to unsaturated hydrocarbon residues are higher than those of the alkyl acid chlorides. Resonance of the acid chloride group with the unsaturated hydrocarbon residue readily accounts for this increase in moment.

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[CONTRIBUTION FROM THE PHYSIOLOGICAL LABORATORY, PRINCETON UNIVERSITY]

The Attempted Use of Crystals as Calcium Electrodes. II

BY RUBERT S. ANDERSON

The concentration cell measurements previously attempted with calcite and fluorite crystals[1] have been repeated after increasing the conductivity of the crystals by the methods of Joffe, Gudden, Pohl and others.[2] Although after such treatments crystals frequently showed detectable photo-currents, only one method gave crystals with a conductivity great enough for easy measurements of e. m. f. with a Lindemann electrometer. So far, however, these e. m. f.'s have fluctuated too rapidly to be usable. This work is being continued and will be reported later.

During these experiments a new source of possible confusion has appeared. Data have been obtained which, although not due to the crystals, may erroneously be ascribed to them. Open Pyrex glass tubes of about 1-cm. diameter which had a crystal sealed over one end with paraffin (m. p. 48–50°), were used for the experiments. The solution inside of the tube, 0.01 M calcium nitrate plus 0.001 M sodium chloride, was connected directly to the electrometer through a silver–silver chloride electrode. The crystal end of the tube dipped into an outer solution from which contact was made either through a saturated potassium chloride bridge to a reference electrode or directly through a silver–silver chloride electrode. Because of the high resistance of the system many hours frequently were required before the electrical effects due to the preparation and handling of the tube had even approximately disappeared. Therefore only the outer solution was varied during a series of measurements.

Since the absolute readings were frequently not reproducible, owing to changing asymmetry potentials, only the changes in e. m. f. produced by changing the concentrations of the outer solutions are given. That is, the data are given either for cell 1

$$\text{Ag} \mid \text{AgCl} \mid \text{KCl (satd.)} \mid \begin{matrix}\text{Ca(NO}_3\text{)}_2\ (C_1)\\ \text{NaCl}\ (C)\end{matrix} \mid \begin{matrix}\text{glass tube}\\ \text{with}\\ \text{crystal}\end{matrix} \mid \begin{matrix}\text{Ca(NO}_3\text{)}_2\ (C_2)\\ \text{NaCl}\ (C)\end{matrix} \mid \text{KCl (satd.)} \mid \text{AgCl} \mid \text{Ag}$$

or for cell 2

$$\text{Ag} \mid \text{AgCl} \mid \text{CaCl}_2\ (C_1) \mid \begin{matrix}\text{glass tube}\\ \text{with}\\ \text{crystal}\end{matrix} \mid \text{CaCl}_2(C_2) \mid \text{AgCl} \mid \text{Ag}$$

The more concentrated solution was negative in the outer circuit.

Tubes 1, 2 and 3 in Table I carried crystalline fluorite plates, 0.1 to 0.2 mm. thick, which had been X-rayed for one-half hour. Each figure of voltage is the average of a number of readings. For purposes of comparison, figures calculated from data in the literature for concentration cells analogous to cells 1 and 2, but with calcium amalgam in the place of the tube and crystal, are given in Table I also. The data from tubes 1, 2 and 3

TABLE I

The changes in e. m. f. in volts given by several tubes when the concentrations of calcium nitrate or of calcium chloride in the outer solutions were alternately C_1 or C_2.

Cell 1						
NaCl, M, C		0.001	0.001	0.01	0.01	0.1
$Ca(NO_3)_2$, M	C_1	.001	.01	.0003	.001	.001
	C_2	.01	.1	.003	.01	.01
Tubes, v.	1	.021	.024		.012	.001
	2	.021	.026		.016	.000
	3	.018	.029			.005
	P_1	.019			.023	.001
	P_2	.019	.023	.017[a]	.019[a]	.009[a]
	P_3	.019	.031		.024	.000
$CaCl_2$, M	C_1		.0012	.01	Contained	
	C_2		.012	.1	no sodium	
Ca amalgam, v.			.021[b]	.027[c]	chloride	
Cell 2						
$CaCl_2$, M	C_1		0.01	0.03	0.01	0.001
	C_2		.03	.1	.1	.01
Ca amalgam,[d] v.			.036	.040	.076	
Tubes, v.	1		.034	.035		
	2		.034	.035	.070	.081

[a] These solutions also contained 0.001 M 1:1 phosphate buffer. [b] This figure is surprisingly small. From Fosbinder, THIS JOURNAL, **51**, 1345 (1929). [c] From Drucker and Luft, *Z. physik. Chem.*, **121**, 307 (1926). [d] From Lucasse, THIS JOURNAL, **47**, 743 (1925).

(1) Anderson, *J. Biol. Chem.*, **115**, 323 (1936).

(2) See review by Hughes, *Rev. Modern Phys.*, **8**, 294 (1936).

in calcium nitrate solutions containing 0.001 *M* sodium chloride, although variable, are of the order of magnitude and of the same sign as the data given by calcium amalgam electrodes in pure calcium chloride solutions. The same approximate agreement is shown for cell 2. Since, except for scattered results, such data had not been observed in the earlier work,[1] they were at first believed to be due to the X-rayed crystal. However, it was soon found that no essential change occurred in the data obtained, when the crystal was completely covered with paraffin.

Tubes were then prepared on the assumption that the conductance and e. m. f. depended on the glass–paraffin interface. The end of the tube was sealed with a paraffin coated crystal or glass plate to make the resistance through it as high as possible. On the other hand, the length of the glass–paraffin interface along the tube between the inner and outer solutions was reduced to less than a centimeter.

The resistance of such a tube was usually very high immediately after its preparation and e. m. f. measurements were not possible. After the tube had stood in a dilute calcium nitrate solution for from a few hours to a few days, the apparent d. c. resistance decreased to between 10^{11} and 10^{12} ohms and the e. m. f. of the system could be measured. One important factor in the variable length of time necessary seemed to be the amount of drying that the glass tube had undergone before paraffining.

Selected data obtained with three such tubes, P_1, P_2 and P_3, are given in Table I. They show that results similar to those from tubes 1, 2 and 3 can be obtained with the crystal entirely covered with paraffin or in its complete absence. In fact, although 0.1 *M* sodium chloride still obliterated calcium concentration effects except with tube P_2, 0.01 *M* sodium chloride interfered less than with tubes 1, 2 and 3. Some tubes gave fairly regular data even at very low concentrations, as shown by the following readings with tube P_2 in pure calcium nitrate solutions referred to 0.001 *M* as zero.

0.0001 *M* $Ca(NO_3)_2$	−0.023
.0003 *M* $Ca(NO_3)_2$	− .012
.001 *M* $Ca(NO_3)_2$	.000
.003 *M* $Ca(NO_3)_2$	.012
.01 *M* $Ca(NO_3)_2$	.024

All of the data are given for purposes of illustration only. If the tubes were kept in solution long enough the resistance dropped to a different order and e. m. f.'s of the same size and sign as those of free diffusion eventually were obtained.

It was pointed out[1] that Tendeloo,[3] who claimed positive results with fluorite crystals, had presented no critical evidence to show that his data were not due to free diffusion potentials. The data presented above show that, with a glass cell of this type, even results quantitatively like those to be expected from a calcium electrode cannot be assumed to arise at the crystal without additional evidence. A glass apparatus was therefore constructed in which the crystal was sealed between the end of a glass tube and a ground off bulb which had been blown in the side of a piece of capillary tubing. Thus there was no continuous glass surface connecting the inner and outer solutions and yet the outer solution could be changed readily. With X-rayed fluorite crystals in it, the system showed a much higher resistance than those observed with the simple glass tubes. Measurements were usually not possible and no data comparable to those in Table I were obtained. Thus, whether or not the postulated path of conductance is correct, none of my e. m. f. data has been proven to be dependent on the calcium crystals and probably none has been obtained so far.

Additional evidence that the data in Table I were due primarily to the glass–paraffin interface was obtained by modifying the interface through the removal or addition of paraffin. In general the resistance changed as would be expected if the interface were the important conducting path, although there were exceptions. Experiments with an all glass tube produced no evidence to indicate that conductance through the glass[4] was a fundamental factor although it may determine the limiting resistance of the system.

The data of Table I, particularly those from tubes P_1, P_2 and P_3, show a marked sensitivity to changes of calcium concentration even in the presence of greater concentrations of sodium chloride. On the other hand, changes in the sodium chloride concentration below 0.01 *M* in the presence of only 0.001 *M* calcium nitrate changed the e. m. f. at most a few millivolts. If the data resulted from the glass–paraffin interface, this specificity for calcium makes them difficult to explain as due to

(3) Tendeloo, *J. Biol. Chem.*, **113**, 333 (1936).

(4) K. Lark-Horovitz [*Nature*, **127**, 440 (1931)] has reported concentration cell measurements at a paraffin surface. No comparison of my results with this earlier work is possible at present, on the basis of the published information. In my experiments the conductivity was too great to have been through paraffin.

a simple immobilization of the negative ions by the usual negative charge of both glass and paraffin. This is true even though this charge is subject to specific ion effects.

A single tube prepared with sealing wax instead of paraffin gave the same type of data. The only difference which was observed was a much slower increase of conductivity and generally more stable properties.

Because of its partial specificity and its response to low concentrations of calcium, even this type of system may have usefulness as an electrode. This and the nature of the effect are being studied.

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[CONTRIBUTION FROM THE DEPARTMENT OF PHYSICS AND THE AGRICULTURAL EXPERIMENT STATION, UNIVERSITY OF FLORIDA]

The Infrared Absorption Spectrum of Vitamin C[1]

BY DUDLEY WILLIAMS AND LEWIS H. ROGERS

The isolation, identification, and synthesis of Vitamin C and its ultraviolet absorption spectrum are well known.[2,3] The spectral region previously studied, however, lies between 200 and 400 mμ. In order to obtain additional information on the molecular structure of this compound, it was decided to make a study of its infrared absorption spectrum in the region from 2 to 8 μ. Although the infrared absorption spectrum of any polyatomic molecule is necessarily complex, the interpretation in terms of atomic groups is more direct than in the case of the visible and ultraviolet regions where electronic energy changes are involved. Complete theoretical analyses of vibrational–rotational spectra have been made only for diatomic and triatomic molecules and for benzene, but semi-empirical explanations of the spectra of organic molecules have proved valuable in many cases. From the study of large numbers of organic compounds it has been found that every organic radical gives rise to a characteristic series of bands, its characteristic frequencies being practically unaffected by other groups present in the same molecule or in neighboring molecules. Thus, for example, one finds a band arising from a C–H vibration which varies in frequency from about 3100 cm.$^{-1}$ in a simple molecule like methane to about 2800 cm.$^{-1}$ in a complex molecule like stearic acid.[4] Those frequencies of particular interest in the present discussion are

$$\nu(\text{C—H}) \leqq 3000 \text{ cm.}^{-1}$$
$$\nu(\text{O—H}) \leqq 3300$$
$$\nu(\text{C=C}) \leqq 1500 \text{ to } 1800$$
$$\nu(\text{C—O}) \leqq 1500 \text{ to } 1800$$
$$\nu(\text{C—C}) \leqq 900$$
$$\delta(\text{CH}_2) \leqq 1440$$

where ν symbolizes a linear vibration and δ a deformation vibration. With the aid of these experimentally obtained results it is possible to gain some general ideas concerning molecular structure from a study of infrared absorption spectra. Lists of other characteristic oscillation frequencies have been given by other workers.[5]

The spectrometer used in the present work was an instrument of the minimum deviation type equipped with a rock salt prism. The effective slit widths ranged from 0.04 μ in the 2.5 μ region to 0.09 μ in the 8 μ region. Fifteen to twenty readings were made per micron of spectral range. The absorption cells were made with fluorite plates separated by mica washers of approximately 0.02 mm. thickness. In obtaining the absorption data the transmission of a vitamin solution was compared with the radiation transmitted by a cell of equal thickness containing water. Other experimental details are given in another paper soon to appear. Baird and his co-workers[6] have pointed out the unstable nature of Vitamin C due to oxidation. Hence, in this work, the material was kept in sealed ampoules until immediately before preparing the solutions used in making the measurements. The samples used were obtained from Merck. Saturated

(1) Presented before the Organic Division, American Chemical Society, Chapel Hill, N. C., April 12, 1937.

(2) Szent-Gyorgi, *Biochem. J.*, **22**, 1387 (1928); Haworth, Hirst, *et al.*, *Chemistry and Industry*, **221**, 482, 645 (1933); *Nature*, **130**, 888 (1932); *J. Chem. Soc.*, 1270, 1419 (1933); *ibid.*, 62 (1934); Reichstein, Grussner, and Oppenauer, *Nature*, **132**, 280 (1933); *Helv. Chim. Acta*, **16**, 561 (1933).

(3) Hirst and Hubert, *Nature*, **129**, 205 (1932); *J. Chem. Soc.*, 1270, 1564 (1933).

(4) Coblentz, Carnegie Inst. of Wash. Pub. No. 35 (1905).

(5) Barnes, *Rev. Sci. Instruments*, **7**, 265 (1936).

(6) Baird, *et al.*, *J. Chem. Soc.*, 63 (1934).