

be solved by standard integration methods, if the radical concentrations are not too low, as Duff did for the H_2-O_2 system (quoted in Emanuel's ref 5). Nevertheless, this calculation was said to require 5000 iterations. Under conditions such that the free-radical concentrations are lower, the number of iterations will increase rapidly.

Emanuel's ref 6-9 concern mathematical techniques for speeding numerical solution of differential equations. Techniques such as forward difference schemes, instability analysis, etc., can speed such calculations by a factor of perhaps 10 or 100, while there are practical problems in chemical kinetics where the calculation speed of standard methods is too slow by a factor of 10^6 , even with the fastest computers. In these cases, the steady-state hypothesis is the answer. My main contribution was to demonstrate a method of applying it in general for any reacting system where it is needed, and to analyze the situations where it is useful.

Emanuel's remark that my paper gives only the constant-temperature case is irrelevant, since methods of generalizing are known.

Emanuel's final comments concern the validity of the steady-state hypothesis. His ref 10 by Libby concerns possible errors in assuming "a partial equilibrium or steady-state treatment of those reactions with specific rate constants considerably higher than the others involved in the series." The comment by Kollrack gives essentially the same information as Libby's publication. Libby essentially says that conservation of matter is satisfied as long as one calculates the extent of each reaction in a mechanism according to the standard rate equations. However, if one abandons some of the rate equations and calculates some components by means of equilibrium expressions, then changes are made in the amounts of certain components without regard to the stoichiometry of the reactions that produce them. He then formulates equations to take into account both equilibria and stoichiometry.

This argument is answered in my paper: the steady-state assumption is valid only when the components that are to be determined from other-than-stoichiometric equations (the free radicals) are present in such small quantities that neglect of the principle of conservation of matter applied to them will introduce negligible error in the amounts of other components present. Libby's warning is not important under the conditions for which the steady-state assumption is valid.

Incidentally, the standard rate expressions can lead to error in numerical integration. An example is the pyrolysis of ethane, where the forward and reverse chain-propagating steps approach equilibrium, and the

difference is beyond the number of significant digits carried by the computer. Libby's method should be of use in this problem.

The work of Giddings and Shin on the hydrogen-bromine system¹ should have been mentioned in my paper.

(1) J. C. Giddings and H. K. Shin, *J. Phys. Chem.*, **65**, 1164 (1961); *J. Chem. Phys.*, **36**, 640 (1962); *Trans. Faraday Soc.*, **57**, 468 (1961); *J. Chem. Phys.*, **39**, 2937 (1963).

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On The Heat Precipitation of

Poly-L-proline¹

Sir: In a recent paper,² a study of the change in certain physical-chemical properties that occur during the well-known heat precipitation^{3,4} of a dilute aqueous solution of poly-L-proline (form II)^{5,6} was reported. The thermodynamic, dimensional, and frictional properties prior to precipitation were interpreted in terms of conventional polymer solution theory appropriate to chains in random-coiled, nonordered conformations.⁷ The experimental evidence⁸ which favors a rigid rod-like structure in dilute solution was ignored. It was concluded in this work² that the precipitation was a consequence of a liquid-crystal transition rather than a liquid-liquid phase separation. These are the two obvious possibilities to choose from when observing precipitation phenomena from a dilute solution of random-coiled polymer.⁷ However, the liquid-crystal transition involving randomly coiled polymers is

(1) This work was supported by a contract with the Division of Biology and Medicine, U. S. Atomic Energy Commission.

(2) A. Ciferri and T. A. Orofino, *J. Phys. Chem.*, **70**, 3277 (1966).

(3) J. Kurtz, A. Berger, and E. Katchalski in "Recent Advances in Gelatin and Glue Research," G. Stainsby, Ed., Pergamon Press, New York, N. Y., 1958, p 31.

(4) W. F. Harrington and M. Sela, *Biochem. Biophys. Acta.*, **27**, 24 (1958).

(5) E. R. Blout and G. D. Fasman, "Recent Advances in Gelatin and Glue Research," G. Stainsby, Ed., Pergamon Press, New York, N. Y., 1958, p 122.

(6) A. Yaron and A. Berger, *Bull. Res. Council Israel*, **A10**, 46 (1961).

(7) P. J. Flory, "Principles of Polymer Chemistry," Cornell University Press, Ithaca, N. Y., 1953.

(8) I. Z. Steinberg, W. F. Harrington, A. Berger, M. Sela, and E. Katchalski, *J. Am. Chem. Soc.*, **82**, 5263 (1960).

invariably accompanied by a major conformation change.⁹

The theoretical approach that was used in the analysis and the conclusion reached² have led to a serious misconception in regard to the actual molecular phenomena that are involved during the heat precipitation of poly-L-proline. We wish to review^{10,11} and introduce new evidence that demonstrates that although this phenomenon is a phase transition there is no accompanying conformational change in the macromolecular component. This process is of the type discussed theoretically by Onsager¹² and by Flory¹³ and results from the geometric asymmetry of the solute molecules with the phase separation being aided by slightly positive interaction free energies between these species.¹³ A dense fibrillar, crystalline phase has been theoretically predicted to occur from a dilute solution of such molecules.¹³ The conclusion that no conformational change is involved during the heat precipitation of poly-L-proline is supported by two main lines of experimental evidence.

It has been reported^{4,14} that the specific optical rotation of form II, $[\alpha]_D = -500^\circ$, remains virtually invariant from room temperature to 60° . Above this temperature, precipitation of the solute prevents any further optical rotation measurements that can be given a molecular interpretation. This large negative specific rotation is characteristic of the ordered helical conformation^{3-6,8} with imide groups in a *trans* orientation. We have also recently observed that the optical rotatory dispersion (ORD), from 600 to 400 $m\mu$, is invariant with temperature up to 63° . However, after standing for 1 min at 63° , the ORD measurements become spurious as a consequence of precipitation. The utilization of optical rotation measurements as a sensitive method of describing conformations and conformational changes for polypeptides has been extensively demonstrated and documented.^{15,16} We can, therefore, conclude from the above results that until the point of precipitation, there is no conformational change in poly-L-proline.

The foregoing conclusion is substantiated by studies of the wide-angle X-ray patterns. In the first column of Table I, there are listed the major d spacings characteristic of poly-L-proline in form II. Detailed crystallographic studies^{17,18} have demonstrated that these spacings are consistent with an ordered helical, *trans* structure. Prior to dissolution at room temperature, freeze-dried poly-L-proline possesses the same major spacings as is indicated in the second column of Table I. After precipitation at 51° , the data in the last column demonstrate that all of these spacings are still present. These results thus confirm

Table I: Spacings (in angstroms) for Poly-L-proline II^a

Ref ^b	Before dissolution	After precipitation
5.80 (s)	5.85 (s)	5.83 (s)
4.92 (s)	4.98 (s)	4.94 (s)
	4.36 (vw)	4.42 (w)
3.61 (s)	3.70 (m)	3.66 (m)
2.74 (m)	2.74 (mw)	2.74 (m)
2.45 (m)		2.47 (mw)
2.28 (m)		2.28 (mw)
2.15 (m)		2.18 (mw)
1.90 (m)		1.98 (w)

^a w = weak; s = strong; m = moderate; vw = very weak; mw = moderately weak. ^b F. Gornick, L. Mandelkern, A. F. Diorio, and D. E. Roberts, *J. Am. Chem. Soc.*, **82**, 5263 (1960).

the conclusions drawn from optical rotation studies in regard to the maintenance of conformation during precipitation.

Quantitative studies that we have made of the precipitation kinetics indicate that the process is one in which nucleation acts play a predominant and rate-determining role. For example, for a 0.16% solution the half-time for precipitation at 51° is 37,500 min. However, at 60° the half-time has been reduced to 1550 min. Similar results are obtained for higher polymer concentrations. This extraordinary variation of rate with temperature can only be attributed to nucleation control, through the dependence of precipitation rate on the supersaturation or undercooling. Theoretically, a first-order phase transition is predicted for this process.¹³ Therefore, nucleation control, from a kinetic viewpoint, is consistent with this equilibrium stipulation. Similar quantitative rate studies, with essentially the same conclusions, have already been published for the heat precipitation of soluble collagen (tropocollagen) in an ordered conformation.^{19,20}

The collagen and poly-L-proline systems that have

(9) L. Mandelkern, "Crystallization of Polymers," McGraw-Hill Book Co., Inc., New York, N. Y., 1964, p 38 ff.

(10) Reference 9, p 66 ff.

(11) L. Mandelkern, *J. Polymer Sci.*, **49**, 125 (1961).

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(13) P. J. Flory, *Proc. Roy. Soc. (London)* **A234**, 60 (1956).

(14) J. Engel, J. Kurtz, E. Katchalski, and A. Berger, *J. Mol. Biol.*, **17**, 255 (1966).

(15) P. Urnes and P. Doty, *Advan. Protein Chem.*, **16**, 402 (1961).

(16) J. A. Schellman and C. Schellman, *Proteins*, **2**, 1 (1964).

(17) P. M. Cowan and S. McGavin, *Nature*, **176**, 501 (1955).

(18) V. Sasisekharan, *Acta. Cryst.*, **12**, 897 (1959).

(19) G. C. Wood and M. K. Keech, *Biochem. J.*, **75**, 588, 598 (1960).

(20) J. M. Cassel, L. Mandelkern, and D. E. Roberts, *J. Am. Leather Chemists' Assoc.*, **42**, 556 (1962).

been described represent a unique situation that is not encountered with random-coiled polymers. A phase transition occurs, wherein a concentrated ordered phase is formed from a dilute solution, without the macromolecular species undergoing any conformational change. The theoretical basis for this phenomenon has already been set forth.^{12,13}

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**Comments on the Note, "On the Heat
Precipitation of Poly-L-proline," by L.
Mandelkern and M. H. Liberman**

Sir: In the preceding letter, Mandelkern and Liberman¹ suggest that no conformational change is involved in the heat precipitation of form II poly-L-proline from aqueous solutions. Essentially, they contend that (i) the model of a rigid rod in solution is more appropriate, and (ii) a liquid-liquid separation resulting in formation of fibrous aggregates, as described by Flory,² is the only reasonable representation of the transition.

In our paper,³ we have also repeatedly called attention to the relatively rigid conformation of this polymer in solution and have invoked, for purposes of qualitative examination, an accordingly appropriate conformational model⁴ based upon the existence of a single minimum in the potential for internal rotation. Nonetheless, it is constructive to recognize that even so-called rigid molecules in dilute solution possess some degree of flexibility.^{4,5} In the case of poly-L-proline, the recent optical rotatory dispersion experiments of Engel, *et al.*,⁶ reveal a decrease in the parameter $[\alpha]_D$ with temperature and with salt concentration (LiBr) which is not at all negligible. Moreover, Blout and Fasman⁷ reported that the value of $[\alpha]$ changes markedly with the solvent medium; *i.e.*, $[\alpha]_{546}^{25} = -768, -694$, and -406 , respectively, in 8 *M* urea, water, and 2 *M* KSCN. Our data³ also indicate that the intrinsic viscosity is strongly affected by salt concentration.

Thus, although a helix \rightarrow coil transformation is not observed, it appears that both temperature and solvents are able to affect considerably the conformation of poly-L-proline II in solution. Clearly, optical rotation and optical rotatory dispersion might well be observed also in a solution of molecules that consist of helical sequences connected by flexible joints for which the

thermodynamic, dimensional, and frictional properties would be normally ascribed to stiff coils. In short, there is certainly no unassailable evidence to support the rod-like model, and consequently, there is no definite evidence that the liquid-liquid separation considered by Flory² is the controlling factor for the precipitation of poly-L-proline. Indeed, even for completely rigid particles, one must allow for the possibility of phase separation as a direct liquid-crystal transformation, rather than the aggregation resulting from liquid-liquid separation involving an anisotropic phase.

The primary object of the work reported by us³ was to provide reasonable evidence for identification of the observed phase change characteristic of poly-L-proline with a direct liquid-crystal transition. For purposes of analysis, we chose to adopt the familiar Flory-Huggins⁸ form for the chemical potential of the dispersed species. On this basis, two sufficient criteria for elimination of liquid-liquid separation as either the ultimate or transient phase separation process are: (i) verification of the ordered nature of the precipitate in the presence of the supernatant liquid (possible complications arising from *subsequent* crystallization of a concentrated liquid phase upon removal of solvent can thus be avoided); and (ii) verification of a positive value for the second virial coefficient just prior to the separation of the solution into two phases. Obviously, our conclusions must be viewed in light of the model invoked to reach them.

The interesting model and the process suggested by Mandelkern and Liberman are, possibly, a better approximation to the case of tropocollagen, although this remains to be seen. In our work,³ the comparison between poly-L-proline and tropocollagen is to be viewed principally in the direction in which the temperature change affects the precipitation.

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(2) P. J. Flory, *Proc. Roy. Soc.*, (London), **A234**, 73 (1956).

(3) A. Ciferri and T. A. Orofino, *J. Phys. Chem.*, **70**, 3277 (1966).

(4) M. L. Volkenstein, "Conformational Statistics of Chain Molecules," Interscience Publishers, Inc., New York, N. Y., 1961, p 189.

(5) G. Cohen and H. Eisenberg, *Biopolymers*, **4**, 429 (1966).

(6) J. Engel, J. Kurtz, E. Katchalski, and A. Berger, *J. Mol. Biol.* **17**, 255 (1966).

(7) E. R. Blout and G. D. Fasman, "Recent Advances in Gelatin and Glue Research," G. Stainsby, Ed., Pergamon Press, London, 1958, p 153.

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