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SIZE-EXCLUSION CHROMATOGRAPHY-LOW-ANGLE LASER LIGHT-SCATTERING PHOTOMETRY OF LIGNITE MACROMOLECULES*

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SUMMARY

The determination of molecular weight distribution in humic acids, derived from lignite, has been successfully carried out by the application of size-exclusion chromatography, coupled with low-angle laser light-scattering photometry. Reduction of soluble derivatives with zinc in acetic anhydride was required to reduce the absorbance so that a linear scattering response was obtained. A benzyl derivative and a methyl derivative exhibited the best chromatographic results, giving weight average molecular weights of about 1 500 000.

INTRODUCTION

One of the important parameters in the characterization of macromolecules derived from coal is the average molecular weight (\bar{M}_w) of the material. Information on the molecular weight distribution of a sample can aid in understanding the behavior of the macromolecule in various chemical and physical processes. Except for examination of the products resulting from the high-temperature reactions of lignite, there has been only one study of molecular weights of lignite-derived macromolecules¹.

The goal of our research in lignite macromolecules is to find a way to apply polymer characterization methods, such as low-angle laser light-scattering (LALLS) photometry in a static mode and also in a coupled mode with size-exclusion chromatography (SEC) to the analysis of macromolecules, derived from coal by oxidation, pyrolysis, supercritical extraction and chemical extraction.

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The macromolecules which comprise low-rank coal structures must be solubilized in order to measure their molecular weights. The most convenient macromolecule to study is the humic acid fraction, obtained by sodium hydroxide treatment of the lignites. Although this fraction comprises less than 5% of the coal structure, it is one obtained under low-temperature conditions, where thermal cleavage reactions have not extensively degraded or depolymerized the macromolecule. Much further work is required to establish how closely the humic acid resembles the remainder of the lignite structure.

A number of methods have been employed to determine molecular weights of humic materials, and a wide range of molecular weights have been reported. Several authors have determined humic acid molecular weights by SEC on Sephadex columns. Posner² measured M_w values for soil humic acids, ranging from 10 000 to 200 000, and Dubach *et al.*³ from 3000 to 100 000. Rashid and King⁴ have reported marine sediment humic acid molecular weights, ranging from 700 to 2 000 000. Aquatic humic acid values from 700 to 26 000 have been reported by Gjessing⁵, and from 3000 to 4000 by Plechanov⁶. High-performance SEC of aquatic humic acid on Bondagel-E125 was reported by Miles and Brezonik⁷. Low molecular weights (<2000) were believed to have been found in that sample.

A low-molecular weight humic acid fraction from Maritzan-Iztok (Bulgarian) lignite was investigated by Sephadex gel filtration, calibrated with protein standards¹. \bar{M}_w values of 2900 to 8600 were reported for four fractions.

Vapor-phase osmometry has been utilized by Reuter and Perdue⁸ to study aquatic humic acid. Values for \bar{M}_w which were less than 2000 were obtained. Also using vapor phase osmometry, Schnitzer and Skinner⁹ measured small \bar{M}_w values (<1500) for soil fulvic acids. Molecular weights of humic acids have also been determined by density-gradient ultracentrifugation¹⁰ which gave large values (730 000). Small angle X-ray scattering¹¹ gave a molecular weight of 1 000 000 for an aqueous humic acid.

Although SEC is an excellent technique for separating polymers on the basis of size and provides information on the distribution of molecular weights, it does not directly provide molecular weight data. The calibrations necessary to determine the molecular weights of eluted macromolecules from the retention volumes are subject to large errors. These errors arise because the polymers used as calibration standards may not be similar enough in shape to the polymer whose molecular weight is being determined.

The effects of differences in shape on molecular weights, determined from elution volumes, have been studied by Chao and Einstein¹², who obtained considerably higher values for flexible proteins using a calibration based on globular proteins than the values obtained by ultracentrifugation. Interactions of polar solutes with the column can also result in errors. The polar functional groups (phenols, amines, quinones and carboxylic acids) present in humic acids can associate strongly with the polar hydroxyl and ether groups on the Sephadex gels¹³. Janson¹⁴ has described two departures from ideal behavior on Sephadex gels: (1) attractive interactions that result in adsorption and, consequently, retardation on the column and (2) repulsive interactions that result in exclusion and more rapid movement on the column. Because of the non-uniform shape and structure of the humic acid molecules, Wershaw and Pinckney¹⁵ have concluded that molecular weight determinations of humic acids

by SEC are of doubtful value. Besides the problem of calibration, changes in analytical conditions, such as variation in flow-rate or pressure, can result in errors.

Rather than examining the molecular weights of fractions collected from SEC of humic acids by another technique, we preferred to determine the molecular weights of the molecules on-line, as they were eluted. Determination of the molecular weight of collected fractions is tedious and may introduce errors because of sample degradation, oxidation, or condensation to higher-molecular-weight species. The only technique for on-line molecular weight determination is LALLS photometry with a flow-cell. Since the LALLS photometer gives absolute molecular weight data, no calibration of the SEC column is necessary. The concentration of molecules in the eluent is determined by a refractive index detector in series with the LALLS photometer.

The major problem involved in utilizing LALLS with coal-derived macromolecules is the intense absorption of light at 633 nm, making scattering difficult to observe and the scattering data more difficult to analyze. The high molar absorptivity is believed to result from extensive conjugation of arene systems, nitrogen heterocyclics, and semiquinone structures.

In a pioneering effort to apply LALLS to coal macromolecules, Hombach¹⁶ has introduced a modification to the virial equation used for calculation of \bar{M}_w from the Rayleigh scattering data. Thus, a term was added to account for the exponential dependence of absorbance and fluorescence on the concentration. Bituminous coal was treated with potassium in glyme, and a pyridine extract of the treated coal was fractionated by ultrafiltration to give two products in low yield. A plot of Rayleigh scattering factors (Kc/\bar{R}_θ) versus C for the two fractions was analyzed by curve fitting to the modified virial equation. Resolution of the non-linear behavior of the plot into a linear and an exponential component gave \bar{M}_w values for the fractions ($1.4 \cdot 10^6$ and $0.58 \cdot 10^6$) consistent with ultracentrifugation results.

EXPERIMENTAL

Humic acids

The lignite (50 g, as received) was stirred with 500 ml of 5% sodium hydroxide for 24 h under nitrogen. The suspension was centrifuged at 2450 g and the supernatant was decanted. The sodium humate solution was used directly in the procedures below for methylation, acetylation and benzylation. For isolation of the humic acids, the sodium humate solution was acidified with hydrochloric acid and the precipitated humic acids were centrifuged at 2450 g. The humic acids were washed several times with water, centrifuging the humic acid precipitate, until the supernatant water was neutral. The humic acids were dried by transferring them to a flask with ethanol and rotary evaporation.

Methylation

To the sodium humate solution described above 25 ml of dimethyl sulfate was added slowly, additional sodium hydroxide being used to keep the solution basic. The solution was refluxed 24 h. The methoxyhumic acid was precipitated with hydrochloric acid, collected by centrifugation, washed several times with water, and dried as above. The methoxyhumic acid was dissolved in N,N-dimethylformamide (DMF) and allowed to react with diazomethane in ether (from Diazald). The ether

was removed by rotary evaporation, and water was added to precipitate the methyl humate. The methyl humate was treated with 100 ml of acetic anhydride and 20 g of zinc dust. Refluxing was carried out for 72 h, with the addition of fresh zinc every 24 h. Acetic acid (50 ml) was added, and refluxing was continued for 15 min. The solution was filtered hot and the filtrate was combined with four volumes of water. After boiling the solution for 30 min and cooling it in ice, the precipitate was centrifuged, washed several times with water, and dried with ethanol.

Acetylation

The sodium humate solution was stirred with 25 ml of acetic anhydride for 24 h at room temperature. The acetoxyhumic acid was then precipitated, methylated with diazomethane, and reductively acetylated, as in the above procedure.

Benzylation

The sodium humate solution was stirred with 25 ml of benzyl chloride for 24 h at room temperature. The benzyl derivative was precipitated and reductively acetylated as in the methylation experiment. The excess benzyl chloride was partially removed during the rotary evaporation at 60°C; the remainder reacted in the last step.

Instrumentation

SEC was carried out with a syringe pump (Model 314, ISCO, Lincoln, NE, U.S.A.), an Altex injector with a 50- μ l loop (Beckman, Irvine, CA, U.S.A.), a 30 cm \times 7.5 mm I.D. 10^5 Å PLgel column (Polymer Labs., Stow, OH, U.S.A.), and tetrahydrofuran (THF) as the solvent. Sample concentration was 2 mg/ml. A KMX-6 LALLS photometer (LDC/Milton Roy, Riviera Beach, FL, U.S.A.) with a flow-through cell and Waters Model 401 refractive index (RI) detector (Millipore, Milford, MA, U.S.A.) were used for measuring the Rayleigh scattering and RI response of the eluent from the SEC column. An Omega A/D converter (Model WB-31, Omega Engineering, Stamford, CT, U.S.A.) with an RS 232C serial output interfaced the detectors with a Tandy 2000 microcomputer (Tandy, Fort Worth, TX, U.S.A.). Data were acquired at 3.5 points/s for each detector. The LALLS and RI peaks were divided into 100 area slices each for calculation of the molecular weights. The dn/dc values (specific refractive index increments) were determined using a KMX-16 differential laser refractometer (LDC/Milton Roy) at several concentrations. The repeatability of the SEC-LALLS determination was investigated for the reduced methyl humate-I. Mean variation of the \bar{M}_w of this sample was less than 1000 daltons.

RESULTS AND DISCUSSION

The specific objective of our work was to reduce the molar absorptivity of the humic acid macromolecules from lignites to a very low value, such that a linear dependence of Kc/\bar{R}_θ on C would be obtained, thus allowing the absolute molecular weights of eluents from a size-exclusion column to be determined. A number of reactions for solubilizing and reducing humic acids were studied. The potassium reduction used by Hombach¹⁶ did not give any significant amount of pyridine solubles from lignite, undoubtedly because of the high content of carboxylic acid groups.

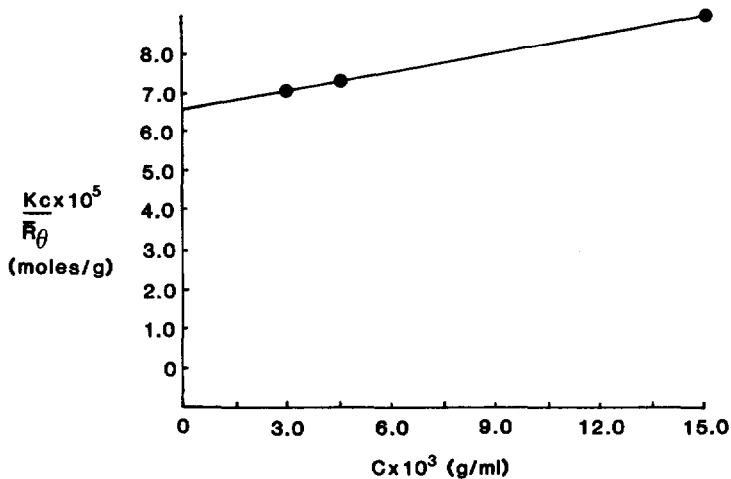


Fig. 1. Kc/R_θ versus C for static \bar{M}_w determination of NaBH_4 - RhCl_3 reduced humic acid.

To obtain material which could be reduced, it was necessary to increase the solubility by alkylating the carboxylate and phenolic groups in the humic acids. Our first experiments utilized a methyl humate, prepared from Beulah lignite humic acids by treatment with methyl iodide in a two phase system with a phase-transfer catalyst.

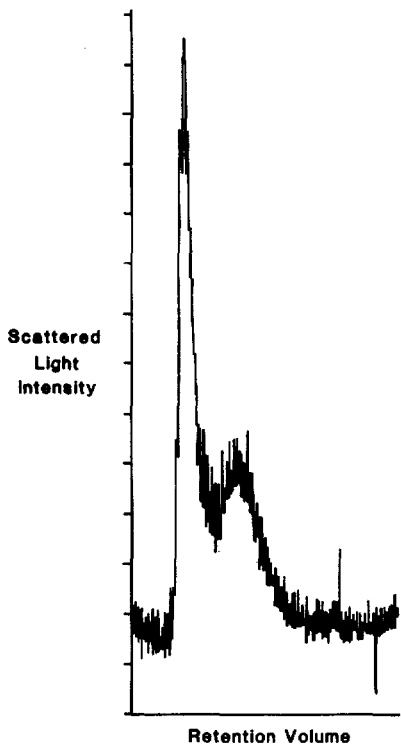


Fig. 2. SEC-LALLS of NaBH_4 - RhCl_3 reduced humic acid.

The initial reduction experiment was a mild hydrogenation with sodium borohydride and a rhodium catalyst. In model compound studies, this reagent was successful in reducing several of the aromatic rings of perylene and pyrene at 20°C and at atmospheric pressure to yield a mixture of hydroaromatics with very low molar absorptivity. The reduction of the arene or semiquinone systems present in the methyl humate did occur to give a low absorbance product. However, the yield varied considerably and was generally poor. The scattering plot (Fig. 1) was quite linear for this material. Difficulties were encountered in the attempted SEC of this reduced methyl humate. A solution of the reduced methyl humate in THF was characterized in the SEC-LALLS-RI system. The chromatogram (Fig. 2) exhibited a distribution consisting of two overlapping peaks. Consistent results were not obtainable; the behavior in SEC was attributed to adsorption of some of the material on the column. Because of the suspected heterogeneity of the samples and the poor yields, this approach was abandoned.

In an effort to reduce the semiquinone and quinone groups of the black humic acids, the following reactions were carried out: (1) refluxing with zinc powder and base, (2) stirring with $\text{Na}_2\text{S}_2\text{O}_4$ and base, (3) stirring with FeSO_4 in triethanolamine, (4) stirring with SnCl_2 in base, (5) refluxing with zinc powder and acetic anhydride, and (6) refluxing with SnCl_2 and acetic anhydride. The reductive acetylation (No. 5) gave a product with the lowest absorptivity. The yield of light tan material was 25% (1% based on starting Beulah lignite). The reductively acetylated humic acid was soluble in DMF but not very soluble in THF. The dn/dc value in DMF was 0.16; a static molecular weight (\bar{M}_w) of $1.5 \cdot 10^6$ was determined in this solvent. The plot of Rayleigh scattering factors was highly linear ($R^2 = 0.96$) with a very small negative slope ($A_2 = -5 \cdot 10^{-5}$). An SEC-LALLS experiment with this material on a polystyrene-divinylbenzene PL gel (10^5 \AA) was unsuccessful because of high background

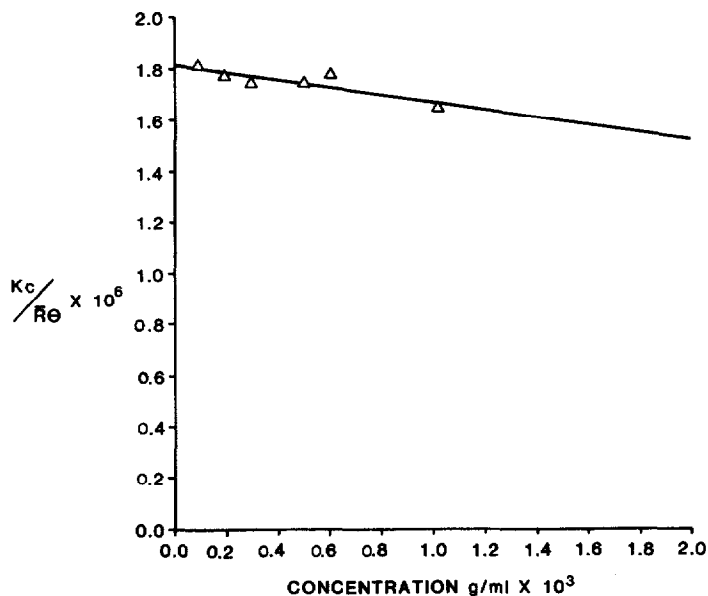


Fig. 3. Kc/R_θ versus C for zinc-reduced humic acid.

scattering due to column breakdown under high back-pressure with this viscous solvent. In contrast, the RI response was not noisy. A second sample of reductively acetylated humic acids obtained from Beulah lignite also exhibited a linear static LALLS plot ($\bar{M}_w = 5.5 \cdot 10^5$, $A_2 = -7 \cdot 10^{-5}$, DMF) (Fig. 3). This sample was dissolved in a THF-DMF mixture (1:1, v/v). SEC in this binary solvent gave a less noisy background with the LALLS detector. However, multiple peaks were eluted over a long time. This behavior was attributed to adsorption; although the LALLS data was believed to be accurate, the chromatography was not ideal.

In order to obtain a material more soluble in THF, the humic acids were methylated first with dimethyl sulfate in sodium hydroxide solution, then with diazomethane. A quantitative conversion to the reductively acetylated methyl humate (reduced methyl humate-I) was obtained with zinc and acetic anhydride. The product was easily soluble in THF. Methylation of both the phenolic groups and carboxylic acid groups was essential to the desired solubility characteristics as well as the improvement in yield. The dn/dc value in THF was 0.22; a linear plot of Kc/\bar{R}_θ vs. C was obtained, which exhibited a small negative slope ($A_2 = -4.2 \cdot 10^{-5}$). \bar{M}_w was $1.3 \cdot 10^6$.

SEC of the reduced methyl humate-I in THF on the 10^5 -Å PL gel column gave smooth, single peaks for both the LALLS and RI detectors (Fig. 4). Values for the molecular weights calculated from the LALLS and DRI curves and polydispersity ratios are given in Table I.

Since the methylation with dimethyl sulfate in the first step of the above sequence involves heating in base, it is a step where degradation of the humic acid macromolecule could occur by hydrolysis of amide or ester linkages or cleavage of ethers of the *p*-hydroxybenzyl type, such as found in lignins. In an attempt to determine whether this degradation was really occurring, the dimethyl sulfate treatment

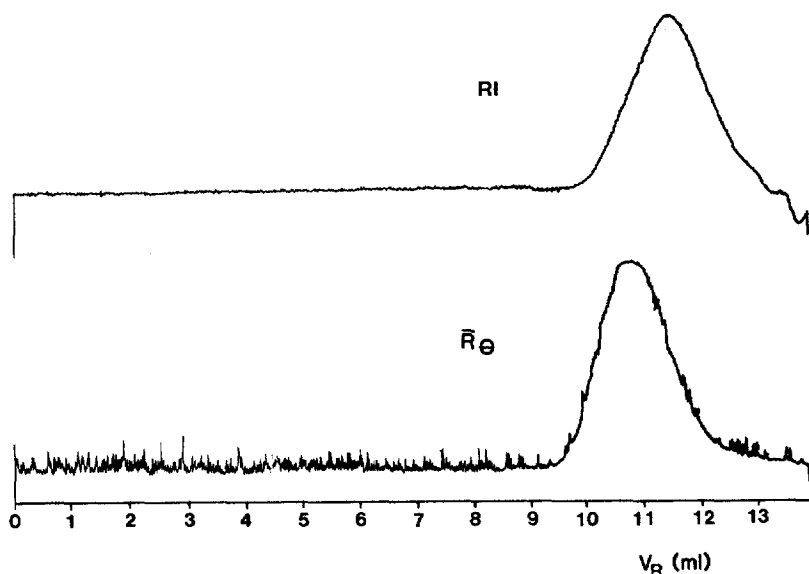


Fig. 4. SEC-LALLS of reduced methyl humate-I, 10^5 Å PL gel, THF (1 ml/min), 22°C.

TABLE I

STATIC LALLS AND SEC-LALLS RESULTS FOR REDUCTIVELY ACETYLATED HUMATE DERIVATIVES

| Humate derivative | \bar{M}_w (static) | \bar{M}_n | \bar{M}_w | \bar{M}_z | $M_z:M_w:M_n$ |
|----------------------------|----------------------|------------------|------------------|------------------|---------------|
| Reduced methyl humate-I | $1.3 \cdot 10^6$ | $9.7 \cdot 10^5$ | $1.4 \cdot 10^6$ | $1.8 \cdot 10^6$ | 1.9:1.3:1 |
| Reduced methyl humate-II | $1.9 \cdot 10^6$ | | | | |
| Reduced acetoxy humate-III | $4.7 \cdot 10^5$ | $3.1 \cdot 10^5$ | $4.3 \cdot 10^5$ | $5.4 \cdot 10^5$ | 1.7:1.2:1 |
| Reduced benzyl humate | $1.8 \cdot 10^6$ | $1.3 \cdot 10^6$ | $1.7 \cdot 10^6$ | $2.0 \cdot 10^6$ | 1.5:1.2:1 |

was carried out at ambient temperature, followed by diazomethane addition and reductive acetylation. Unfortunately, a low yield of material (reduced methyl humate-II) was obtained. The static LALLS gave an \bar{M}_w value of $1.9 \cdot 10^6$ ($A_2 = 8.7 \cdot 10^{-5}$, $dn/dc = 0.20$, $r^2 = 0.94$).

There are two explanations for the higher value of \bar{M}_w for the sample which was methylated at ambient temperature: either the heating in base during methylation simply degrades the macromolecules or else the substantial amount of material which was lost during the ambient temperature preparation has a lower average molecular weight, resulting in a higher value for the molecular weight of the recovered product.

Two alternatives to the methylation procedure for increasing the solubility were investigated: in the first study, and acetylation step was carried out on the solution of the humic acid in sodium hydroxide at ambient temperature. The acetylated humic acid was then methylated with diazomethane and finally treated with zinc in acetic anhydride to give the reduced acetoxy humate-III. A yield of about

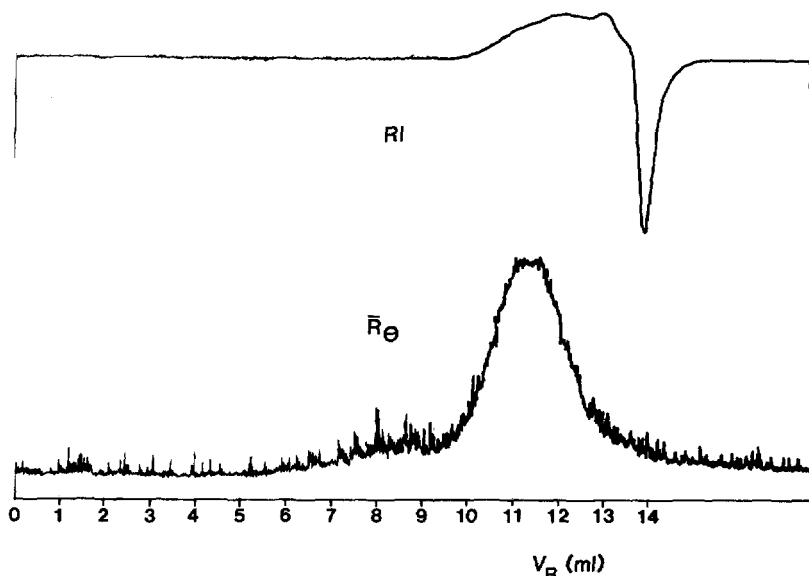


Fig. 5. SEC-LALLS of reduced acetoxy humate-III, 10^5 \AA gel, THF (1 ml/min), 22°C. (A large negative peak was consistently observed in the RI curve. The origin is unknown.)

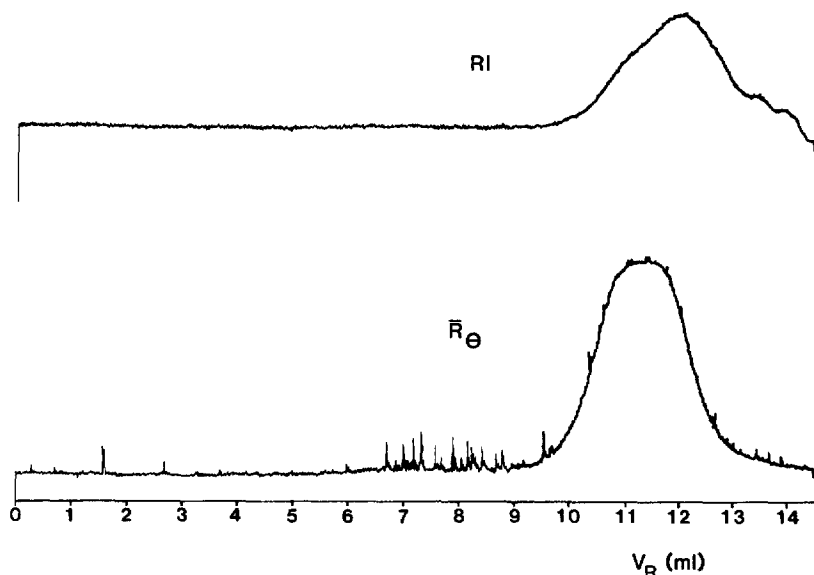


Fig. 6. SEC-LALLS of reduced benzyl humate, 10^5 Å PL Gel, THF (1 ml/min), 22°C.

35%, based on humic acid was obtained. The static LALLS measurements in THF gave an \bar{M}_w value of $4.7 \cdot 10^5$ ($dn/dc = 0.25$, $A_2 = -5 \cdot 10^{-5}$). The SEC-LALLS data (Table I) (Fig. 5) for this material were similar to the static data and also to those for the reduced methyl humate-I. Because of the loss of material in this experiment, no conclusion regarding the possible degradation during the methylations could be drawn.

A more successful experiment was the benzylation of humic acid with benzyl chloride in sodium hydroxide at ambient temperature. The benzyl humate was reductively acetylated to give a quantitative yield of the reduced benzyl humate. This material was very slightly colored and highly soluble in organic solvents. The static LALLS determination in THF gave an \bar{M}_w value of $1.8 \cdot 10^6$ ($dn/dc = 0.19$, $A_2 = -4.1 \cdot 10^{-5}$). The SEC-LALLS curve (Fig. 6) was a single symmetrical peak; the calculated \bar{M}_w value of $1.7 \cdot 10^6$ was close to the value from the static measurement.

The polydispersity ratios for the Z average, weight average and number average molecular weights ($M_z:M_w:M_n = 1.5:1.2:1$) are lower than those obtained for the other reductively acetylated products, and are lower than that obtained for the NBS 706 polystyrene standard (2.3:1.5:1).

Since the benzylation reaction did not involve heating and gave a molecular weight similar to that obtained by methylation (with heating), it is likely that the heating in base did not significantly degrade the macromolecules. All the materials were heated with zinc and acetic anhydride; the effects of this treatment on molecular weight is unknown.

CONCLUSIONS

A study of the application of LALLS and SEC-LALLS to derivatives of humic

acids has demonstrated the feasibility of determining molecular weight distributions of coal macromolecules. The most effective methods for carrying out these determinations are to convert the humic acids to a form highly soluble in THF by benzylation or exhaustive methylation and then to acetylate reductively with zinc in acetic anhydride in order to reduce the semiquinone and quinone moieties to a nearly colorless form.

Plots of the Rayleigh scattering factors (Kc/\bar{R}_θ) versus C were linear, indicating minimal light absorption effects. The molecular weights of the humate derivatives are 1 500 000 and the distributions are unimodal. Polydispersity ratios are relatively low.

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