(c) The production of currents by the use of organic compounds as the source for the escape of electrons in oxidation-reduction cells.

9. It is pointed out that in the oxidation and reduction of organic compounds at ordinary temperature the same general laws hold as for inorganic compounds, namely, that *exposed* valences in organic compounds on double and triple bonds, in bivalent and trivalent carbon, etc., are the common seat of oxidation and reduction reactions, especially at ordinary temperature, exactly as the exposed valences of free ions, free atoms, etc., of inorganic compounds have been found to be.

10. While polarity in organic compounds is insisted upon as giving us an invaluable guide in following organic reactions, it is pointed out that the polarity need not be of the extreme character shown by common salts but may very well be of the character proposed by Bohr, Lewis and Kossel, where the transfer of electrons from atom to atom is not as complete as in the case of common electrolytes. Polarity exists, nevertheless, and the application of the theory of polar valence represents a decided advance in the interpretation of reactions of organic compounds.

CHICAGO, ILLINOIS

THE SOL-GEL EQUILIBRIUM IN PROTEIN SYSTEMS[1]

BY ROBERT HERMAN BOGUE[2]

Introduction

The significance of a transitional temperature in gelatin solutions recently has been receiving much attention from chemists. In a general sense it has long been recognized that whereas very dilute solutions (1.0%) of pure gelatin would gel at low temperatures (10°), yet that above certain temperatures, roughly placed at about 35°, gelation would not take place at any concentration. Exceedingly viscous solutions might be obtained, but the ability of these to congeal to a jelly was not observed above this temperature.

In a sense, the melting point of a gelatin or glue has been taken as the critical temperature, as is the case with crystalloids, but melting point is not at all easily obtained or even defined when such substances as gelatin are under consideration. Many attempts have been made to determine this property, but none of them may be regarded as absolute melting-

point determinations in the classical conception of the term. A very appreciable time factor enters into the above determinations which prevents an exact coincidence of the melting and solidification or setting points. The fact must be met that in such systems as these the transition from the hydro-sol to the hydro-gel condition is continuous, as far as our ability to measure the "point" of change is concerned, and to quote Sheppard,[2] "both the 'melting point' and the 'setting point' are more or less arbitrary conceptions, and their determination depends mainly upon standardized experimental conventions."

In a study upon the mutarotation of gelatin C. R. Smith[4] has shown that at temperatures above 33° to 35° the specific rotation of gelatin is practically constant at about −123°, while at temperatures below 15° the specific rotation is practically constant at about −266°. At all temperatures intermediate between 35° and 15° the rotation varies between these two limits. Smith arrives at the conclusion that gelatin in aqueous solution exists in two modifications; the one stable at temperatures above 33–35° which he denotes as Sol form A, and the other stable at temperatures below 15° which he denotes as Gel form B. "Between these temperatures a condition of equilibrium between the two forms exists and the mutarotation observed seems to be due to the transformation of one form into the other by a reaction which is reversible with temperature."

Bingham and Green[5] have made exhaustive studies of the laws and measurement of plastic flow, and the applications of plastic flow to industrial processes, and Bingham[6] has described a variable pressure method for the measurement of viscosity.

It has been shown that a *viscous liquid* will start to flow no matter how small a pressure is applied. With *plastic materials* no flow takes place until after the pressure has exceeded a certain definite value. Bingham points out that when viscosity determinations are made by noting the volume of outflow of the liquid in a given unit of time, and this volume is plotted against a variable but rigidly controlled and accurately measured pressure, an extension of the curve to the axes will pass through the origin, or zero point of the axes, provided the substance obeys the laws of a truly viscous liquid, but that the extension of the curve will fall upon the pressure axis at a finite distance (*f*) from the volume axis if the substance is a plastic solid. This distance (*f*) he calls the *yield value* and defines as the force required to start the flow.

It seems that equally comparable, although perhaps less sensitive measurements for the determination of the viscosity-plasticity relations may be made by the use of a torsional viscosimeter of the MacMichael type. By varying the speed of rotation of the cup the same effect is produced as by varying the pressure in the capillary tube type of instrument. A study of gelatin solutions was conducted by this method.

[3] Sheppard and Sweet, *J. Ind. Eng. Chem.*, **13**, 423 (1921).

[4] Smith, THIS JOURNAL, **41**, 146 (1919); *J. Ind. Eng. Chem.*, **12**, 878 (1920).

[5] Bingham, Bur. Standards *Bull.*, **13**, 309 (1916–17). Bingham and Green, *Proc. Am. Soc. Testing Materials*, **19**, 640 (1919). Green, *ibid.*, **20**, 451 (1920).

[6] Bingham, *ibid.*, **18**, Pt. 11, 373 (1918).

Viscosity-plasticity Studies

The procedure adopted was as follows. Several lots of the highest quality of granulated gelatin were employed in the tests. These were made up accurately into 10, 20 and 25% solutions by soaking in cold water for 1 hour, dissolving in a water-bath at 70°, again making up accurately all water lost by evaporation and, with no delay, introducing into the cup of the viscosimeter. The latter was at the same temperature as the
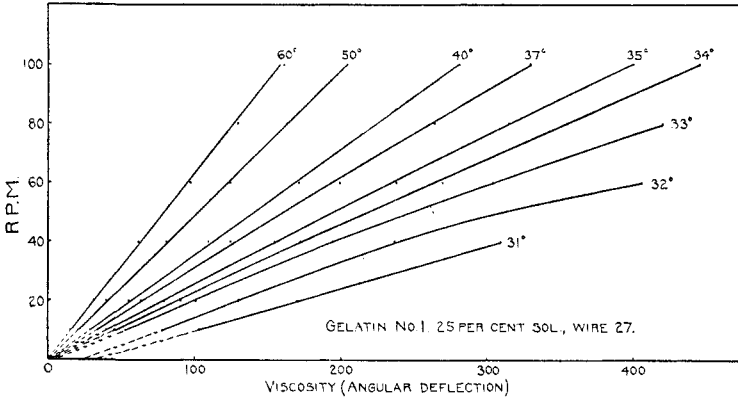

Fig. 1.—Viscosity-plasticity curves.

gelatin solution, and was immersed in the water-bath, which is a part of the instrument, at a temperature of 70°. The heating process for bringing the gelatin into solution was made as brief as possible. The cover of the instrument was kept on the cup to prevent evaporation during the measurements.

The solution was kept thoroughly stirred by lifting the plunger up and down, and the temperature permitted (by use of the electric heating unit)
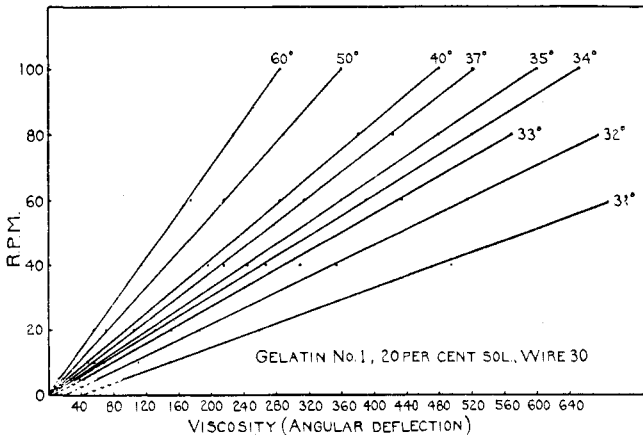

Fig. 2.—Viscosity-plasticity curves.

to fall very slowly. The viscosity was taken intermittently at definitely indicated temperatures as the solution cooled until it became too viscous to measure.

The velocity of rotation of the cup was very carefully adjusted before and after the measurements. Each series, as above, was measured at
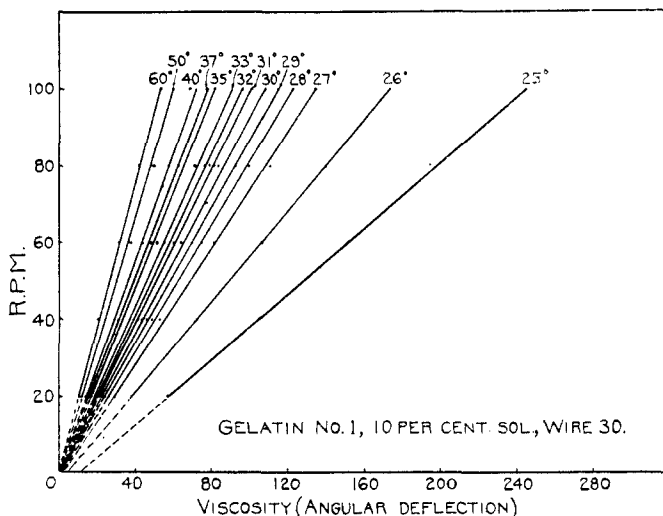

Fig. 3.—Viscosity-plasticity curves.

the same velocity of rotation throughout the temperature range from 60° to 31° or lower, and then the velocity changed. Speeds from 5 to 100 r.p.m. were used.

The whole process was repeated for the three concentrations used, and again repeated with the employment of differently sized wires in the instrument.
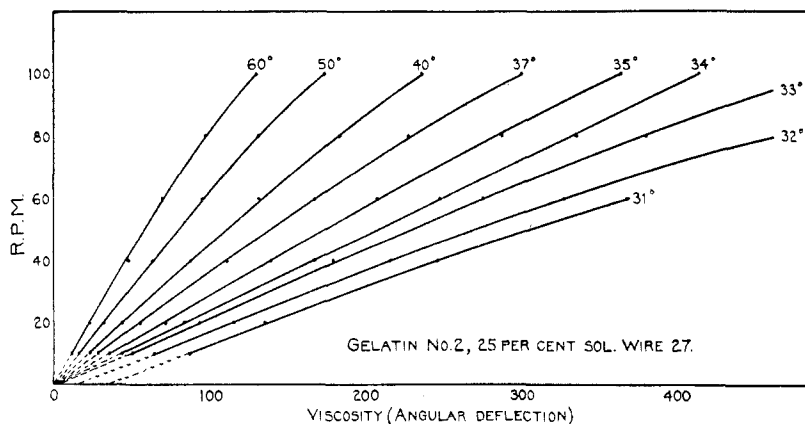

Fig. 4.—Viscosity-plasticity curves.

Some of the data obtained are shown in graph form in Figs. 1 to 4.

In the curves the velocity of rotation is plotted against angular deflection. An examination of these graphs shows that by continuing each curve downward until it intercepts the axis two conditions are made manifest. In one of these conditions the origin of each curve is the zero point of the axes. In general, all curves plotted from temperatures higher than 34° are of this type. In the other condition, the origin of the curves lies at some point on the viscosity axis at a varying distance from the ordinate representing r.p.m. The lower the temperature, the further is the point of interception with the abscissa removed from the convergence point of the axes.

This seems to mean, arguing from the geometry of the graphs, that in those cases where the intercept lies on the abscissa an infinitely small velocity of rotation will result in a viscosity deflection of finite magnitude. That is, the gelatin, under those conditions, offers a permanent and fixed resistance to deformation. It is an elastic body; it possesses a measurable degree of rigidity; and deformation may not occur until after a certain minimum of pressure, exerted against it, has been exceeded. These are, as a matter of fact, the very attributes which are characteristic of plastic substances.

If it were necessary to carry the analogy further we might say that the distance from the origin of the axes to the point of intersection corresponds very closely to, although it is not identical with, the yield value, f, as obtained by Bingham's method. The magnitude of this distance may correctly be taken as a measure of the plasticity of the material.

It will be observed that at velocities of rotation above 60 r.p.m. there is in some cases a slight bending of the curves, away from the velocity axis. That is, at the higher speeds of rotation, the observed viscosity is somewhat greater than should obtain if the lower (straight) portion of the curve may be regarded as most correctly expressive of the true theoretical values. The reason for this bending is undoubtedly to be found in an instrumental error by which eddy currents are set up within the liquid when the velocity exceeds a certain value. There is also very probably produced at the higher velocities a slipping of the liquid along the sides of the cup causing it to move to a greater degree *en masse* rather than with the telescopic shear of a truly viscous flow.

Above a certain temperature (at any given concentration) the curves follow the laws of truly viscous flow, *e. g.*, they converge, when extrapolated to the axes, at the origin. In other words the observed angular deflection is directly proportional to the speed of rotation. At a given temperature (for a given concentration), however, and at all temperatures below this point, the curves follow the laws of plastic flow as above pointed out. Our "solution" of gelatin behaves, therefore, as a viscous liquid

at elevated temperatures, and as a plastic solid at low temperatures (but still above the solidification point).

If we may accept C. R. Smith's conclusions that above 33–35° the sol form only may exist, while below that temperature increasing amounts of the gel form are in equilibrium with the former until at 15° the gel form only is stable, then it seems to follow from the data here presented that gelatin sol is a viscous liquid while very small amounts of gelatin gel are sufficient to impart to the "solution" the property of plastic flow.

The Sol-gel Equilibrium

The writer[7] has already pointed out that gelatin sols appear to consist of molecules which, upon a lowering of the temperature, tend to cohere into aggregates in the form of catenary threads of more heavily hydrated molecules. Further evidence in support of this viewpoint is given in the following paper on "The Structure of Elastic Gels."[8] We will add at this place, however, that the resiliency or elasticity of the jelly seems to be dependent upon the length of these threads. And apparently elasticity in the gel state is synonymous with plasticity in the fluid condition, for, on account of the amicroscopic or ultramicroscopic size of these particles and the short threads characteristic of the sol state, any displacement of them in the fluid would meet with so great a frictional resistance that the property of elasticity, or plastic flow, would be transmitted to the whole mass. This is what is observed when the curve of viscous flow changes to one of plastic flow.

None of the instruments that have been devised for measuring molecular or molecular group elasticity (plasticity) is in any sense absolute; e. g., there is a sensitivity coefficient below which they cease to function. In other words, while we can say definitely that paint for example, is a plastic solid or shows properties of plastic flow, we cannot say as positively that water exhibits no such properties. All that we may say is that, as far as the most delicate sensitivity of our instrument reveals, there is no indication of plastic flow in water. Theoretically there is no reason to believe that liquid water (dihydrol) should not possess intermolecular elasticity.

With the instrument used in the foregoing experiments (the Mac-Michael viscosimeter) the highest temperature at which evidences of plastic flow were observed (in 25% concentration) was about 34°. A more delicate instrument might show this property at a higher temperature. As the concentration of the gelatin solution was decreased, the maximum temperature at which plastic flow was first observed became lower, e. g., about 33° in the 20% concentration, and 29° in the 10% concentration.

[7] Bogue, Chem. Met. Eng., **23**, 62 (1920).

[8] Bogue, This Journal, **44**, 1343 (1922).

This is in entire conformity with the argument presented above; for while it was stated that the plasticity was probably an expression of inter-fibrillar elasticity, and that elasticity seemed to be determined by the length of the fibrils, it also follows, from the limited sensitivity of our apparatus, that the measurability of this property must depend upon the actual concentration of fibrils in the solution, and this is proportional to the total concentration of gelatin in the solution at any given temperature.

Davis[9] has reported that at the temperature of $38.03°$ gelatin sol and gel can exist in equilibrium, while this is not true for any other temperature. That is, a "seeded" solution (one to which a little gelatin gel had been added) showed no change in viscosity with time at the temperature of $38.03°$. At any temperature below this a regular increase in viscosity with time was observed. At higher temperatures a decrease occurred until the viscosity equaled that of a similar unseeded portion at the same temperature. Sheppard[10] has been able to corroborate this value very closely.
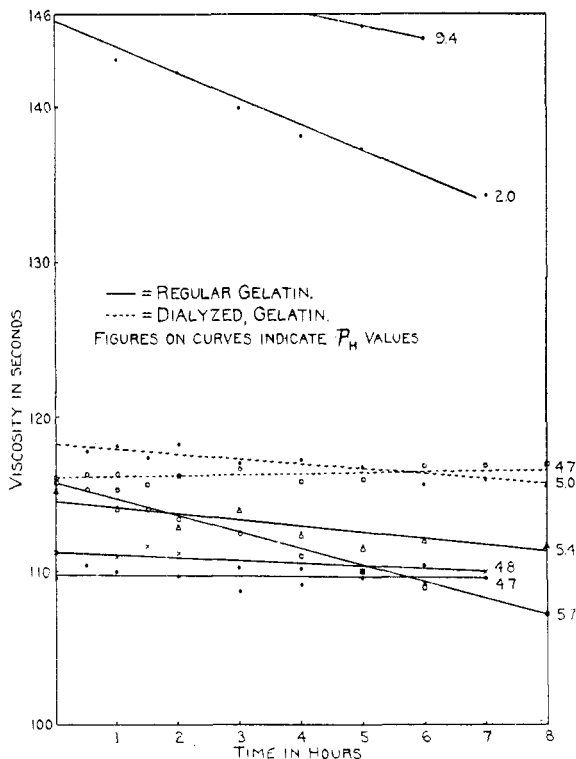

Fig. 5.—Change in viscosity with time at varying P_H; 2% solution, 35°.

[9] Davis and Oakes, THIS JOURNAL, **44,** 464 (1922).

[10] S. E. Sheppard, discussion at 62nd Meeting, American Chemical Society, New York, Sept. 6-10, 1921.

but Loeb[11] has reported that at any temperature above 35°, the viscosity (of a 2% solution of gelatin chloride of Sörensen value, P_H 2.7) *decreases* on standing.

In order to bring more data to bear upon this point a series of experiments was performed with the object of noting the changes in viscosity with time of gelatin solutions of varying hydrogen-ion concentration and of varying gelatin concentration. The varying degrees of hydrogen-ion concentration were obtained by soaking the granulated gelatin in cold hydrochloric acid or sodium hydroxide solutions of the proper strength, for 1 hour, then washing out the excess electrolyte as directed by Loeb, and making up to 2% concentration. Portions of this were also diluted to 1 and 0.5% concentrations and viscosity tests made with an Ostwald viscosimeter at frequent intervals for 8 hours. The temperature was rigidly controlled at 35.0°. The data, shown in part in Fig. 5, indicate a decrease in viscosity with time at every hydrogen-ion value tested, from P_H 2.0 to P_H 9.4, with the exception of the sample at P_H 4.7 in which case there was no change. The sample at P_H 4.8 was "seeded," but no alteration in the slope of the curve was observed. The nearer the hydrogen-ion concentration of the samples to the iso-electric point, the less was the variation in viscosity with time. There was in no case, however, an increase in viscosity with time.

A gelatin that had been purified by dialysis was next subjected to the same treatment, and although the curves were in most instances very similar to the previous ones, yet at P_H 4.7 there was a slight tendency for an increase in viscosity with time as indicated by the dotted curves in Fig. 5. At 37.0° the curve was again horizontal.

The significance of these data is now apparent. There are obviously many factors which influence the effective volume of the gelatin in the solution. Of these the hydrogen-ion concentration seems to be most important. The amount and nature of the inorganic ions with which the gelatin is associated is another. The presence of the hydrolysis products of gelatin is a third factor, and the measurability of these influences will be determined by the concentration. At low temperatures, *e. g.*, 25°, the tendency in the system is for an increase in the size of the molecule aggregates; hence an increase in viscosity with time. At high temperatures, *e. g.*, 40°, the tendency is for a decrease in the size of these aggregates. Hence a decrease in the viscosity with time. At any specific temperature, *e. g.*, 35°, whether the aggregates will become larger or smaller is determined by the hydrogen-ion concentration of the solution, and the presence of inorganic ions and protein hydrolysis products. Under any given set of conditions there will be some temperatures at which neither increase nor decrease will occur. This point was found in gelatins studied

[11] J. Loeb. *J. Gen. Physiol.*, **4**, 107 (1921).

by Davis and by Sheppard to be at about $38°$; in gelatins studied by Loeb to be at $35°$ (in solution of P_H 2.7); and in gelatins studied by the writer to be at $35°$ and $37°$ (in solution of P_H 4.7).

It appears that the colloid fibril consists at elevated temperatures of but a few partially hydrated molecules attached to each other, and float-ing about as discrete particles in the solvent. An increase in viscosity with time would then signify either an increase in the size (length) or number of the threads, or an increased volume due to the absorption of water or hydration. At elevated temperatures, the equilibrium is evi-dently rapidly attained. This seems to be due to the relatively small changes that are induced in the particle size and degree of water absorption or hydration by variations in temperature at *elevated temperatures*, and to the high mobility of the free solvent. But as the temperature falls, the amount of change per unit drop in temperature rapidly increases, and with this is a rapid decrease in the mobility of the solution through the withdrawal of the solvent by absorption or hydration. The time required for the colloidal molecule-fibrils to reach a state of complete equilibrium with the solvent is consequently vastly increased. In other words the solution will show an increase in viscosity with time.

Under any given condition of temperature and hydrogen-ion concen-tration there will be a certain condition of viscosity which the system will attain at equilibrium. A temperature at which no change in viscosity with time occurs indicates an immediate equilibrium condition, but this temperature will vary with different hydrogen-ion concentrations and with different degrees of purity of the sample. It is in no way indicative of a critical equilibrium temperature between the sol and gel forms, but is rather only a point on a continuous curve. This may be expressed by the equation

$$\eta_{P_H} = K/f(T)$$

where η_{P_H} is the viscosity at equilibrium at any given hydrogen-ion con-centration, $f(T)$ is some function of the temperature, and K is a constant. On account of the length of time required to attain equilibrium, and the difficulty of eliminating completely all other influences such as hydrolysis due to the prolonged action of water, electrolytes, or bacteria, the exact measurement of η_{P_H} is uncertain, except where the conditions have been met for the existence of an equilibrium *immediately*. This is the condition encountered where that temperature is obtained at which no change in viscosity with time is observed.

The idea of a gradual rather than an abrupt change on passing from the sol to the gel form is further indicated by the finding of Walpole[12] that the refractive index of a gelatin-water system is a linear function of the

[12] Walpole, *Kolloid-Z.*, **13**, 241 (1913).

concentration, and if plotted against the temperature shows no discontinuity at any point between the liquid sol and the rigid gel. McBain[13] has reported that the sol and gel of soaps differ only through the mechanical rigidity and elasticity of the gel form.

Summary

Experiments have been conducted upon the viscosity-plasticity relations in gelatin solutions which have indicated (1) that gelatin in aqueous solution as measured by the MacMichael viscosimeter follows the laws of viscous flow at elevated temperatures, and that the same exhibits the properties of plastic flow at lower temperatures (above the solidification point); and (2) that the transition between the sol and gel form does not take place at any given *point* of temperature, but rather extends throughout a rather indefinite *period* of temperature.

The variation in viscosity with time was studied and it was found that at a given temperature (35°) the increase or decrease in viscosity with time was dependent upon the hydrogen-ion concentration, the nature of the inorganic ions present, and the amount of hydrolyzed protein in the system. It is urged that the temperature at which no change in viscosity with time is observable may not be taken as a critical temperature between the sol and gel forms, but rather that, given sufficient time under aseptic conditions, there may be conditions reached at any temperature at which there will be no change in viscosity with further lapse of time. The expression, "no change in viscosity with time," means only that the relative volume of the particles of gelatin or gelatin aggregates in the system tends neither to increase nor decrease under the conditions attained. A lowering of the temperature will cause an increase in this relative volume and hence an increase in viscosity, but this increase being attained (after the lapse of time), the viscosity will then again become constant. A rise in temperature will produce the opposite effect. The equation is, therefore, written

$$\eta_{P_H} = K/f(T)$$

indicating that the viscosity of pure gelatin at any given hydrogen-ion concentration is inversely proportional to some function of the temperature, and that, at equilibrium, there will be some viscosity (at any given hydrogen-ion concentration) which will correspond with every point of temperature.

The conclusions indicate that there is no sharp transition point between the sol and gel forms in protein systems, but that the transition is continuous and reversible over a somewhat indefinite period.

PITTSBURGH, PENNSYLVANIA

[13] Laing and McBain, *J. Chem. Soc.*, **117**, 1506 (1920).