

CHROM. 5138

## INDUSTRIAL PREPARATIVE LIQUID CHROMATOGRAPHY

J. L. WATERS

*Waters Associates Inc., Framingham, Mass. 01701 (U.S.A.)*

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SUMMARY

This paper discusses the uses and requirements for narrow polymer fractions and pure chemical compounds separated by liquid chromatography. The ready availability of material fractions opens new opportunities for systematic investigation and research. It is now practical to separate quantities of several grams at extremely high resolution up to quantities of 20,000 g at moderately high resolution.

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## INTRODUCTION

Polymers and large molecules have, for many years, been preparatively fractionated by electrophoresis, sand column fractionation, and liquid chromatography employing affinity or size. Recent technical advances in liquid chromatography permit the fractionation of larger quantities of material with higher resolution. Waters Associates Inc. is now offering a custom fractionation service for liquid chromatography separations. In this paper the uses and applications for separated materials and the separation resolution attributable will be discussed.

## USES AND APPLICATIONS FOR SEPARATED FRACTIONS

There are many needs and uses for narrow polymer fractions, individual chemical separations and pure chemical compounds. Table II briefly outlines some of these uses. The sources of material to be fractionated and uses for separated materials are listed.

## SEPARATION TECHNOLOGY

Preparative liquid chromatography separations are required when a separation is difficult. This occurs when the components are degraded under the conditions for other separation methods or where they are very similar and, as a result, require the ultimate in resolution. Examples are separations where the temperature required degrades the molecule or where complex isomers or polymers must be separated. Liquid chromatography separations are generally made from a distribution of polymers or chemical mixtures. These mixtures are complex and contain similar compounds differing in either functionality or size and often in both.

The separation of a mixture of two or three compounds is a special distribution case and is generally an easier separation than one requiring the separation of many components.

There are four frequently used modes of liquid chromatography: (A) Molecular size separation is based on the relative size of the solute molecules in relation to the pore size of the column packing. (B) Liquid-liquid partition separation utilizes the relative solubilities of the sample components in the stationary and mobile liquid phase. (C) Liquid-solid separation involves the relative adsorptivity of the sample components on an active solid support. (D) Ionexchange separation is based on the reversible exchange of ions between the solid ionexchange resin and the eluting liquid.

The yield and resolution and hence the complexity of a separation is affected by the following factors: (A) A narrower polydispersity of a polymer fraction or higher purity of a separated chemical species increases the complexity. (B) A lower sample viscosity permits a higher sample concentration. Thus, lower molecular weight materials can be fractionated with higher yield and less complexity. (C) A greater compound similarity increases the complexity of a separation. Materials similar in size and functionality are difficult to separate. (D) A lower concentration of the desired material in the mixture increases complexity. If the desired component is present in a concentration of 0.1 % and several grams are required, it is necessary to fractionate several thousand grams to obtain the desired quantity of material.

The separation resolution (available from Waters Associates) is shown in Table I.

TABLE I

	<i>Easy</i>	<i>Difficult</i>	<i>Very difficult</i>
$M_1/M_2$	1.4	1.02	1.005
$M_w/M_n$	1.3	1.005	1.001
$\alpha$	1.1	1.006	1.0015

$M_1/M_2$  represents the molecular weight ratio of two compounds separated by molecular size.  $M_w/M_n$  is the heterogeneity index of the resulting polymer fractions.  $\alpha$  is the ratio of the chromatographic elution volumes of two compounds separated by affinity chromatography. For example, two compounds having elution volumes of 10 and 11 liters have an  $\alpha$  of 1.1.

For comparative purposes, preparative gas chromatography of  $\alpha$ 's of 1.1 or less is considered difficult. The increased resolution for preparative liquid chromatography separation is the result of the use of a liquid phase separation instead of a gas phase separation.

Separations of 2 to 20,000 g quantities are available now. Larger quantity separations will be available in the near future.

Table II is an outline of the uses for fractionated material. The uses listed in the table can be divided into two general classes: those involving the separation of a known material and those involving an unknown material. The separations of known materials result in an improved understanding of the material, an improvement in the end product for specific applications, or the direct use of the fractions.

TABLE II

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*Typical sources of material*

- (A) Research material:
  - 1. Polymer synthesis
  - 2. Chemical compound synthesis
- (B) Production material:
  - 1. Impurity removal
  - 2. Manufacture of special property materials
  - 3. High purity compounds
- (C) Materials from other sources:
  - 1. Natural products
  - 2. Vendor products
  - 3. Competitor products

*Uses for separated materials*

- (A) Analysis by the identification of fractions using:
    - 1. Infrared spectra
    - 2. Gas chromatography
    - 3. Mass spectra
    - 4. NMR spectra
    - 5. Ultra violet spectra
    - 6. Thermal analysis
    - 7. Elemental analysis
    - 8. Atomic absorption
    - 9. Thin-layer chromatography
    - 10. Liquid chromatography
  - (B) Evaluation of properties of a single fraction and of a blend of fractions:
    - 1. *Mechanical properties*
      - (a) Molding
      - (b) Milling
      - (c) Extruding
      - (d) Toughness
      - (e) Plasticity
      - (f) Melt index
      - (g) Creep
      - (h) Hardness
      - (i) Impact strength
      - (j) Tensile strength
      - (k) Viscosity
    - 2. *Thermal properties*
      - (a) Heat degradation
      - (b) Cold resistance
      - (c) Flow characteristics
    - 3. *Electrical properties*
      - (a) Dielectric constant
      - (b) Conductivity
      - (c) Electrical breakdown
    - 4. *Permeability properties*
      - (a) Oxygen
      - (b) Moisture
    - 5. *Chemical and physical properties*
      - (a) Solubility
      - (b) Melting point
      - (c) Infrared spectra
      - (d) Ultra violet spectra
      - (e) NMR
      - (f) Mass spectra
    - 6. *Biological activity*
      - (a) Toxicity
      - (b) Metabolism
      - (c) Pharmaceutical activity
      - (d) Insecticidal activity
      - (e) Herbicidal activity
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*(Continued on p. 216)*

TABLE II (continued)

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- 7. *Degradation properties*
    - (a) Time
    - (b) Temperature
    - (c) Radiation
    - (d) Electrical discharge
    - (e) Oxidation — ozone
    - (f) Mechanical
      - (i) Molding
      - (ii) Milling
    - (g) Chemical
  - 8. *Miscellaneous properties*
    - (a) Uniformity of degree of copolymerization
    - (b) Degree of branching
    - (c) Adhesion
    - (d) Rheology
  - 9. *Uses for separated materials as intermediates*
    - (a) Chemical synthesis
    - (b) Polymer synthesis
    - (c) New product engineering for optimum performance
    - (d) Rules of mixture for properties of combinations
    - (e) Degradation of fractions
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Two direct uses for fractions are as synthesis intermediates and as standards. Standards are vital for the identification of unknowns and the calibration of analytical instruments, such as mass spectrometers, infrared, NMR, GPC and other instrumental techniques.

Standards for molecular weight are an example of the usefulness of fractions. Until recently, the principal standards available for GPC were a series of polystyrene standards from 900 to 2 million molecular weight. Because of the lack of availability of other polymer standards, there has been a considerable effort to develop a universal calibration which will relate the polystyrene standards to all other polymer systems. This has been difficult and time-consuming. With the availability of preparative separation techniques having high resolution, it is now possible to prepare GPC calibration standards in a wide variety of polymer materials. Waters Associates will offer, in the near future, an extensive line of GPC calibration standards in many difficult polymer systems.

A series of linear polyethylenes are being fractionated by Waters for the National Bureau of Standards for characterization and distribution by NBS as primary polymer standards.

A more specialized standard is the ultra narrow distribution polymer. Using high resolution recycle preparative chromatography technology<sup>1, 2</sup> extremely narrow polymer standards have been prepared. A set of polystyrene standards is now available from Waters Associates having molecular weights from 900 to 800,000. Typical polydispersities for these materials range from 1.0025 to 1.009. These narrow polystyrene standards are used to determine the properties of very narrow distributions, cross-calibrate osmotic pressure and light scattering instruments and investigate the mechanism of gel permeation chromatography.

In the past, there have been no high molecular weight standards above 2 million. Material separations make possible the preparation of extremely high molecular weight standards. Anionic polymers of 2 million molecular weight or greater are very

broad. GPC is used to separate at high resolution polymers greater in molecular weight than 2 million.

A particularly interesting field for separated materials is the degradation of compounds and polymers. Usually, degradation is studied by degrading a product and measuring the change in its overall properties. Although this method is often adequate, there are many cases when due to the complexity of the product, it is impossible to obtain an adequate understanding of the degradation process by this method.

Several techniques are used to overcome this problem. The starting material is fractionated and the individual fractions are degraded. The material resulting from the degradation of each fraction is measured as a whole material and it can, in turn, be fractionated and further measured as fractions. Alternatively, the whole material is degraded and fractionated. The original mixture fractions are then compared with the degraded material fractions to identify changes.

Obviously, degradation is a complex investigation. Fractionation of either the starting material or the degradation product offers a powerful tool to increase the understanding of the degradation mechanism. An example of this type of problem is the determination of the resistance of a polymer to radiation or chemical degradation. The polymer is first fractionated into reasonably narrow distribution fractions. Each fraction is then subjected to radiation and the resulting molecular weight distribution determined. On the other hand, when degradation is by chemical means, it is often desirable to fractionate the degraded material. The end group composition of each fraction of the degraded material is then measured to develop a better understanding of the mechanism.

Narrow polymer distribution materials and pure species are also important in the determination of physical properties. The properties listed in Table II can be examined for both pure materials and narrow polymer distributions. In this way, the interrelationship between chemical structure and the property of interest can be developed.

The study of complex mixtures is another important area. Mixtures such as asphalts, natural oils, polymers and resins have been optimized for performance by synthesizing different distributions, or combining material from different sources. The fractionation of a broad distribution and the blending of the individual fractions to obtain a desired blend of properties provides a greater range of adjustment and control of end product properties than the adjustment of a distribution or the blending of different distributions. As a general rule, the blending of individual fractions enables the research worker to obtain more combinations and develop more data in a given period of time. His examination of the range of properties and the optimization of performance can, as a result, be greatly expedited.

An example of this application is the fractionation of a broad distribution of lubricating oil. Appropriate blends of individual fractions are then run in an engine to determine the optimum combination of properties such as wear resistance, chemical stability; temperature resistance, etc.

Another example is the development of a new polymer where the synthesis technology is difficult to develop. For example, when preliminary data indicates that the high molecular weight end of the polymer has a valuable property and when it is difficult to make a reasonably narrow high molecular weight distribution, it is much easier to fractionate a broad polymer to obtain high molecular weight fractions.

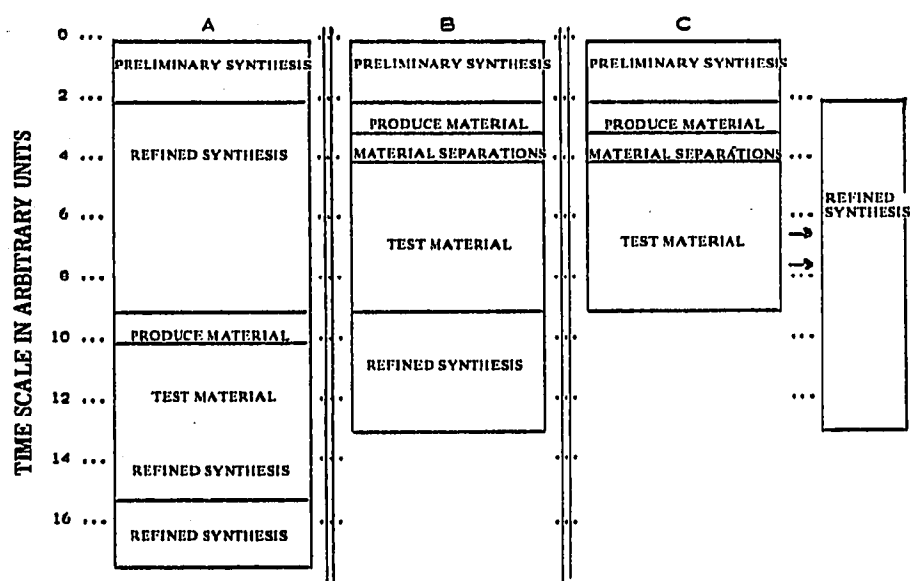


Fig. 1. Time sequence diagram.

Testing the fractions then indicates whether or not further work on the project will yield a useful product.

Fig. 1 is a time sequence diagram for a typical development project. The sequence is: (1) Development of a preliminary synthesis method. (2) Refinement of the synthesis method to produce a desired material. (3) Synthesis to obtain the test material. (4) Testing of the new compound or polymer to identify and optimize desirable properties. (5) Continuing refinement of the synthesis method to produce the desired product.

Most developments of this type require that these operations be done in sequence because each operation is dependent upon success in the previous one. This method has two major disadvantages: (1) It requires the time-consuming development of a refined synthesis method in order to produce enough material to determine whether or not the end product will be useful. If the end product is not useful, the development of a synthesis method represents a loss of development effort. (2) The fact that the synthesis method must be improved in order to produce test material requires that the two stages must be conducted in series. Hence, the total time between conception of the project and introduction to the marketplace is substantially longer than if the two are conducted in parallel.

Material separations permit two modifications in the normal development procedure. These are shown in Figs. 1B and 1C. In each of these cases, as soon as preliminary synthesis method has been developed, enough material is produced to prepare fractions for end product testing. If the end product evaluation is negative, the time-consuming development of a refined synthesis method can be omitted.

If a crash project is appropriate, the refinement of the synthesis method can proceed in parallel with the testing programme. In this way, the results of the test can be fed back into the refinement of the synthesis method development in order to direct it quickly and profitably to the best result.

The advantages of the use of separated materials are that they reduce the total

cost of the project. (1) If the end product is unsuccessful, a saving of unnecessary development efforts is made by reversing the order of development. (2) If the testing indicates a successful product, the refinement of the synthesis method will be better directed to the actual product desired. (3) If parallel development is appropriate, the material separation will shorten the time of development by permitting simultaneous material testing and refinement of the synthesis method.

A second major area for fractionations is in the examination of unknown materials. Natural products, new synthesis compounds, defective production batches and products manufactured by competitors and vendors are the usual sources of unknowns. The identification of the individual components of these materials leads to a better understanding of the nature of the mixture and to subsequent product improvement.

The first major classification for this type of product is the identification of the components of the mixture. The identification is made by standard instrumental techniques. Chemical structure involves the use of standard laboratory procedures, such as infrared spectra, NMR, GPC, gas chromatography and mass spectrometry.

One of the major applications for fractionation of unknown materials is copolymers. Copolymers can be extremely complex. In addition to having different degrees of copolymerization with change in size of the polymer molecule, they can also have differences in the degree of randomness, blocking and tactic properties. The mechanism of reaction as well as the temperature and reaction vessel geometry affect these properties.

Material separation makes possible the separation of a copolymer distribution into individual size fractions which are then further examined by analytical techniques to increase the knowledge of the reaction mechanism. In some cases, it is desirable to take the individual size fractions and further fractionate them using an affinity separation. In this case, they will be separated according to the chemical nature of the reactive groups on the polymer molecule. The complexity of copolymers requires the use of a separation fractionation for the development of a complete understanding of the mechanism.

In a biologically active product, the determination of toxicity and activity of each component present in a mixture are of vital concern. The development of a new drug requires the analysis of all of the unknown impurities present in the final product. The concentration of many of these components is very low. They must be identified and if their properties have not previously been determined, their properties must be determined. Material separations are used to rapidly separate and identify trace impurities which might have a deleterious effect on the product.

#### REFERENCES

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