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Column-Fractional Precipitation

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REVIEW ARTICLE

Column-Fractional Precipitation

Since the early attempts of Mark and Saito (*1*) and Levi and Giera (*2*) to fractionate cellulose acetate and natural rubber by adsorption chromatography on animal charcoal, this method has been employed for other polymer separations (*3–6*). The bulk of polymer fractionation, however, was done in batch operations and was based on the differences in solubility behavior of the various molecular-weight species. In suitable solvents, the solubility of a homogeneous polymer fraction decreases with increasing molecular weight. Two general possibilities exist. Precipitation of polymer fractions from dilute solutions can be brought about by (1) the addition of a nonsolvent to the solution, (2) the lowering of the temperature of the solution, or (3) the volatilization of the solvent. Second, polymer fractions may be brought into solution either by increasing the solvent power of the solvent at constant temperature or by slowly raising the temperature of the system at constant solvent composition. Both of these techniques, fractional precipitation and fractional extraction, lend themselves to column methods. These methods and their applications have been reviewed in part by Hall (*7*) and Schneider (*8*).

POLYMER FRACTIONATION

In 1948 Desreux (*9*) reported the fractional extraction of polyethylene from a Celite column with a stepwise changing of the solvent–nonsolvent mixture at constant temperature. Fractions of narrow-molecular-weight distribution were obtained with good reproducibility. A continuous gradient-elution technique was applied by the same author to a column of Celite impregnated with polyethylene over its entire length (*10*). A variation of this technique was made in which the solvent composition was kept constant and the temperature was raised at intervals (*11*). In addition to polyethylene, cellulose nitrate and cellulose acetate were fractionated. The author critically evaluated the various fractionation techniques and their accompanying theories (*12*).

The Desreux technique was used by Francis et al. (*13*) to fractionate high-density polyethylenes. The column was filled with glass beads or sand and surrounded by a constant-temperature jacket maintained either by vapor heating or by circulation of thermostatted water. At a temperature of 127°C and a flow rate of 300 ml/hr, 1.2 g of the polymer was eluted stepwise with preheated cellosolve-*p*-xylene mixtures. Henry (*14*) was able to collect larger polymer fractions by a 40-fold scaleup of the above apparatus and by substituting Celite for the glass beads or sand. The efficiency of this fractionation was determined by rerunning single eluted fractions and combinations of fractions.

Kenyon and Sayler (*15*) applied the elution method to atactic polystyrene and Ziegler-type polyethylene and investigated several variables, notably sample size and elution rate. They found improved fractionation, especially toward the high-molecular-weight end, if the polymer was "selectively" deposited onto the column support prior to elution. This deposition was effected by lowering the column temperature at intervals of 5° every 15 min over an 8-hr period. The deposit was believed to consist of shell-like polymer fractions of decreasing molecular weight on the particles of the column packing.

Shyluk (*16*) reversed the solvent flow and found that an upward flow gave better control of flow rate and reduced channelling in the column. In the absence of selective deposition, atactic polypropylene could not be fractionated.

Tiselius (*17*) suggested a method for increasing the eluant concentration in a continuous manner. Alm et al. (*18*) investigated this new technique of gradient elution analysis. The separation of the two substances resulted from (1) the different rate of travel of the substance in the gradient of eluting concentration, and (2) the different strength of adsorption on the column packing. The apparatus consisted of a solvent chamber connected to a mixing vessel with magnetic stirrer, then the column. The solvent chamber contained a solution of the solvent to be used for development, while the mixing vessel was filled with the nonsolvent, which also filled the column. The application of pressure to the solvent chamber caused gradual mixing and movement of the solution down the column. A similar approach was described for the separation of organic acids in a silica gel column (*19*). The stem of the solvent reservoir extended almost to the bottom of the mixing vessel. Mixing occurred only by taking advantage of the difference between the density of the solvent and the nonsolvent. The resultant progressive increase in the polarity of the influent caused the separation, which would not take place using a solvent of fixed composition. Drake (*20*) presented a mathematical derivation to illustrate the principles involved in gradient elution analysis. Bannister et al. (*4*) used a 200-ml mixing vessel in their procedure for the separation of silicone polymers by gradient elution; animal charcoal was the adsorbant in the column. After a certain amount of effluent had been collected, the solvent was changed from ethanol to consecutively more concentrated solutions of diethyl ether in ethanol, and finally to pure ether. The effectiveness of these methods is limited, because only a single parameter is being employed to effect the separation. If more than one parameter is varied, the possibility of more effective separations is sometimes realized.

COLUMN-FRACTIONAL PRECIPITATION

Baker and Williams (*21*) developed a new chromatographic procedure for polymers, known as column-fractional precipitation. It involved equilibration between a stationary precipitated phase and a moving solution. The method was devised so that a multistage fractional extraction and precipitation process could be applied to the column. The polymer was supported in the column by inert material such as glass beads and subjected to both a thermal and elution gradient. The temperature of the packing varied linearly down the jacketed length of the column. This was brought about by heating the top of the insulated aluminum jacket with Nichrome wire and by cooling the lower part of the jacket with a circulating solution. By means of a drop capillary, "good" solvent entered the mixing vessel. This mixing vessel was filled initially with nonsolvent, in which only a negligible quantity of the material would dissolve, even at the highest temperature of the column. The "poor" solvent was thus gradually enriched with a solvent of higher elution power, so that the solution entered the column in the form of a logarithmic solvent gradient from the mixing vessel. The polymer was normally precipitated on the support at the top of the column. As the solvent power increased, the most soluble species of the material dissolved, moved down the column, and was reprecipitated by the cooler temperature. Reextraction was then achieved by equilibration as the mobile phase of further improved solvent power passed over it. In this manner each fraction underwent a sequence of fractional extraction and precipitation steps during its travel down the column. It emerged as a saturated solution at a temperature equal to that of the bottom of the column.

The method was applicable only if the substance had a positive temperature coefficient of solubility. Baker and Williams fractionated industrial types of polystyrene of large-molecular-weight distribution and polymers of narrow distribution, prepared under controlled conditions. The near-homogeneity of the collected fractions was demonstrated by a number of tests.

POLYMER FRACTIONATION BY COLUMN-FRACTIONAL PRECIPITATION

The method of Baker and Williams received immediate attention and was used to fractionate a variety of polymers. A number of modifications of the basic apparatus were made. An improved design to control the flow rate and composition gradient of the solvent was presented by Schneider and co-workers (*22*). Glass pipe fittings and Teflon joints were used to prevent solvent leakage. The solvent was purged of dissolved gas in a boiling flask before it entered the column. To make the heat transfer from the heating wire and to the cooling coils more efficient, the cooling water was carried through a helical groove cut into the wall of the aluminum jacket and sealed with a press-fitted sleeve. In addition, an annular space between the jacket and the column was filled with mineral oil, and the voltage on the heating wire was kept constant with a voltage regulator.

Jungnickel and Weiss (*23*) employed 3-ft columns made of copper, with thermostats at each end. Nearly constant solvent flow was achieved with a micropump, delivering from 1 to 50 ml/hr. Large preparative columns for polymer loads of 5 g (*24*) and 8 g (*25*) were reported. Caplan (*26*) miniaturized the column for the microanalysis of polymers. A column was made from a 1-ml pipette. The fractions were collected continuously with a paper-strip collector, and the polymer material was identified on the paper by isotopic labeling or dyeing. Polysarcosines, labeled with C^{14}, were fractionated into three species. Other column improvements were made by Cooper et al. (*27*) and by Pepper and Rutherford (*25*).

Column-fractional precipitation has been employed by Schulz (*28*) in a recent study of polyethylene glycol polymers. The technique gave inconclusive results, since any possible separation was obscured by the decomposition of the polymers. Schulz also modified the apparatus so that the fractions were automatically collected in a fraction collector located at the base of the column. Schneider et al. (*29*) fractionated two high-molecular-weight polystyrenes with and without a thermal gradient and found increased efficiency with the thermal gradient, especially toward the high-molecular-weight end of the polymer mixture. Guillet and co-workers (*24*) came essentially to the same conclusion. Similarly, Weakley et al. (*30*) fractionated polymethyl methacrylate both by the precipitation and elution methods. However, the solvent flow was inverted, and the temperature increased from the top to the bottom of the column. The advantage of the inverted column was that polymer gel, which was denser than the polymer solution in equilibrium with it, flowed into column regions of better solvent properties at higher temperatures. The likelihood of gel blocking the solvent flow was reduced. The efficiency of the two methods was found to be about equal, with the Baker-Williams' method slightly better.

Krigbaum and Kurz (*31*) investigated the fractionation efficiency of the two methods for amorphous and crystalline polymers. Both methods yielded poor results with crystalline polyacylonitrile. Better fractionation was obtained for crystalline isotactic polystyrene with the precipitation method. Both methods were effective with artificially composed amorphous polymer mixtures of varying degrees of heterogeneity.

FACTORS AFFECTING EFFICIENCY OF COLUMN-FRACTIONAL PRECIPITATION

By comparing the exit temperature of polymer fractions with their critical temperatures in solvents of the same composition as those in which they emerged from the column, significant differences were observed; these differences were attributed to adsorption effects on the presumably inert packings of sand or Celite. Adsorption became more pronounced as the molecular weight of the polymer was increased and as the particle size of the support was reduced (*31*).

Variables, such as sample size, flow rate, solvent gradient, column packing, and solvent–nonsolvent systems, were explored by several workers (*24,25,32*). Investigating high-molecular-weight polystyrene, Schneider et al. (*32*) found that the highest polymer concentration consistent with satisfactory fractionation depended on the molecular weight of the largest fraction and varied approximately inversely with the square root of the molecular weight.

Cooper et al. (*33*) investigated the fractionation of polybutadiene of narrow-molecular-weight distribution and observed that later fractions did not always follow the general increase in viscosity which occurred in initial fractions. This regression was attributed to back-diffusion of lower-molecular-weight species, caused by too rapid a change in solvent power along the column. It was concluded that the most important factor controlling efficient column fractionation was the establishment of the correct solvent power gradient.

The precipitation method was applied to study the changes in molecular-weight distribution due to thermal depolymerization of vinyl polymers (*34*). In the case of polyester, fractionation revealed that the experimental distribution agreed with that deduced from polymerization kinetics, but the agreement was not so convincing for some polypeptides. McLeod and Hulme (*35*) fractionated polyisobutylene and Scholtan (*36*) determined the molecular-weight distribution of polyvinyl pyrrolidone. The deviation of the distribution of "monodisperse" polystyrene samples from the Poisson distribution was ascribed to the formation of low-molecular-weight polymers by the action of contaminants initially present (*37*). Through fractionation, Cooper et al. (*38*) were able to correlate the polydispersity of butadiene polymers and copolymers with the degree of branching.

THEORY OF COLUMN-FRACTIONAL PRECIPITATION

The theory for general polymer fractionation describes the phase relationships of polymer-solvent systems and has been dealt with in papers by Scott (*39,40*), Cragg and Hammerschlag (*41*), and in the treatises of Flory (*42*) and Tompa (*43*).

An interpretation of precipitation chromatography is complicated by the lack of a relationship equivalent to an isotherm, since any quantity of the precipitated phase may be in equilibrium with the saturated mobile phase at a given temperature. Caplan (*26*) offered a semiempirical treatment of this system for the case of amorphous polymers. However, several assumptions had to be made:

1. When a polymer in a dilute solution of two solvents is cooled to the point corresponding to the binodial of its phase diagram, a small quantity of a highly viscous gel phase precipitates out in equilibrium with a much larger quantity of virtually pure solvent.
2. The composition of the dilute solution is close to the critical value. As the zone passes down the column, a single species of polymer will travel the length of the column in a zone corresponding to its critical temperature in the solvent in which it finds itself.
3. At the top of the column, the ternary system—polymer, solvent, nonsolvent—can be treated as a binary system, containing polymer and a single solvent, intermediate in properties between the two solvent components. A continuous series of solvents, having steadily decreasing Flory temperatures, enter the column.
4. The Flory temperature can be related to V, the volume of solvent that entered the column, by an empirical relation. V, in turn, is related to the volume fraction of good solvent by the exponential mixing function in the case of a constant-volume mixing vessel.

The resulting expression relates the molecular weight of a homogeneous species leaving the column to the exit temperature and the solvent composition,

$$\frac{1}{M^{1/2}} = \frac{\sigma}{KT_2}(1 - v_1) + \frac{1}{K}\left(\frac{\tau}{T_2} - 1\right) \tag{1}$$

where M is the molecular weight, T_2 the exit temperature, v_1 the volume fraction of good solvent, K a constant, and σ and τ parameters describing the solvent–precipitant system employed. However, this equation applies only to a single species at the exit of the column and represents nothing more that the description of extracting this species at the temperature of the critical zone. To include a distribution of species at each point of the column would lead to an equation too involved to be useful at this stage.

Caplan used data from Baker and Williams' (*21*) original paper on the fractionation of polystyrene to check Eq. (1). At constant T and with a constant-volume mixing vessel,

$$v_1 = 1 - e^{V/G} \tag{2}$$

where G is the volume of the mixing vessel. A plot of $KM^{-1/2}$ vs. $e^{V/G}$ should result in a straight line, which was found to be the case.

Theoretical curves, based on Eq. (1), for either an exponential or linear solvent gradient showed that the exponential gradient was much better for polymers of broad-molecular-weight distributions. For narrow distributions, the linear gradient offered greater sensitivity, but the maximum breadth of distribution should not be more than a hundredfold increase over the lowest molecular weight.

FRACTIONATION OF STEREOISOMERS

A novel new use of column-fractional precipitation was reported by Schulz and Purdy (*44*). They applied the technique to the separation of certain stereoisomers. With slight modification of the Baker and Williams' apparatus (*45*), these workers successfully separated 1,3,5-tri-(4-bromophenyl)benzene from 1,3,5-tri-(3-bromophenyl)benzene and *o,p*-quaterphenyl from *m,p*-quaterphenyl. Isomeric steroid mixtures of 5β-androstan-3α-ol-17-one and 5α-androstan-3α-ol-17-one and 3α-hydroxycholestane and 3β-hydroxycholestane were separated in 100-mg amounts. Schelz (*46*) has continued this work by applying the technique to the separation of androst-5-ene-3β, 17β-diol and androst-5-ene-3β,17α-diol. The degree of separation was determined by using the Rosenkrantz reagent (*47*) to develop the deep blue-violet color with both isomers.

The technique of column-fractional precipitation has also been applied to the sterol family of steroids (*46*). The sterols chosen were monohydroxysteroids having 27 to 29 carbon atoms. The naturally occurring compounds have a 3β-hydroxy group. Most of the members of this steroid family have one or more double bonds. Ergosterol is the most important 28-carbon sterol. It is readily converted by ultraviolet irradiation to lumisterol, a provitamin.

The lumisterol was in the form of irregularly shaped, clear crystals. The ergosterol crystals were smaller and yellow in color. From a study of the solubility characteristics using turbidimetric titrations (*44*), it was apparent that lumisterol was the more soluble in dioxane. The addition of water caused a fine lumisterol precipitate which did not settle on continued standing. The ergosterol precipitate was more coarse and settled rapidly. The solubility curves were rather steep, so that a mixing vessel of 250-ml capacity could be used. This vessel was initially filled with a 40% mixture of dioxane in water, the solvent–nonsolvent pair of choice. After fractionation the steroid distribution curve was determined with the help of the colorimetric reaction involving antimony trichloride in chloroform (*48*). The distribution curve showed two distinct peaks, the smaller of which was assigned to the lumisterol on the basis of the solubility characteristics of that isomer. This experiment indicated that column-fractional precipitation could be employed successfully for the separation of 50-mg amounts of ergosterol and lumisterol.

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