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THE USE OF A 'CYTOPHEROMETER' IN ELECTROPHORETIC STUDIES OF MINERALS AND OF MINERAL-LEACHING BACTERIA

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MINERAL SCIENCES DIVISION

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THE USE OF A 'CYTOPHEROMETER' IN ELECTROPHORETIC STUDIES OF MINERALS AND OF MINERAL-LEACHING BACTERIA

by

Syed M. Ahmed*

ABSTRACT

The application of a 'Cytopherometer' (Carl Zeiss Inc.) to electrokinetic studies of mineral suspensions in aqueous electrolyte solutions has been investigated. The Cytopherometer was modified by incorporating palladium electrodes with electrochemically occluded hydrogen. Thus modified, the Cytopherometer enables fast and accurate electrokinetic measurements to be made on mineral suspensions in liquids. Precautions to be taken in the use of palladium electrodes are given.

It was found from electrokinetic studies that the mineral-leaching bacteria, thiobacillus-ferrooxidans did not acquire any surface charge in acidic solutions of KNO_3, KCl, Fe^{2+}, Fe^{3+}, NH_4^+, Mg^{2+}, and Ca^{2+}. This electrical neutrality of the bacterial surface is probably due to the protective action of the bacterial membrane. However, the bacteria developed a strong negative charge in alkaline solutions of KNO_3 and KCl which is attributed to the rupture of protective membrane and the formation of $R<^{NH_2}_{COO^-}$ groups on the cellular surface. Any ionic surfactant that may be used to enhance the bacterial leaching of minerals (in acid solutions) should have a charge that is opposite to the charge on the mineral surface. The amount of surfactant to be used may also be critical.

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Direction des mines

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L'UTILISATION D'UN "CYTOPHEROMETRE" POUR L'ETUDE ELECTROPHORETIQUE DES MINERAUX ET DES BACTERIES INTERVENANT DANS LA LIXIVIATION DES MINERAUX

par

Syed M. Ahmed*

RESUME

L'auteur a fait des recherches sur l'application d'un "cytophéromètre" (Carl Zeiss Inc.) à l'étude, du point de vue de l'électrocinétique, des minéraux en suspension dans des solutions électrolytiques aqueuses. Il a modifié le "cytophéromètre" en lui incorporant des électrodes de paladium contenant de l'hydrogène occlus par un procédé électrochimique. Ainsi modifié, le "cytophéromètre" permet d'effectuer des mesures rapides et précises des caractéristiques électrophorétiques des minéraux en suspension dans des liquides. L'auteur indique les précautions à prendre pour l'utilisation des électrodes en palladium.

L'auteur a découvert, au cours de ces recherches, que la bactérie thiobacillus-ferrooxidans, qui intervient dans la lixiviation des minéraux, n'acquiert aucune charge superficielle dans des solutions acides de: KNO_3, KCl, Fe^{2+}, Fe^{3+}, NH_4^+, Mg^{2+}, et Ca^{2+}. L'absence de charge électrique superficielle chez cette bactérie et dans ces conditions est due, probablement, à l'action protectrice de sa membrane cellulaire. Cependant, la même bactérie présentait, dans des solutions alcalines de KNO_3 et de KCl, une forte charge négative que l'auteur attribue à la rupture de la membrane protectrice et à la formation de groupements $R\langle^{NH_2}_{COO^-}$ sur la surface cellulaire. Tout surfactif ionique pouvant être utilisé pour améliorer la lixiviation bactérienne des minéraux (en solution acide) devrait avoir une charge de signe opposé à celui de la charge apparaissant sur la surface du minéral. Il est possible que la quantité de surfactif à utiliser soit critique.

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CONTENTS

	Page
Abstract	i
Résumé	ii
Contents	iii
Introduction	1
Apparatus and Method	3
The Cytopherometer	3
The Electrode System	6
Preparation and Use of the Palladium Electrodes	7
pH Variations During Electrophoresis Using Pd Electrodes	9
Stationary Planes and Measurement Parabola of the Electrophoretic Mobility of Particles	12
Electrokinetic Studies with the Mineral-Leaching Bacteria	15
Conclusions	15
References	17

FIGURES

No.		Page
1	Photograph showing different parts of the Cytopherometer...	4
2 and 3	Photographs showing different parts of the Cytopherometer with the $Cu/CuSO_4$ reversible electrodes..	5
4	Apparatus for electrophoretic studies of suspensions using palladium electrodes with the Cytopherometer.....	8
5	Variation of pH of the electrolyte solution (~10 ml) in the anode and cathode compartments with time during electrophoresis	10
6	Variation of the total cell-resistance with time during electrophoresis in a cylindrical tube.....	11
7	Figure showing parabolic variation of the electrophoretic mobility of quartz suspensions in 0.1M KNO_3 solution, as a function of the cell depth	14

INTRODUCTION

Electrokinetic studies constitute a standard method of obtaining information on several interfacial properties of solids such as the zeta-potential, the zero point of charge of the solid-solution interface, the nature of the surface charge of the solid, and the adsorption of inorganic ions and of surface-active agents on the solid surface. Such information on the mineral-solution interface, for example, essentially determines the nature of chemical treatment to be given to the mineral surface in ore-dressing operations such as flotation, agglomeration, and selective flocculation of minerals (1-4). The theory and experimental details of electrokinetic studies of suspensions have been discussed in several recent publications (5-11). A commercially available apparatus (Zeta Meter) for electrophoretic studies of particles has been described (11). The Zeta Meter employs a cylindrical capillary cell with non-reversible metal electrodes (platinum and molybdenum) and has no provision for the temperature control of the cell. Further, while using platinum electrodes at high current densities, the electrolytic evolution of gases interferes with the electrokinetic work. If a molybdenum anode is used, oxide formation occurs that necessitates frequent cleaning of the electrode surface. The pH of the solution in the electrode compartments also changes during electrophoresis. This variation of pH in electrophoresis is common to all cells that use immersion-type electrodes without an intermediate salt bridge to separate the electrode from the test solution. Alternate designs using flat observation chambers have also been used (5, 6, 10, 12). A laterally oriented, flat, observation chamber for micro-electrophoresis has been described in detail by Neihof (13) and by Parreira (14). The Cytopherometer (manufactured by Carl Zeiss, Inc., Oberkochen, Wuertt, West Germany) that was originally designed for electrophoretic studies in clinical work has all the special features of apparatus described in literature for research studies in electrophoresis (13, 14). Also, this apparatus has

several advantages over the equipment described by the above two authors (13, 14) including a water jacket surrounding the observation chamber and a high precision mechanical and optical system. The Cytopherometer, as supplied, employs $Cu/CuSO_4$ reversible electrodes with several intermediate salt bridges. In practice, it was found that the preparation and use of these electrodes was difficult and time consuming and it was also difficult to make duplicate electrodes with identical electrical behaviour. In investigations of the electrophoretic properties of mineral surfaces, particularly in the mineral industry, a fast and a fairly accurate commercially available apparatus is highly desirable. In a recent investigation (13), the use of palladium electrodes with electrochemically occluded hydrogen has been recommended for electrophoretic measurements. The first part of the present work was undertaken to investigate the use of the Cytopherometer in electrokinetic investigations of minerals and the possibility of modifying this apparatus by adopting palladium electrodes. A description of the apparatus, and the standard procedure of using it, is provided elsewhere (15) and will not be repeated here except where necessary.

The second part of this report consists of electrophoretic investigations of mineral-leaching bacteria. The leaching of many sulphide ores of Cu, Ni, Fe and Zn using thiobacillus-ferrooxidans is well established (16-20) and has been used commercially for copper sulphide ores. Uranium (21-23) has also been leached commercially by the acidic solutions of ferric iron produced by microbiological leaching of pyrite usually associated with uranium ores. In addition to the ability of the bacteria to oxidize ferrous iron to ferric iron, bacteria have also been shown to attack sulphur (24) and the ferrous iron of minerals directly (16, 25). It was also found by Duncan et al.(26) that the use of certain surface-active agents (Tween-20) enhances the bacterial leaching of some sulphide minerals considerably. A wetting agent, inositol phosphotide has also been shown (27) to be produced by the thiobacillus-thiooxidans and this wetting agent is believed to assist the bacterial attack on sulphur. The oxidation of sulphur by thiobacillus-thiooxidans

has also been shown by Adair (28) to increase considerably in the presence of wetting agents. Elementary sulphur, which is usually hydrophobic in nature, was found to become finely divided by the bacterial attack in the presence of the wetting agent. It has been proposed (29) that ferrous iron is bound to the cell walls of the bacteria before being oxidized into ferric complexes. The oxidation of ferrous iron to ferric iron occurs by an electron transfer process which involves complex membrane equilibria (28, 29) at the bacterial-cell walls. The electrokinetic behaviour of the microorganisms thiobacillus-ferrooxidans, was studied in this work to find whether the bacteria in electrolyte solutions possess any surface charge. The result of the above investigation, whether the bacterial surface is neutral or charged ($\pm$), would then suggest if the surface-active agent to be used in ore leaching operations should be neutral or ionic in nature.

APPARATUS AND METHOD

The Cytopherometer

The main parts of the Cytopherometer are shown in Figures 1, 2 and 3. It consists of four sections mainly: i) a power supply (0-50 mA or 0-900 V), ii) the electrophoresis system, iii) an optical system, and iv) a thermostat and a pump for circulating water from a constant-temperature bath.

The electrophoresis part of the apparatus consists of an electrode system and an observation chamber to hold the particle suspension. The observation chamber is enclosed in a transparent jacket made of Lucite through which water is circulated from a constant-temperature bath. The observation chamber is a flat cell (depth x height = 0.7 ± 0.1 mm x 14 ± 0.5 mm) with engraved lines on the inside of the walls for focussing and for exact measurements of the cell depth. The front lens of the objective is permanently fixed to the temperature-controlled Lucite jacket and is in close proximity with the observation chamber containing the suspensions. The main

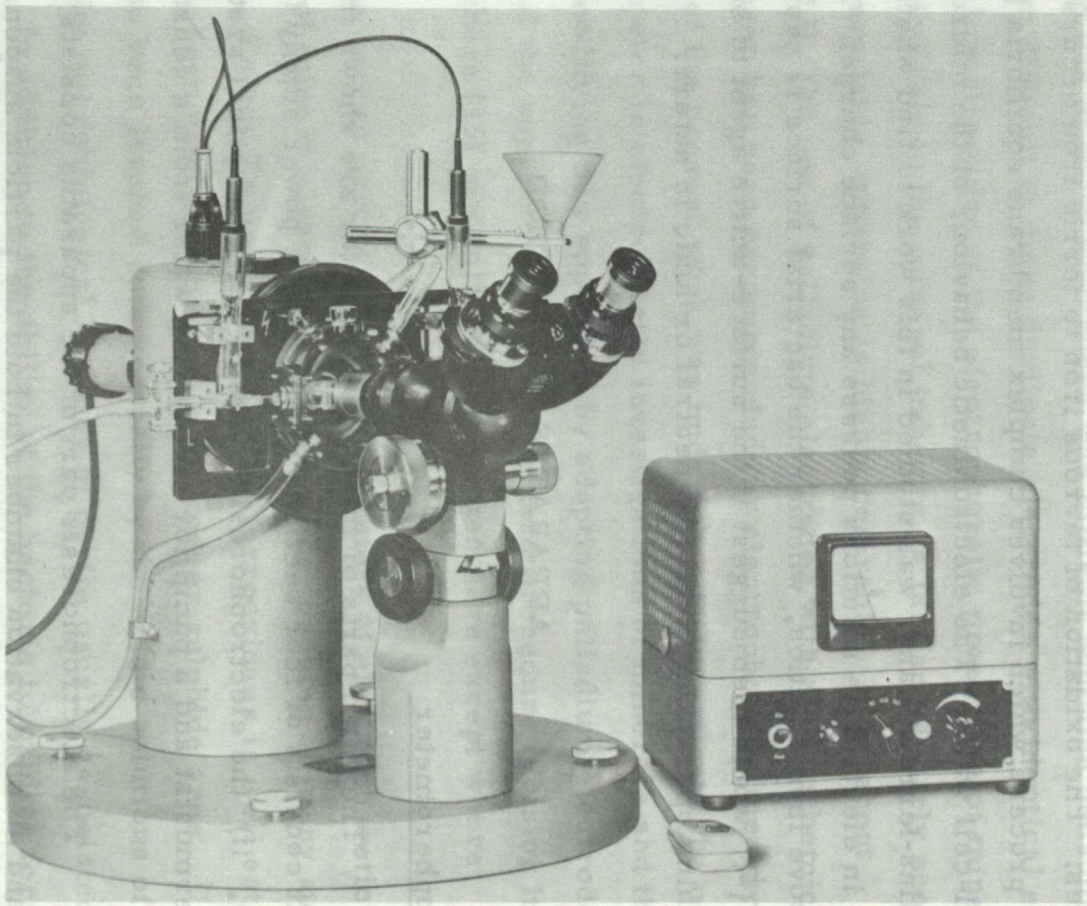
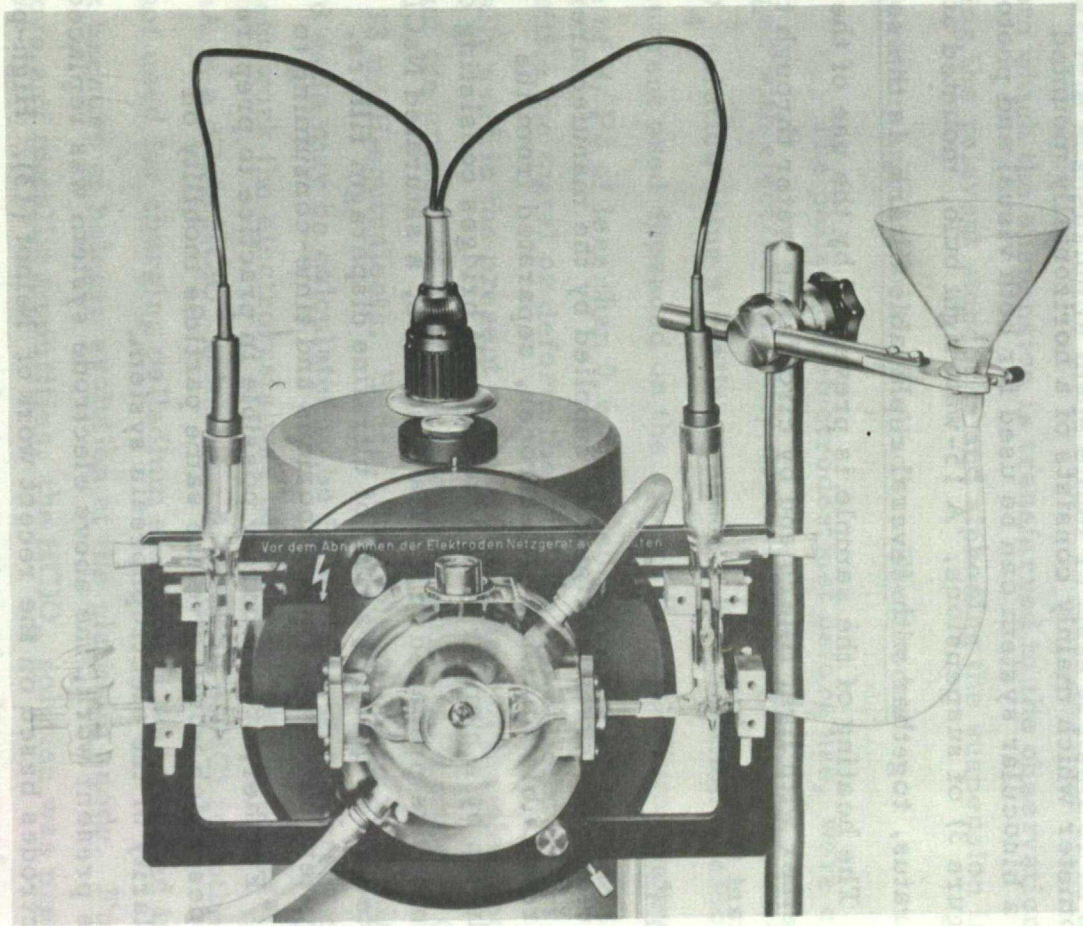


Figure 1. - Photograph showing different parts of the Cytopherometer.



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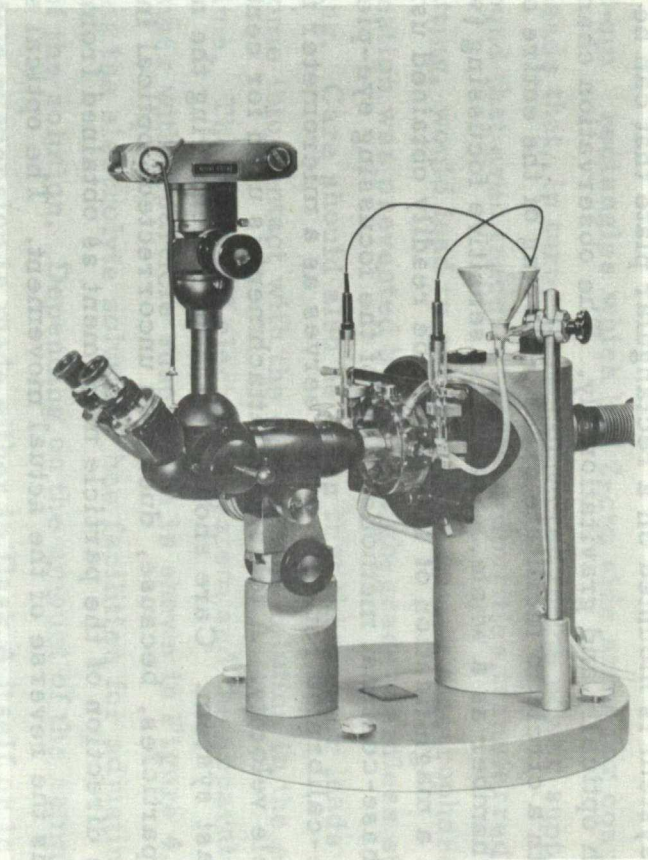

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Figures 2 and 3: Photographs showing different parts of the Cytopherometer with the $Cu/CuSO_4$ reversible electrodes.

electrophoresis system is mounted on a rectangular plate that can be rotated and centered both optically and gravitationally. The observation chamber is also provided with a circle to facilitate exact centering of the entire optical system and the chamber as a whole. Sharp and sensitive focussing (within one micron) with a magnification of 1000 X can be readily obtained using either bright-field or phase-contrast methods. One of the focussing eye-pieces has a special pre-calibrated reticule which serves as a micrometer for measuring particle velocity. A telescopic attachment is used for centering the phase constrast system. Care should be taken in identifying the nature of charge on the particles, because, due to an uncorrected, optical inversion of the image, the direction of the particle movement as obtained from the Cytopherometer is the reverse of the actual movement. The optical system of the Cytopherometer which mainly consists of a horizontally mounted microscope and a binocular system can be used for both visual and photographic examination (Figure 3) of suspensions. A 15-watt light bulb, mounted at the back of the apparatus, together with several replacable filters, is the source of illumination. The heating of the sample is prevented by the use of the low-wattage bulb together with light filters and by circulating water through the jacket at a constant temperature.

The Electrode System

The electrode system originally supplied by the manufacturer consisted of the reversible $Cu/CuSO_4$ electrodes, separated from the experimental solution by a number of conducting salt bridges consisting of plaster, a gelatin bridge prepared in 1M NaCl solution, a saturated NaCl solution in a fritted glass cell,and a pair of ultrafine diaphragm filters. This electrode system was found to be laborious and time-consuming to prepare and use. Further, it was hardly possible in practice to prepare duplicate electrodes which would give the same particle mobility on reversing the polarity of the electrophoresis system.

In the present work, the above electrode system was replaced by palladium electrodes based on the recent work of Neihof (13). High-purity palladium foil (0.125 mm thick) for the electrodes was obtained from

Johnson Matthey and Mallory Ltd., Montreal. The palladium was cut into 1-cm x 2-cm rectangles which had 2-cm side arms for connection to copper leads that, in turn, were connected to the power supply. It was found that even traces of metal impurities, transferred to palladium while cutting, would lead to gas evolution during electrophoresis. This contamination was prevented by using high-grade stainless steel blades for cutting. Care should also be taken that the copper leads of the electrodes do not come into contact with the electrolyte solution in the electrode chamber at any time. The modified electrode system and the apparatus for filling the chamber with a particle suspension is shown in Figure 4. The flask A, containing the electrolyte solution, has facilities for adjusting and measuring the pH of the solution. Depending on the amount of the sample available, the particle suspension is made either in flask A itself or in a small reservoir B from which the suspension is transferred to the observation chamber by opening the relevent stopcocks and siphoning the suspension into the chamber.

Preparation and Use of the Palladium Electrodes

The palladium electrodes, just before use, were cathodically charged with electrochemically occluded hydrogen by electrolysis in a dilute $HClO_4$ solution using a platinum anode. The duration of electrolysis and the current used depended on the size of the palladium electrode. An atomic ratio of H/Pd of less than 0.6, as recommended by Neihof (13), does not embrittle the metal or deform the surface. A ratio of H/Pd of 0.1 was initially used in the present work. The weight of the electrode was 0.35 g so that the amount of hydrogen required for a H/Pd ratio of 0.1 is equal to 0.33 milli-equivalents. Hence, the time required to obtain the desired H/Pd ratio may be calculated to be 50 minutes at a current of 10 mA. In the present work the electrolysis was performed for 10 minutes at a current of 50 mA in a dilute $HClO_4$ solution. A solution of HCl (or other halides) that Neihof used for charging palladium electrodes was avoided in this work because of halogen evolution at the platinum anode. Further, to remove any metallic impurities, the $HClO_4$ solution was pre-electrolyzed for several hours using a platinum gauze of a large surface area as the

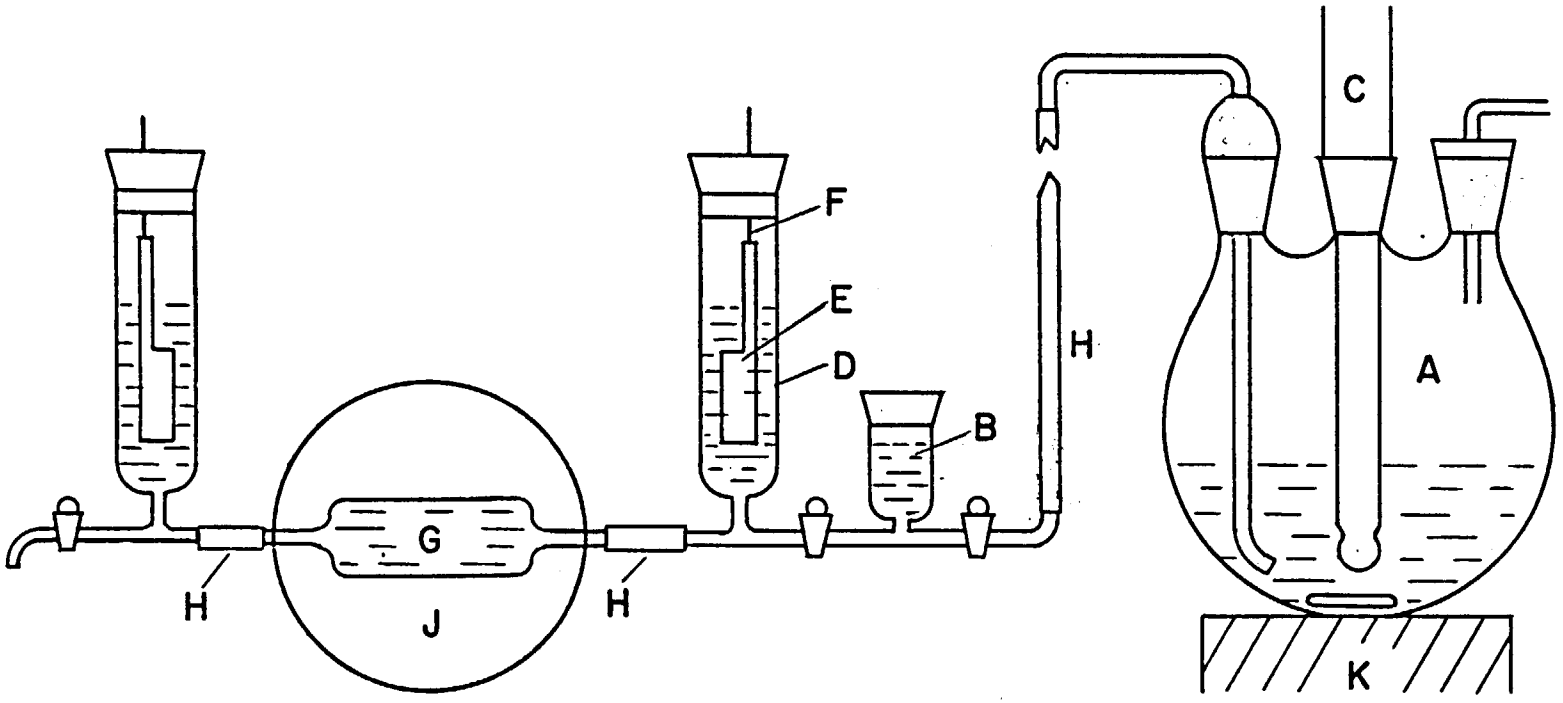

Figure 4. - Apparatus for electrophoretic studies of suspensions using palladium electrodes with the Cytopherometer.

A: Flask with the electrolyte solution, B: Small reservoir for adding suspensions, C: Glass-Calomel, combination electrode, D: Electrode chamber, E: Palladium electrode, F: Copper lead, G: The electrophoresis, observation chamber, H: Silicone flexible tubing; J: Transparent water jacket made of lucite; K: Magnetic stirrer.

cathode. While recharging the palladium electrodes after a previous run, there is a possibility of excessive inclusion of hydrogen in the metal, because of an uncertain amount of hydrogen already present. To avoid this possibility, the palladium electrodes, before the cathodic treatment, were pre-anodized just until the oxygen evolution was seen to occur on the surface.

pH Variations During Electrophoresis Using Pd Electrodes

Palladium electrodes are easy to prepare and use and do not result in gas evolution during electrophoresis. However, these electrodes, being reversible to H^+, cause significant changes in the pH of the solution in the anode and cathode compartments due to the following reactions,

$$\text{(Cathode)} \quad Pd + x\,H_2O + x\,e^- = PdH_x + xOH^- \text{ (basic)}, \qquad \ldots(1)$$

$$\text{(Anode)} \quad PdH_x - x\,e^- = Pd + x\,H^+ \text{ (acidic)}. \qquad \ldots(2)$$

Thus, if the original solution is neutral, then a few seconds of electrolysis in electrophoretic studies would make the solution considerably basic or acidic depending on the electrode polarity. The electrolysis that occurs without any apparent gas evolution produces concentration gradient of the acid in the electrophoretic system. Such changes in pH can affect the cell conductivity and the electrokinetic properties of suspensions if the H^+ ions diffuse into the measurement area of the cell (G, Figure 4) or into the narrow capillary ends of the cell. The above possibilities were tested in a cylindrical cell (12 cm long, 1.5 mm diameter) by measuring, as a function of time, the pH of a 0.1M KNO_3 solution during electrolysis. The results of these measurements are shown in Figure 5. Changes in the conductivity of the cell were also measured. It was found that the pH in the central portion of the capillary was unaltered during electrolysis for a short time, indicating that the net amount of acid transferred into the central portion of the capillary, as a result of pH difference in the two compartments, is negligible. However, the resistance of the cylindrical cell decreased with time during electrolysis as shown by curve A in Figure 6. The cell resistance

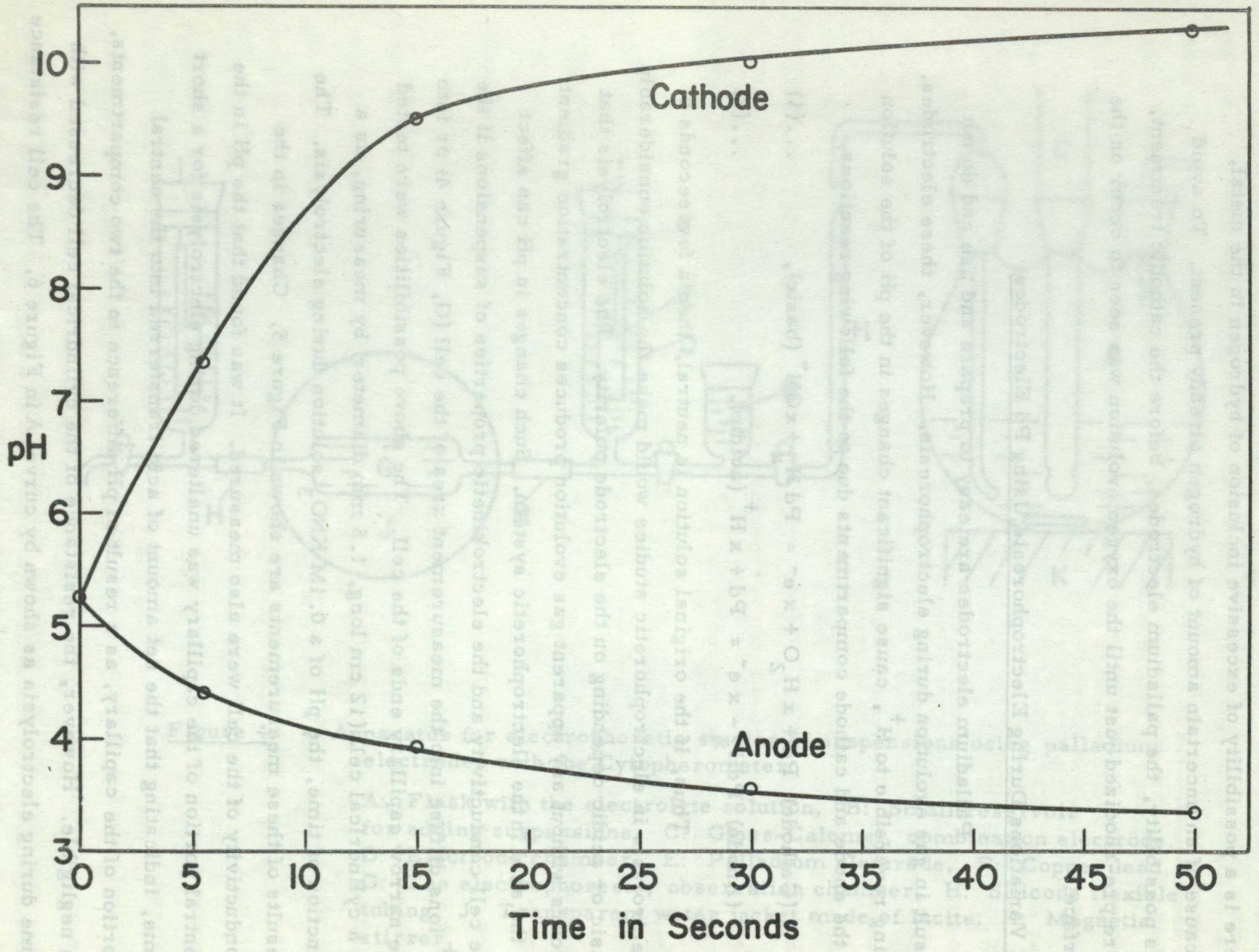

Figure 5. - Variation of pH of the electrolyte solution (~ 10 ml) in the anode and cathode compartments with time during electrophoresis.

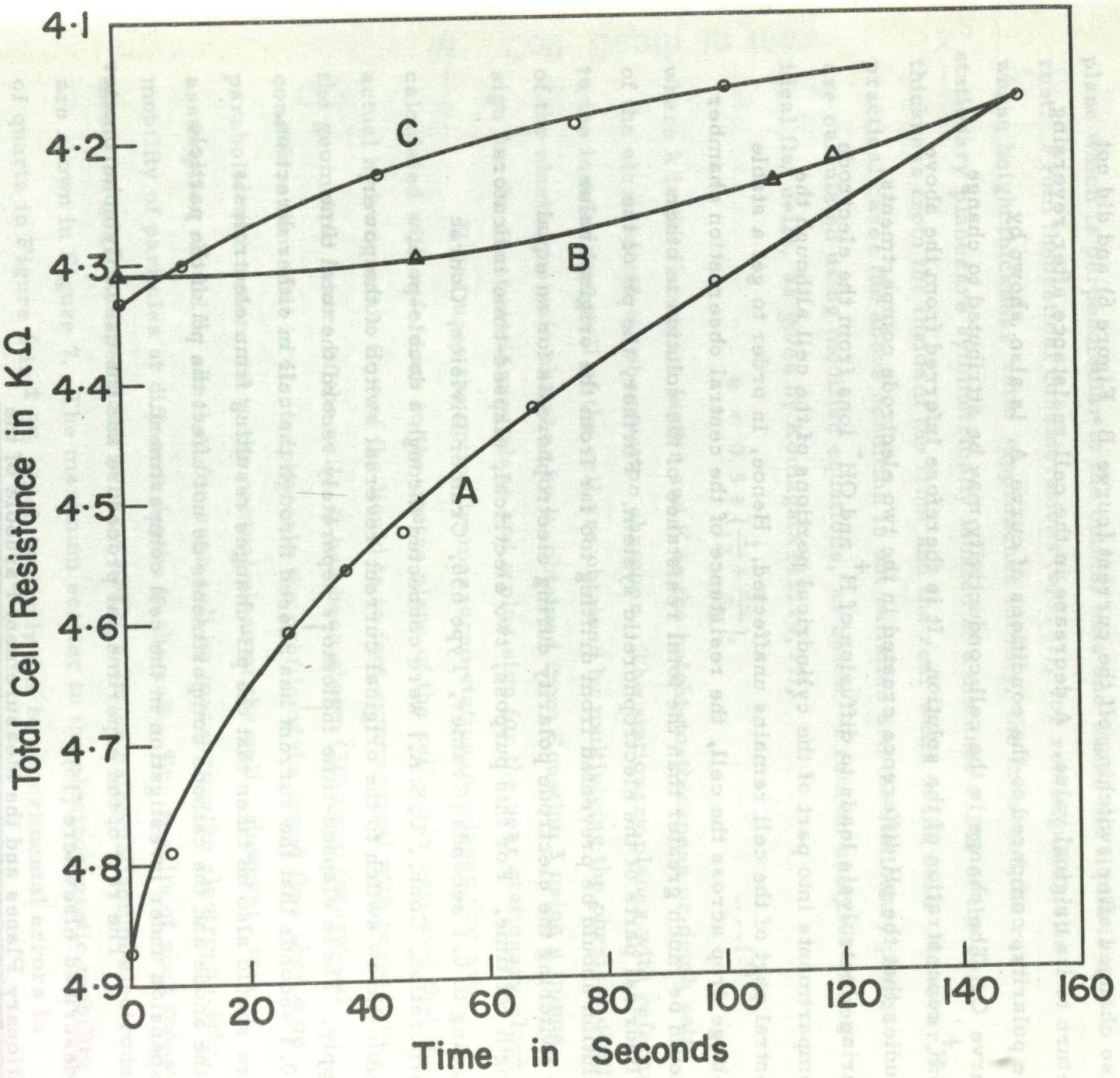

Figure 6. - Variation of the total cell-resistance with time during electrophoresis in a cylindrical tube.

also changed after switching off the current (curve B, Figure 6) and did not return to its original value. A decrease in the cell resistance after reversing the polarity, compared to the conditions of curve A, is also shown by curve C. The change in the cell conductivity may be attributed to change in H^+ concentration of the solution. It is therefore inferred from the above studies that the pH difference created in the two electrode compartments during electrolysis leads to diffusion of H^+ and OH^- ions from the electrode compartments into part of the cylindrical portions of the cell, although the central part of the cell remains unaffected. Hence, in order to get a stable voltage drop across the cell, the resistance of the central observation chamber should be much greater than the total resistance of the solution in other cylindrical parts of the electrophoretic system. Further, the pH of the solution should be prevented from drifting too far from the original value by switching the electrode polarity during electrophoresis for an equal length of time. For this purpose, two electrical, elapsed-time indicators reading to 0.1 seconds (Cramer, Type 636, Cramer Division, Conrac Corporation, Conn., U.S.A.) were connected through a double-pole, double-throw switch to the original current reversal switch of the power supply. These elapsed-time indicators separately record the total time to 0.1 seconds that the current has passed through the cell in either direction. Care should also be taken that the pH-changes resulting from electrolysis in the anode and the cathode compartments do not affect the pH of the particle suspension under investigation in the cell compartment.

The rest of the experimental procedure and the use of Cytopherometer is described elsewhere (15).

Stationary Planes and the Measurement Parabola of the Electrophoretic Mobility of Particles

In electrophoretic measurements, the particle velocity that is observed is the sum of the electrophoretic velocity of particles and the superimposed laminar flow of the liquid resulting from electro-osmosis (30). The true electrophoretic mobility of particles is obtained by measuring the electrophoretic velocity at a particular cross section of the cell, known as the stationary

plane which is perpendicular to the optical axis and where the liquid is at rest. From theoretical considerations (6, 7, 31), for rectangular cells whose height:thickness ratio approaches infinity, the front and the rear stationary planes are symmetrically located at a distance 0.211 of the cell thickness from the inside of the front and the rear walls of the cell. However, in practical cases the above condition is not satisfied and the stationary planes are calculated using modified equations, as below. From an analysis of non-ideal flat cells, as given by Komagata (12, 14, 31),

$$k = 0.5 \mp \left(\frac{1}{12} + \frac{32}{\pi^5 K}\right), \qquad \ldots(3)$$

where k is the Komagata factor and K is the height-to-thickness or depth ratio of the electrophoretic observation chamber. The stationary planes with reference to the inside front wall of the cell are obtained by multiplying the physical depth of the electrophoretic chamber by k, obtained from Equation 3 using a minus sign for the front stationary plane and a plus sign for the rear stationary plane.

If one uses the optically-measured stationary planes that are calculated with reference to the centre plane of the cell, it is assumed that the actual hydrodynamic flow of the liquid is in fact symmetrical with respect to the geometric centre of the cell. It has been shown (30) from theoretical considerations that in electrophoresis the profile of the liquid flow should be parabolic with reference to the central plane of the cell. However, this assumption should be checked for each cell by measuring the electrophoretic mobility of particles at different depths in the cell. The results of such measurements on quartz suspensions in 0.1M KNO_3 solution using palladium electrodes are shown in Figure 7. The maximum scatter in the electrophoretic mobility of quartz in Figure 6 is $\pm$ 3% which is partly due to experimental errors in velocity measurements. As mentioned before, scatter can be minimized by reversing the current for equal lengths of time during the measurements. The parabola of the electrophoretic mobility of quartz (Figure 7) is seen to be symmetrical with reference to the central plane M of the cell, and x_1 and x_2 in Figure 6 refer to the front and the rear stationary planes. The electrophoretic mobility of quartz suspensions in the x_1 and x_2 planes is seen to be in good agreement ($\sim 3.3 \times 10^{-4} cm^2 V^{-1} sec^{-1}$).

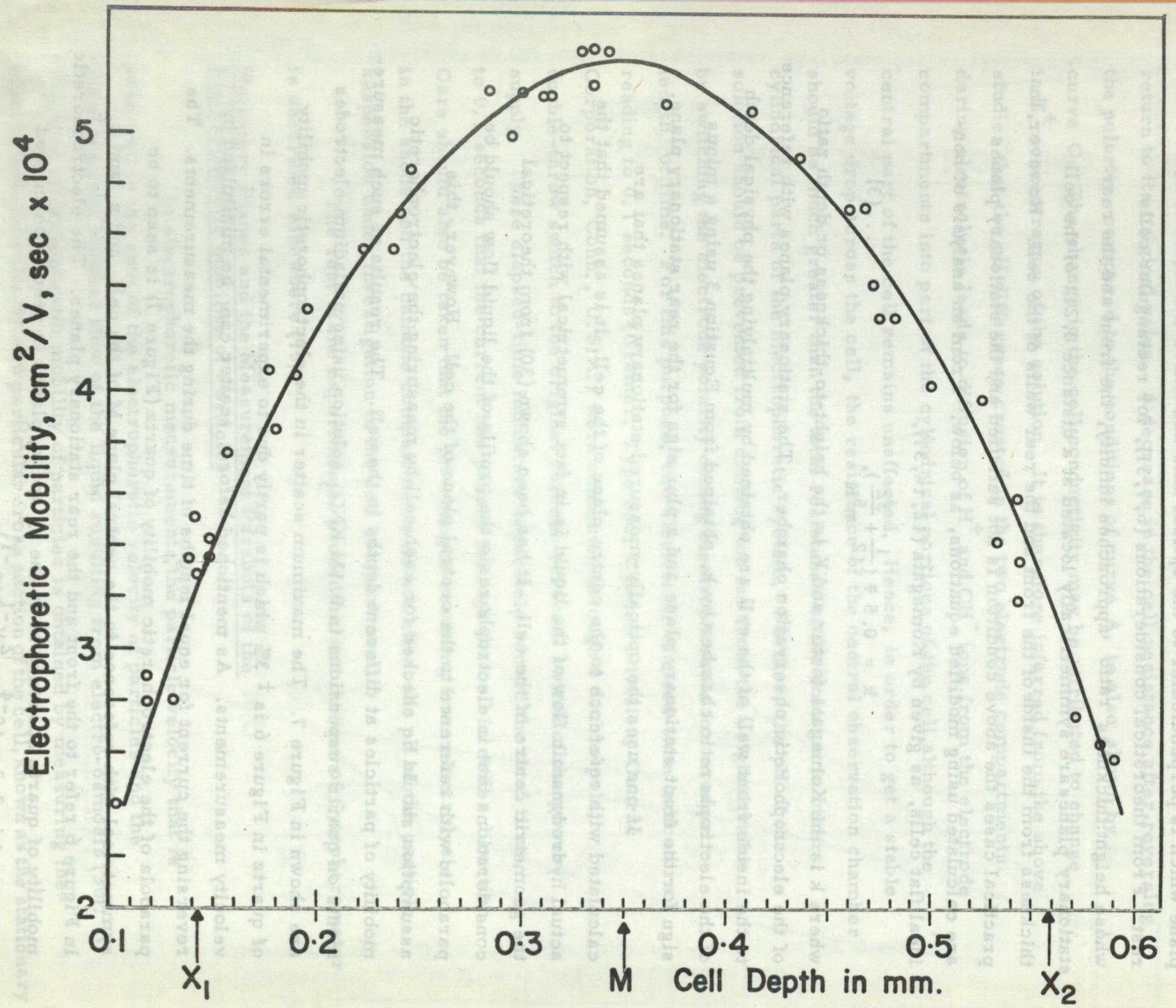

Figure 7. - Figure showing parabolic variation of the electrophoretic mobility of quartz suspensions in 0.1M KNO_3 solution, as a function of the cell depth.

Electrokinetic Studies with the Mineral-Leaching Bacteria

The bacteria, thiobacillus ferrooxidans, cultured by standard procedure (19, 20) were obtained from the Extraction Metallurgy Division of the Mines Branch. The bacteria were washed free of iron just before electrophoretic measurements. Electrokinetic studies of the bacteria were carried out in acidic and in neutral solutions of KNO_3 and KCl, and also separately in the acidic solutions of Fe^{2+}, Fe^{3+}, NH_4^+, PO_4^{3-}, Mg^{2+}, and Ca^{2+} ions that are usually provided as neutrients for the bacteria in the mineral-leaching solutions. The bacteria did not exhibit any surface charge in any of the above solutions. However, in alkaline solutions (pH$\gtrsim$9), the bacteria developed a strong negative surface charge. From structural considerations, proteins are known to acquire a positive surface charge in an acid medium due to the formation of $R\langle^{NH_3^+}_{COOH}$ groups and a negative charge in an alkaline medium due to formation of $R\langle^{NH_2}_{COO^-}$ groups. The mineral-leaching bateria have been shown to carry a protective, cytoplasmic membrane surrounding their cellular structure (28, 29). The absence of a positive surface charge on bacteria in acid medium is probably due to the protective membrane which isolates the bacterial cell from the solution. The survival of bacteria in highly acidic solutions has also been attributed to the protective nature of this membrane. In alkaline solutions, however, the membrane appears to rupture, exposing the negatively charged $R\langle^{NH_2}_{COO^-}$ groups of the cells to the solution. The origin of negative surface charge on the bacteria was not investigated further because the bacteria are known to survive and to leach minerals only in a strongly acid medium.

Figure 1. - Figure showing parabolic variation of the electrophoretic mobility of quartz suspensions in 0.1M KNO_3 solution, as a function of the cell depth.

CONCLUSIONS

The 'Cytopherometer', after modification by incorporating palladium electrodes with electrochemically occluded hydrogen, enables fast and accurate electrokinetic measurements to be made of particle suspensions in liquids. However, drastic pH variations in the electrode

compartments during electrophoresis may lead to inaccurate results and, hence, care should be taken in designing the electrophoresis system to avoid major conductivity variations in the cell and pH deviations in the electrode compartments. Deviation of the pH from the original value can be minimized by reversing the electrode polarity for equal lengths of time during electrophoretic measurements.

On the basis of these electrokinetic investigations of mineral-leaching bacteria, it is suggested that any ionic surface-active agent that is used to accelerate the leaching of minerals should have a charge opposite to that of the mineral surface. Under such conditions, the non-ionic group of the surfactant would adhere to the bacteria. The concentration of surfactant to be used may also be critical.

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