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Concentrated Macromolecular Solutions

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DILUTE solutions were covered in the papers of Rice (p. 69), Stockmayer (p. 103), and Doty (p. 61). This paper first discusses concentrated solutions in contrast to dilute solutions. Many properties in concentrated solutions are determined by the fact that the interactions between the molecules are of primary importance, whereas they are of secondary importance when the dilution is high.

In most macromolecular solutions, one may never neglect completely the interactions between the large molecules. The nearest exception to this is probably solutions of the globular proteins at high ionic strength and at reasonable concentrations—i.e., concentrations in which it is quite easy to do physical experiments, such as the determination of intrinsic viscosity, and so on. In any solution, interactions can be ignored fairly well, providing highly precise measurements are not desired. As soon as extended particles—like fibrinogen, or especially the nucleic acids, or the randomly coiled polymers such as the synthetic ones, or starch or other polysaccharides—become involved, it is practically impossible to make them sufficiently dilute to eliminate the interactions between the molecules, and, at the same time, to have enough material remaining in solution to measure by the standard techniques. In these cases, it might be said that one is dealing always with concentrated solutions.

For practical purposes, the interaction can be characterized by the following. The osmotic pressure *versus* the concentration is plotted. The ideal-solution laws predict that the osmotic pressure divided by the concentration should be practically constant. The van't Hoff law says that the osmotic pressure is proportional to the concentration. For an ideal solution, one obtains, then, the curve *a* in Fig. 1. Most of the time, however, curve *b* is obtained. As soon as the curve has deviated from the ideal law by about as much as the ideal law itself predicts, one has a concentrated solution.

This upward, or positive, deviation usually results from repulsion between the molecules, and the fact that the molecules are large means that there are unavoidably a lot of repulsions just from their space-filling properties. This is the common type of behavior in a macromolecular solution; in effect, the molecules elbow each other apart. There is the same type of behavior in the theory of imperfect gas where one must consider the "co-volume" of the molecules. The pressure is raised in consequence.

A similar effect is observed on the scattering of light. If there are these repulsive interactions, the fluctuations of concentrations can not take place as easily or as independently of each other, and with less fluctuation there is less scattering of light.

These are manifestations of the intermolecular forces. There is one trap into which the uninitiated easily fall, and that is to consider these intermolecular forces as acting as though the molecules were in a vacuum. The molecules are in a solvent, and the forces between the molecules and the solvent molecules and other solvent molecules are as important as the direct interaction between the macromolecules. What is seen here actually is a balance of forces.

Figure 2 shows some data obtained by Doty and Yang[1] on polybenzylglutamate. The fraction of internal hydrogen bonds in a molecule are shown. When they are all intact, the helical form results, and when none of them are intact, the random-coil form results. The transition region between these two forms depends upon the solvent composition, and this particular solvent is a mixture of dichloroacetic acid and dichloroethane. Taking the transition temperature as the reference point, it is seen that, when the temperature is reduced, one goes to the unbonded form. If the temperature is raised, one goes to the bonded form. It is incorrect to consider solely the interactions between parts of the macromolecule itself because, in that case, an increase in temperature should melt it— that is, break the bonds. In fact, just the opposite is done in raising the temperature; the bonds are formed. Obviously, there is competition between the bonding to the solvent and the internal bonding of the macromolecule. The solvent here actually is the determining factor. In fact, the entropy of this transition has the wrong sign. Instead of the entropy of the random coil being the greater and that of the helix the lesser, the reverse is true. The helical

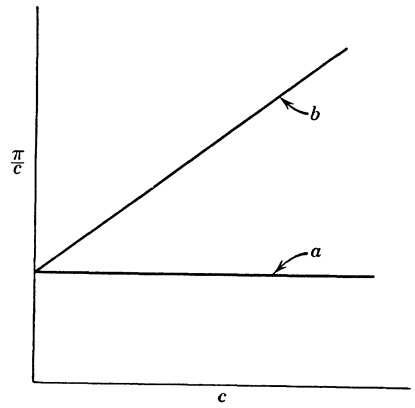


FIG. 1. Osmotic pressure π as a function of concentration c for real and ideal macromolecular solutions. See text.

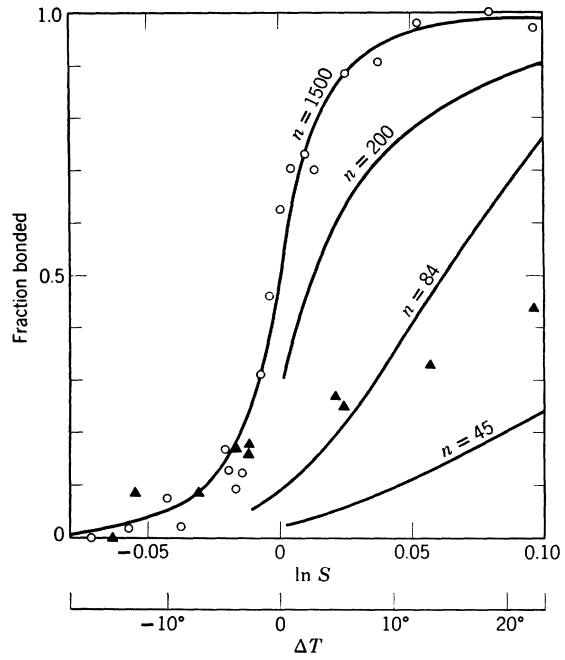


FIG. 2. Fraction of intramolecular hydrogen bonds in poly-γ-benzyl-L-glutamate; points calculated from optical-rotation data of Doty and Yang[1]; the curves represent an unpublished theory developed by J. K. Bragg and the author.

form is the one that is stable at high temperatures and the entropy of the helix is greater and that of the random coil is less. This means that the solvent is participating and that there is considerable entropy change in the solvent. This illustrates the pitfalls possible when the solvent is overlooked.

Consider highly concentrated solutions, such as 10 to 50% (or possibly more) dissolved material in solvent. What can be said about them? The kinetics of such systems are treated in the paper by Ferry (p. 130). The concern here is with statics. One of the first items of interest is, "What is the absorptive capacity of such solutions?" They can be called gels, for convenience. What then is the absorptive capacity of gels for foreign solvents, such as ions, etc.? For gels composed of long-chain macromolecules, the thermodynamic behavior with respect to the absorption of solvent is described by a theory advanced by Flory, Huggins, and Guggenheim.[2,3] The derivation contains numerous obviously wrong assumptions, over-simplifications, and so on; however, since it is a classical theory, one need say little about it here. If one simply wants to calculate an absorption isotherm, it is useful. It has an adjustable parameter that allows one to make it work fairly well in many different cases.

Another point of view should be mentioned. As shown in Fig. 3, one can plot the volume fraction of a solute ϕ_1 as a function of the activity of the solvent a_1 ,defined in such a way that, in a pure solvent, a_1 has the value of unity. The activity a_1 is really little more than the relative vapor pressure—the vapor pressure of the solvent in the mixture over that of the solvent. To be very careful, gas-law corrections and such things must be included.

An ideal solution should give the perfectly straight diagonal of curve *c*. This shows the activity of the small molecule component proportional to the volume fraction. Raoult's law actually was stated in terms of mole fractions, but if it is modified slightly, it states that the ideal solution follows curve *c*. However, a real macromolecular solution usually gives something of the type of curve *d*. Sometimes the curve is more complicated, as is shown later.

Two characteristics are to be noted. At the upper right-hand corner, the slope of curve *d* is, as is usual, very much smaller than the ideal one. This is merely a manifestation of the repulsive interaction mentioned before, i.e., a positive deviation. The osmotic pressure is higher; the vapor pressure, therefore, also is higher in dilute solution than in the ideal case. At the lower left-hand corner, curve *d* usually rises much more steeply than the ideal. This slope, in cases where there are no specific interactions, is of the order of three to five times that of the ideal.

There is a very simple interpretation for this marked steepness in slope. Malcolm Dole probably was the first to apply it to any extent.[4] It is based upon the proposed mechanisms for rigid absorbing systems, like clays, inorganic catalysts, carbonblack, etc. It says that, in the absorbing material—in this case, the dry polymer—there are a number of sites which can take up the solvent, and that the rate with which the activity rises is an inverse function of the number of sites. If there is a large number of sites, then much absorbed material can be taken up without raising the activity very much. If there are a very few sites, however, they will fill up very fast, and the activity will rise much more rapidly than the volume fraction.

A mixture of two pure liquids, toluene and benzene, gives the straight line, curve *c* in Fig. 3. The number of

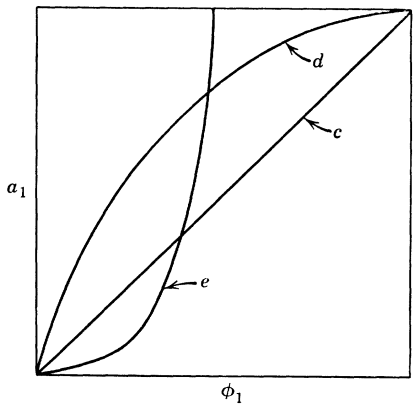


FIG. 3. Activity a_1 against volume fraction ϕ_1 of solvent sorbed in a high polymer. See text.

sites in the mixture apparently increases in proportion to the amount of toluene put in, with one site available for every molecule added. The whole volume remains accessible to these molecules and curve *c* results.

The macromolecular system is different. There are not nearly as many available sites in which to put solvent molecules; there appear to be about one-fourth as many. This suggests a very simple and reasonable hypothesis. Figure 4(a) shows the absorption of toluene in benzene. The benzene molecules can be elbowed out of the way, presenting little resistance to the added toluene, since the whole volume is accessible to its admission.

For macromolecules, the volume initially consists of intertwined chains [Fig. 4(b)]. If a toluene molecule is introduced at position *a*, a long cavity will have to be made with considerable wasted space at its ends, since the macromolecular chains are quite rigid. Therefore, the toluene molecule enters position *b* preferentially where it can cause with greater ease the necessary rearrangements in the neighboring macromolecules. It appears obvious that material of this kind is relatively unreceptive to small molecules; it can fit them into certain specific places only.

One cannot predict the number of specific places, but the experiments quoted in the following indicate that about one-fourth of the volume is occupied by sites into which a small molecule easily can be introduced. This discussion refers to unactivated absorption where no strong specific forces are involved. An example is toluene in polystyrene where the forces are fairly well in balance.

In systems permitting hydrogen bonding, specific interactions are likely, with pronounced deviations resulting (curve *e*, Fig. 3). This curve, which is typical of water absorbed in protein, starts off with a very low, rather than a high, slope, indicating that some of the sites are very receptive to the water molecules. After these specific places have been filled up, the curve rises rapidly. It even may come up and hit the axis, which means that the protein is saturated and cannot take up any more water.

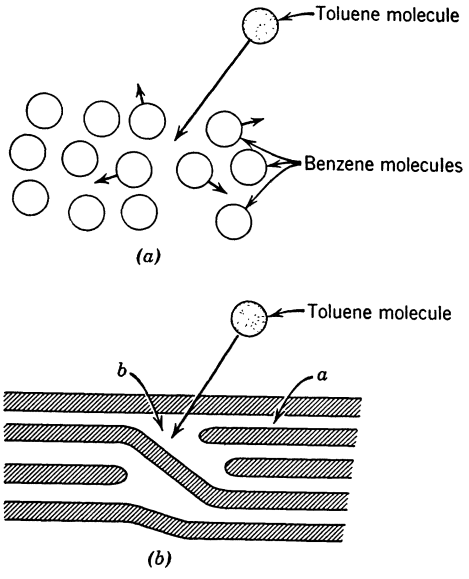


FIG. 4. Sorption of a small molecule, toluene, (*a*) in an ordinary liquid and (*b*) in a high polymer.

In this case, there are a few very specific active sites as compared with the previous case in which there are many inactive sites. But, when one attempts to apply the simple site hypothesis quantitatively, it is not very successful, because the manner in which the absorbed molecules affect the protein has been ignored. Apparently, the protein rearranges its state considerably, and it is difficult to describe the situation in simple terms. The whole concept of an inert absorptive site loses its applicability.

There is, however, another approach to this problem.[5] Define a function that measures the tendency of the absorbing molecules to cluster. This function, the "clustering function," has an exact molecular definition. It is given by an integral,

$$G_{11}=(1/V)\int\int[F_2(i,j)-1]d(i)d(j),$$

where i and j are molecules of component 1, the solvent; V is the total volume; and $F_2(i,j)$, the distribution function, is defined by the statement that $(1/V^2)F_2(i,j)d(i)d(j)$ is the probability that the molecules i and j are each at the positions specified by the coordinates (i,j) in the range of these coordinates $d(i)$ and $d(j)$. This distribution function is familiar in the case of spherical molecules where it is called the radial distribution function. It is simply the probability of finding one molecule in a given position with respect to another.

The quantity $\phi_1 G_{11}/v_1$, where v_1 is the molar volume of the solvent, is the mean number of solvent molecules *in excess of the random expectation* in the neighborhood of a given solvent molecule. Thus, it measures the clustering tendency of solvent molecules, and, for this reason, G_{11} is called the clustering function. If G_{11} is positive, the solvent molecules cluster; if it is negative, as frequently happens, they do not.

It should be noted that this clustering function has a simple and exact relation to the isotherm. There are no assumptions except for a very minor approximation of neglecting the compressibility of the system. The relation is as follows:

$$\frac{G_{11}}{v_1}=(\phi_1-1)\frac{\partial}{\partial a_1}\left(\frac{a_1}{\phi_1}\right)-1.$$

Similar relations were known to Willard Gibbs,[6] but they were neglected until interest was revived recently by Mayer[7] and by Kirkwood[8] and their collaborators.

The clustering function can be determined from the isotherms, and they immediately reveal whether the

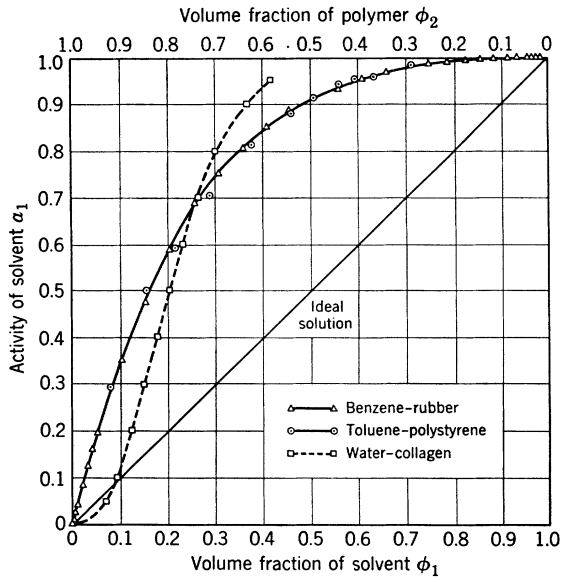


FIG. 5. Activities of solvents *vs* volume fractions of solvents and polymers for benzene-rubber, toluene-polystyrene, and water-collagen at 25° [from B. H. Zimm and J. L. Lundberg, J. Phys. Chem. **60**, 425 (1956)].

absorbing molecules tend to crowd into a few sites, or whether they tend to occupy isolated sites and prevent other molecules from entering. This would depend upon whether G_{11} is positive or negative, and upon how much so.

Figure 5 shows the data for three systems, two of which are simple nonpolar systems. The heavy solid curve represents the activity of the solvent in the systems benzene-rubber and toluene-polystyrene, in which the intermolecular forces are neither specific nor strong. The two curves lie almost on top of one another. The initial slope is quite high, which indicates that the number of sites available for the absorption of the incoming solvent molecules is much smaller than it would be in a mixture of two ordinary liquids. A water-collagen system exhibits another type of behavior.[9] First, there is a region of very strong attraction for the first few molecules of water absorbed, with the water hardly showing any vapor pressure. Then, after the initial sites are filled, the activity rises very rapidly until it is seen to come up to that of liquid water. Dole and McLaren[9] found that the first region has a strong negative heat of absorption; that is, heat was evolved to the amount of about 6 kcal/mole of water in the region of ϕ_1 less than 0.1.

Figure 6 shows the clustering function for these same systems. A clustering function of zero would mean no interactions at all between the molecules. The ideal solution has G_{11} equal to -1. In the ideal solution, there are interactions; the molecules still have volume. One molecule cannot be placed on top of another, so the clustering function is -1; in other words, the first molecule excludes one molecular volume to the other molecules. For the hydrocarbons, a positive clustering tendency appears. G_{11} is fairly uniformly $+1$ for all values of ϕ_1. This means that the molecules of the liquid tend to cluster in the polymer, which is probably a manifestation of the same geometrical problems described in Fig. 4(b). It is not easy for the polymer to make room for the liquid molecules, but when one has been admitted and a cavity is opened, it is apparently easier to put in other liquid molecules by extending the same cavity than by opening up new cavities. Therefore, there is this mild clustering tendency which is not a manifestation of any special attractive properties between the benzene and rubber or between the toluene and polystyrene. Actually, in the former case, the heat of absorption is mildly negative, and, in the latter case, it is mildly positive. This contrast leads to the tentative conclusion that G_{11} is affected more by the geometry of the packing than by the heat of sorption.

The Flory-Huggins-Guggenheim theory predicts values of G_{11} of less than unity for the systems previously referred to. In general, it underestimates the amount of clustering. In other words, it does not take sufficiently into account the intrinsic heterogeneity of the mixture. This, in fact, is very difficult to do.

The water-collagen system presents a different picture. Initially, the clustering function is markedly negative. The first experimental point seems to be at about -8, and the curve is very steep. This means that the first molecule of water that is adsorbed excludes at least eight times its own volume to other molecules. One can interpret this as meaning that the first molecule occupies a special site, and that there are no other special sites

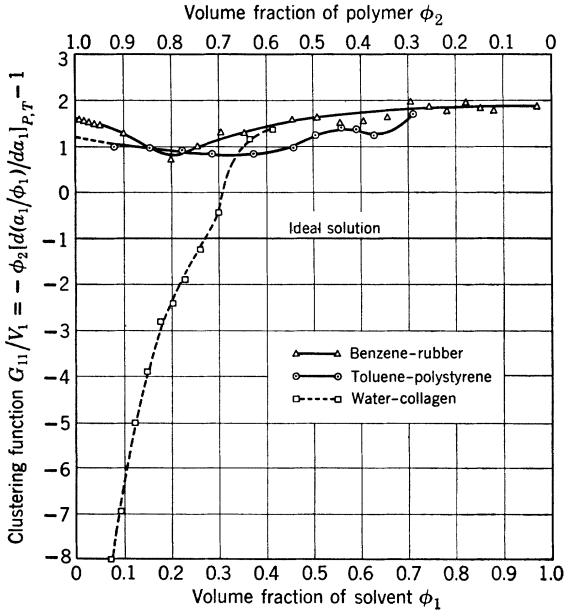


FIG. 6. Clustering functions of solvents *vs* volume fractions of solvents and polymers for benzene-rubber, toluene-polystyrene, and water-collagen at 25° [from B. H. Zimm and J. L. Lundberg, J. Phys. Chem. **60**, 425 (1956)].

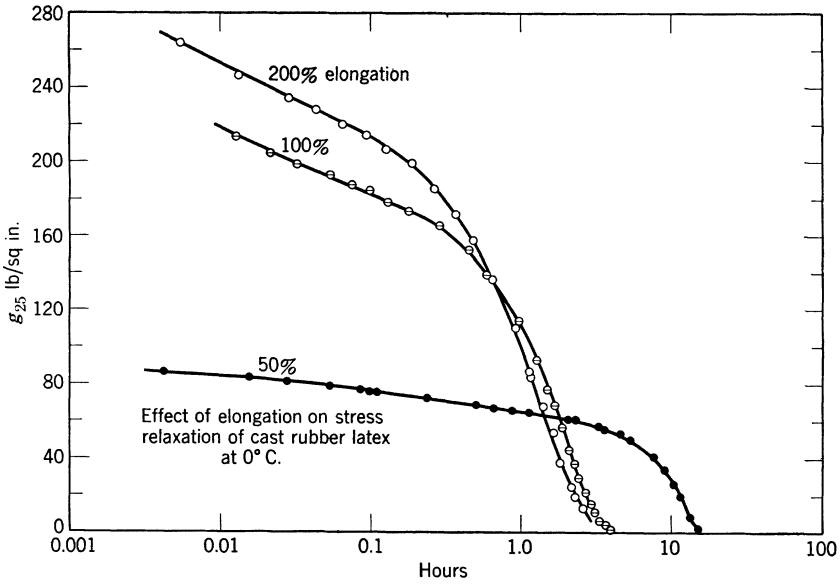


FIG. 7. Crystallization of stretched rubber [from A. V. Tobolsky and G. M. Brown, J. Polymer Sci. **17**, 547 (1955)].

within at least the cube root of eight molecular diameters around. Other molecules that enter will have to find a site elsewhere. After these special sites are filled, the system behaves more normally, and just before the collagen is saturated with water, it acts like an ordinary unspecific high polymer in which the water molecules show a mild tendency to cluster.

From the curve of the clustering function, it is apparent that the initial excluded volume for water on collagen is at least 10 water volumes, or 180 ml, which amounts to less than 0.8 mole of absorption sites per 100 g of collagen. By use of the Langmuir isotherm, Dole and McLaren estimated about 0.6 mole,[9] which seems to be reasonable agreement in view of the fact that the curve is still climbing steeply at the lowest point. If the Langmuir isotherm is used, one makes special assumptions, whereas, with the clustering function, none are made.

Consider now the elasticity of macromolecular systems. Most concentrated macromolecular solutions are not simple liquids. They show some kind of structural elasticity or structural viscosity. The following discussion is restricted to the equilibrium manifestations. [For further discussion, see Ferry (p. 130).] Rubber is classically the best-known example. When a rubber band is stretched, it warms up; the work of stretching is converted into heat. In contrast, when a steel spring is stretched, there is practically no thermal effect; in steel, the work of stretching is converted into internal energy. The obvious explanation is that in rubber the coiled macromolecular chains are straightened and their entropy is reduced. If this is done reversibly, an output of heat must result. Alternatively, the retractive force of the stretched rubber band is caused by the thermal vibration of the macromolecular chains which try to return to a state of increased entropy.

This is the entropic theory of rubber elasticity, and it works very well at elongations of less than 200 to 300%. At small elongations, the change of entropy accounts for nearly all of the retractive force. This is discussed by Flory.[2]

Beyond about 300% elongation, the material stiffens rapidly and the internal energy contributes heavily to the retractive force. The energy of extension becomes negative, which means that there is a decrease in internal energy on extension. With further extension, the entropy also becomes more negative than theory predicts. This marked decrease in entropy and energy is suggestive of the decreases that occur during crystallization where the molecules are ordered with the result that the entropy decreases; at the same time, they are placed in energetically favorable configurations so that the energy decreases also.

Direct evidence for this is shown in Fig. 7 from work by Tobolsky and Brown.[10] It is known that rubber does crystallize, since x-ray patterns are obtainable. If rubber is stretched at 0°C, which is well below the freezing point for the initially amorphous rubber, the retractive force decreases with time; the curve approaches the axis. (The apparatus is incapable of measuring a negative retractive force. In many cases, the rubber band extends spontaneously.) The macromolecular chains, which initially are lined up somewhat by the stretching, are crystallizing now into an even better linear array, and the band extends.

At present, polyethylene thread is available which, when stretched, oriented, and then cross-linked by irradiation, exhibits a striking phenomenon. If heated, it melts and contracts about 10 to 20%. When cooled and frozen at about 125°C, it extends again spontaneously. This phenomenon is the result of crystallization of oriented chains. A similar process occurs in biological

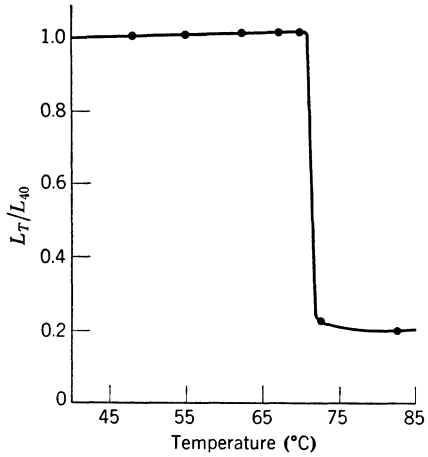


FIG. 8. Length as a function of temperature for formaldehyde-tanned rat-tail tendon under a constant small load [from P. J. Flory, J. Cellular Comp. Physiol. **49**, Suppl. 1, 175 (1957)].

materials, for example in collagen. Figure 8 illustrates some interesting data obtained by Dumitru, and discussed by Flory.[11] Collagen, normally, is in crystalline form, but, if heated to its melting point, it suddenly contracts. Unfortunately, when annealed below its melting temperature, collagen does not re-extend very markedly on cooling, although the density does go up somewhat. This striking change of length accompanying the melting of the oriented crystal is similar to the phenomenon observed in rubber.

A similar interesting calculation can be made for α-helices. Instead of plotting the usual stress-strain diagram with length as a function of force, two convenient dimensionless variables can be introduced. In place of length L, substitute $L/n\delta$, where n is the number of residues in the chain and δ is the length along the helix axis of one residue. Similarly, in place of force f, substitute $fb^2/3\delta kT$, where b^2 is the square of the effective length of a residue in the random form; that is, b^2 is the mean-square end-to-end length divided by n. These variables are plotted in Fig. 9.

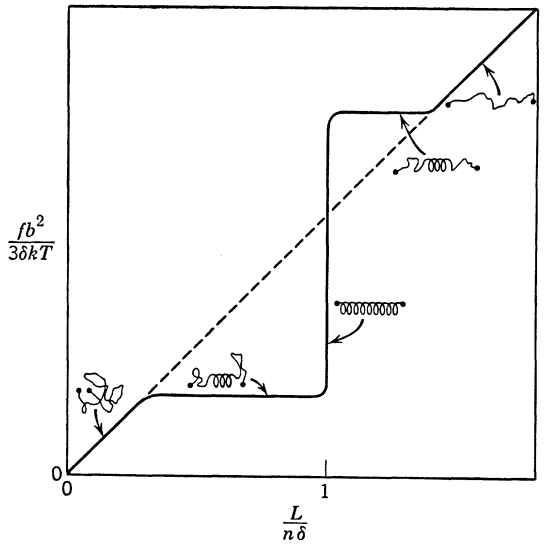
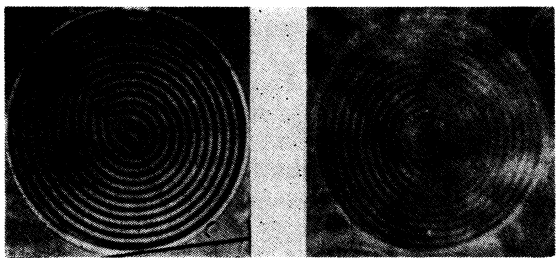


FIG. 9. Hypothetical stress-strain diagram for a polypeptide chain that can "crystallize" into a helix on stretching. See text for symbols.

Suppose that the material initially is in the random form. The stress-strain curve is linear and somewhat simpler than the curve for rubber, because only one molecule is considered rather than an array of molecules with different orientations. Initially, the slope of the curve is unity. Upon stretching, the helical form is favored because the helix is oriented in the direction of stretch. The coil-to-helix transition commences with an essentially two-phase situation. In this situation, considerable change in length results from a small change in force, since the proportion of helical to random form rapidly increases. Upon complete conversion into an α-helix, the structure becomes very rigid, and large changes in the force produce little change in length. As



(a) (b)

FIG. 10. Two spherulites of an anisotropic solution. (a) Large regular spherulite, showing radial fault. *R*1 in methylene chloride; spacing about 0.015 mm×154. (b) Large regular spherulite, fault not visible. Phase contrast; *R*1 in methylene chloride; spacing about 0.015 mm×131 [from C. Robinson, Trans. Faraday Soc. **52**, 571 (1956)].

illustrated in Fig. 9, the 45° line is crossed, and the random form represented by the dashed diagonal line would extend more readily than the helical form in the direction of stretching. Thus, the random form again becomes more favorable, and a second transition from the helical to the random coil occurs. As shown in the diagram, both of the horizontal regions of the curve (the transition regions) represent a mixture, with a segment of random coil followed by one of helix. There is probably always a random segment at the end, because of the disordering effect of the end.

An additional subject of interest is that of liquid crystals. Many macromolecular solutions contain a considerable amount of order. Tobacco mosaic virus particles are random in dilute solution, but, in a more concentrated form, the solution is intrinsically anisotropic and exhibits colors between crossed polaroids. Its x-ray diffraction pattern consists of arcs of circles. This pattern originally was investigated by Bernal and Fankuchen.[12] There are also many mixtures of soaps

and water and of various lipids in water which have similar properties. There is, in fact, a large and rather neglected field of what are generally called liquid crystals, or crystalline liquids, or *mesophases*—different names for the same phenomenon.

Figure 10, a recent photograph taken by Conmar Robinson,[13] shows a liquid crystal, a drop of a solution of polybenzylglutamate in methylene chloride. The concentration is roughly 20% by weight. In this solvent, the molecules are in the α-helix form, and the presence of the long extended structures promotes formation of liquid crystals. In a concentrated solution, the liquid crystals separate and form small drops of a separate phase. Figure 10 illustrates one of the drops surrounded by the ordinary isotropic solution. The diameter of the drop is approximately 300 μ. Each illustrated layer is about 10 to 15 μ thick. This onion-like structure is remarkable. In Fig. 10(a), one sees a single spiral with something of the nature of a stem, or radial fault, on one side. Looked at from a different angle, Fig. 10(b), the stem radius is not apparent; instead, a double spiral is seen. The structure is very striking. For further reference, the reader is referred to Robinson[13] and to papers by Robinson and Frank.[14] Onsager[15] and Flory[16] have proposed quantitative theories which try to explain the formations of the liquid crystal as resulting from the anisotropic interaction between long rod-like molecules. These theories indicate very nicely the essential feature that, if too many long rods are packed in a given space, they pack very poorly unless they are aligned; if aligned, they can be packed more economically. Hence, a concentrated solution of rod-like molecules tends to form liquid crystal structures, but, if diluted, isotropic structures occur. In fact, phase boundaries can result between two solutions of the same materials at different concentrations.

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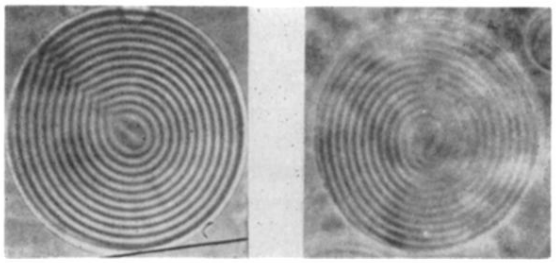

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