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CHROMATOGRAPHIC BEHAVIOUR OF AROMATIC COMPOUNDS ON SEPHADEX LH GELS

CALIBRATION OF GEL COLUMNS FOR DETERMINATION OF MOLECULAR WEIGHT DISTRIBUTIONS

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SUMMARY

The chromatographic behaviour of phenols, phenolic acids, aromatic carbonylic compounds and cinnamic compounds on Sephadex LH gels is determined, and the dependence of the elution characteristics on the composition of the solvent, dioxane–water are reported. The results show that a dioxane–water composition of 7:3 causes the distribution coefficients to lie in a narrow range, in spite of the chemically different character of the investigated compounds. In pure dioxane strong adsorption effects are observed. In water–dioxane mixtures, however, partition effects occur when the water content is considerably higher than 30%.

The results are used to design a gel chromatographic system for molecular weight determination using Sephadex LH gels and dioxane–water as eluent.

INTRODUCTION

Gel chromatography is a widespread analytical method for the determination of molecular weights and molecular weight distributions of polymers. In our work the separation of lignin fractions was of major importance. For this purpose we required a simple method for determining in a short time the molecular weight distributions of hydrothermally degraded lignins[1,2].

Several authors have reported investigations of lignin by gel chromatographic methods[3–12]. The authors of two recent studies[13,14] obtained good results using alkylated dextran gels (Sephadex LH-20 and Sephadex LH-60*) as stationary phases and dimethylformamide as solvent.This solvent was unsuitable for our hydrothermally degraded lignin products for the following reasons:

Investigations of the solubility of these lignin derivatives showed that the best results were obtained in dioxane–water and acetone–water mixtures. Because of the high UV absorption at low wavelengths (UV cut-off at 330 nm), acetone was not suitable as eluent.

* Sephadex LH gels are hydroxypropylated cross-linked polysaccharides manufactured by Pharmacia, Uppsala, Sweden.

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Hydrothermally degraded lignin is very reactive. Even the removal of the solvent at temperatures above 50°C causes considerable changes in the sample by forming insoluble precipitates. Dioxane–water mixtures are easy to remove from lignin, either under reduced pressure or by freeze-drying.

Preliminary investigations showed that the chromatographic behaviour of lignin degradation products with different functional groups needs to be examined intensively. The relatively stable dioxane–water mixtures were found to be particularly suitable for these investigations.

The present paper deals with the selection of good separation systems and the calibration of these systems for the determination of molecular weight distributions.

EXPERIMENTAL

Materials

The investigations were carried out with commercially available solvent-resistant columns (SR 10/50, 50 × 1 cm I.D., Pharmacia). The chromatographic characteristics of the separation systems were determined using 25 monomeric lignin degradation products and related compounds (E. Merck, Darmstadt, G.F.R., and Fluka, Buchs, Switzerland), polystyrene and polypropylene glycol molecular weight standards (Waters Assoc., Milford, MA, U.S.A.). LH-20 and LH-60 were selected as stationary phases.

Methods

The columns were packed with the gels swollen in several solvents, according to the method of Determann[15]. Before packing, however, the gels were treated in an ultrasonic bath for *ca.* 10 min to remove air.

The column data are given in Table I or in the legends of Figs. 4, 7 and 8. Dioxane or its mixtures with water were used as mobile phases. The void volume (V_0) was determined using the excluded part of the hydrothermally degraded lignin. The solvent was delivered by a reciprocating piston pump with a pulse dampener (Model 110, Altex, Berkeley, CA, U.S.A.) and passed down the columns. The flow-rates were 0.3 and 0.5 ml/min for the LH-20 columns and 0.1 ml/min for the LH-60 columns, respectively.

TABLE I

CHROMATOGRAPHIC DATA FOR THE SEPARATIONS ON SEPHADEX LH-20

Eluent composition dioxane–water (mobile phase)	*Flow-rate (ml/min)*	*Bed volume V_t (ml)*	*Void volume* V_0 (ml)*	*Elution volume of acetone, V_{ac} (ml) $V_e = V_{ac}$*	*K_{av} of acetone***	*Composition of the solvent in the stationary phase*[16]
1:0	0.5	27.5	10.0	22.5	0.714	1:0
9:1	0.5	27.5	8.5	21.5	0.684	9:2
7:3	0.5	31.4	9.1	24.3	0.681	7:4
1:1	0.5	27.5	8.8	—	—	1:1

* V_0 was determined with the excluded part of hydrothermally degraded lignin[1,2].

** K_{av} is defined in eqn. 1.

The UV absorption at 254 nm and the refractive index were continually measured with a detector (Dualdetektor No. 103.07, Knauer, Oberursel, G.F.R.), the absorption at 280 nm was monitored with the detector model 770 (Spectra Physics, Santa Clara, CA, U.S.A.). The samples were applied to the column by a sample loop valve (Altex) using a 0.1-ml loop. Sample aliquots were 0.14 mg of each lignin and 0.1 mg of the model compounds.

RESULTS AND DISCUSSION

Selection of the phase

In preliminary investigations, a series of commercially available packings (Sephadex G-types, polystyrene gels, etc.) were tested. Thereby Sephadex LH gels were found to be most suitable for hydrothermally degraded lignins.

Dependence of the gel chromatographic behaviour on the solvent composition

The chemical analysis of the hydrothermally degraded lignin showed compounds of different substance classes. Most of these compounds are aromatics, the larger part of them containing several functional groups (phenolic carbonyl compounds, phenolic acids, etc.). Aliphatic substances were also found.

The elution behaviour of some already identified lignin degradation products as well as related compounds was investigated with different solvents and solvent mixtures to find a method which guarantees gel chromatographic separation of these chemically different substances according to their molecular size.

As hydrothermally degraded lignin showed a high solubility in dioxane, the experiments were carried out with this solvent and its mixtures with water up to a ratio of 1:1. Acetone, ethanol and other solvents were rejected because of their unfavourable UV cut-off, or their unspecific separation results or their high boiling points. In each case 25 monomeric compounds were applied to the LH-20 gels. The corresponding column data are summarized in Table I. As can be seen, the K_{av} values of acetone show only slight deviations in dioxane–water mixtures, whereas in pure dioxane the value is somewhat higher. To a rough approximation, however, it is independent of the solvent composition and thus can be used as a reference to the accessibility of the solutes to the pore volume.

To describe the elution behaviour the distribution coefficients, K_{av}, were calculated according to the method of Laurent and Killander[17]:

$$K_{av} = \frac{V_e - V_0}{V_t - V_0} \tag{1}$$

where V_e is the elution volume of the solute, V_t is the bed volume and V_0 is the void volume.

Figs. 1–3 show the dependence of the K_{av} values on the solvent composition. Most of the distribution coefficients decrease considerably as the solvent is changed from pure dioxane to dioxane–water mixtures up to a ratio of 9:1. They increase with further addition of water to the eluent. This tendency is particularly pronounced with the carbonyl compounds, the cinnamic alcohols and the phenols (Figs. 2 and 3). The differences of the K_{av} values in pure dioxane are very useful for analytical separa-

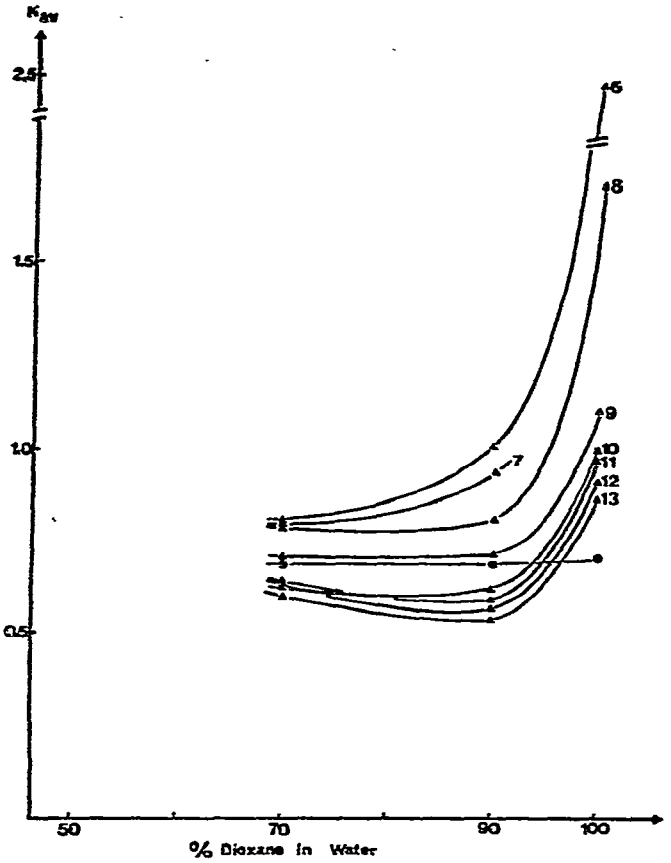

Fig. 1. Dependence of the distribution coefficients for aromatic acids on the eluent composition. Mobile phase, dioxane–water; stationary phase, Sephadex LH-20. 6 = 3,4-Dihydroxybenzoic acid; 7 = 4-hydroxybenzoic acid; 8 = 4-hydroxycinnamic acid; 9 = vanillic acid; 10 = ferulic acid; 11 = syringic acid; 12 = veratric acid; 13 = sinapic acid; ● = acetone.

tions. For instance, a mixture of three components was separated and in succession vanillin, phenol and 4-hydroxybenzaldehyde were eluted (Fig. 4). Molecular weight determinations, however, are not possible in this system.

The considerable retardation of many compounds in pure dioxane is due to adsorption effects in which the hydrogen-bonding plays the most important part. This has already been shown by Gelotte[18], Somers[19], Determann and Walter[20] and Brook *et al.*[21–23], who found that phenols and other aromatics are strongly adsorbed by hydrogen-bonding between the solute and the ether or hydroxyl groups on the Sephadex gels.

A comparison between the distribution coefficients and the structures of the compounds investigated in the present work revealed (Figs. 1–3) that there is a clear relationship between the K_{av} values and the number of free phenolic hydroxyl groups, the influence of keto or aldehyde groups and the number of methoxy groups, which are *ortho* to the 4-hydroxy group.

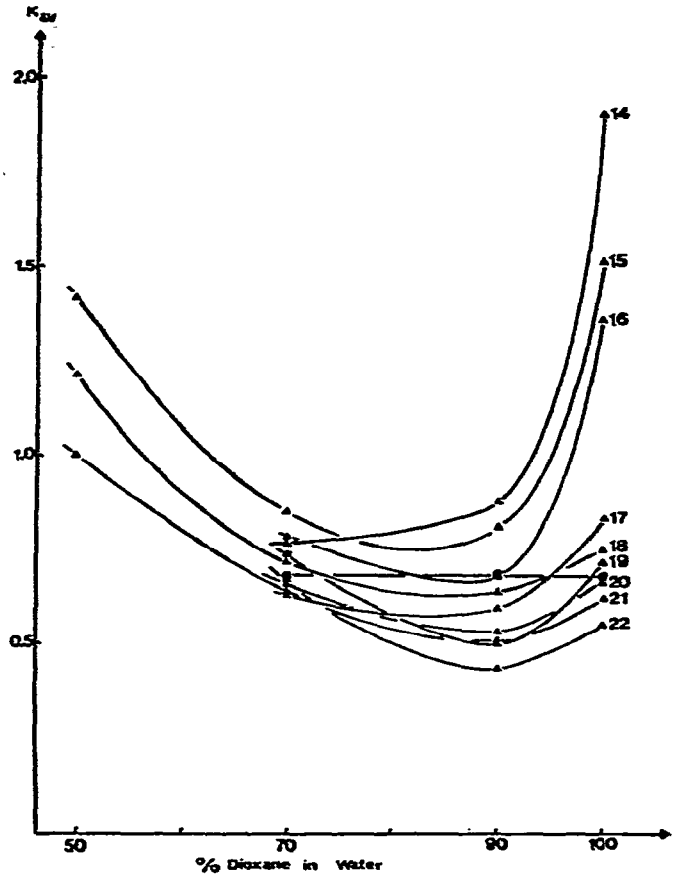

Fig. 2. Dependence of the distribution coefficients for carbonylic aromatics and cinnamic alcohols on the eluent composition. Mobile phase, dioxane–water; stationary phase, Sephadex LH-20. 14 = 3,4-Dihydroxybenzaldehyde; 15 = 4-hydroxybenzaldehyde; 16 = 4-hydroxyacetophenone; 17 = coniferyl alcohol; 18 = vanillin; 19 = cinnamic alcohol; 20 = acetovanillon; 21 = syringaldehyde; 22 = veratraldehyde; ● = acetone.

Therefore, compounds without a free hydroxyl group (*e.g.* veratrole) have low distribution coefficients. These effects can also be observed with the acids (Fig. 1). Owing to the presence of the carboxylic group the minimum region of the K_{av} values is larger. In several cases it was shown that the adsorption of phenols on Sephadex gels obeys a linear free energy relationship of the Hammett type[21,23,24]. In the present work the substances were mostly di- or tri-substituted. For some compounds the necessary measurements are lacking and therefore only in some individual cases (*e.g.* phenol, 4-hydroxybenzaldehyde, 4-hydroxybenzoic acid and 4-hydroxycinnamic acid) a good correlation with the Hammett equation was found.

The high distribution coefficients in pure dioxane can be much reduced by adding water to the eluent. In the case of 10% water, for example, the K_{av} value of 3,4-dihydroxybenzaldehyde decreases by 1.1 units. With a dioxane–water ratio of *ca.* 9:1 most of the K_{av} measurements show the lowest value. If the water content is further

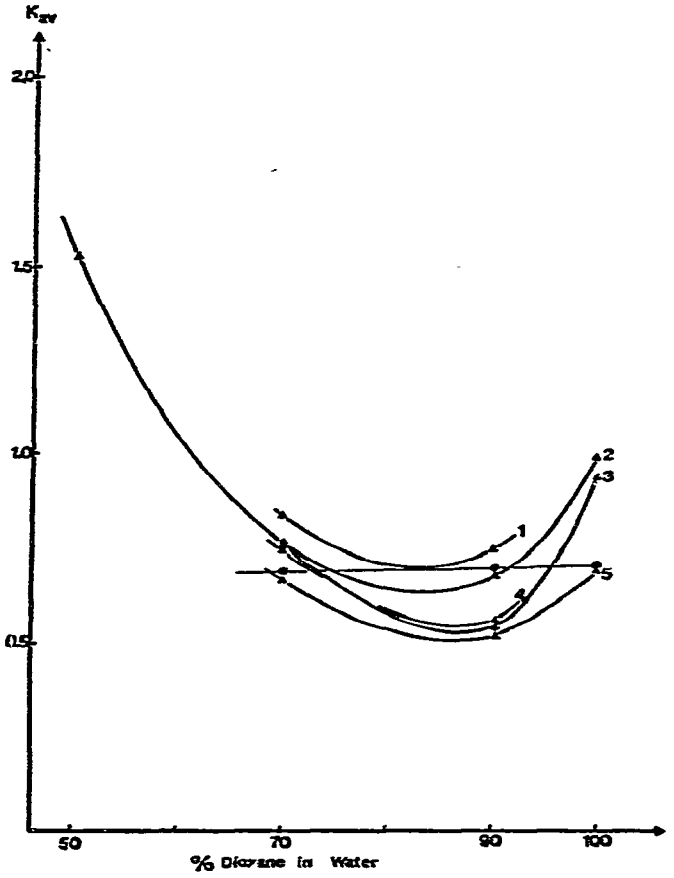

Fig. 3. Dependence of the distribution coefficients for phenols and phenolic ethers on the composition of the eluent. Mobile phase, dioxane–water, stationary phase, Sephadex LH-20. 1 = Catechol; 2 = phenol; 3 = *o*-cresol; 4 = *p*-cresol; 5 = guaiacol; ● = acetone.

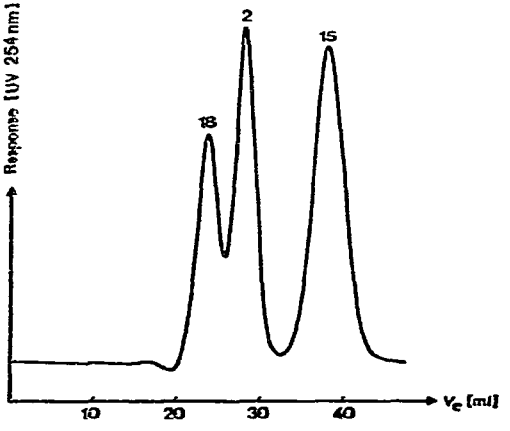

Fig. 4. Gel chromatographic separation of vanillin (18), phenol (2) and 4-hydroxybenzaldehyde (15) on a Sephadex LH-20 column 35 × 1 cm I.D. Eluent, dioxane; flow-rate: 0.5 ml/min.

increased, the distribution coefficients increase again. The elution volumes of almost all compounds were measured up to a dioxane–water ratio of 7:3, and four compounds were chromatographed up to a ratio of 1:1, where the tendency to higher K_{av} values was clearly confirmed.

The behaviour observed with different solvent compositions can be explained by two results of a more detailed investigation of the mobile and the stationary phases. Table I gives the solvent composition determined by Bush *et al.*[16] in the gel phase, at a certain ratio in the mobile phase. The excess of water in the stationary phase compared with the mobile phase (*e.g.* an eluent composition of 9:1 becomes in the gel phase a composition of 9:2) is due to the formation of hydrated hydroxyl groups at the hydrophilic sites of the gel. These adsorption sites are no longer available for the solute and thus a lower distribution coefficient results. With increasing water content in the eluent, however, the water content in the stationary phase decreases and the distribution coefficients increase. This effect was observed by Bush *et al.*[16] with *p*-benzoquinone.

Optimization of the flow-rate

Determination was made using the 9:1 dioxane–water solvent, as at this ratio the distribution coefficients of most compounds show a minimum. As can be seen from Fig. 5, the resolution (R_s) of the separation of 4-hydroxybenzaldehyde and vanillin reaches the highest value at 0.3 ml/min.

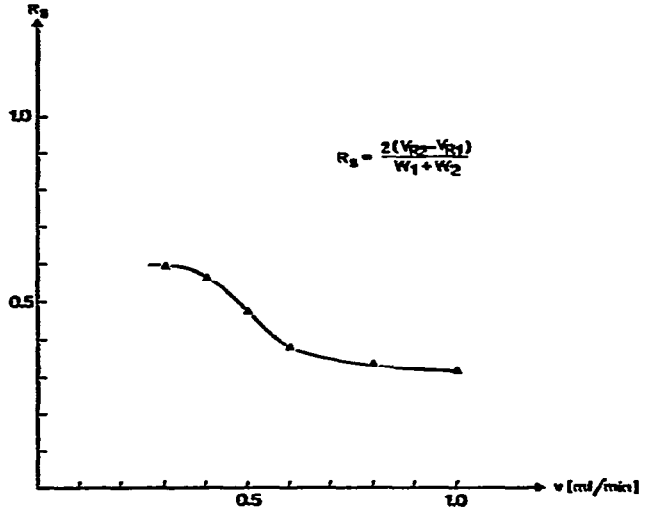

Fig. 5. Resolution *versus* flow-rate. Stationary phase, Sephadex LH-20; mobile phase, dioxane–water 9:1; R_s = resolution. V_{R1} and V_{R2} are the retention volumes of vanillin and 4-hydrobenzaldehyde (start to peak maximum). W_1 and W_2 are the base widths measured between two tangents drawn on the points of inflection of the curve and extended to the base-line.

Selection and calibration of a gel chromatographic system for the determination of molecular weight distributions

The separation of the substances according to their size is only possible using a

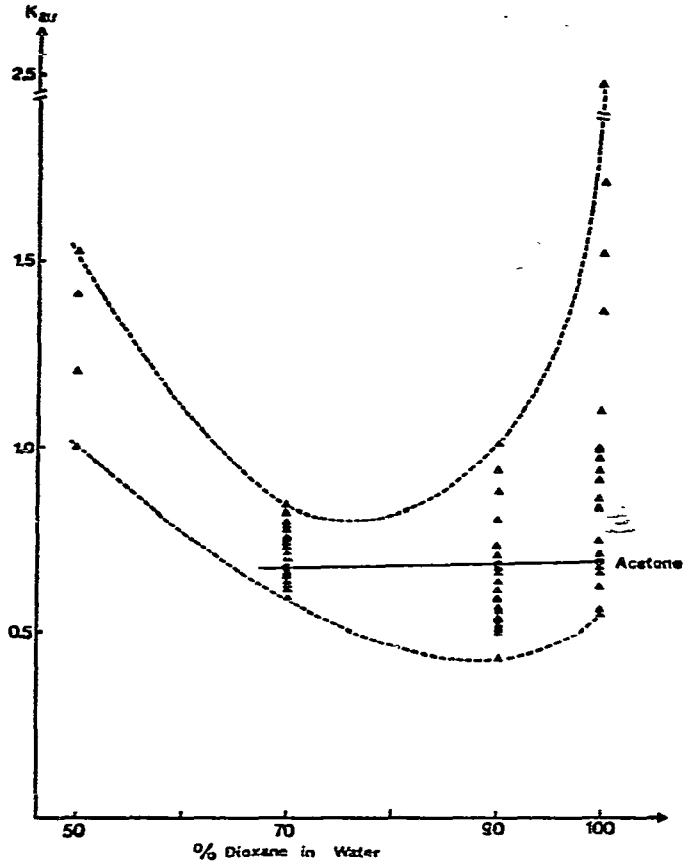

Fig. 6. Summary of the dependence of the K_{av} values of the investigated compounds on the composition of the eluent.

solvent which elutes compounds of a certain size in the narrowest range possible, regardless of their chemical character and thus of adsorption and distribution effects. Fig. 6 summarizes the K_{av} values of the compounds investigated with four solvent compositions. The eluent dioxane–water 7:3 meets the above requirements for the gel Sephadex LH-20. The flow-rate should not exceed 0.3 ml/min.

In addition to Sephadex LH-20, LH-60 gels were used for calibration. The calibration curves for Sephadex LH-20 and LH-60 columns are shown in Figs. 7 and 8. Both systems offer an extensive range where a linear dependence of the distribution coefficients on the logarithm of the molecular weight can be observed. With LH-20 this ranges from a molecular weight of *ca.* 200 to 1200; the exclusion limit is *ca.* 1900.

In spite of the chemically different calibration substances (glycols, polystyrenes, aliphatics), all substances obey the molecular weight–elution parameter (K_{av}) relationship: $K_{av} = k \cdot \log \overline{M}$.

In contrast to LH-20 gels, which show a greater resolving power in the low

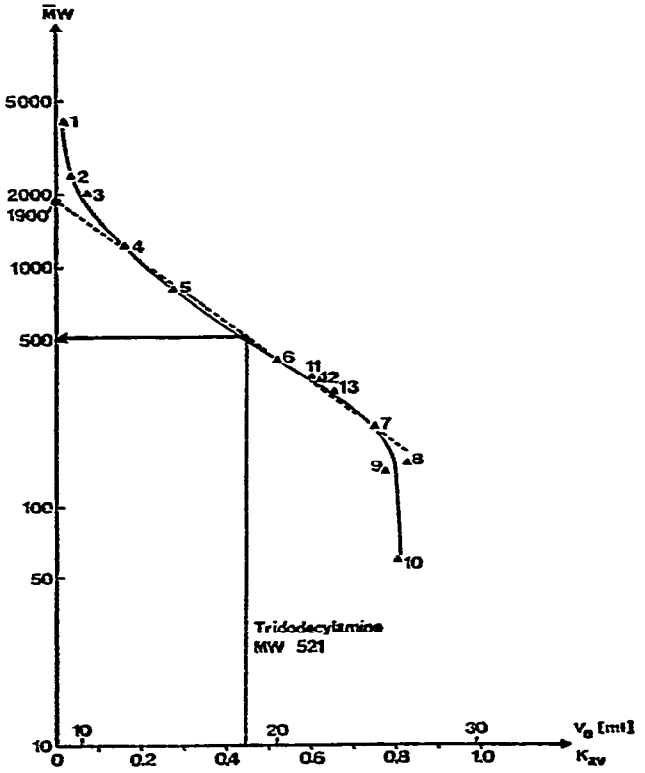

Fig. 7. Semilog plot of molecular weight *versus* K_{av} values and elution volume (V_e) of polymer standards and lignin degradation products. Stationary phase, Sephadex LH-20; column, 38.7 × 1 cm I.D.; mobile phase, dioxane–water (7:3). 1 = Poly(propylene glycol), $\overline{MW}$ 4000; 2 = polystyrene, $\overline{MW}$ 2350; 3 = poly(propylene glycol), $\overline{MW}$ 2000; 4 = poly (propylene glycol), $\overline{MW}$ 1200; 5 = poly (propylene glycol), $\overline{MW}$ 800; 6 = squalene; 7 = salicylic acid phenyl ester; 8 = vanillin; 9 = veratrole; 10 = acetone; 11 = pinoresinol; 12 = lariciresinol; 13 = dehydrodivanillin.

molecular weight region, the LH-60 gels accumulate compounds with molecular weights up to 300. The linear range, however, is considerably greater: 400–8000. The excluded molecular weight is *ca.* 16,000.

Contrary to the LH-20 gels, with the LH-60 gels the K_{av} values of the low molecular weight compounds are greater than unity. It seems that the distribution effects are more pronounced, which can be explained by the larger pores of LH-60 and the resulting stronger hydration of the hydroxyl groups in the gel.

CONCLUSION

The results show that a thorough investigation of the elution behaviour of chemically different substances permits the determination of the predominant mechanism of separation in gel permeation chromatography. This can be achieved if a change in separation effects can be obtained by varying the solvent composition.

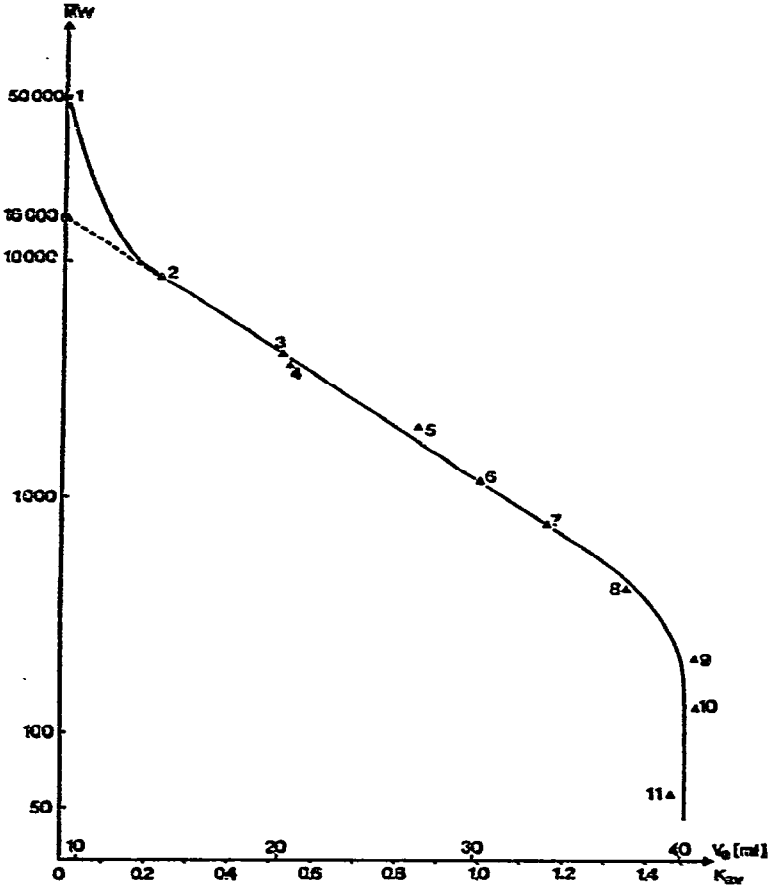

Fig. 8. Semilog plot of molecular weight *versus* K_{av} values and elution volume (V_e) of polymer standards and lignin degradation products. Stationary phase, Sephadex LH-60; column, 35.4 × 1 cm I.D.; mobile phase, dioxane–water (7:3). 1 = Polystyrene, $\overline{MW}$ 50,000; 2 = polystyrene, $\overline{MW}$ 3500; 3 = poly (propylene glycol), $\overline{MW}$ 4000; 4 = polystyrene, $\overline{MW}$ 3600; 5 = poly (propylene glycol), $\overline{MW}$ 2000; 6 = poly (propylene glycol), $\overline{MW}$ 1200; 7 = poly (propylene glycol), $\overline{MW}$ 800; 8 = squalene; 9 = salicylic acid phenyl ester; 10 = veratrole; 11 = acetone.

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