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On the Isomerism of Hydroxyurea

V. Polarographic Behaviour of the Lower-Melting Isomer

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The lower-melting hydroxyurea (m.p. 72 °C) has been found to produce a single, well-developed and reproducible diffusion-controlled cathodic wave at approximately −1.5 V (S.C.E., 25° C) in neutral, buffered and unbuffered solutions, 0.1 to 0.2 *N* in potassium ion. In acidic buffers the hydrogen wave interferes, in alkaline solution, pH> 9, the wave is independently developed, but it decreases rapidly with time due to instability of the substance. The variation of wave height with concentration is linear over the range 0.5 to 5 millimoles/l. The observed influence of the pH upon the half-wave potential together with the shape and the height of the wave seem to indicate that hydroxyurea (m.p. 72°) is reduced according to two distinctly different mechanisms, a pH-dependent one in acid solution and a pH-independent one in neutral and alkaline solution, both being irreversible two-electron processes.

A concurrent polarographic examination of the hydroxylammonium ion confirmed that the lower melting hydroxyurea is not identical with hydroxylammonium cyanate.

Since the higher melting isomer (140° C) proved to be polarographically inactive in the same voltage region, the method developed in this paper is an adequate analytical tool for a study of the formation and interconversion of the isomerides.

The polarographic method, when applicable to an organic compound, not only constitutes an analytical tool of high sensitivity, but very often valuable structural inferences may be drawn from the experimental data. Both potentialities are relevant to the studies dealt with in the present series, the former with a view to a kinetic examination of interconversions of the isomers. This will be the subject of a separate paper. In the following a general polarographic examination of the hydroxyureas is presented and the requisite analytical conditions are reported.

Introductory experiments revealed that in neutral unbuffered electrolyte solution (0.5 *M* lithium chloride) the lower melting isomer (m.p. 72°C) produced a cathodic wave at approximately —1.5 V *vs* S.C.E. ("saturated calomel electrode"), whereas the higher melting isomer (m.p. 140° C) gave neither a

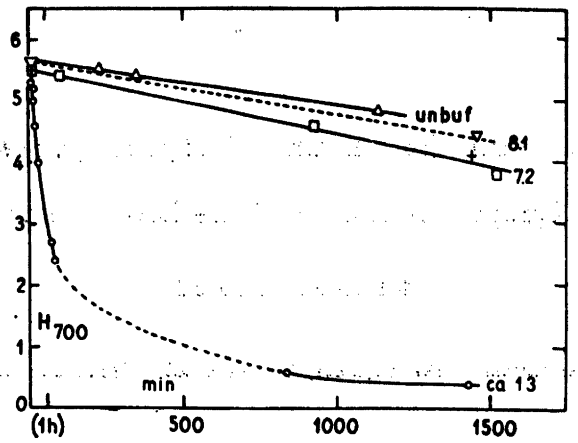

Fig. 1. Lability of hydroxyurea (m. p. 72°) in aqueous solution at room temperature. Wave-height (1 unit = 2.33 microamp.) against minutes from dissolution of the solid. △ 10.0 mM *stock solution (diluted with 4 volumes of buffer at pH 7.2 immediately before recording of the polarogram). 2.0* mM *solutions in buffer pH 7.2 (□), 8.1 (▽), 9.2 (+) and in 0.1* N *potassium hydroxide (○) respectively.*

cathodic nor an anodic wave in the voltage region + 0.2 to —2.0 V (S.C.E.) covered by the experiments. A typical polarographic wave of the lower melting hydroxyurea is given in Fig. 2, pH 7.2, upper curve. The current-voltage curve produced by the higher melting isomer under similar conditions was identical with that of the supporting electrolyte (lower curve) even at high galvanometer sensitivities. This proves the polarographic inactivity of the higher melting isomer and also indicates that it does not contain detectible amounts of the lower melting isomer, when prepared according to the previously reported directions [1].

EXPERIMENTS ON THE STABILITY OF THE LOWER-MELTING HYDROXYUREA IN SOLUTION

Although no quantitative information is available, the lower melting isomer is known to be relatively labile [2, 3, 4] and at the same time the polarographic method of analysis is a rather slow one, the recording of a polarographic wave requiring 2—3 minutes at least. It was therefore imperative to ensure that the error due to the drop in concentration during the time of recording did not seriously exceed the normal error in quantitative polarographic analysis.

Some important results are summarized in Fig. 1, giving the wave height against a time basis. A freshly prepared 0.01 *M* aqueous stock solution was kept at room temperature (20° ± 1°). 1 ml samples were withdrawn at intervals, mixed with 4 ml buffer solution at pH 7.2 (see the last section) and polarographed after appropriate deaeration. The experimental points (△) indicate a drop of approximately 1 % during the first hour from the preparation of the stock solution. This is within the probable error in wave heights (2—4 %).

The first analytical dilution in the above experiment was set aside at room temperature and measured from time to time, Fig. 1, □. It is seen that the stability of this 2.0 m*M* solution in buffer at pH 7.2 is very nearly the same as that of the unbuffered stock solution. Similar orienting experiments were carried out at pH 8.1 (▽) and 9.2 (+) with similar results. The deviations between these curves are probably not significant.

In strongly alkaline medium the situation is different. If a freshly prepared 2.0 m*M* solution in 0.1 *M* potassium hydroxide (pH *ca.* 13) is kept at room temperature, the wave height (Fig. 1, ○) is reduced to approximately half the initial value in the course of the first hour.

In acid solution the lower melting hydroxyurea is perfectly stable. A 10.0 m*M* solution in 0.52 *N* hydrochloric acid was stored in a closed vessel at room temperature. 0.2 ml samples were withdrawn at intervals, diluted with 5 ml buffer to give a final pH of ca. 7 and polarographed under the standard conditions specified in the last section ($s = 150$, $t = 3.2$ sec.). The results are given in Table 1, τ being the time elapsed from the dissolution of hydroxyurea in the hydrochloric acid and until the recording of the polarogram. There is no detectible drop in wave height i_d over a period of 72 hours, on the contrary there appears to be a slight increase. A satisfactory explanation of this phenomenon has not so far been found.

A rough test was made of the lability of neutral solutions at higher temperatures. 5 ml samples of a 10.0 m*M* solution in 0.5 *M* lithium chloride were exposed to heat as indicated in Table 2 and subsequently polarographed at room temperature. *H* is the wave height in arbitrary units.

Table 1.

Stability of hydroxyurea (m.p. 72°) in acidic medium

τ min.	i_d μamp.
5	3.0
13	3.0
25	3.0
37	3.0
79	3.1
96	3.1
116	3.1
153	3.1
205	3.1
280	3.1
365	3.3
4 320 (72 h)	3.2

Table 2.

Thermolability of hydroxyurea (m.p. 72° C) in aqueous solution

	H
not heated	34
heated to the boiling point, quickly cooled	32
boiled for 2 minutes	17
boiled for 5 minutes	5
boiled for 10 minutes	0

The hydroxyurea wave rapidly decreases. After 10 minutes boiling no trace of the original substance is detectible even when applying high galvanometer sensitivity.

To summarize the above results it has been shown that acidic aqueous solutions of the lower melting hydroxyurea are perfectly stable at room

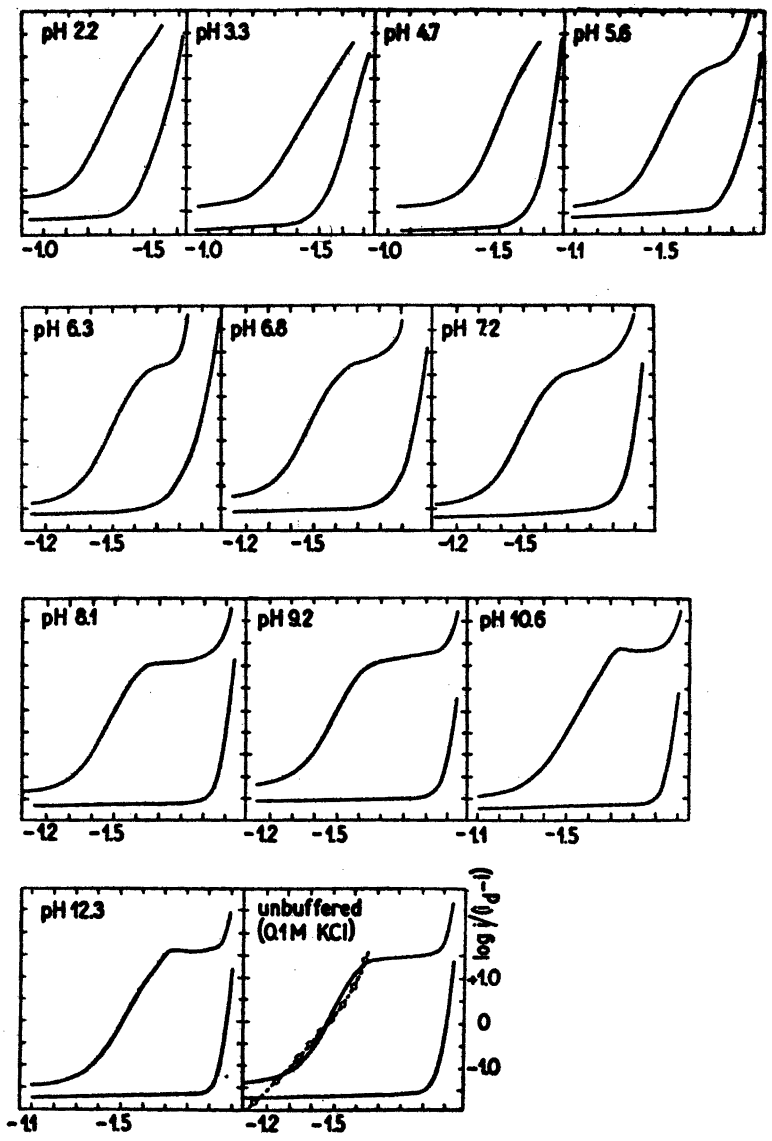

Fig. 2. Polarographic wave of hydroxyurea (m.p. 72°) at various pH values; 2.0 mM *solutions in buffers approximately 0.1—0.2* N *in potassium ion (upper curve). Blank polarograms of the supporting buffers (lower curve). The potential on the abscissa is in volts* vs. *S. C. E., one unit on the ordinate represents 2.33 microamps.*

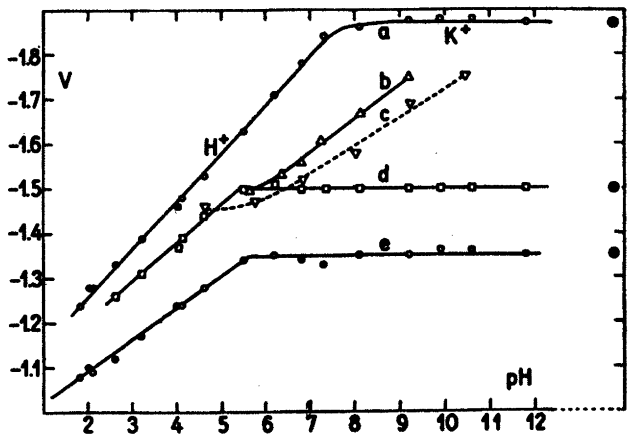

Fig. 3. Variation of half-wave and tangent potentials with the pH. a. tangent potential of the supporting buffer solution. b. 2.0 mM *hydroxylammonium chloride in buffers. Half-wave potential. c. as b., data reported by Petru (Ref.[5] p. 626) 1.0* mM*/ d. 1.0* mM *hydroxyurea (m. p. 72°) in buffers, half-wave potential. e. as d. but tangent potential. The full dots refer to an unbuffered 1.0* mM *solution of hydroxyurea in 0.1* N *potassium chloride.*

temperature. In neutral and slightly alkaline, buffered and unbuffered solutions it is reasonably stable for experimental work carried out within a few hours from the dissolution, but freshly prepared solutions should always be used. Satisfactory stability was also found under conditions extant in polarographic work, *i.e.* in the presence of mercury and maximum suppressor (methylcellulose). Strongly alkaline solutions, pH > *ca.* 11, are very unstable at room temperature and at higher temperatures this also applies to neutral solutions.

The actual process responsible for the deterioration of hydroxyurea (m.p. 72° C) in neutral and alkaline solutions and the kinetics of this reaction from more exact data will be considered in a subsequent paper.

THE INFLUENCE OF THE pH ON THE WAVE OF HYDROXYUREA (m.p. 72° C)

A 10.0 m*M* solution of hydroxyurea in redistilled water was freshly prepared and kept at room temperature. 1 ml samples of this stock solution were mixed with 4 ml of buffer solution and polarographed at 25.0° C after appropriate deaeration. The general technique and the composition of the buffers are given in the last section.

The appearance of the hydroxyurea wave at various pH-values is illustrated in Fig. 2. The lower curve in each graph is that of the supporting buffer solution, polarographed under identical conditions immediately before the hydroxyurea was added. The rise of these blank curves represents the hydrogen discharge in the acidic buffers and the reduction of the supporting potassium ion in the neutral and alkaline buffers.

On the acid side the hydroxyurea wave is poorly developed, it is more or less masked by the hydrogen wave. Measurements of the independent wave can be undertaken with fair accuracy from pH 5.6 onwards, more accurately still from pH 7 and far into the alkaline region. However, in view of the notable instability of hydroxyurea in alkaline solutions, demonstrated in the preceding section, pH 7—8 should be preferred for analytical work.

It should be noted that the wave height is essentially the same over the pH-range investigated and that only one wave appears.

In neutral unbuffered solution with potassium chloride as a supporting electrolyte, last graph in Fig. 2, the wave is of a similar shape and appears to be equally reproducible.

The variation of the half wave potential $E_{0.5}$ with the pH is demonstrated in Fig. 3, curve d (the experimental data are taken from a series different from the one illustrated in Fig. 2). $E_{0.5}$ is shifted to more negative values with rising pH until at pH 5.5 it becomes constant. The implications of this result is discussed later in this paper.

THE INFLUENCE OF CONCENTRATION ON THE WAVE OF HYDROXYUREA

As a consequence of the results reported in the preceding section the buffer having pH 7.2 was chosen for a detailed investigation of the concentration influence. From a 2.0 mM stock solution in the buffer a series of dilutions were prepared as indicated in Table 3 column 2, using the buffer as a diluent. Each

Table 3 (cf. Fig. 4)

Polarogram No.	c millimoles/l	s	H cm	i_d microamp.	E volt
26/7/19	2.0	700	6.9	16.1	−1.45
21	0.01	15	wave undeveloped		
23	0.02	30			
25	0.04	50	2.7	0.5	−1.42 *
26	0.1	50	5.2	0.9	−1.43 *
27	0.2	100	4.9	1.6	−1.43 *
28	0.4	150	7.5	3.8	−1.46
31	0.6	300	5.1	5.1	−1.46
32	0.8	500	4.0	6.7	−1.46
33	1.0	500	4.9	8.2	−1.46
35	1.2	500	5.9	9.8	−1.46
36	1.4	500	6.9	11.5	−1.46
37	1.6	500	7.8	13.0	−1.46
38	1.8	700	6.3	14.7	−1.47
39	2.0	700	6.8	15.9	−1.45
40	2.0	700	6.9	16.1	−1.44
41	3.0	1 000	7.2	24.0	−1.45
42	4.0	1 500	6.4	32.0	−1.45
43	5.0	2 000	6.0	40.0	−1.44
44	6.0	2 000	7.1	47.3	−1.44
45	8.0	3 000	6.1	61.0	−1.45
47	10.0	3 000	7.5	75.0	−1.45

* wave poorly developed.

dilution was deaerated by passing a nitrogen stream through it for 6 minutes, whilst at the same time the following dilution was being prepared and the foregoing one was being recorded. In that way the error due to the lability was reduced to a minimum. Polarogram No. 39 served as a control. It was recorded with the same cell-solution as No. 19 at the end of the series, 2 hours after the preparation of the stock solution. Comparison of the diffusion currents shows that the error due to the lability of hydroxyurea is less than 1.5 % in all the i_d-values.

The higher concentrations were investigated in a separate series, polarograms Nos. 40 to 47. The dilutions were prepared from a fresh 10.0 mM stock solution of hydroxyurea and the operations were carried out exactly as above. The last polarogram in this series was recorded less than one hour from the dissolution of the solid.

In the table s is the current multiplier, H is the wave-height actually measured at the applied galvanometer sensitivity (full sensitivity divided by s), i_d is the diffusion current calculated from the wave-height by means of the expression $i_d = Hs/300$ and $E_{0.5}$ is the half-wave potential in volts *vs* S.C.E., corrected for the iR drop across the polarographic cell, as indicated in the last section.

Apart from the most dilute solutions in which the wave was poorly developed and the experimental error abnormally large, the half-wave potential is virtually independent of concentration. The best value is probably 1.46 ± 0.02 V.

The relation between wave-height (diffusion current) and concentration is graphically represented in Fig. 4. It is perfectly linear up to 5 millimoles/l but shows a slight deviation from linearity above this concentration. The optimal concentration region for analytical work is from 0.5 to 2 millimoles/l, provided the supporting electrolyte is approximately 0.1 M. At higher concentrations the buffering capacity is inadequate, and this cannot be improved by increasing the buffer concentration because the hydrogen wave and potassium wave would then start at a more positive voltage and interfere with the hydroxyurea wave. Concentrations below 0.5 millimoles/l are inconvenient because much greater attention must then be given to the removal of oxygen from the solution.

The slope of the straight line in the optimal concentration region is 8.0. Concentrations may therefore — under standard conditions, see Fig. 4 — be calculated from the experimental diffusion currents i_d by means of the equation $i_d = 8.0\ c$, in which c is given in millimoles/l and i_d in units of microamperes (10^{-6} amp.).

Fig. 5 illustrates the variation of wave-height with concentration in unbuffered solution. 0.5 M lithium chloride was used as a supporting electrolyte. There is strict linearity over the concentration range 0.4 to 10 millimoles/l. The ordinate is the wave-height in cm for a galvanometer sensitivity reduced 1 : 30, but these data were measured some years ago with an older polarographic instrument about 20 times less sensitive, and the results are therefore not directly comparable with other ones reported in this paper. They do, however, support the view that buffering of the cell solution is not strictly necessary in analytical work with hydroxyurea in the neutral region.

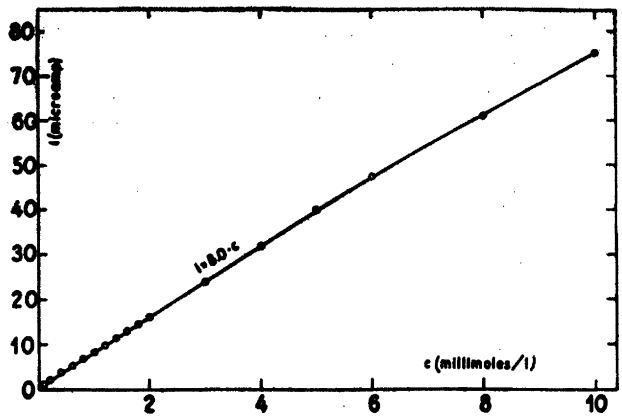

Fig. 4. Hydroxyurea, m. p. 72°. Relation of diffusion current to concentration. Supporting electrolyte 0.1 M *potassium phosphate buffer at pH 7.2. Temp. 25.0° C, drop time* t = *3.33 sec, height of mercury column* h = *45 cm, rate of mercury outflow* m = *2.66 mgsec*$^{-1}$.

THE ELECTRODE PROCESS

The conventional logarithmic analysis of the current-voltage curve of the lower melting hydroxyurea clearly indicates an irreversible electrode process. The general equation of a wave due to a cathodic, *reversible* electrode reaction in a well buffered solution is [6]

$$E = E_{0.5} - \frac{0.0591}{n} \log \frac{i}{i_d - i}$$

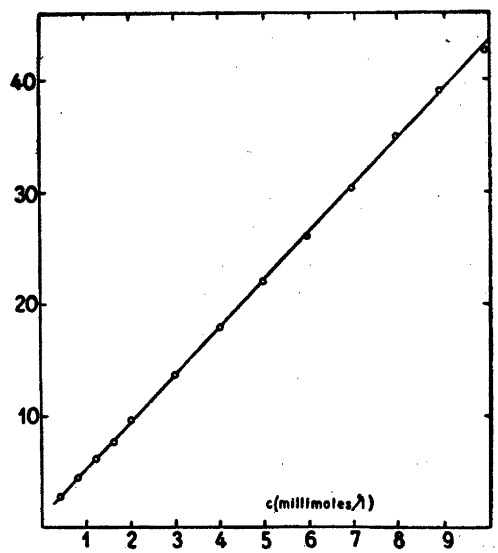

Fig. 5. Hydroxyurea, m. p. 72°. Relation of diffusion current to concentration. Supporting electrolyte 0.5 N *lithium chloride. 20° C.*

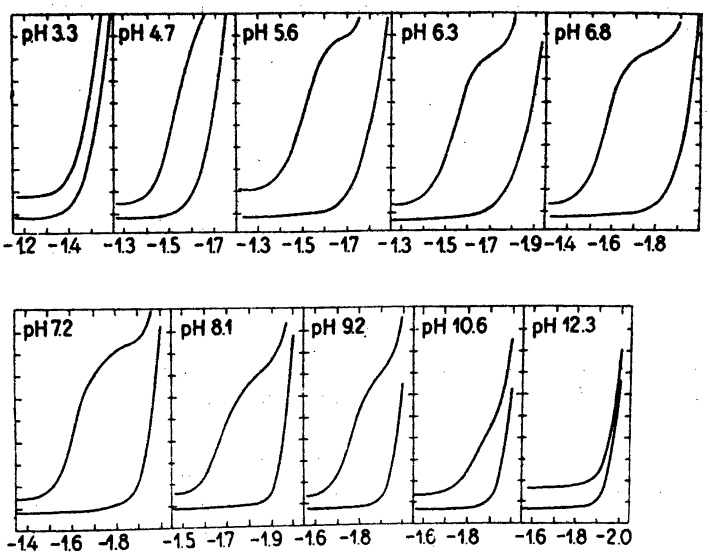

Fig. 6. Polarographic wave of hydroxylammonium chloride at various pH values. 2.0 mM *solutions in buffers approximately 0.1* N *in potassium ion (upper curve). Blank polarograms of the supporting buffers (lower curve). Units and experimental conditions as in Fig. 2.*

i being the diffusion current at any time during the reduction, i_d the limiting diffusion current and n the number of electrons. It follows that a plot of log $\frac{i}{i_d - i}$ against the potential E of the dropping electrode should be linear.

Since n has been estimated to 2 (see below) the theoretical slope would be approximately —30 mV. The actual plot (Fig. 2, last graph) is not linear and the slope in the half-wave point is approximately —190 mV, and thus considerably at variance with the theoretical value. This indicates the irreproducibility of the process.

Since the diffusion coefficient of the lower-melting hydroxyurea is unknown, the number of electrons involved in the cathodic reduction can only be estimated by comparison of the wave height with that produced by some suitable substance, for which the electrode process is established. Hydroxylammonium chloride was chosen for this purpose, firstly because it has a similar molecular size, secondly because it is one of the starting materials in the preparation [1] of hydroxyurea and hence may possibly occur as an impurity, and thirdly because a comparison with the hydroxylammonium wave might provide additional evidence as to whether the lower-melting hydroxyurea is identical with hydroxylammonium cyanate or not.

Although some previous information on the polarographic behaviour of hydroxylammonium chloride is available [5] it was deemed necessary to reinvestigate the substance under conditions strictly identical with those applied to hydroxyurea in Fig. 2.

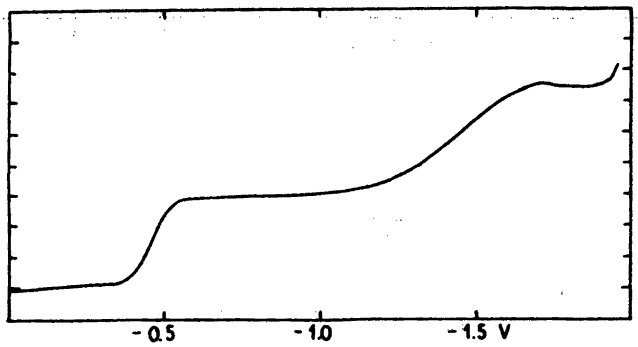

Fig. 7. Polarogram of a solution 1.0 mM *in hydroxyurea, 1.0* mM *in thallous ion and 0.1* M *in potassium chloride. 0.02 % methylcellulose added as a maximum suppressor.*

Freshly prepared 2.0 m*M* solutions of hydroxylammonium chloride in a series of buffers (*cf.* p. 469) were polarographed with the standard technique described in the last section. The results, which on the whole confirm those of Petru [5], are reproduced in Fig. 6. There is a relatively well developed wave in the pH-range from about 6 to 7; in acid solution the wave is masked by the hydrogen wave, in alkaline medium the wave due to the discharge of the supporting potassium ion interferes, in contradistinction to the hydroxyurea wave, which is independently developed far into the alkaline region (*cf.* Fig. 2). In neutral solution, where the wave is well developed, its height is very nearly identical with that of the hydroxyurea wave under equal conditions, *cf.* Fig. 2. Since, according to Petru [5] hydroxylamine is reduced by a two-electron process, it is a reasonable assumption that the cathodic reduction of hydroxyurea also involves two electrons per molecule and this seems to apply over the entire pH-range investigated.

A calculation of n from the Ilkovic equation, using an estimated value of the diffusion coefficient also gave a result close to 2. From polarogram No. 43 (Table 3) the following set of experimental data were drawn; diffusion current $i_d = 40.0$ microamperes, concentration $c = 5.0$ millimoles/l, mercury flow rate $m = 2.66$ mg/sec and drop time $t = 3.33$ sec, temperature 25° C. By inserting these figures in the Ilkovic equation $i_d = 607 \cdot n \cdot D^{1/2} \cdot c \cdot m^{2/3} \cdot t^{1/6}$ we get at 25° C $nD^{1/2} = 0.0056$. The diffusion coefficient D of most organic compounds is of the order of magnitude 1×10^{-5} cm^2 sec^{-1} which gives a value $n = 1.75$.

Finally the wave height of hydroxyurea has been compared with that produced by thallous ion, which is known to react according to a one-electron process. Fig. 7 shows a polarogram of a solution containing equimolar amounts of hydroxyurea and thallous ion. The limiting diffusion currents are 8.4 and 6.3 microamperes, respectively. Since the two species are polarographed under strictly identical conditions we get from the Ilkovic equation

$$\frac{(i_d)_{hy}}{(i_d)_{Tl^+}} = \frac{n_{hy} D_{hy}^{1/2}}{n_{Tl^+} D_{Tl^+}^{1/2}}$$

the index hy standing for hydroxyurea. The diffusion coefficient of thallous ion is $D_{Tl^+} = 2 \times 10^{-5}$ cm^2 sec^{-1}. Introducing this, as well as $n = 1$, the above experimental diffusion currents and the assumed diffusion coefficient $D_{hy} = 1 \times 10^{-5}$ cm^2 sec^{-1} we get $n_{hy} = 1.7$. There seems to be every reason to believe, therefore, that the reduction of hydroxyurea, m.p. 72°, involves two electrons per molecule.

In the dependency of the half wave potential upon the pH hydroxylamine and hydroxyurea show a remarkable difference (Fig. 3, b and d). Whereas $E_{0.5}$ of the former is strongly dependent upon the pH in the region pH 5—10 that of the latter is constant in the same region. The $E_{0.5}$ values for hydroxylamine are somewhat larger than those reported by Petru [5] (Fig. 3 c) but it should be remembered that the absolute values can only be roughly estimated because the wave is more or less masked by the hydrogen wave in all the buffer solutions.

For hydroxyurea the relation between the half-wave potential and the pH has the form of two straight lines intersecting at pH 5.5. The linear dependency in acid solution is substantiated by supplementary measurements (e) of the tangent potential (45°) (Ref.[6], p. 623) in strongly acidic medium, where the half-wave potential cannot be determined with any great accuracy. The general relation between $E_{0.5}$ and pH for a *reversible* reduction $R + nH^+ + ne^- \rightleftharpoons RH_n$ is given by [6]

$$E_{0.5} = E^\circ_{0.5} - \frac{0.0591}{n} \log \frac{k_R}{k_{RH_n}} - 0.0591 \text{ pH} \qquad (1)$$

in which $E^\circ_{0.5}$ is the standard potential of the reaction and k a constant characteristic of the species. The second term on the right is usually negligibly small. It follows that the theoretical shift of the half-wave potential due to one pH-unit is —59 millivolts for the above *reversible* reaction. The acidic branch of the curve Fig. 3 d is strictly linear as required, but its slope is —87 millivolts.

The slope —87 millivolts is so close to the average slope found for the discharge of the hydrogen ion (Ref.[12], p. 123; *cf.* Fig. 3 a) that the possibility should be considered whether the hydroxyurea wave in acid medium be due to hydrogen discharge from the hydroxyuronium ion functioning as a weak acid, the discharge for some reason taking place slightly before the hydrogen wave of the buffer starts. This explanation is, however, unlikely, because the hydrogen wave is much higher than that due to normal organic compounds. Consequently a considerable change in wave height should have been observed for hydroxyurea when shifting from acid to alkaline medium. As reported above (*cf.* Fig. 2) no such change was observed, the wave height is constant over the entire pH-range.

The deviation of the slope from the theoretical value may then be explained either as a result of the irreproducibility of the process or by assuming an anomalous number of hydrogen ions to take part in the electrode process [12]. Equation (1) is valid only for a process involving the same number (usually $n = 2$) of electrons and hydrogen ions. If the numbers were n and m respectively, the constant in the last term in eq. 1 would be $\frac{m}{n}$ (—59) mV. It is seen that

an electrode process involving two electrons and three hydrogen ions per molecule

$$R + 2e^- + 3H^+ \rightarrow RH_3^+ \qquad (2)$$

would give a shift of $3/2 \cdot (-59) = -88$ mV in close agreement with the experimental value.

In neutral and alkaline solution the half-wave potential of hydroxyurea is completely independent of the pH. The distinct break at pH 5.5 suggests that hydroxyurea is reduced at the dropping mercury-electrode by two distinctly different mechanisms. A pH-dependent one, in which hydrogen ions take part in the potential-determining step, operates in acid solution. As the hydrogen ion activity decreases, the reduction by this mechanism finally becomes so difficult (*cf.* the rising $E_{0.5}$) that a different, pH-*in*dependent mechanism is favoured. That hydrogen ions do not take part in the second mechanism seems to be substantiated by the fact that hydroxyurea produces a well-defined wave of exactly the same height in neutral *unbuffered* solutions (*cf.* Fig. 2, last graph) in distinct contrast to hydroxylamine, which gives ill-defined double waves under such conditions, as expected with the mechanism suggested by Petru [5] involving hydrogen ions. It is quite certain, therefore, that in neutral and alkaline medium hydroxyurea and hydroxylamine are reduced by entirely different mechanisms and the question as to whether the lower-melting hydroxyurea is simply hydroxylammonium cyanate has at the same time received a clear negative answer. Whether the pH-dependent mechanisms are of a similar type remains to be settled.

The regions of pH-dependency fall in the vicinity of the dissociation constants, $pK_a = 8.0$ for hydroxylammonium ion [10] and ca. 2.3 for the hydroxyuronium ion [11]. It seems probable that the species, which is reduced at the dropping electrode in the case of hydroxyurea is the uncharged basic molecule (B) in neutral and alkaline medium and the conjugate acid, hydroxyuronium ion (BH^+) in acid solution. The fact that only a single wave of constant height is obtained over the whole pH-range indicates that the protolytic reaction $BH^+ \rightleftharpoons B + H^+$ proceeds with infinite velocity (*cf.* Ref.[7]) so that the hydroxyuronium ions consumed by the electrode process are immediately replaced by newly formed ones. Under these circumstances (*cf.* Ref.[8]) no separate reduction of the basic form takes place until at about pH 5.5 the pH-dependent mechanism has become so difficult that the pH-*in*dependent reduction of the uncharged molecule, taking place at a slightly more negative potential, is favoured. As a result of these combined mechanisms the limiting current (wave height) would be independent of pH and proportional to the total analytical concentration of hydroxyurea, as indeed we find.

The possibilities that the limiting current is either kinetically controlled or of a catalytic nature have been ruled out since the relation $i_d = k \cdot h^{1/2}$ (Ref.[6], p. 85) characteristic of a purely diffusion-controlled current has been found to hold for hydroxyurea. Table 4 contains the experimental material. h_{corr} is the height of the mercury column, corrected for the back-pressure $-\frac{3.1}{(mt)^{1/3}}$ due to interfacial tension (Ref.[6], p. 79) which for the employed capillary ($mt = 7.68$ mg) amounted to -1.6 cm Hg.

Table 4. Variation of i_d *with the height* $h_{corr.}$ *of the mercury column. 2.0* mM *hydroxyurea in 0.1* N *potassium chloride, 0.02* % *methylcellulose.*

$h_{corr.}$ cm	t sec	i_d μamp	$i_d / h_{corr}^{1/2}$
28.4	3.7	14.5	2.72
38.4	2.8	16.6	2.68
43.4	2.5	17.8	2.70
48.4	2.2	18.7	2.69
58.4	1.9	20.3	2.67
68.4	1.6	22.0	2.66

The values in the last column are virtually constant. The apparent decrease is due partly to the approximative nature of the equation $i_d = k \cdot h^{1/2}$ (Ref.[5], p. 85) and partly to the lability of the substance in aqueous solution. The last polarogram in Table 4 was recorded 1 ½ hour after the first one. According to previous results a drop of 1—2 % in the "constant" is to be expected in this period, due to the lability of hydroxyurea. Much larger deviations in $i_d/h_{corr}^{1/2}$ would have been found if the limiting current were kinetically controlled. Frequently the current is quite independent of the height of the mercury column (Ref.[5], p. 274). Adsorption waves usually show a linear relationship between the limiting current and h.

No attempt will be made at the present time to formulate definite electrode processes. When dealing with irreversible reductions of organic molecules this cannot normally be achieved on the basis of polarographic data alone.

It seems likely, however, that urea must be taken into account as a possible reduction product. This would be compatible with a two-electron process and, since urea has been found to be inactive towards the dropping mercury electrode, also with the apparent irreversibility of the electrode process. It should be added that the reaction scheme (2), p. 466, must be discarded, if the reduction product is shown to to be urea, because this substance is a *weaker* base than hydroxyurea, whereas the hypothetical process

$$O = C\begin{matrix}\diagup NHOH \\ \diagdown NH_2\end{matrix} + 2e^- + 3H^+ \rightarrow O = C\begin{matrix}\diagup NH_3^+ \\ \diagdown NH_2\end{matrix} + H_2O$$

corresponding to (2), requires that the reduction product be a stronger base than hydroxyurea. Isolation of urea from a large scale controlled-potential electrolysis is being attempted.

The higher-melting hydroxyurea (140°) produces neither a cathodic nor an anodic wave in the usual voltage region + 0.2 to —2.0 V *vs* S.C.E. It may be assumed that a much more negative potential is required for the reduction of the higher melting isomer; this would be parallelled by the greater robustness of that isomer. Alternatively the reducible group in the lower melting isomer may be absent in the higher melting one.

Methoxyurea, which only exists in one form, is not reduced at the dropping mercury electrode. This is compatible with the view that the —NHOH group is engaged in the polarographic reduction of the lower melting isomer.

MATERIALS, APPARATUS, AND GENERAL TECHNIQUE

The preparation and characterization of the isomeric hydroxyureas have been reported in a previous paper [1].

The lower melting hydroxyurea was stored at 0° C in a dry atmosphere, and all measurements reported in this paper were carried out within 2 weeks from the preparation. Comparison of the wave-height produced under standard conditions at various instants indicated a satisfactory stability under these circumstances.

Hydroxylammonium chloride as well as all chemicals used for the preparation of the supporting solutions were analytical grade products. The solvent was water, carefully redistilled in a Pyrex apparatus. The composition of the various stock solutions used for preparing the buffers are indicated in Table 5.

Table 5. Composition of buffer stock-solutions.

A	1 *M* phosphoric acid 1 *M* potassium chloride
B	1 *M* citric acid 1 *M* potassium chloride
C	1 *M* acetic acid 1 *M* potassium chloride
D	1 *M* potassium hydrogen carbonate
E	1 *M* boric acid 1 *M* potassium chloride
F	1 *M* potassium di-hydrogen phosphate
G	1 *M* potassium mono-hydrogen phosphate
H	1 *M* potassium di-hydrogen borate
I	1 *M* potassium hydroxide

The set of stock solutions used for each individual buffer is indicated in Table 6. Appropriate volumes were mixed and diluted to the tenfold volume with water containing 0.05 % methylcellulose as a maximum suppressor. In most experiments 4 to 4.5 ml of the buffer were mixed with 1 to 0.5 ml of a stock solution of the depolarizer, so that the final volume of cell solution was 5 ml, and the final concentration of potassium ion was approximately 0.1 to 0.2 *M*. By employing a relatively large number of buffer types (Table 6, col. 4) and using each system only in the immediate neighbourhood of the relevant p*K*-value a sufficient buffer capacity was secured in all solutions. This is a point of great importance often neglected in polarographic work [9]. The final pH of each cell-solution was measured after the polarographic recording by means of a "Radiometer" pH-meter, type PHM 221, against a standard phosphate buffer of pH 6.78.

The polarographic cell was a cylindrical glass tube, 17 by 50 mm, fitting into a brass water-jacket through which water at 25.0 ± 0.1 was circulated from a Höppler ultra-thermostat. The anode was a saturated potassium chloride-calomel electrode with a saturated potassium chloride liquid junction. Unless otherwise stated the capillary characteristics of the dropping mercury electrode were as follows, height of mercury column h = 45 cm, rate of mercury outflow into distilled water m = 2.66 mg sec^{-1}, drop time 3.0 to 3.3 sec.

Table 6.

Final pH	Stock solutions		Buffer type
	acid	base	
1.0	A	F	phosphate
1.6	A	F	
2.2	A	F	
2.4	A	F	
2.9	B	I	citrate
3.6	B	I	
4.0	C	I	acetate
4.7	C	I	
5.6	F	G	phosphate
6.3	F	G	
6.8	F	G	
7.2	F	G	
8.1	F	G	
9.2	E	H	borate
9.7	D	I	carbonate
10.5	D	I	
11.9	G	I	phosphate

Oxygen was removed from the cell-solution prior to any polarographic recording by passing a stream of oxygen-free nitrogen through the cell. 6 to 10 minutes were required to reduce the oxygen wave to a negligible height, depending upon the applied galvanometer sensitivity. The absence of the oxygen wave and of waves due to other impurities was checked by running a blank polarogram of the supporting electrolyte solution before the addition of the depolarizer.

The polarographic instrument was a "Radiometer" PO 3e. The voltage scale was checked against thallous ion, the half-wave potential of which was taken as -0.46 V *vs* S.C.E. Half-wave potentials were corrected for the iR-drop across the cell, when necessary. The resistance of the polarographic cell under average experimental conditions was determined by measurement of a series of widely different concentrations of thallous ion. The plot of the apparent half-wave potentials against the diffusion current in the half-wave point was linear with the slope $\triangle E_{0.5} / \triangle i_{0.5} = 2.0 \times 10^3$, which is equal to R. The correction thus amounts to $+0.002\ i_{0.5}$ volts, $i_{0.5}$ being in units of microamperes. It is seen that the correction is significant only for diffusion currents $i_d > 20$ microamperes ($i_{0.5} > 10$), since the probable error in polarographic half-wave potentials with the employed technique is estimated to $\pm$ 0.02 V.

The full galvanometer sensitivity was maintained at a constant value of 7.8 cm deflection for a current of 2.6×10^{-8} amperes by frequent adjustment. In most measurements this sensitivity was reduced to 1/300 to 1/500 (current multiplyer s = 300 to 500). The diffusion currents i_d were calculated from $i_d = H \cdot s/300$, H being the wave height in cm and i_d the current in microamperes (10^{-6} amperes). The wave-height H was measured as the vertical distance between the tangent intersection points and has thus been corrected for the residual current.

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