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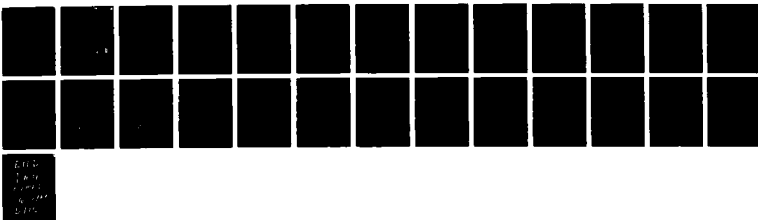
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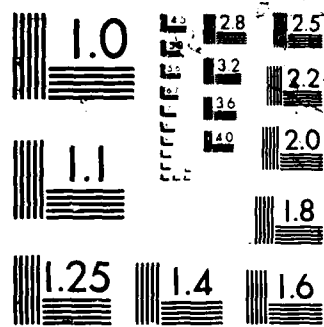
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

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EXAMINATION OF IONTOPHORETIC TRANSPORT OF IONIC
DRUGS ACROSS SKIN I. BASELINE STUDIES WITH
THE FOUR-ELECTRODE SYSTEM

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Abstract

A four-electrode system for systematically studying iontophoresis of charged drugs across skin has been investigated. This system is clearly superior to the conventional two-electrode system since it allows us to determine and control the actual electrical potential drop across a membrane. The applicability of the following equation relating the iontophoretic flux enhancement ratio (E) and the applied voltage ($\Delta\phi$) has been studied using two model compounds (tetraethylammonium bromide and citric acid) with hairless mouse skin and a cellulose acetate membrane.

$$E = \frac{J}{J_0} = - \frac{FZ\Delta\phi}{RT \left[\exp \left(\frac{-FZ\Delta\phi}{RT} \right) - 1 \right]}$$

where, E = flux enhancement ratio; J = flux with an electric field; J_0 = flux without an electric field; $\Delta\phi$ = applied voltage; Z = molecular charge; F = Faraday constant; R and T have their usual meanings. The results with the cellulose acetate membrane were generally in good agreement with the flux enhancement equations. In the case of hairless mouse skin, the results were consistent with Eq. 2 only at low applied voltages, significant positive deviations were observed at higher applied voltages.

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INTRODUCTION

Recently (1,2,3) there has been increased interest in the possibility of utilizing iontophoresis for drug transport across skin. Although previous studies with conventional two-electrode systems have shown that it is feasible to obtain flux enhancement of drugs across membranes by iontophoresis, they have not provided data relating the actual potential drop across a membrane with the iontophoretic flux. The principal difficulty with the two-electrode system is that it does not permit the direct determination of the potential drop across the membrane.

The purpose of the present communication is to examine the validity of an equation derived from the Nernst/Planck relationship describing flux enhancement of charged molecules across a membrane or skin caused by an electric field. In order to test this equation with experimental data, a four electrode system for a two chamber diffusion cell assembly was developed which allows for the first time determination and control of the actual potential drop across a membrane.

Background

The movement of ions in an electric field can be described by the following fundamental iontophoresis equation:

$$J = -D \left(\frac{dC}{dx} \right) - \frac{ZF}{RT} DC \left(\frac{d\psi}{dx} \right) \quad \text{Eq. 1}$$

where J is the flux of ions, $\frac{dC}{dx}$ is the concentration gradient, Z is the molecular charge, D is the diffusivity, F is the Faraday Constant, RT has the usual meaning, and $\frac{d\psi}{dx}$ is the electrical potential gradient. For a linear potential drop across a membrane the solution to Eq. 1 in the steady state case yields:

$$E = \frac{J}{J_0} = \frac{-FZ \Delta\phi}{RT \left[\exp \left(\frac{-FZ \Delta\phi}{RT} \right) - 1 \right]} \quad \text{Eq. 2}$$

E is defined as a flux enhancement ratio, i.e., the ratio of flux with applied electric field, J, to flux at zero field, J_0 , $\Delta\phi$ is the applied voltage. The experimental flux enhancement ratio, E may be related to experimental parameters by

$$E = \frac{J}{J_0} = \frac{PA \cdot \Delta C}{P_0 A \cdot \Delta C} = \frac{P}{P_0} \quad \text{Eq. 3}$$

where P is the effective permeability coefficient with an electric field and P_0 the permeability coefficient without electrical field, A is the diffusional area and ΔC the concentration differential across the membrane.

P and P_0 may be determined in a two chamber diffusion cell experiment. The slopes in the steady state case give the fluxes, J and J_0 , in the presence of and in the absence of the electric field and P and P_0 , are calculated from the slope and the concentration differential ΔC , for each species from

$$P = \frac{J}{\Delta C} = \frac{\left(\frac{V}{A} \right) \cdot \text{slope}}{\Delta C} \quad \text{Eq. 4}$$

$$\text{and } P_0 = \frac{J_0}{\Delta C} = \frac{\left(\frac{V}{A} \right) \cdot \text{slope}}{\Delta C} \quad \text{Eq. 5}$$

where V is the volume of the receiver solution and A is the diffusional area of the membrane.

Experimental Section

Materials - Tetraethylammonium bromide (TEAB) and citric acid were selected as model ionic drugs for this study. $[1-^{14}\text{C}]$ TEAB (4.7 mCi/mmol) and $[1,5-^{14}\text{C}]$ citric acid (54.5 mCi/mmol) were obtained from New England Nuclear Co., Boston, MA, with stated radiochemical purity of greater than 98%. Ethylalcohol (200 proof dehydrated alcohol U.S.P.) was obtained from U.S. Industrial Chemicals Co., Tuscola, IL. All other reagent-grade chemicals were obtained from American Scientific Products, McGaw Park, IL and were used as received.

Membrane - Full-thickness skin was freshly obtained (used within 30 hours of isolation) from the abdomen of 2-4 month old male hairless mouse (SKH/Hr1) as reported elsewhere⁴). Cellulose acetate membrane (25 μm thickness) was obtained from Sargent-Welch Co., Denver, CO.

Apparatus - The four-electrode system for these studies was a modified version of the system developed by Z. Samec and co-workers⁵). The system essentially consists of three main components, a diffusion cell with four electrodes, a potentiostat (Type DT2101, Hi-Tek Instruments, England) and a recorder (Omni Scribe®, Houston Instrument). Figure 1 shows one-half of the two chamber diffusion cell assembly consisting of the two sections, the Luggin capillary and a stopcock. The temperature of the jacketed half cells was controlled by circulating constant temperature water. A ring shaped platinum wire, 0.6 mm diameter, served as the counter electrode in the diffusion cell. Each half cell was fitted with a flange, inside diameter 10 mm, and a syringe (2 cc Interchangeable Syringe, Becton-Dickinson, NJ) tube at both open ends. The flange allowed tight sealing of the membrane, and the syringe tube allowed tight sealing of the Luggin capillary with stopcock. Luggin capillary, a long thin capillary with an open tip, and stopcock were connected with same size

syringe piston tube orthogonally. The long thin capillary was bent horizontally to keep it from touching the stirrer. The Luggin capillary and the reservoir above the stopcock was filled with the medium and a reference electrode (calomel) was then immersed in the reservoir allowing the potential across the membrane to be conducted from the tip of the capillary into the reservoir. The current flowing throughout the system was then monitored using a recorder.

Experimental Procedure - The membrane was assembled between the cell halves using a No. 18 ball-joint clamp. Luggin capillaries with stopcock, filled with appropriate medium, were inserted into the assembled half cells such that the tips of Luggin capillaries were positioned very close to both sides of membrane. The donor and receiver compartments were then filled with 6 ml of the medium through their respective sampling ports. Sodium chloride solutions and ethanol (for TEAB) and pH 8.0 isotonic buffer solution (for citric acid) were used as the transport medium. The cell contents were constantly stirred (150 rpm) by motors mounted above the cell system, care being taken to center the stirrer propellers from contact with the Luggin capillaries and cell walls. Reference and counter electrodes were connected to the potentiostat such that, in cationic drug transport studies the donor electrode would be the anode and the receiver electrode would be the cathode and vice versa for anionic drug transport. After the contents were mixed for 30 min, the donor side was charged with radiolabeled drug and the system was allowed to achieve steady-state without applied voltage. The time needed to achieve steady-state transport conditions was predetermined for each drug and the membrane. After the end of this period a constant voltage was applied and changed stepwise at predetermined time intervals. The electric current was recorded during the iontophoretic transport studies. At various times, aliquots from both

receiver and donor compartments were sampled (and replaced with same medium) and transferred to vials containing 10 ml of Beckman Ready-Solv HP scintillation cocktail and were counted in a Beckman LS 1801 scintillation counter. Radioactive counts were automatically corrected for quenching by the instrument.

Results and Discussion

Iontophoretic Transport of Monovalent Positively Charged Ion - Raw

Iontophoretic permeation profiles of the tetraethylammonium (TEA) ion in saline at 37°C are shown in Fig. 2, for hairless mouse skin and the cellulose acetate membrane as a function of time and the applied voltage. In both cases enhanced permeation of the TEA ion was observed with increased applied voltage. Permeability coefficients (P) calculated from the slopes of these plots along with the flux enhancement ratio (E) at each applied voltages are presented in Table 1 and Figure 3. The theoretical relationship as predicted from Eq. 2 is also shown in Fig. 3.

The cellulose acetate membrane showed quite good agreement with Eq. 2. The hairless mouse skin, however, though slightly higher, showed reasonably good agreement with theoretical predicted values up to 1.0 volt, and then deviated significantly at higher voltages.

Ionic Strength of Medium - The effects of ionic strength on iontophoretic transport was studied by changing sodium chloride concentration in the medium. Figure 4 shows the effect of ionic strength ($\mu = 0.075, 0.15, 0.30$) on TEAB flux enhancement ratio for hairless mouse skin. It is clear from the plots that there appear to be no significant effect of ionic strength on the iontophoretic transport at least up to 0.5 volts. However, at higher voltage,

increasing the ionic strength seems to cause a greater positive deviation from the predicted flux enhancement ratio values.

Skin Damage - In order to test if the hairless mouse skin is damaged at higher voltages and hence observed large positive deviations, voltage were applied in cycles (0-0.25 volt and 0-1.5 volt). A cycle consisted of 4.0 hours of total duration with 3.0 hours without voltage and 1.0 hour with voltage. Results as shown in Fig. 5 indicate that almost same flux pattern were observed in each of 0-0.25 volt cycle. In case of 0-1.5 volt cycle, however, the flux at 1.5 volt in the second cycle increased remarkably and P_o in the third cycle was about 185 times larger than P_o in the first cycle indicating an irreversible skin damage to the hairless mouse skin at higher voltages. These data also indicated that the observed skin damage is not only the result of the applied higher voltage but also appear to depend on the duration of the applied voltage. The physical integrity of the cellulose acetate membrane showed good correlation with Eq. 2 (Fig. 3) to be preserved at all voltages up to the maximum of 1.5 volt. The ionic strength of medium seems to have an enhanced skin damaging effect with increasing ionic strength, as seen from larger than expected deviations (due to higher voltage alone) then theoretically predicted (Fig. 4).

Electric Current - Fig. 6 shows the relationship between applied voltage and the electric current during the iontophoretic transport studies of TEAB in saline at 37°C for hairless mouse skin and cellulose acetate membrane. A steady increase in the current was observed during constant applied voltage periods and hence the current value plotted in Fig. 6 are those recorded at mid time points of the duration. The current, which increased rapidly up to 0.125 volt and gradually above 0.125 volt, did not follow Ohm's law ($I = V/R$). In case of hairless mouse skin above 1.0 volt, the electric current

increased rapidly again indicating skin damage. It is interesting to note from the plot of permeability versus current in Fig. 7 that the flux of TEAB is not proportional to the current.

Non Aqueous Medium - Results of iontophoretic transport through hairless mouse skin in a non aqueous medium (ethanol) are shown in Fig. 8. P_o of TEAB at 37°C in ethanol is 5.12×10^{-6} (cm/sec) (177 times larger than that observed in saline). The flux enhancement followed the predicted values reasonably well up to 1.5 volt with no apparent damage to the skin as seen with aqueous medium.

Iontophoretic Transport of a Trivalent Negative Ion - As a model of trivalent ion, citric acid ionizes in pH 8.0 isotonic buffer ($\mu = 0.30$) used as medium. The citric acid flux enhancement results with hairless mouse skin and the cellulose acetate membrane as a function of applied voltages are plotted in Fig. 9. The cellulose acetate membrane results showed good agreement with Eq. 2 over the entire range of applied voltage, whereas in case of hairless mouse skin an abrupt departure from the theoretical predictions began at low applied voltages (~ 0.125 volt) showing significant positive deviations apparently due to skin damage.

Conclusion

These studies have demonstrated the validity of a recently derived equation for predicting the transport enhancement of ions across membranes. The present work has also shown the usefulness of a newly developed four-electrode system for carrying out iontophoretic studies under conditions where the voltage drop across the membrane is well-defined.

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Table 1 - Iontophoretic permeability coefficient (P) and the flux enhancement ratio (E) through the hairless mouse skin and cellulose acetate membrane in saline at 37°C.

Figure 1 - Shema of diffusion half cell with four-electrode system for iontophoretic transport studies.

Figure 2 - Raw data for iontophoretic permeation of TEA in saline at 37°C through (●) hairless mouse skin and (■) cellulose acetate membrane.

Figure 3 - The relationship between the applied voltage and the flux enhancement ratio (E) of iontophoretic transport of TEA in saline at 37°C. The dotted line is the theoretical line from Eq. 1. Key: (●) hairless mouse skin; (■) cellulose acetate membrane. The error bars represent the SD with n=3.

Figure 4 - The effect of ionic strength (μ) of medium on the flux enhancement ratio (E) of iontophoretic transport TEA through hairless mouse skin at 37°C. The dotted line is the theoretical line from Eq. 1. Key: (▲) $\mu = 0.15$, NaCl 0.9%; (■) $\mu = 0.30$, NaCl 1.8%; (●) μ is 0.075, NaCl 0.45%.

Figure 5 - Cyclic iontophoretic permeation of TEA in saline through the hairless mouse skin at 37°C. Key: (A) 0-0.25 volt cycle test; (B) 0-1.5 volt cycle test.

Figure 6 - The relationship between the applied voltage and the electric current during the iontophoretic transport of TEA in saline at 37°C. Key: (●) hairless mouse skin; (▲) cellulose acetate membrane.

Figure 7 - The relationship between the electric current and permeability coefficient (P) of the iontophoretic transport of TEA in saline at 37°C. Key: (●) hairless mouse skin; (■) cellulose acetate membrane.

Figure 8 - The relationship between the applied voltage and the flux enhancement ratio (E) of the iontophoretic transport of TEA through the hairless mouse skin at 37°C in ethylalcohol. The dotted line is the theoretical line from Eq. 1.

Figure 9 - The relationship between the applied voltage and the flux enhancement ratio (E) of the iontophoretic transport of citric acid in pH 8.0 isotonic buffer solution ($\mu = 0.30$) at 37°C. The dotted line is the theoretical line from Eq. 1. Key: (●) hairless mouse skin; (■) cellulose acetate membrane.

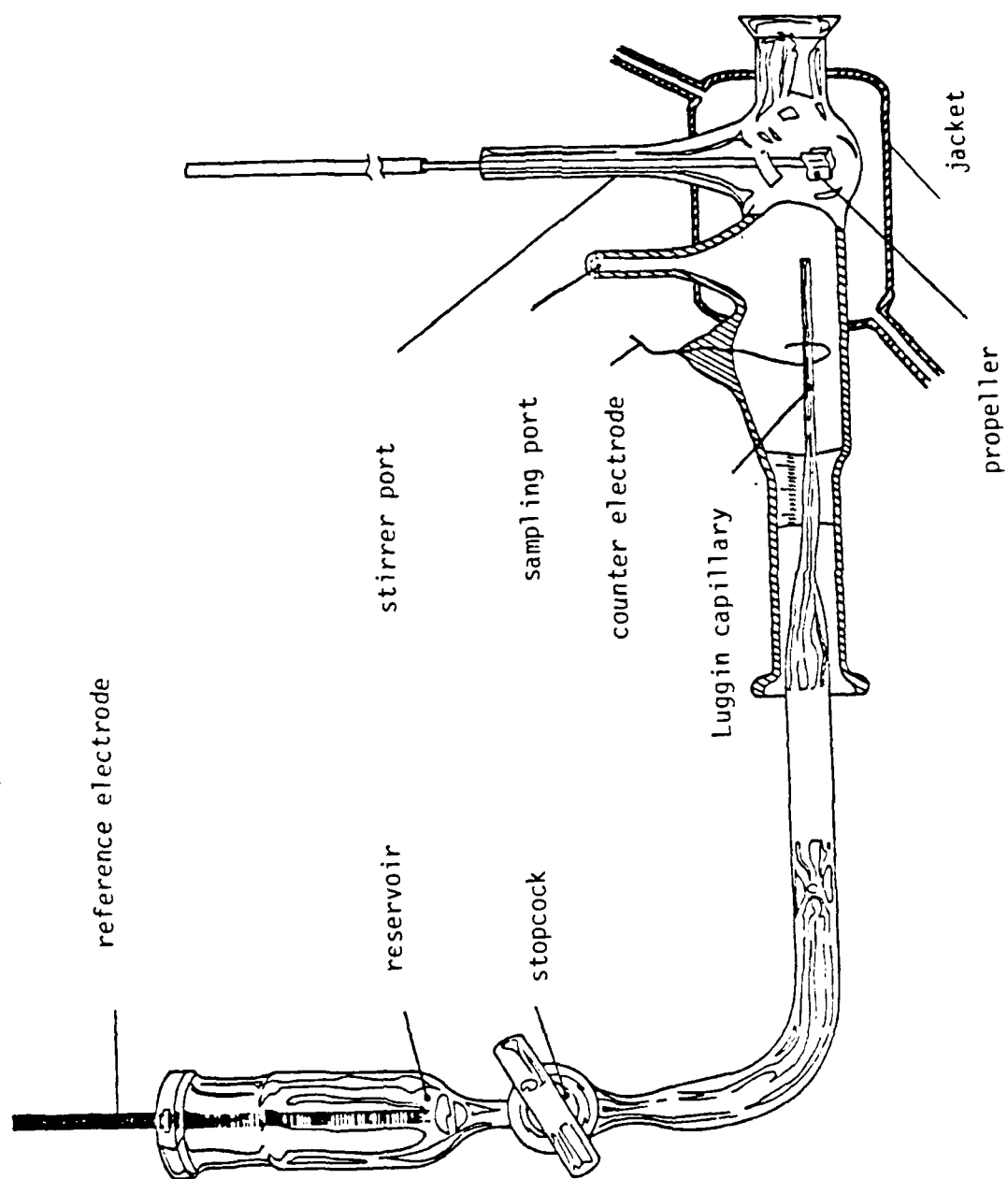


Fig. 1

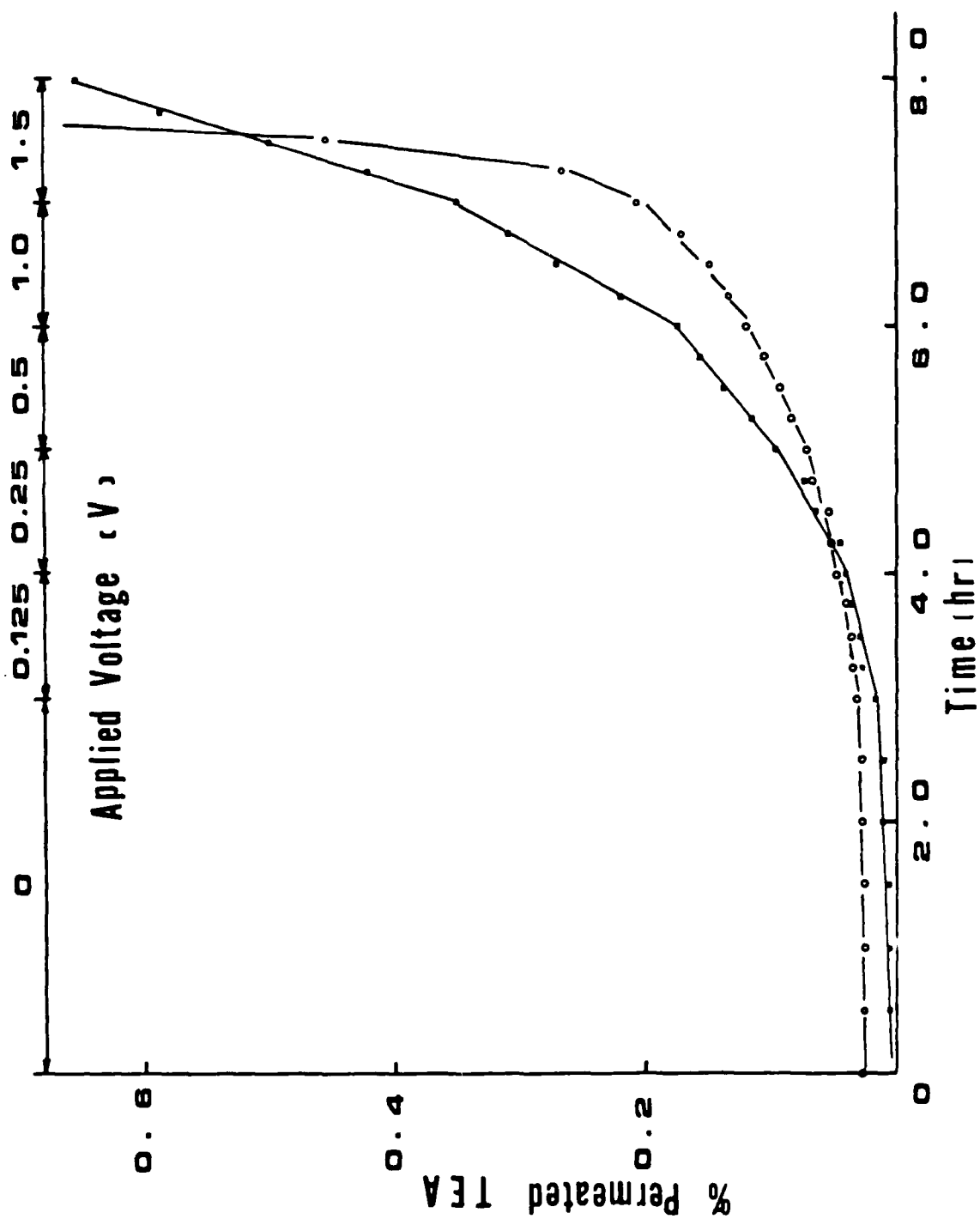


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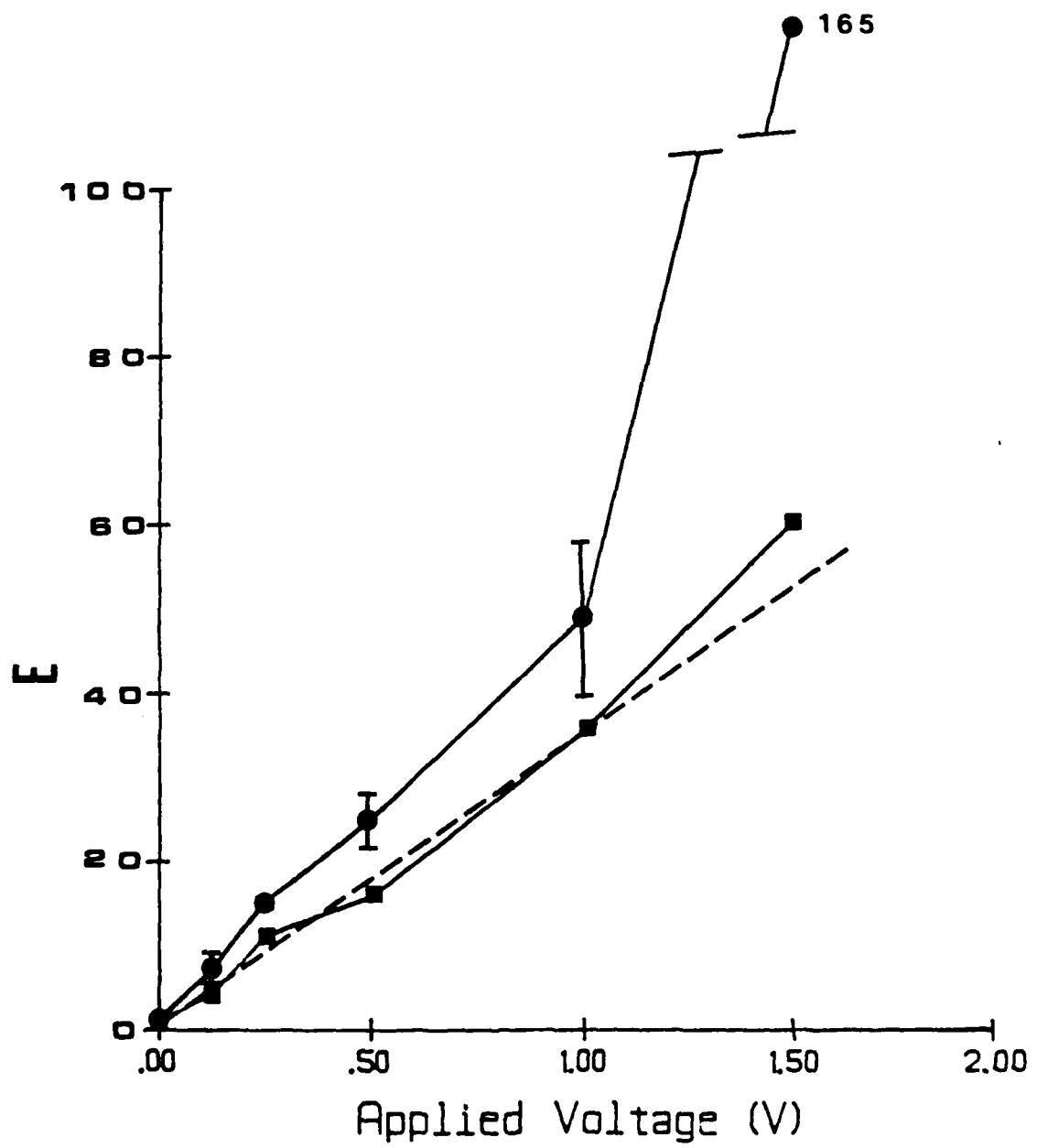


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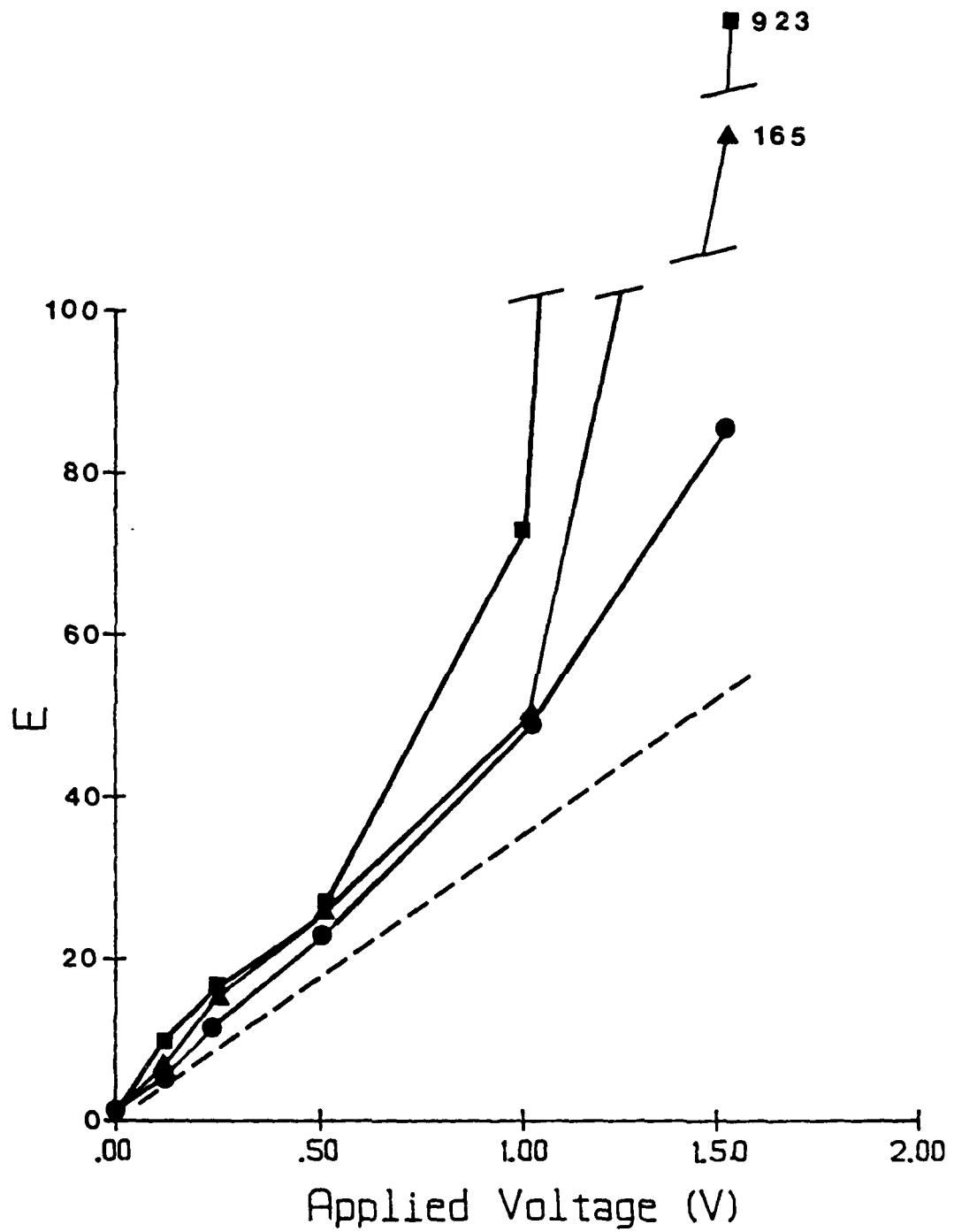


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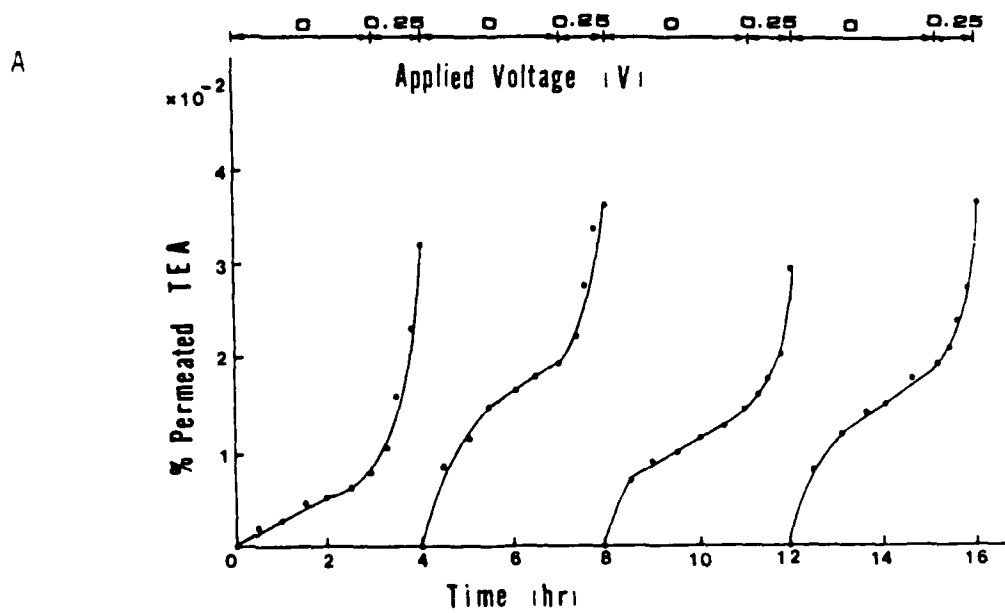
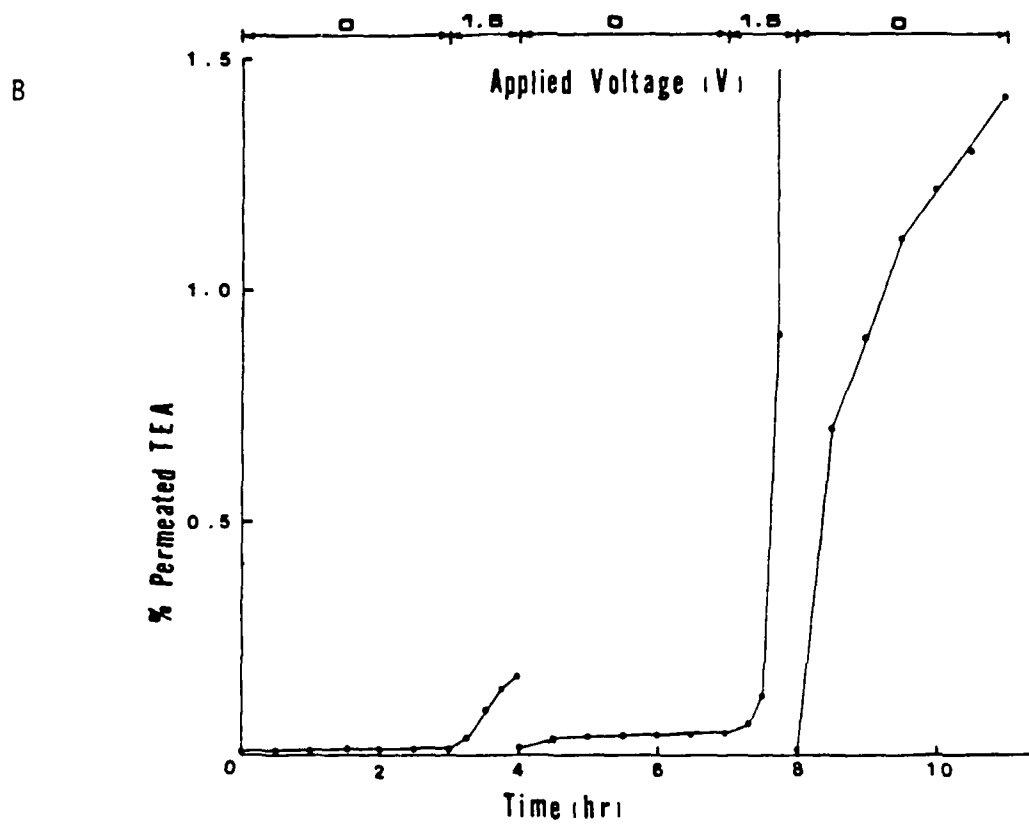


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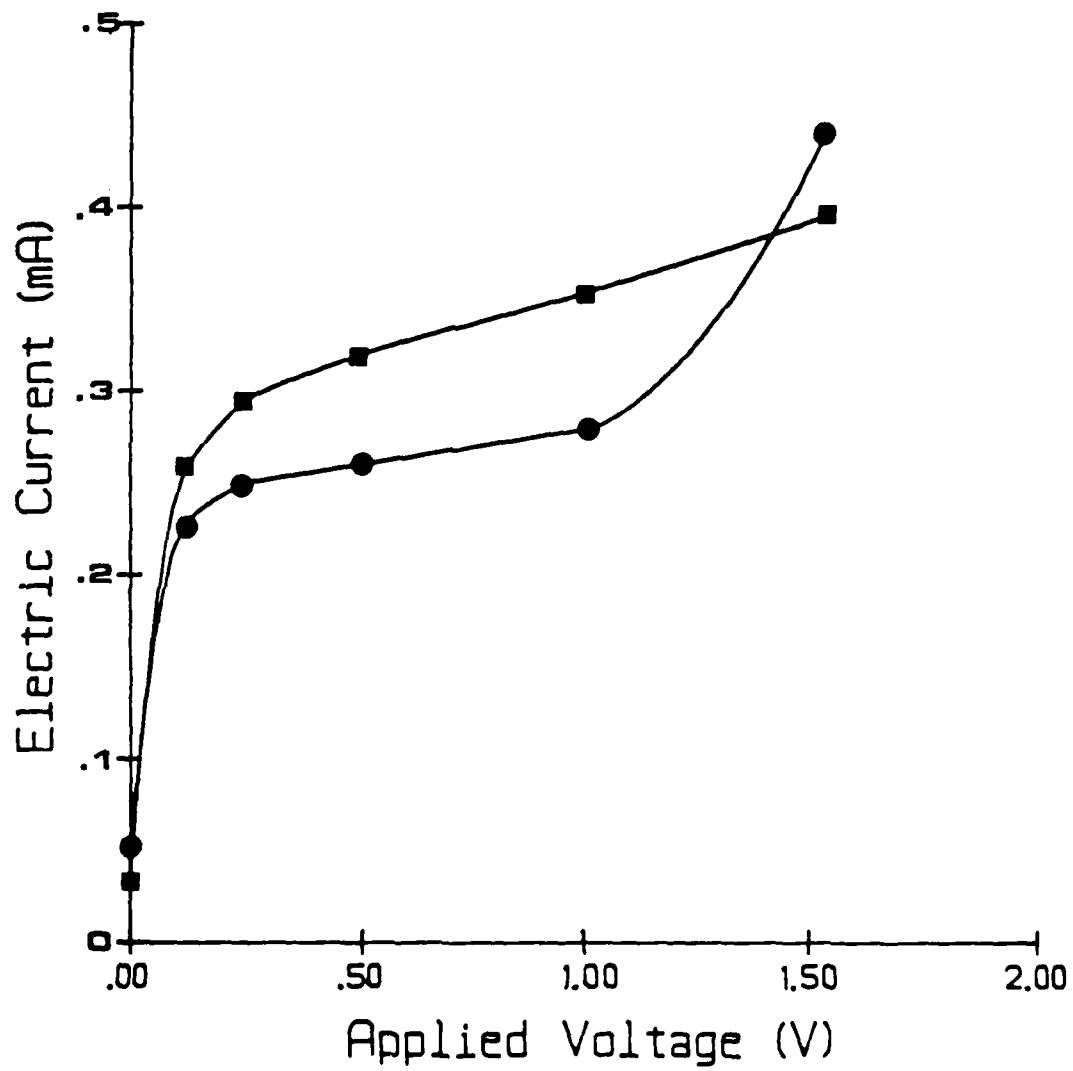


Fig.: 6

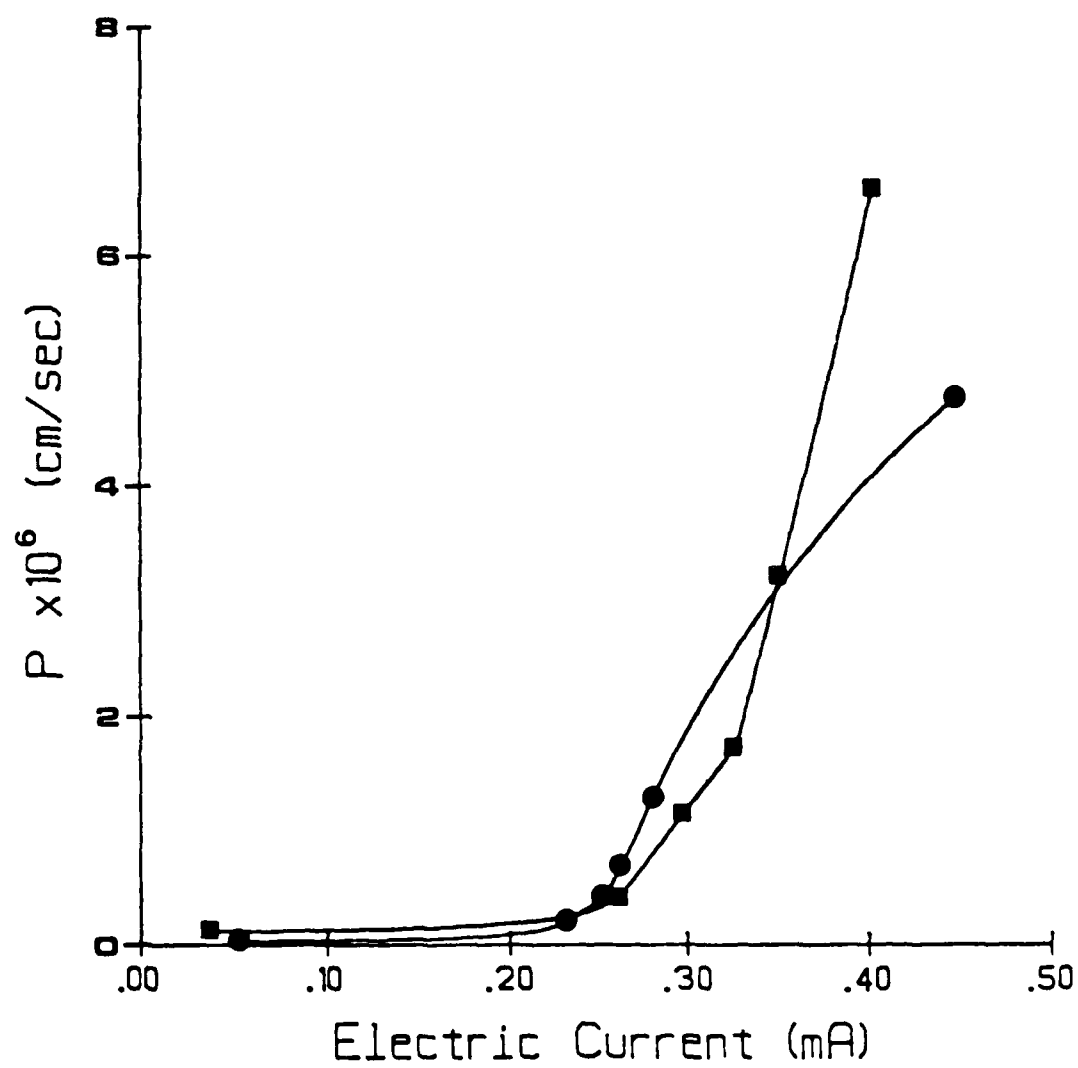


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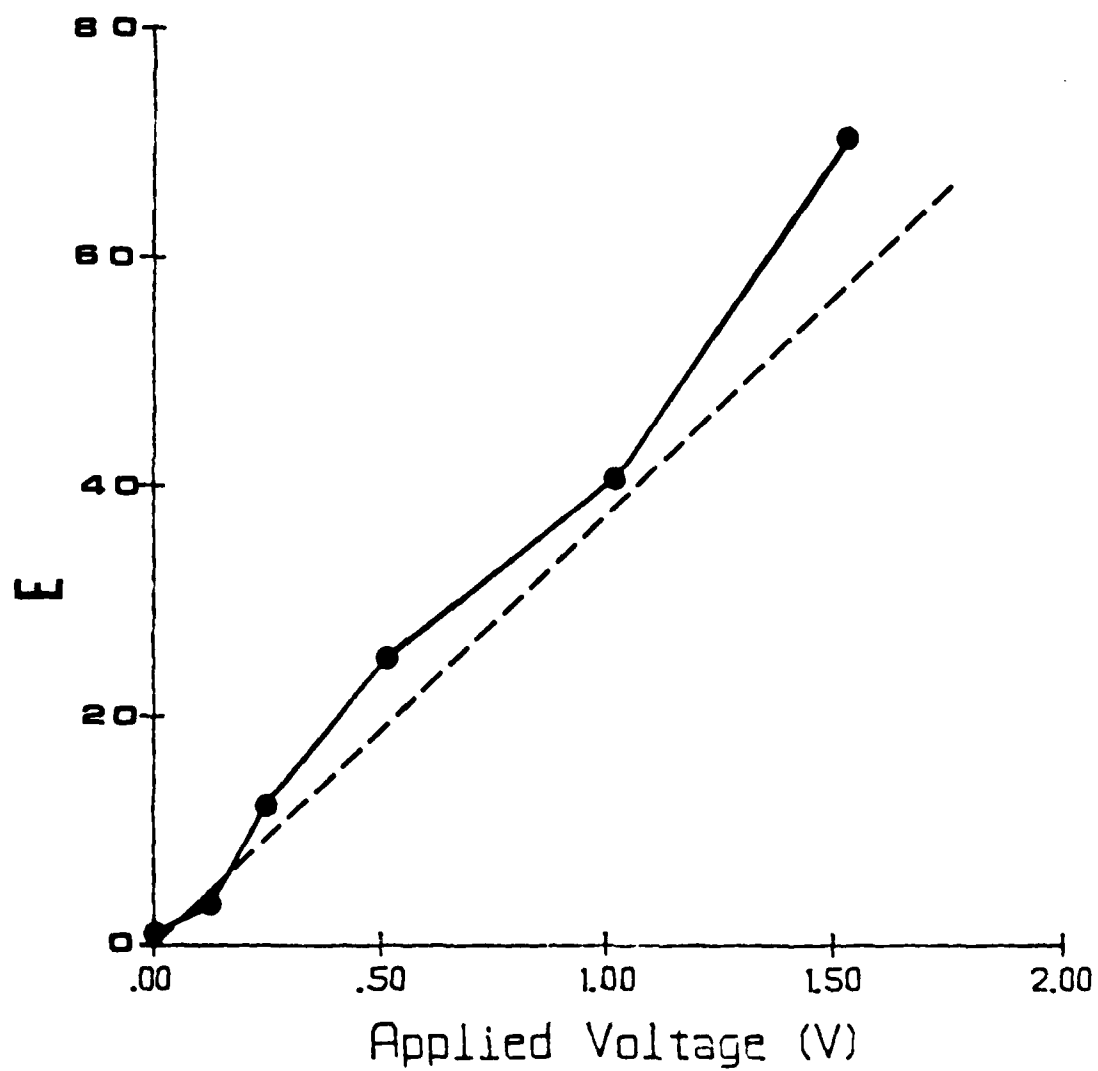


Fig.: 8

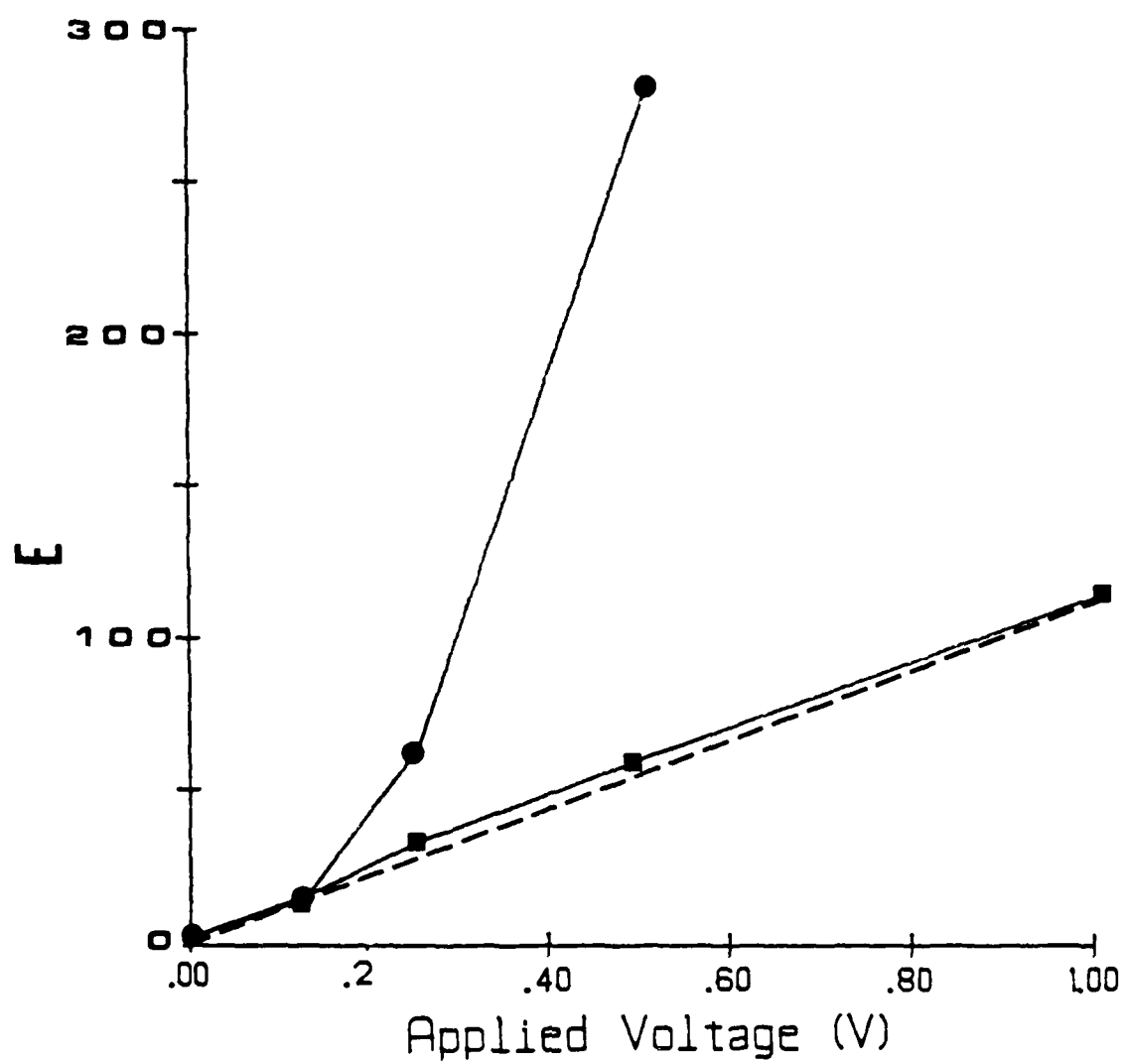


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