KETOACID POLYMERS AS GAS–LIQUID CHROMATOGRAPHY SUBSTRATES

R. G. ACKMAN AND J. C. SIPOS

Fisheries Research Board of Canada, Technological Research Laboratory, Halifax, N. S. (Canada)

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INTRODUCTION

Early analyses of volatile fatty acids depended on the addition of an organic acid to a relatively neutral substrate to reduce tailing of the peaks. Acids such as stearic[1] or sebacic[2] were employed, although bleeding of these materials led to short column life at higher temperatures[3]. Subsequently the introduction of phosphoric acid[4,5] as an additive, and of dimer and trimer acids as a substrate[6] permitted higher temperature operation. More recently formic acid vapor in the carrier gas has been shown to reduce adsorption of fatty acids on the support or in the substrate at both moderate[7] and elevated[8] temperatures.

Prior to the introduction of the latter procedure, and of the non-volatile acid additives or substrates, the need for a material combining high thermal stability with an acidity suitable for the reduction of tailing was evident. The development of keto-acid polymers, formed by techniques such as heating the cadmium salt of sebacic acid[9-12] suggested that this combination of a purely organic polymer with attached carboxyl groups would solve the problem of substrate thermal stability. This paper presents some results of an examination of a ketoacid polymer, primarily in the analysis of volatile fatty acids.

MATERIALS AND APPARATUS

A small sample of ketoacid polymer was prepared from the cadmium salt of sebacic acid by heating at 330° as described by PAQUOT *et al.*[9-12]. The first material crystallized from acetic acid (m.p. 156–159°) was not employed, but instead a second fraction (m.p. 103–110°), probably of much lower molecular weight, was precipitated by the addition of water to the acetic acid mother liquor. The latter sample was preferred since it should have a higher proportion of carboxyl groups. Only a small amount of material was available and the entire sample was used in the preparation of columns for gas–liquid chromatography. The molecular weight and the ratio of ketone to carboxyl groups could thus not be determined.

Two six-foot columns were prepared, of stainless steel tubing 1/8 in. O.D. One contained 2.15 g of packing made up of 20% polymer on 60–80 mesh Chromosorb W, the other 3.59 g made up of 10% polymer on 60–80 mesh Gas-pack F (a teflon-impregnated support obtained from Chemical Research Services, Inc., Addison, Ill.).

Chloroform was used as a solvent in coating the support and was removed with stirring on a steam bath.

The columns were used in an apparatus fabricated in this laboratory based on a Wilkens flame ionization detector and electrometer, with an injection port of the type employed in the Wilkens A-90. Peak areas were measured by a pipping-pen integrator fitted to a 1 mV Minneapolis-Honeywell recorder. Owing to the fewer counts available than in a previous study[7] the deviations from average, although reasonably low in quantitative studies, were in some cases higher than those previously reported.

RESULTS AND DISCUSSION

Analysis of volatile fatty acids

Two mixtures of volatile fatty acids, at two different levels of concentration in water–acetone (2:1) solution, were analysed in triplicate on the two columns, in each case both with and without formic acid vapor in the carrier gas. Very good separations with minimal tailing of the peaks were obtained in all cases (Figs. 1 and 2), with no indication of double peak formation[7]. The average relative area responses given in Table I show that the ketoacid polymer gives quantitative results, comparable with previous analyses[7], on both columns when formic acid vapor is used in the carrier gas, and that with the teflon-impregnated support results can be obtained comparable with the previous data even without formic acid vapor in the carrier gas. The more
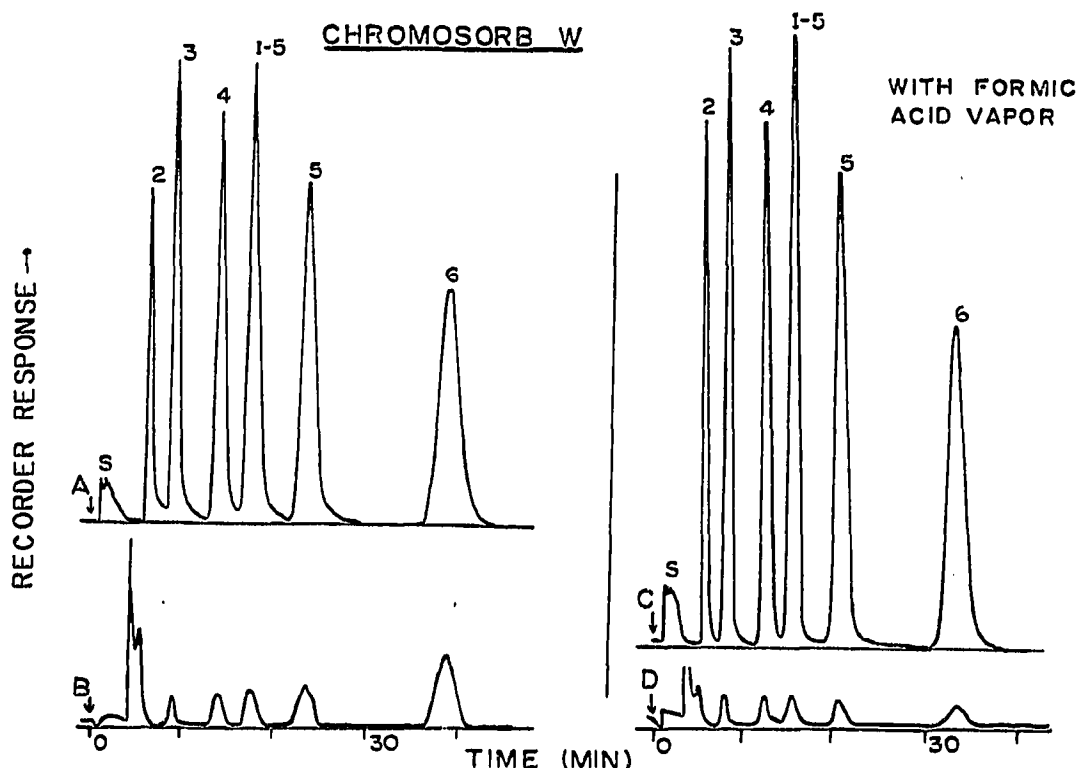

Fig. 1. Separation of C_2–C_6 normal acids, plus isovaleric acid, (*ca.* 1 % each in water–acetone) on Chromosorb W column. A: plain carrier gas, 0.001 ml sample. B: subsequent injection of 0.001 ml 1:1 formic acid–water mixture. C: carrier gas containing formic acid vapor, 0.001 ml sample. D: subsequent injection of 0.001 ml 1:1 formic acid–water mixture. Helium carrier gas at approximately 15 p.s.i. in all analyses, column at 150°. Solvent peak (S) attenuated.
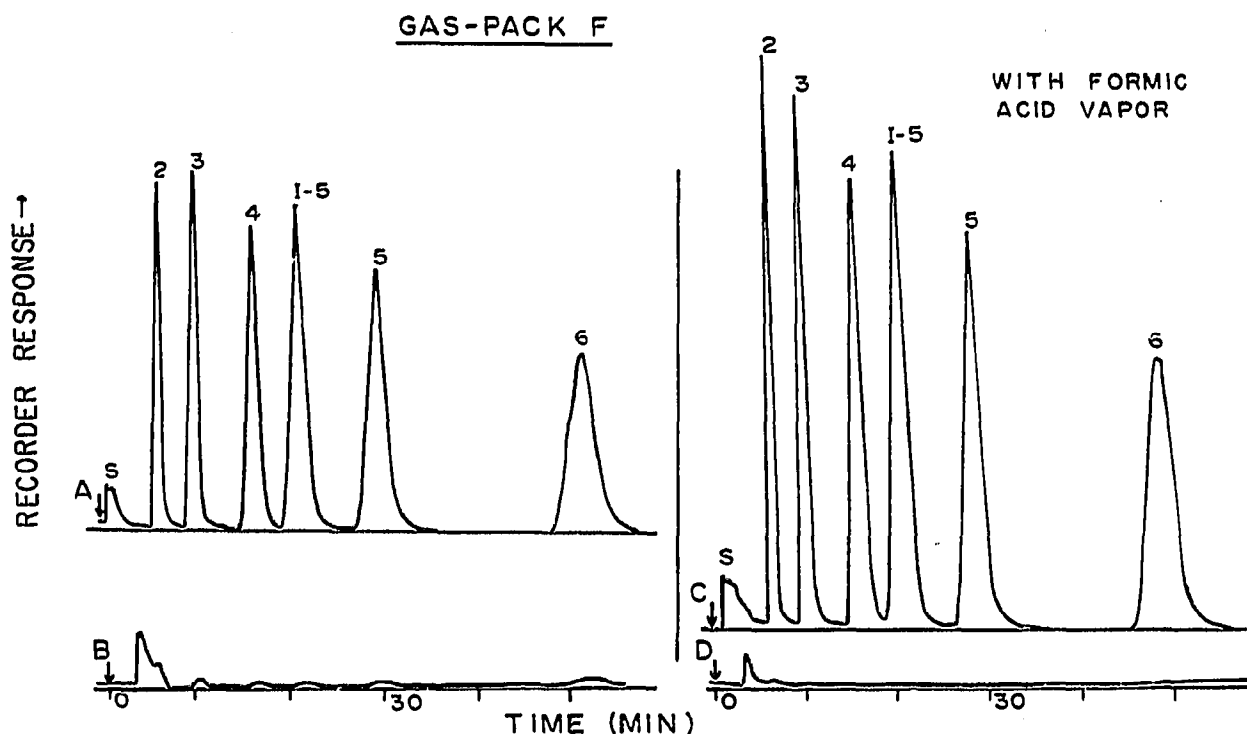
Fig. 2. Separation of C_2–C_6 normal acids, plus isovaleric acid, (*ca.* 1 % each in water–acetone) on Gas-pack F column. A: plain carrier gas, 0.001 ml sample. B: subsequent injection of 0.001 ml 1:1 formic acid–water mixture. C: carrier gas containing formic acid vapor, 0.001 ml sample. D: subsequent injection of 0.001 ml 1:1 formic acid–water mixture. Helium carrier gas at approximately 15 p.s.i. in all analyses, column at 130°. Solvent peak (S) attenuated.

TABLE I

AREA RESPONSE, RELATIVE TO ISOVALERIC ACID AS 1.00, OF EQUAL WEIGHTS OF C_2–C_6 VOLATILE FATTY ACIDS, AT TWO DIFFERENT LEVELS OF CONCENTRATION

Analyses on two different ketoacid polymer columns as indicated by support, both with (F) and without (NF) formic acid vapor in the carrier gas

	Carrier gas	Acid					
		Acetic	*Propionic*	*Butyric*	*Valeric*	*Caproic*	*Isovaleric*
Acid (g/100 ml)		*1.0096*	*0.8496*	*0.8399*	*1.0286*	*1.0206*	*0.9992*
Chromosorb W	NF	0.33	0.72	0.89	0.97	0.99	1.00
	F	0.41	0.70	0.89	0.98	1.05	1.00
Gas-pack F	NF	0.39	0.70	0.87	0.96	1.05	1.00
	F	0.43	0.71	0.86	0.96	1.08	1.00
Acid (g/100 ml)		*0.061*	*0.053*	*0.065*	*0.083*	*0.089*	*0.077*
Chromosorb W	NF	0.22	0.53	0.89	0.98	1.03	1.00
	F	(0.30)*	0.68	0.86	1.00	1.13	1.00
Gas-pack F	NF	0.36	0.66	0.84	1.00	1.03	1.00
	F	0.35	0.72	0.82	0.97	1.10	1.00

* See discussion in text.

dilute acid solution ($< 0.1\%$) cannot be satisfactorily analysed for acetic and propionic acids on the Chromosorb W column unless formic acid vapor is present, although the more concentrated acid solution (*ca.* 1.0%) gives reasonably satisfactory results in repeated analyses or this support without formic acid.

The injection of formic acid–water ($1:1$) mixture subsequent to each series of analyses (Figs. 1 and 2) shows the extent to which the presence of formic acid vapor suppresses adsorption of acids on the column.

The behavior of the ketoacid polymer on the Chromosorb W support in this respect is somewhat similar to that previously observed with the silicone oil (with stearic acid) column[7]. The displacement of acids, on injection of formic acid–water mixture, even with formic acid vapor in the carrier gas, indicates that slightly more adsorption of acids has occurred. On the other hand, the employment of formic acid vapor in the carrier gas does not give a significant reduction in retention time, suggesting that most of the support is "covered" as with the more polar substrates. With acid solutions of varying concentration the acids displaced by formic acid–water injection are roughly in constant proportion to the preceding normal peak area, indicating that whatever adsorption takes place on the column is proportional to the amount of acid injected. For this reason precautions should be observed, as in all such analyses, to eliminate extraneous peaks from "ghosting"[13] when using a ketoacid polymer on Chromosorb W support in the analysis of volatile fatty acids. Prior to injection of unknown samples the column may be cleared by injection of formic acid–water mixture. With the addition of formic acid vapor to the carrier gas such a column should, however, give satisfactory quantitative results. Moreover the injection of water alone, or of weaker acids such as acetic, as a 1% solution (0.001 ml) failed to displace any acids subsequent to analyses of the mixture carried out with formic acid vapor in the carrier gas. Analysis of mixtures *not* containing formic acid should not therefore present any "ghosting" problems. Both columns contain nearly the same weight of ketoacid polymer. Consequently the fact that far more reversible adsorption occurs on the Chromosorb W column (Figs. 1 and 2) suggests that the substrate itself does not retain any appreciable amount of acid in "solution" or through hydrogen bonding.

After completion of the quantitative analyses the calculations indicated an anomalous relative area response for acetic acid (of 0.66) in the analysis of the weaker acid solution on the Chromosorb W column with formic acid in the carrier gas. Careful examination of the chromatogram (Fig. 3) indicated that this figure, obtained if the approximate baseline was taken as line L_2 (dashes) was erroneous, and the true baseline was actually line L_1 (dots). The same effect could be detected in the analyses of the stronger solution carried out at the same time, but owing to the attenuation of signal and larger acid peak the baseline dip had no serious effect on the interpretation of the chromatogram (Fig. 1). The approximate value for acetic acid of 0.30 given in Table I for the analysis under discussion is based on line L_1, although it is not possible to include that portion of the peak actually below line L_1.

The gradual dip in the chromatogram line after the solvent peak S (acetone) had not previously been noted, and re-examination of earlier analyses[7] indicated that it was not present. A more familiar phenomenon, a sharp dip below baseline, had on occasion been seen after large peaks with vertical trailing edges, or any material coming off the column at a very high concentration. This is also characteristic of carbon disulfide peaks when this material is employed as a solvent, and it has been

suggested that combustion products may in the latter case inhibit ionization[14]. Curiously similar dips in the baseline are shown in certain chromatograms[15] obtained with an argon ionization detector, although water desensitization may be responsible in the latter case. Various experiments apparently ruled out insufficient air supply as
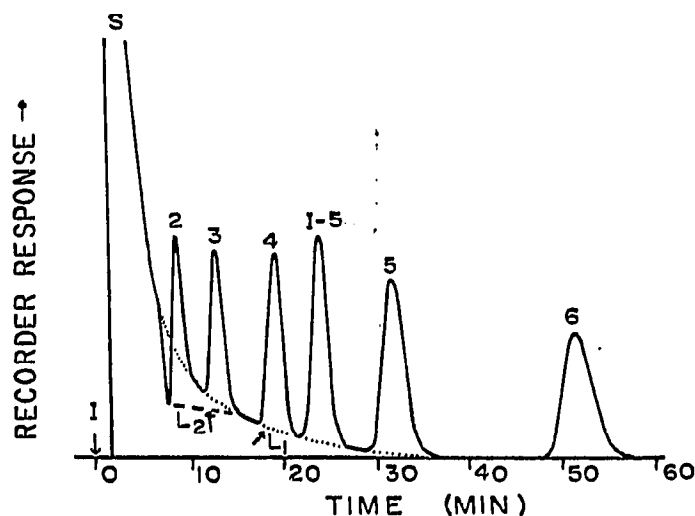

Fig. 3. Injection of 0.001 ml of C_2–C_6 acid mixture (< 0.1% in water–acetone solvent) on Chromosorb W column. L_1 is approximate true baseline, L_2 an erroneous apparent baseline due to dip in curve after acetone peak (S).

a cause of the chromatogram dip under discussion. The injection of the formic acid–water mixture on this column (cf. Fig. 1) does, however, give material peaks, the trailing edge of which is very similar in shape and position to the leading edge of the chromatogram dip. It must therefore be concluded that this was due to formic acid, or, more probably, an impurity in formic acid, which desensitized the detector under the particular operating conditions.

The column prepared with the teflon impregnated support approaches ideality of operation for the analysis of volatile fatty acids in that *even with no formic acid in the carrier gas* adsorption of acids, as shown in Fig. 2, was minimal. This type of column would then be suitable with aqueous solutions for use with thermal conductivity detectors, provided the water peak, which should come through rapidly on this combination of hydrophobic support and substrate, does not interfere with the acids under study. Non aqueous solvents such as acetone are also eluted very rapidly and this type of column could therefore be useful for similar analyses with argon ionization detectors.

Analysis of hydrocarbons

The analysis of hydrocarbons does not fall within the normal scope of this laboratory, and the column dimensions were perhaps not entirely satisfactory for this type of work. Adequate separations of decalins and naphthalene[16] were obtained with the teflon-impregnated support (Fig. 4-A) but considerable tailing occurred with the Chromosorb W column (Fig. 4-B). Cyclohexane appeared before benzene, and on both columns the benzene peak tailed appreciably. When temperature or gas flow were varied to give the cyclohexane the same retention time, the tailing of the latter was
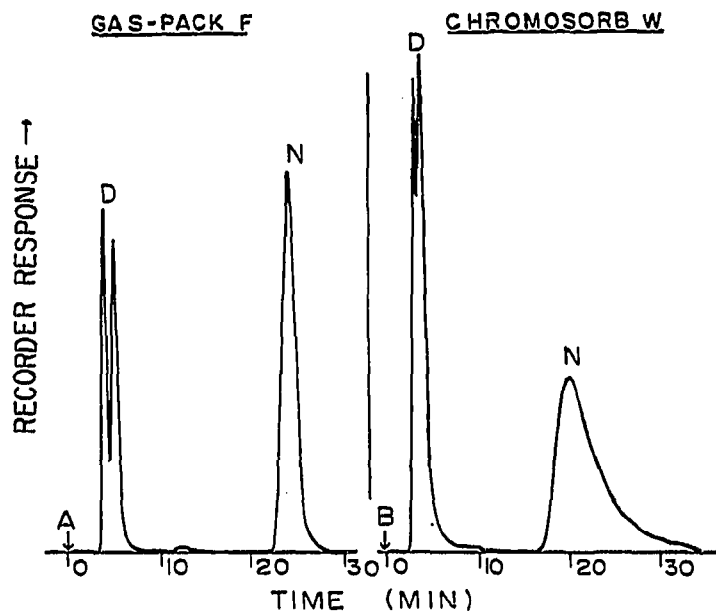
Fig. 4. Separation of decalins (D) and naphthalene (N). A: Gas-pack F column, helium carrier gas at 15 p.s.i., column at 135°. B: Chromosorb W column, helium carrier gas at 15 p.s.i., column at 135°. Sample 0.001 ml CS₂ solution (1 %).
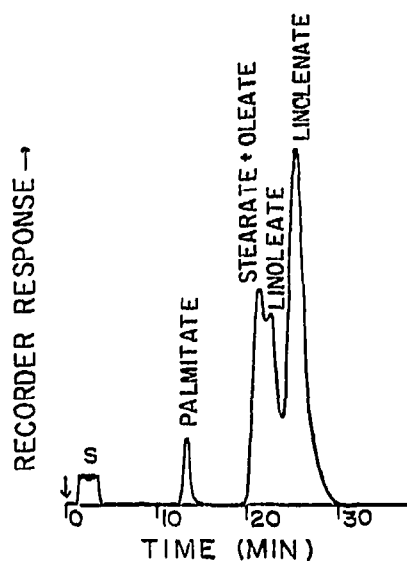
Fig. 5. Separation of methyl esters of linseed oil fatty acids on Chromosorb W column. Helium carrier gas at 15 p.s.i., column at 200°. Sample 0.001 ml CS₂ solution (1 %).

markedly less than that of the benzene. Interaction of the ketone or carboxyl groups with the aromatic double bonds therefore seems possible.

Analysis of higher methyl esters

The performance of this substrate in respect to methyl esters of saturated and unsaturated fatty acids (Fig. 5) is similar to that of one of the less polar polyesters such as neopentyl glycol adipate. In this respect it differs from silane polymers such as SE-30, and Apiezon greases, where the more highly unsaturated materials appear first. There was no appreciable difference in the performance of the two columns with this mixture.

Stability

At temperatures up to 150° the freshly prepared columns did not bleed noticeably or change properties over several weeks of operation. On heating to 220° heavy bleeding lasting several hours was observed. This may have been due in part to removal of cyclic monomeric ketones reportedly formed in association with the polymer, and possibly traces of sebacic acid. Baseline stability thereafter was excellent, but over a period of operation extending over several weeks at temperatures in excess of 200°, the columns gradually lost resolving power with respect to the volatile fatty acid mixture when tested at 150°. A corresponding drastic reduction in retention time indicated that an appreciable proportion of the substrate had been lost from the columns.

The stability of the particular columns tested is probably not typical of this material as a gas–liquid chromatography substrate, since the degree of polymerization

as indicated by the melting point was low. Virtually any range of molecular weight or ketone–carboxylic acid ratio could be obtained by fractionation of a large quantity of polymerization product. The complete removal of all traces of metal used in the preparation of the ketoacid polymer is probably necessary for maximum polymer stability and elimination of side reactions affecting the material under analysis.

CONCLUSIONS

Although particularly suited to analysis of volatile fatty acids, the potentialities of ketoacid polymers in gas–liquid chromatography are extensive. In addition to polymers of different molecular weights obtained from different dicarboxylic acids, chemical modification of the ketone or carboxyl groups, particularly through the insertion of other substituents or functional groups at these sites, could extend the useful range of the basic material into fields other than that of the volatile fatty acids.

SUMMARY

A low molecular weight ketoacid polymer prepared from the cadmium salt of sebacic acid has been demonstrated to be a satisfactory substrate for the separation of the volatile fatty acids. With the addition of formic acid vapor to the carrier gas quantitative results may be obtained employing conventional supports. With a teflon-impregnated support the use of formic acid vapor, while probably helpful at very low concentrations of acids, is not strictly necessary. Separations of certain other materials and the thermal stability of the polymer are discussed. An instance of desensitization of a flame ionization detector has been noted.

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