

Disassociation of Polyphenolics Studied by Dynamic Light Scattering and Size Exclusion Chromatography

INTRODUCTION

Southern pine bark contains approximately 30% by dry weight of alkali-soluble polyphenols. Interest in this polyphenolic material stems from an increased emphasis on bark utilization relating to our current energy needs. The chemical industry converts petroleum by-products into plastics, man-made fibers, resins, fertilizers, solvents, paints, and a host of other important speciality products. Due to the ever tightening supply and accompanying higher price of petroleum, it may become economically feasible to develop alternate sources such as waste bark for the raw materials required to manufacture such products.

Previous works^{1,2} have indicated that bark polyphenols were made up of a repeating series of flavonoid units shown in Figure 1. Other work³ has shown that polyphenols had a strong tendency to associate and that molecular weights of polyphenol derivatives depend upon extraction conditions. The objective of this study was to develop a solvent system in which the alkali-soluble polyphenols showed a minimum degree of self-association while in the free phenolic form. Various solvent systems were evaluated using dynamic light scattering, and the molecular weight (MW) and molecular weight distribution (MWD) of the polyphenols were estimated by high-performance size exclusion chromatography. Knowledge of the MW and MWD of the polyphenols in their least associated state is of fundamental importance if they are to be used in reactions to prepare, for example, resins with specific properties.

EXPERIMENTAL

Preparation of Alkali-Soluble Polyphenols

Polyphenolic material was obtained by stirring 20 g of bark previously extracted by benzene, methanol, and petroleum ether for 1½ hr with 1 liter 1% sodium hydroxide solution at 90–95°C under a nitrogen atmosphere. After filtering the solution, the filtrate was deionized using Rexyn 101 (H⁺) from Fisher and then evaporated to dryness under reduced pressure at 45–50°C. The yield was 5–6 g polyphenolic material. The water-soluble portion of this polyphenolic material was used in all subsequent experiments.

Size Exclusion Chromatography

The MW and MWD of the polyphenols were investigated by size exclusion chromatography (SEC) using a Waters Associates Model 202/401 high-pressure liquid chromatograph with du Pont size exclusion columns SE 60, 100, 500, and 1000 Å. Samples were injected at a concentration of approximately 1%. The elution flow rate was 1.2 ml/min, and the ultraviolet detector was employed for sample detection. Polyethyleneglycols from Waters Associates and sodium polystyrenesulfonates from Pressure Chemical were used to standardize the columns in methanol and aqueous sodium sulfate solutions, respectively.

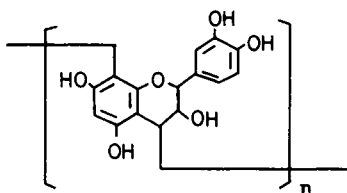


Fig. 1. Proposed molecular structure for polyphenols.

Dynamic Light Scattering

Dynamic light scattering (DLS) was performed using a Spectra Physics Model 124 A He-Ne laser and a Honeywell SAI-42A digital correlator. This light scattering technique provides a measure of the z -average translational diffusion coefficient (\bar{D}_z) of the scattering particles.⁴ A cumulant analysis⁵ of the DLS data also gave a measure of sample polydispersity. All solutions for light scattering were filtered through 0.8- μm Nuclepore membrane filters. A jacketed cell from Hellma allowed the temperature of the scattering solution to be controlled to within $\pm 0.02^\circ\text{C}$.

RESULTS AND DISCUSSION

The SEC chromatogram of the water-soluble portion of the polyphenols revealed that the sample was eluted at the void volume for a du Pont SE 1000 column having a molecular weight exclusion limit of approximately 1 million. A cumulant analysis of light scattering data on the same polyphenolic solution gave $\bar{D}_z = 2 \times 10^{-9} \text{ cm}^2/\text{sec}$ at $T = 25^\circ\text{C}$ and revealed marked sample polydispersity. A temperature dependence study showed that the polyphenols maintained their high degree of self-association in water over the temperature range of 10–70°C.

Aqueous solvents containing low molecular weight alcohols were found to be effective in reducing the degree of self-association of the polyphenols. The light scattering results using a series of water-alcohol solvents differing in composition are shown in Figure 2. The ratio of the average diffusion coefficient of the polyphenols in water compared to that in a particular solvent is plotted versus solvent composition. For solvents containing up to about 35% by volume of alcohol there was no apparent change in the \bar{D}_z of the polyphenols. If the amount of methanol in the solvent was increased beyond about 35%, there was an abrupt increase in the \bar{D}_z of the scattering particles, indicating a marked decrease in the degree of association of the polyphenols. Similar results were obtained for aqueous solvents of ethanol, propanol, and isopropanol, except that somewhat higher percentages of these alcohols were required before disassociation of the polyphenols was observed. Using methanol as the mobile phase and solvent for the polyphenol sample gave the SEC results shown in Figure 3. This chromatogram verified that in methanol the polyphenols were not as highly associated as in pure water. A polydisperse system described by a bimodal distribution having one predominant molecular weight of about 35,000 but also including smaller molecular weight aggregates was present.

Further investigation by light scattering as to the effects of other solvents revealed that aqueous solutions of sodium hydroxide, sodium sulfate, sodium bicarbonate, and sodium lauryl sulfate were effective in causing disassociation of the polyphenols. The average diffusion coefficient was de-

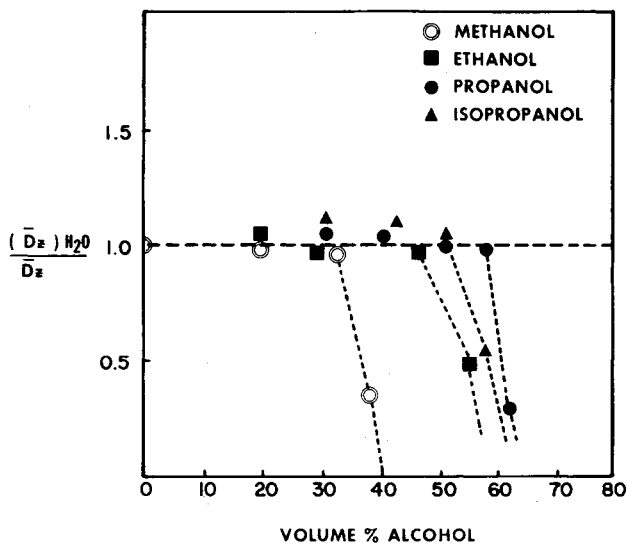


Fig. 2. Ratio of the average diffusion coefficient of polyphenols in water compared to that measured in various aqueous alcohol solvents.

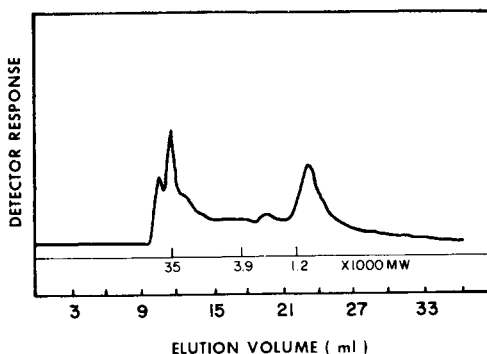


Fig. 3. SEC chromatogram of polyphenols dissolved in methanol.

terminated for the polyphenols in various sodium sulfate solutions. The results in Figure 4 show that for a solvent composition of about 0.2% by weight of sodium sulfate, a very sharp decline in the size of the scattering particles was observed. At slightly higher concentrations of sodium sulfate, the molecular size of the associated polyphenols was below that detectable by our DLS experiment ($MW \approx 10,000$). Such an abrupt change in the average diffusion coefficient of the polyphenols suggested that there was a critical solvent composition beyond which solvent-polyphenol interactions exceeded intramolecular forces of the self-associated polyphenols. Once the large polyphenol aggregates began to disassociate, they quickly collapsed to a much smaller and more uniform size rather than forming stable intermediate sized aggregates. Evidence for the existence of such low-order associated polyphenols was given by the SEC chromatogram shown in Figure 5. The polyphenols were in 4.4% sodium sulfate solution, and the molecular weight estimates from these data were $\bar{M}_w = 1050$ and $\bar{M}_n = 530$. These molecular weights were the lowest obtained for the polyphenols in any of the solvents studied.

In a separate study,⁶ the acetate derivative of the water-soluble polyphenols was prepared and subjected to SEC using tetrahydrofuran as solvent and polystyrenes as standards. The acetate derivative should have approximately 58% higher molecular weight than the parent free phenolic form. The chromatographic data gave an estimate of the molecular weight for the acetate derivative of $\bar{M}_w = 2080$ and $\bar{M}_n = 870$. Using these values to calculate the corresponding molecular weight of the free phenolic form gave $\bar{M}_w = 1320$ and $\bar{M}_n = 550$. These results indicated that the polyphenols in sodium sulfate solution had a degree of association comparable to that estimated for the derivatized polyphenols. However, our molecular weight estimates for the polyphenols in aqueous sodium sulfate could be somewhat low because of association of the polyphenols with the column

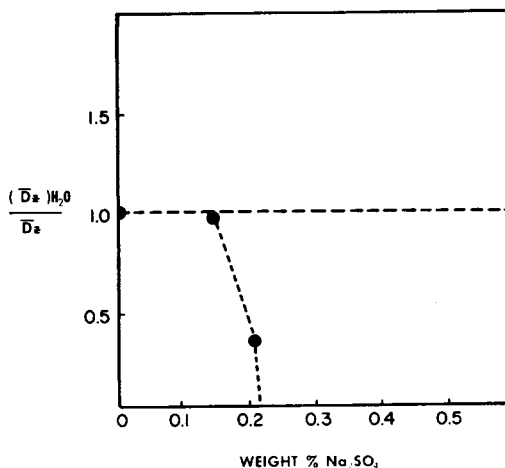


Fig. 4. Ratio of the average diffusion coefficient of polyphenols in water compared to that measured in various aqueous sodium sulfate solvents.

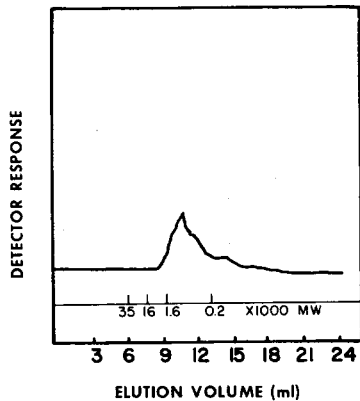


Fig. 5. SEC chromatogram of polyphenols in aqueous sodium sulfate.

packing as well as comparison of the polyphenols to polystyrenesulfonates regarding elution characteristics.

We conclude that in aqueous sodium sulfate, alkali-soluble polyphenols will disassociate to form lower-order aggregates that approach a least-associated state. For the synthesis of polymers from polyphenols, it will be advantageous to have as starting material polyphenols that are in such a least-associated state and that also are in free phenolic form.

References

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