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Received for review September 14, 1972. Accepted April 9, 1973. Presented at Symposium on Non-Vapor Phase Techniques for Isolation and Identification of Flavor Compounds, 164th National Meeting of the American Chemical Society, New York, N. Y., September 1972.

Liquid Chromatography Applications in Analysis and Quality Control of Flavor Chemicals

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A simple gel permeation chromatographic method has been applied to the determination of the extent of oligomer or polymer formation in flavor chemicals. The method is illustrated for Δ-dodecalactone, 2,4-pentadienal, and *p*-vinyl guaiacol. In addition, a procedure for calibration of the uv detector is described which requires the preparation of a pure sample component only once, after which the detector sensitivity may be calculated using a stable reference standard. The significance of the gel permeation method in relation to internal standard and area normalization gas chromatographic methods is discussed.

In the course of our evaluation of currently practiced liquid chromatographic techniques, we searched both for methods which could be used in the isolation of natural product constituents and for methods which could be made a routine part of our quality control procedures for pure flavor chemicals. For the latter application, a method was sought which would provide an estimate of chemical purity in addition to presently used gas chromatographic methods, especially for those compounds with a history of polymer formation. Gel permeation chromatography, which elutes components essentially in order of decreasing molecular weight, seemed particularly promising. With this paper, we wish to present some preliminary results which illustrate the type of information obtainable by a simple gel permeation method.

EXPERIMENTAL SECTION

Apparatus. *Lc System 1.* Initial experimentation and analyses, including Figures 1 and 2, were performed using a Varian Aerograph Model 4100 liquid chromatography system equipped with ultraviolet absorption (uv, 254 nm) and refractive index (ri) detectors. The column was ¼ in. o.d. × 6 ft stainless steel, packed with Poragel 60Å (Waters Associates).

Lc System 2. Subsequent analyses, including Figures 3–7, were done with a system consisting of a Waters C903 pumping system, a 5-μl volume high-pressure injection valve (Hamilton part no. 77503), and a Varian Aerograph uv detector. The column used with this system was ¼ in. o.d. × 12 ft stainless steel packed with Poragel 60Å. In both systems 1 and 2, the eluting solvent was reagent grade dichloromethane flowing at the rate of 100 ml/hr. At that flow rate, all analyses were complete within 30 min. Chart speed on the 1 mV Varian Model 20 recorder was 0.25 in./min for both systems 1 and 2.

Preparative gas chromatography was accomplished using a ⅜ in. o.d. × 12 ft stainless steel column packed with 20% SE-52 on 30–60 mesh Chromosorb W in a Varian Aerograph Model 712 preparative gas chromatograph.

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Analytical gas chromatography was performed using a 500 ft × 0.03 in. i.d. open-tubular column coated with SF-96 for 2,4-pentadienal. For *p*-vinyl guaiacol and Δ-dodecalactone, a ⅛ in. × 12 ft column packed with 12% SF-96 on 80–100 mesh Chromosorb W-HP was used. Both columns were operated in a Hewlett-Packard Model 5751B gas chromatograph with area measurements and internal standard calculations performed by an on-line gc computer system (Craven *et al.*, 1971).

Uv Detector Calibration. The relationship between peak area, A, and sample weight, W, may be expressed as $A = KW$, where K is the response factor. Since the detector measures absorbance at 254 nm, the response factor may be considered equal to a detector constant, X, divided by the absorptivity, a, at 254 nm: $K = X/a_{254}$. Since X reflects any changes in detector sensitivity and is independent of the compound being analyzed, it can be determined using a pure, stable reference standard such as 1,4-dimethylnaphthalene (Aldrich Chemical Co., λ_{max} = 289 nm), as in the present case. The absorptivities at 254 nm were determined using a Beckman DBG spectrophotometer. Peak area measurements from gel permeation chromatography were made using a planimeter. Sample and standard solutions were made on a weight/volume basis using dichloromethane as the solvent.

RESULTS AND DISCUSSION

The formation of polymeric material is often suspected on the basis of changes in properties such as solubility, color, viscosity, and flavor or aroma intensity. Among the first chemicals checked were two samples of Δ-dodecalactone, one of which had a lower aroma level than a fresh sample. Area normalization gas chromatography had indicated that the two samples were virtually identical, but it was observed that the suspected sample did not completely dissolve in propylene glycol at the 10% level as did the fresher sample. Figure 1 shows the gel permeation chromatogram obtained from the suspected sample using lc system 1. No attempt was made to calibrate the detector response at this time, but, for purposes of comparison, we assumed that absorptivities would not be very different for the higher molecular weight materials and calculated

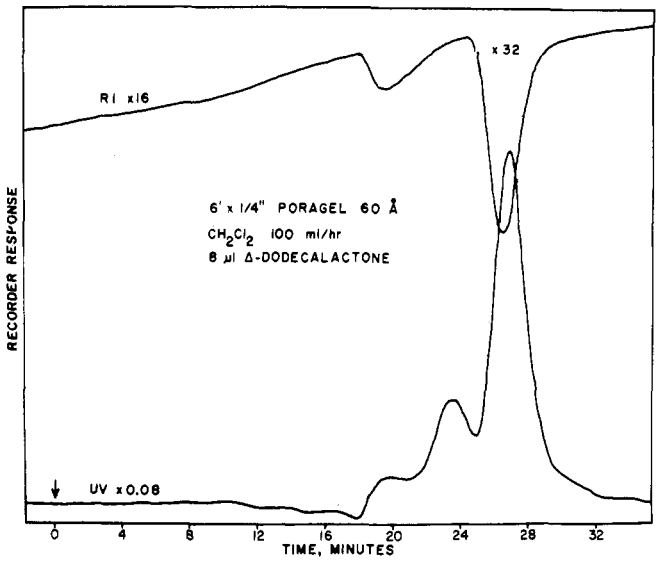

Figure 1. Gel permeation chromatogram of Δ-dodecalactone sample with decreased aroma intensity; lc system 1.

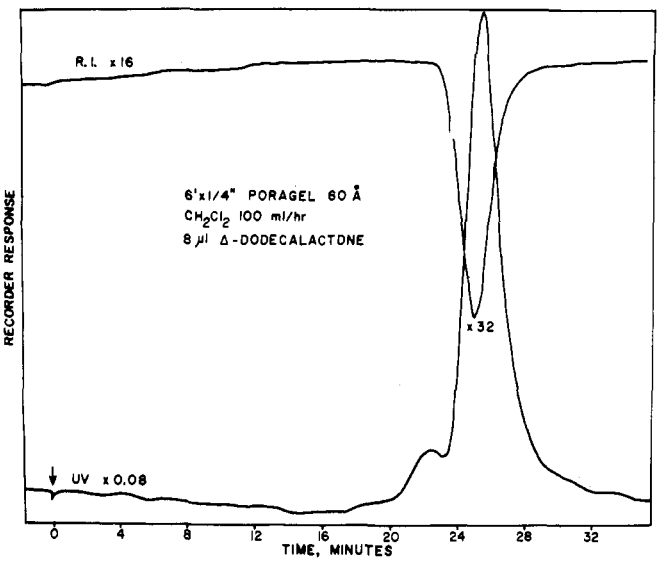

Figure 2. Gel permeation chromatogram of fresh Δ-dodecalactone; lc system 1.

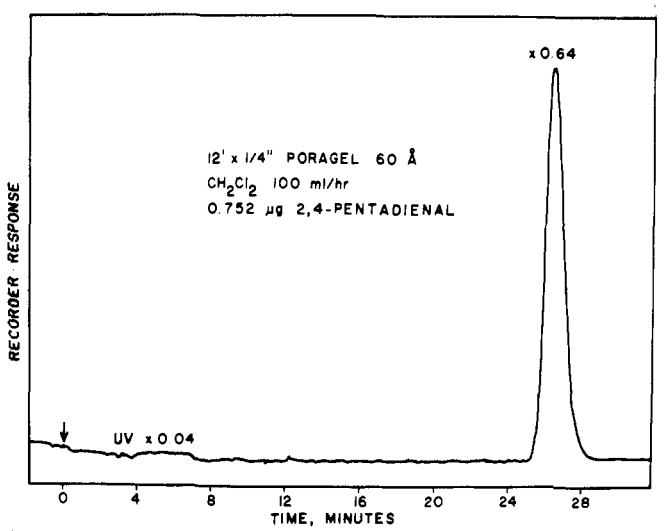

Figure 3. Gpc of 2,4-pentadienal purified by preparative gas chromatography; lc system 2.

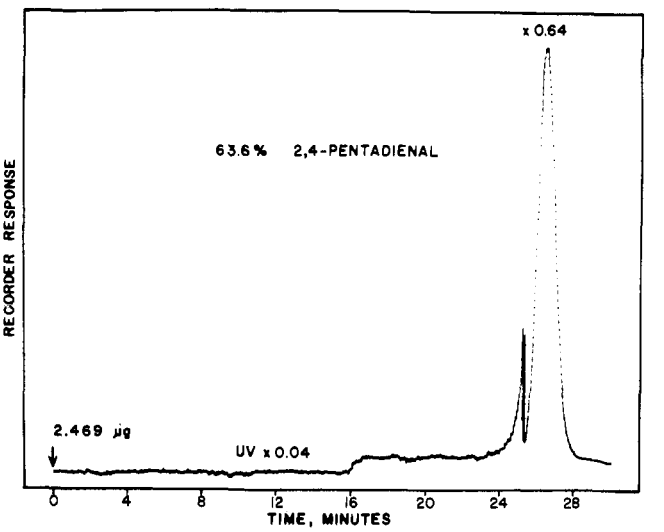

Figure 4. Gpc of 2,4-pentadienal stored at 4° for 7 months as a 10% solution in ethanol; lc system 2.

the approximate percentage of area represented by each peak on the uv detector trace. In order of elution, these quantities were 5, 22, and 73%. Also note that the first peak gives a refractive index response. Figure 2 shows an odor-approved sample of Δ-dodecalactone analyzed under identical conditions in which the area percentages are approximately 0, 10, and 90%. The early peak observed on the ri detector in the former sample is not seen in this one. This example, among others, suggested to us that gel permeation chromatography could be a useful tool in the study of chemicals which form higher molecular weight products in processing or storage.

Obviously, to be of real value, this method must be made quantitative. Using the calibration approach outlined in the Experimental Section, the absorptivity of the pure sample component need only be determined once. Subsequently, the detector constant can be determined simply by analyzing a sample of the standard (1,4-dimethylnaphthalene in this case), which eliminates the need of storing or preparing a pure reference sample of the chemical being analyzed.

A comparison of X values determined using 1,4-dimethylnaphthalene, 2,4-pentadienal, and p-vinyl guaiacol with lc system 2 is shown in Table I. The absorptivities at 254

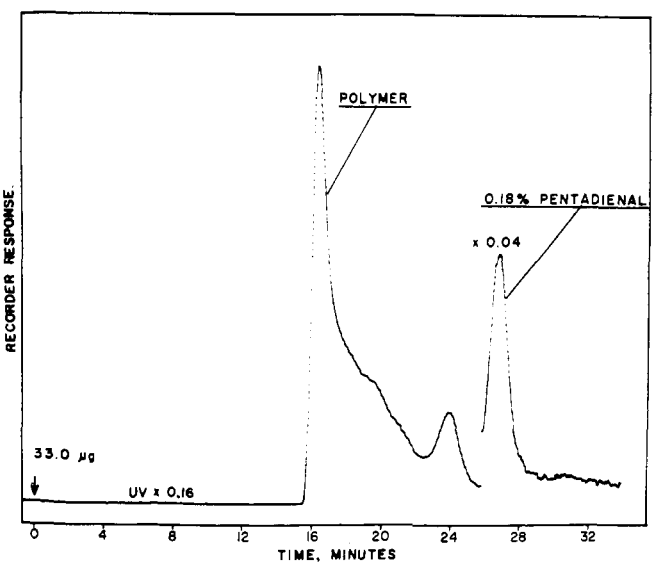

Figure 5. Gpc of 2,4-pentadienal stored neat at room temperature for 15 months; lc system 2.

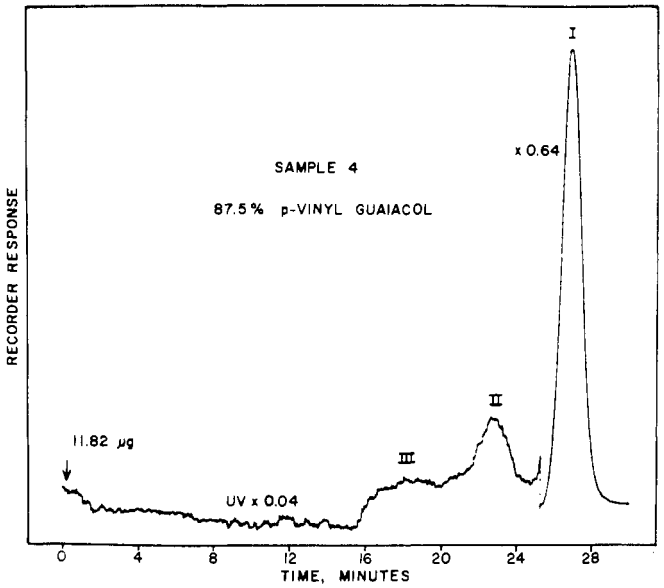

Figure 6. Gpc of *p*-vinyl guaiacol sample 4 (see Table II); lc system 2.

Table I. Calculation of Detector Constants[a]

Compound	a_{254}	X
1,4-Dimethylnaphthalene	6.67	2.58
2,4-Pentadienal	297.9	2.58
p-Vinyl guaiacol	50.9	2.58

[a] Where $X = A \cdot a_{254}/W$.

nm were used, along with the sample weight and peak area from gel permeation analyses, to calculate the detector constants. Experience has shown that solvent flow rate and accumulation of material in detector cells have the most influence on the value of X. The samples of 2,4-pentadienal and *p*-vinyl guaiacol used for these calculations were purified to eliminate polymeric materials as well as any low molecular weight impurities which would alter the absorptivities of the pure chemicals.

The pure 2,4-pentadienal was obtained by preparative gas chromatography and was verified to be 99.9% pure by rechromatography on the open tubular gc column. No polymer peak could be observed when analyzed on the gel permeation column using lc System 2, as shown in Figure 3. The base line was obtained with the sensitivity on the 0.04 range, while the peak was recorded at a sensitivity of 0.64 absorbance units full scale.

Dilutions of pure 2,4-pentadienal were prepared in CH_2Cl_2 and analyzed to verify detector linearity over the usable sensitivity range. A plot of sample weight *vs.* peak area for duplicate analyses showed the response to be linear up to 1.5 μg of sample. The response factor was calculated for each dilution analyzed and the average was used for computation of the pentadienal content in two of several samples on hand.

Figure 4 shows a sample of 2,4-pentadienal which analyzed 63.6% by gel permeation. This sample had been stored at 4° for 7 months as a 10% solution in ethanol. Area normalization gas chromatography indicated 88.6% of 2,4-pentadienal. The difference between 88.6% by gas chromatography and 63.6% by gel permeation is probably due principally to the polymer present which is not eluted from the gas chromatograph. It should also be pointed out that gas chromatography showed two low molecular weight impurities, accounting for 10% of the area (including the diethyl acetal of 2,4-pentadienal), which are not resolved from the 2,4-pentadienal peak by the gel permeation column. Other samples stored under similar conditions have yielded similar results, while a sample stored neat at room temperature for 15 months contained less than 0.2% 2,4-pentadienal, as shown in Figure 5. This latter sample was tacky and nearly solidified at the time of analysis.

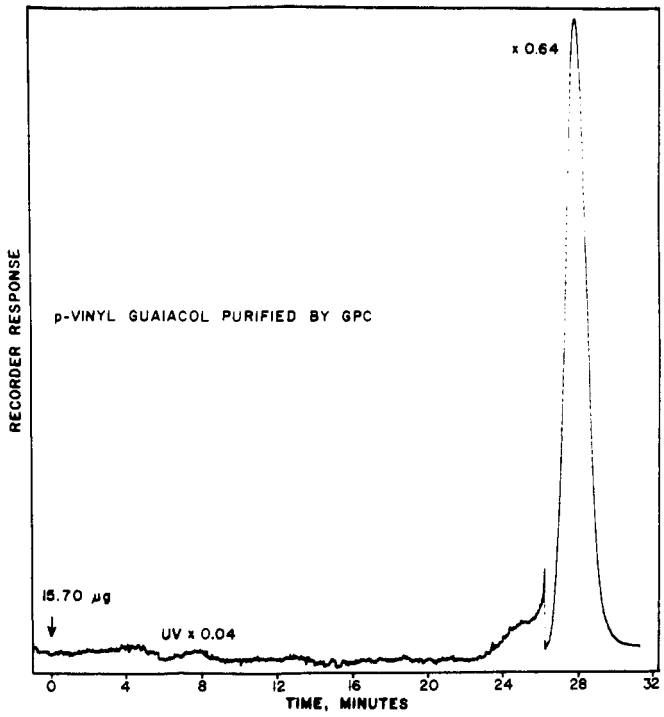

Figure 7. Gpc of *p*-vinyl guaiacol purified by gpc and used for detector calibration; lc system 2.

p-Vinyl guaiacol is another chemical with a history of polymerization problems. In 1971 a paper was published on the formation of *p*-vinyl guaiacol oligomers in the thermal decarboxylation of ferulic acid (Klaren-DeWit *et al.*, 1971). These authors separated the dimer and trimer by column chromatography on silica gel and showed by means of nmr, ir, and mass spectra that they were formed by head-to-tail condensation.

Figure 6 is a *p*-vinyl guaiacol sample which had been stored neat for 13 months under refrigeration with occasional sampling. Mass spectra of fractions collected from peaks II and III indicate that peak III contains the MW450 trimer, while the second peak consists of a mixture of the dimer and trimer, with the dimer predominating.

We attempted to obtain "pure" *p*-vinyl guaiacol for calibration purposes by preparative gas chromatography, but gel permeation analysis still showed a small amount of material eluting on the leading edge of the monomer peak. We then collected the monomer peak from three successive 100-μl injections of *p*-vinyl guaiacol on the Poragel column, removed the solvent under vacuum, and reinjected the "purified" monomer. Figure 7 shows the gpc record with the base line recorded at 0.04 and the peak at 0.64 absorbance units full-scale sensitivity. The leading shoulder is only slightly smaller than that in samples purified by preparative gc. The detector was calibrated using dilutions of the *p*-vinyl guaiacol purified by gel permeation. A plot of peak area *vs.* sample weight was linear up to 16 μg of *p*-vinyl guaiacol.

Several samples of *p*-vinyl guaiacol with various histories were analyzed by gel permeation chromatography and internal standard gas chromatography for comparison. The results are summarized in Table II. The number in parentheses is the range between two values averaged to give the indicated purity. Response factors for the internal standard gas chromatographic analyses were determined

Table II. Comparison of Gpc and Internal Standard Gc Values for p-Vinyl Guaiacol Samples

Sample	Storage conditions	Gpc, %	Gc, %
1. Old gc standard	9 mo at 4° + 4 mo at −15°	80.1 (0.0)	75.4
2. 10% in ethanol	34 mo at 4°	69.5	82.2
3. Sample C	7 mo at 4°	95[a]	98.6
4. Sample C	11 mo at 4°, sampled occasionally	64.8	62.6
5. Sample C	11 mo at 4°, sampled frequently	58.4 (6.3)	57.8
6. Fresh, fraction 10	1 week at 4°	101	
7. Fresh, fraction 8	1 week at 4°	102	99.8
8. Fresh, fraction 5	1 week at 4°	101	
9. Fresh, fraction 7	1 week at 4°	104.5 (1.0)	99.3
10. Fresh, fraction 7	After preparative gc cleanup	99.2	
11. Storage control	9 days at 40° + 4 days at 50°	90.7	88.4

[a] Value is an estimate from noncalibrated analysis.

using freshly prepared *p*-vinyl guaiacol (sample 9 in the table). The first sample, which had been used previously for internal standard gc calibration, showed one of the pitfalls of that method—a changing calibration standard.

Analysis of sample 2 indicated that simple dilution in alcohol did not prevent polymerization in refrigerated storage. The gel permeation value for sample 3, indicated by an asterisk, was only estimated based on comparison of the chromatogram with more recent quantitative analyses. The results for samples 4 and 5 compared to sample 3 indicate that serious deterioration has occurred in the additional 4 months of storage. We suspect that this deterioration was accelerated by the sampling procedure, which included standing at room temperature for several hours to soften the material.

All fractions of the recent preparation represented by samples 6–9 were greater than 99.4% *p*-vinyl guaiacol by gas chromatography and, likewise, analyzed virtually pure by gel permeation. Sample 10 is the same as sample 9 after preparative gas chromatography. Various storage conditions are currently being evaluated using freshly prepared material from fraction 8. Sample 11, the control for that study, was analyzed after 9 days at 40° and 4 days at 50°. Gpc showed it to be 90.7% and internal standard gas chromatography gave 88.4% *p*-vinyl guaiacol. For the most part, agreement between gel permeation and internal standard gc values is good.

CONCLUSIONS

Obviously, no single analytical method can provide all the answers, but we believe that the simple gel permeation chromatography method outlined here will be of considerable value in the study of unstable low molecular weight chemicals. It offers advantages over internal standard gas chromatography in that polymerization or decomposition during analysis is less likely and, after the initial calibration, no pure sample component is required for subsequent quantitative analyses.

The results obtained by this method should be checked by other applicable techniques such as gas chromatography or high speed liquid chromatography (partition or adsorption) whenever possible to insure against the presence of unresolved low molecular weight impurities. This was demonstrated in the case of 2,4-pentadienal, which contained the diethyl acetal and other low molecular weight impurities not resolved by gel permeation chromatography. High speed liquid–liquid or liquid–solid chromatography are also capable of greater resolution but require more time to establish optimum parameters than the simple, routine gel permeation method.

In any quantitative method, sampling accuracy is of prime importance. We are convinced that inclusion of a sampling valve is imperative in any liquid chromatographic system. The reproducibility of the Hamilton valve which we used was as good as the area measurement accuracy using a planimeter. Since this work was completed, we have succeeded in interfacing the uv detector output with our gas chromatography data system (Craven *et al.*, 1971) using a method similar to that for external standard gas chromatography. This has resulted in improved area measurement accuracy and has reduced the required manual computation to a single operation.

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Received for review November 24, 1972. Accepted May 3, 1973. Presented at Symposium on Non-Vapor Phase Techniques for the Isolation and Identification of Flavor Compounds, 164th National Meeting of the American Chemical Society, New York, N. Y., September 1972.