Appropriate collaboration of the Weather Bureau, the Atomic Energy Commission, the Public Health Service, and the Department of Agriculture in the development and use of such preventive procedures could thus provide real safeguards for our populace.

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13 January 1965

Microelectrophoresis with Alternating Electric Fields

Abstract. *Electrophoretic mobility of microscopically visible particles is measured from photographs of their oscillatory migration in a low frequency electric field. The measurements are inherently free of gravitational or thermal-convective drifting. Many measurements may be made from one photograph. An automatic timing circuit insures reproducibility and determines the sign of the particles' charge.*

A method, which is free of drift, for measuring the electrophoretic mobility of microscopically visible particles has been developed. In a cell of simple design, the suspension of particles is exposed to an alternating, electric field (1 to 10 cy/sec). Particles having a net charge experience a force varying with time. The resulting movement of the particles is photographed in such a way that the mobility can be calculated from measurements on the photographic record.

In the d-c method of microelectrophoresis, particles, suspended in a liquid and subjected to a d-c electric field, are observed microscopically as they move a measurable distance in a fixed length of time, say 5 seconds. However, the same displacement may be due partially to gravity or to thermal convection in the liquid. The thermal convection is caused by drafts in the room, insufficient thermal equilibration of the apparatus, or uneven heating of the sample by the microscope lamp. At best, the d-c method requires many serial measurements, each on one particle, to obtain a consistent average.

In the a-c method of microelectrophoresis, problems of drifting are obviated by measuring the amplitude of the particle's oscillatory migration in an alternating electric field. Gravitational and thermal drifts, being nonoscillatory, do not contribute to the quantity being measured. Observation is made photographically, so that measurements for many particles may be obtained from one picture.

At the low frequencies used, 1 to 10 cy/sec, forces of electric origin are very large compared to inertial forces on micron-sized particles (*1*). Therefore, one may consider that the particles instantaneously assume a velocity proportional to the time-varying, applied electric field, $\mathcal{E}(t)$. Thus, if $v(t)$ is the particle's velocity and $x(t)$ is its displacement,

$$v(t) = \mu\mathcal{E}(t)$$

and

$$x(t) = \mu\int\mathcal{E}(t)\,dt$$

where μ is the mobility. If $\mathcal{E}(t) = E \sin \omega t$, where E is the amplitude and ω is the angular frequency of the applied, sinusoidal electric field, then

$$x(t) = -\frac{\mu E}{\omega}\cos \omega t,$$

where $A = \mu E/\omega$ is the amplitude of the oscillatory migration. Thus

$$\mu = \omega A/E.$$

The value of A is measured from a photograph of the motion. However, the entire cell must be moved perpendicularly both to the applied field and to the line of view in order to display the oscillatory migration on a time base (Fig. 1). This artifice resolves the motion into an oscillatory migration about a slowly drifting origin, thus separating electric from nonelectric migrations.

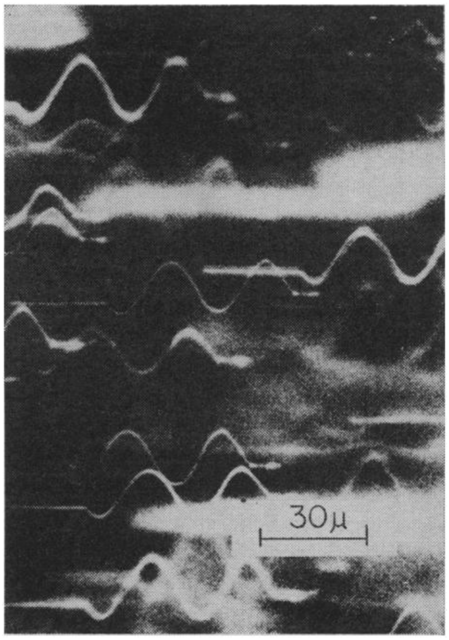

Fig. 1. Electrophoresis patterns of polystyrene spheres (1 micron in diameter) subjected to 340 volt/cm, 10 cy/sec. The "artificial" horizontal component of the motion is the result of moving the stage of the microscope during the exposure. Each pattern has two "tails," since the field is applied for less time than the illumination.

The frequency of the applied voltage should be high enough to cause the particle to oscillate rapidly about an origin which may be slowly drifting. It should also be low enough to give a large, easily measured amplitude, A. When these requirements are balanced and E is kept small to minimize heating, frequencies between 1 and 10 cy/sec are usually the most useful.

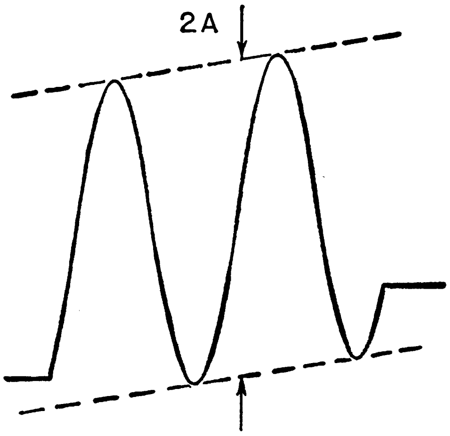

Fig. 2. Method for measuring amplitude, *A*, of a particle's alternating migration from the photographic record.

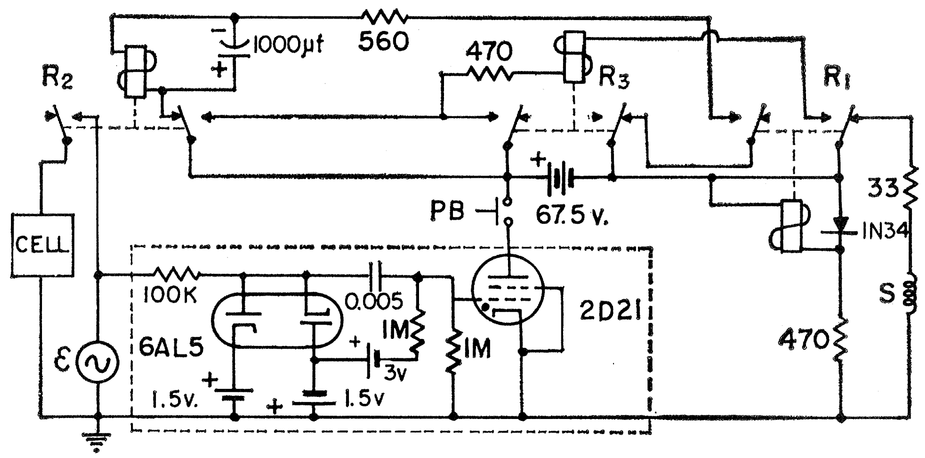

Fig. 3. Sequencing and timing circuit. Operation starts with the push button (center). At the first transition from a negative to a positive voltage, $\mathcal{E}$, the phase selector (dotted box) initiates the sequential operation of the illumination-shutter solenoid, S, and the relays R_1, R_2, and R_3. About 90 msec after S and R_1 are energized, R_2 applies $\mathcal{E}$ to the cell for 220 msec. The illumination shutter is set to stay open for a total of about 350 msec. The 300 ohm, 24 volt, d-c relays are shown in their unenergized state.

Waveforms other than sinusoids may also be used. Triangular waveforms, in fact, give the same amplitude of particle motion with 17 percent less heating (*2*).

The field strength is calculated from the electrode spacing and the applied voltage. But if the conductivity of the sample is high, the applied voltage may be partially lost at the electrode surfaces (electrode polarization). In that case $V_{\mathrm{eff}} = IZ$, where V_{eff} is the effective value of the applied voltage, I is current through the cell, and Z is sample impedance. The sample impedance may be obtained as the ratio of applied voltage to resulting current at frequencies sufficiently high to minimize electrode polarization and yet not high enough to affect the cell impedance by uncontrolled capacitive currents. The appropriate frequency range is usually between 1 and 100 kcy and is recognized from the frequency independence of the cell's impedance within this range.

For most measurements so far, a very simple cell has been used. Two parallel Pt wires (60 microns in diameter), serving as electrodes, pass between two parallel cover glasses. The glasses are held together by Parafilm (*3*), which also immobilizes the wires, and are held about 150 microns apart by the thickness of the Parafilm sheet. The center of the Parafilm layer is removed before assembly, thereby forming a sample chamber (about 3 mm^3) through which the wires pass. The entire layered assembly is made in a jig which holds the wires taut and accurately parallel (± 1 percent) while the Parafilm selectively is heated (60°C) and cooled. This heating cycle seals in the still-cool sample (less than 45°C) and the wires. The cell, which must be refabricated for each new sample, comes to thermal equilibrium rapidly and is easily photographed. The small space between electrodes (about 1 mm) facilitates the production of high field strengths. For highly conductive liquids, a more intricate cell can eliminate the electrolytic effects of an excessive current density at the wire electrodes.

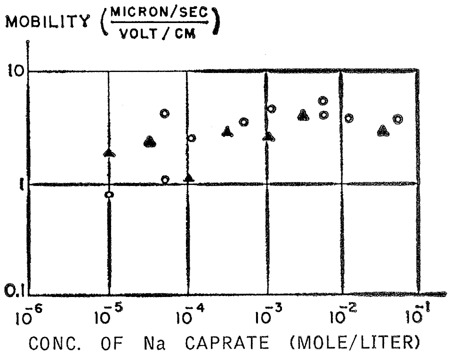

Fig. 4. Electrophoretic mobility of polystyrene spheres in an aqueous solution of sodium caprate. Open circles represent data obtained by a d-c technique (*5*); solid triangles, our new data.

Dark-field illumination is obtained by converging a strong light beam onto the sample from beneath the horizontal stage, at an angle of about 30° from the vertical. An electrically actuated, photographic-type shutter, interposed in the light beam, serves to determine the length of the photographic exposure (on Polaroid film type 47). This oblique (asymmetrical) dark-field illumination facilitates the observation of small movements of large particles, since each large particle appears as two small points of light which leave well-defined photographic tracks.

The potential is applied after the illumination comes on and is removed before it goes off. Between two and three cycles of the potential waveform are applied, since two are required for a measurement (Fig. 2) and three are unnecessary (unnecessary temperature rise in the sample). The moment of application of the applied potential is selected by a phase selection circuit so that the particles are subjected to a known initial polarity. This refinement establishes the sign of the particles' net charge. Phase selection, sequencing, and timing are accomplished automatically (Fig. 3). Manual movement of the stage provides a sufficiently uniform velocity.

Measurements from a photomicrograph may very accurately be converted to real distances with a similar calibrated photomicrograph. Correction for the electro-osmotic effect may be made in the same way as in the usual d-c technique (*4*).

An example of the resolution of this technique is the mobility measurement of polystyrene spheres, 1.17 microns in diameter, as a function of the concentration of the suspending detergent, sodium caprate (Fig. 4). The data compare favorably with measurements on polystyrene spheres 1.7 microns in diameter done by the d-c method (*5*). There is virtually no size dependence of mobility in this range of sizes (*6*). The comparison substantiates the feasibility and potential utility of the a-c technique. It may also provoke some thought on the possible extension of these ideas to the microelectrophoretic measurement of macromolecular suspensions.

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8 February 1965

Structure of 9,9,10,10-Tetrachloroanthracene

Abstract. *The crystal structure of 9,9,10,10-tetrachloroanthracene has been determined by x-ray analysis. The structure is characterized by dovetailed packing of crystallographically independent molecules. The anthracene skeletons are planar, and the molecules have approximately* D_{2h} *symmetry. Bond distances and angles are summarized and the stereochemical implications of the results are discussed.*

We have completed the x-ray structure analysis of a tetrasubstituted derivative of anthracene, 9,9,10,10-tetrachloroanthracene. This study is part of a program devoted to analysis of the role played by molecular packing in the electronic properties of solids. Furthermore, this analysis, as well as that of the structure of 10,10-dibromoanthrone *(1)* illustrates the extent to which dihalo-substitution at the carbon No. 9 or No. 10 position of anthracenes may affect the expected conformation.

This compound was prepared by the method of Meyer and Zahn *(2)*. Cream-colored crystals (mp 153° to 157°C) were obtained from the reaction mixture. The specimen selected for x-ray study was rod-shaped, 1.27 mm in length, and 0.1 mm in diameter (average).

The crystallographic data are: $a = 10.93$ Å, $b = 13.90$ Å, $c = 9.89$ Å, $\beta = 116.25°$; monoclinic; space group $C2/m$; $Z = 4$; molecular weight = 317.80; density (obs.), 1.55 g/cm^3; density (calc.), 1.56 g/cm^3; linear absorption coefficient, μ (CuKα) = 76.53 cm^{-1}, μ (MoKα) = 8.67 cm^{-1}.

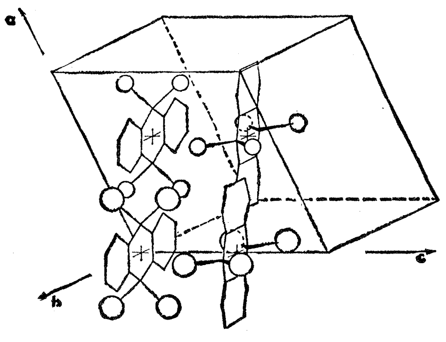

Fig. 1. Packing arrangement of the molecules.

The structure was solved by inspection of a sharpened three-dimensional Patterson function. Despite the presence of well-resolved heavy-atom interactions in this function, the solution was somewhat complicated by the usual space-group ambiguity among $C2/m$, $C2$, and Cm, and by unusual occupancy of special positions. The centers of the four molecules in the unit cell occupy two sets of twofold special positions of point-group symmetry $2/m$; namely positions $2a$ and $2c$ of space group $C2/m$ (No. 12).

A refinement of the structure based on 791 reflections has led to an R factor of 13.6 percent. The parameters involved in the refinement were position and anisotropic thermal parameters for all atoms and one scale factor. Figure 1 illustrates the arrangement of the four molecules whose centers lie at 0,0,0; ½,½,0; 0,0,½; and ½,½,½. These molecules are positioned so that the carbon skeletons are all nearly perpendicular to the c-axis direction. Neighboring molecules are turned, with respect to each other, by 90 deg about the c-axis direction. As a result, chlorine atoms from each molecule "nest" in the terminal rings of adjacent molecules and form infinite rows along the c-axis.

Figure 2 shows the bond distances and bond angles of the two crystallographically independent molecules. Representative standard deviations are 0.03 Å for the distances and 1.5 deg for the angles. The dimensions of the outer rings of both molecules approximate closely those found for benzene.

Within the experimental error the dimensions of both molecules are identical, the carbon skeletons are planar, and the carbon-atom skeleton is perpendicular to the plane formed by the four chlorine atoms in each molecule. Thus the molecule approximates very closely the symmetry of three mirror planes intersecting at right angles (D_{2h}), even though this condition is not demanded by the symmetry constraints of the space group.

The planarity of the carbon framework represents a strained form of the cyclohexa-1,4-diene ring in the center of the molecule. This conformation tends to relieve tight intramolecular contacts between the chlorine atoms and the hydrogen atoms at the 1 : 8 and 4 : 5 anthracene positions *(3)*. For the present compound, these contacts are about 2.9 Å, only slightly shorter than the sum of the van der Waals radii, 3.0 Å. On the other hand, tentative results on 10,10-dibromoanthrone *(1)* indicate a slight buckling of the carbon framework, presumably a result of the greater halogen-halogen repulsions in this molecule as compared to tetrachloroanthracene.

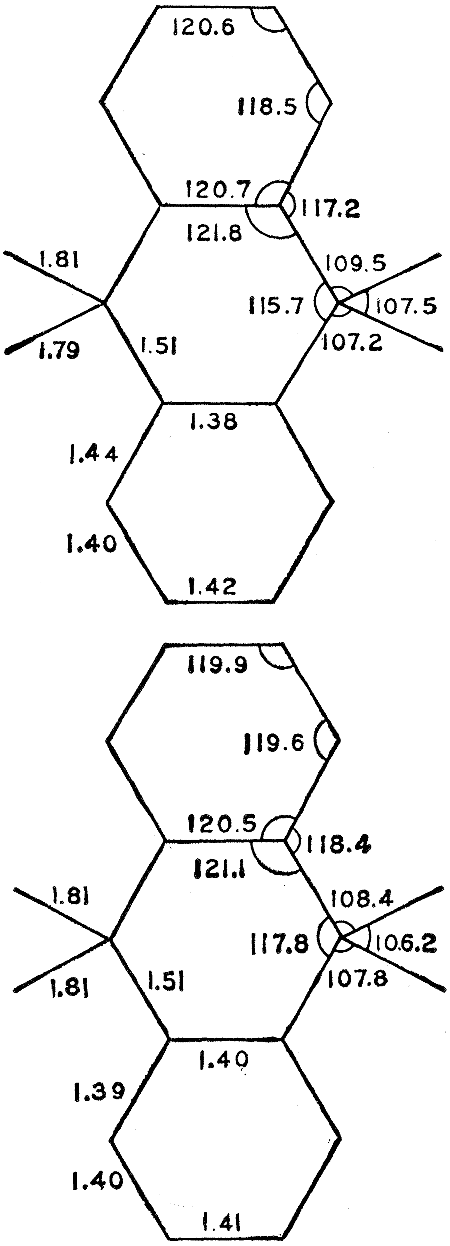

Fig. 2. Bond distances (Å) and bond angles (deg) for 9,9,10,10-tetrachloroanthracene.

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