STUDIES ON THE PHOSPHORESCENCE OF GELATINE AND FLUORESCEIN AT LOW TEMPERATURES.

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It is already known that many organic compounds are phosphorescent at low temperature, first discovered by Dewar(1) and further studied by Nichols, Meritt(2) and Kowalski(3) and others.(4) Chemically pure substances show, generally, after-glow of very short duration at low temperature, while they show after-glow of rather long duration (say more than 1 sec.) when dissolved or suspended in a suitable medium and are exited by a ray of suitable wave-length at suitable temperature. Gelatine itself shows brilliant phosphorescence at the temperature of liquid air as shown by Dewar. Moreover, gelatine prolongs duration of after-glow of some substances mixed with it. Though it is not sure whether " pure " gelatine is phosphorescent or not, its phosphorescence depends largely upon the temperature and the water content.

It was also known that fluorescein is phosphorescent when it is dissolved (or suspended) in boric-(5) tartaric-(6) and citric acid.

Experiments on the phosphorescence of gelatine are described in the first part of this paper, and of fluorescein in various mediums are described in the second part.

Part 1. Phosphorescence of Gelatine. As gelatine is phosphorescent only at low temperature, it was sealed in a small test-tube and laid in a cryostat during the experiment. The cryostat was composed of a Dewar's vessel containing pentane or petroleum ether and cooled by a regulating flow of liquid air in a spiral tube within the vessel. A 100 volt, 250 watt incandescent lamp was used as the light source and the light was condenced and filtered by a flask containing ca. 1% cupric sulphate solution before entering the Dewar's vessel.

Time duration of after-glow, denoted by t in the following, was determined subjectively, the accuracy of which was ca. 1 second even when the eyes were rested fully in the darkness.

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(1) Dewar, " Collected Papers ", Vol. I (1927), p. 409.

(2) Kayser, " Handbuch der Spektroskopie ", Vol. IV, p. 1027.

(3) Kowalski, *Physik. Z.*, **12** (1911), 956.

(4) For instance, Pringsheim, " Fluorescenz und Phosphorescenz ", 3ed. (1928).

(5) E. Tiede and P. Wuff, *Ber.*, **55** (1922), 588; R. Tomaschek, *Ann. Physik*, **76** (1922), 612.

(6) B. Batscha, *Ber.*, **58** (1925), 187.

Nelson's No. 2 Photographic Gelatine was used in the experiments. Gel was prepared in the following manner: gelatine was swelled for about an hour in distilled water, and then warmed to 30–40°C. until it dissolve into a sol. Now one part was sealed off in a small test-tube for using in the experiment, while another part was dried at 110°C. in an air bath to determine the composition of the gel. A gel thus dried was noted as 100% gelatine. The composition of the ordinary gelatine was found to be 82%. It coloured slightly yellow and contained ca. 3.5% ash.

When gel of 15 to 12% gelatine was frozen, test-tube was broken frequently, probably due to expansion of gel as it frozen. If carefully cooled from the bottom of tube, breakage might be got rid off, but even if bear surface of a gel came in contact with the liquid of the bath (pentane or petroleum ether) no change in t was observed.

Colour of the phosphorescence is white, but in cases of 82% and 100% gels, bluish green colour appeares distinctly. Change of colour in the course of after-glow cannot be observed definitely.

Before measuring t, we must know its relation to the time of excitation. General behaviour is shown in Fig. 1. Thus if excited longer than 5 seconds a constant value of t is obtained. Excitation more than 5 minutes showed no change of the value of t. In the following experiments, therefore, the time of excitation was always 5 to 10 seconds. The results of the experiments are shown in Table 1 and Fig. 2. The hysteresis of these after-glow duration, t, is small and if the sample be kept 10 minutes or more in the cryostat t-value becomes quite constant.

Table 1.

T=temperature in absolute scale. t=duration of after-glow in seconds.

100%		82%		58.5%		35%	
T	t	T	t	T	t	T	t
290	12	265	8	233	6	225	3
265	15	260	13	213	18	220	6
244	21	242	17	203	27	213	15
233	23	235	20	193	30	205	20
213	26	219	25	183	32	190	30
203	27	205	30	173	34	170	34
175	27	185	33			146	34
158	29	173	30			128	32
131	30	136	26			84	35
123	31	123	25				
84	35	84	35				

18.6%		4%		0.8%		0.04%	
T	t	T	t	T	t	T	t
213	13	213	5	204	2		
203	19	203	7	183	27	203	7
193	24	188	27	174	29	193	12
183	29	174	30	166	29	183	15
173	31	166	33	155	29	173	22
		159	34	152	32		
		140	34	135	29		
		131	32				
		84	34				

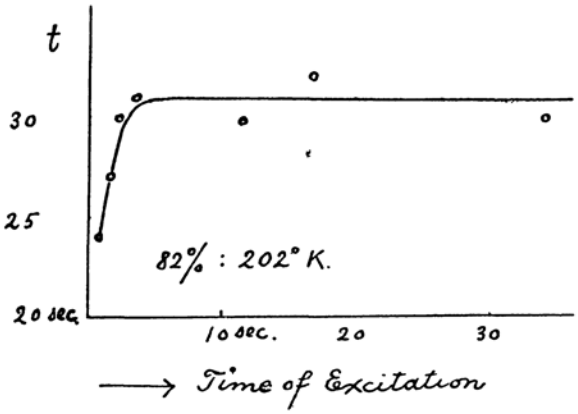
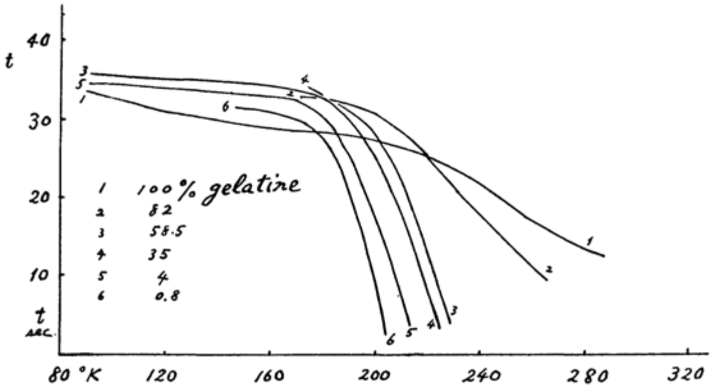


Fig. 1



Temp. in absolute scale.

Fig. 2

The t-value of the gels containing less than 20% gelatine varies markedly by the heat treatment of them. It was observed that a dilute gel (gelatine content less than 20%) is heated for several hours at 80° to 90°C. after the preparation as described before, the value of t changes markedly. The gel was again melted and heated, then again different t-value will be obtained. This cannot be explained as a result of change in composition due to evaporation. Gel of initial composition 20% showed hardly the heating effect.

Some examples of t-values when excited by a 100 volt, 1000 watt lamp are shown in Table 2.

Table 2.

Excited by a 1000 watt lamp.

T	35%	82%	100%
250	—	15	22
233	—	26	29
220	15	—	—
213	—	—	33
195	32	—	34
183	—	41	—
155	46	—	—
150	—	36	30

It is clear from Fig. 2, etc. that, as a rule, t suddenly increases at about 220°K., and further cooling has no remarkable effect on it. Moreover, as concentration increases, the temperature where it begins to be phosphorescent becomes higher and, besides, t gradually decreases from its nearly constant value as the temperature rises.

Then, to answer the question about the relation between phosphorescence and composition of gels, it seems best to compare the temperature and the inclination of t-temperature curves of Fig. 2 where t increases suddenly. As a rough epproximation we may take the temperature where t takes a half of its maximum value. Because it may be considered that these temperature is rather independent of the quantity, form of surface, etc. of the gel. Fig. 3 shows the results thus obtained.

Frozen gels can be divided into three classes from their appearances: gels containing 4 to 5% or less geletine separates laminae of ice and gelatine alternately which reminds of Liesegang's ring, more concentrated but less

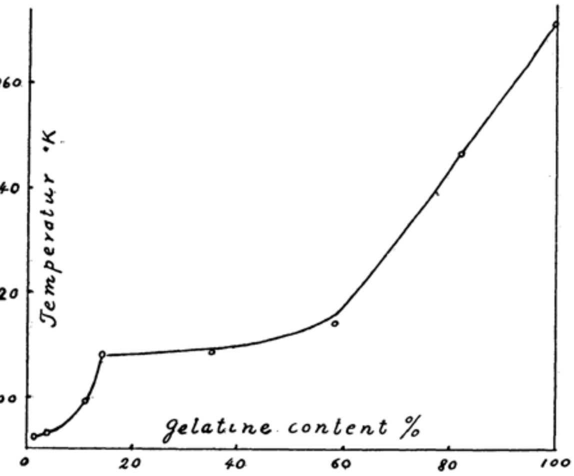


Fig. 3.

than about 60% gelatine freeze to a white solid probably due to irregularly separated small flocks of ice, and more concentrated gels retain their appearance at the room temperature even in the liquid air.

Returning to the present problem, we see here also analogous phenomena. Nearly horizontal part in the middle of the curve in Fig. 3 may be attributed to the separation of gelatine of constant structure, say identical degree of hydration, etc. The inclination in the right side of the curve may be explained as the change in structure, say degree of hydration decreases and thus disturbance of water molecule to the mechanism of phosphorescence is removed. Rather sharp "Knick" in the left side of the curve may be undue, but it is probable that in this region the structure of the gel is distinctly different from the gels containing more than 15% gelatine. Remarkable heating effect may partly due to chemical change like hydrolysis and partly due to the change of degree of dispersion of hydrated gelatine. Of cource the extreme left side of the curve must fall to very low temperature because distilled water is not phosphorescent even in the liquid air in the region of wave length of this experiment.

Part 2. Phosphorescence of Fluorescein. It is well known that fluorescein is phosphorescent when it is buried in boric-, tartaric- and citric acid, and grape sugar containing several organic acids in it. Fluorescein may be dissolved in these substances or in a state of colloidal suspension but we are not sure to which it belongs. B. Batscha(1) has suggested that fluorescein is phosphorescent only when substances of acidic nature co-

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(1) Loc. cit.

exists with it. The writer found that fluorescein is also phosphorescent when it is buried in sulphuric acid, hydrogen chloride, nitric-, formic, acetic-, butylic-, oxalic-, and succinic acid at low temperatures. All these acids give coloured solid matter.

Experiment is described in the followings. Exciting light is the same which was used in Part 1. Fluorescein is dissolved in concentrated sulphuric acid considerably. The solution shows slight surface fluorescence and is light to dark orange in colour and when cooled below -37°C. it freezes to yellow silky crystal or brown glassy mass as the quantity of fluorescein increases. 0.005, 0.05 and 2.6 mol/litre solutions of fluorescein were frozen and found to be intensively phosphorescent. Colour and duration of afterglow was nearly the same for them all. Duration was about 15 seconds at -37°C., but if it is cooled below -100°C., then after the cease of orange yellow intense phosphorescence, very weak phosphorescence lasts more than 3 minutes.

Next, a small quantity of sulphuric acid is added to ca. 0.0001 mol/litre aqueous fluorescein solution. On freezing this solution a brilliant phosphorescence was appeared. To the same 0.0001 mol/litre solution hydrochloric-, nitric-, acetic-, formic-, and butylic acid were added respectively and again found to be phosphorescent below their freezing temperature, Fluorescein is soluble in liquid hydrogen chloride to a small quantity and by cooling it gives yellow transparent solid. This is also phosphorescent but the colour of it is rather yellowish.

It was known that fuming nitric acid gives tetra-nitro-fluorescein. This substance was prepared and dissolved in water and frozen, but the result was negative, showing that the substitution cannot be the cause of phosphorescence.

Oxalic- and succinic acid containing fluorescein showed very weak phosphorescence even at the temperature of liquid air in contrast with the result of Batscha, where oxalic acid gave rather intense phosphorescence (in grape sugar). Molten succinic acid dissolves fluorescein and gives coloured crystal but showed none.

Some salts of the above mentioned acids like KCl, $CaCl_2$, $Ca(CH_3CO_2)_2$, etc. and some alkalies like NH_4OH, NaOH, KOH, $Ba(OH)_2$ were added to the aqueous fluorescein solution and frozen, but the result was rather negative, neverthless some of them seem to show phosphorescence of short duration. The same for picric acid, acid potassium phthalate, benzoic acid and glycerine.

Substances like acids probably go into complex molecule (even if lablie as a complex compound) with fluorescein, and in this complex molecule the positive part of the polar group is directed to fluorescein. In a complex molecule we understand that molecules of acid and fluorescein are situated so near that the polar group serves nearly as a positive charge to fluorescein. In the interior of this complex molecule the mechanism analogous to that of "crystalo-phosphore"(1) may occur and the phosphorescence is observed. As formerly described NaCl, etc. show no phosphorescence with fluorescein. This may partly due to spare solubility of fluorescein in them or the formation of complex molecule is difficult, but even these conditions are fulfilled large ionic volume of sodium, etc. compared with that of hydrogen ion may give a negative result. Further the structure of the fluorescein in the phosphorescent state should be akin to that in the ionized state in liquid solution, and the spectra of phosphorescence may be in the same system of that of fluorescence, because the positive part of the polar group situated so nearly as supposed above will force fluorescein to take analogous structure as in a ionized state.

In conclusion the writer wishes to express his cordial thanks to Prof. J. Sameshima for his kind guidance throughout this experiment.

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(1) R. Tomaschek, *Ann. Physik*, **76** (1922), 612.