Short Communications

Impurities in commercial formamide as critical factors in the paper chromatography of the violarin–mycetin antibiotics

Systems containing formamide are often used in the paper chromatography of antibiotics[1–7], steroids, glycosides and alkaloids[8–9]. LEES, DEMURIA AND BOEGELMANN[6] have shown that the degree of separation in these systems depends on the temperature conditions, as well as on the type of chromatographic chamber. We have found that the impurities present in commercial formamide are a decisive factor in the quality of the paper chromatograms of the mycetin–violarin antibiotics when this solvent is used for treatment of the paper. These antibiotics[10–13] are members of the same group of substances as actinorhodin, rhodomycin, granaticin, etc. A comparison of the latter and the mycetin–violarin antibiotics will be the subject of a further investigation.

It was shown in a previous paper[7] that the antibiotics of this group could be separated by paper chromatography in the system benzene/formamide into several components: A, B_1, B_2, C, D and E. However, discrepant results were obtained with different lots of formamide. These lots varied in their acid content, and the inconsistency of the results was attributed to this fact. The higher the acid concentration in the formamide, the lower was the mobility of the B_1, B_2, C and D components. The R_F values increased on adding sodium hydroxide to the acid formamide. For example, good separation was obtained when 0.8 % sodium hydroxide was added to formamide containing 1.84 % formic acid (Fig. 1a).

It was decided to purify the formamide carefully. The pure solvent, of freezing point 2.5°, was prepared by VERHOEK's method[14]. However, the purified formamide did not give a satisfactory separation (Fig. 1b). These results pointed to the presence in commercial formamide of substances that exert a decisive influence on the quality of the separation. According to VERHOEK[14] commercial formamide is usually acid and contains ammonium formate.

We added various quantities of ammonium formate, sodium formate and formic acid to pure formamide. As control we used commercial formamide, containing 1.84 % formic acid, to which 0.8 % sodium hydroxide was added. As the result of the neutralization the formamide now contained 1.36 % sodium formate, 0.92 % formic acid, and an unknown quantity of ammonium formate.

In the first series of experiments the ammonium formate content was varied from 0 to 10 %, while the contents of sodium formate and formic acid were kept constant at 1.36 % and 0.92 %, respectively. It can be seen from Fig. 2, that an increase in the salt content raised the R_F's of B_1, B_2, C and D. The same separation as with the control was obtained on using formamide with an ammonium formate content of 5 %.

In the second series of experiments the effect of varying the sodium formate content from 0 to 5 % was investigated. The ammonium formate and formic acid

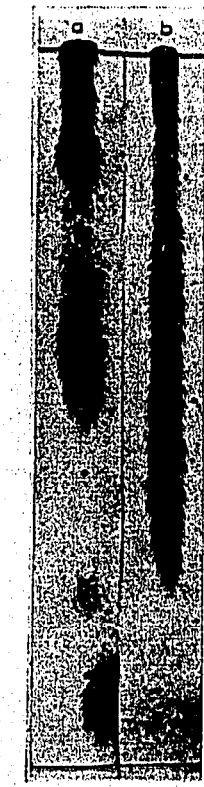

Fig. 1. Chromatography of mycetin–violarin antibiotics on formamide-treated paper. The chromatograms were developed with formamide-saturated benzene. (a) Chromatography with commercial formamide containing 1.84 % formic acid with 0.8 % NaOH added. (b) Chromatography on paper treated with pure formamide.

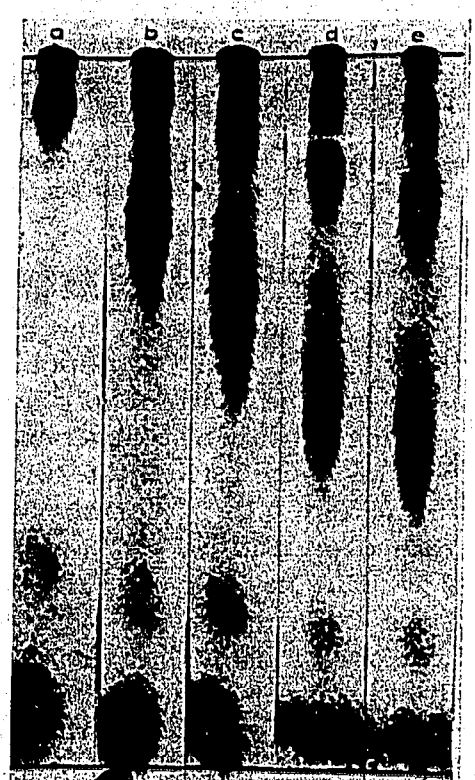

Fig. 2. Effect of ammonium formate on the separation. Sodium formate: 1.36 %; formic acid: 0.92 %; ammonium formate: (a) 0 %; (b) 2.5 %; (c) 5 %; (d) 7.5 %; (e) 10 %.

contents were now kept constant at 5 % and 0.92 %, respectively. The influence of sodium formate was in general the same as that of ammonium formate. Good separation was obtained with a sodium formate concentration of 1.36 %.

In the third series of experiments the sodium formate and ammonium formate contents were constant and equal to 1.36 % and 5 %, respectively. The formic acid

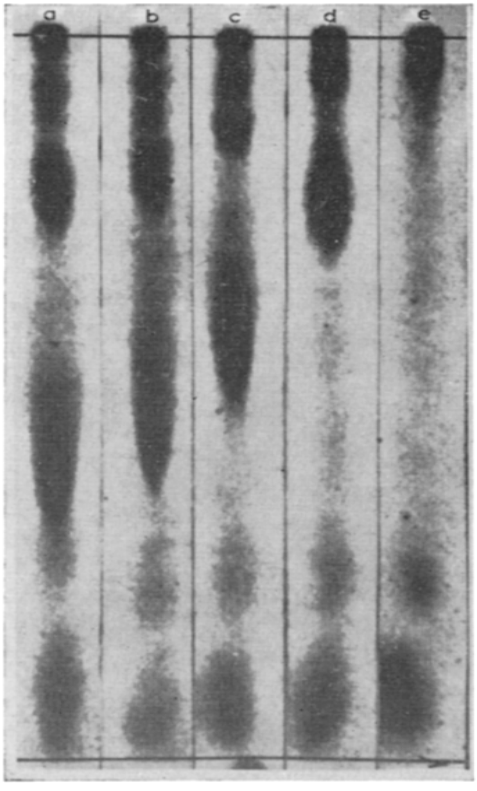

Fig. 3. Effect of formic acid on the separation. Ammonium formate: 5 %; sodium formate: 1.36 %; formic acid: (a) 0.016 %; (b) 0.4 %; (c) 0.92 %; (d) 1.2 %; (e) 5 %.

content was varied from 0.016 % to 5 %. The acid had an opposite effect to that of its salts. As can be seen from Fig. 3, an increase in the acid concentration leads to a decrease in the mobility of the B_1, B_2, C and D components. The same separation as with the control was observed at an acid content of 0.92 %.

These data show that in order to obtain a good separation of the violarin–mycetin antibiotics on paper chromatograms using formamide, one should add 5 % ammonium formate, 1.36 % sodium formate and 0.92 % formic acid to the pure formamide. Good results can also be obtained on adding 5 % ammonium formate, 2 % sodium formate and 0.46 % formic acid. But in that case the R_F values will differ slightly from those obtained in the former experiment. We did not examine all possible combinations, limiting ourselves to the observation that the impurities in commercial formamide play a decisive role in the paper chromatography of mycetin–violarin antibiotics with this solvent and to finding conditions that would give good separation. It follows from this that when commercial formamide is employed in paper chromatography, the degree of its purity must be taken into account. This is especially

important when the results are at variance with each other, as well as when attempts to use formamide-containing systems are unsuccessful.

The complete data will be published in Russian.

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Relative response of the flame ionization detector*

The relative response of the flame ionization detector for different organic substances has been the subject of considerable discussion[1–8]. For hydrocarbons, it is generally accepted that the responses per unit weight of the individual compounds (above about C_5) differ only slightly from each other[9–11] and that the relative molar responses seem to be directly proportional to the carbon number of the molecule[2–4]. However, it was demonstrated recently[13] that this rule is valid only in the first approximation; actually, the isomers with the same carbon number have different relative molar responses, but the relative molar responses of homologous series (*e.g.* normal paraffins, substituted cyclopentanes or substituted benzenes) follow a linear relationship with the carbon number.

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