input voltage and ouput voltage by using the variable potentiomenter in the circuit.

3. The observation of the cell rotation rate is made by either using a stopwatch to time one revolution of the cell or by using a stroboscope if spinning is too fast for an accurate determination by eye.

4. Following the reading, the stopwatch is reset, the frequency generator is switched to the next desired frequency and the circuit is rebalanced as in step 2.

5. In this way a series of spin rates versus frequency measuremets are obtained for the CSR spectrum.

Temperature. The effect of temperature was also studied. This was done in two ways. In the first, Saccharomyces cerevesiae was grown at room temperature for a day and then split into two groups. One group was placed in a refrigerator for 24 hours at approximately $3^{o}C$ and the second group was left growing at room temperature. In the second procedure, cells were placed in the chamber and then cooled down using the apparatus shown in Figure 6 and 7.

Figure 6
Cooling chamber.

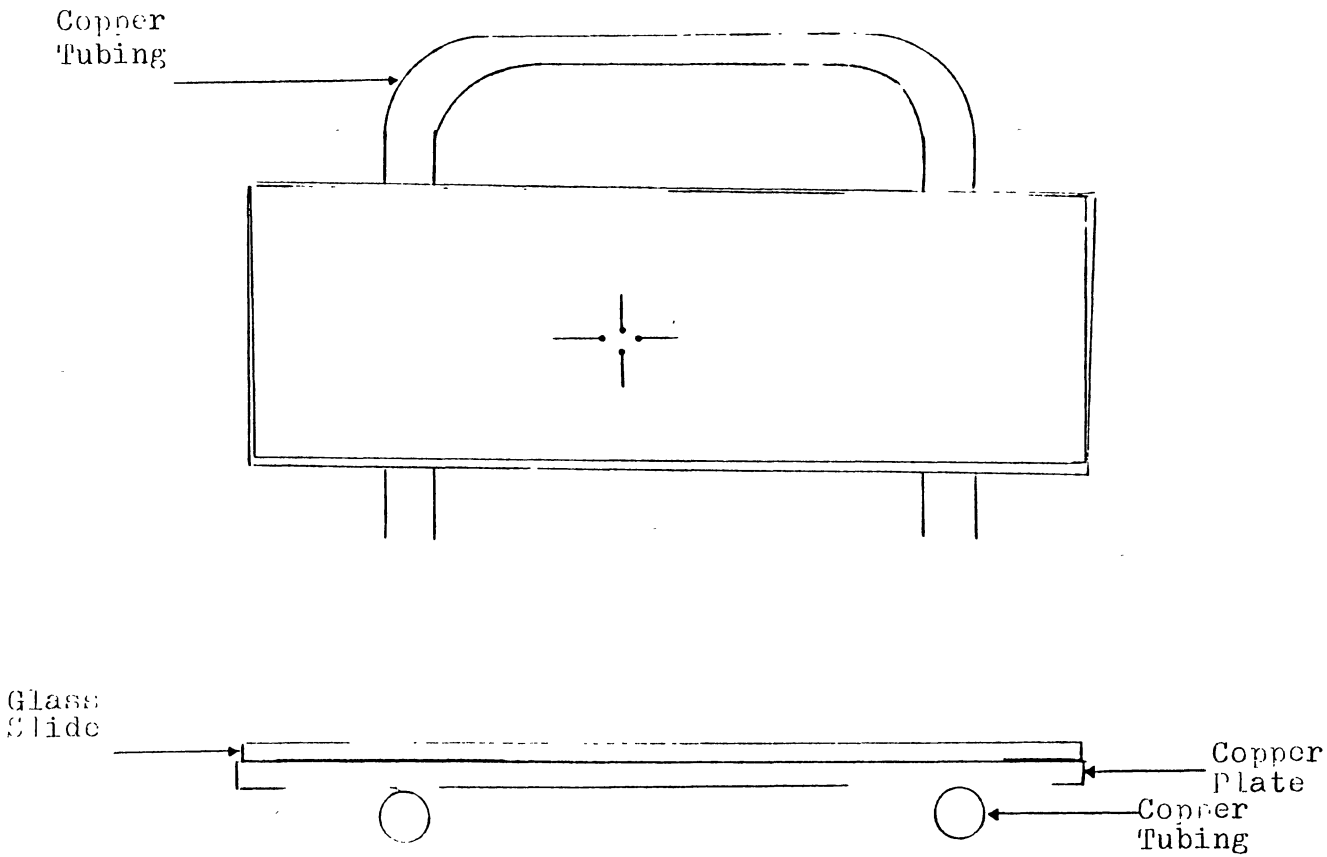
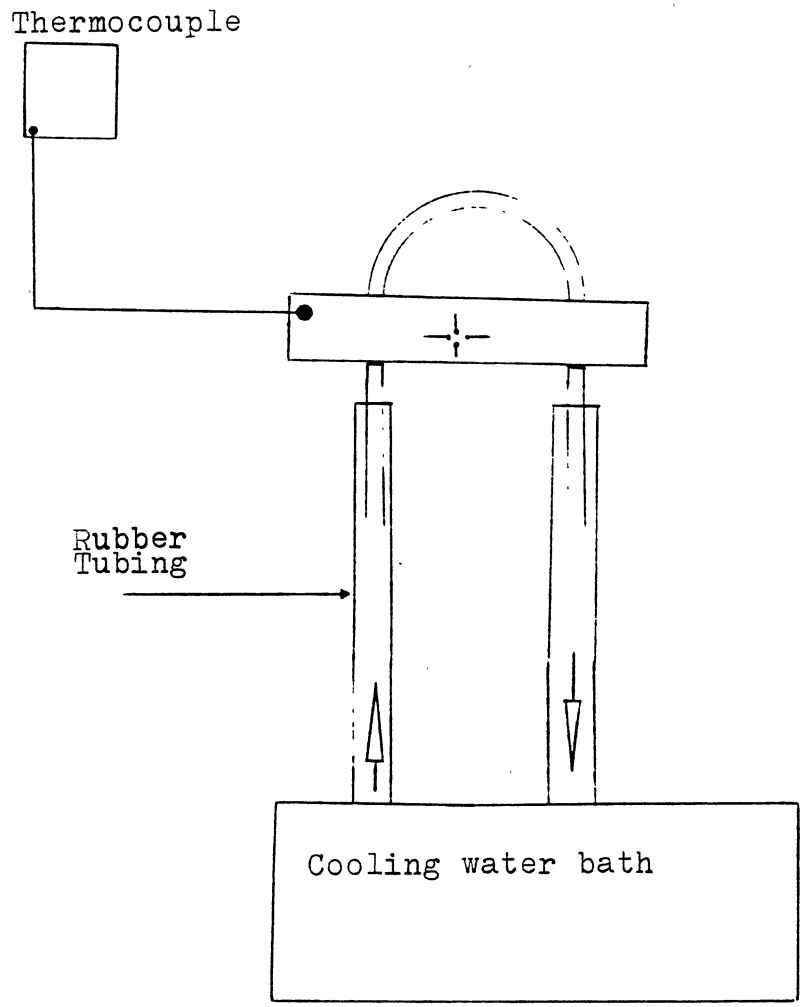

Copper
Tubing
Glass
Slide
Copper
Plate
Copper
Tubing

Figure 7
The chamber was cooled via the use of a cooling/heating bath which pumped liquid through the copper tubing to get to the desired temperature. A thermocouple was set on the glass slide to monitor the temperature.


Thermocouple
Rubber
Tubing
Cooling water bath

CHAPTER III

RESULTS

Cellular spin resonance can be appreciated to be a very sensitive measurement by studying Fig. 8 (in the ensuing figures, the log of frequency is in Hertz and the rotation rate in seconds $^{-1}$.) which shows the effect of a polyelectrolyte sodium salt of poly (acrylic acid) (commercially known as Darvan) on $BaSO_4$. Almost incredibly, the addition of approximately 10 ppb is sufficient to cause an appreciable change in the CSR spectrum. This is a very encouraging result since the subject under study is an inanimate object, so variation which might be seen in a living organism cannot account for the change in spectrum. A change due to the polyelectrolyte in the spectrum of $BaTiO_3$ can also be seen in Fig. 9.

Figure 10 and 11 illustrate the type of sensitivity that can be achieved when using CSR as a method to detect living cells among dead cells. A complete reversal in the direction of spinning direction between a dead and living cell can be observed. This phenomenon suggests the use of CSR as a "vital" test. Mixtures of live and dead cells were tested with this technique. Dead cells were stained deep blue with Nile Blue A and live ones were left colorless. Upon examination of the spectrum the live cells were seen to spin against the field while the dead cells were seen to go with the field. The signal applied was that of 100KHz. This gives

Figure 8
Effect of Darvan on the CSR of $BaSO_4$

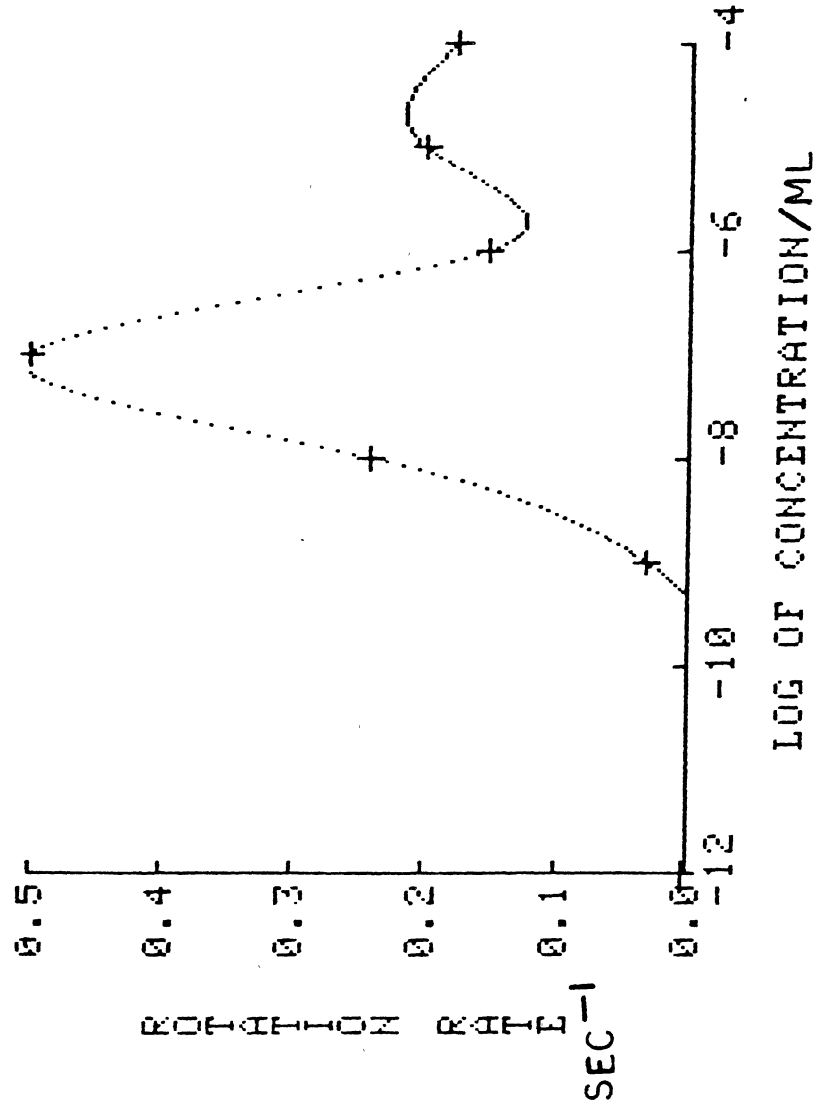

0.5
0.4
0.3
0.2
0.1
0.0
-12
-10
-8
-6
-4
LOG OF CONCENTRATION/ML
REACTION RATE
SEC-1

Figure 9
Effect of Darvan on the CSR of $BaTiO_3$.

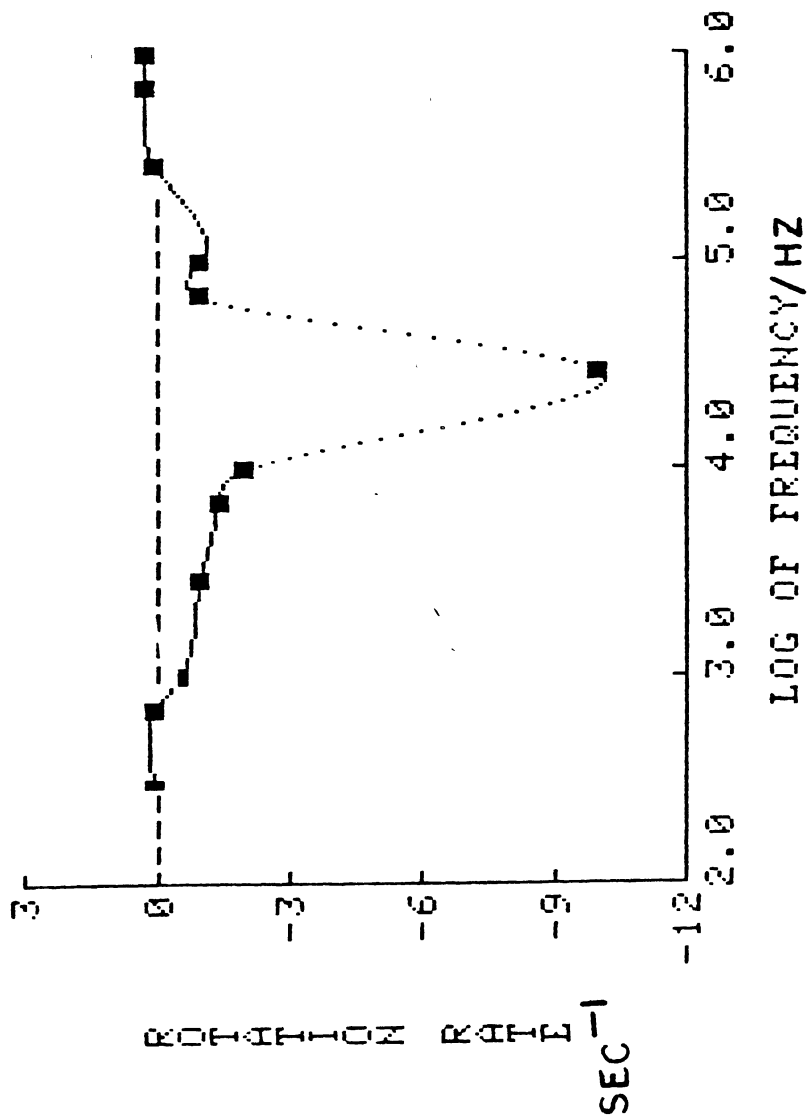

3
0
-3
-6
-9
-12
2.0
3.0
4.0
5.0
6.0
LOG OF FREQUENCY/HZ
ROTATION RATE
SEC-1

Figure 10
CSR of a live yeast cell.

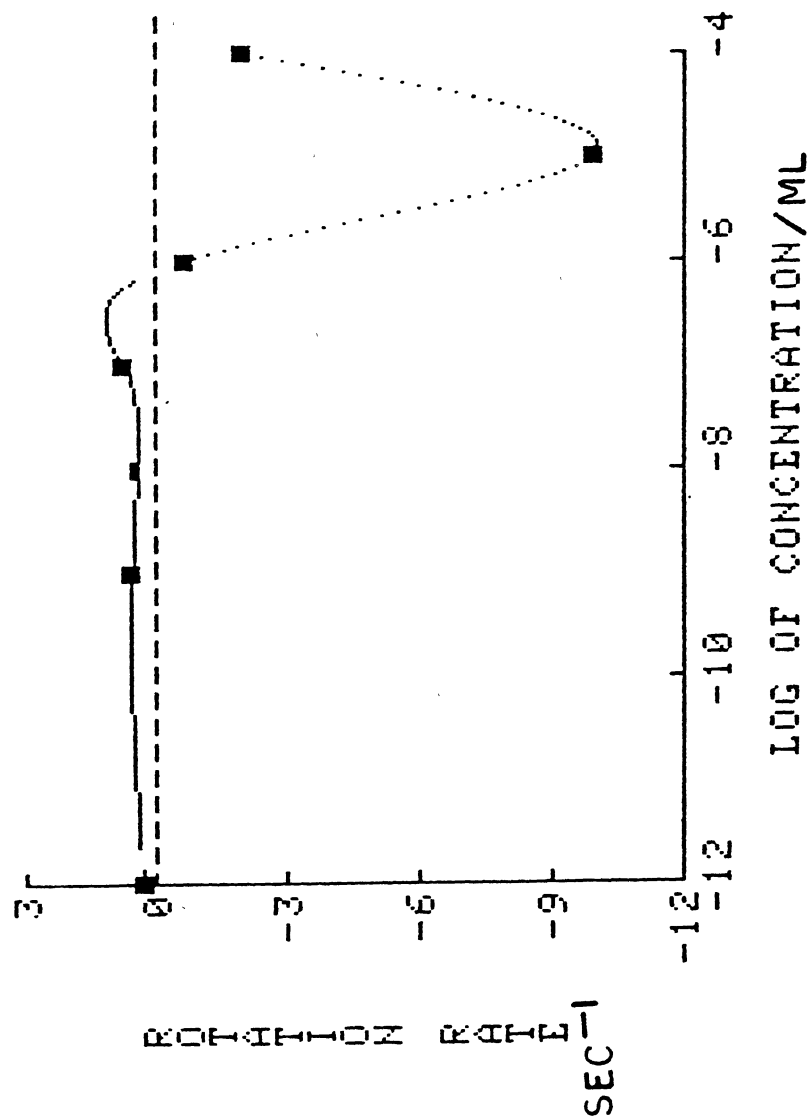
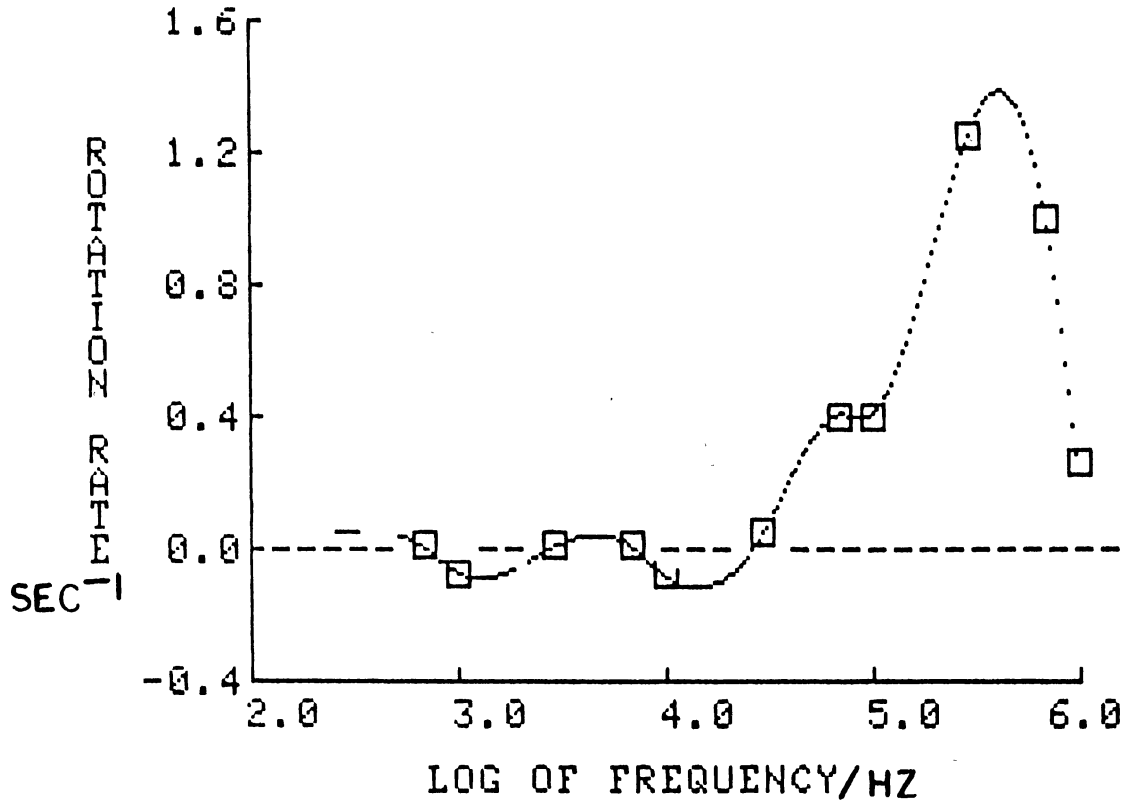

3
0
-3
-6
-9
-12
-12
-10
-8
-6
-4
LOG OF CONCENTRATION/ML
REACTION RATE
SEC⁻¹

Figure 11
CSR of a dead yeast cell (killed by heat treatment).


1.6
1.2
0.8
0.4
0.0
-0.4
ROTATION RATE
SEC⁻¹
2.0
3.0
4.0
5.0
6.0
LOG OF FREQUENCY/HZ

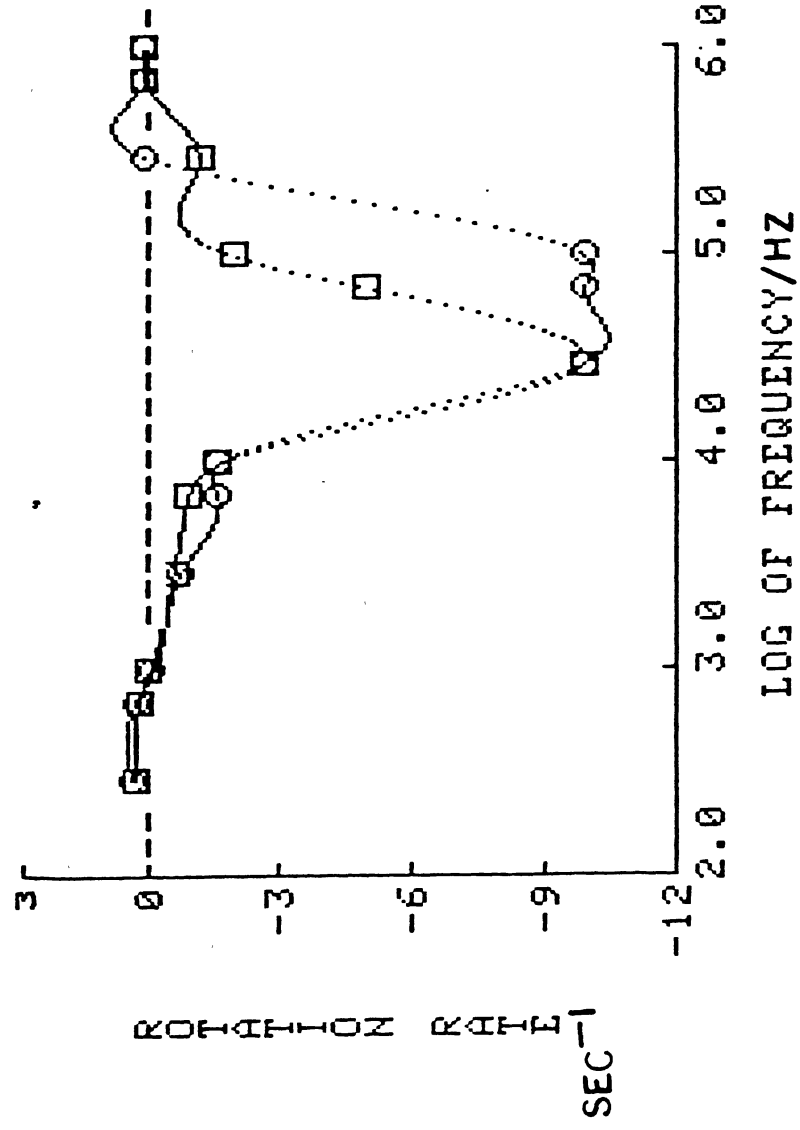
definite proof that the change in spectra is due to the difference in cells and not to an experimental error.

Culture age also has a significant effect on the CSR spectrum. Figure 12 demonstrates that in Saccharomyces cerevesiae there is a measurable difference between culture age of 16 hours which are in the growth phase, and culture age of 48 hours which are in the stationary phase. This is particularly readily observable at 300 Khz. Three types of response to the applied field of 300Khz is seen in the young culture. These are: (1) spinning clockwise, (2) spinning counter-clockwise, and (3) chaining. Cells that have been in a culture medium for 48 hours or more are seen on the other hand to only spin counter-clockwise at this particular frequency.

Appendix A has the CSR spectrum of several cell lines showing the differences between them.

Another phenomenon that was observed was that of cells spinning in a two-pole field. These cells were seen to spin while out in solution without any other cell or object nearby to cause any kind of interaction. This type of spinning is totally different from the 4-pole spinning. In the two-pole spinning it is theorized that the interaction of an internal ac field being produced by the cell is interacting with the externally applied field to create a net torque which then causes the spinning. It has been observed that a cell can spin in one direction while under the influence of a two-pole field and will change direction of spinning when a 4-pole field is applied. This leads to speculation that the 4-pole spinning is one that is produced by an overwhelming effect of polarization by the field on the cell, whereas two-pole spinning is probably due more to an interaction of the natural ac oscillations of the cell with applied field.

Figure 12
CSR of 16 hour culture (circles)
versus that of a 48 hour culture
(squares).


3
0
-3
-6
-9
-12
2.0
3.0
4.0
5.0
6.0
LOG OF FREQUENCY/HZ
ROTATION RATE SEC⁻¹

Cellular spin resonance of yeast is also affected by temperature. It was observed that cells in the stationary phase (old culture) which spun counter-clockwise at 300Khz were not much affected by changes in temperature. This was found to be true while they were being cooled down while in the chamber or chilled in a refrigerator overnight. However, cells in the growth phase (young culture) that responded to an applied field of 300Khz in a variety of ways, such as the 16 hour culture age sample, were affected by changes in temperature. Cooling these cells down to $2^{o}C$ while in the chamber caused them to all start spinning counter-clockwise at 300Khz. Cooling takes approximately 25 minutes in the metallic chamber. Upon seeing this effect the cells were then gradually warmed up to $20^{o}C$. It was thought that the cells might now revert to their original spinning state. This however, was not the case. The cells all remained spinning in the counter-clockwise direction as if they had aged into the stationary phase.

Cooling down of the cells while in their medium produced the following results. Cells that were left at room temperature for two days were all observed to spin counter-clockwise at 300Khz. However the cells that had been put in the refrigerator for cooling down after one day of growth were observed to spin just as if they were a very young culture, even though now they have been in culture for a total of two days. This series of experiments was done to determine if cells would respond differently to a temperature change in relation to the type of cell. The type of cells chosen were those that spun in a particular direction at 300Khz. Remembering that the young culture spun clockwise while the old culture spun counter-clockwise it might be argued that any effect seen on the cells because of temperature change is due in fact to the interaction between

temperature and culture age. This in fact was observed to be true. It seems that a culture which is relatively young (cells spin clockwise) will react to the change in temperature. In our case, the cooling down of the sample on the microscope stage seems to age the culture irreversibly. Those that are old cultures are not affected. Also, cultures that were refrigerated while still young seemed to be halted at that stage, because when allowed to warm up they displayed a young culture's CSR while a control group that was allowed to grow, changed its CSR spectrum to that of an old culture.

These observations lead us to speculate on the following. Young cultures are still very active since they still have an abundance of nutrients in their medium. Upon being cooled down they start preparing themselves for adverse conditions and shut down some of their activity. Upon being warmed up to ideal growing conditions they now start activities again. This only happens though, if they are still in a suitable growing environment. This is substantiated by the fact that the cells that were cooled down on the microscope stage (while in low conductivity water) never reverted to their previous CSR spectrum even after being warmed back up. Cultures that are "old" on the other hand already have shut down activities because of a lack of a suitable growing environment and are not affected by cooler temperatures, at least where a CSR spectrum is concerned.

CHAPTER IV

CONCLUSION

Cellular spin resonance as we know it now is a very useful and powerful tool. It allows us to study the reaction of cells to age, physiological state, temperature, and type. Eventually the effect of certain contaminants to the medium will be studied. These studies might allow us to use CSR as a method for testing potentially hazardous chemicals before they are approved for human or animal use. CSR potentially will be able to detect differences in cells that appear to be normal using standard tests but that may actually be the early stages of disease. CSR could be used to differentiate between organisms. For instance, it could be used to identify a particular strain of yeast which might be infecting a human or animal patient. Current methods of culturing take several days before a positive identification can be made. CSR could be used as single cells or on a clump of cells, say a micro-biopsy, to be tested for some malignant condition.

An enormous improvement to CSR as a practical tool would be through automation. Automating the system to the point where it will be able to take a reading while under computer control would make it much speedier and more objective. Speed is important for two reasons. First, the condition of the cell is going to start degrading as soon as it is placed in the test

solution. Second, the cell itself will start to leak out ions which will change the parameters of the solution making it difficult to relate changes of spectra to the cell or to the conductivity of the solution.

Looking Ahead

There is still much to be done with CSR even if it means taking the measurements by eye. Additional avenues of study are:

1. The effect of chemicals on cells.

2. The difference in spectra between normal mammalian cells and their oncogenic state.

3. Differences, if any, found between the spectra of one single cell and a clump of cells as in a biopsy.

4. Continued study between CSR in a four pole configuration and two pole system.

5. Effects (correlation) with cell age or cell life cycle phase.

These are but a few of the possible fields to study and explore with the use of Cellular Spin Resonance.

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APPENDIX A

CELLULAR SPIN RESONANCE (CSR)

A chapter to be published in the book titled Modern Bioelectrochemistry.

Figure captions are at the end of this appendix.

CELLULAR SPIN RESONANCE (CSR)

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CELLULAR SPIN RESONANCE (CSR)

ABSTRACT

Small objects such as suspended live cells, organelles, tissue fragments, or even inanimate powder particles may be made to spin in an electromagnetic field. The spinning occurs in a resonant response to the applied frequency and reflects the dielectric properties (permittivity) of the suspension.

There are three special cases:

Spinning

(1) in a static (dc) field

(2) in a simple oscillatory field, and

(3) in a rotating field.

The theory and examples for several interesting cases and their probable mechanisms are presented.

The technique of cellular spin resonance (CSR) has several interesting applications. It sensitively detected alterations in surface properties due to a polyelectrolyte at concentrations of ca 100 ppb. The CSR spectra of cells reflect their type and physiological state. Data to date indicates that live cells spin oppositely from dead ones at some frequency, even in mixtures. The dielectric properties of tiny particles

can be readily determined.

Introduction

Living cells and other small particles in suspension can be observed to spin while in the presence of an alternating current (ac) electric field. The cellular spin rates-phenomenon can be correlated with various characteristics such as cell age, culture age, health of the cell (normal verses tumor, etc), and cell type. The spinning of inanimate particles can be informative as to their dielectric properties. The present account describes the studies being done to reach understanding of this exciting new technique.

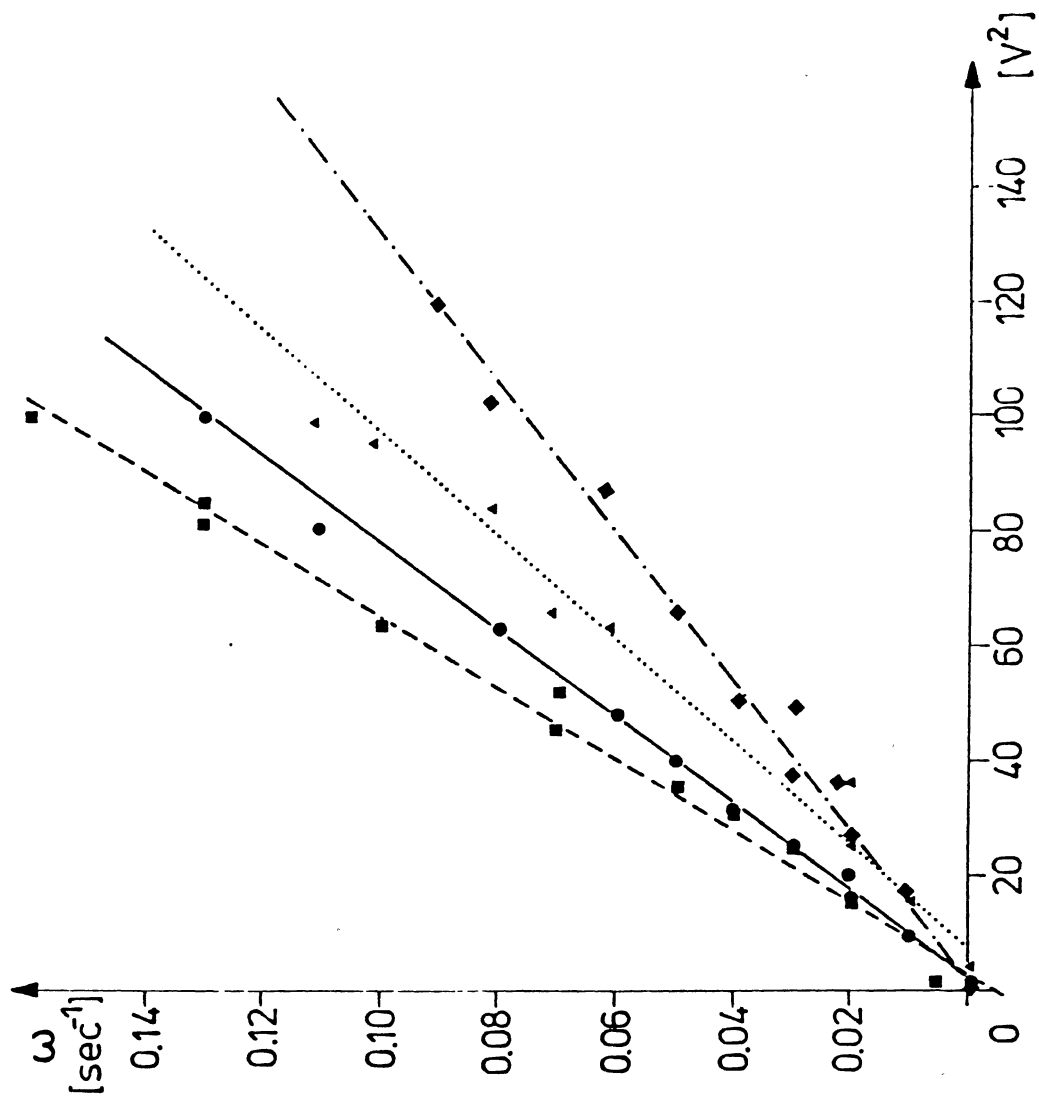
1. CELLULAR SPINNING

1.1. In a Static Field

Cells or other small particles may be observed to spin in an electric field that is either static, oscillatory, or rotating in direction. [1-6] The spinning of more or less spherical bodies in a static field has been known for some time. The theory and confirming experiments for that type due to surface charge depostion by ambipolar (bi-directional) current have been given. [4]

1.2 In a Simple Oscillatory Field

The spinning of cells while in a two-pole oscillatory field has been reported by a number of researchers [1-12]. The first account of resonant spinning was that of Pohl and Crane. [3] Their observations stemmed from the dielectrophoretic studies of baker's yeast, Saccharomyces cerevisiae. These studies were done while the cells were in between two parallel wire electrodes and subject to an ac field. The cells spun in a sharply resonant manner, in that each cell responded and spun only at a rather sharply defined specific applied frequency. Typically the cells were seen to spin at about 0.1 to 10 Hz while the applied field might be oscillating at, say 100 Hz to 10,000,000 Hz. The cells were observed to spin rapidly either against one or the other of the electrodes or even out in the suspension. The frequency being applied could be adjusted so as to stop the spinning of some cells while starting others, and to slow the spinning rate or even to change the direction of rotation. Later studies found that the spin rate was proportional to the intensity of the applied field (Fig. 13). The sharply resonant nature of the spinning response led to the use of the descriptive term "cellular spin resonance" (CSR). Since then, various investigators have quantitated the spin rate of budding yeast cells, [8] and the characteristic CSR of various cell lines, including human erythrocytes, Friend cells, and mesophyll protoplasts of Avena sativa. [11]


ω
[sec⁻¹]
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0
20
40
60
80
100
120
140
[V²]

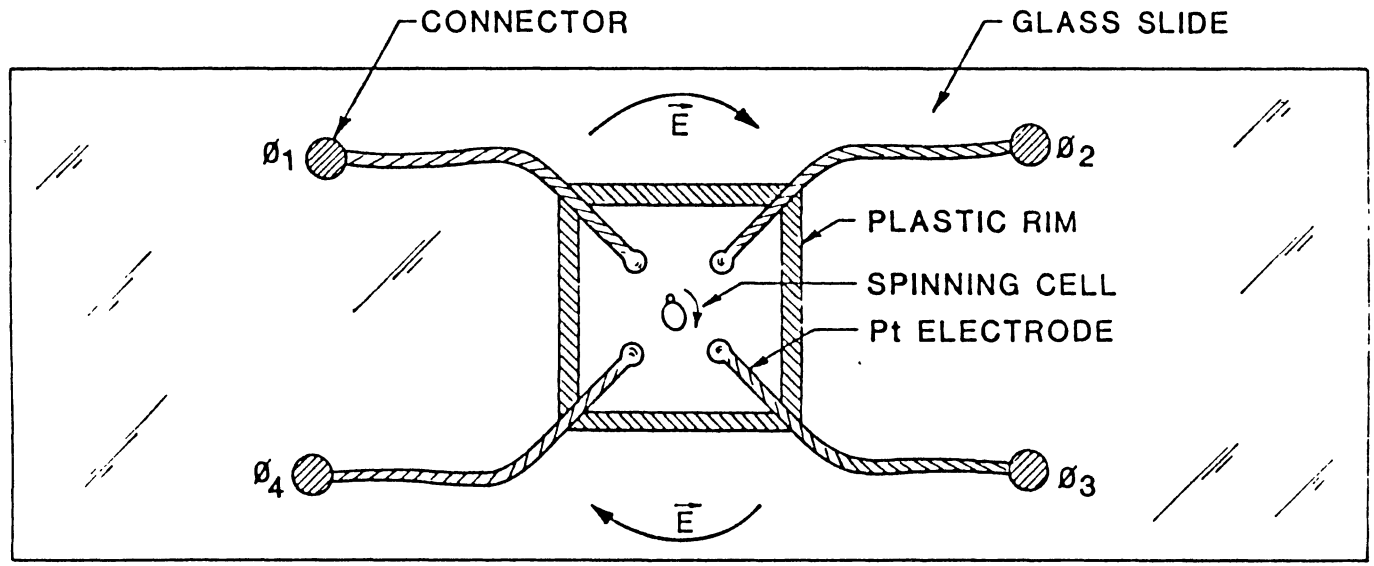
Cellular spin resonance in the simple oscillatory field can be broken down into two major types. One, a common type in which cells spin while in close proximity to another cell or other polarizable objects; second, a rarer type in which cells spin while alone in suspension or against a smooth electrode. The observation of lone cells spinning out in the middle of the suspension is a rarer event than that of spinning alone against a smooth electrode or while interacting with other cells. Even so, there have been several research groups report observing and studying this event. [3, 12, 13, 14] The fact that lone cell rotation is a rarer event can be attributed in part to dielectrophoresis.[4] Dielectrophoresis (DEP) is the motion if bulk or particulate matter induced by the action of non-uniform electric fields. The movement of the particle will be towards the region of highest field intensity (positive dielectrophoresis) if the effective dielectric constant of the particle (or cell) exceeds that of the suspending medium. Conversely, the movement of the particle will be away from the region of higher intensity if the particle is of a lower dielectric constant of the medium. Normally, cells perturb the field and create a region of strong field intensity nearby, thereby attracting and linking other cells and forming "pearl chains". Because of this phenomenon of "mutual dielectrophoresis", any cell that is alone out in a suspension will tend to be attracted to other cells, thus preventing prolonged close examination of lone cell rotation. Also, there exist physical contraints such as thermal upsets, ionic injection, etc. causing field streaming.

There exists however, another reason for the rarity of observation of lone cell rotation in a two-pole field. This laboratory has recently

done multiple field studies with Saccharomyces cerevisiae at dilute concentrations where only a few (2-10) cells were present in a rotating field provided by a four-pole electrode arrangment (Fig.14). Once a lone cell was observed to rotate freely in the rotating field, an ac field was then applied to only two poles while the remaining two were shorted to avoid field induction. Only a small percentage of the cells (ca. 1%) are observed to be capable of spinning in the two-pole field in this arrangement while out in suspension. Furthermore the type of cells so far observed to be spinning while under these conditions was at the stage in its life cycle just before the splitting of the mother cell into two daughter cells. The frequency of the applied field at which the cells were observed to rotate was between 10kHz and 100kHz. This raises the question; is lone cell rotation linked to a particular stage of the life cycle?

What are the possible explanations for lone cell rotation in a simple oscillating field? The most compelling reason seems to be that of an internal dipolar oscillation within the cell.[15] This oscillation would be present only with live cells since upon the death of the cell spinning ceases. This seems to be supported by the fact that the cells spin at a much lower rate than that being applied by the external field. The cell rotates at somewhere between 0.1-30.0 Hz while the external field oscillates at, say, 600kHz. The presence of an internal dipolar field oscillation would interact with the externally applied field to provide a rotational torque and thus induce the spinning.

The cellular oscillations are not necessarily dipolar, but may oscillate as linear quadripoles or higher multipoles. In view of the

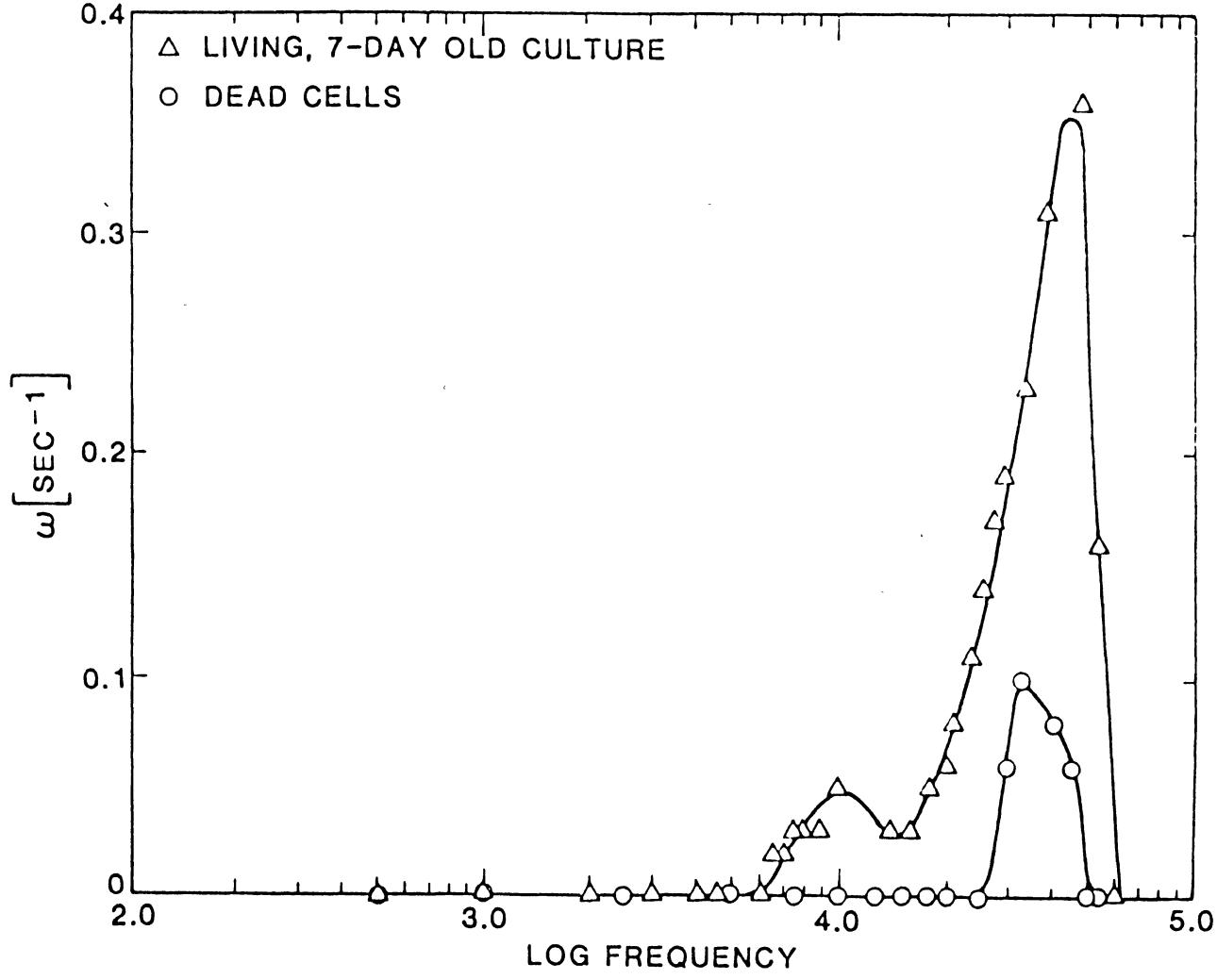

CONNECTOR
GLASS SLIDE
$\vec{E}$
ϕ_1
ϕ_2
PLASTIC RIM
SPINNING CELL
Pt ELECTRODE
ϕ_4
ϕ_3
$\vec{E}$

relatively weak character of the cellular oscillations it would also be expected that the externally applied oscillatory fields would serve to "pull" or change the frequency into resonance with that of the applied field. This would cause the CSR spectrum to be broadened by the external frequency pulling.

1.3. In a Rotating Field:

Finally, there is the rotation of cells and other particles in rotating electric fields. If, for example, three or more electrodes are arranged in a ring and pulsed sequentially to produce a rotating electromagnetic field in the intervening space this produces a polarization on a particle in the mid region. Moreover, this polarization takes a finite time to establish. The angular lag between the direction of the dipole thus created and the direction of the exterior rotating field now gives rise to a torque. The spin of the particle can thus be correlated with the field and frequency dependent dielectric properties, or permittivity. The CSR technique has potentially broad applications in minerological, as well as biophysical and medical problems.

Experimental evidence shows that there are correlations between the physiological state of the cell and its CSR spectrum. A comparison of the CSR of normal and cancerous fibroblasts was shown in an earlier paper.[16] Fig. 15 shows the difference, for example, between a live yeast cell and a dead one. It may turn out that the technique of CSR at


0.4
0.3
0.2
0.1
0
ω [SEC⁻¹]
△ LIVING, 7-DAY OLD CULTURE
○ DEAD CELLS
2.0
3.0
4.0
5.0
LOG FREQUENCY

a particular low frequency range will be useful as a "vital" test of cells. Each cell type has its own characteristic spectrum which identifies its state of being, in this case live or dead. Fig. 16 illustrates the dependency of colony age. Fig. 17 through Fig. 19 show several examples of CSR spectra for different cell lines.

2. PARTICLE SPINNING

Inanimate particles can also spin while in the presence of ac electrical fields. The use of particles provides a model with which to test theories on spin resonance without having to be concerned with the ever-changing state of live cells. As can be seen from Fig. 20 the conductivity of the solution is a critical factor in determining an accurate CSR. This is especially true at lower frequencies.

The general assumption made about particles is that they will spin in the direction of the ac field if they are more polarizable than the medium they are suspended in, and will spin against the field if they are less polarizable. In our case, if spinning with the field is clockwise, it is denoted by a (+) value, and if spinning counter-clockwise it is denoted with a (-), that is, spinning against the rotation of applied field. As model particles of high polarizability we have used crystals of $BaTiO_3$ (ca. 2000) and of a low polarizability those of $BaSO_4$ (ca. 40). Fig. 21 shows the CSR spectrum of $BaTiO_3$ while in pure water. It can be observed that the particles follow the field as the frequency increases until approximately 10kHz. Thereafter it crosses over to the negative

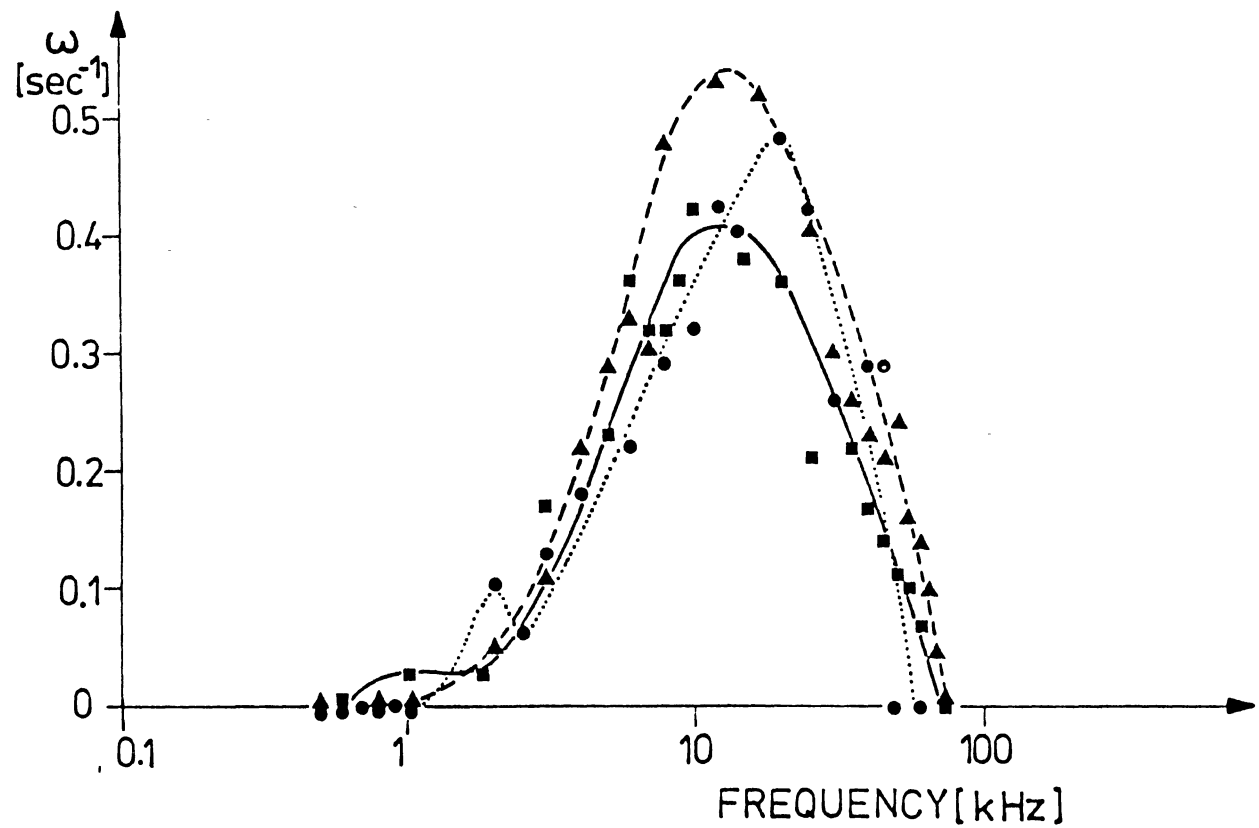
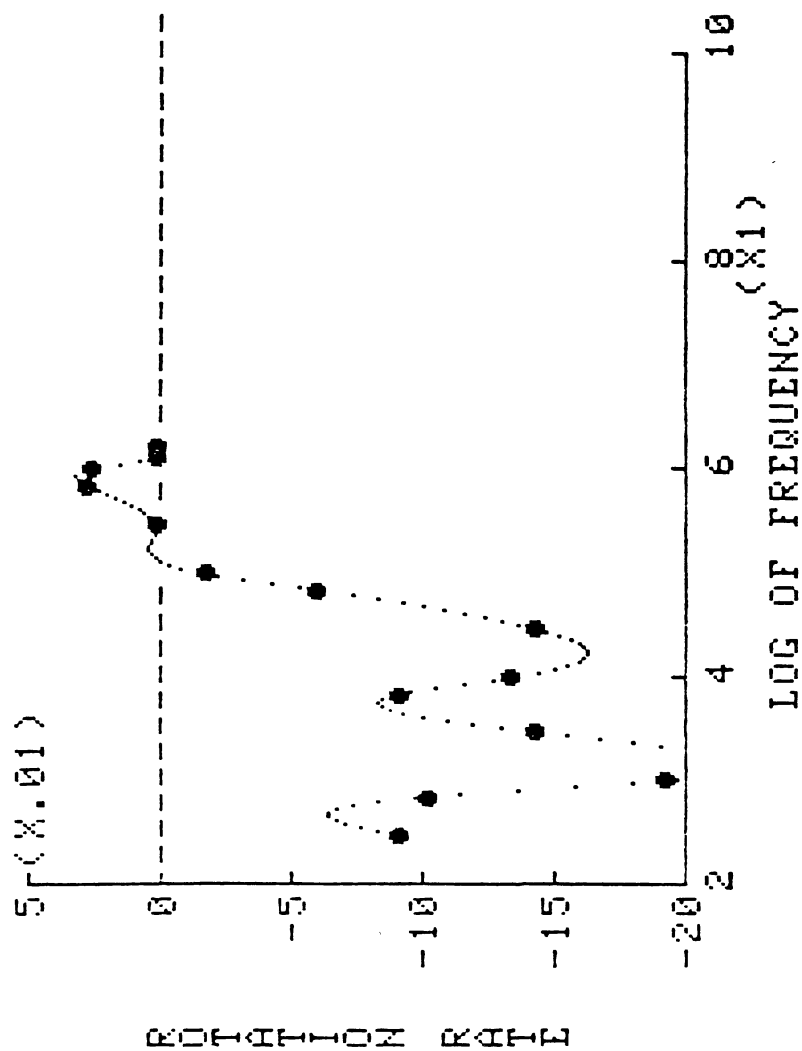
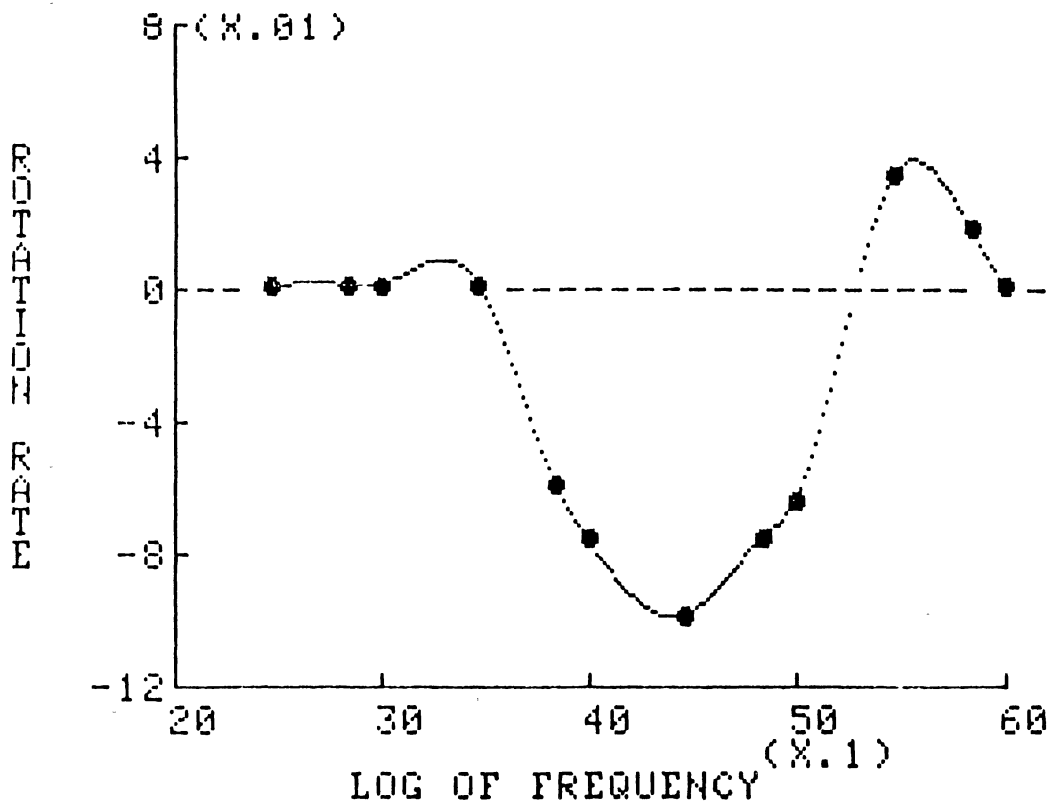
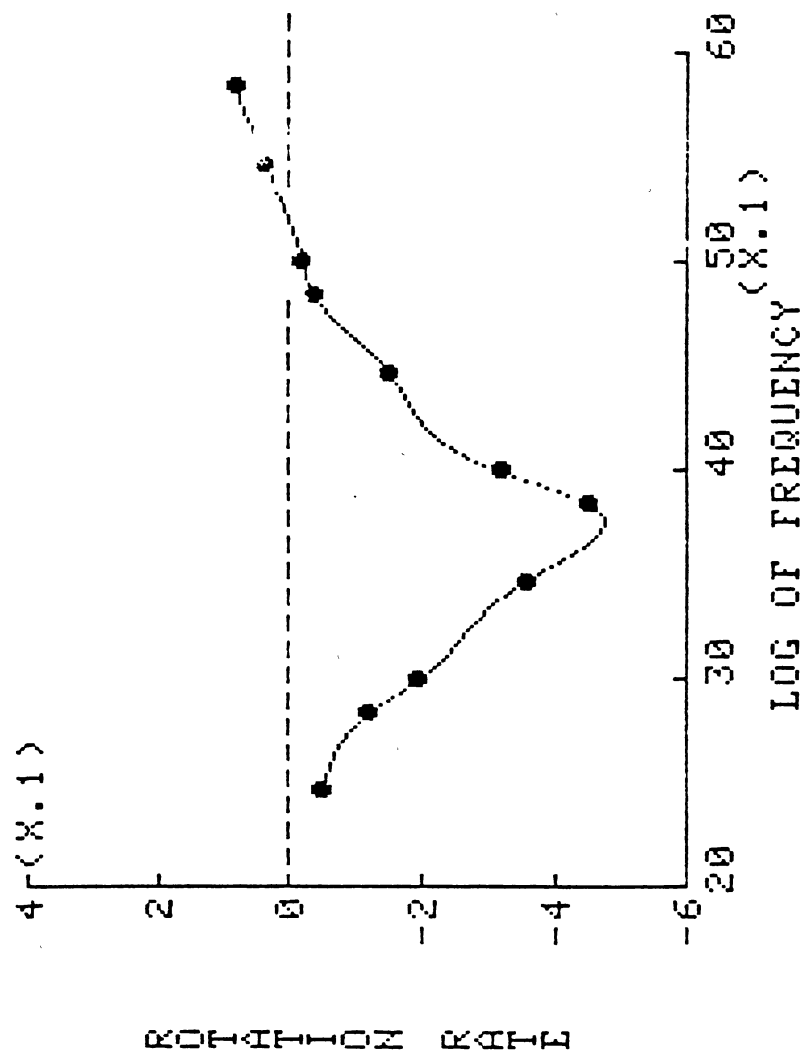
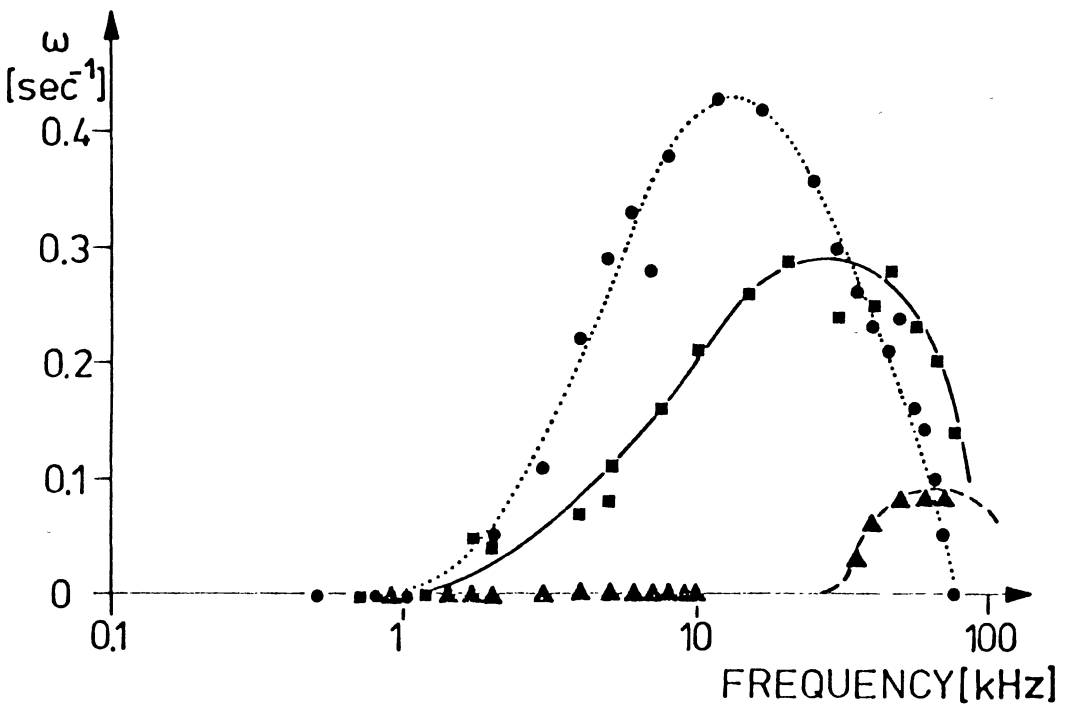

ω
[sec⁻¹]
0.5
0.4
0.3
0.2
0.1
0
0.1
1
10
100
FREQUENCY [kHz]

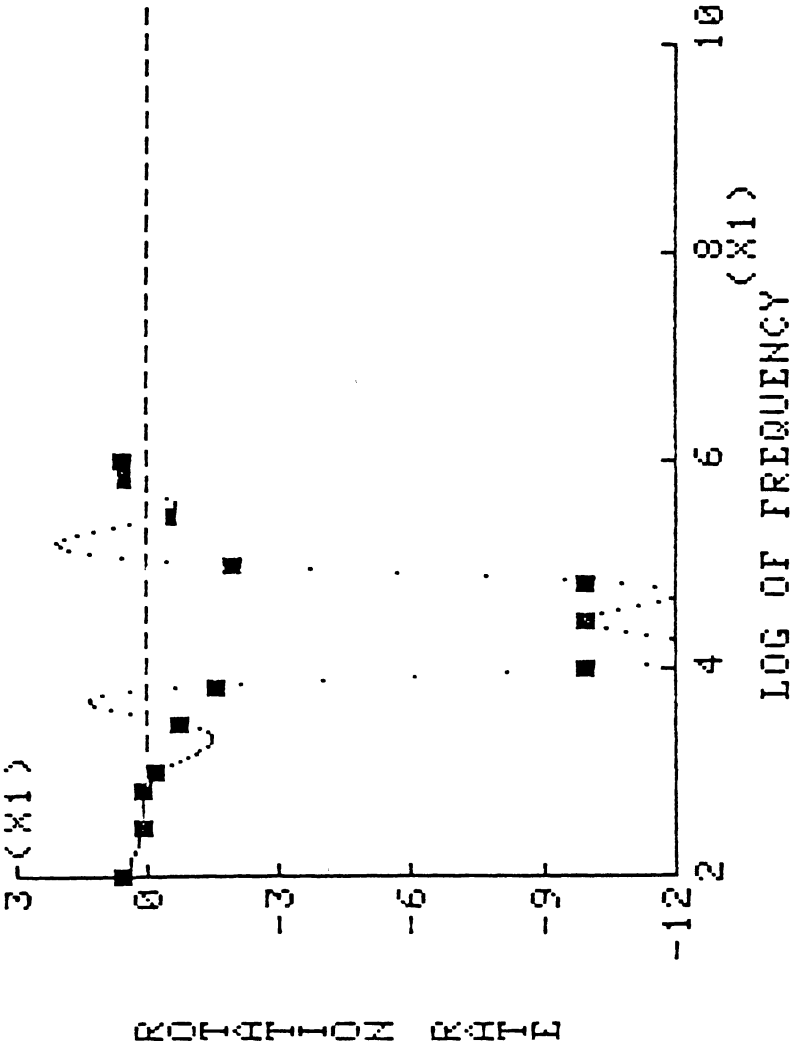
Figure 17


5 (X.01)
0
-5
-10
-15
-20
2
4
6
8
10
LOG OF FREQUENCY (X1)
GAIN MODULATION


8
(X.01)
4
0
-4
-8
-12
ROTATION RATE
20
30
40
50
60
(X.1)
LOG OF FREQUENCY


4 (X.1)
2
0
-2
-4
-6
20
30
40
50
60
(X.1)
LOG OF FREQUENCY
GAIN MODULATION

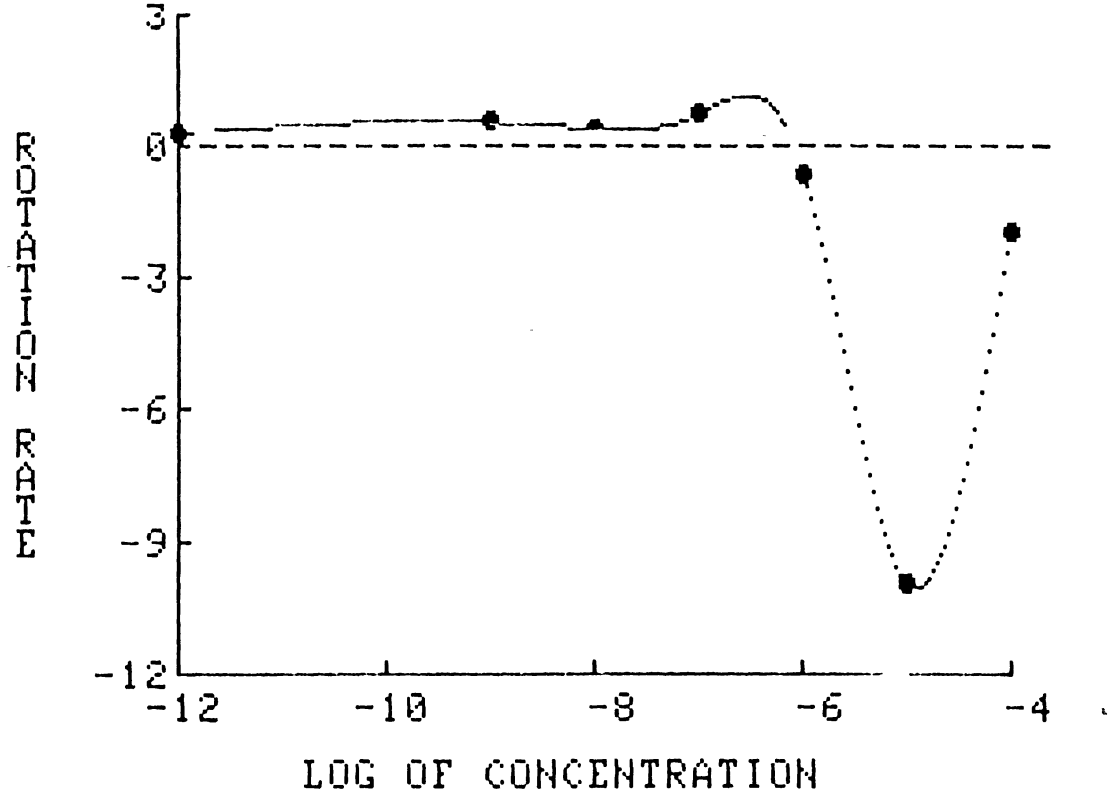

ω
[sec⁻¹]
0.4
0.3
0.2
0.1
0
0.1
1
10
100
FREQUENCY[kHz]

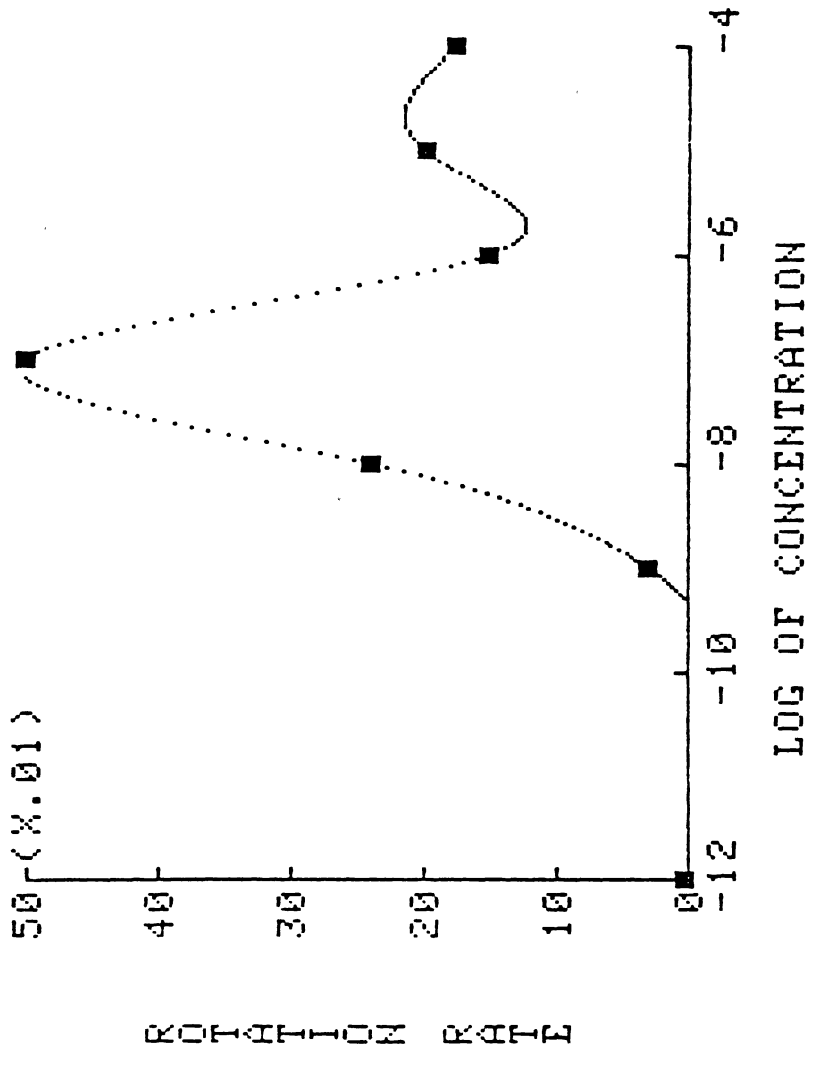

PHASE ROTATION RATE
3 (X1)
0
-3
-6
-9
-12
2
4
6
8
10
LOG OF FREQUENCY (X1)

region of spinning until about 600kHz where it again goes back to the positive side of the spectrum. $BaSO_4$ in pure water, conversely is not observed to spin at any frequency. As seen by Fig. 22 and 23 the addition of Darvan No. 7, a polymeric polyelectrolyte based upon polyacrylic acid (manufactured by R.T. Vanderbilt Company, Inc.) is shown to alter the CSR spectrum of both the $BaTiO_3$ and $BaSO_4$. Almost incredibly low concentrations of it suffice to affect the CSR of suspended particles. It appears that concentrations as low as 100 parts per billion will substantially alter the CSR spectrum. This observation most readily points out two things. First, that the CSR spectrum technique is extremely sensitive and that it will detect small changes in the object being tested. Second that, the intrinsic properties of the models being tested can be altered at least as far as the CSR spectrum is concerned. We must conclude that the surface absorption of the polyelectrolyte, Darvan, appreciably modifies the exterior of the particles, causing a new set of parameters for the models to be set.

3. THEORY

To facilitate the application and the understanding of CSR a simplified analysis of the theory is presented below. Briefly stated, it is found that the CSR spectrum gives spin rates proportional to the magnitude and sign of the effective (differential) polarization of the body in the suspending medium. From the observed CSR spectra, then, the size and course of the effective permittivity spectra of small bodies can


3
0
-3
-6
-9
-12
ROTATION RATE
-12
-10
-8
-6
-4
LOG OF CONCENTRATION


50 (X.01)
40
30
20
10
0
-12
-10
-8
-6
-4
LOG OF CONCENTRATION
AREA NORMALIZED

be determined and the nature of the polarizabilities can be interpreted. In the following analysis, friction between the body and the floor of the chamber is neglected as a first approximation since we are dealing with tiny objects usually visible in Brownian motion.

Torque on a Polarizable Sphere in a Rotating Field

$$\vec{T} = \vec{\mu} \times \vec{E} \tag{1}$$

$$T = \mu E_o \sin \delta; \quad \delta = \text{phaselag angle of the polarization behind the phase of the field } E_o(t). \tag{2}$$

For a sphere the induced moment when in a medium is, after $t \to \infty$,

$$\vec{\mu} = 2\pi a^3 \, \mathrm{Re} \left\{ \varepsilon_1^* \frac{(\varepsilon_2 - \varepsilon_1)}{\varepsilon_2 + 2\varepsilon_1} \right\} \vec{E}_0 \tag{3}$$

Assuming a simple Debye dielectric of one characteristic relation time, τ, of the form

$$\varepsilon = \varepsilon' - j\varepsilon'' = \varepsilon_\infty + \frac{(\varepsilon_s - \varepsilon_\infty)}{1 + \omega^2 \tau^2} - j \frac{(\varepsilon_s - \varepsilon_\infty)\omega\tau}{1 + \omega^2 \tau^2} \tag{4}$$

where

ε = absolute dielectric permittivity (complex)

$\varepsilon = \varepsilon' - j\varepsilon''$

ε' = in-phase absolute dielectric constant

ε'' = out-of-phase dielectric constant

$j = \sqrt{-1}$

ω = angular frequency of applied field

μ = induced moment

D = dielectric replacement

we may write

$$D=\varepsilon E \tag{5}$$

$$\begin{aligned} D(t) &= \varepsilon_\infty E(t) + (\varepsilon_s - \varepsilon_\infty) E(t) e^{-i\delta} = (\varepsilon' - j\varepsilon'')E(t) \\ &= \varepsilon_\infty E(t) + (\varepsilon_s - \varepsilon_\infty) E(t) \left[\cos \delta - j \sin \delta\right] \end{aligned} \tag{6}$$

From Eqs. 4, 5, and 6 we find

$$\sin \delta = \frac{\omega\tau}{(1+ \omega^2 \tau^2)^{\frac{1}{2}}} \tag{7}$$

for the angle of the phase lag, δ.

In a rotating field as from a four-electrode system with potentials $V_x = V_o \sin$ and $V_y = V_o \cos \omega t$ applied to the x and y electrode pairs, the magnitude of the maximum field, E_o, and the potential differences remain constant in the mid-region of the symmetric electrodes.

Combining Eqs. 2, 3, and 7, we obtain as an expression for the torque, and using $K = \varepsilon / \varepsilon_o$

$$T = \mu E_o \sin\delta = 2\pi a^3 \, Re \left\{ \frac{K_1^* (K_2 - K_1)}{K_2 + 2K_1} \right\} \frac{\omega\tau}{(1+\omega^2\tau^2)^{1/2}} \qquad (8)$$

where ε_0 is the permittivity of free space. For a sphere slowly rotating in a fluid medium we may compute the frictional drag from Strokes formula. (17) The electrical torque and the hydrodynamic drag will equal in steady state.

$$T_{hydro} + T_{el} = 0$$

$$8\pi a^3 \eta \omega_c = 2\pi a^3 \epsilon_0 \left\{ \frac{\omega\tau}{(1+\omega^2\tau^2)^{1/2}} \right\} Re \left\{ \frac{K_1^* (K_2 - K_1)}{K_2 + 2K_1} \right\} E_o^2$$

or

$$\omega_c = \frac{\epsilon_o}{4\eta} \left\{ \frac{\omega\tau E_o^2}{(1+\omega^2\tau^2)^{1/2}} \right\} Re \left\{ \frac{K_1^* (K^2 - K_1)}{K_2 + 2K_1} \right\}; \qquad (9a)$$

$$\omega_c = \frac{\epsilon_o}{4\eta} \left\{ \frac{\omega\tau E_o^2}{(1+\omega^2\tau^2)^{1/2}} \right\} K_{eff} \qquad (9b)$$

where:

η = viscosity of the medium

ω_c = rotational (angular speed) of spherical body

a = radius

ω = angular frequency of the applied field

τ = characteristic relaxation time of the body dielectric K_1 and K_2 are the complex constants of the medium and sphere, resp.

$$K_i = K_i' - j K_i'' = K_i' - j \frac{\sigma_i}{\epsilon_o \omega}$$

$$K_{eff} = Re \left\{ \frac{K_1^* (K_2 - K_1)}{K_2 + 2K_1} \right\} \qquad (10)$$

For the special case of a sphere of insulating character in a conductive fluid

$$\mu = 4\pi a^3 \epsilon_0 K_1' \left(\frac{\sigma_2 - \sigma_1}{\sigma_2 + 2\sigma_1} \right) \qquad (11a)$$

and if $\sigma_2 \approx 0$ then

$$\mu \cong - 2\pi a^3 \epsilon_0 K_1'$$

(11b)

and the rotational speed (in a direction opposite to the rotation of the field) is

$$\omega_c = \frac{\epsilon_0 K_1'}{4\eta} \frac{\omega\tau E_0^2}{(1+\omega^2\tau^2)^{1/2}} \qquad (12)$$

In this case, can be evaluated in advance. We can expect the relaxation time of the insulating sphere in the conducting medium to approximate $\tau = RC$ where $R \cong \rho_1/2a$; $c = 4\pi\epsilon_0 a$ or $\tau \approx 4\pi\epsilon_0\rho_1$

We conclude that the simplified theoretical analysis predicts the rate of cellular rotation, ω_c, to be proportional to the square of the applied field intensity (as observed, Fig. 13); to be inversely proportional to the viscosity; to be proportional to the field frequency; to the relaxation time of the (presumed Debye-type) cell; and to the "effective polarizability", K_{eff}. We expect and, so far, find ω_c to reflect the magnitude and sign of K_{eff}.

For example, it is known from earlier studies of the DEP of yeast

the K_{eff} is generally negative in the region of 500 to 70kHz for live cells, and positive for dead ones. This agrees with the observed sign of ω_c. A plot of ω_c/ω_E versus ω_E can be expected to provide a convenient method for obtaining dielectric spectra for single cells, to give relative values of K_{eff} as a function of the applied frequency.

ACKNOWLEDGEMENTS

The authors acknowledge with thanks their support by the Pohl Cancer Research Laboratory, Inc., and to Mrs. Jill F. Dotson for her generous contribution of cell cultures and valuable advice.

FIGURE CAPTIONS

Figure 13: Spin rate of yeast (Saccharomyces cerevisiae) in a four-pole rotating electric field as a function of the square of the applied voltage on electrodes with a 1 mm gap. The frequency of the applied field was 60 kHz. Measurements of the spin rate (w_c) for cells in various concentrations of sucrose in water. (Squares, circles, triangles, and diamond designate data for 0, 100, 200, and 300 g sucrose per liter resp.) The resistivity of the solutions was adjusted to 133 kOhm cm. The cells examined were from 10-day-old culture, and were classified as 98% viable by methylene blue stain test.

Figure 14: The four-electrode CSR chamber. The distance between opposed (i.e. N-S or E-W) tips is 1.2 mm. The inner least width of the well is 8.2 mm. It is 1.0 mm deep. The Pt tips are ca. 130 m in diameter on 75 m diameter Pt wire. All are mounted on a standard glass microscope slide.

Figure 15: Spin rate spectra of living (triangles) and dead (circles) yeast (Saccharomyces cerevisiae). The live cells were from a 7-day-old culture; the dead cells were heat-killed by exposure to 70°C for 3 min. The applied voltage was 10 V p-p, and the resistivities of the suspensions were 250 to 460 kOhm cm.

Figure 16: Dependence of the CSR spectrum of yeast (Saccharomyces cerevisiae) upon the colony age. (Circles, Triangles, and squares refer

to data for colony ages of 2, 6, and 8 days respectively.) Note the shift of the 2-and the 20-kHz peaks to lower frequency as the colony age increases.

Figure 17: The CSR spectrum of one day old culture of bovine kidney cells.

Figure 18: The CSR spectrum of CRFK (Crandall feline kidney) cells from a four-day-old culture.

Figure 19: The CSR spectrum of green monkey (VERO) kidney cells from a 5-day-old culture.

Figure 20: Effect of the conductivity of the suspension upon the CSR spectrum of single yeast (Saccharomyces cerevisiae) cells bearing a small bud and from a six-day old colony. Voltage 10 V p-p. Spinning was counter-field in direction. Dotted curve, cells in pure water, 2.4 S/cm. Full curve, cells in 8.9 S/cm. Dashed curve, cells in 0.025 S/cm. The conductivity was adjusted by adding NaCl. Note that the effect of increasing the conductivity is to shift the peaks to a higher frequency.

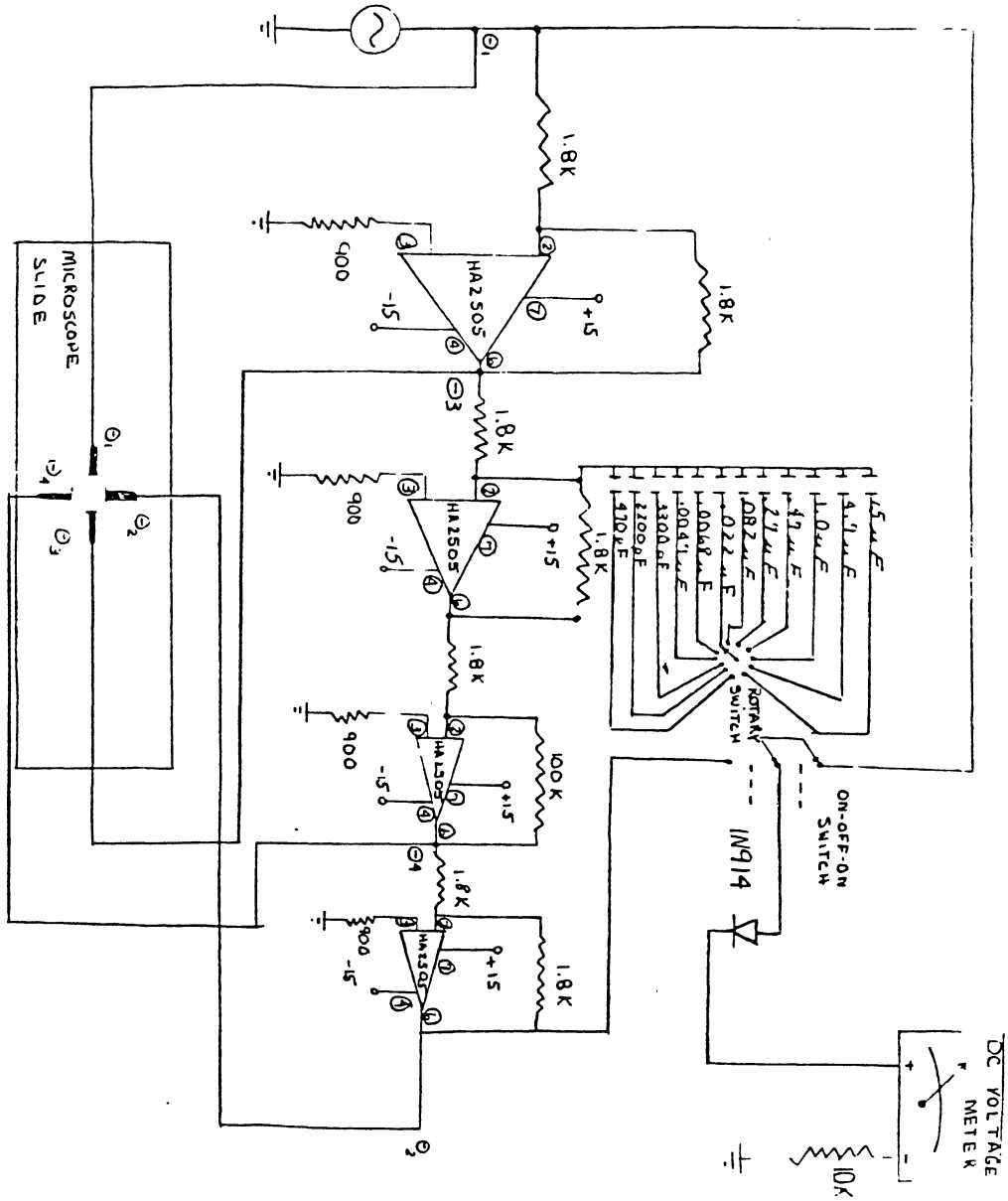
Figure 21: The CSR spectrum of barium titanate particles in high purity (5 micromho/cm) water.

Figure 22: The CSR of barium titanate as affected by polyacrylate polyanions. The particle spin rate when driven by a rotating field at 600 kHz is seen to be affected even by very dilute solutions.

Figure 23: The CSR of barium sulfate as affected by polyacrylate polyanions. The particle spin rate when driven by a rotating field at 600 kHz is seen to be affected even by very dilute solutions.

APPENDIX B

ELECTRICAL SCHEMATIC FOR FOUR POLE CIRCUIT


MICROSCOPE SLIDE
1.8K
900
HA2505
-15
+15
100K
IN914
ON-OFF-ON SWITCH
ROTARY SWITCH
DC VOLTAGE METER
10K

VITA

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