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Investigation of Isotope Effects with the Aid of Kinetic and Catalytic Polarographic Currents

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Two types of chemical reactions, used to investigate isotope effects, are considered: reactions preceding the electrochemical stage proper and reactions accompanying this stage. The results of studies on the dehydration of formaldehyde are presented as examples of reactions of the first type and those on the oxidation of titanium(III) to titanium(IV) by hydroxylamine and the decomposition of hydrogen peroxide by catalase are presented as reactions of the second type. The results of a polarographic determination of the rate constants for reactions involving isotopic molecules, on the basis of which it is possible to determine the isotope effect for the corresponding reaction, are described together with the results of the determination of the equilibrium constants with the aid of both classical polarography and oscillographic polarography with rapid potential scanning. The equilibrium constants of the acid dissociation and dehydration reactions involving formaldehyde were determined and were employed to determine the corresponding thermodynamic isotope effects. It is shown that a direct isotope effect obtains in the acid dissociation reaction and the inverse effect obtains in the dehydration reaction.
The possibility of using the results of the study of isotope substitution in relation to catalytic currents in the cobalt-sodium nitrohydroxylaminate-cysteine sytem in an ammonia buffer for the determination of the isotopic composition of water is demonstrated. It is concluded that the polarographic method has many possibilities for the inverstigation of both kinetic and thermodynamic isotope effects and the isotope effects of the solvent.
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I. INTRODUCTION

The polarographic method makes it possible to determine the rate constant for any chemical reaction, provided that it is coupled with a charge transfer process. Taking into account the sequences in relation to the electrode process, it is possible to distinguish the following coupled reactions: reactions preceding and accompanying catalysis and subsequent reactions. A theoretical analysis has been carried out for all three cases and the tabulated values of special functions make it possible to calculate the corresponding rate constants from experimental data for kinetic waves (a general literature review may be found in the book by Heyrovský and Kuta[1]). If the polarographic method is used to investigate a chemical reaction involving isotopic molecules, atoms, or ions, it is possible to determine the rate constant for the reaction corresponding to the given isotopic species; furthermore, the kinetic isotope effect may be determined from the ratio of the observed rate constants.

The first study devoted to the use of polarographic kinetic currents for the determination of isotope effects was published by the present author in 1965.[2] The decomposition of hydrogen (deuterium) peroxide by catalase in the course of the reduction of oxygen at a mercury dropping electrode was investigated. Later kinetic waves were also used to study certain chemical reactions involving isotopic molecules in light and heavy water[3-5].

Polarographic currents of another type, namely catalytic currents associated with the discharge of hydrogen ions[6-11], were used to study certain special isotope effects[12,13]. Although in this case it is impossible to determine the reaction rate constants from the experimental data, nevertheless the observed isotope effects may be referred to kinetic phenomena, particularly in connection with protonation–deprotonation reactions. In this instance the isotope effect is expressed by the ratio of the numbers of hydrogen and deuterium ions participating in the protonation–deprotonation reaction[12].

This review presents the results obtained in the study of certain typical chemical reactions with participation of isotopic molecules. The classification unsually employed in polarographic studies is adhered to in the description of the results.

II. KINETIC CURRENTS

In order to determine isotope effects, studies were made of only two types of chemical reactions: reactions preceding charge transfer and reactions accompanying this process. However, the number of reactions investigated is very limited in precisely these two instances. The difficulties encountered in the synthesis or deuteriation of large and complex organic molecules may be regarded as the main cause of this situation.

1. PRECEDING REACTIONS

In the determination of isotope effects, we chose only the dehydration of formaldehyde as the preceding reaction[4]:

$$X_2C(OX)_2 \underset{k'}{\overset{k}{\rightleftarrows}} X_2C{=}O + X_2O, \qquad (1)$$

where X is the protium (H) or deuterium (D) and k and k' are the rate constants for the forward and reverse reactions. This process is most characteristic of preceding reactions and makes it possible to determine both thermodynamic and kinetic isotope effects. Among the components of reaction (1), only the aldehydic form X_2CO is

reducing, because the hydrated form [methylene glycol $X_2C(OX)_2$] is not a depolariser. In conformity with Eqn. (1), the dehydration reaction therefore precedes the electrochemical reduction of the aldehyde.

In the first study on the polarographic reduction of formaldehyde, Jahoda[14] found that formaldehyde is reduced to methanol via a two-electron process. This result was confirmed by other investigators[15-18].

The overall electrode reaction may be described as follows:

$$\underbrace{X_2C(OX)_2 \underset{k'}{\overset{k}{\rightleftarrows}} X_2CO}_{\text{chemical stage}} \underbrace{\overset{+2e}{\rightarrow} X_3COX}_{\text{electrochemical stage}} \quad . \qquad (2)$$

The chemical stage may undergo acid–base catalysis and for higher values of pX (pX > 13) account must be taken of the dissociation of the hydrated form. The study of reaction (2) requires the use of certain deuteriated substances. Clearly the most important factor is the preparation of $D_2C(OD)_2$; this substance has been synthesised[4] by the acid catalysis of the exchange of hydrogen bound to the carbon atom. For acid catalysis, solid paraformaldehyde was dissolved in 53% D_2SO_4. A concentrated solution of deuteroformaldehyde was obtained by distillation with dissolution of gaseous formaldehyde in 99.5% D_2O. This solution was redistilled and finally a solution with an overall formaldehyde concentration of 1 M was obtained. NaOD solutions were prepared by the reaction of metallic sodium with heavy water. Deuteriated boric acid D_3BO_3 was synthesised by dissolving boron trioxide in heavy water. The concentration of protons was determined by NMR and proved to be fairly low compared with that in the heavy water usually employed (99.5% D_2O).

(a) Thermodynamic Isotope Effects

In order to determine the rate constant for the chemical reaction preceding the electrode stage proper, it is necessary to know the equilibrium constant for the reaction[1]. As a result of the kinetic effect described by Eqn. (2), it is impossible to determine by classical polarography the equilibrium concentrations of the non-hydrated form of formaldehyde. It is therefore necessary to eliminate the contribution of the chemical reaction itself to the overall current. This problem was solved for the usual non-deuterated formaldehyde by Valenta[19], who used a single-cycle oscillographic polarographic method with a very high rate of change of potential. Using the same procedure, the present author and his coworkers[4] studied systems incorporating both protium and deuterium. i–E curves are presented in Fig. 1 for protium (*a*) and deuterium (*b*); evidently, for a rate of change of potential $s = 4$ V s^{-1} in light water, there is no maximum in the current, while in heavy water there is a small peak, indicating a contribution of diffusion to the reduction of non-hydrated formaldehyde. At a higher rate of change of potential, the diffusional current increases in both light and heavy water. It was found unexpectedly that the diffusion-controlled current in heavy water is reached for lower rates of change of potential than in light water. For very high rates of change of potential ($s = 320$ V s^{-1}, Fig. 1*c*), the maximum currents correspond to the analytical concentration of the free aldehyde form, since the kinetic component has been fully eliminated. Knowing the equilibrium concentration of formaldehyde, it is possible to calculate the equilibrium constant for its dehydration in both light and heavy water.

The concentration of the non-hydrated form in the bulk of the solution can be found from the results obtained by the single-cycle oscillographic polarographic method, treating the height of the diffusion-controlled current peak as a function of $s^{1/2}$ (Fig. 2). For low values of s, the variation of the current is of the kinetic type and the difference between the currents corresponding to systems with light and heavy water is small, while for high rates of change of potential, the difference becomes considerable; as we predicted, the currents are greater in heavy water than in normal water.

The corresponding concentrations of the free aldehydic form can be calculated from the slopes of the straight lines illustrated in Fig. 2. The calculation is possible if it is assumed that the reduction reaction has a

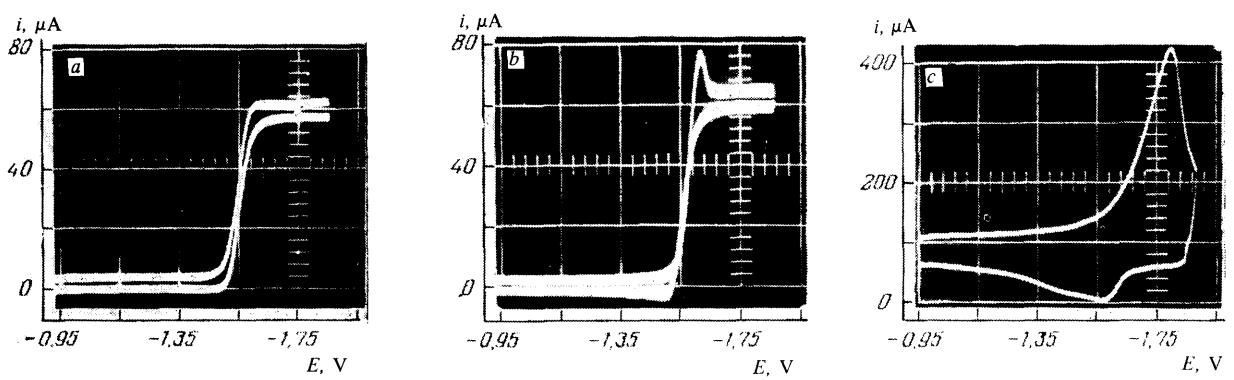


Figure 1. Oscillographic i – E curves for the reduction of formaldehyde at rates of change of potential of 4 V s^{-1} (*a* and *b*) and 320 V s^{-1} (*c*); initial potential −0.95 V; anode–saturated calomel electrode; triangular pulse. Solution: 0.03 M borax and 1.3 M KCl: *a*) 0.217 M H_2CO in H_2O; *b*) and *c*) 0.555 M D_2CO in D_2O. Characteristics of the capillary: $m = 0.227$ mg s^{-1}; $t_1 = 48$ s for $h = 46$ cm (m is the flow rate and t_1 is the drop time).

two-electron mechanism and the following equation derived by Delahay[20] is used:

$$i_p = 3.03 \cdot 10^5 n (\alpha n_\alpha)^{1/2} A D_b^{1/2} c_b s^{1/2},$$

where i_p is the peak current (A), n the number of electrons involved in the overall reaction, n_α the number of electrons involved in the rate-determining stage, α the transfer coefficient, A the drop area (cm^2), D_b the diffusion coefficient of the electrochemically active form ($cm^2\ s^{-1}$), c_b the concentration (mole cm^{-3}), and s the rate of change of potential ($V\ s^{-1}$). The slope, i.e. $\tan\varphi$, can be found from the data in Fig. 2:

$$\tan\varphi = \Delta i_p / \Delta s^{1/2},$$

so that the concentration of the non-hydrated form is

$$c_{b,X_2O} = (\tan\varphi)_X / 3.03 \cdot 10^5 n (\alpha n_\alpha)^{1/2} A D_b^{1/2}. \quad (3)$$

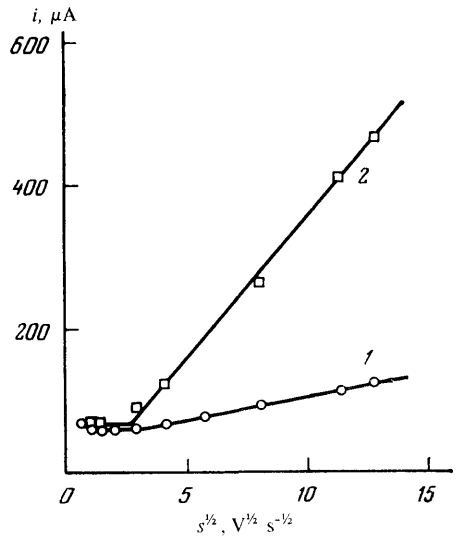


Figure 2. Dependence of the current peak on the rate of change of potential s. Solution: 0.03 M borax and 1.3 M KCl: 1) 0.217 M H_2CO in H_2O; 2) 0.555 M D_2CO in D_2O.

We obtain the following values from Fig. 2: $(\tan\varphi)_H = 6.3 \times 10^{-6}$ and $(\tan\varphi)_D = 41.3 \times 10^{-6}$. Other numerical values are as follows: $n = 2$; $\alpha = 1.48$;[19] $n_\alpha = 1$; $D_{b,H_2O} = 1.22 \times 10^{-5}\ cm^2\ s^{-1}$;[16] $D_{b,D_2O} = 1.09 \times 10^{-5}\ cm^2\ s^{-1}$;[21] $A = 2.67 \times 10^{-2}\ cm^2$ for the flow rate $m = 0.227$ mg s^{-1} and the drop time $t = 20$ s. On substituting these values in Eqn. (3), we obtain the formaldehyde concentrations $c_{b,H_2O} = 0.94 \times 10^{-1}$ M and $c_{b,D_2O} = 6.4 \times 10^{-4}$ M. The analytical concentrations are $c_{b,t,H_2O} = 0.217$ M and $c_{b,t,D_2O} = 0.555$ M. The equilibrium constants for the dehydration reactions then assume the following values: $K_{h,H_2O} = 4.33 \times 10^{-4}$ and $K_{h,D_2O} = 1.15 \times 10^{-3}$.

The thermodynamic isotope effect can be obtained from the ratio of these two constants:

$$K_{h,H_2O}/K_{h,D_2O} = 0.376\ .$$

Evidently this is in fact an inverse isotope effect, which shows that formaldehyde is more solvated in heavy water than in light water.

This result may be interpreted taking into account the difference between the energies of the chemical bonds of the isotopic molecules. The overall energy of the molecule can be expressed as the sum of the following quantities:

$$E = E_\nu + E_{R,e} + E_{R,i} + E_{Tr} + E_{R,\nu} + E_{El} + E_k,$$

where the subscripts denote vibrations (ν), external rotations (R, e), internal rotation, namely the vibration of a molecule bound to another molecule (R, i), translation (Tr), rotational-vibrational interaction (R, ν), electron shell (El), and atomic nucleus (k).

Analysis of atomic and molecular spectra permitted the conclusion that the difference between the electronic energies of protium and deuterium cannot be responsible for the isotope effect, which exceeds 0.5%. Consequently molecular vibrations may be regarded as the only possible cause of isotope effects[22]. Isotopic molecules have the same chemical formula but, owing to the difference between their masses, their vibration frequencies and "zero-point" energies also differ. A molecule containing a heavy isotope has a "zero-point" energy smaller than the molecule containing the light isotope. Clearly the energy required for the dissociation of each isotopic molecule is different and the difference depends both on the enthalpy and entropy of reaction.

In the formaldehyde dehydration reaction the chemical bond between oxygen and hydrogen is of greatest importance. In water and formaldehyde these bonds are different. In water each molecule has three vibration frequencies, while in formaldehyde their number is greater ($n > 3$). In a water molecule the frequencies ν_i are higher than in a formaldehyde molecule[23]. The zero-point energy is given by the formula

$$E^0 = \sum_{i=1}^{n} \frac{1}{2} h\nu_i.$$

It follows from experimental dehydration constants that

$$E^0(H_2O) - E^0[H_2C(OH)_2] > E^0(D_2O) - E^0[D_2C(OD)_2]$$

or

$$E^0[H_2C(OH)_2] - E^0[D_2C(OD)_2] < E^0(H_2O) - E^0(D_2O) = 3.55\ \text{kcal mole}^{-1},$$ (Ref. 21),

Eqn. (4) agrees well with the rule known from isotope chemistry, which states that the heavy isotope enters preferentially the chemical compound in which it is bound more strongly. This rule is applied on the hypothesis that the molecules are in their lowest vibrational energy level and that the entropy change is insignificant.

The hydrogen isotope effect at room temperature depends mainly on the type of chemical bond. The separation fractors, defined as $(D/H)_{compound}/(D/H)_{water}$, are close for compounds with similar bond types. For alcohols, the separation factor depends on the given molecule: whereas for methanol the separation factor is 1.0, for ethanol it is 1.1. In this connection, mention should be made of an important postulate, namely that the greatest isotope effect is noted when the isotopes are considered in compounds where they form a strong chemical bond with a free monatomic element.

We have already noted that, for high pX values, the degree of dissociation of hydrated formaldehyde molecules becomes considerable:

$$X_2C(OX)_2 \rightleftarrows X_2C(OX)O^- + X^+ \quad . \qquad (5)$$

It is possible to calculate the dissociation constant for this reaction, which occurs at high pX values, using the kinetic currents determined by the classical polarographic method.

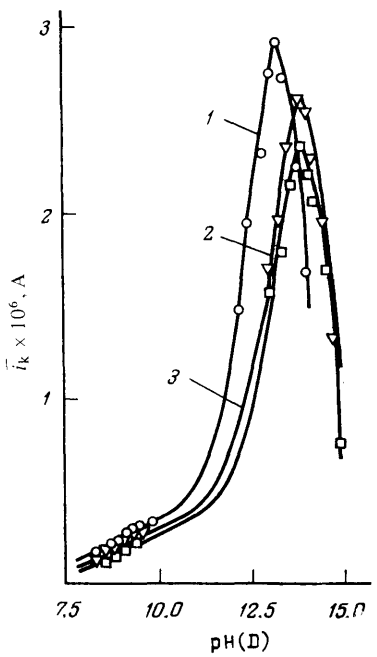


Figure 3. Average limiting current $\bar{i}_k$ as a function of pH(D) for the polarographic reduction of formaldehyde (4×10^{-3} M): 1) H_2CO in H_2O; 2) H_2CO in D_2O; 3) D_2CO in D_2O. A borate buffer was used in the pH range 7.5–10.0 and sodium hydroxide was used in the pH range 10.0–15.0; temperature 20°C.

Fig. 3 [4] illustrates the height of the kinetic waves $\bar{i}_k$ as a function of pH(D) for the reduction of formaldehyde in an alkaline medium. Evidently, in contrast to diffusion currents, kinetic currents are smaller in heavy water than in light water for H_2CO and smaller still for D_2CO. The validity of this result was tested by adding in succession increasing amounts of heavy water at a constant H_2CO concentration and at pH close to the value corresponding to the maximum kinetic current (Fig. 4). Extrapolation of the curve to 100% D_2O agrees, within the limits of experimental error, with the corresponding maximum value on curve *2* in Fig. 3.

In order to determine the dissociation constant K_a for reaction (5), we used the formula derived by Vesely and Brdička[16]:

$$K_a = \frac{[X^+]_M^{1/2}}{[X^+]_M^{1/2} + 2a} \quad ,$$

where $[X^+]_M$ is the H^+ or D^+ concentration for maximum $\bar{i}_k$ (Fig. 3) and a is a constant[16] defined by the formula

$$a = \frac{\bar{i}_k}{(\bar{i}_d)_{b,aq} - \bar{i}_k} [X^+]^{1/2}.$$

Here $(\bar{i}_d)_{b,aq}$ is the diffusion current corresponding to the overall constant concentration of the undissociated form $X_2C(OX)_2$. In the region of low pX values, where the degree of acid dissociation of formaldehyde is very low, the diffusion current corresponds to the analytical concentration of formaldehyde and may be calculated by the Ilkovič equation. Ultimately the following values were obtained[4]: $K_a = 2.5 \times 10^{-14}$ ($pK_a = 13.6$) for H_2CO in H_2O and $K_a = 10^{-14}$ ($pK_a = 14$) for H_2CO and D_2CO in D_2O. The thermodynamic isotope effect is defined as the ratio of these equilibrium constants:

$$K_a(H_2O)/K_a(D_2O) = 2.5 \quad . \qquad (6)$$

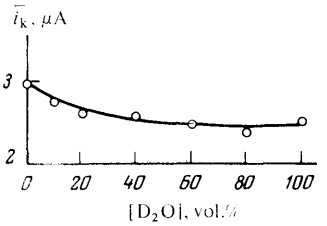


Figure 4. Influence of the content of D_2O on the kinetic current for the reduction of 4×10^{-3} M H_2CO in 0.15 M sodium hydroxide solution (which corresponds to the current maximum in Fig. 3).

In contrast to the dehydration reaction, in the dissociation of formaldehyde the isotope effect is direct, as expected. This result can be accounted for on the basis of the difference between the ionic products of light and heavy water: $pK_{H_2O} = 14.167$ at 20°C [24] and $pK_{D_2O} = 14.963$ at 20°C (calculated from the value of pK obtained by Abel et al.[25] at 25°C). In general the acid dissociation constants of OH, NH, SH, and CH in D_2O are smaller than in H_2O.[26] For weak acids, it has been found experimentally that the ratio K_H/K_D is in the range from 2.5 to 3.9. This agrees well with the value obtained using Eqn. (6) for X_2COXO^-.

(b) Kinetic Isotope Effects

On the basis of data obtained by classical polarography, it is also possible to determine the product of the equilibrium constant K_{h,X_2O} and the dehydration rate constant k_{i,X_2O}. K_{h,X_2O} was defined above and the corresponding rate constant may be found from the formula derived by Weber and Koutecký[27]:

$$\frac{\bar{i}_k}{\bar{i}_d} = \frac{0.886\,(K_h k_i t_1)^{1/2}}{1 + 0.886\,(K_h k_i t_1)^{1/2}} \quad ,$$

where t_1 is the drop time and $\bar{i}_d$ the calculated current determined by diffusion and corresponding to the analytical concentration of undissociated formaldehyde.

The overall rate constant is the sum of the partial rate constants for the dehydration of formaldehyde with participation of water, hydrogen ions, hydroxide ions, and acid (A) and alkaline (B) components:

$$k = k_{X_2O} + k_{X^+}[X^+] + k_{OX^-}[OX]^- + \sum k_{XA}[XA] + \sum k_B[B],$$

In a weakly alkaline borate buffer it is possible to neglect the contribution of the ions X^+ and acid components, and the rate constant is then obtained from a simpler formula:

$$k = k_{X_2O} + k_{X_2BO_3^-}[X_2BO_3^-] + k_{OX^-}[OX^-].$$

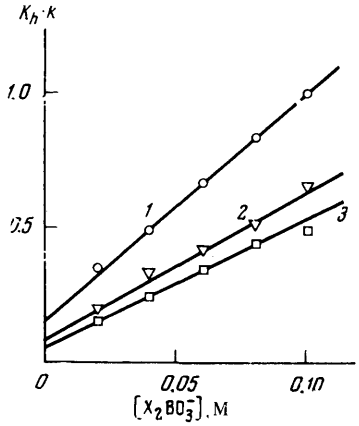


Figure 5. Variation of $K_h k$ with borate concentration. Formaldehyde concentration 4×10^{-3} M: 1) H_2CO in H_2O; 2) H_2CO in D_2O; 3) D_2CO in D_2O; temperature 20°C.

This expression shows that the dependence of $K_h k$ on $[X_2BO_3^-]$ is linear {[the term $k_{OX^-}[OX^-]$ can also be neglected (Fig. 5)]}. The products $K_h k_{X_2O}$ and $K_h k_{X_2BO_3^-}$ can be determined from the intercept made by the straight line on the ordinate axis and from its slope respectively. Their values at 20°C are listed below:

Solution	$10^4 \cdot K_h \cdot k_{X_2O}$, s^{-1}	$10^4 \cdot K_h \cdot k_{X_2BO_3^-}$, litre mole^{-1} s^{-1}	$K_h \cdot k_{OH^-}$, litre mole^{-1} s^{-1}
H_2CO in H_2O	15	8.5	0.279
H_2CO in D_2O	8	5.5	0.6666
D_2CO in D_2O	5.5	4.8	0.496

The corresponding rate constants calculated using the equilibrium constants for the dehydration reaction, determined previously, are:

X	$10^3 \cdot k_{X_2O}$, s^{-1}	$k_{X_2BO_3^-}$, litre mole^{-1} s^{-1}	$10^{-3} \cdot k_{OX^-}$, litre mole^{-1} s^{-1}
H	3.46	1.69	6.44
D	0.478	0.417	4.31

The kinetic isotope effect k_H/k_D assumes the following values at 20°C:

k_i	k_{X_2O}	$k_{X_2BO_3^-}$	k_{OX^-}
k_H/k_D	7.25	4.7	1.5

The maximum theoretically possible kinetic isotope effect for hydrogen isotopes at 25°C is 18,[28] but the experimental values proved to be smaller by a factor of 3. The results can be explained taking into account both the electrode reaction and the reaction medium. Among the three rate constants quoted above, that which corresponds to the solvent (k_{X_2O}) is determined by its nature. In the dehydration of formaldehyde the solvent behaves both as a catalyst and a reactant. A greater isotope effect is therefore to be expected for the rate constant k_{X_2O}, as has been confirmed experimentally. The OH or OD bonds are dissociated in the hydration–dehydration reaction. The maximum theoretical value of k_H/k_D expected for this process at 25°C is 10.6. In practice this value may be largely compensated by other isotope effects. For the given reaction and the constant k_{X_2O} under consideration, the main isotope effect is determined by the transfer of a hydrogen atom and a hydroxy-group from the bound state to water. In order to examine the process more completely, account must also be taken of the changes in the vibration frequency of the system, the anharmonicity of the phenomenon, and the liberation of liquid water molecules.

The isotope effect corresponding to the catalytic constant $k_{X_2BO_3^-}$ probably depends on the dissociation constant of boric acid in light and heavy water. There are no literature data for this constant in heavy water. The relatively high isotope effect is determined by the transfer of a hydrogen ion between the water molecule and the species undergoing reduction, i.e. the process constituting the stage which determines the rate of the entire reaction. This result may be compared with the isotope effect[4,5] in the alkaline catalysis of the ionisation of acetylacetonate[29].

For the constant k_{OX^-}, the isotope effect is determined by the dissociation and hence the strength of the alkali which catalyses the dehydration of formaldehyde. Fig. 3 shows that pD > pH for this process. The relatively small isotope effect in this instance can be accounted for by the two-step mechanism of this reaction. If there is no bond dissociation in the first step, which determines the rate of the entire reaction, and the system undergoes only a slight change, then only a secondary isotope effect of the overall reaction may be noted[30]. A more modern explanation of this mechanism was given by Westheimer[31], who believes that the high asymmetry of the transition state in this reaction, where the proton transfer is the rate-determining step, reduces the isotope effect to a mass coefficient, i.e. $k_H/k_D = 1.4$.

Evidently other chemical reactions preceding the electrode reaction proper can be investigated similarly. Among the substances containing a hydrated aldehyde group and behaving similarly to formaldehyde, mention may be made of the following: pyridine-2-aldehyde, pyridine-3-aldehyde, pyridine-4-aldehyde, glyoxalic acid, pyruvic acid, etc. All these substances have already been investigated in normal water[1]. There also exists a group of substances belonging to the same class, which have not been investigated quantitatively: acetaldehyde, propionaldehyde, glycolaldehyde, alloxan, tetroses, halogenoaldehydes, phthalic aldehyde, and mesoxalic, phenylpyruvic, trimethylpyruvic, and phenylglyoxalic acids.

Another preceding chemical reaction involves the recombination of anions with hydrogen ions. Phenylglyoxalic acid is reducible, while boric acid is non-reducible. The dissociation reactions of complex compounds, among which those of cadmium complexes with nitrilotriacetic acid have been most thoroughly investigated, are analogous. Monosaccharides (particularly aldoses) constitute an important class of organic substances which give rise to kinetic waves. In the study of the isotope effects in all the above instances the greatest

difficulty is the synthesis of the corresponding isotopic molecules.

2. REACTIONS ACCOMPANYING ELECTRON TRANSFER

In contrast to preceding reactions, which the rate constants for the reactions accompanying electron transfer are determined, there is no need to know the equilibrium constants. This facilitates significantly the experiment and classical polarographic data are sufficient for the calculation of the rate constant. In order to determine the kinetic isotope effects, the following reactions have been studied: the oxidation of titanium(III) to titanium(IV) by normal and deuteriated hydroxylamine; similar reactions involving the oxidation of iron(II) to iron(III) and of chromium(II) to chromium(III); decomposition of hydrogen and deuterium peroxide by catalase.

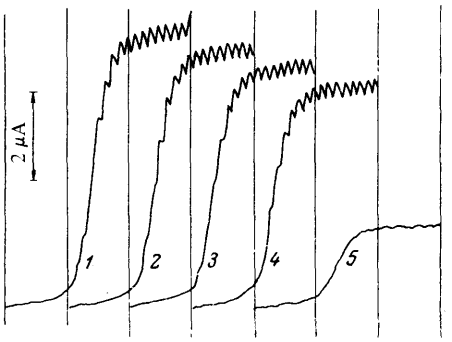


Figure 6. The influence of the concentration of heavy water on the titanium reduction wave in the presence of hydroxylamine; 10^{-3} M $TiCl_4$; 0.2 M $H_2C_2O_4$; 0.0855 M NH_2OHHCl; $[D_2O]$ in 10 ml of solution (ml): 1) 0; 2) 2; 3) 4; 4) 5.8; 5) without hydroxylamine; $t_1 = 3.8$ s; initial potential 0 V; scan along abscissa axis 200 mV; anode–saturated calomel electrode; temperature 24.5°C.

(a) Oxidation of Titanium(III) by Hydroxylamine[3]

In the presence of hydroxylamine the change in the limiting current accompanying the reduction of titanium(IV) to titanium(III) has a kinetic mechanism[32] and is due to the chemical oxidation of titanium(III) by hydroxylamine. The kinetics of this reaction were first studied polarographically by Blažek and Koryta[32]. The rate constant determined by the authors is $k_{25°C} = 42.1 \pm 1.5$ litre mole^{-1} s^{-1} and the activation energy is E_H 7.9 kcal mole^{-1}. Delahay et al.[33], who studied this reaction by the chronopotentiometric method, found that the rate constant is $k_{30°C} = 30$ litre mole^{-1} s^{-1}. Saveant and Vianello[34] obtained by the same method $k = 42.0 \pm 1.7$ litre mole^{-1} s^{-1}. Using an improved chronopotentiometric method, other investigators[35] found $k_{27°C} = 32.0 \pm 2$ litre mole^{-1} s^{-1}. Lingane and Christie[36] used a two-cycle chronopotentiometric method and found $k_{25°C} = 43.4 \pm 1.1$ litre mole^{-1} s^{-1} and the activation energy $E_H = 17.3$ kcal mole^{-1}.

In the study of the isotope effect in the above reactions all the substances were deuteriated in the following way: $TiCl_4$ was dissolved in D_2O and deuteriated oxalic acid was then added to the solution. The acid and hydroxylamine were deuteriated by isotope exchange in three successive recrystallisations from 99.9% D_2O. As the heavy water gradually substitutes normal water, the height of the kinetic wave decreases appreciably (Fig. 6). Fig. 7 shows that the dependence of the ratio i_l/i_d on the amount of D_2O is linear (i_l is the limiting current of titanium in the presence of hydroxylamine and i_d is the diffusion current in the absence of hydroxylamine). The kinetic current increases with hydroxylamine concentration and with the ratio i_l/i_d (Fig. 8). It follows from the data in Fig. 8 that, at the same hydroxylamine concentration, the current ratio is always smaller in the deuterium system than in the hydrogen system, but the overall form of the curves is the same in the two systems.

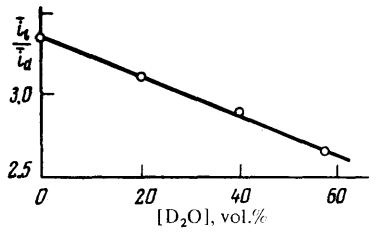


Figure 7. Influence of the concentration of deuterium on the current ratio at 24.5°C.

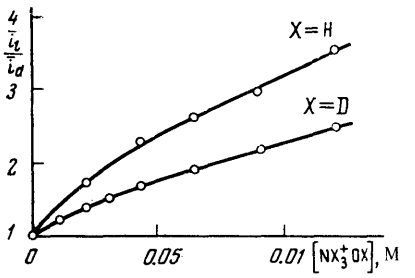


Figure 8. Dependence of the current ratio on hydroxylamine concentration at 23°C.

The kinetic current is determined by the rate of reaction, which explains the rapid increase of its limiting value with increase of temperature. The extent of this change depends on the activation energy, which can be calculated with the aid of the familiar Arrhenius equation:

$$\lg k = -0.43E/RT + \text{const.}$$

Fig. 9 presents plots of $\lg k$ against $1/T$ for the oxidation of titanium(III) to titanium(IV) by hydroxylamine in the hydrogen and deuterium systems. The following activation energies were obtained from the slopes of these straight lines: $E_H = 16.7$ kcal mole^{-1} and $E_D = 17.6$ kcal mole^{-1}; these values are comparable to those published

by Lingane and Christie[36]. It is noteworthy that the activation energies for the hydrogen and deuterium systems are very close, which can be used to elucidate the reaction mechanism.

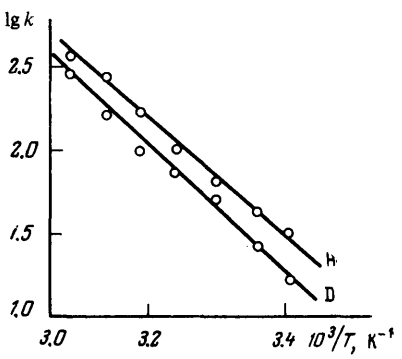


Figure 9. Dependence of $\lg k$ on $1/T$ for the oxidation of titanium(III) by hydroxylamine in the hydrogen and deuterium systems.

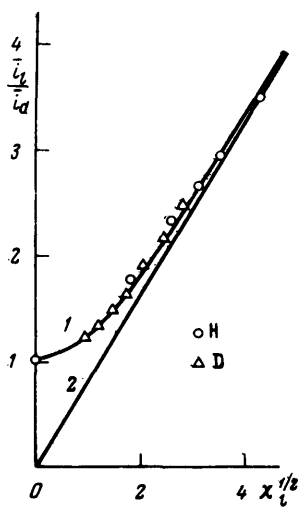


Figure 10. Variation of the ratio $\bar{i}_l/\bar{i}_d$ with $\chi_l^{1/2}$ (curve *1*) and the asymptotic solution (curve *2*); $t_1 = 3.8$ s; $k^H = 41.3 \pm 1.5$ litre mole^{-1} s^{-1}; $k^D = 18.2 \pm 0.8$ litre mole^{-1} s^{-1}; temperature 23 °C.

The following reactions are involved in the given process:

$$Ti^{IV} + e \rightarrow Ti^{III} \text{ (electrochemical stage)}, \quad (7)$$

$$Ti^{III} + NX_3^+OX \rightarrow Ti^{IV} + X_2O + \dot{N}X_2 \text{ (chemical stage)}. \quad (8)$$

The current corresponding to reaction (7) increases owing to reaction (8), which determines the kinetic nature of the limiting current. Since the measurements are made in the presence of an excess of hydroxylamine, reaction (8) is pseudounimolecular and Koutecký's method of calculation[37] may be used to determine the rate constants for the chemical reactions accompanying the electrode process. The calculation shows that the ratio i_l/i_d is a function of χ_l defined by the equation

$$\chi_l = k_X [NX_3^+OX] t_l.$$

The dependence of the ratio i_l/i_d on $\chi_l^{1/2}$ is illustrated in Fig. 10; the experimental data[3] for the oxidation of titanium(III) to titanium(IV) by normal and deuteriated hydroxylamine were used. The calculation yielded the following rate constants at 23 °C: $k_H = 41.3 \pm 1.5$ litre mole^{-1} s^{-1} and $k_D = 18.2 \pm 0.8$ litre mole^{-1} s^{-1}. The kinetic isotope effect is

$$k_H/k_D = 41.3/18.2 = 2.26.$$

The isotope effect in the activation energy for reaction (8), found from the experimental values already indicated, is

$$E_H/E_D = 16.7/17.6 = 0.946.$$

On the basis of the Arrhenius equation in the form

$$k_X = A_X \exp(-E_X/RT)$$

it is possible to determine three isotope effects: in the rate constant, in the activation energy, and in the frequency factor A_X. The first two isotope effects can be calculated from the experimental values of k_X and E_X. The isotope effect associated with the frequency factor A_X can be obtained from the formula

$$\frac{A_H}{A_D} = \frac{k_H}{k_D} \frac{\exp(-E_D/RT)}{\exp(-E_H/RT)} = 1.95.$$

Its value shows that the difference between the rate constants for the reactions of isotopic molecules depends little on the difference between their activation energies. At the same time it is seen that the lower collision frequency in the presence of the heavy isotope leads to a decrease of the rate constant.

The isotope effects in the oxidation of iron(II) to iron(III) and of chromium(II) to chromium(III) by hydroxylamine[5] were determined by the same methods as for the oxidation of titanium(III) to titanium(IV).[3] The reaction medium consisted of an alkaline solution of triethanolamine, which forms complexes with iron and chromium. The test substances were deuteriated as in the case of titanium[3]. Triethanolamine was recrystallised several times from heavy water (clearly only the mobile hydrogen was deuteriated).

The following values were obtained for the oxidation of iron(II): $k_H = 205 \pm 25$ and $k_D = 151 \pm 25$ litre mole^{-1} s^{-1}. Hence the kinetic isotope effect is $k_H/k_D = 205/151 = 1.36$; the activation energies are $E_H = 14.15$ and $E_D = 15.55$ kcal mole^{-1} and the corresponding isotope effect is $E_H/E_D = 14.15/15.55 = 0.909$. This value does not compensate the isotope effect in the rate constant. If the isotope effect in the frequency factor is calculated from the Arrhenius equation, we obtain $A_H/A_D = 1.35$.

The situation is the same in the oxidation of chromium(II) to chromium(III) by hydroxylamine. The observed values are $k_H = 274 \pm 29$ litre mole^{-1} s^{-1} and $k_D = 168 \pm 17$ litre mole^{-1} s^{-1}, $E_D = 12.4$ kcal mole^{-1}, $E_H = 9.74$ kcal mole^{-1}, and the corresponding isotope effects are $k_H/k_D = 274/168 = 163$, $E_H/E_D = 9.74/12.4 = 0.78$, and $A_H/A_D = 1.62$. Hence it follows that the collision frequency plays a decisive role in the kinetic isotope effects for reactions of this type.

(b) Decomposition of Hydrogen Peroxide by a Catalase

In the study of isotope effects the decomposition of hydrogen peroxide by catalase was also examined as a reaction accompanying the electrode process. Polarographic investigation of haemoproteins[38,39] showed that these substances react with the hydrogen peroxide formed in the cathodic reduction of oxygen. One group of haemoproteins reacts with hydrogen peroxide and the resulting compounds can be reduced at potentials intermediate between those of the oxygen and hydrogen peroxide waves. The following haemoproteins belong to this group: haemin[39], haemoglobin, and cytochrome[28]. Catalase differs from haemoproteins by its behaviour[38], because it decomposes hydrogen peroxide. In this process the height of the first oxygen wave increases, while that of the hydrogen peroxide wave diminishes and ultimately the two waves merge into one.

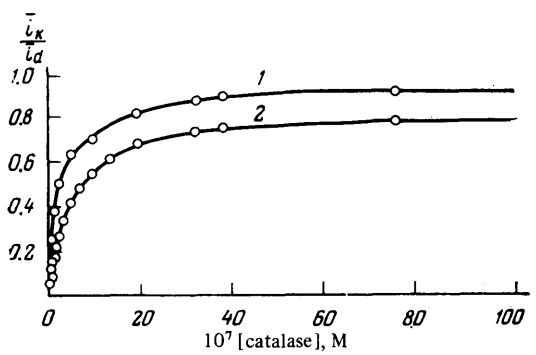


Figure 11. Dependence of the ratio $\bar{i}_k/\bar{i}_d$ on the catalase concentration in Sorensen phosphate buffers in H_2O (curve *1*) and D_2O (curve *2*); pH = pD = 7.2; t_1 = 2.56 s.

Koutecký et al.[40] determined polarographically the rate constants for the decomposition of hydrogen peroxide by catalase. The theoretical interpretation was based on the hypothesis that catalase decomposes into two components the hydrogen peroxide formed during the reduction of molecular oxygen: water and oxygen[2,40]:

$$O_2 + 2X^+ + 2e \rightarrow X_2O_2 \xrightarrow{\text{catalase}} X_2O + \frac{1}{2}O_2 \quad .$$

The study of this reaction in heavy water requires the deuteriation of the test substances[2]. The authors synthesised the deuteriated compounds in the following way. Deuterophosphoric acid was obtained by dissolving P_2O_5 in heavy water and NaOD was synthesised from metallic sodium and heavy water. Since it is clearly impossible to obtain fully deuteriated catalase, we recrystallised catalase twice from a buffer with deuteriated phosphate. In this procedure only the mobile hydrogen was deuteriated. The dependence of the ratio i_k/i_d on the catalase concentration in the range from zero to 1.05×10^{-5} M is illustrated in Fig. 11. Evidently curves having a similar overall shape are obtained in both systems, although the process is slower in D_2O.

The displacement of the second oxygen wave until it coincides with the first wave in the presence of catalase can be accounted for by two causes[41]: the decomposition of hydrogen peroxide by catalase results in the formation of molecular oxygen, which is reduced in the region of the first wave; the hydrogen peroxide formed reacts on the electrode surface with catalase and the resulting compound is then reduced at the electrode, also in the region of the first wave. Koutecký et al.[40] believe that the first mechanism is more probable, since a better agreement can be obtained in this way between the polarographic and oscillographic polarographic methods.

According to this mechanism, the kinetic current $\bar{i}_k$ is determined by the rate of formation of oxygen during the catalytic decomposition of hydrogen peroxide and deuterium peroxide. If the rate constant for the bimolecular decomposition of the peroxides by catalase is denoted by k_X, one can write

$$-\frac{d[X_2O_2]}{dt} = k_X\,[\text{cat}]\,[X_2O_2]_0,$$

and the rate of formation of oxygen is then expressed in the form

$$\frac{d[O_2]}{dt} = \frac{1}{2}k_X\,[\text{cat}]\,[X_2O_2]_0 \,.$$

The rate constant may be calculated from the formula[40]

$$\frac{i_l}{i_d} = 1 + \frac{\chi_l}{7.42 + 1.25\chi_l}, \qquad (9)$$

where

$$\bar{i}_l = \bar{i}_d + \bar{i}_k \text{ and } \chi_l = k_X\,[\text{cat}]\,t_1 \,.$$

The rate constant is then given by

$$k_X = \frac{7.42}{t_1\,[\text{cat}]} \cdot \frac{\bar{i}_k}{(\bar{i}_d - 1.25\bar{i}_k)} \,.$$

Fig. 12 illustrates the dependence of the ratio $\bar{i}_k/\bar{i}_d$ on χ_l. The average rate constants found are[2] $k_H = 1.72\times10^7$ litre mole^{-1} s^{-1} and $k_D = 5.29\times10^6$ litre mole^{-1} s^{-1}. There is good agreement between our value of k_H and that found by Koutecký et al.[40]

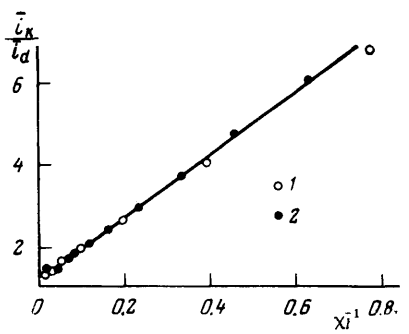


Figure 12. Variation of the ratio $\bar{i}_k/\bar{i}_d$ with χ_l^{-1}; continuous line—calculation by Eqn. (9). The abscissae of the experimental points were calculated from the following values: 1) H_2O_2, $k^H = 1.72\times10^7$ litre mole^{-1} s^{-1}; 2) D_2O_2, $k^D = 5.29\times10^6$ litre mole^{-1} s^{-1}; t_1 = 2.56 s.

Finally the ratio of these constants $k_H/k_D = 1.72\times10^7/0.529\times10^7 = 3.25$ characterises the kinetic isotope effect in the decomposition of hydrogen peroxide by catalase.

III. CATALYTIC CURRENTS

Although the catalytic discharge of hydrogen ions is a very important electrochemical process, the explanation of its mechanism remained for a long time unsatisfactory. The cobalt–nitrohydroxylaminate anion system was the first in which the formation of a cobalt–dinitrohydroxylamine complex, undergoing discharge [oxidation?] and having catalytic properties, was investigated[41]. On the basis of the model of such a dischargeable catalytic complex, mathematical equations were derived for the catalytic waves and a very satisfactory agreement was obtained between the calculated and experimental values[42]. The equations for the catalytic waves in the cobalt–cycloserine system[7,8] were derived on the assumption that the active principle in the catalytic discharge of hydrogen ions is a cobalt complex with a zero charge. In the mechanisms with and without discharge account was taken only of the number of neutralisable protons in a single molecule of the catalyst-complex. Thus the catalytic current depends on the catalyst concentration at the given potential and on the catalytic activity, expressed in terms of the number of protons discharged by a single molecule. This mechanism proved to be useful for the prediction of conditions under which it is possible to obtain the catalytic double wave characteristic of proteins. The equation for the catalytic nth degree wave[6–8,42] suggests that, when the same solution contains two complexes with different catalytic properties, a double catalytic wave will be recorded. The theory was tested experimentally for solutions containing cobalt(III), sodium nitrohydroxylaminate, the cysteine in an ammonia buffer[43].

It is well known that, for substances exhibiting catalytic properties in the presence of cobalt (giving rise to the so called Brdička waves), the height of the hydrogen wave is independent of pressure, in contrast to the nitrohydroxylaminate ion. This behaviour is characteristic of kinetic waves, i.e. waves determined by the chemical reactions accompanying the electron transfer process. However, the study of the cobalt–cycloserine system[7,8] showed that the independence of the overall catalytic hydrogen wave of pressure is due to the superposition of two catalytic hydrogen waves of different types: of the type of the "pre-sodium" wave and the wave determined by diffusion induced by the discharge of hydrogen ions under the influence of the cobalt–cycloserine complex. The resolution of the complex wave into the above components can be achieved at very low cobalt concentrations. At high cobalt concentrations the decrease of the first wave as the height of the mercury column is varied is compensated by the increase of the second. Ultimately the resulting overall hydrogen wave is independent of pressure. Clearly such independence is not evidence for the kinetic nature of the polarographic waves of the Brdička type. Any mechanisms in which such complex waves are assumed to be of the kinetic type must therefore be completely excluded from the interpretation of the Brdička type hydrogen ion discharge process.

Another important type of the catalytic discharge of hydrogen ions was discovered recently[9–11] in acetate buffer solutions containing nickel and selenocysteine[9,10] or normal cysteine[11] as the catalyst in weakly acid or neutral media. Such discharge of hydrogen proceeds in the region of the catalytic pre-wave with a half-wave potential of approximately –1.14 V relative to the saturated calomel electrode (sat.c.e.).[9] It was shown[9] that in this case catalysis is due to the uncharged partly protonated form of the nickel complex of selenocysteine or cysteine.

If the half-wave potential is adopted as a criterion in the classification of the hydrogen ion discharge processes at a mercury dropping electrode, the following waves may be indicated: (1) the catalytic hydrogen pre-wave at –1.14 V;[9] (2) the normal hydrogen wave at –1.67 V;[1,9] (3) the Brdička hydrogen wave between –1.67 and –1.70 V;[6,11] (4) the catalytic pre-sodium hydrogen wave at –190 V (sat.c.e.);[6] (5) secondary discharge of hydrogen ions beginning at –2.63 V (relative to a stationary mercury electrode in 10^{-3} M KCl, after the subtraction of the ohmic potential drop[44]). Thus the discharge of hydrogen ions at a mercury dropping electrode is a fairly complex process.

Since the catalytic discharge of hydrogen ions involves protonation–deprotonation reactions and more or less protonated catalyst molecules in a free or bound form, it follows that the catalytic hydrogen waves should be influenced by the substitution of hydrogen ions by deuterium ions. On the other hand, as shown in the first part of this review, the dissociation constants of normal and heavy water differ. Despite the complexity of the processes occurring in the catalytic discharge of hydrogen, it is possible to characterise the overall isotope effect (I) by the ratio of the numbers of protons (N_H) and deuterons (N_D) discharged by one molecule of the catalytic complex[12]:

$$I = N_H/N_D;$$

N_H and N_D can be calculated from the formulae for the catalytic waves[8,42].

When a non-dischargeable complex or a complex with a zero charge is taken into account in the mechanism of the catalytic discharge of hydrogen ions, the equation for the waves assumes the following form[8]:

$$\bar{i}_t = \frac{\bar{i}_{d,\mathrm{Co(II)}}}{1+\exp[(\varepsilon-\varepsilon_{1/2,\mathrm{Co(II)}})\,\alpha_l n_l F/RT]} + \frac{\bar{i}_{l,\mathrm{H(0)}}}{1+\exp[(\varepsilon-\varepsilon_{1/2,\mathrm{H(0)}})\,\alpha_3 n_3 F/RT]} + \frac{\bar{i}_{l,pv}}{1+\exp[(\varepsilon-\varepsilon_{1/2,pv})\,\alpha_4 n_4 F/RT]} + \bar{i}_{el}\,, \tag{10}$$

where $\bar{i}_t$ is the overall average current, $\bar{i}_{d,\mathrm{Co(II)}}$ the Co(II) diffusion current, ϵ the electrode potential, $\epsilon_{1/2,\mathrm{Co(II)}}$ the experimental half-wave potential of the cobalt wave, α_l the effective transfer coefficient, n_l the valence of the electrode reaction, F the Faraday, and T the absolute temperature; $\bar{i}_{l,\mathrm{H(0)}}$ has the value stated above, but refers to the uncharged zero-valent cobalt complex catalysing the discharge of hydrogen ions. The subscript pv refers to the catalytic pre-sodium wave and $\bar{i}_{el}$ is the current corresponding to the discharge of the ions of the indifferent electrolyte.

The quantity N_X can be calculated from $il,\mathrm{H(0)}$ by Eqn. (10), provided that the concentration of the uncharged complex on the electrode surface is known.

When the complex undergoing discharge is involved in the mechanism of the catalysis of the discharge of hydrogen ions, the equation for the wave can be written in the following form[2,4,6,8]:

$$\bar{i}_t = \frac{\bar{i}_{l,\mathrm{Co}}}{1+\exp[(\varepsilon-\varepsilon_{1/2,\mathrm{Co}})\,\alpha_l n_l F/RT]} + \frac{\bar{i}_{l,k}}{1+\exp[(\varepsilon-\varepsilon_{1/2,k})\,\alpha_2 n_2 F/RT]} + 1 - \frac{1}{1+\exp[(\varepsilon-\varepsilon_{1/2,k})\,\alpha_2 n_2 F/RT]} \cdot \frac{N\,(\bar{i}_{l,k}/n_2)}{1+\exp[(\varepsilon-\varepsilon_{1/2,c})\,\alpha_3 n_3 F/RT]} + \frac{i_{l,pv}}{1+\exp[(\varepsilon-\varepsilon_{1/2,pv})\,\alpha_4 n_4 F/RT]} + X\cdot c\cdot\exp[-(\varepsilon-\varepsilon_{1/2,el})\,\alpha_{el} n_{el}\cdot F/RT]\,.$$

In this equation the subscripts k and 2 correspond to the complex being reduced and c and 3 correspond to the wave associated with the discharge of hydrogen ions by

the catalytic complex; X is the constant of the Ilkovič equation and c is the concentration of the indifferent electrolyte. In practice only the third term of Eqn. (11) need be taken into account in the calculation of N_X. This type of calculation has been carried out for hydrogen[42] and deuterium[12] and for cobalt–cysteine solutions in an ammonia buffer.

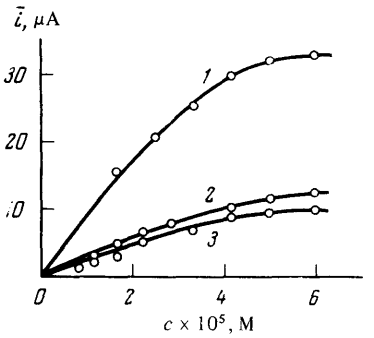


Figure 13. Variation of the height of the catalytic wave with cysteine concentration; 10^{-3} M $CoCl_2$; 10^{-1} M NX_4Cl–NX_4OX: 1) X = H; 2) and 3) X = D; 1) in the presence of 0.01% of gelatin; 3) in the presence of deuteriated gelatin.

A marked isotope effect was shown in the cobalt–cysteine system for waves corresponding to the catalytic discharge of hydrogen ions. As in previous instances, deuteriated compounds are necessary for the investigation of the isotope effect. ND_4OD was synthesised by the reaction

$$Mg_3N_2 + 8D_2O = 2ND_4OD + 3Mg(OD)_2.$$

ND_3 was then separated from $Mg(OD)_2$ by a partition and by bubbling with gases. DCl was synthesised by the reaction

$$SiCl_4 + 4D_2O = 4DCl + Si(OD)_4.$$

$DCl.D_2O$ was separated from $Si(OD)_4$ by the distillation of an azeotropic mixture. (Both ND_4OD and DCl are also available commercially.) The substance ND_4Cl was obtained either by the reaction of ND_4OD with DCl or by isotope exchange between N–H and D_2O with repeated recrystallisations. The mobile hydrogen in gelatin was deuteriated by repeated dissolution in D_2O and evaporation. Cysteine for the deuterium system was recrystallised three times from solution in D_2O.

Waves corresponding to the catalytic discharge of deuterium ions can be obtained with the aid of these deuteriated compounds and compared with the corresponding hydrogen waves. Fig. 13 illustrates the experimental variation of the current maximum on the catalytic waves as a function of cysteine concentration. The currents are much smaller in the D_2O system. $N_H = 740$ and $N_D = 238$ were calculated from the experimental data. Hence

$$I = N_H/N_D = 740/238 = 3.2.$$

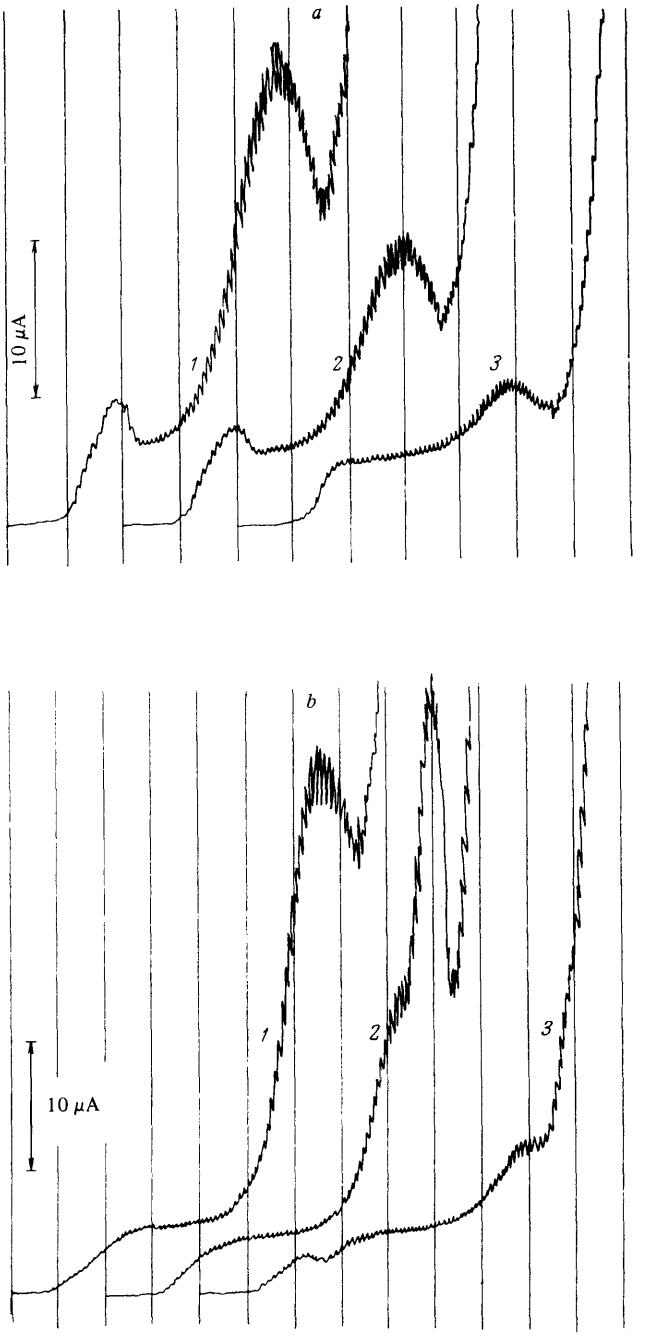


Figure 14. The catalytic isotope effect in the presence of 10^{-3} M Co^{2+} (*a*) and Ni^{2+} (*b*); 5×10^{-5} M (*a*) and 3×10^{-4} M (*b*) cysteine solutions; 0.1 M NX_4Cl; 0.1 M NX_4OX (X = H or D); initial potential −0.8 V (*a*) and −0.4 V (*b*); scan along the abscissa axis 200 mV; anode–saturated calomel electrode; deuterium content (vol.%): 1) 0; 2) 60; 3) 100.

The isotope effect is even greater in solutions containing cobalt or nickel (Fig. 14) in the absence of gelatin. A polarographic determination of the D_2O content in

enriched systems has been suggested as an application of this very intense isotope effect[13]. Calibration plots for the cobalt system are illustrated in Fig. 15*a* and that for the nickel system in Fig. 15*b*. A straight line is obtained when the maximum catalytic current is plotted against the deuterium content. The accuracy of the analytical determinations is equal to the accuracy of the polarographic method (1–3%). An advantage of the method is the simplicity of the procedure and apparatus.

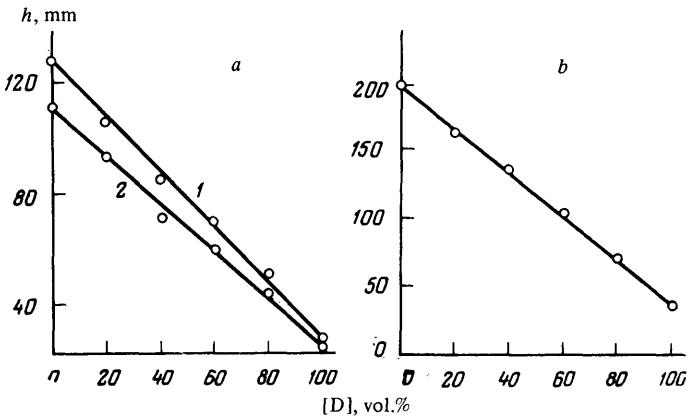


Figure 15. Variation of the height of the catalytic wave (*h*) with the deuterium content in the presence of cobalt (*a*) and nickel (*b*). Solutions the same as Fig. 14: 1*a*) first recording; 2*a*) second recording.

The study of isotope substitution in relation to catalytic currents constitutes an important procedure for the determination of the mechanism of this main type of polarographic currents. The intense isotope effect demonstrates the fundamental role of protonation–deprotonation processes and the importance of this stage, which determines the rate of the overall reaction. Isotope substitution also demonstrates the importance of hydrogen catalysis when other criteria are unsuitable for the determination of the nature of the polarographic wave.

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In conclusion one must emphasise that, despite the complexity of the phenomenon under consideration, the study of the isotope effect with the aid of kinetic and catalytic waves gives rise to much scope for the understanding of the mechanisms of many reactions and at the same time demonstrates the advantages and possibilities of the polarographic method in this field.

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