AUTOMATIC TURBIDIMETER FOR
DETERMINATION OF MOLECULAR WEIGHT
 DISTRIBUTION OF POLYMERS

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The methods commonly used for estimation of the molecular weight distribution of a polymer usually involve a preliminary fractionation of the polymer by means of fractional precipitation, isolation of a series of fractions and subsequently an average molecular weight determination of each fraction with the aid of, e.g. viscosimetry, osmometry, ultracentrifugation or light scattering. A complete molecular weight distribution analysis of this type is usually very laborious and time-consuming, large quantities, not always available, of the polymer sample are usually required, and the result will be a fractional histogram, where small irregularities of the "true" distribution of the polymer will be "smoothed out" by a final interpolation. Furthermore, the problem of co-precipitation will cause a shift of the curve in the high-molecular direction.

A more suitable principle, from this point of view, is that devised by Morey and Tamblyn [2] for analysis of cellulose acetate-butyrate and which has since been used on different polymers by other investigators. With some modification of this principle we have developed a method which enables the determination of the molecular weight distribution of a polymer—in our case degraded dextran preparations—using only 5 μg of the sample, an amount that easily can be recovered from, e.g., blood samples in physiological experiments.

PRINCIPLE

When adding a non-solvent to a dilute solution of a polymer—in our case ethanol to a water solution of dextran—the larger molecules, being less soluble, precipitate first, followed by the smaller molecules as more alcohol is added. The turbidity provoked by each increment of alcohol is measured in a sensitive turbidimeter, that is, a continuous "optical weighing" of the cumulative precipitate. The lower the original polymer concentration is in the precipitation vessel, the more accurate the turbidimetric estimation of the amount of the precipitate will be. A dextran concentration of 5 μg per ml is used here.
The basis of the method is the "saturation limit law" which relates the solubility limit of a molecular species of the polymer in a solvent–non-solvent system to its concentration at the point of precipitation and its molecular weight, the temperature being kept constant. The solubility limit is defined as the quantity of non-solvent that has to be added to cause initial precipitation.

These relationships were studied for dextran on a series of "narrow" fractions using different polymer concentrations and observing the concentration of the precipitant at which precipitation just started (Fig. 1). The results were in accordance with the theories of Schulz [3] and the relation for each fraction was found to be

\[ P = K \log C + f(M), \]

\( P \) being the precipitant concentration, \( C \) the polymer concentration and \( f(M) \) a function indirectly correlated to the molecular weight (Fig. 2).

By this calibration procedure with well-defined, narrow fractions, the necessary data are supplied to permit the analysis of a complex mixture of different molecular species. Necessarily, this involves a series of assumptions and approximations. Thus, for instance, the increasing alcohol concentration in the precipitation vessel will influence the physical nature of the precipitate flocules, and the refractive index of the clear fluid and the precipitate. By testing a series of mixtures of high- and low-molecular fractions an empirical factor (Fig. 3) with different numerical values at different alcohol concentrations (or \( f(M) \) values) was found and could be incorporated into the system (for a full description of the calculation procedure, cf. Wallenius [4]). The calculation of the final results involves the use of a series of nomograms, which can be combined into a convenient slide ruler.
APPARATUS

The instrument which we have developed for this purpose consists in principle of three different units (Fig. 4): an automatic "feeder" of non-solvent, a sensitive turbidimeter and a recording device.

The automatic non-solvent feeder has been constructed to deliver precipitant in such a manner that the increment of alcohol concentration in the precipitation vessel (i.e. the cuvette of the photometer) is kept constant. This is accomplished by a helicoidal cam, driven by a synchronous motor and pushing the plunger of an all-glass syringe. In this way, the time axis of the recorder will represent the alcohol concentration in the cuvette. Furthermore, this type of alcohol addition to the water solution, being very slow
in the beginning, avoids unnecessary development of mixing heat. A rapid mixing of the cuvette contents is secured by a stirrer and the temperature is kept constant at +20°C by immersion of the cuvette in a thermostated water-bath.

A light beam, generated by a tungsten lamp and passing a condensor lens and a monochromatic filter at 4360 Å, enters the cuvette through an entrance slit and illuminates a small section of the cuvette contents. The resulting scattered light from the precipitate is picked up at an angle of 90° by a lens system after first passing an exit slit, and is focussed on to the cathode of a photomultiplier tube. The exit slit prevents the phototube from seeing other than the relevant parts of the cuvette.

The stabilized high voltage, necessary for energizing the dynodes of the photomultiplier is delivered by a power supply, which at the same time powers the tungsten lamp and a D.C. amplifier. This amplifier, which is highly stable and linear, prepares the photomultiplier output to fit the input of a Varian recorder. As a turbidity reference source a polished Plexiglass prism of the same shape as the cuvette is used and all turbidity values are relative to this standard. The actual set up of the apparatus appears on Fig. 5.
EVALUATION OF RESULTS

The recording thus achieved consists of a relative turbidity vs. non-solvent concentration plot (Fig. 6). This curve has to be corrected for the dilution caused by the addition of alcohol and calculated as per cent of the final turbidity. Starting from the point where the precipitation begins, the turbidity increment corresponding to each alcohol increment (usually 1 per cent) is calculated. These data are via the nomographical procedure or via the slide ruler transferred into an integral relative concentration vs. molecular weight plot, the derivative of which is shown on the lower part of Fig. 6. This figure shows schematically the outcome for a high-molecular and a

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Fig. 6.—Schematic representation of the calculation procedure for different dextran preparations.

Fig. 7.—Distribution curve for two dextran fractions analyzed separately and as a mixture of equal parts.
low-molecular dextran preparation and a mixture of equal parts of these two preparations. An example of such a mixing experiment is shown in Fig. 7.

A complete analysis of this type requires about 90 min.

**DISCUSSION**

The theoretical deductions of Morey and Tamblyn require a series of assumptions and approximations, the permissibility of which is difficult to evaluate. As far as we have been able to test the system objectively, the results seem to be rather valid and reproducible. An alternative solution that clarifies some of the problems involved has been found by Claesson [1].

Turbidimetric methods are usually considered as being relatively inaccurate ones. In our experience turbidimetry, as outlined here, gives good and reproducible results. It is very important that the temperature is kept constant and that the polymer concentration is kept as low as possible and constant according to the standardization procedure. This requires the use of a very sensitive photometer and a very careful, dust-free handling of all solutions.

**SUMMARY**

A convenient and rapid method for the estimation of the molecular weight distribution of dextran has been developed, requiring only 5 μg of the dextran sample and about 90 minutes for a complete analysis.

**REFERENCES**