695. *Distribution of Molecular Weights in Some Polyesters and Polypeptides.*

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A recently proposed method for the fractionation of high polymers has been used successfully in the analysis of the molecular-weight distribution in polyesters and polypeptides. In the case of the polyesters the distributions found agree with those deduced from the polymerisation kinetics but the agreement is not so convincing for some of the polypeptides.

The essential features of a new method for the fractionation of high polymers have been described.[1] The method requires that two miscible solvents for the polymers, one good and one poor, should be found in which the polymers have a positive temperature coefficient of solubility. As the suitablity of a given pair of solvents depends upon the polymers, we will discuss separately the two polymers which we have studied. The first polymer fractionations to be described are of polyesters prepared by Flory's method.[2]

The dependence on temperature of the solubility of these polymers in a number of solvents was determined by turbidity titration. Some typical results for one of the most

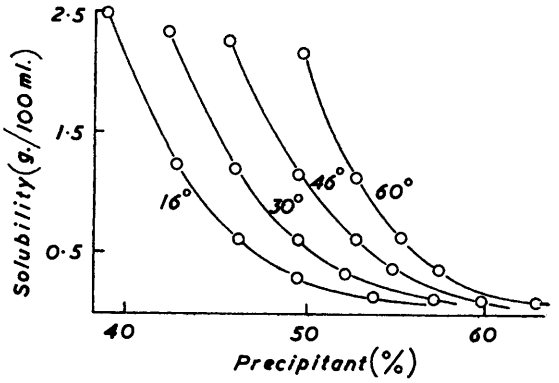

Fig. 1. *The variation of solubility with percentage of precipitant and temperature. Good solvent, ethyl methyl ketone; poor solvent, cyclohexane; molecular weight of polymer,* 1750.

promising solvent systems are shown in Fig. 1. A further requirement of the method [1] is that the solubility of the polymers in the solvents should increase (or decrease) with decreasing (or increasing) molecular weight over the whole range of molecular weight. From the point of view of the fractionation of polyesters then it is inconvenient that the lower-molecular weight members, below about 1000, are decreasingly soluble in non-polar solvents, such as hexane, as the molecular weight decreases, whereas the highest molecular weight polymers are decreasingly soluble in the same solvent as the molecular weight increases. Nor is the order of solubility of fractions of different average chain length simple in polar solvents such as acetone. The suitability of given solvents for fractionation depends then on the average molecular weight of the polymers. We have found that the following solvent systems are most useful:

Number-average molecular weight	Poor solvent	Good solvent
<500(*A*)	Cyclohexane	30% Ethanol in cyclohexane
>500(*B*)	Cyclohexane	30% Ethanol in cyclohexane. Change to ethyl methyl ketone after 20 fractions
>800(*C*)	Cyclohexane	Ethanol. Change to ethyl methyl ketone after 25 fractions
$>1,500$(*D*)	50% Ethanol–cyclohexane	Ethyl methyl ketone

N.B. No two-solvent system is suitable for analysis in the range $M_n = 500—1500$.

[1] Baker and Williams, *J.*, 1956, 2352.
[2] Flory, *J. Amer. Chem. Soc.*, 1940, **62**, 1057.

Details of Method

In the analysis of polystyrenes [1] small samples (*ca.* 500 mg.) were put on the column. In the present analyses up to 5·0 g. of polyesters were analysed in a single experiment. This alteration of conditions was enforced by the difficulty of determining the molecular weights of the fractions. The titration method used for the determination of the number-average molecular weights of both whole polymers and fractions is to be described.[3] In all other respects the method was that described.[1]

Results

The efficiency of fractionation of a polymer can be tested in three ways. The first is to refractionate the material and observe the spread in the fractions. It is usual to test the efficiency of any chromatographic procedure in this manner. Fig. 2 illustrates the fractionation of a polyester of number-average molecular weight, $M_n = 2500$, and the refractionation of some sample fractions. It can be seen that the method is satisfactory. A given analysis can be accurately reproduced (Table 1).

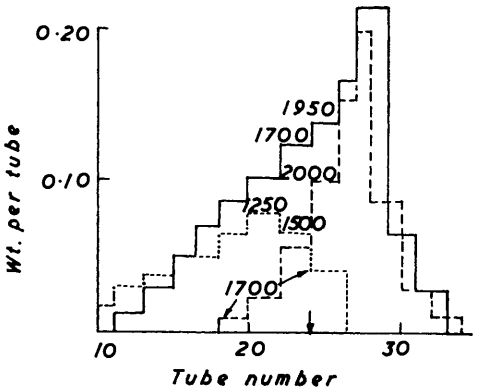

Fig. 2. *The fractionation of a polyester (full line) and the refractionation of two artificial polymers formed from mixing together the first fractions up to tube number 22 and the fractions from this tube number to the end of the chromatogram. Numbers in the Figure refer to molecular weights.*

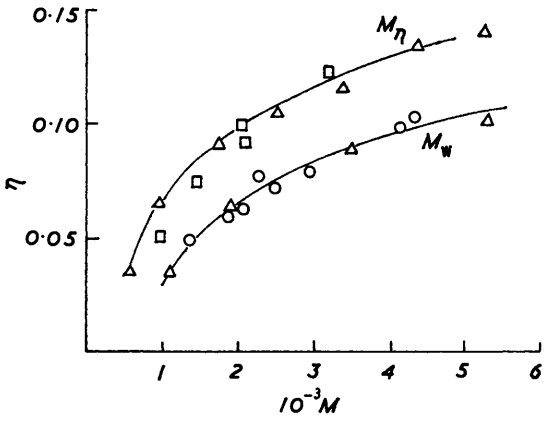

Fig. 3. *M_n and M_w of some whole polymers plotted against intrinsic viscosity, △, compared with M_n for column fractions, ○, and batch fractions, □.*

A second method is to compare those physical properties of the fractions, *e.g.*, bulk and intrinsic viscosity, which depend more nearly on weight-average than number-average molecular weight, with the properties of the bulk polymers. Typical data are in Fig. 3. The properties of the fractions indicate that the ratio of $M_w : M_n$ does not significantly exceed 1·0. The properties of fractions prepared by a conventional fractional precipitation are also given for comparison.

[3] Pope and Williams, *J.*, in the press.

TABLE 1. *Reproducibility of fractionations of polyesters.*

Chromato-gram tube No.	Weight per tube (1)	Weight per tube (2)	M (1)	M (2)	Chromato-gram tube No.	Weight per tube (1)	Weight per tube (2)	M (1)	M (2)
14,15	0·03	0·02	590	590	24,25	0·14	0·14	2150	2420
16,17	0·05	0·04	860	790	26,27	0·19	0·17	2950	3400
18,19	0·075	0·07	990	—	28,29	0·21	0·21	3550	4200
20,21	0·095	0·09	1270	1260	30,31	0·15	0·10	4000	5000
22,23	0·12	0·12	1680	—					

A third method is to determine the distribution of molecular weight in a polymer for which the distribution is well established by kinetic studies. The kinetics of the formation of polyesters of high molecular weight only are sufficiently understood for this purpose.[2,3] An analysis of a high molecular weight polyester is shown in Fig. 4. The full curve was

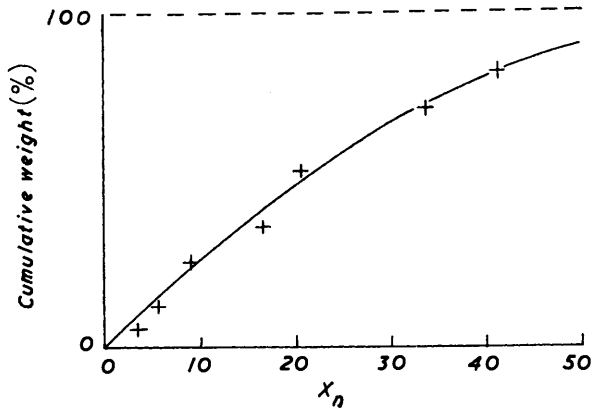

FIG. 4. *The distribution of molecular weight in a high-molecular weight polyester compared with the theoretical distribution given by the full line.*

obtained theoretically from the known number-average molecular weight by assuming the most probable distribution. The agreement is good and supports the method of analysis.

Some analyses of polyesters of unknown distribution will be given in a paper devoted to the kinetics of formation of polyesters of *low* molecular weight.[3]

DISTRIBUTION OF MOLECULAR WEIGHTS IN POLYPEPTIDES

The polypeptides which we will discuss were prepared for us through the courtesy of Dr. C. H. Bamford (Courtaulds, Maidenhead). They were made from cyclic *N*-carbonic anhydrides through the action of (*A*) pre-formed polymers, (*B*) lithium chloride–methylhydantoin, and (*C*) aniline. It has been supposed that the molecular-weight distribution in these polymers is dependent on the initiator because the kinetics of the reaction vary greatly from one initiator to another. For polymerisation in the presence of prepared polymer the polymer has been stated to have a Poisson distribution.[4] The Poisson distribution is extremely narrow as compared with the most probable distribution which is found in polyesters. It was therefore of great interest to see if our method of fractionation could lead to a fractionation of these polypeptides. The lithium chloride–methylhydantoin- and aniline-initiated polymers should have a molecular-weight distribution more nearly like that of the polyesters according to the kinetics, but the actual distribution is complex.[5]

DETAILS OF FRACTIONATION METHOD

The apparatus was as described elsewhere.[1] When we attempt the fractionation of new polymer the only feature that must be changed is the solvent gradient. The solvent gradient used in the fractionation of the polypeptides was produced by using cyclohexane as non-solvent

[4] Ballard and Bamford, *Proc. Roy. Soc.*, 1954, *A*, **223**, 495.
[5] Ballard, Bamford, and Weymouth, *Proc. Roy. Soc.*, 1955, *A*, **227**, 155.

in the mixing vessel, and a mixture of 20% methanol in ethanol as the good solvent in the reservoir. This system was chosen as the polymers showed a positive temperature coefficient of solubility in it and their solubility decreased regularly with increasing molecular weight. The fractions were characterised by a determination of their viscosity in aqueous solution with an Ubbelohde viscometer. The molecular weight of the fractions could then be found by using the expression of Fessler and Ogston.[6] This expression was not likely to be greatly in error for fractions of high homogeneity as the expression itself was obtained empirically from a study of polymers having a Poisson distribution. (The results below confirmed this distribution for the particular polymers used by Fessler and Ogston for their calibration.)

Results

Fig. 5 shows the analysis of an artificial mixture of two polymer-initiated polypeptides of specific viscosity 0·051 and 0·075 in 0·5% solution. Not only has there been a separation of the two polymers but there is also a fractionation within each polymer. Fig. 6 gives the

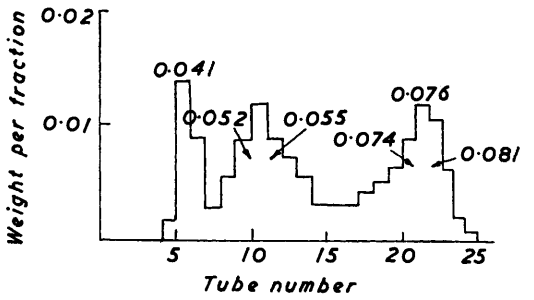

FIG. 5. *The fractionation of a mixture of two polysarcosines. The three concentration peaks imply no more than that the polymer is fractionated. Numbers refer to specific viscosity.*

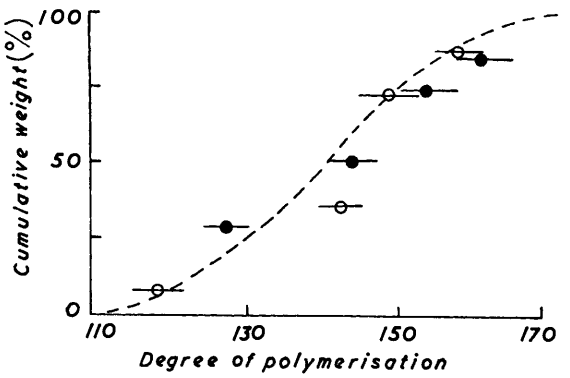

FIG. 6. *Distribution of molecular weights in a polysarcosine compared with the Poisson distribution.*

distribution of molecular weight in one of the polymers. It was obtained from two analyses. The broken line is a calculated distribution from the known molecular weight of the polymer, a distribution according to the Poisson equation being assumed.[7] There can be little doubt that this equation correctly represents the distribution. These observations add further strength to the postulated kinetic scheme.

The kinetics of the lithium chloride–methylhydantoin-initiated polymerisation of *N*-carbonic anhydrides lead to a very complex distribution function.[5] We analysed polymers prepared in this way with the result shown in Fig. 7. The distribution is certainly narrower than that calculated on the kinetic scheme suggested [5] but is not quite so narrow as in the pre-formed polymer-initiated polymers. Because of the discrepancy between the kinetically determined and observed distributions a further sample of lithium chloride–methylhydantoin-initiated polymer was prepared under very carefully controlled conditions

[6] Fessler and Ogston, *Trans. Faraday Soc.*, 1951, **47**, 667.
[7] Flory, " Principles of Polymer Chemistry," Cornell Univ. Press, New York. 1953, 337.

by Dr. Bamford, and this polymer was analysed by the column method. It was again shown to have a narrow distribution very much as in Fig. 7 in which $M_w : M_n$ did not exceed 1·2. (As we observed no very low molecular weight material we concluded that it was absent.) The distribution suggested for this polymer from the kinetics should have a ratio $M_w : M_n$ in the region of 2·0.[5] The discrepancy between the observations is so large that additional checks were attempted on the $M_w : M_n$ ratio by measuring the number-average molecular weight of the polymer by two methods: (*a*) from the osmotic pressure of the polymer in chloroform solution and (*b*) from the number of basic end-groups. In chloroform the osmotic head did not give a steady equilibrium value but fell slowly. This showed that some polymer was diffusing through the membrane and the method cannot be considered to be very reliable. However the fall in the head was sufficiently gradual

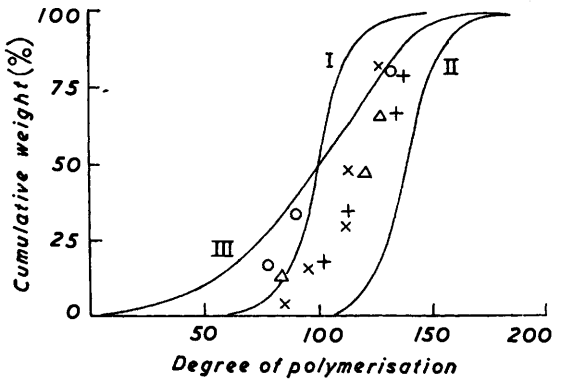

FIG. 7. *Distribution of molecular weights in a* LiCl–*hydantoin-initiated polymerisation of sarcosine* (three separate experiments) *compared with two Poisson distributions* I *and* II *and the distribution suggested*[5] *for such polymers* (III).

to permit extrapolations to zero time. The number-average molecular weights obtained by extrapolation to infinite dilution, which may be too high, are given in Table 2 where the molecular weights from other methods are also given. $M_w : M_n$ is close to 1·2 and is certainly not larger than this.

TABLE 2. *Molecular weights of polysarcosines.*

Initiator	Viscosity	Osmotic	End group
Preformed polymer	(*a*) 7100	—	—
	(*b*) 10,300	—	—
LiCl–Methylhydantoin	(*c*) 6750	5500	5700
	(*d*) 7470	—	7500
Aniline	(*e*) 7100	Method failed	6750
	(*f*) 8300	,,	7300

The position of travel of the polymer in the chromatograms indicates that all the polymers have very similar molecular weights except (*b*) which is obviously much higher.

A sample of polysarcosine was next prepared by aniline initiation. A broad distribution was expected from the kinetics but the determined distribution was again closer to the Poisson distribution than any other. Confirmation of the distribution is provided by the molecular weights in Table 2. It appears that all the synthetic polysarcosines prepared from *N*-carbonic anhydrides have narrow distributions.

In conclusion we comment that the fractionation achieved in the analyses of the polypeptides is a separation of polypeptides which differ in molecular weight by about 10%. It may well be that a similar technique will fractionate natural peptides in the same molecular-weight range, approximately 10,000, and having similar differences in molecular weight.

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