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Instytut Chemii UMCS
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Kierownik: prof. dr hab. Kazimierz Sykut

Joanna BASAK, Kazimierz SYKUT

Studies in the Catalytic Effect of Histidine on Acetanilide Bromination by the Method of Diffusion Layer Titration on a Rotating Ring-disc Electrode

Badania katalitycznego wpływu histydyny na reakcje bromowania acetanilidu metodą miareczkowania w warstwie dyfuzyjnej na wirującej elektrodzie dysk-pierścień

Исследования каталитического влияния гистидина на реакцию бромирования ацетанилида методом титрования в диффузном слое на вращающемся дисковом электроде с кольцом

The paper presents new methods of applying diffusion layer titration on the rotating ring-disc electrode for investigations on homogeneous catalysis. The usefulness of this method for studies of this kind has been shown on the example of acetanilide bromination. From among a number of the compounds examined it has been found that histidine shows the catalytic activity. It is proved that the catalytically active form is the amphoteric ion of histidine. A mechanism of the catalytic process is proposed as well as its experimental verification.

Keywords: ring-disc electrode
homogeneous catalysis
diffusion layer titration

Untersuchungen über den katalytischen Einfluss von Histidin auf die Bromierungsreaktion von Acatanilid mittels Titrationsmethode in diffus-er Schicht an der rotierenden Ring-Scheiben-Elektrode

In der Arbeit wird eine neue Anwendung der Titrationsmethode in diffu-ser Schicht an der rotierenden Ring-Scheiben-Elektrode zur Untersuchung der homogenen Katalyse bechrieben.
Die Eignung dieser Methode für diesen Typ von Untersuchungen wird auf der Basis der Bromierung des Acetanilids gezeigt.
Aus der Reihe der untersuchten Verbindungen zeigte Histidin eine katalytische Wirkung. Es wurde nachgewiesen, dass die eigentliche katalytische Wirkung vom Zwitterion des Histidins ausgeht.
Ein Mechanismus des Katalyseprozesses wurde vorgeschlagen und experimentall verifiziert.

INTRODUCTION

The diffusion layer titration on a rotating ring-disc electrode has been designed by Albery and Hitchman [1]. The method makes it possible to investigate only those reactions in a solution in which one of the substrates may be electrogenerated. The method allows a determination of the rate constants of homogeneous reactions within the interval from 3.10^{-2} l mol^{-1} s^{-1} to 10^{7} l mol^{-1} s^{-1}. Traditionally accepted methods for the investigations of the kinetics of homogeneous reactions are characterized by comparable intervals.

Let us consider by way of example a homogeneous reaction described by the stoichiometric equation:

$$S \longrightarrow X \qquad (1)$$

and the equation of the rate

$$-\frac{d[S]}{dt} = k[S] \qquad (2)$$

and the reaction accelerated by the action of catalyst K:

$$S + K \longrightarrow X + K \qquad (3)$$

Then the dependence

$$-\frac{d[S]}{dt} = k_k [K][S] \qquad (4)$$

is valid.

Since $[K]$ = constans, the rate constant of the catalyzed reaction is described by equation

$$k = k_K [K] \qquad (5)$$

from which one may calculate k_K, that is, the rate constant for the catalyzed reaction at catalyst concentration equal to a unity. This value is called a catalytic constant.

EXPERIMENTAL

Measurements were taken by means of an apparatus of our own design [2,3] . The kinetics of acetanilide bromination was examined on a rotating ring-disc electrode [4] . It was additionally found out that at a rise in the pH of the solution, the rate of acetanilide bromination slightly decreased. Measurements of the rate constants were taken for solutions whose pH was within the range of about 0.5 - 5.3. The results obtained are shown in Fig. 1.

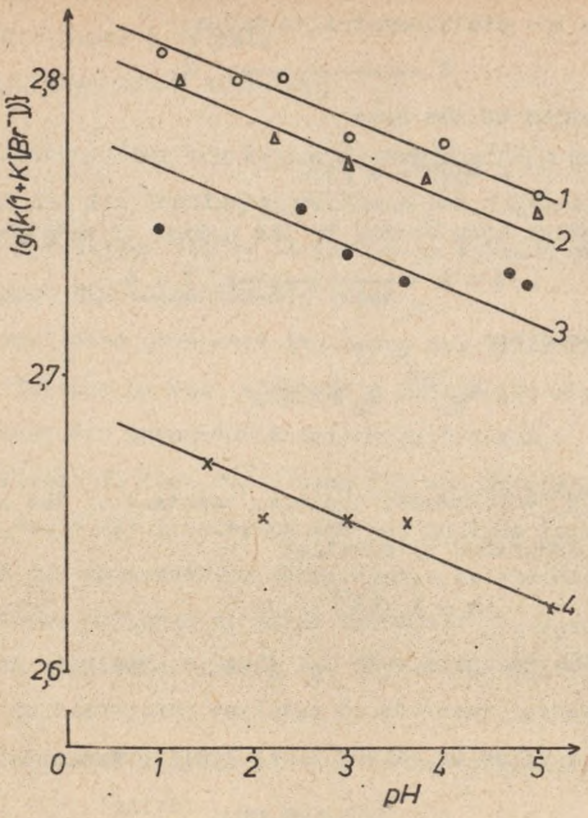

Fig. 1. Dependence of the logarithm of the rate constant of acetanilide bromination on pH. T = 298 K; μ = 1,05; $[Br^-]$ = 1 - 0.045, 2 - 0.100, 3 - 0.182, 4 - 0.363 mol l^{-1}

In view of the employed method of measurement, the substances whose catalytic effect was to be examined, had to satisfy the following conditions:

1 - they could not undergo the electrode process on the ring and the disc;

2 - they could not react with the titrating substance;

3 - they should not form stable compounds with other substances present in the solution.

The simplest test to ensure that conditions 1 and 2 are met is to check if an addition of these substances to the solution does not cause a change in the collection efficiency of the ring-disc electrode. On the other hand, condition 3 results from the properties of the system.

The total of about 100 compounds were checked, of which

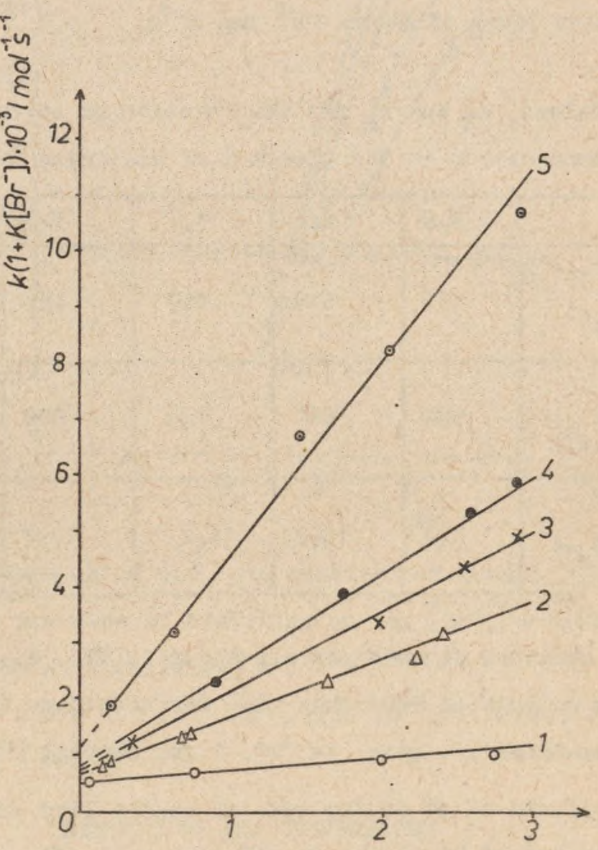

Fig. 2. Dependence of the rate constant of acetanilide bromination on the squared level of histidine concentration. $[Br^-]$= 0.182; μ = 1,05; T = 298 K; pH = 1 - 3.0, 2 - 3.6, 3 - 3.7, 4 - 3.9, 5 - 4.4

only about one quarter satisfied the conditions mentioned above. Only in the case of histidine a considerable acceleration of acetanilide bromination was achieved.

The measurements of the rate constants of acetanilide bromination in the presence of histidine was carried out in a solution $[Br] = 0.182$, $\mu = 1.05$, $T = 298$ K, and acetanilide concentration level of about 10^{-2} mol l^{-1}.

Tab. 1. Values, k_N and k_K for the reaction of acetanilide bromination in the presence of histidine

pH	3,0	3,6	3,7	3,9	4,4
$k(1+K[Br^-])$ $l.mol^{-1}s^{-1}$	551	539	539	537	529
k_N $l.mol^{-1}.s^{-1}$	500	660	740	800	1150
$k_K.10^{-10}$ $l^2.mol^{-2}s^{-1}$	2,3	10,3	14,2	17,2	34,6

The obtained dependences are shown in Fig. 2. Catalytic constants calculated from this plot for histidine in the process examined are given in Tab. 1 for several pH values.

The effects of pH on the rate of acetanilide bromination in the presence of histidine is illustrated in Fig. 3.

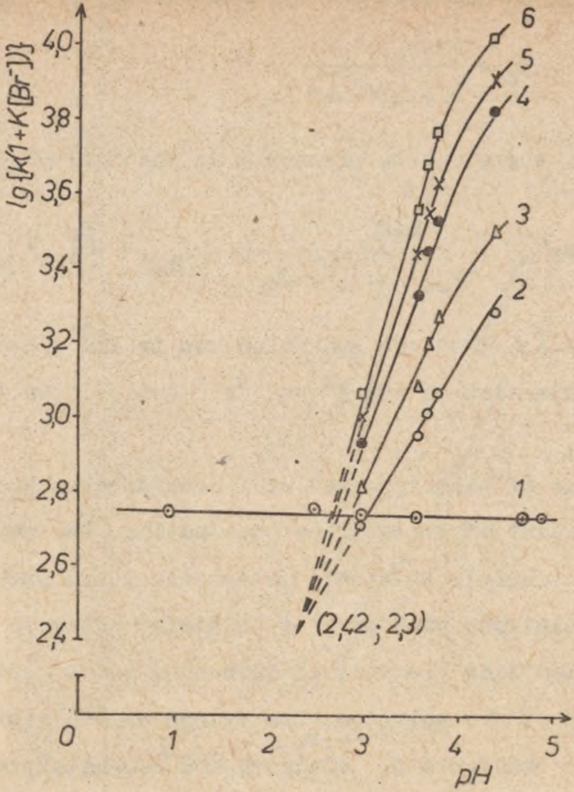

Fig. 3. Dependence of the rate constant of acetanilide bromination in the presence of histidine on pH. $[Br^-] = 0.182$; $\mu = 1.05$; $T = 298$ K; [histidine] = 1 - 0.
2 - 4.6 10^{-4}, 3 - 7.9 10^{-4}, 4 - 12.0 10^{-4}, 5 - 14.3 10^{-4}, 6 - 17.1 10^{-4} mol l^{-1}

DISCUSSION AND CONCLUSIONS

Within the pH interval of $\sim 0.5 < pH < 5.3$ the rate constant of acetanilide bromination linearly decreases with rising pH. Within this range the following equation is valid:

$$k\,(1+K\,[Br^-]) = k_N + k_{H_3O} + [H_3O^+] \qquad (6)$$

Hence, in this case we may speak of acidic catalysis.

The value of k_N is expressed by equation (3) :

$$k_N = \frac{k_1 k_3}{k_{-1}\left[Br^-\right] + \bar{k}_3} \qquad (7)$$

Equation (6) may therefore be presented in the following form:

$$k\ (1+K\left[Br^-\right]) = \frac{k_1 k_3}{k_{-1}\left[Br^-\right] + k_3} + k_{H_3O} + \left[H_3O^+\right] \qquad (8)$$

The catalytic constant of the hydronium ion in the process of acetanilide bromination is 609 $l^2\ mol^{-2}s^{-1}$ (calculated from Fig.)1 .

The presence of histidine causes a considerable increase in the rate constant of acetanilide bromination. The magnitude of this increase depends on histidine concentration level and the pH of the solution. With growing histidine concentration the rate of acetanilide bromination increases quicker, the higher is the pH of the solution. The values of the ratio between the rate constants of catalyzed and non-catalyzed (t) reactions at various histidine concentration levels and pH values are presented in Fig. 4.

In the case of homogeneous catalysis one does not observe such high accelerations as in that of heterogeneous catalysis. Most often the rate constants increase by several times. The obtained values of the rise in the rate constant of acetanilide bromination in the presence of histidine (t) , especially at higher pH values, are therefore quite considerable.

Histidine is an alkaline aminoacid. In a strongly acidic environment it occurs in the form of an ion with a positive charge. With increasing pH, there appears an amphoteric ion in the solution. When pH rises still further, an aminoacid

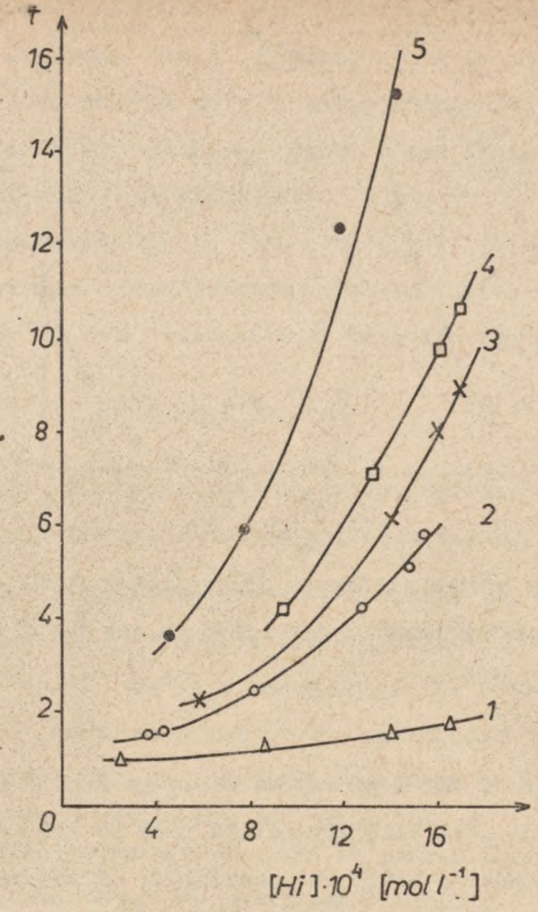

Fig. 4. Dependence of the value of the increase in the rate constant of acetanilide bromination on histidine concentration. $[Br^-] = 0.182$; $\mu = 1.05$; T = 298 K; pH = 1 - 3.0, 2 - 3.6, 3 - 3.7, 4 - 3.9, 5 - 4.4

anion occurs in the solution. The values of subsequent constants of histidine acidic dissociation are $pK_1 = 1.82$, $pK_2 = 6.08$, $pK_3 = 9.16$, respectively [5,6] . A plot of the intervals of the existence of particular histidine forms at various degrees of protonation up to pH = 6 is presented in Fig. 5.

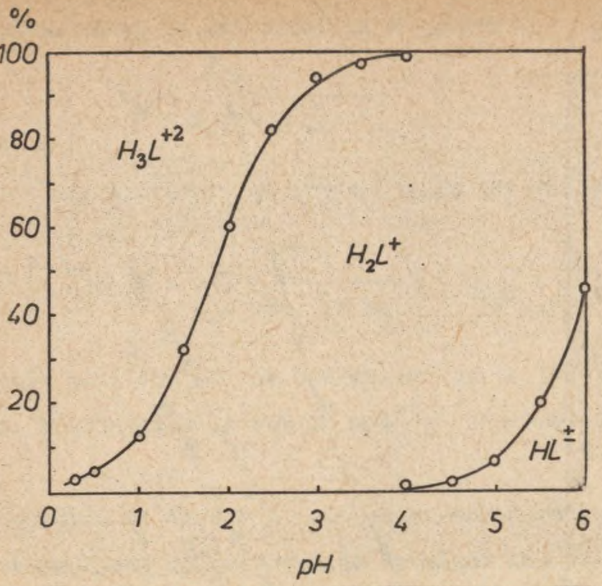

Fig. 5. Intervals of the existence of histidine forms at varying degrees of protonation depending on the pH of the solution

A comparison of the dependences shown in Fig. 3 and 5 indicates that the catalytically active form is the amphoteric ion if histidine which, under the conditions of the measurements, is likely to appear at somewhat lower pH value of the solution.

Apart from histidine other aminoacids which assume the form of an amphoteric ion already in acidic solution include glutamic acid, asparaginic acid, cysteine, ornithine (the latter two only at pH = 6.5).

The catalytic effect of cysteine on bromination process was not examined because its presence causes a lowering in the collection efficiency of the electrode. The remaining three aminoacids were examined but showed no catalytic effects.

Apart from histidine, other alkaline aminoacids include lysine and arginine. These compounds occur in the form of amphoteric ions only at pH < 7, and probobly for this reason no catalytic effect of these compounds was observed on the rate of acetanilide bromination.

The dependence of the rate constant of acetanilide bromination on histidine concentration level indicates that the kinetic equation of this reaction has the following form

$$-\frac{d[S]}{dt} = k_N + k_k \cdot [K_k]^{2} \qquad (9)$$

where: $[S]$ - denotes substrate concentration

$[K_K]$ - denotes catalyst concentration.

The value of k_N increases along with rising pH, while the rate constant (k) of the reaction slightly decreases.

The values of the rate constant of the reaction without catalyst (k), rate constant of the spontaneous reaction (read from Fig. 2), and catalytic constant of histidine for acetanilide bromination are set up in Tab. 1.

The obtained dependences make it possible to propose the following mechanism of the catalyzed reaction:

$$HL^{+} + Br_2 \xleftrightarrow{K_4} HL^{\pm} . Br_2 \qquad (10)$$

$$2\,(HL^{\pm} . Br_2) \xleftrightarrow{K_5} (HL^{\pm})_2Br^{+} + Br_3^{-} \qquad (11)$$

$$(HL^{\pm})_2Br^{+} + ArH \xrightarrow[\text{slowly}]{k_4} (ArHBr)^{+} + 2\,HL^{\pm} \qquad (12)$$

$$(ArHBr)^{+} \xrightarrow[\text{quickly}]{} ArBr + H^{+} \qquad (13)$$

where $HL^{\pm}$ denotes the amphoteric ion of histidine.

Reaction (11) is probable, especially in such a polar solvent as water. Ginn et al. [7] have provided by means of spectroscopy a proof of the existence of such a reaction in polar solutions for pyridine.

On the basis of this mechanism the rate constant equation has the following form:

$$v_1 = k_4 \left[(HL^{\pm})_2 Br^+\right]\left[ArH\right] \tag{14}$$

Taking into account the fact that

$$K_4 = \frac{\left[HL^{\pm} \cdot Br_2\right]}{\left[HL^{\pm}\right]\left[Br_2\right]_f} \tag{15}$$

and that

$$K_5 = \frac{\left[(HL^{\pm})_2 Br^+\right] Br_3^-}{\left[HL^{\pm} \cdot Br_2\right]} \tag{16}$$

and

$$K = \frac{\left[Br_3^-\right]}{\left[Br_2\right]_f \left[Br^-\right]} \tag{17}$$

where

$$\left[Br_2\right]_f = 1 + K\left[Br^-\right]$$

we obtain the following formula:

$$k_K\left(1 + K\left[Br^-\right]\right) = k_4 K_4^2 K_5 K^{-1}\left[HL^{\pm}\right]^2\left[Br^-\right]^{-1} \tag{18}$$

The overall rate constant of acetanilide bromination consists of the rate of non-catalyzed reaction (K_N) and the rate constant of the catalyzed reaction (k_K). The overall rate constant may thus be expressed by equation:

$$k_c\left(1 + K\left[Br^-\right]\right) = \frac{k_1 k_3}{k_{-1}\left[Br^-\right] + k_3} + k_{H_3O} + \left[H_3O^+\right] +$$

$$+ k_4 K_4^2 K_5 \frac{\left[HL^{\pm}\right]^2}{K\left[Br^-\right]} \tag{19}$$

The above indicates that the dependence curves of the rate constants of acetanilide bromination on the squared level of histidine concentration in solutions of a constant concentration of the Br^- ions, should be straight lines intersecting the y axis at the point corresponding to the value of the rate constant of the non-catalyzed reaction (k). Yet, in practice, these curves intersect the y axis at various points. This implies that the rate of the process is also affected by another factor which may be called k_6.

The concentration of the amphoteric ion of histidine depende on the pH of the solution and is expressed by equation

$$[HL^{\pm}] = \frac{10^4 K_1 K_2 [H_3O^+]}{K_1K_2K_3 + K_1K_2 [H_3O^+] + K_1 [H_3O^+]^2 - [H_3O^+]^3} [Hi] \qquad (20)$$

where: $[Hi]$ - denotes the summary concentration of histidine molecules at different degrees of protonation.

For small pH values, at which the amphoteric ion of histidine begins to appear, eqn. (20) may be expressed by

$$[HL^{\pm}] = \frac{10^4 K_2 [Hi]}{[H_3O^+]} \qquad (21)$$

The summary equation for the rate of acetanilide bromination in the presence of histidine has the form

$$k_c (1+K [Br^-]) = K_6 + \frac{k_1k_3}{k_{-1} [Br^-] + k_3} + k_{H_3O} + [H_3O^+] +$$

$$+ 10^8 k_4 K_2^2 K_4^2 K_5 K^{-1} [Br^-]^{-1} [H_3O^+]^{-2} [Hi]^2 \quad . \qquad (22)$$

The value of the rate constant of the spontaneous reaction (k_N) is expressed by equation

$$k_N = K_6 + \frac{k_1 k_3}{k_{-1}\left[Br^-\right] + k_3} + k_{H_3O} + \left[H_3O^+\right] \qquad (23)$$

The dependence of k_6 on pH is shown in Fig. 6. For pH = 3.2 the value of $k_6 = 0$.

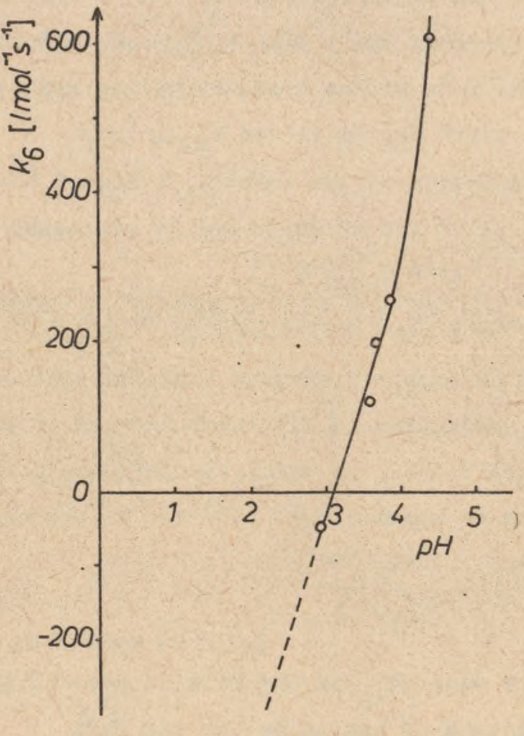

Fig. 6. Dependence of the constant k_c on pH

The catalytic constant is expressed by

$$k_k = 10^8 k_4 K_2^2 K_4^2 K_5 K^{-1} \left[Br^-\right]^{-1} \left[H_3O^+\right]^{-2} \qquad (24)$$

The logarithm of histidine catalytic constant is thus directly proportional to pH and inversely proportional to the concentration of the Br$^-$ ions. The dependence of the logarithm of the catalytic constant on pH in presented in Fig. 7.

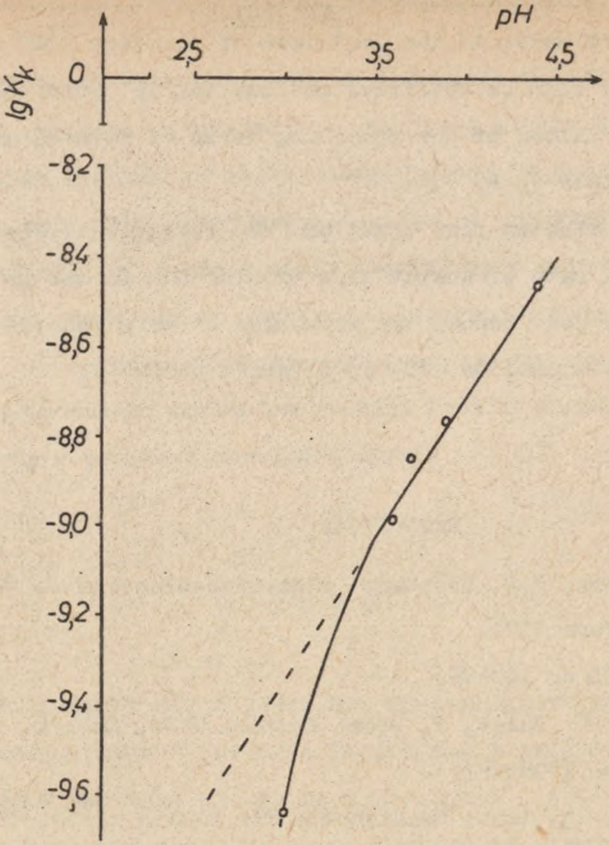

Fig. 7. Dependence of the logarithm of histidine catalytic constant on pH

The linear dependence of the catalytic constant logarithm on pH occurs only above pH = 3.5, while for its lower values the decrease in the catalytic constant is greater.

Fig. 2 and the summary equation (22) for the rate constant of acetanilide bromination in the presence of histidine indicate that the last term of this expression is equal to zero for pH = 2.3. It results from the fact that at this pH value practically no histidine occurs in the form of the amphoteric ion which acts catalytically.

The zero value of the last term of equation (22) for pH = 2.3 may also be confirmed mathematically (from the approximate values of the remaining terms of this equation read from plots 1, 6, 3).

This indicates that equation (22) fairly well describes the constant rate of acetanilide bromination in the presence of histidine and, hence, the mechanism of this reaction as described by equations (10-13) is quite probable.

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STRESZCZENIE

W pracy przedstawiono nowe zastosowanie metody miareczkowania w warstwie dyfuzyjnej na wirującej elektrodzie dysk - pierścień do badania katalizy homogenicznej. Przydatność tej metody do tego typu badań przedstawiono na przykładzie bromowania acetanilidu. Spośród wielu przebadanych substancji stwierdzono, że histydyna wykazuje właściwości katalityczne. Wykazano, że czynnym katalitycznie jest jon obojnaczy histydyny. Zaproponowano mechanizm procesu katalitycznego, który jest zgodny z wynikami doświadczalnymi.

РЕЗЮМЕ

Исследование каталитического влияния гистидина на реакцию бромирования ацеталинида методом титрования в диффузионном слое на вращающемся дисковом электроде с кольцом.

В работе представлено новое применение метода титрования в диффузном слое на вращающемся дисковом электроде с кольцом для исследования гомогенного катализа. Пригодность этого метода для данного рода исследований представлена на примере бромирования ацетанилида.

Установлено, что среди многих исследованных веществ каталитические свойства проявляет гистидин.

Доказано,что каталитически активным является амфион гистидина.

Предложено механизм каталитического процесса, согласующийся с результатами измерений.