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Application of Gas Chromatography to Resin Acid Analysis

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APPLICATION OF GAS CHROMATOGRAPHY TO RESIN ACID ANALYSIS

by

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1 INTRODUCTION

For many years resin acids have been finding their way into the aquatic environment through the waste waters of industries processing wood and its related products. Questions have been raised as to the effectiveness of the colorimetric procedure used for the measurement of these acids. Research has shown that gas-liquid chromatography may be used to detect and quantify resin acid content. It is the purpose of this report to illustrate some of the problems inherent in the colorimetric procedure and to describe the selection and evaluation of an alternative gas chromatographic method.

2 ASPECTS OF RESIN ACID METHODOLOGY

2.1 The colorimetric Method

The colorimetric method used in the past for the measurement of resin acids depended on the color formed by the action of sulphuric acid on the conjugated carbon - carbon double bonds, in the presence of acetic anhydride (1). This method, however, was not designed originally for resin acids but as a spot test for sterols (2). Using this technique, β-sitosterol, a wood sterol, was found to give a positive test (1, 2). Terpenes and humus may also give a positive color reaction (1).

Abietic and neo-abietic acids gave intense color reactions using this method but essentially no reaction was found with dehydroabietic and pimaric-type acids (1, 2, 5). In pulp mill effluents, the latter group are frequently more abundant than the abietic type of resin acid (2). Variable test responses and lower color intensities relative to pure abietic acid were obtained from commerical grades of abietic acid recommended for use as standards in this procedure (2).

2.2 The Gas Chromatographic Method

Gas-liquid chromatography has been used for the determination of resin acid content for a number of years (1-6). Prior to chromatography and detection by flame ionization, resin acids are converted to their methyl esters using diazomethane (1 - 3, 5, 6). A liquid phase that will separate all seven of the major resin acids has not yet been found (3) but a variety of liquid phases have been used such as QF-1 (1), EGSS-X (2, 5, 6), DEGS (2, 3) and Versamide 900 (3, 4) in loadings ranging from 4 to 20 percent. Separations have been carried out in the isothermal (1, 3, 4) as well as in the temperature programmed mode (2, 6).

Isolation of resin acids has been effected by solvent extraction from acidfied samples (1, 6) and also by use of XAD-2 or XAD-7 ion exchange resin in combination with alkaline samples (2, 5). Problems in the former method of isolation were the formation of stable emulsions as well as the possible isomerization of resin acids due to acidic conditions. The acid isomerization of levopimaric acid has been used to produce abietic acid (7). Levopimaric acid has been observed to isomerize on a Versamide 900 column (3).

3 METHOD OF ANALYSIS BY GAS CHROMATOGRAPHY

The following is the step-wise procedure used for the analysis of resin acid samples:

1. Adjust a 500 ml portion of sample to pH 12 with 50% sodium hydroxide.
2. Extract once with 100 ml of petroleum ether, discarding the organic phase.
3. Adjust the aqueous phase to pH 2 using 50% sulphuric acid.

4. Extract three times using 50.0 ml portions of petroleum ether, retaining and combining the organic layers.
5. Measure the combined volume of the organic layer recovered (used in the final calculation). Centrifugation may increase this recovery where emulsions are encountered.
6. Place the ether extract in a 250 ml round bottom flask and remove the solvent over a steam bath using a rotary evaporator.
7. Using a 9:1 ether - methanol mixture, transfer the residue to a glass vial containing a known weight of internal standard. (nonadecylic acid)
8. Methylate the mixture using diazomethane.
9. Remove the solvent under a stream of nitrogen.
10. Reconstitute the residue with 1 ml of benzene.
11. Analyse a 2 µl portion by gas chromatography on a 6' x 1/4" glass column containing 4% QF-1 on 100/120 mesh Chromsorb W, acid washed and treated with dimethyldichlorosilane (N_2 flow rate 35 ml/min., isothermal 190 C).
12. Measure the areas under the peaks of the internal standard and resin acid methyl esters.
13. Calculate resin acid content by the following procedure.
    (a) Total the areas of peaks eluting in the retention interval 1.00 to 2.25 (retention time of internal standard = 1.00).
    (b) Ratio this total to the area of the internal standard peak.

(c) Using this ratio and the calibration curve (see Figure 2), determine the amount of resin acid present as dehydroabietic acid.

(d) Using the value obtained in step C, calculate the final resin acid content by the following formula:

Resin acids (mg/l) as dehydroabietic acid =

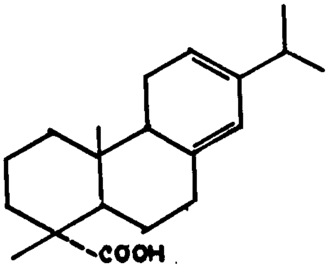
$$\text{mg resin acid (C)} \times \frac{1000\ \text{ml}}{\text{vol of sample used}} \times \frac{140\ \text{ml*}}{\text{ml of ether recovered}}$$

* Due to the volatile nature of petroleum ether and its solubility in water, 140 ml was all that could be recovered from the original 150 ml of ether used in the extraction procedure carried out on distilled water blanks.

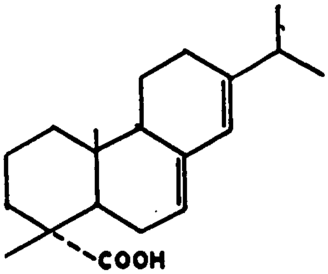
4 DISCUSSION

4.1 Colormetric Method

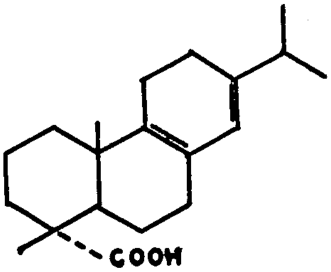
A number of compounds fall within the group known as resin acids, some of which are shown in Figure 1. These acids are similar in structural form but differ as to the location and number of carbon - carbon double bonds. Some of these acids have a conjugated diene system while the remainder have either no conjugation or else an aromatic ring. The colorimetric method will detect the former type, but little or no response is obtained from the latter group; for example, dehydroabietic acid gave no color reaction.



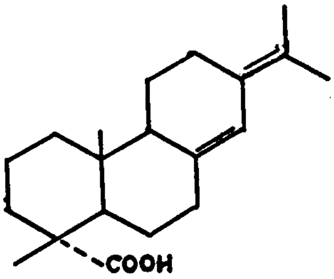
LEVOPIMARIC



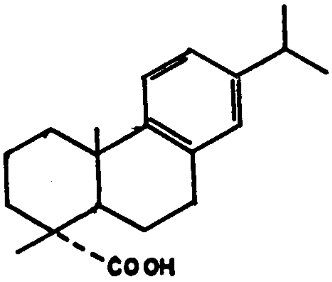
ABIETIC



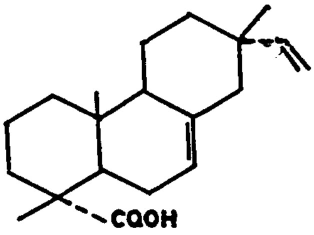
PALUSTRIC



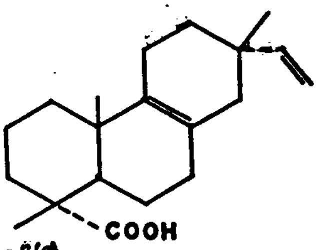
NEOABIETIC



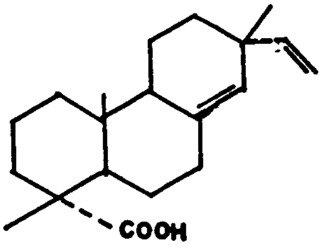
DEHYDROABIETIC



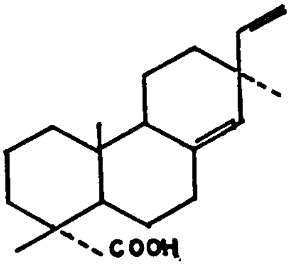
ISOPIMARIC



$\Delta^{8(9)}$ ISOPIMARIC



SANDARACOPIMARIC



PIMARIC

FIG I COMMON RESIN ACIDS

Technical grades of abietic acid have been recommended for use as standards in the colorimetric procedure. Two grades of abietic acid were subjected to the colorimetric mode of analysis with the results as listed in Table 1. These standards were also subjected to gas chromatography, which showed them to be a mixture with the dehydroabietic and abietic acids forming the major constituents within the resin acid fraction (Figure 2). Both grades of abietic acid were estimated to be between 60 and 75 percent resin acid in content. The difference in response between the two grades of abietic acid supports data from similar work (2).

Table 1 COLORIMETRIC ANALYSIS OF TECHNICAL GRADES OF ABIETIC ACID

Resin acid (mg/l)	Absorbance	
	Technical grade 1	Technical grade 2
10	0.094	0.143
30	0.269	0.455
50	0.460	0.779

4.2 Chromatographic Method

Figure 3 illustrates a typical resin acid trace using the gas chromatographic method described. As pure standards of various resin acids were not available, their individual retention times could not be established. Peak designations were arrived at by a comparison of the experimentally determined relative retention times with corresponding literature values (maenpaa, Hynninen, Tilla - 1968) as shown in Table 2. The value obtained by analysing pure dehydroabietic acid agreed with this data.

Table 2 COMPARISON OF RELATIVE RETENTION DATA

Acid	Relative retention time to C_{19}	
	Published	Laboratory
C_{19} internal standard	1.000	1.000
Pimaric acid*	1.285	1.240
Levopimaric & palustric acid*	1.485	1.430
Isopimaric acid*	1.655	1.570
Dehydroabietic acid*	1.785	1.696
Abietic acid*	2.035	1.950
Neoabietic acid	2.270	2.165

* methyl esters

Response factors for the various acids could not be determined due to the lack of pure standards. However, other workers (Brooks, Fisher, Joye - 1965) have found similar response factors for the main resin acids with the exception of levopimaric acid. For the purposes of this method, the contribution of response factors in the final calculations was neglected.

Resin acids were found to elute within the relative retention interval from 1.00 to 2.25. Resin acid concentration was based on the total area of peaks appearing in this interval. Other peaks in this interval, probably due to the fatty acids, were observed in samples containing large amounts of resin acids but their contribution to the final result was minimal.

As dehydroabietic acid was available in a pure form, a calibration curve was constructed using it as the standard (Figure 4) and resin acids were therefore calculated on the basis of the analytic behavior of this acid.

FIG 2 TECHNICAL ABIETIC ACID, BY G.C.

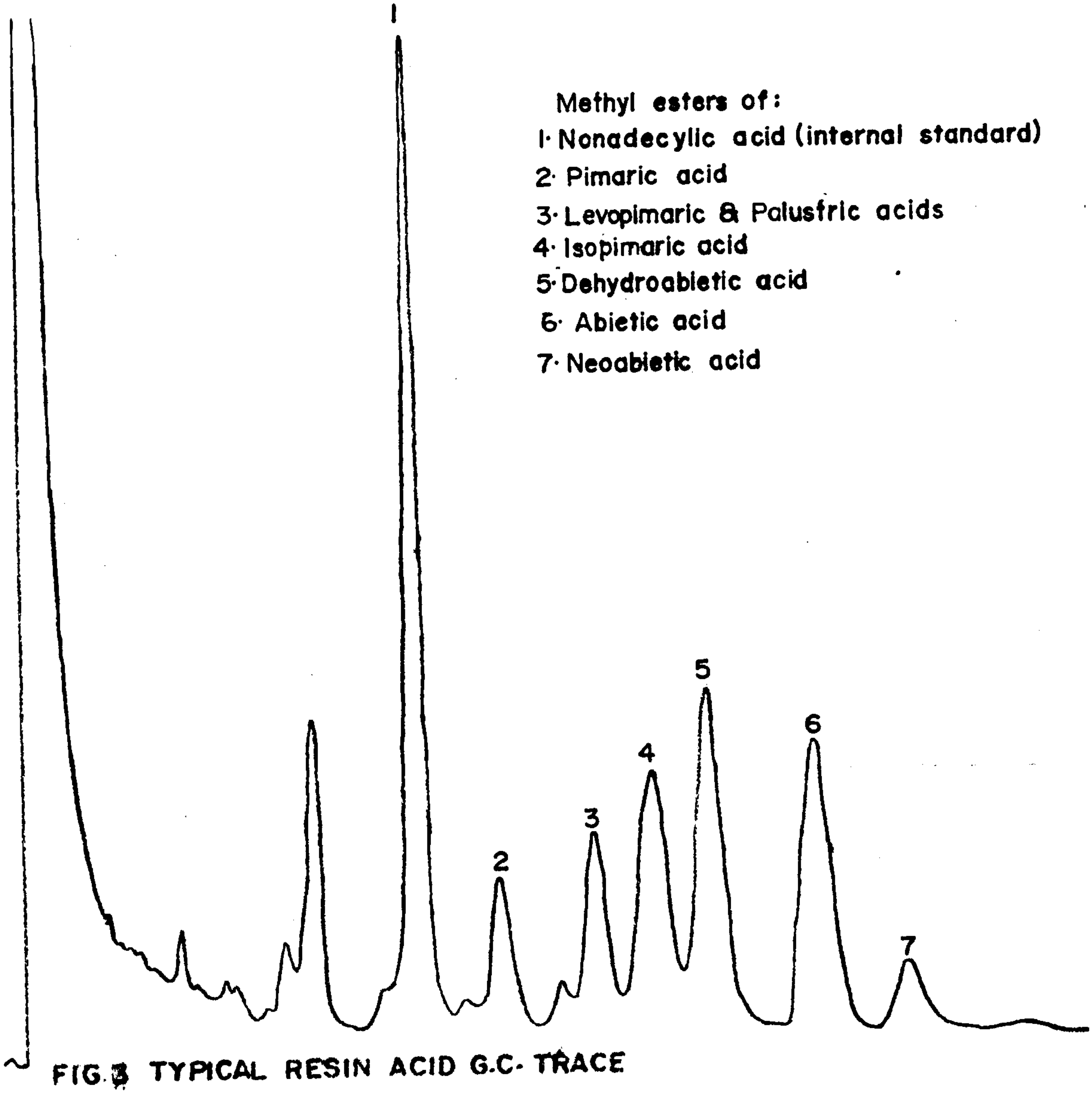


FIG.3 TYPICAL RESIN ACID G.C. TRACE

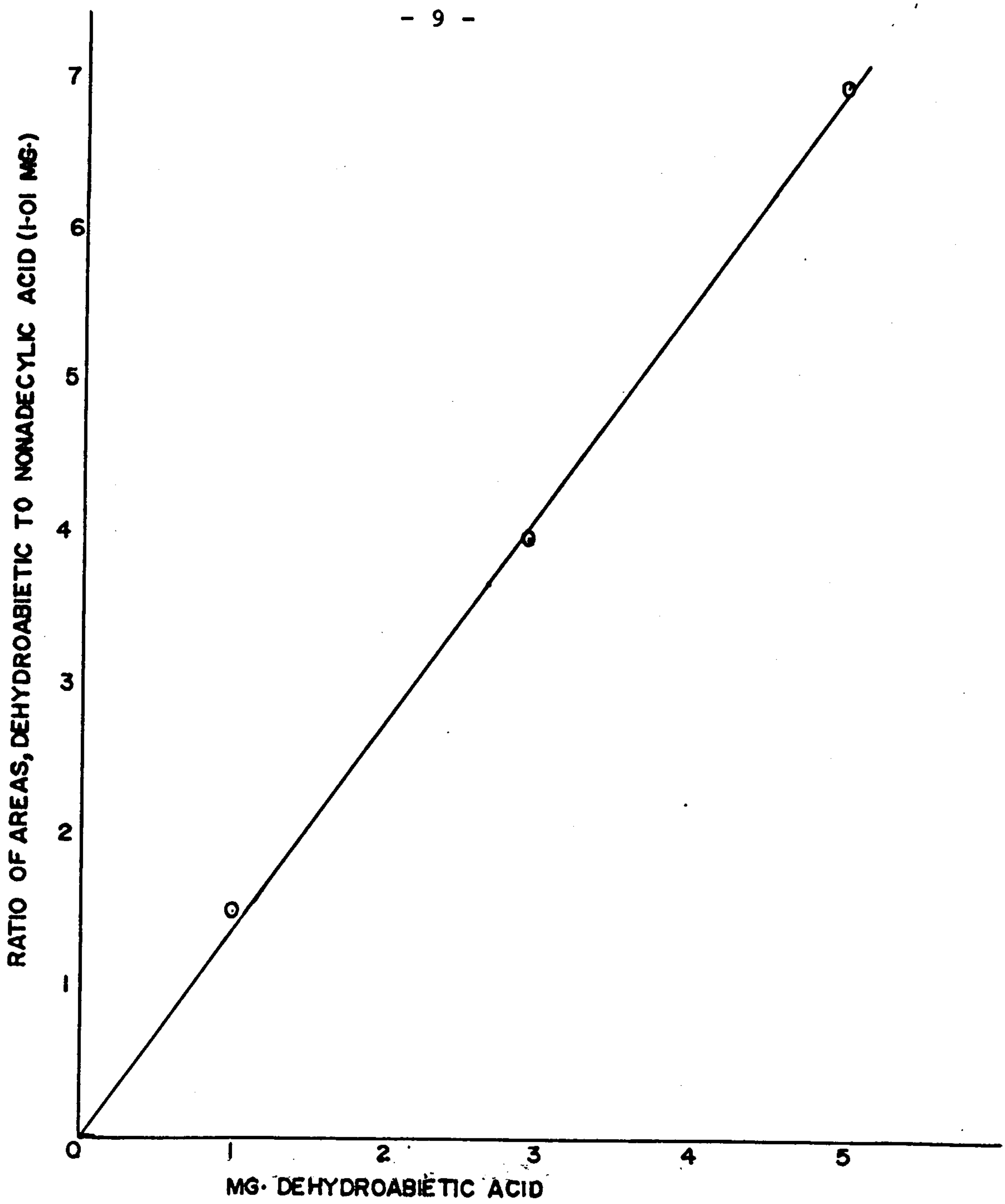


FIG 4 TYPICAL CALIBRATION CURVE

With areas being calculated by the formula of height times peak width at half height, a range of ratio values of 1.28 - 1.34 was obtained for 0.96 mg samples of dehydroabietic acid.

In the course of testing, an artificaal agueous resin acid sample was prepared by dissolving some technical grade abietic acid in water aided by the use of 50% sodium hydroxide. Six 500 ml sub-samples were analysed with the results shown in Table 3. The results gave an average resin acid content of 0.406 mg/l, a standard deviation of 0.096 with a precision of 23.7%.

Table 3 RESIN ACID DETERMINATION ON REPLICATE SAMPLES.

Sample Number	Resin Acid (mg/l)
1	0.427
2	0.403
3	0.320
4	0.319
5	0.580
6	0.385

In order to establish whether a relationship existed between the colorimetric and chromatographic determinations, a number of samples, taken from a pulp and paper mill, were analysed by both methods. The results are illustrated in Table 4 and graphically displayed in Figure 5.' From these results there appears to be no direct correlation between the two methods and this is probably due to problems connected with the colorimetric procedure.

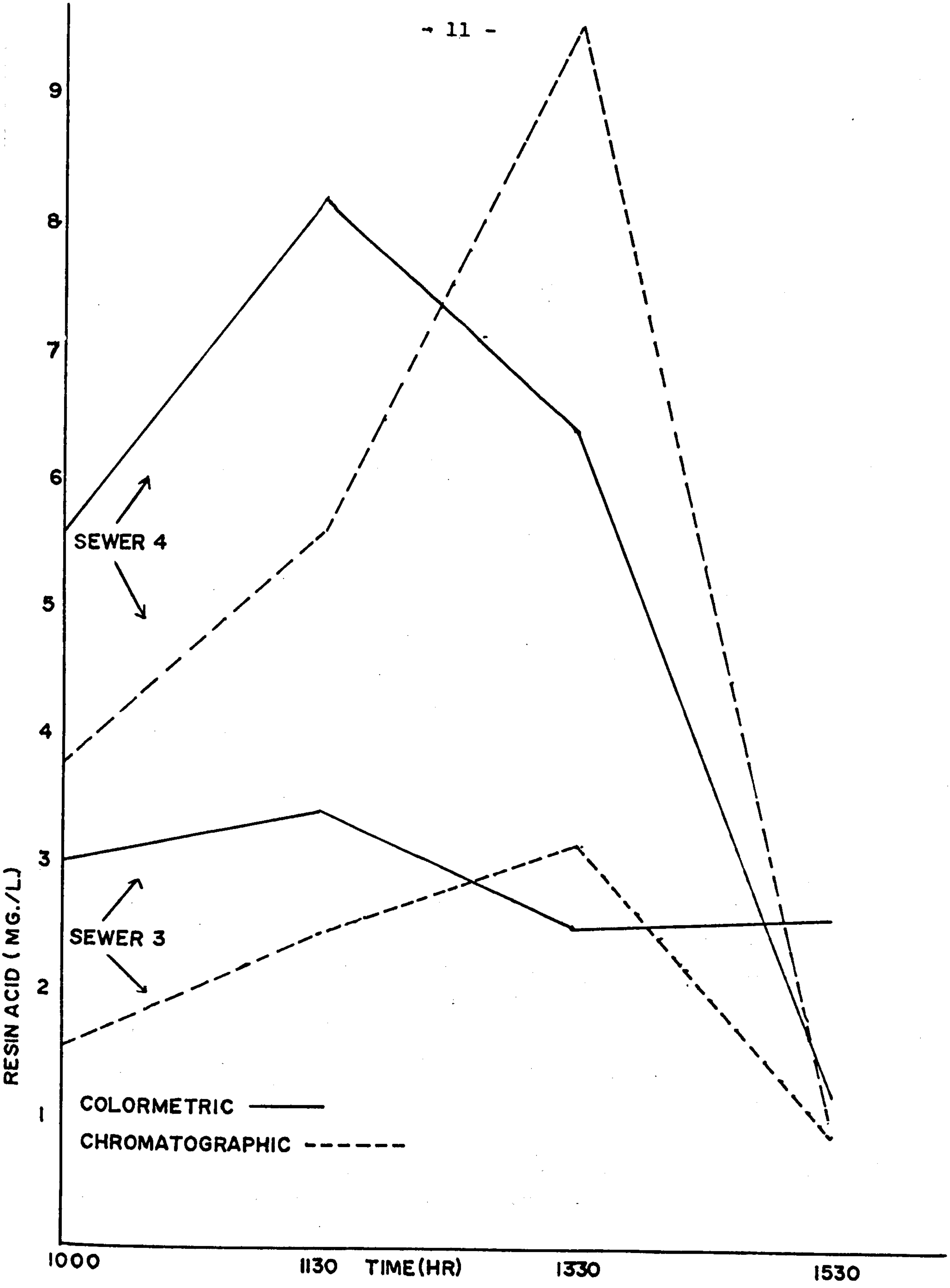


FIG. 5 GRAPHICAL REPRESENTATION OF DATA (TABLE 2)

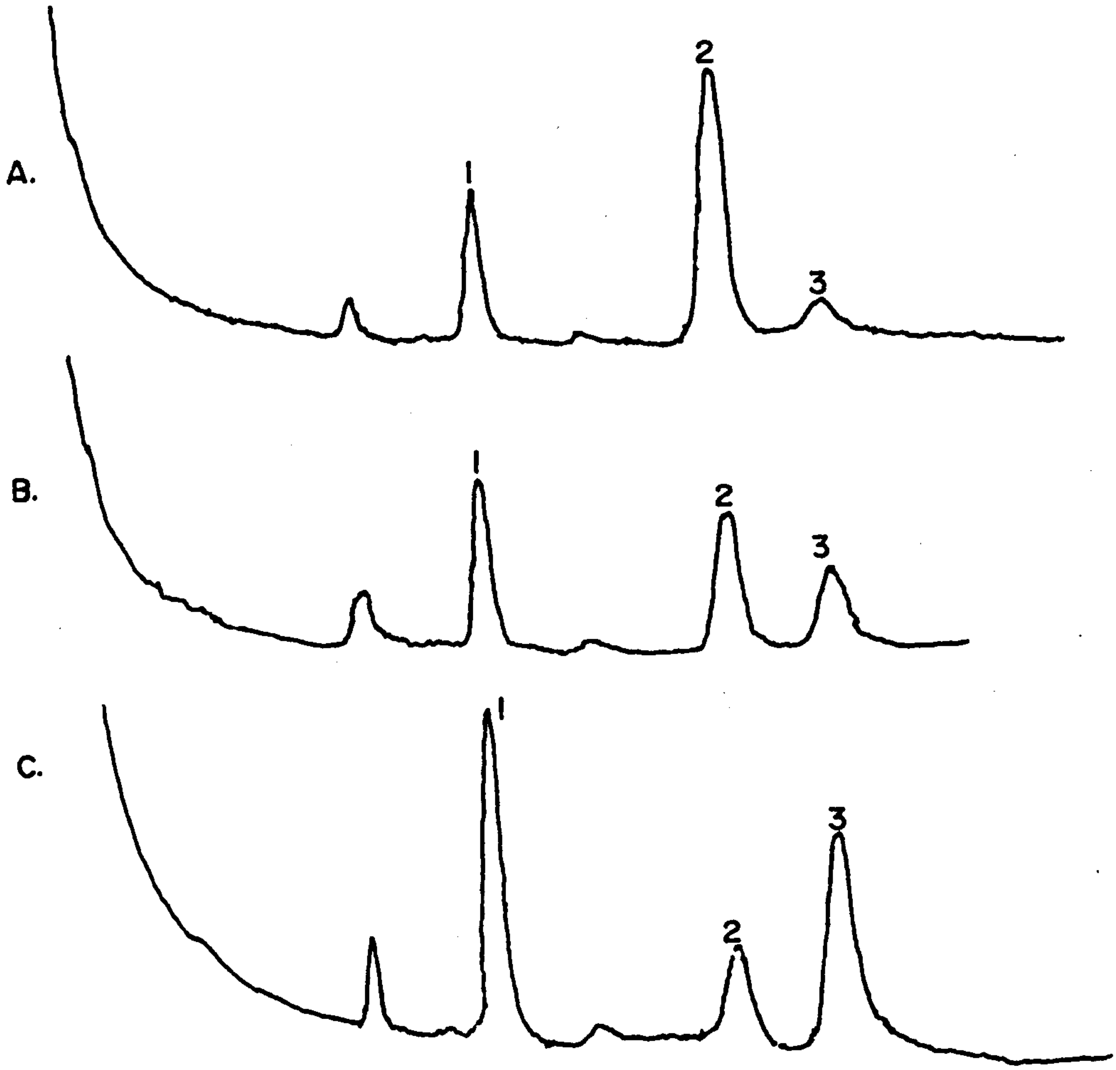
Table 4 RESIN ACID CONCENTRATION BY THE COLORIMETRIC AND GAS CHROMATOGRAPHIC METHODS IN SAMPLES FROM A PULP AND PAPER OPERATION

Source	Total resin acids (mg/l)	
	Colorimetric	Gas chromatographic
Sewer I, II, III (composite)	5.0	2.67
Sewer III 1000 hrs.	3.0	1.55
Sewer III 1130 hrs.	3.4	2.45
Sewer III 1330 hrs.	2.5	3.14
Sewer III 1530 hrs.	2.6	0.87
Sewer IV 1000 hrs.	5.6	3.77
Sewer IV 1130 hrs.	8.2	5.60
Sewer IV 1330 hrs.	6.4	9.57
Sewer IV 1530 hrs.	1.2	1.03

A sample of methylated levopimaric acid was subjected to gas chromatography in order to establish its response factor. Three consecutive injections of the same sample were made with the resulting GC traces as shown in Figure 6. From the first injection (A) to the third (C), the area of levopimaric methyl ester peak decreased while the area of a peak corresponding to the retention time of methylated dehydroabietic acid was observed to increase. This behavior was also noted in some analytical samples.

During the summer months, resin acid determinations were carried out using the GC technique. Samples processed from within the pulp mill operations to the outfalls showed good resolution of peaks on the GC trace. Poor resolution, however, was noted on some samples taken from the receiving waters. This resulted in poor quantification of peak areas which was reflected in the final resin acid concentration. This poor resolution appears to be caused by some interference(s) that tends to mask the resin acid peaks.

Values of dehydroabietic acid recovered from aqueous solution ranged between 84 and 91 percent.



1. INTERNAL STANDARD
2. LEVOPIMARIC ACID
3. DEHYDROABIETIC ACID

FIG. 6 ANALYSIS OF LEVOPIMARIC ACID

5 CONCLUSIONS

To date, the data indicates that the colorimetric method has some serious drawbacks in the determination of resin acid concentrations. In view of this, a chromatographic method such as the one tested appears to be a more viable form of measurement. It also offers the selectivity for measuring individual resin acid content.

6 RECOMMENDATIONS

1. Due to the problems associated with the colorimetric method, it is recommended that comparison data between it and the chromatographic method be discontinued.
2. The use of XAD-2 or XAD-7 resin to increase resin acid recoveries from aqueous samples should be tested.
3. Other liquid phases such as EGSS-X should be tested to effect better resin acid separation and perhaps resolve them from possible interferences found in some receiving water samples.

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