[CONTRIBUTION FROM THE MASSACHUSETTS INSTITUTE OF TECHNOLOGY, THE HARVARD MEDICAL SCHOOL, AND THE UNIVERSITY OF WISCONSIN]

Size Distribution in Gelatin Solutions.[1] Preliminary Report

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In our studies of the physico-chemical behavior of solutions of degraded gelatin proposed as transfusion fluids, we have derived the following simple picture of the nature of such solutions, which explains their behavior to a good approximation. Collagen consists of long chains of polypeptide residues. The bonds between these residues are usually hydrolyzed at about the same rate, but there are a very few bonds equally spaced along the chain which hydrolyze very much more rapidly. In the preparation of gelatin, nearly all the more reactive bonds and a small fraction of the less reactive bonds are hydrolyzed. The degradation of gelatin consists largely in the hydrolysis of the less reactive peptide bonds. There is thus an ideal parent undegraded gelatin molecule, which is the length of chain between two reactive bonds. Real gelatin consists of a mixture of such molecules with the products of their degradation, which include every possible peptide from single amino acids to chains containing only one less residue than the parent molecule.

If p is the number of amino acid residues per undegraded molecule, α is the fraction of the less active bonds which are hydrolyzed, and c_i is the weight fraction of the molecule with i residues, the weight fraction of unchanged molecules ($i = p$) is $c_p = (1 - \alpha)^{p-1}$ and the weight fraction of the ith species ($i < p$) is $c_i = [2\alpha + (p - i - 1)\alpha^2](1 - \alpha)^{i-1}\, i/p$. The first term in square brackets is the probability of a link being broken i units from either end, the second term is the probability of two links i units apart being broken, and $(1 - \alpha)^{i-1}$ is the probability that the intermediate links are all unbroken.[2]

The number average molecular weight is $M_n = M_0 (\Sigma c_i)/(\Sigma c_i/i)$, if M_0 is the average residue weight,[3] the weight average molecular weight is $M_w = M_0 (\Sigma c_i i)/(\Sigma c_i)$, and the Z average molecular weight is $M_z = M_0 (\Sigma c_i i^2)/(\Sigma c_i i)$. The sum Σc_i is obviously unity, and $M_0/M_n = \Sigma\, c_i/i = \alpha + (1 - \alpha)/p$ is the number of molecules per amino acid residue. The other sums are obtained by graphic summations, as is the sum $\Sigma c_i H_i = H_0$, in which H_i is the intrinsic viscosity of the ith species. M_n is determined by extrapolating to zero concentration the ratio of concentration to osmotic pressure. For the gelatins we have studied M_n/M_0 varies from 0.002 to 0.007.

The rate of hydrolysis, $d\alpha/dt$, is proportional to $(1 - \alpha)$, and as long as α is small it is practically independent of the time, at least in neutral solutions. The variation of rate with temperature yields an energy of activation of 23,000 calories.

M_s, which is directly determined from the distribution in the equilibrium ultracentrifuge with measurements of refraction, together with the number average molecular weight, permits the calculation of p and α individually. Since p is independent of the degradation, a single measure of the Z average molecular weight is sufficient. For bovine ossein gelatin we find $p = 1170$, so α varies from 0.0012 to 0.0054. Our picture explains quantitatively the details of the distribution in the equilibrium ultracentrifuge. The ultracentrifuge equilibrium was measured in 2 M potassium thiocyanate, in which the intrinsic viscosity and the number average molecular weight are the same as in 0.15 M sodium chloride.

For such very asymmetric molecules, the sedimentation rate in the ultracentrifuge depends upon the cross-section and only very slightly upon the length.[4] These measurements give a cross-sectional diameter of about 17 Å. for gelatin, nearly independent of the degradation.

The intrinsic viscosity, H_0, is most easily obtained as the limit of $(1/c)\ln(\eta/\eta_0)$ as c approaches zero; η is the viscosity of the solution and η_0 that of the solvent. The intrinsic viscosity is proportional to the number average molecular weight for these solutions. The cross-sectional diameter may also be calculated from the intrinsic viscosity using H_i values from the viscosity theory of Simha[5] and our size distribution of the molecule. At a pH about 7 in 0.15 molal sodium chloride, the viscosity corresponds to a diameter of 17 Å. and to a length of 800 Å. for the undegraded molecule with 1170 units. At a lower pH, the viscosity is somewhat smaller, indicating a slight flexibility which disappears as the charges increase at the higher pH.

Our picture is also consistent with measurements of the double refraction of flow,[6] which indicate very little orientation in thiocyanate solutions even at high gradients and high solvent viscosities, and therefore very few, if any, molecules longer than 1000 Å. There is appreciable double refraction in 0.15 molal sodium chloride due to aggregation, even at temperatures at which the gel rigidity is zero.

In Table I are listed "undegraded" gelatin, and gelatin of four stages of degradation which have been proposed for transfusion fluids. For each is given the fraction of bonds in the parent molecule which are broken, α; the weight fraction of unchanged parent molecules, c_p; the number average molecular weight, M_n; the ratios of the weight average, M_w, and the Z average, M_z, to the number average minus one,

(1) Presented before the Division of Physical and Inorganic Chemistry, American Chemical Society, September 12, 1944. These studies were made at the request of the Subcommittee on Blood Substitutes of the Division of Medical Sciences of the National Research Council, and partly under contracts recommended by the Committee on Medical Research between the Office of Scientific Research and Development, and Harvard University and the University of Wisconsin.

The gelatine was obtained through the courtesy of Dr. D. Tourtelotte of the Knox Gelatine Co.

(2) The mathematical treatment of the distribution function is given by E. W. Montroll and R. Simha, *J. Chem. Physics*, **8**, 721 (1940).

(3) The average residue weight is taken as 94 from E. O. Kraemer, *J. Phys. Chem.*, **45**, 660 (1941).

(4) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Oxford, 1940, pp. 434–435.

(5) R. Simha, *J. Phys. Chem.*, **44**, 25 (1940).

(6) J. T. Edsall and J. F. Foster, unpublished.

and the intrinsic viscosity, H_0. The values of M_n and H_0 are experimentally determined, as is M_z for the first solution. The other quantities are calculated on the basis of our picture.

TABLE I

PHYSICO-CHEMICAL CHARACTERISTICS OF DEGRADED GELATINS

	α	c_p	M_n	$M_w/M_n - 1$	$M_z/M_n - 1$	H_0 at 55°
A	0.00120	0.244	45,700	0.61	0.92	0.47
B	.00175	.129	36,000	.73	1.17	.36
C	.00227	.071	30,000	.81	1.38	.31
D	.00409	.008	19,000	.92	1.59	.18
E	.00540	.002	15,000	.96	1.82	.14

The quantity $(M_z/M_n - 1)$ is a good measure of the size spread. If all the molecules are of the same size, it is zero, and for random splitting of an infinitely long chain, it becomes two. The ratio $(M_w/M_n - 1)$, which increases from zero to one, could also serve. As the fraction of parent molecules decreases, the size spread increases to approach that which would be obtained from infinitely long parent molecules.

Figure 1 shows the size distribution in another way for the same five solutions. The abscissas are molecular weight and the ordinates are the weight fraction of the solute with molecular weights greater than the abscissa, calculated by summing weight fractions from c_{i+1} to c_p. The length of the ordinate at the end of the curve is c_p. For all the curves, about 25% of the material has a molecular weight less than the number average,

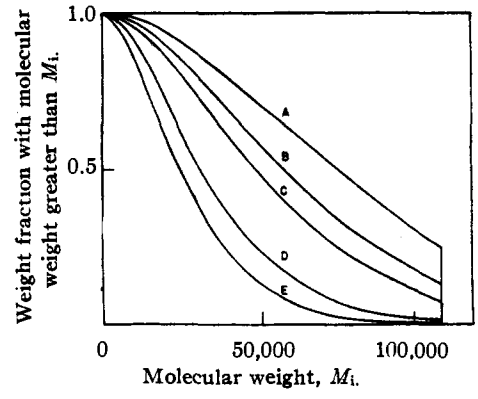

Fig. 1.

and about 40% has a molecular weight greater than twice the number average. If the abscissas were given as multiples of M_n, the curves would be very similar except that each would be lopped off at a different point.[7]

Our studies of calfskin and porcine gelatins are as yet less complete. They show no great difference from ossein gelatin. However, we have not yet measured any quantity which depends greatly upon the size of the parent molecule.

(7) A similar picture, which assumes parent molecules so long that they could be taken as infinite, is inconsistent with the measurements of ultracentrifuge equilibrium and double refraction of flow in thiocyanate solution but explains successfully the practically important quantities such as the rate of hydrolysis, the viscosity, sedimentation and diffusion.

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NOTES

m-Trifluoromethylphenyllithium and its Addition to Some Quinolines

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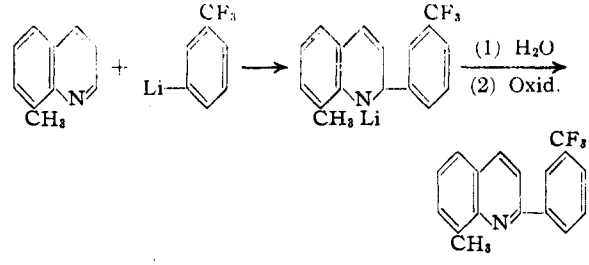
In connection with the pharmacological examination of some fluorine-containing heterocycles, a need arose for a reactive organometallic compound containing a trifluoromethyl group which would add directly to the azomethylene group of heterocycles like quinoline.

m-Trifluoromethylphenyl bromide does not react under conventional conditions with metallic lithium. However, the desired organolithium compound is conveniently prepared by a halogen-metal interconversion reaction with *n*-butyllithium.

m-$CF_3C_6H_4Br$ + *n*-C_4H_9Li → *m*-$CF_3C_6H_4Li$ + *n*-C_4H_9Br.—It is interesting to note that the smooth formation of a Grignard reagent[1] from *m*-trifluoromethylphenyl bromide is another illustration of the complementary use of magnesium and lithium: some RX compounds which do not react smoothly with one of these metals do react with the other.[2]

The resulting *m*-trifluoromethylphenyllithium adds to the azomethylene group in quinoline and 8-methylquinoline to give the corresponding 2-trifluoromethylphenyl derivatives in satisfactory yields.



This supplements a procedure described recently[3] in which an organolithium compound was

(1) Simons and Ramler, THIS JOURNAL, **65**, 389 (1943).

(2) Gilman, Zoellner and Selby, *ibid.*, **55**, 1252 (1933).

(3) Gilman and Blume, *ibid.*, **65**, 2467 (1943).