LII. THE PROPERTIES OF DIBENZOYLCYSTINE.

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NEARLY all the organic substances exhibiting gel formation in water are compounds of complex structure and high molecular weight.

In order to elucidate the mechanism of gel formation, in recent years search has been made for substances of relatively low molecular weight and simple constitution, and the dye-stuffs, benzo-purpurin, chrysophenone, thionin blue, the soaps, the organic sulphonic acids and camphorylphenylthio-semicarbazide have been used. As Gortner and Hoffman have recently pointed out [1921], benzoylcystine is the simplest known type of organic substance which exhibits gelation with water and it therefore appeared to present a suitable material with which to study some of the elementary properties of gels in greater detail.

The preparation of dibenzoylcystine used in this work was essentially that of Brenzinger [1892] and Gortner and Hoffman [1921], except that the substance, after recrystallisation from dilute alcohol, was extracted for several hours with benzene in a Soxhlet apparatus to remove completely all traces of benzoic acid and benzoyl chloride. The melting-point of our product was 189° (uncorrected) which is some eight degrees higher than that given by the older authors.

While the method of dispersion in water from an alcoholic solution is well known, yet for many purposes the presence of a second component is undesirable, and accordingly attempts were made to obtain direct dispersion of the material in water. It was found that aquagels may be prepared by triturating the solid in small quantities at a time with warm water and completing peptonisation by stirring the solution for long periods at a temperature of 80°–90°. The rigidity of the gels obtained by this method was essentially of the same order as that of those described by Gortner and Hoffman using alcohol for dispersion.

The sol-gel transformation was found to be perfectly thermally reversible, thus differing from that of silicic acid, and decomposition does not appear to occur as in the case of gelatin. Dilute gels are perfectly transparent; but at higher concentrations, 0.2 % and over, an opalescence makes its appearance, increasing with the amount present in the liquid. Dilute gels, on standing,

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become somewhat cloudy. On prolonged standing syneresis occurs with contraction of the gel to a core and the exudation of water. Simultaneously small nuclei make their appearance in the gel, which continue to grow to form spherites. Microscopic examination of the spherites shows them to consist of minute elongated radiating crystals. The appearance of these crystalline spherites in the gel, differing from the shot-like spheroids formed in the gelation of gelatin [Bradford, 1921], lends additional support to the two-phase theory of gel structure. The thread or fibrillar nature of the spherite group, in addition, supports the assumption that the natural crystal growth of dibenzoylcoystine in water is in the form of poly-molecular crystalline rods, the gel consisting of a number of intersecting fibrils built up of these rods in which the water is enclosed. The fibrils in the gel are evidently relatively coarse, since the gel is both moist and elastic and exhibits syneresis.

The examination of the spherites thus classifies dibenzoylcoystine as belonging to the fibrillary crystalline gels, which include nearly all the gels which have been examined, with the possible exception of dilute gelatin, agar and silica. These latter, although generally regarded as a class apart, may be brought into the same category, if one assumes, in the disperse phase, the existence of highly elastic or mobile fibrils undergoing a process analogous to crystal twinning.

Dibenzoylcoystine is a relatively strong acid, a gel containing 2.66 g. per litre showing a p_H of 3.05 at 20°, the value being obtained both by indicators and the hydrogen electrode. This value, if the acid be considered as monobasic, corresponds to a dissociation constant of 0.3×10^{-3} . The degree of dissociation was also determined by the conductivity method. The molecular conductivities of a 0.1 % solution and solutions more dilute were determined at 25° in a conductivity cell of the usual type, with the aid of a potentiometer, telephone and coil. The value of λ_{∞} was found to be 250, a limit to which the molecular conductivity had almost attained at a dilution of 0.00014 molar. The value of K , the dissociation constant, varied from 1.4×10^{-3} to 1.5×10^{-3} with a mean value of 1.48×10^{-3} . The acid is thus comparable to monochloroacetic acid and monobromosuccinic acid. No discontinuity in conductivity in the 0.1 % solution, which exhibits a feeble tendency to set, was observed, whether the liquid was agitated or kept at rest prior to the determination. This is a phenomenon which has been observed by McBain [1919] and his co-workers in their studies of soap solutions. The discrepancy between the dissociation constant value as calculated from the hydrogen ion concentration and determined by the conductivity method is probably to be attributed to a liquid junction potential in the former due to the non-diffusibility of the anion in the dibenzoylcoystine. This discrepancy is of the order of 10 millivolts.

On the alteration of the hydrogen ion concentration of the medium by the addition of sodium hydroxide or hydrochloric acid several interesting points were noted. On the addition of increasing amounts of alkali the gel formation gradually became less, until the contents of the tube were entirely liquid.

On the addition of acid, the rate of setting increased and the gels were distinctly more opalescent in character, while on the addition of relatively large quantities of acid the gels became distinctly granular and less resistant to penetration. The gel possessed the maximum stability at the isoelectric point. The marked change in the physical characteristics on the alteration of the p_H of the solution was made evident by the following experiments.

Water Retention. One gram of dibenzoylcystine was dissolved in 6.0 cc. of hot alcohol and poured into 250 cc. of boiling water as quickly as possible with constant stirring. From this stock solution 20 cc. were run into boiling tubes which contained 10 cc. of solutions of alkali and acid of varying concentration as given in Table I. 20 cc. of the mixtures were then pipetted into Petri dishes for determinations of their setting power. The remaining 10 cc. were used for hydrogen determinations. At the end of 72 hours the jellies contained in the Petri dishes were removed and allowed to drain under their own gravity on filter papers in funnels. The liquid running through was measured. The results of these determinations are given in Table I.

Table I. 0.30 % *dibenzoylcystine*.

No.	$N/20$ H_2SO_4 cc.	$N/10$ $NaOH$ cc.	Water cc.	Dibenzoyl- cystine solution cc.	p_H 20° C.	Water drained in 2 hours from 20 cc. of mixture after setting for 3 days cc.	Observations made on mixing and cooling
1	0	2	8	20	3.65	16.5	Quite fluid
2	0	1	8	20	3.48	16.5	" "
3	0	0.5	9.5	20	3.36	15.5	Moderately set, not very opalescent
4	0	0.25	9.75	20	3.26	13.0	Not quite stiff, pours
5	0	0	10	20	3.05	13.0	Stiff, does not pour
6	0.5	0	9.5	20	2.65	13.0	Very stiff
7	1.0	0	9.0	20	2.59	14.0	Full of air bubbles, set at once
8	1.5	0	8.5	20	2.52	13.0	" "
9	2.0	0	8.0	20	2.41	13.0	Opalescent, set at once
10	3.0	0	7.0	20	2.29	11.0	" "
11	4.0	0	6.0	20	2.19	11.5	Very opalescent
12	8.0	0	2.0	20	1.93	11.5	Solid had separated out

It is evident as the hydrogen ion concentration decreases below p_H 3.0 the water-retaining power of the gel is very seriously diminished. In the more acid solutions a slight alteration takes place but the curve of drainage is not so regular.

In the determination of the p_H of the alkaline solutions it was noticed that the internal resistance of the cell became extremely high, possibly owing to the formation of a pellicle at the interface of the solution and the saturated potassium chloride junction fluid. It was not, however, sufficient to interfere with the readings.

Effect of Electrolytes. In common with other gels, the water-retaining power of the fibrils is much affected by the presence of neutral electrolytes. The effect of alteration of the osmotic pressure and the reduction in the free water content and of ion hydration naturally varies with the nature of the added electrolyte. Quantitative investigations on the effect of the addition of ammonium thiocyanate to a typical emulsoid albumin to gelatin and to silicon hydroxide gels are to be found in the literature.

Ammonium thiocyanate exerts a similar lyotropic effect on the dibenzoyl-cystine gels. Syneresis is more pronounced in the presence of small quantities of this salt and relative instability of the gel structure may be obtained in fairly dilute solutions as is indicated by the following draining experiments performed in a manner similar to those previously described.

Table II. *Effect of Ammonium Thiocyanate on the setting of Dibenzoylcystine.*

Number	0.4 % dispersion of dibenzoylcystine. 5.0 % solution of ammonium thiocyanate.			
	Ammonium thiocyanate cc.	Water cc.	Dibenzoylcystine solution cc.	Filtrate cc. in 1 hour
1	1	9	20	14.5
2	2	8	20	17.5
3	3	7	20	18.0
4	4	6	20	19.0
5	6	4	20	21.0
6	8	2	20	21.0
7	10	0	20	21.0
8	0	10	20	14.0

Nos. 1 and 8 went solid in about 10 minutes. The others some time afterwards.

Resistance to Penetration. In order to obtain some information on the tenacity of the gels as affected by change in the p_H of the dispersion medium a simple penetration test was devised. This consisted essentially in measuring the rate of fall of a hemispherically ended plunger through a column of the gel maintained at room temperature. It was noted that a uniform velocity was attained after a fall of two or three centimetres. The time in seconds for a penetration of 1 cm. is plotted in the following curve.

It will be noticed that the influence of the alkali is extremely marked, whilst a distinct reduction in the internal viscosity is produced in relatively acid solutions. The conclusion that one may draw from these experiments is that on the addition of alkali the number of fibrils decreases, presumably because the sodium salt has no gelatinising qualities and is entirely soluble, while, on the addition of acid, the fibrils become relatively much coarser and consequently fewer in number.

Protective Action. An attempt was made to determine the protective power of dibenzoylcystine, using red colloidal gold prepared according to Zsigmondy's method. This gold gave correct values when tested against gelatin. Owing to the sparing solubility of the compound, it was impossible to carry out the test in exactly the way which Zsigmondy [1920] prescribes.

With a 0.2 % solution of the compound partial protection of 10 cc. of gold suspension occurred with 4.0 cc. of the gel. Complete protection was afforded by 6.0 cc. The gold number should therefore be about 10, which is close to that of dextrin and is nearly a negligible quantity.

Diffusion Experiments. In dilute concentrations gels such as gelatin and agar offer no abnormal resistance to the diffusion of electrolytes. With more concentrated gels the coefficient of diffusion is somewhat reduced. Attempts to estimate the fibril mesh of such gels by observations on the rate of diffusion of electrolytes, semi-colloids and colloids of varying particle diameter have frequently been made. The results, however, have been extremely variable. The same abnormalities have been noticed with dibenzoylcystine which being of much simpler chemical constitution permitted partial interpretation of the results obtained.

The experiments were made by taking a gel of 0.2 % and allowing it to set in tubes of uniform size. As soon as the gel was fairly stiff, solutions of dyes were carefully pipetted on top of it and the level marked. At intervals the entrance of the dye into the gels was estimated with calipers. All the dyes used were of Gröbler's make. The results are given in Table III.

It will be noted that the gel offers very slight resistance to an electrolyte such as potassium dichromate, a distance of 100 mm. being traversed in a few hours.

Dibenzoylcystine is a negative colloid, being itself a relatively strong acid, and although the ion, as evidenced by the highly dissociated sodium salt, possesses feeble if any gelatinising properties, yet there is no doubt that the fibrils are partially ionised. In the presence of positive crystalloid or colloid dyes adsorption or mutual precipitation is to be anticipated. For such basic dyes, diffusion far into the interior is not to be looked for, and the fibrils should be markedly stained, the partition coefficient between fibril and surrounding liquid being very high. The low apparent diffusibility is for these dyes to be ascribed to adsorption and not to a small fibrillary mesh. The zone of precipitation naturally varies with the colloidal character of the dye and its diffusibility.

Table III.

Diffusion of	Nature	Adsorption	Precipitation	Diffusion rate mm. in 24 hrs.
Potassium dichromate	Crystalloid	—	—	135
<i>Basic dyes, cationic:</i>				
Night blue	Colloid	× ×	× ×	0
Neutral red	Semi-colloid	× × ×	×	0.2
Methyl violet 5b	„	× ×	× ×	1.0
Bismarck brown	Crystalloid	× ×	—	1.6
Toluidine blue	Colloid	× ×	×	2.0
Methylene blue	„	×	×	2.0
Malachite green	„	×	—	2.0
Thionin	„	× ×	—	2.4
Safranin	Crystalloid	× ×	—	3.0
Fuchsin	Semi-colloid	—	—	3.0
<i>Chemically reacting dyes:</i>				
Eosin	Colloid	× ×	× ×	0.2
Rose bengale	„	× ×	× ×	1.0
<i>Acid dyes, anionic:</i>				
Azo blue	„	× —	—	1.1
Congo red	„	× —	—	1.6
Benzopurpurin	„	× —	×	1.6
Alkali blue	„	—	—	2.0
Nigrosin	„	—	—	2.4
Carminic acid	„	—	—	3.3
Water blue	„	—	—	4.2
Light green F.S.	„	—	—	4.4
Acid fuchsin	Semi-colloid	—	—	5.0

POSSIBLE STRUCTURE OF THE GEL FIBRIL.

Evidence is collecting to show that the union of molecules to form molecular complexes is brought about by forces identical with, though generally more feeble in character than, those uniting atom to atom within the molecule; further that these residual force fields, the product of these internal molecular asymmetries, are localised within certain definite areas in the molecule itself. These forces are electrical in character, and for convenience the various residual affinities may be designated as positive or negative relative to other such fields. The active areas on the molecules are associated with the presence of specific chemical groups which are electrically unsaturated.

The results on the diffusion of the dyes indicate that mutual precipitations of the cationic dyes and the anionic gel do actually take place, and that with the more colloidal dyes the precipitation zone is more limited and the colour more intense, confirming the work of Biltz and Teague and Buxton.

The halide-containing dyes, eosin and rose bengale, appear to react chemically with the dibenzoylcystine. But feeble, if any, adsorption was observed in the case of the anionic dyes. Those that were distinctly colloidal in character were feebly adsorbed. Benzopurpurin, a dye very similar to electrolytes, was apparently relatively easily precipitated. In general, the order of diffusibility observed with dyes and benzoylcystine was very similar to that observed by Bechold and others [1906, 1919].

Some idea of the nature of the linkages involved in the gel fibril may be obtained from a survey of the effect of substitution in dibenzoylcystine of various groups on the gel formation.

SUMMARY.

Gels of dibenzoylcystine may be prepared by peptisation with hot water. The substance is a relatively strong acid with a dissociation constant of 1.49×10^{-3} . The gel structure appears to be fibrillary and relatively coarse. The sodium salt of the acid exhibits no gelatinising properties. The presence of acids greatly reduces the solubility of the compound. This was confirmed by observations on the water-retaining capacity and internal viscosity of the gel. Lyotropic salts, such as ammonium thiocyanate, reduce the water-retaining power of the gel, producing ultimate liquefaction. Cationic dyes are adsorbed and precipitated by the gel. Anionic dyes diffuse in a normal manner, whilst halogen dyes apparently react chemically with the sulphur group. Di-*m*-nitrobenzoylcystine has similar properties to the simple compound. A possible structure for the gel fibril based upon the effect of chemical substitution on the gel formation is suggested.

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