PAPER CHROMATOGRAPHY OF AMINO ACID-COPPER(II) COMPLEXES

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The separation of amino acid-copper complexes was studied by paper chromatography. The best results were obtained with phenol and o-cresol solvents. For developments the dried chromatograms were sprayed firstly with ruebanic acid (0,2 per cent ethanolic solution) and then with ninhydrine.

At simultaneous chromatography of different amino acid-copper complexes, no changing of the R_F values of each complexes could be observed. Consequently, the elimination of the disturbing interaction between amino acids can be achieved through complex formation with cupric ion. The chromatography of amino acid-copper complexes instead of the free amino acids is one of the most promising methods for the more exact paper chromatography of amino acids.

Many papers were published in connection with paper chromatography of amino acid metal complexes. The formation of amino acid-metal complexes was used for chromatographic determination of amino acids by WIELAND and FISCHER [1] (retention analysis) and for determination of cations by FLOOD [2] (chromatographic titration).

Recently, ERLENMEYER and his co-workers [3] and ERDEM [4] dealt with the retention analysis. The application of copper complexes was suggested especially for the determination of lysine by ALBANESE and LEIN [5]. The paper chromatographic properties of amino acid-copper complexes was studied by Miura, Tamaoki and Honda [6] in connection with the physico-chemical investigations of these complexes.

The present investigations were initiated by the experiences of paper chromatography of amino acids [7].

Namely, it is proved by numerous observations that the paper chromatographic behaviour (R_F value) of amino acids depends on the composition of the chromatographed solution too [7]—[3].

It was concluded that the reason of this phenomenon is the interaction of amino acids [14] which is the consequence of the presence both of the amino and the carboxyl groups.

In the formation of metal complexes both the amino and the carboxyl groups take part. Consequently, it may be expected that at the paper chromatography of amino acid-metal complexes in contrast to the free amino acids the above mentioned disturbing moment — which makes difficult the interpretation of chromatograms — does not appear. As our preliminary experiments [15] had verified this presumption, we carried out detailed investigations in this field.

Experimental

For preparation of amino acid-copper(II) complexes the aqueous, about neutral (p_H 8) solutions of amino acids and mixtures of amino acids were shaken with copper(II) carbonate under moderate heating. The reaction took place in 15 minutes quantitatively. Then the solution was filtered and 0,003 ml of it was dropped on the paper. The amount of amino acid dropped was about 6γ. For the experiments Schleicher Schüll 2043/a filter paper was used proving the best during the previous experiments. Among the common solvents used in our experiments the best were phenol and o-cresol saturated by water. All the experiments were carried out by the ascending method at 27° C.

Methods for development. There are three methods for development:

1. The development of amino acid component by ninhydrine, but in this case the complex must be previously decomposed because the complexes do not give reaction with ninhydrine. The ethylenediaminetetraacetic acid is very suitable for this purpose.

2. The development of metal component. For this purpose among the numerous reagents tested the rubeanic acid and 8-oxychinolin in 0,2 per cent ethanolic solution were found the most suitable.

3. The development both of the components. On the course of reaction with rubeanic acid or oxine amino acid liberates and can be developed with ninhydrine, too. This latter double development is the most sensitive.

With combination of the above mentioned methods, the accidental existence of free amino acids, or copper ion can be detected.

In Table I the R_F values of amino acid-copper(II) complexes are summarized with phenol and o-cresol as solvents.. For comparison there are shown the R_F values with lutidine as solvent from the paper of MIURA and his co-workers.

Chromatographying the mixtures of amino acid-copper(II) complexes the fluctuation of R_F values does not exceed the experimental errors ($\pm$ 0,02), consequently the disturbing interaction at the paper chromatography of free amino acids can be eliminated by the complex formation.

The possible formation of mixed complexes was investigated by chromatography of pairs of amino acid-copper(II) complexes. The existence of mixed complexes could not be detected in accordance with the results of MIURA and his co-workers.

Table 1.

Amino acid	R_F values		
	Phenol	o-cresol	Lutidine
glycine	0,59	0,36	0,33
alanine	0,84	0,67	0,49
alanine	0,69	0,35	—
serine	0,54	0,89	—
valine	0,93	0,95	0,64
threonine	0,76	0,46	—
lysine	0,54	0,00	0,05
proline	0,95	0,98	0,58
oxyproline	0,70	0,34	0,48
arginine	0,70	0,08	0,07
histidine	0,09	0,53	0,17
glutamic acid	0,20	0,00	0,45
glycil-glycine	0,04	0,16	—

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ON THE MEANING OF THE INFLUENCING FACTOR OF THE HOMOGENEOUS CHAIN CATALYSIS AND INHIBITION

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The authors investigated the role of the influencing factor of the homogeneous chain catalysis and inhibition in the kinetic behaviour of the process. They established a connection between the half period of the conversion and the influencing factor, and explained how the connection can be applied in the kinetic characterization of the influencing. The comparison of the results of theoretical considerations with the experiments led to good agreement.

In the recent years SZABÓ and his co-workers dealt with the investigation of the homogeneous chain catalysis and inhibition [1], [2]. They took for basis the scheme of the reaction — reduced by means of the restriction- and selection-principles of the four stage mechanism —

1. $A \rightarrow E+X$ or $A+A \rightarrow E+E,$ $\quad k_1$
2^{I}. $A+X \rightarrow E+X'$ $\quad k_2^{\mathrm{I}}$
4^{I}. $X+X \rightarrow E$ $\quad k_4^{\mathrm{I}}$

and its extension with the reaction steps opened by the addition of a reactant I — called influencing substance — to the reaction mixture. This reactant forms new radicals with the intermediates of the process according to the very fast equilibrium reaction

$$I+X \underset{k-}{\overset{k+}{\rightleftarrows}} IX = Y$$

and these radicals also participate in the set of the chain and rupturing reactions of the transformation in the way as follows

2^{II}. $A+Y \rightarrow E+Y',$ $\quad k_2^{\mathrm{II}}$
4^{II}. $X+Y \rightarrow E+I,$ $\quad k_4^{\mathrm{II}}$
4^{III}. $Y+Y \rightarrow E+2I,$ $\quad k_4^{\mathrm{III}}$

The rate equations of the process can be established based on the assumption that the concentrations of the intermediare radicals are very insignificant related to those of the reacting and influencing substances, from which the stationary rate equation of the reaction can be deduced by a rather simple calculation in the form

$$-\frac{dc}{dt}=k_1c^p+k_2^{\rm I}\sqrt{\frac{k_1}{k_4^{\rm I}}}\,c^{1+\frac{p}{2}} \tag{1_0}$$

for the non-influenced, and in the form

$$-\frac{dc}{dt}=k_1c^p+k_2^{\rm I}\sqrt{\frac{k_1}{k_4^{\rm I}}}\,F(I)\,c^{1+\frac{p}{2}} \tag{$1_{\rm I}$}$$

for the influenced process. Here $p=1,\ 2$ depending on whether the starting reaction is of first or second order, and

$$F(I)=\frac{1+\dfrac{k_2^{\rm II}}{k_2^{\rm I}}\dfrac{I}{K}}{\sqrt{1+2\dfrac{k_4^{\rm II}}{k_4^{\rm I}}\dfrac{I}{K}+\dfrac{k_4^{\rm III}}{k_4^{\rm I}}\dfrac{I^2}{K^2}}}=\frac{1+\beta I}{\sqrt{1+2\delta_1 I+\delta_2 I^2}}. \tag{2}$$

(where $K=k^-/k^+$ and I denotes the concentration of the reactant I too) is the so-called influencing factor of the reaction defined as the ratio of the stationary chain component of the rate of the influenced reaction to that of the non-influenced one. (It is to note that the scheme given above is not the only possibility of the influencing, because one or both of the rupturing reactions $4^{\rm I}$. and $4^{\rm III}$. may be of first order, but in this case a similar treatment is possible with an influencing factor of similar type.)

For the purpose of the following consideration the form $(1_{\rm I})$ of the stationary rate equation is to be taken for basis which includes also (1_0) — due to $F(I)_{I=0}=1$ —. This form of the rate equation of the process can be further simplified in a very significant set of the cases included. In fact, when the rate of the starting reaction is negligible in comparison with the chain component, the rate equation of the process reduces to

$$-\frac{dc}{dt}=k_2^{\rm I}\sqrt{\frac{k_1}{k_4^{\rm I}}}\,F(I)\,c^{1+\frac{p}{2}}. \tag{$3_{\rm I}$}$$

When the validity of such a rate equation, especially the negligibility of the starting reaction in the conversion can be established (the criterion of which is *e. g.* the 3/2-order of the reaction or the very shortness of the induction period and its insignificant participation in the conversion), then the kinetic characterization of the influencing can be carried out in an other way as mentioned and developed in the paper of SZABÓ and his co-workers.

In fact, equation $(3_{\rm I})$ can be integrated in the closed form

$$\frac{2}{p}\left(\frac{1}{c^{\frac{p}{2}}}-\frac{1}{c_0^{\frac{p}{2}}}\right)=k_2^{\rm I}\sqrt{\frac{k_1}{k_4^{\rm I}}}\,F(I)\cdot t \tag{4}$$

which enables us to express the half period of the reaction also by means of closed formulae instead of numerical integration of the system of the rate equations of the reaction. As a matter of fact, we get easily by the substitution $c=\frac{c_0}{2}$ in equation (4)

$$\frac{2}{p}\frac{1}{c_0^{\frac{p}{2}}}(\sqrt{2^p}-1)=k_2^1\sqrt{\frac{k_1}{k_4^1}}F(I)\,t^{(I)}_{1/2}, \tag{5_I}$$

the division of which by the corresponding equation of the non-influenced process ($F(I)=1$ for $I=0$)

$$\frac{2}{p}\frac{1}{c_0^{\frac{p}{2}}}(\sqrt{2^p}-1)=k_2^1\sqrt{\frac{k_1}{k_4^1}}\,t^{(0)}_{1/2} \tag{5_0}$$

gives simply

$$t^{(I)}_{1/2}=\frac{1}{F(I)}\,t^{(0)}_{1/2}, \tag{6}$$

i. e. a closed expression for the half period of the influenced process as a function of the concentration of the influencing substance.

For our purposes it is more adequate to write equation (6) for the expression of $F(I)$ by means of experimental data in the form

$$F(I)=\frac{t^{(0)}_{1/2}}{t^{(I)}_{1/2}}, \tag{6'}$$

because this expression of $F(I)$ can be very easily compared with the experiments.

The aim of this comparison is to bring into covering the theoretical expression of the influencing factor (2) by a suitable choice of its constant-parameters β, δ_1, δ_2 with that obtained from the experiments. For this end, based on the initial slope of the curve $F(I)$ at $I=0$

$$m=\left.\frac{\partial F}{\partial I}\right|_{I=0}=\beta-\delta_1 \tag{7}$$

and on the limiting value for $I\rightarrow\infty$

$$F_\infty=\lim_{I\to\infty}F(I)=\frac{\beta}{\sqrt{\delta_2}}, \tag{8}$$

equation (2) has been transformed into a quadratic equation relating β

$$(1+\beta I)^2=\left(\frac{t^{(0)}_{1/2}}{t^{(I)}_{1/2}}\right)^2\left(1+2(\beta-m)I+\frac{I^2}{F_\infty^2}\beta^2\right) \tag{9}$$

and this latter has been solved at several values of I, especially in the neighbourhood of the minimum of the influencing curve. This way of the determination of characteristic quantities β, δ_1 and δ_2 can be carried out practically only by means of trials, because the limiting value $F(\infty)$ could be estimated only instead of a precise determination. This fact, however, does not cause significant difficulty as the variation of $F(\infty)$ in the procedure outlined led in fairly few trials to the result desired.

To illustrate the above considerations we give at first the comparison of STAVELEY'S and HINSHELWOOD'S experiments [3] with the theoretical form of the influencing factor. These authors dealt in several papers with the thermal decomposition of different organic compounds [4]—[6], and in the case of thermal decomposition of propylaldehyde influenced by nitric oxyde they chose the reciprocal value of the half period of the reaction as a quantity proportional to the measure of the influencing. From their data the influencing factor of the reaction can be immediately determined, thus it seemes to be advantageous to bring their results into covering with the statements of the present paper.

We give therefore in Table I the results of their experiments, the change of the influencing factor established from the half periods of the reaction and the calculated influencing factor obtained with suitable values of the constants β, δ_1 and δ_2. These data are illustrated in Fig. 1 which contains also the results of the paper of SZABÓ and his co-workers. It can be stated that the latter calculations may be regarded as a more exact and more uniform interpretation of the experiments.

Similar comparison can be made also on the basis of the experiments carried out in the Institute of Inorganic and Analytical Chemistry of the University, Szeged. These experiments are directed to investigate the thermal decomposition of propylaldehyde influenced by nitric oxyde with the aim of a more detailed establishment of the kinetics under the purest possible

Table I

The influencing factor according to STAVELEY'S and HINSHELWOOD'S experiments and its representation by expression (2) with $\beta = 0{,}3{,}6$ $\delta_1 = 2{,}31$ and $\delta_2 = 0{,}001$.

NO mm	$F(\mathrm{I})_{\mathrm{exp.}}$	$F(\mathrm{I})_{\mathrm{calc.}}$
0,00	1,00	1,00
0,18	0,86	0,79
0,38	0,68	0,68
0,51	0,60	0,64
1,00	0,55	0,57
1,53	0,54	0,54
1,95	0,50	0,53
2,90	0,51	0,53
3,90	0,54	0,55
4,90	0,56	0,56
6,90	0,60	0,60
9,40	0,63	0,65
10,10	0,70	0,67
12,70	0,71	0,72
14,70	0,78	0,78

conditions to be able to bring into accordance the statements of several authors investigating the question — HINSHELWOOD *et al.*, BOYER, NICLAUSE and LETORT [7]—[9] — with our results. The experiments have established the whole kinetics of the decomposition and led to the conclusion that the above considerations can be applied in its treatment. According to these establishments the reaction is a chainpropagated one wherein the starting process can be neglected in the conversion and in which the order of the reaction varies about $^3/_2$. Moreover, the experiments have in a significant relation a considerably different face compared with STAVELEY's and HINSHELWOOD's results. This discrepancy can be attributed to the differences in the method applied. As a matter of fact the experiments of the authors have been carried out in pyrex glass vessel instead of quartz tube and by

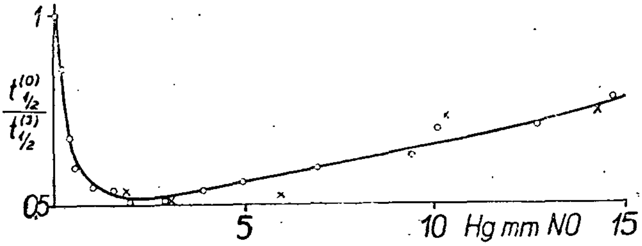

Fig. 1. The comparison of the calculated influencing factor with STAVELEY's and HINSHELWOOD's experimental results and with the calculations of Z. G. SZABÓ and co-workers. 0 STAVELEY's and HINSHELWOOD's results, — calculated curve, *x* values of Z. G. SZABÓ and co-workers

Table II

The influencing factor according to the experiments of the authors and its representation by expression (2) with $\beta = 0{,}65$, $\delta_1 = 2{,}75$ and $\delta_2 = 0{,}001$.

NO mm	$F(I)_{\text{exp.}}$	$F(I)_{\text{calc.}}$
0,00	1,00	1,00
0,20	0,77	0,78
0,38	0,68	0,70
0,65	0,62	0,66
0,90	0,60	0,64
1,00	0,63	0,64
1,30	0,64	0,64
1,60	0,68	0,65
3,80	0,80	0,74
7,70	0,93	0,91
16,40	1,24	1,22
21,50	1,40	1,37
27,50	1,55	1,52
60,00	2,12	2,15

glass valve technique, thus — owing to the latter — under considerably purer circumstances than available by application of lubricated stopcocks. These differences give rise to the discrepancies in the structure of the influencing factor established in our experiments, especially of the more significant catalysis at higher concentrations of the influencing nitric oxyde. In spite of these differences the characteristic quantities of the influencing do not differ to a high extent from those of STAVELEY's and HINSHELWOOD's experiments. The change of the influencing factor and its comparison with the calculated one is given in Table II and Fig. 2.

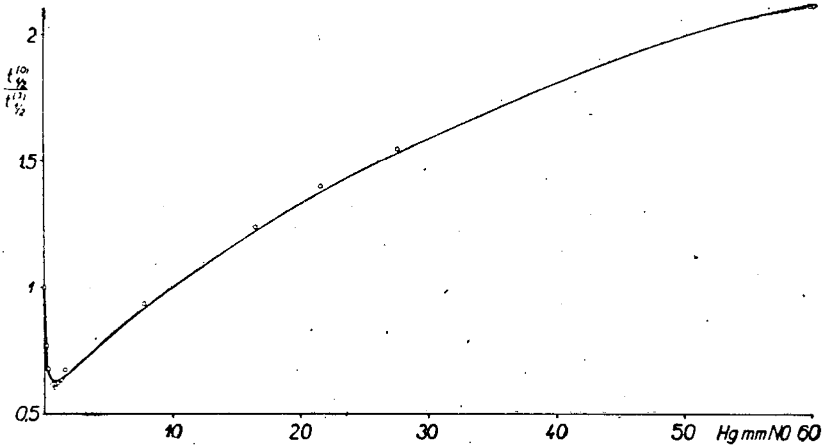

Fig. 2. The comparison of the calculated influencing factor with the experiments of the authors. O measured data, — calculated curve

The comparison shows the very good agreement of the experimental results and the calculated date, further the applicability of the considerations of the paper in treating the structure of the influencing factor. It must be mentioned here that this treatment realizes an idea of the preceding paper of SZABÓ and his associates, whereby the empirical determination of the influencing factor and its bringing into covering with the theoretical form (2) allows to draw some conclusions on the kinetic structure of the influencing. Further must be mentioned that the experiments resulted in the determination of several influencing curves too, and they are being continued to establish the influencing factor at more general conditions of initial pressure and temperature as well as for further substances. The authors hope that by the evaluation of these experiments new statements can be made on the kinetic characteristics and on the mechanism of the influencing by nitric oxyde.

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