ethanol and allowed to cool. Orange-yellow crystals were obtained which after recrystallization from ethanol did not melt under 340°.

Calcd. for $C_{20}H_{12}N_2O_4$: N, 8.14. Found: N, 8.16.

N-2-(9-Oxo)-fluorenylbenzamide.—A mixture of 0.5 g. of 2-aminofluorenone, 0.36 g. of benzoyl chloride and 10 ml. of pyridine was heated to boiling. While still hot, the solution was filtered, diluted with 50 ml. of ethanol and allowed to cool. Vermillion crystals were obtained which after recrystallization from ethanol melted at 242° uncor.

Calcd. for $C_{20}H_{13}NO_2$: N, 4.68. Found: N, 4.89.

2-(9-Oxo)-fluorenylphenylurea.—A mixture of 0.5 g. of 2-aminofluorenone, 0.6 g. of phenyl isocyanate and 50 ml. of absolute ethanol was heated slowly to boiling. Crystals formed immediately but the mixture was allowed to cool. After three recrystallizations from ethanol the bright orange needles melted at 268°, resolidified and remelted at 323–324° (all uncor.).

Calcd. for $C_{20}H_{14}N_2O_2$: N, 8.92. Found: N, 9.18.

2-(9-Oxo)-fluorenylphenylthiourea.—A mixture of 0.5 g. of 2-aminofluorenone, 0.4 g. of phenyl isothiocyanate and 50 ml. of ethanol was brought to the boiling point and then allowed to cool. After 3 recrystallizations from dilute ethanol, the yellow-orange crystals melted at 185°.

Calcd. for $C_{20}H_{14}N_2SO$: N, 8.49. Found: N, 8.40.

2-Aminofluorenone oxime, prepared in the usual way and recrystallized from dilute ethanol, separated as orange yellow needles melting at 216–217° uncor.

Calcd. for $C_{13}H_{10}N_2O$: N, 13.33. Found: N, 13.29.

2-Aminofluorenone semicarbazone, prepared in the usual way, did not melt under 325° when repeatedly crystallized from ethanol.

Calcd. for $C_{14}H_{12}ON_4$: N, 22.22. Found: N, 21.69.

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The Role of Water Vapor on the Stoichiometric Reactions of Solid Proteins with Polar Gases[1]

BY SIDNEY W. BENSON, ROBERT L. ALTMAN,[2] RYDEN L. RICHARDSON[2] AND JERROLD M. SEEHOF[2]

RECEIVED JULY 28, 1953

In a very interesting paper,[3] Czarnetsky and Schmidt (henceforth C. and S.) reported the reversible addition of acidic and basic gases such as CO_2, H_2S, NH_3 to proteins in the solid state. Such results, if verified, would have great importance both as an analytical tool in establishing the number and type of functional groups in the protein molecule and as a structural tool in clarifying the configuration, thermodynamic properties and spatial availability of these groups in the protein molecule.

C. and S. reported that on measuring the sorption isotherms of the above gases on solid proteins, isobaric regions were observed (*i.e.*, regions in which the proteins adsorbed the gases at constant partial pressures). Such isobaric regions of adsorption can be interpreted by means of the phase rule as corresponding to the formation of new, pure, solid phases and the amount of gas adsorbed would then correspond quantitatively to the amount of the new phase, in short, a stoichiometric, gas phase titration.

These results are, however, in contradiction to the results obtained in our own[4,5] and other laboratories.[6–8] The discrepancies may be summarized as follows: 1. The vapor pressures of the isobaric regions reported by C. and S. are in general different from those found in our own[4,5] and other laboratories.[6,7] 2. Only one pressure flat is found by C. and S. and this is ascribed to chemical combinations of some 5 or more different components of the protein (*e.g.*, arginine, lysine, etc.). One would expect each hydrochloride to be formed at a different equilibrium pressure. 3. An anomalous type of hysteresis is found by C. and S. in the adsorption of HCl on gelatin. 4. C. and S. find completely reversible sorption of HCl on gelatin. This is again in direct conflict with the previous findings of ourselves and others.[4–7]

Because of the above inconsistencies it was decided to repeat the experiments with gelatin and dry HCl gas. For these purposes a conventional apparatus similar to the one used by C. and S. was employed with certain modifications which have been described elsewhere.[4] The results which were obtained were consistent with those we had obtained previously with other proteins. There was no region of stable, reproducible[9] isobaric sorption. The desorption curve lay considerably above the sorption curve and was typical of hysteresis found with other proteins. Finally the desorption did not continue to zero HCl pressure but was completed (at 10^{-6} mm. pressure) at an amount of HCl sorbed, quantitatively equal to the sum of the arginine, histidine and lysine residues present in the gelatin sample.

These experiments, however, differed in one important respect from those reported by C. and S. The latter reported that in order to obtain reproducible results it was necessary to have catalytic traces of water vapor present. To achieve this they attached a side arm to their sorption buret containing $Na_2SO_4\cdot 10H_2O$–Na_2SO_4 mixtures. At constant temperature this would provide a constant vapor pressure of water in the system. While they gave none of the specific details of the procedure followed they did mention having to then correct their pressure readings by a constant amount (*ca.* 1 mm.),[10] presumably that due to the water vapor thus introduced. They did not say whether or not the side arm with the $Na_2SO_4\cdot 10H_2O$ was left open to the system during the sorption runs, although this seems implied by some of their subsequent discussion.

To check on these presumed effects of water

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[1] This work has been supported by a Grant (G-3541) from the United States Public Health Service.

[2] Graduate Research Fellows on United States Public Health Service Grant (G-3541).

[3] E. Czarnetsky and D. Schmidt, *J. Biol. Chem.*, **105**, 301 (1934).

[4] S. W. Benson and J. Seehof, THIS JOURNAL, **73**, 5053 (1951).

[5] Benson and Seehof, *ibid.*, **75**, 2427 (1953).

[6] W. D. Bancroft and C. J. Barnett, *J. Phys. Chem.*, **34**, 449 (1930).

[7] B. Belden, *ibid.*, **35**, 2164 (1931).

[8] R. Green, *Trans. Proc. Roy. Soc. New Zealand*, **78**, 291 (1950).

[9] Transient isobaric regions have been found which disappear if sufficient time is allowed to elapse to reach equilibrium.[4,5]

[10] This vapor pressure correction is rather mysterious since it does not correspond to any vapor pressure of the decahydrate easily accessible under laboratory conditions.

vapor we made two different types of measurements. In the first we measured the sorption of HCl gas on a $Na_2SO_4 \cdot 10H_2O$–Na_2SO_4 mixture in the absence of protein.[11] We found in these experiments that the decahydrate reacted stoichiometrically and irreversibly at 25° with HCl and the sorption curves could be interpreted as corresponding to the consecutive reactions[12]

$$HCl(g) + Na_2SO_4 \cdot 10H_2O(s) \longrightarrow NaCl(s) + NaHSO_4(s) + 10H_2O(g) \quad (1)$$

$$HCl(g) + NaHSO_4(s) \longrightarrow NaCl \cdot H_2SO_4 \text{ (solid solution)} \quad (2)$$

The first reaction occurs at constant pressure indicating the formation of a new phase, while the second takes place with rising pressure indicating solid solution. The region of constant-pressure sorption is very close to the pressure observed by C. and S. and it seems reasonable to thus interpret their results as arising from the reaction of HCl with the decahydrate rather than with the protein. This conclusion is not certain since C. and S. nowhere state their experimental method explicitly.

In order to then check further we performed an experiment in which the gelatin was first allowed to come to equilibrium with a constant vapor pressure of water. The side arm with decahydrate was then closed off and the sorption of HCl measured directly on this sample. The results of this experiment were similar to those found in the absence of H_2O and showed none of the characteristics reported by C. and S.

While the above experiments do not explain all of the features of the sorption processes observed by C. and S., in particular the results obtained with CO_2 and H_2S (similar to HCl) or the lengths of the isobars (with all gases) they do indicate that their results with HCl and gelatin are inconsistent with results obtained in these and other laboratories, and they indicate a probable source of the anomalies: namely, the reaction of HCl with the $Na_2SO_4 \cdot 10H_2O$–Na_2SO_4 mixtures introduced into the system to maintain constant vapor pressure of water.

[11] These experiments were done by weighing the sample *in situ* using as a balance a helical quartz spring.

[12] The H_2O in reaction 1 may be displaced as a vapor as indicated or may under some conditions be partially bound in the form of hydrates of the NaCl and/or $NaHSO_4$.

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Dielectric Measurement of Heptacosafluorotributylamine

BY FIELDING BROWN

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The dielectric constant and loss tangent of heptacosafluorotributylamine, $C_{12}F_{27}N$, has been measured over the temperature range of 140 to −52°. The measurements were carried out at frequencies of 60 cycles, 1000 cycles, 15 kc., 30 kc., 60 kc. and 100 kc., and the results are shown in the accompanying graph.

The liquid was measured in a cell originally described by Horsch and Berberich[1] and the values of capacity and power factor were determined by means of a General Radio 716-C bridge using generators and detectors appropriate to the frequencies in question. A General Radio 716-P2 guard circuit also was employed to balance the guard ring of the measuring cell.

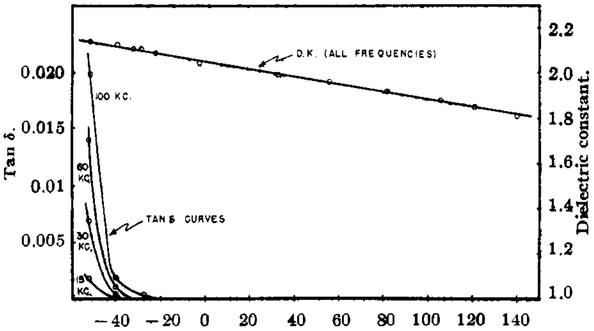

Fig. 1.—Dielectric properties of $C_{12}F_{27}N$.

The points on the dielectric constant curve are accurate to ±4% and those of the tan δ curve to ±0.00007. As can be seen, the losses in the liquid are extremely small above −20° but rise sharply below that temperature. The values shown on the graph are further in approximate agreement with those given earlier.[2] Unfortunately it was not possible to carry the measurements low enough in temperature to calculate the electric moment of the molecule. Various members of this laboratory have assisted in performing the measurements.

[1] W. G. Horsch and L. J. Berberich, *Rev. Sci. Instr.*, **5**, 194 (1934).

[2] Technical Report No. 57, Laboratory for Insulation Research, M. I. T.

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NORTH ADAMS, MASS.

Reaction of Methyl *p*-Nitrobenzoate with Phenylmagnesium Bromide

BY DAVID Y. CURTIN AND JAMES C. KAUER

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Phenylmagnesium bromide has been shown to react readily with nitrobenzene to give diphenylamine as the principal product.[1]

$$4C_6H_5MgBr + C_6H_5NO_2 \longrightarrow (C_6H_5)_2NH + C_6H_5C_6H_5 + C_6H_5OH$$

Newman and Smith,[2] however, have found that at −70° the reaction of *m*-nitrobenzaldehyde with phenylmagnesium bromide occurs preferentially at the carbonyl group to give *m*-nitrobenzhydrol in 77% yield.

In connection with other work, we have had occasion to examine the reaction of phenylmagnesium bromide with methyl *p*-nitrobenzoate at −70° and found it leads to *p*-carbomethoxydiphenylamine in a yield of 45% (based on Grignard reagent). None of the product to be expected from reaction at the ester carbonyl could be isolated.

[1] H. Gilman and R. McCracken, THIS JOURNAL, **51**, 821 (1929).

[2] M. S. Newman and A. S. Smith, *J. Org. Chem.*, **13**, 592 (1948).