

Quantitative Paper Chromatography of Fatty Acids

III. The Chemical Interaction with Cellulose

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The experiments were based on the titration of combinations of cellulose powder, copper acetate, and fatty acids with dilute sodium hydroxide. The results showed that the formation of pure copper soaps in stoichiometric amounts could only take place in the pH interval $5.4 \leq \text{pH} \leq 5.8$. Below $\text{pH} = 5.4$ copper formed fatty acid compounds also containing acetic acid and cellulose in compositions depending on the type of fatty acid. Above $\text{pH} = 5.8$ the formation of copper soaps was disturbed by the further precipitation of basic copper acetate. The Cu-fatty acid-acetic acid-cellulose complexes found were not stable at $\text{pH} = 7.5$, which is the value usually encountered during the rinsing of paper chromatograms.

The previously shown¹ influence of pH in the copper acetate bath on the quantitative transformation of fatty acids into their copper soaps after paper chromatography could not be explained in a simple manner. A change in pH from 6.0 to 5.0 in the copper bath resulted in a substantially reduced addition of Cu^{2+} to areas on the paper with low concentrations of myristic acid, a reduction that was not nearly as marked in connection with stearic acid. It was suggested that the factors involved could be the solubility and dissociation of the fatty acid and the solubility and dissociation of the copper soap or perhaps an association between the fatty acid or soap and the paper itself.

Seher² has shown that various long-chain fatty acids will react with Cu^{2+} in stoichiometric amounts at spot sizes between 100 and 800 μg . In the present investigation we have studied the formation of this copper compound, using a model mixture where combinations of cellulose powder, Cu-acetate or Cu-sulphate, and myristic or stearic acid were titrated with 0.1 N NaOH. The limitations of such an investigation, based on macro-amounts that are treated in aqueous solutions, are obvious. However, the results do give quite a clear general picture which suggests a spot model where an important chemical role is played by the cellulose moiety.

MATERIALS AND METHODS

Experiments were run as titrations with 0.10 N NaOH in 100 ml of water to which 5.00 ml 0.10 N acetic or nitric acid had been added in advance. They were primarily designed according to a 2³ factorial scheme with three factors at two levels combined in eight different ways. The three factors were:

Fatty acids (Fa): 1.0 mmole myristic or stearic acid

Copper salt (Cu): 0.6 mmole (CH₃COO)₂Cu·H₂O or
0.4 mmole CuSO₄·5H₂O

Cellulose (Cel): 10 g cellulose powder, Machery and Nagel MN 300.

Further investigations covered the effect of the addition of calcium ions:

Calcium salt (Ca): 0.6 mmole CaCl₂·2H₂O.

We measured the hydrogen ion concentrations with a pH meter, Radiometer type 22, using a glass electrode G 202B. It took a great deal of time to achieve equilibrium when cellulose or a fatty acid was involved. In most cases a standard procedure was adopted for pH readings: mechanical stirring was continued for about 3 h after addition of a portion of 0.10 N NaOH, the solution was left overnight, the next day stirring was resumed for about half an hour before a final measurement was made. Even then, control measurements showed that it could be weeks before a true equilibrium was reached. In the case of cellulose, however, gradual hydroxylations or Cu-catalyzed oxidations might also have caused the drift in pH.

The titrations were plotted with added mequiv. NaOH as the abscissa and pH as the ordinate. The excess consumption or release of base at constant pH following factor combinations could be determined graphically. If we call the blank 0 and let (A) stand for the consumption by A alone, (B) for the consumption by B alone and (A+B) for the consumption by a combination of A and B, then the excess consumption {AB} (with sign) appears from the expression

$$\{AB\} = (A+B) + 0 - (A) - (B)$$

The calculated excess consumption, positive, negative, or zero, can be regarded as a measure of chemical interaction. One sees directly that it will be zero if the added factors behave independently, in which case the titration of (A+B) will be the simple sum of the titration values for A and B alone. Any deviation from zero means that the factors involved affect each other. In case of positive consumption extra base is used to bring (A+B) to a certain pH as compared with what was needed for A and B alone, which implies that A and B have formed a proton-deficient chemical compound. Similarly it can be said that negative consumption indicates the formation of a chemical complex between A and B without a simultaneous release of protons. It should be noted that the absolute value of {AB} also depends on the relative concentrations of A and B, and its variation with pH must thus be interpreted with care.

The results of the present investigation will mainly be presented as a comparison of the interaction {FaCu} between fatty acids and cupric ions with the corresponding interaction {FaCu-Cel} after further addition of cellulose. In agreement with the terms mentioned above we have

$$\{FaCu\} = (Fa + Cu) + 0 - (Fa) - (Cu)$$

and

$$\{FaCu-Cel\} = (Fa + Cu + Cel) + (Cel) - (Fa + Cel) - (Cu + Cel)$$

We will also discuss the interaction {FaCel} between fatty acids and cellulose alone:

$$\{FaCel\} = (Fa + Cel) + 0 - (Fa) - (Cel)$$

RESULTS

The results are shown in Figs. 1–8.

Fig. 1 shows the titration values for the four factors and factor combina-

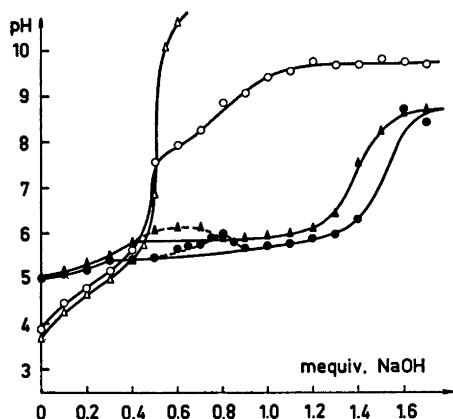


Fig. 1. Titration curves. Δ Blank (0). \circ Myristic acid (Fa). \blacktriangle Copper acetate (Cu). \bullet Myristic acid + copper acetate (Fa + Cu). Concentrations as described in the text.

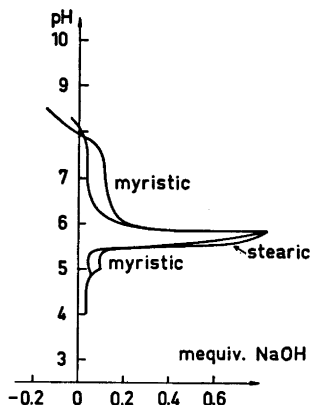


Fig. 2. Excess consumptions {FaCu} for titrations of combinations with myristic or stearic acid based on the results from Fig. 1 or similar.

tions that make up the investigation of the interaction {FaCu} between myristic acid and Cu-acetate. The corresponding graphically calculated {FaCu} curve is shown in Fig. 2 together with the similar curve for stearic acid. At pH=5.4 both fatty acids start forming Cu-salts, the nature of these salts cannot be derived directly from the titration values, but it is seen from Fig. 1 that the consumption of base corresponds only to the amount of added fatty

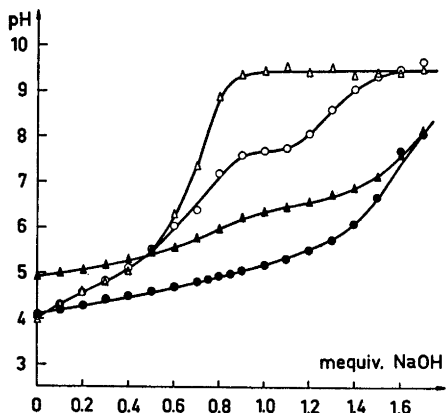


Fig. 3. Titration curves. Δ Cellulose (Gel). \circ Myristic acid + cellulose (Fa + Cel). \blacktriangle Copper acetate + cellulose (Cu + Cel). \bullet Myristic acid + copper acetate + cellulose (Fa + Cu + Cel). Concentrations as described in the text.

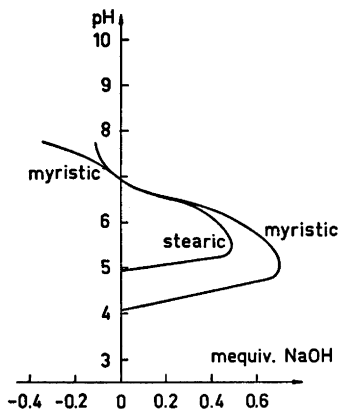


Fig. 4. Excess consumptions {FaCu-Cel} for titrations of combinations with myristic or stearic acid based on the results from Fig. 3 or similar.

acid, regardless of the excess amount of cupric ions, which means that we must be dealing with mixed Cu-salts of fatty acid and acetic acid. The decrease of $\{FaCu\}$ at $pH=5.8$ in Fig. 2 arises from the subtraction of the amount of base used to precipitate basic Cu-acetate. It does not necessarily correspond to any true change in the Cu-fatty acid interactions. In Fig. 1 one further notices that there is a tendency to "over-titrate" the precipitation of copper salts, even though the addition of base is extended over many days.

Figs. 3 and 4 correspond to Figs. 1 and 2 with further addition of cellulose powder. It appears directly from the smoother course of the curves between $pH=4$ and $pH=7$ in Fig. 3 that we are dealing with another chemical mechanism than in Fig. 1. There is no evidence of Cu-salt precipitation, but a gradual release of protons following the addition of base resembles the titration of weak ion exchangers; acetic acid, myristic acid, copper acetate, and cellulose seem to unite into a polymeric complex. In Fig. 4 one notices the difference between the myristic and stearic interaction curves. Myristic acid interaction is more marked, starting already at $pH=4.1$ and showing the largest maximum excess consumption of base. The two curves meet at $pH=6.8$, indicating that both Cu-fatty acid-cellulose compounds include the same Cu-cellulose subunit. Above $pH=7$, differences in the saponification of the various compounds seem to lead to new types of interactions.

Fig. 5 shows the interaction $\{FaCel\}$ between myristic or stearic acid and cellulose. It is characterized by a small constant initial value which gradually goes up above $pH=6$, reaching its maximum around $pH=8$. The positive $\{FaCel\}$ base consumption indicates a preferential adsorption of fatty acid anions. There are only minor differences between the myristic and stearic curves.

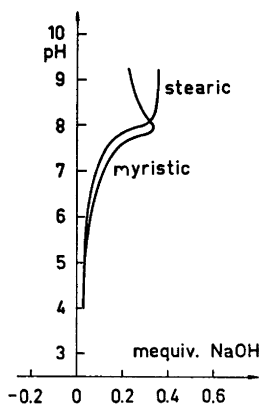


Fig. 5. Excess consumptions $\{FaCel\}$ for titrations of combinations with myristic or stearic acid based on the results from Figs. 1 and 3 or similar.

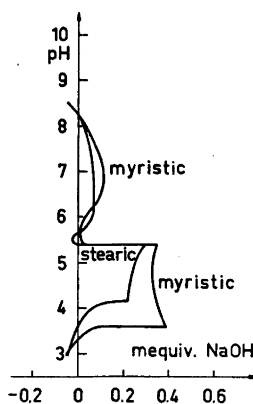


Fig. 6. Excess consumptions $\{FaCu\}$ for titrations of combinations with myristic or stearic acid as in Fig. 2 except that nitric acid was added to the blank instead of acetic acid and copper sulphate was added as copper salt instead of copper acetate.

All the results presented so far have had 0.5 mmole acetic acid as the blank and 0.6 mmole Cu-acetate as the copper salt. The curves shown in Figs. 6 and 7 are based on 0.5 mmole nitric acid as the blank and 0.4 mmole Cu-sulphate as the copper salt. In principle they represent the same {FaCu} and {FaCu-Cel} interactions as Figs. 2 and 4. Nevertheless one is struck by the marked difference between the two sets of corresponding interactions. Fig. 6 shows Cu-salts formed at much lower pH values than in Fig. 2 and at different points for myristic and stearic acid. Fig. 7 differs from Fig. 4 mainly in the relative position of the myristic and stearic curves. These differences indicate clearly that other anions than those from the fatty acids are involved in the Cu-fatty acid compounds.

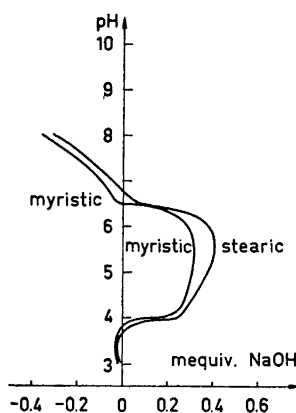


Fig. 7. Excess consumptions {FaCu-Cel} for titrations of combinations with myristic or stearic acid as in Fig. 4 except that nitric acid was added to the blank instead of acetic acid and copper sulphate was added as copper salt instead of copper acetate.

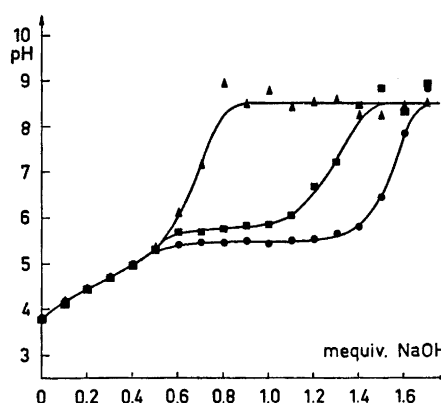


Fig. 8. Titration curves. \blacktriangle calcium chloride + cellulose (Ca+Cel). \bullet Myristic acid + calcium chloride + cellulose (Fa+Ca+Cel). \blacksquare Stearic acid + calcium chloride + cellulose (Fa+Ca+Cel). Concentrations as described in the text.

A supplementary investigation of the interaction {FaCa-Cel} between myristic or stearic acid and calcium ions in connection with added cellulose powder is finally shown in Fig. 8. Here there seem to be no further interactions with the cellulose moiety. One notices especially the well-defined precipitation of what the stoichiometry points out as very nearly pure calcium myristate at pH = 5.5. Both the Ca-salts formed are stable at least up to pH = 8.5.

DISCUSSION

Seen in relation to the reaction between cupric ions and fatty acids separated by paper chromatography, the most significant result of the present investigation is the demonstration of the possibility of Cu-fatty acid-cellulose interactions.

The titrations of the model mixtures with 5 mM acetic acid as the blank and 6 mM Cu-acetate as the copper salt represent the conditions during the colouring of chromatographic strips as far as the concentrations of the acetic anions and the cupric cations are concerned. They also cover the concentration of fatty acid anions in the copper bath since we are dealing with saturated solutions of the fatty acids throughout. The titrations shown differ from the colouring conditions in one important respect. A ratio of about 250 mg fatty acids to 10 g cellulose in the model mixture should be compared with an average chromatographic spot content of about 25 μg spread over an area of about 2 cm^2 of paper weighing 13 mg/cm^2 , thus: $25 \times 10^{-3}/26 \sim 1$ mg fatty acid to 1 g cellulose. This means that there is a relatively greater probability of fatty acid-cellulose interactions after chromatography than in the present investigation. Furthermore, the fatty acids are deposited within the spots in amounts that are two-dimensionally Gauss distributed, which leads to extremely low fatty acid/cellulose ratios along the spot boundaries.

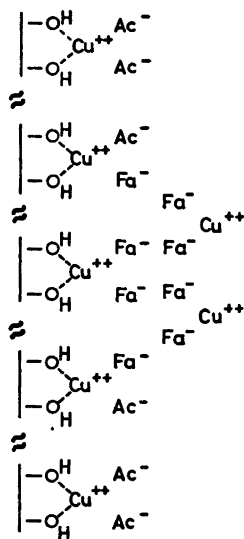


Fig. 9. Suggested spot model. Ac^- stands for acetate ions. Fa^- stands for fatty acid anions. The OH groups come from the cellulose moiety.

Our results suggest a spot model based on a chemically non-uniform fatty acid moiety after treatment in the copper acetate bath. The Cu-fatty acid-cellulose compound is of the type $\text{Cu}_x\text{Fa}_y\text{Ac}_z\text{Cel}$. Fig. 9 shows a general view of our model. Outside the fatty acid spot we have the simple Cu-acetic acid-cellulose complex $\text{Cu Ac}_2\text{ Cel}$: as we pass the spot boundary, we start meeting Cu FA Ac Cel complexes: the centre of the spot is dominated by $\text{Cu Fa}_2\text{ Cel}$, supplemented with copper soaps in amounts depending on the pH in the copper acetate bath. Referring to Fig. 2, pH must be above 5.4 before the precipitation of Cu-salts starts, and below 5.8 in order that further formation of basic Cu-acetate may be avoided.

After treatment in the copper bath the chromatograms are rinsed in flowing water at $\text{pH}=7.5$. Practical experience has shown that this washes out all excess copper unless pH has been 6.0 or higher during Cu-salt formation. An investigation of the copper cellulose interaction based on the results from Figs. 1 and 3 shows a gradual drop of $\{\text{CuCel}\}$ down to zero at about $\text{pH}=8$, which means that the effect must be rather weak. Viewing this in relation to the spot model presented above, we therefore conclude that Cu^{2+} can be washed out from $\text{Cu Ac}_2 \text{ Cel}$, but not from Cu Ac OH , and likewise that Cu^{2+} can be washed out from areas of Cu Fa Ac Cel , but not from areas of $\text{Cu Fa}_2 \text{ Cel}$ or from Cu Fa_2 . This would be in full accordance with the previous findings¹ concerning the advantage of keeping pH at 5.8 in the copper bath. Thereby one makes possible the formation of copper soaps with a stoichiometry more or less independent of the type of fatty acid, without on the one hand being disturbed by the precipitation of insoluble basic Cu-acetate or, on the other hand, risking the formation of too many Cu-fatty acid-cellulose compounds, which differ in composition according to the type of fatty acid and which can be washed out during rinsing. Our titration results show that the latter compounds will dominate altogether if excess acetic acid from the mobile phase makes the pH in the copper bath lower than 5.4.

The work of Kaufmann and Ahmad³ mentions that calcium ions in the rinsing water have a favourable effect on the retainment of Cu-fatty acid compounds. The results shown in Fig. 8 cannot explain this effect since they point to the formation of simple Ca-soaps independently of the cellulose moiety. We know from previous experience¹ that the washing out of fatty acid spots does not affect the acids themselves. The transformation of the Cu-fatty acid-cellulose compounds into the corresponding insoluble Ca-soaps could explain this. It might, however, also be due to direct fatty acid-cellulose interactions as shown in Fig. 5.

The presentation of a spot model based on the general formula $\text{Cu}_x \text{ Fa}_y \text{ Ac}_z \text{ Cel}$ is apparently in disagreement with the findings by Seher,² who claims the formation of pure copper soaps, Cu Fa_2 , on the paper after treatment in the copper acetate bath. His experiments are based on a polarographic determination of the copper contents of individual spots containing fatty acids in the range from 100 to 800 μg . The results show that Cu^{2+} has reacted with the various acids in stoichiometric amounts. In the present investigation the $\{\text{FaCu}\}$ interactions shown in Fig. 2 point to the formation of mixed copper soaps. This stresses the limitations of our aqueous titrations of model mixtures, which might well have led to the precipitation of pure Cu-soaps had the circumstances resembled practical colouring conditions to a greater degree. However, a washing out of Cu^{2+} due to the involvement of further acetic acid anions in various Cu-fatty acid-cellulose compounds would probably not have been noticed by Seher.² The relatively large spot contents used in his investigation, together with the fact that the fatty acids were pipetted directly onto the paper and thus not given a real change to equilibrate with the cellulose moiety, could have prevented any substantial Cu-fatty acid-cellulose interactions.

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