GAS–LIQUID CHROMATOGRAPHY OF VOLATILE FATTY ACIDS FROM FORMIC ACID TO VALERIC ACID

II. THE INSTABILITY OF SILICONE OIL–FATTY ACID STATIONARY PHASES

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JAMES AND MARTIN[1] reported that silicone DC550–stearic acid columns retained their resolving power for volatile fatty acids indefinitely when operated at temperatures up to 137°. However, several workers have reported rapid loss of efficiency under these conditions[2,3,4,5].

Both MCINNES[3] and BOER[2] noticed a loss of stearic acid from the column; if loss of stearic acid had caused deterioration of the columns the retention volumes of the acids should have decreased, but this was not the case. Behenic acid was lost at a slower rate than stearic acid but complete suppression of acid bleeding was obtained by using a fatty acid mixture prepared from Carnauba wax (BOER[2]). This permitted about 20 determinations before resolution became unsatisfactory. MCINNES[3] replaced stearic acid with varying proportions of monocarboxylic acids (C_{20} and C_{22}) and dicarboxylic acids (C_{13}, C_{18} and C_{20}). However, replacing stearic acid by less volatile acids did not increase the life of the columns to any appreciable extent.

The importance of water in the sample and carrier gas has been noticed by several workers[1,6,7,8]. GRAHAM[8] reported that some batches of Celite gave poor resolution of C_1–C_5 fatty acids when using dry nitrogen. This was improved by saturating the carrier gas with water at room temperature and he found that the columns tolerated a considerable proportion of water in the samples.

HAWKE[6], using wet carrier gas, found no deterioration of a silicone MS550–behenic acid–phosphoric acid column at 137°.

In view of the conflicting reports about the stability of silicone–fatty acid columns and the effect of water on their performance, a systematic study of these aspects has been made.

APPARATUS AND MATERIALS

Chromatograph

The glass column, 120 cm long and 4 mm inside diameter, was supported inside an electrically heated air jacket. Temperature was controlled by means of a variable transformer. The column terminated in a ground-glass joint which fitted into a titration cell. Acids emerging from the column were detected by automatic titration

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with a Radiometer Titrigraph[10]. The carrier gas was commercial dry nitrogen which was either passed through water, or dried by passing through a column of activated Union Carbide Molecular Sieve, Type 5A, before entering the column.

Materials for preparing columns

Chromosorb W, acid-washed, 80–100 mesh; batch I was obtained from F & M. Scientific Corporation, batch II from Johns-Manville Products Corporation; Haloport F (F & M. Scientific Corporation); Paraffin oil (British Pharmacopoeia grade); Silicone DC 550 (Dow-Corning); Stearic acid (Eastman-Kodak); Behenic acid (Hopkin & Williams), recrystallized from acetone; Phosphoric acid (B.D.H. Analar); Acetone and chloroform (May and Baker reagent grade).

Organic acids

The carboxylic acids used were commercial samples. The mixture used for testing the columns contained approximately equi-molar amounts of formic, acetic, propionic, isobutyric, *n*-butyric, isovaleric and *n*-valeric acids. In some cases formic, isobutyric and isovaleric acids were omitted from the test mixture.

EXPERIMENTAL PROCEDURE AND RESULTS

Preparation of columns

The materials for the liquid phases were dissolved in acetone except for that containing paraffin oil; the packing containing paraffin oil was prepared in chloroform. The solid support was added and the solvent removed on a rotary evaporator.

Operating conditions

The temperature of the columns was maintained at 130 ± 1°. The nitrogen flow-rate was 10, 15 or 30 ml/min depending upon the retention times of the acids on the column being tested.

The performance of a column was tested by applying 1 μl of the mixture of acids to a plug of glass wool at the top of the packing.

Chromosorb W–silicone DC 550–stearic acid packing

This packing consisted of acid-washed Chromosorb W, batch I (10 parts), silicone DC 550 (4 parts) and stearic acid (0.4 parts). Curves A to D (Fig. 1) show the separation of the mixture of acids after the column had been operating with dry nitrogen at 15 ml/min for 0, 4, 10 and 24 h, respectively. The first noticeable change was loss of resolution between iso- and *n*-butyric acids after 4 h. After 10 h all the acids showed increased tailing and there was little separation of the butyric acid isomers from one another. Even greater loss of resolution was apparent after 24 h. At that stage the carrier gas was passed through water instead of the column of molecular sieve. There was no immediate change in the resolving power of the column, but 4 h after changing to wet gas some reduction of tailing was evident (curve A, Fig. 2). The performance of the column 8, 24 and 48 h after changing to wet gas is shown by curves B, C and D (Fig. 2). The separation of the butyric isomers was not quite as good as that obtained with the freshly prepared column but otherwise the resolving power was restored. Operation of the column for a further 28 h produced no further

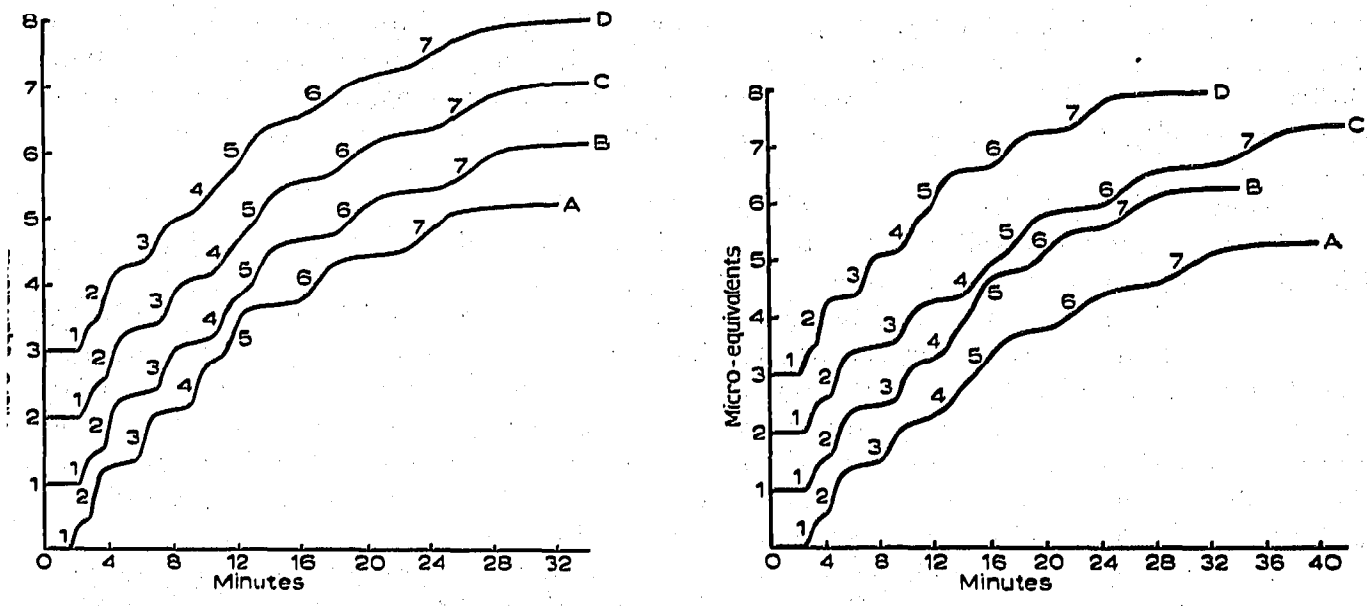

Fig. 1. Chromatograms of a mixture of C_1 to C_5 acids showing deterioration of a column of 40% (w/w) silicone DC 550 and 4% (w/w) stearic acid on acid-washed Chromosorb W (batch I) when operated with dry carrier gas for (A) 0 h, (B) 4 h, (C) 10 h and (D) 24 h. Column temperature: 130 ± 1°. Flow rate: 15 ml/min. Acids: 1 = formic; 2 = acetic; 3 = propionic; 4 = isobutyric; 5 = *n*-butyric; 6 = isovaleric; 7 = *n*-valeric.

Fig. 2. Restoration of the column described in Fig. 1 (D) when operated with wet nitrogen for (A) 4 h, (B) 8 h, (C) 24 h and (D) 48 h. Temperature and flow rate as for Fig. 1. Acids: as in Fig. 1.

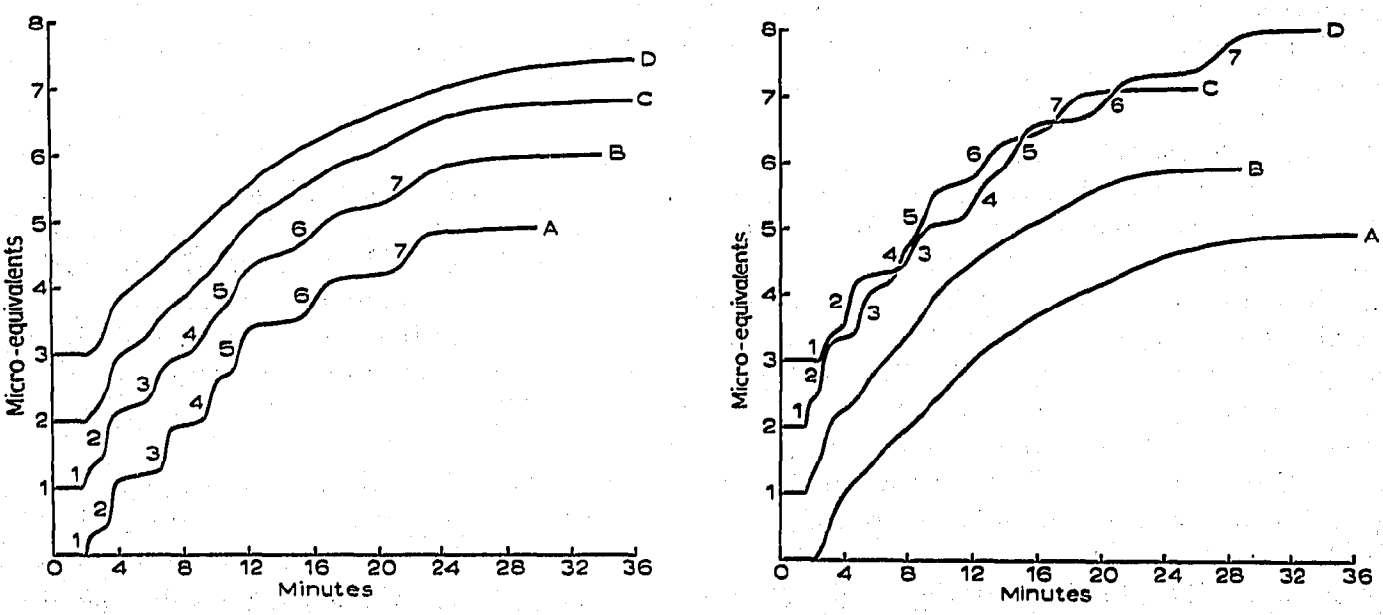

Fig. 3. Chromatograms of a mixture of C_1 to C_5 acids showing deterioration of a column of 40% (w/w) silicone DC 550 and 4% (w/w) behenic acid on acid-washed Chromosorb W (batch I) when operated with dry nitrogen for (A) 0 h, (B) 24 h, (C) 76 h and (D) 193 h. Column temperature: 130 ± 1°. Flow rate: 15 ml/min. Acids: as in Fig. 1.

Fig. 4. Restoration of the resolving power of the column described in Fig. 3 (D) when operated with wet nitrogen for (A) 0 h, (B) 2 h, (C) 24 h and (D) 48 h. Column temperature and flow rate as in Fig. 3. Acids: as in Fig. 1.

change in performance. At this stage the column had been run for a total of 100 h and had lost an appreciable amount of stearic acid. The performance was improved slightly by repacking the column with the addition of more stearic acid and then was comparable with that of a freshly prepared column run with wet carrier gas.

Chromosorb W–silicone DC 550–behenic acid packing

This packing consisted of acid-washed Chromosorb W, batch I (10 parts), silicone DC 550 (4 parts) and behenic acid (0.4 parts).

The performance of this packing with dry carrier gas is illustrated in Fig. 3. The pattern of change was very similar to that found for silicone–stearic acid columns operated with dry gas. Curve B (Fig. 3) shows the result obtained after 24 h; excessive tailing of all acids is apparent and there is practically no separation of iso- and *n*-butyric acids. After 76 h the loss of separating power was almost complete (curve C, Fig. 3). The column was run for an extended period prior to testing the reversibility of the changes. After 193 h curve D (Fig. 3) was obtained.

At this stage the carrier gas was changed to wet nitrogen; there was no immediate change in performance (curve A, Fig. 4), but after 2 h a slight separation was apparent (curve B, Fig. 4). After 24 h operation with wet gas (curve C, Fig. 4), all components of the mixture except the butyric acid isomers were identifiable although they still tailed considerably. After 48 h (curve D, Fig. 4) the resolving power of the column was only slightly inferior to that of a freshly prepared column, except that iso- and *n*-butyric acids overlapped considerably. The column was run for an additional 70 h without further change in behaviour being apparent. For comparison, a freshly prepared silicone–behenic acid column was run with wet carrier gas from the beginning. Performance after 0, 4, 10 and 84 h is shown in Fig. 5. There was an early loss of some resolving power for iso- and *n*-butyric acids and a slow increase in the tailing of formic acid, otherwise the behaviour of the column was stable.

Effect of phosphoric acid

JAMES AND MARTIN[1] found that the addition of orthophosphoric acid to a Celite–silicone–stearic acid column eliminated tailing and improved the separation of formic and acetic acids. HAWKE[9] also obtained an excellent separation of formic and acetic acids on a Celite–silicone oil–behenic acid–phosphoric acid column using wet carrier gas. As this seemed to be a useful packing, its stability under wet and dry conditions was investigated. The packing consisted of acid-washed Chromosorb W, batch I (10 parts), silicone DC 550 (4 parts), behenic acid (0.4 parts) and orthophosphoric acid (0.4 parts). The nitrogen flow rate was 30 ml/min.

Curve A (Fig. 6) was obtained with a freshly prepared column using dry carrier gas. Formic and acetic acids were not separated but the separation of the remaining acids was good. After 9 h some resolution of formic and acetic acids became apparent. Thereafter little further change took place, curves B, C, and D (Fig. 6) being obtained after 24, 48 and 336 h, respectively. Wet carrier gas was then used and the retention volumes of the acids decreased immediately (curve A, Fig. 7). There appeared to be no separation of formic and acetic acids but any separation may have been obscured because the addition of alkali for this run was too slow for the rate at which these acids were eluted. The decrease in retention volumes continued over the first 3–4 h; thereafter, the performance of the column showed little change, curves B and C

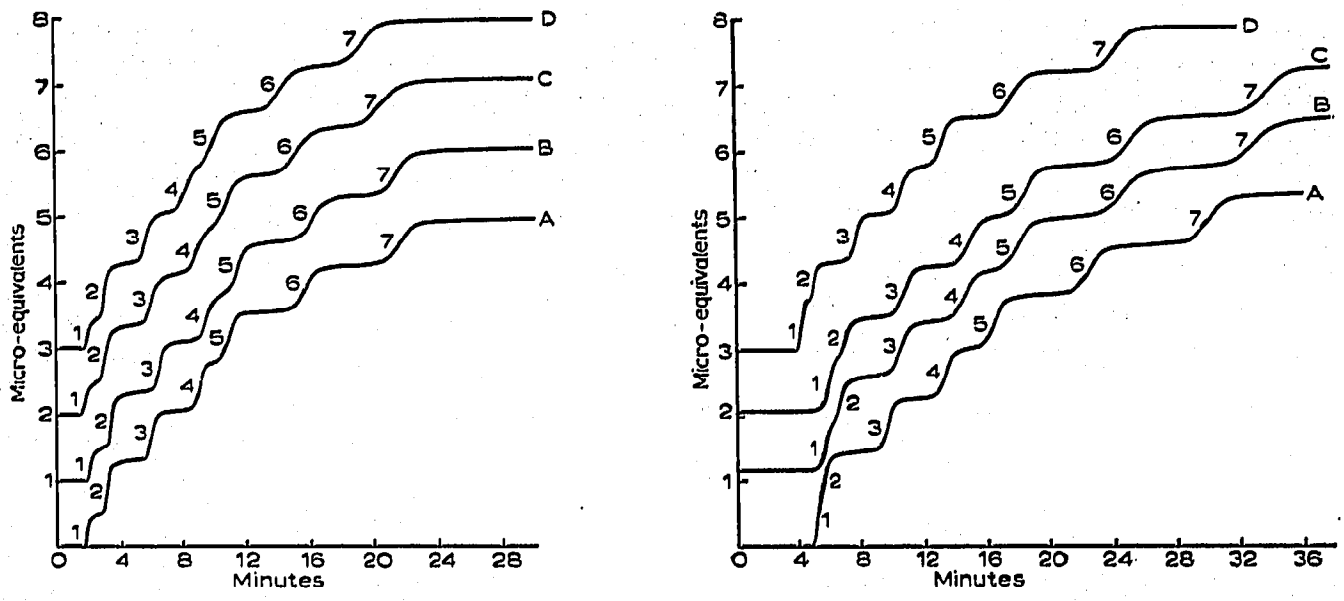

Fig. 5. Separation of a mixture of C_1 to C_5 acids on a column of 40% (w/w) silicone DC 550 and 4% (w/w) behenic acid on acid-washed Chromosorb W (batch I) after operating with wet nitrogen for (A) 0 h, (B) 4 h, (C) 10 h and (D) 84 h. Column temperature: 130 ± 1°. Flow rate: 15 ml/min. Acids: as in Fig. 1.

Fig. 6. Separation of a mixture of C_1 to C_5 acids on a column of 40% (w/w) silicone DC 550, 4% (w/w) behenic and 4% (w/w) orthophosphoric acids on acid-washed Chromosorb W (batch I) after operating with dry nitrogen for (A) 0 h, (B) 24 h, (C) 48 h and (D) 336 h. Column temperature: 130 ± 1°. Flow rate: 30 ml/min. Acids: as in Fig. 1.

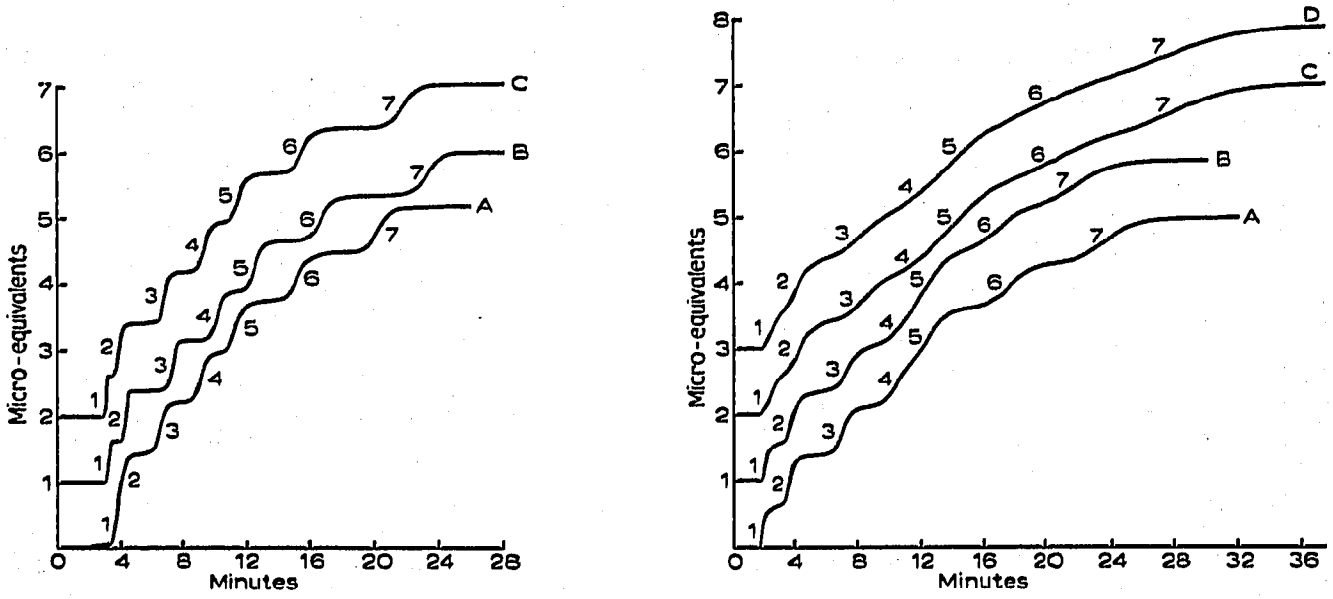

Fig. 7. Separation of a mixture of C_1 to C_5 acids on the column described in Fig. 6 (D) after operating with wet nitrogen for (A) 0 h, (B) 24 h and (C) 180 h. Column temperature and flow rate as in Fig. 6. Acids: as in Fig. 1.

Fig. 8. Chromatograms of a mixture of C_1 to C_5 acids showing deterioration of a column of 13.3% (w/w) silicone DC 550 and 1.3% (w/w) behenic acid on Haloport F after operating with dry nitrogen for (A) 0 h, (B) 24 h, (C) 72 h and (D) 144 h. Column temperature: 130 ± 1°. Flow rate: 15 ml/min during separations, otherwise 30 ml/min. Acids: as in Fig. 1.

(Fig. 7) being obtained after 24 and 180 h, respectively. At this stage of the investigation the poor performance of the silicone–fatty acid phases was attributed to tailing caused by adsorption of the volatile acids by the Chromosorb support. Addition of water to the carrier gas was thought to prevent tailing by saturating the adsorption sites. The resolution obtained under dry conditions when phosphoric acid was added to the liquid phase was likewise attributed to suppression of adsorption.

Haloport F–silicone DC 550–behenic acid packing

To obtain further evidence on this point, Chromosorb W was replaced by the perfluorocarbon, Haloport F. There was negligible adsorption on this material since

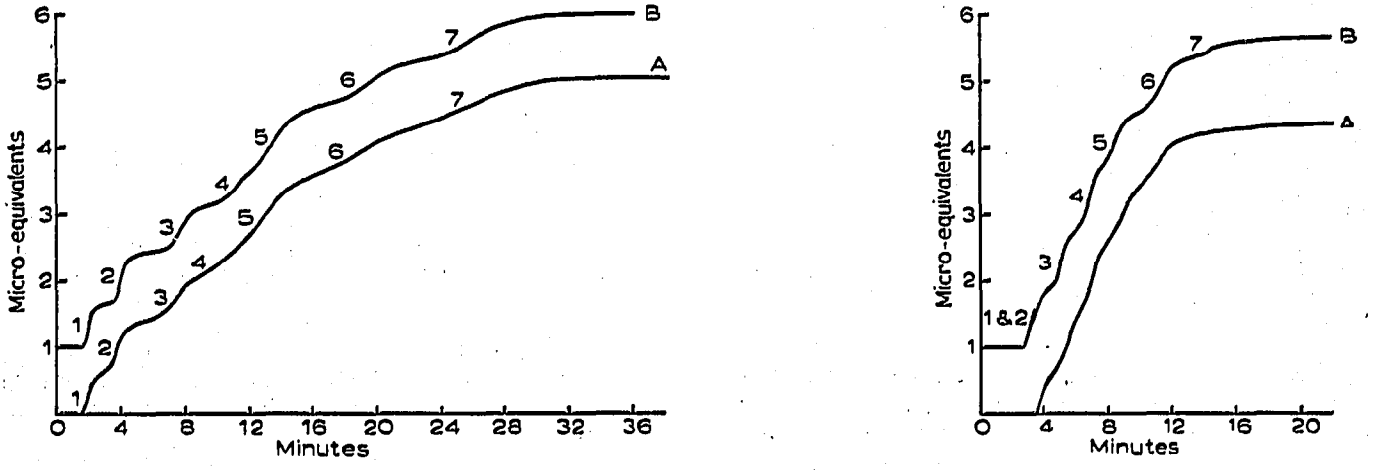

Fig. 9. Restoration of the column described in Fig. 8 (D) when operated with wet nitrogen for (A) 5 h and (B) 48 h. Temperature and flow rates as in Fig. 8. Acids: as in Fig. 1.

Fig. 10. Chromatograms of a mixture of C_1 to C_5 acids on a column of 40% (w/w) silicone DC 550 on acid-washed Chromosorb W (batch II). (A) Dry nitrogen, freshly prepared column; (B) after 144 h with wet nitrogen. Column temperature: 130 ± 1°. Flow rate 10 ml/min during separations, otherwise 30 ml/min. Acids: as in Fig. 1.

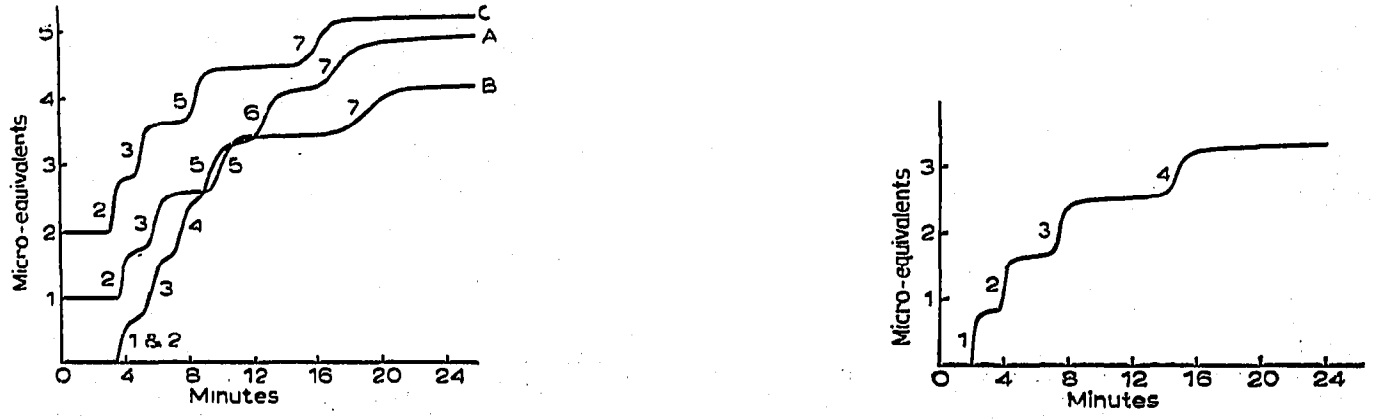

Fig. 11. Chromatograms of mixtures of C_1 to C_5 and C_2 to C_5 acids on a column of 4% (w/w) behenic acid on acid-washed Chromosorb W (batch II). (A) wet nitrogen, freshly prepared column; (B) after 72 h with wet nitrogen; (C) after 24 h with dry nitrogen. Column temperature: 130 ± 1°. Flow rate: 10 ml/min during separations, otherwise 30 ml/min. Acids: as in Fig. 1.

Fig. 12. Chromatogram of a mixture of C_2 to C_5 acids on a column of 40% (w/w) paraffin oil and 4% (w/w) behenic acid on acid-washed Chromosorb W (batch II) after 72 h operation with dry nitrogen. Column temperature: 130 ± 1°. Flow rate: 30 ml/min. Acids: as in Fig. 1.

when the acids were run on a column with no liquid phase, they were completely eluted in less than 15 ml. The column packing consisted of Haloport F (15 parts), silicone DC 550 (2 parts) and behenic acid (0.2 parts) and contained about the same weight of liquid phase as that prepared on Chromosorb W. This column was run with a flow rate of 30 ml/min except that 15 ml/min was used for testing performance. The curves obtained after 0, 24, 72 and 144 h with dry gas are shown in Fig. 8. Wet nitrogen was then passed through the same column. There was no immediate change but some improvement was evident after 5 h (curve A, Fig. 9) and after 48 h (curve B, Fig. 9) the performance was almost as good as when freshly prepared. Further evidence that the support was not involved in the deterioration of the Chromosorb W–silicone oil–behenic acid packing was provided by the observation that wetting the carrier gas had no apparent effect on the adsorption of acids by a Chromosorb W column with no liquid phase.

The results obtained with the Haloport F column and the column packed only with Chromosorb W implicated the liquid phase as the source of instability when dry carrier gas was used. To determine whether one or both components were involved, columns were prepared in which each component of the liquid phase was omitted in turn.

Chromosorb W–silicone DC 550 packing

This packing consisted of acid-washed Chromosorb W, batch II (10 parts) and silicone DC 550 (4 parts). Performance was tested at a flow rate of 10 ml/min, otherwise the flow rate was 30 ml/min. Curve A (Fig. 10) was obtained with the freshly prepared column and there was no apparent change in performance after 48 h. Wet nitrogen was then used and the performance slowly improved. After 144 h there was some resolution of six of the seven acids (curve B, Fig. 10). Thereafter no further improvement took place.

Chromosorb W–behenic acid packing

This packing consisted of acid-washed Chromosorb W, batch II (10 parts) and behenic acid (0.4 parts). The flow rate was maintained at 30 ml/min but performance was tested with a flow rate of 10 ml/min. Curve A (Fig. 11) was obtained when the freshly prepared column was run with wet nitrogen. Formic and acetic acids were not resolved and most of the acids appeared to tail badly. Later it was found that formic acid tailed so badly on columns prepared with batch II Chromosorb* that it overlapped all the acids of the test mixture except *n*-valeric acid. Consequently a mixture containing acetic, propionic, *n*-butyric and *n*-valeric acids was used for testing performance and curve B (Fig. 11) was obtained after 72 h. Dry carrier gas was then used and after 24 h operation curve C (Fig. 11) was obtained. The column was run for a total of 168 h without any apparent deterioration.

Chromosorb W–paraffin oil–behenic acid packing

Although the experiments described above clearly show that the deterioration

* This material was later found to contain appreciable quantities of acid-soluble iron which may have caused tailing of formic acid. With the exception of the behaviour of formic acid, columns prepared with silicone oil–behenic acid on batch II Chromosorb W exhibited the same loss of resolving power under dry conditions and restoration with wet carrier gas, as those prepared with batch I.

of silicone oil–fatty acid packings under dry conditions is attributable to the silicone oil, further confirmation was sought by re-placing the silicone with paraffin oil. The packing consisted of acid-washed Chromosorb W, batch II (10 parts), paraffin oil (4 parts) and behenic acid (0.4 parts). The column was operated with dry carrier gas at a flow-rate of 30 ml/min. No change in performance was apparent after 72 h and excellent separation as shown in Fig. 12 was obtained.

Effect of water in the sample

In view of the improvement effected by adding water to the carrier gas and the conflicting reports about the effects of water in the sample, a few experiments were carried out to determine the effect of water in the sample on the separation by a silicone oil–behenic acid–phosphoric acid column, using wet carrier gas. A freshly prepared column was used and curves A, B, C and D (Fig. 13) were obtained when 0, 1, 2 and 4 μl of water, respectively, were added to 1 μl samples of fatty acid mixture.

That the effects observed were not due to deterioration of the packing was demonstrated by subsequent application of an anhydrous sample, which gave a curve practically identical to curve A, Fig. 13. Amounts of water up to 1 μl did not interfere with the separation but amounts in excess of this caused poor separations, particularly of the lower acids.

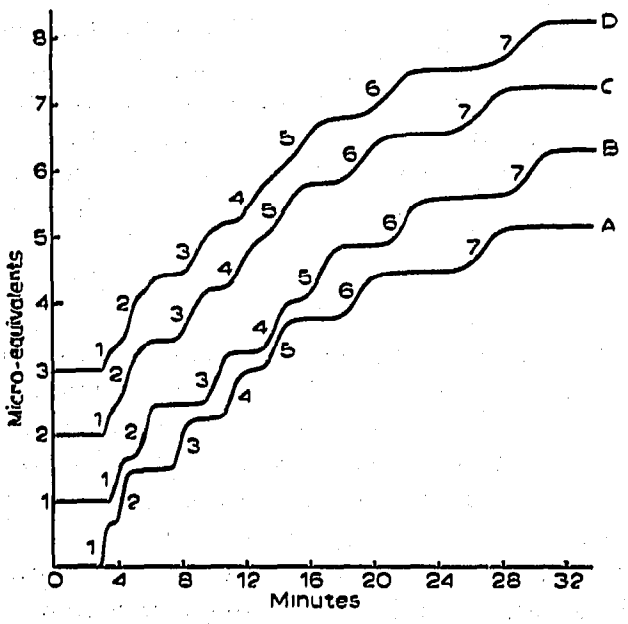

Fig. 13. Effect of water in the sample on the separation of a mixture of C_1 to C_5 acids on a column of 40% (w/w) silicone DC 550, 4% (w/w) behenic and 4% (w/w) orthophosphoric acids on acid-washed Chromosorb W (batch II). Samples: 1 μl mixture of acids without added water (A) or with the following amounts of water added: 1 μl (B), 2 μl (C) and 4 μl (D). Wet nitrogen flow rate: 15 ml/min. Column temperature: 130 ± 1°. Acids: as in Fig. 1.

DISCUSSION

The results of the present study clearly show that silicone oil–stearic acid and silicone oil–behenic acid stationary phases have very short useful lives under dry conditions at 130°. Deterioration can be prevented by continuously adding a small

amount of water to the carrier gas or by incorporating a small amount of orthophosphoric acid in the stationary phase. The latter modification, however, may cause partial decomposition of any formic acid present in the sample[5]. The satisfactory performance of freshly prepared columns is probably due to the presence of small amounts of water which, however, are eluted from the column during the first few hours of operation[8].

The experiments reported above clearly show that the silicone oil component of these packings is a source of instability under dry conditions. The possibility that the Chromosorb W support is also involved has not been eliminated completely. However, the performances of the paraffin oil–behenic acid and the lightly loaded behenic acid columns strongly suggest that the support is not involved.

KELLER, BATE, COSTA AND FORMAN[11] have reviewed the literature dealing with changes which occur in the immobile liquid phase during gas–liquid chromatography. The effect of these changes on retention volumes was discussed further by KELLER AND STEWART[12]. The stationary phase of a gas chromatographic column may undergo physical and chemical changes during use. KELLER AND STEWART[12] classified as physical changes, those which change the total amount of liquid and/or its distribution on the support. It is conceivable that physical changes as defined by KELLER AND STEWART[12] could, on the Chromosorb W support, give rise to adsorption effects which would be reversed by addition of water to the carrier gas[13]. However, the fact that the resolving power of the silicone–behenic acid phase was lost also on the non-adsorptive support, Haloport F, under dry conditions, shows that deterioration was not due to unmasking of adsorption sites by movement of liquid phase.

KELLER *et al.*[11] cited a number of reports of chemical changes in silicone liquid phases but most of these changes were observed at temperatures considerably higher than 130°. The loss of resolving power under dry conditions and its restoration by water suggest that the changes observed in the present study may involve dehydration and hydration reactions in the liquid phase. MARTIN[14,15] proposed that adsorption of the solute can occur on the surface of the liquid phase as well as on the surface of the support.

If carboxylic acids are adsorbed at the liquid–gas interface under dry conditions, then addition of water or phosphoric acid to the system presumably prevents this by saturating the adsorption sites. It might be expected that even under dry conditions the adsorption sites would be masked by the behenic acid in the stationary phase. However, the long hydrocarbon chains of the behenic acid molecules may prevent them reaching adsorption sites at the gas–liquid interface. Some evidence consistent with this is MCINNES' observation[3] that longer chain volatile acids (heptanoic to decanoic) can be separated on DC 550 silicone columns.

The necessity for using a wet carrier gas with silicone oil–behenic acid (or stearic acid) columns for separation of volatile fatty acids precludes their use with detectors that respond to, or are dampened by water (*e.g.* thermal conductivity and argon ionisation detectors*): If formic acid is absent from the samples, loss of resolving power may be prevented by including phosphoric acid in the stationary phase. Some of the difficulties associated with the separation of mixtures containing formic acid have been discussed[5,10].

* FOSTER AND MURFIN[16] recently reported that the response of the hydrogen-flame ionization detector too, is depressed by water.

Although only a limited investigation of the effect of water in the sample was carried out, the results support the contention of JAMES AND MARTIN[1] that even small amounts of water in the sample upset the separation. However, this appears to be the case only when the sample is applied directly to the column. The experiences of HAWKE[9], GRAHAM[8] and LANIGAN AND JACKSON[10] indicate that, provided the water is vaporized before entering the packing, considerable amounts of water can be tolerated.

ACKNOWLEDGEMENT

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SUMMARY

The stability of silicone DC 550–stearic acid and silicone DC 550–behenic acid stationary phases has been investigated. Columns containing these liquid phases rapidly lost their resolving power for volatile fatty acids when operated with a dry carrier gas. Resolving power was restored by passing the carrier gas through water for about 48 h. Deterioration could be prevented by using a wet carrier gas or by incorporating orthophosphoric acid in the liquid phase; the latter, however, may cause some decomposition of formic acid when the carrier gas is dry.

The silicone oil was shown to be the source of instability in the stationary phase.

Some possible reasons for the deterioration under dry conditions have been discussed.

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