Kinetics and mechanism of oxidation of hydroxyurea with hexacyanoferrate(III) ions in aqueous solution

Budimir, Ana; Bešić, Erim; Biruš, Mladen

Source / Izvornik: **Croatica Chemica Acta, 2009, 82, 807 - 818**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

CROATICA CHEMICA ACTA
CCACAA, ISSN 0011-1643, e-ISSN 1334-417X
Croat. Chem. Acta **82** (4) (2009) 807–818.
CCA-3375

Original Scientific Paper

Kinetics and Mechanism of Oxidation of Hydroxyurea with Hexacyanoferrate(III) Ions in Aqueous Solution

Ana Budimir,* Erim Bešić, and Mladen Biruš**

Faculty of Pharmacy and Biochemistry, University of Zagreb, A. Kovačića 1, 10000 Zagreb, Croatia

RECEIVED OCTOBER 6, 2008; REVISED APRIL 9, 2009; ACCEPTED JUNE 4, 2009

Abstract. Hydroxyurea (HU) effectively reduces $Fe(CN)_6^{3-}$ to $Fe(CN)_6^{4-}$ species in neutral and basic aqueous solution via an electron transfer process that includes the formation and subsequent fading out of a free radical, $U^•$ ($U^• \equiv H_2N{-}C({=}O)N(H)O^•$). The EPR spectrum of $U^•$ in H_2O solutions suggests that the unpaired electron is located predominantly on the hydroxamate hydroxyl-oxygen atom. Visible spectrophotometric data reveal HU as a two-electron donor. Stoichiometry of the studied reaction can be formulated as: $2Fe(CN)_6^{3-} + NH_2CONHOH + ½H_2O \rightarrow 2Fe(CN)_6^{4-} + CO_2 + NH_3 + ½N_2O + 2H^+$. Lack of evidence for the formation of NO probably is a consequence of fast dimerization of HNO in comparison with the rate of its oxidation, which is slow due to the low reduction potential of the $Fe(CN)_6^{3-}$/ $Fe(CN)_6^{4-}$ couple.

The kinetic of oxidation of HU by $Fe(CN)_6^{3-}$ was studied using stopped-flow technique, as a function of H^+, HU, $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$ concentrations, as well as a function of ionic strength and temperature. The kinetic results reveal that oxidation of HU by $Fe(CN)_6^{3-}$ proceed via an outer-sphere electron-transfer process. The effect of ionic strength on the reaction rate reveals that $NaFe(CN)_6^{2-}$ is the reacting species rather than $Fe(CN)_6^{3-}$ ion. The rate of the redox process was found to be first order with respect to both redox reactants while the H^+ concentration dependence make clear that U^- is about four orders of magnitude more reactive than HU. The formal reduction potentials for $U^•/U^-$ and $HU^•/HU$ couples were estimated from the kinetic results as +0.47 V and +0.84 V, respectively.

Keywords: hydroxyurea, hexacyanoferrate(III), kinetics, redox reaction, oxidation, mechanism

INTRODUCTION

Despite its simple molecular structure (Figure 1) hydroxyurea, HU, shows many biological activities and its pharmacology has drawn considerable attention of many scientists.

HU is a highly specific low-molecular inhibitor of ribonucleotide reductase and therefore of DNA synthesis[1] with a broad spectrum of anti-tumor effects.[2] It has been reported that HU effectively improves clinical outcomes in patients with sickle cell disease.[3,4] Nowadays, hydroxyurea represents a new treatment for sickle cell anemia.

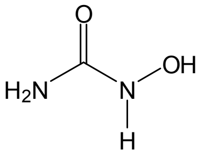


Figure 1. Chemical structure of hydroxyurea, noted as HU.

The main benefit from the treatment of patients with sickle cell anemia with HU arises from an increased production of fetal hemoglobin, genetically distinct hemoglobin that prevents the polymerization of deoxy sickle cell hemoglobin. However, some patients appear to benefit from HU even before the production of fetal hemoglobin is increased, indicating other mechanisms that can account for the HU activity.

It was reported that HU, as many other hydroxamic acids, also acts as a nitric oxide donor under oxidative conditions *in vitro*[5,6] and that direct nitric oxide producing reactions of HU and hemoglobin, myoglobin, or hemin may contribute to the overall pathophysiological properties of this drug.[7] Chemically, the treatment of HU with hydrogen peroxide and copper(II) sulfate produces an "NO-like" species capable of *N*-nitrosating morpholine.[8] Oral administration of HU in the treatment of sickle cell disease produced *in vivo* detectable nitrosyl hemoglobin.[9–11] On the other hand, investigation of inhibitory effects of nitro-vasodilators and HU on DNA synthesis in cultured human aortic smooth muscle

---

* Taken in part from A. B.'s PhD Thesis, University of Louis Pasteur – Strasbourg, and University of Zagreb, 2007.
** Author to whom correspondence should be addressed. (E-mail: birus@pharma.hr)

cells, indicates that NO does not mediate the inhibitory action of HU in this system.[12]

These few examples demonstrate the diversity of the mechanisms of HU action which may account for its therapeutic activity, whereas its NO-unit structure gave rise to a serious question whether HU takes effect via an NO mechanism? Therefore, clear molecular mechanisms describing the actions of HU remain to be established.[13,14] In line with the last observation, we have recently published a paper dealing with the oxidation of HU with dioxovanadium(V) ions. The results published herein are continuation of our previous studies on HU[15–17] to broaden the general knowledge on the reactivity of HU by studying its oxidation by various oxidants by employing the stopped-flow kinetic methods. Here we present the results on kinetics and mechanism of oxidation of HU with hexacyanoferrate(III) ions, $Fe(CN)_6^{3-}$, within a wide pH span that covers physiological acidities as well. $Fe(CN)_6^{3-}$ is a single-electron oxidant which due to its extreme inertness is expected to follow the outer-sphere electron transfer mechanism. It is capable of oxidizing under both acidic and alkaline conditions and has been utilized extensively in the oxidation of organic compounds.[18,19]

EXPERIMENTAL

Materials

Hydroxyurea (SIGMA, 98 %), potassium hexacyanoferrate(III) (MRECK), potassium hexacyanoferrate(II) (MERCK), sodium perchlorate (SIGMA), sodium nitrate (MERCK) perchloric acid (MERCK, 70 %), sodium hydroxide (MERCK), tetramethylammonium hydroxide pentahydrate (FLUKa, ≥ 97 %), tetramethylammonium nitrate (FLUKA, ≥ 97 %), MES (SIGMA, 99.5 %), HEPES (SIGMA, 99.5 %) were purchased from commercial suppliers and used without further purification. $[(CH_3)_4N]_3[Fe(CN)_6]$ was prepared[20] by mixing water solutions of $K_3[Fe(CN)_6]$ and $(CH_3)_4N_2O_3$, and cooling to 0 °C for 15 min. The precipitate was washed with ice-cooled water and methanol and dried in vacuum over silica gel. The solution of $Na_3[Fe(CN)_6]$ was prepared by dissolving appropriate amount of $K_3[Fe(CN)_6]$ in water and than passing trough an DOWEX® 50 WX strong cation-exchanger column in Na^+ form. No traces of K^+ could be detected in the solution.

All solutions for thermodinamic and kinetics studies were prepared with twice distilled and deionized water boiled for 1 hour and cooled down under argon atmosphere (purified by a SIGMA OXICLEAR cartridge) in order to exclude CO_2 and O_2. The ionic strength was maintained constant with sodium perchlorate, sodium nitrate or tetramethylammonium nitrate. Temperature control was maintained with the use of a HAAKE DC10-K10 Refrigerated Circulator Bath with temperature accuracy of 0.1 °C over range 10−100 °C.

Care was taken in the preparation and manipulation of hexacyanoferrate(III) solutions in order to prevent decomposition upon light exposure and long standing. Fresh solutions of hexacyanoferrate(III) were prepared before each experiment. The concentration of hexacyanoferrate(III) solutions was calculated by using the molar absorptivity of 1050 mol^{-1} dm^3 cm^{-1} at 420 nm.

Instrumental measurements

The potentiometric titrations of HU (5 mmol dm^{-3}) were performed using an automatic titrator system (METTLER TOLEDO DL55 Titrator) with a combined glass electrode (METTLER TOLEDO DG 111-SC) filled with 3 M aqueous NaCl. The combined glass electrode was calibrated as a hydrogen concentration probe by titrating standard solution of $HClO_4$ with standard CO_2-free NaOH solution.[21,22] A stream of argon was passed over the surface of the solution. The potentiometric data (about 100 points collected over pH range 7.8–11.8) were refined with SUPERQUAD[23] program. The electron paramagnetic resonance (EPR) spectra were recorded at room temperature on a Varian E-109 spectrometer. The spectra were recorded immediately after mixing reactants at a frequency 9.28 GHz with a microwavepower of 10 mW. The UV-vis absorption spectra were recorded on a VARIAN CARY 50 spectrophotometer, and the FT-IR spectra by using a BRUKER EQUINOX 55 FT-IR spectrometer equipped with gas cell. The resolution of FT-IR spectra was 4 cm^{-1}. To avoid interference of CO_2 contained in the air, the FT-IR equipment was flushed with nitrogen gas before measurements. Kinetic measurements were performed by using an OLIS USA RSM stopped-flow spectrophotometer coupled to an on-line data acquisition system. The kinetic traces were evaluated using the OLIS GLOBALWORKS™ program.

RESULTS

A yellow colored $Fe(CN)_6^{3-}$ anion is a an extremely weak Brønsted base in aqueous solution. Its conjugate acid, $H_3Fe(CN)_6$, ferricyanic acid, is a very strong acid with $pK_1 = -6.25$, $pK_2 = -3.23$, $pK_3 = -0.60$.[24] Since the protonation of $Fe(CN)_6^{3-}$ occurs at much higher acidities than used throughout our experiments, only the acid-base behavior of HU has to be considered to elucidate the influence of proton concentration on the rate of the redox reaction between $Fe(CN)_6^{3-}$ and HU. Hydroxyurea possesses three ionizable protons but only a single-proton dissociation was observed in aqueous medium.

$$\mathrm{HU} \xrightleftharpoons{K_a} \mathrm{H^+ + U^-} \qquad (1)$$

The ionization constant of HU at two ionic strengths, corresponding $pK_a = 10.71(2)$[25] at 0.04 mol dm^{-3} and $pK_a = 10.82(2)$ at 2 mol dm^{-3} ionic strength, was determined by the potentiometric titrations and well agrees with previously published data.[26]

Stoichiometry of the redox reaction

In order to analyze the obtained kinetic results, the stoichiometric coefficients of $Fe(CN)_6^{3-}$ and HU for the redox reaction were determined spectrophotometrically at $p[H^+] = 6.8$ (MES buffer) and $p[H^+] = 12.0$ (0.01 mol dm^{-3} NaOH), by measuring absorbance of reaction solutions at 420 nm, where only $Fe(CN)_6^{3-}$ absorbs considerably. Independently of the $p[H^+]$ value, for 0.5 mmol dm^{-3} $Fe(CN)_6^{3-}$, two straight lines shown in Figure 2 intersect at 0.25 mmol dm^{-3} HU indicating that HU acts as a two-electron donor in the reduction of $Fe(CN)_6^{3-}$ ions.

In order to identify the other products and to determine their stoichiometric coefficients, gas evolved during the redox reaction was analyzed by FT-IR spectroscopy as well as by chemical analysis. The latter was performed by passing the gaseous products through a standard solution of $Ba(OH)_2$ at 25 °C.[27] The titration of the excess $Ba(OH)_2$ revealed 0.96 ± 0.02 moles of CO_2 produced per mole of HU. The presence of CO_2 in the gaseous products accounts for a medium-strong peak observed at 2350.8 cm^{-1} in the FT-IR spectrum (Figure 3), whereas the peak at 2223.5 cm^{-1} could be assigned to $N_2O(g)$.[28]

Ammonia was determined via the indophenole formation reaction, by the standard analytical procedure.[29] Analysis of the reaction solution containing 0.01 mol dm^{-3} $Fe(CN)_6^{3-}$, 5 mmol dm^{-3} HU and 1 mmol dm^{-3} NaOH reveal formation of 0.91 ± 0.05 moles of ammonia per mol of HU. Furthermore, the reaction solutions were tested for nitrite and nitrate as the reaction products by the reactions with sulfanilic acid and naphthilamine[30] and results show that these two species were not formed under our experimental conditions. Therefore, from the obtained results the reaction stoichiometry that can be proposed with certainty is: $2Fe(CN)_6^{3-} + NH_2CONHOH \rightarrow 2Fe(CN)_6^{4-} + 0.96\ CO_2 + 0.91\ NH_3 + xN_2O$, from which the stoichiometrically balanced reaction equation can be written as: $2Fe(CN)_6^{3-} + NH_2CONHOH + ½H_2O \rightarrow 2Fe(CN)_6^{4-} + CO_2 + NH_3 + ½N_2O + 2H^+$.

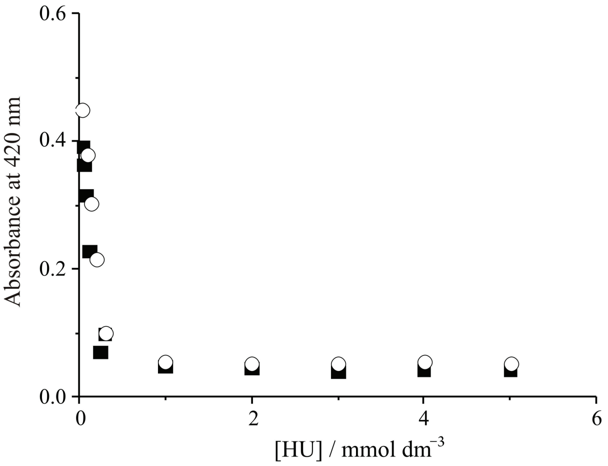


Figure 2. Absorbance of the equilibrated solutions of HU and $K_3Fe(CN)_6$ at 420 nm as a function of concentration of HU. Conditions: $[K_3Fe(CN)_6] = 0.5$ mmol dm^{-3}, $I = 2$ mol dm^{-3} ($NaClO_4$), $l = 1$ cm, $\theta = (25 \pm 0.1)$ °C. Squares: $p[H^+] = 12.0$; circles: $p[H^+] = 6.8$.

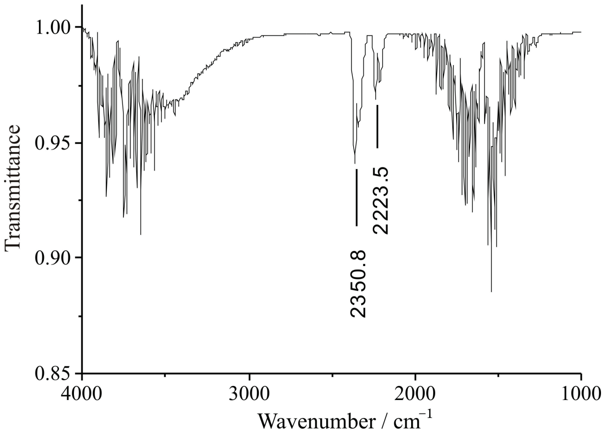


Figure 3. FT-IR spectrum of the gaseous products of the oxidation of HU by $Fe(CN)_6^{3-}$.

Free radical characterization

The formation of free radical in the reaction of $Fe(CN)_6^{3-}$ and HU at room temperature was demonstrated by the EPR spectroscopy. The formation and subsequent fading of the EPR free radical signal was monitored on the time scale of the redox reaction. The EPR signal of the free radical consist of six resonance lines characteristic of the aminocarbonyloaminoxyl radical $H_2N{-}CO{-}NHO^{\bullet}$. The observed spectrum (Figure 4) is in excellent agreement with the previously published EPR spectra of the same free radical produced by the oxidation of HU either with Cu(II) and H_2O_2,[31] Fe^{3+} [32,33] or VO_2^+.[16]

Kinetics of the redox reaction

Kinetics of redox reaction between $Fe(CN)_6^{3-}$ and HU was studied using the stopped-flow technique. The redox reaction was monitored by following a decrease in absorbance of hexacyanoferrate(III) at 420 nm with time. Figure 5 shows a typical example of absorbance changes in time during the reaction.

The observed rate constants were calculated by fit-

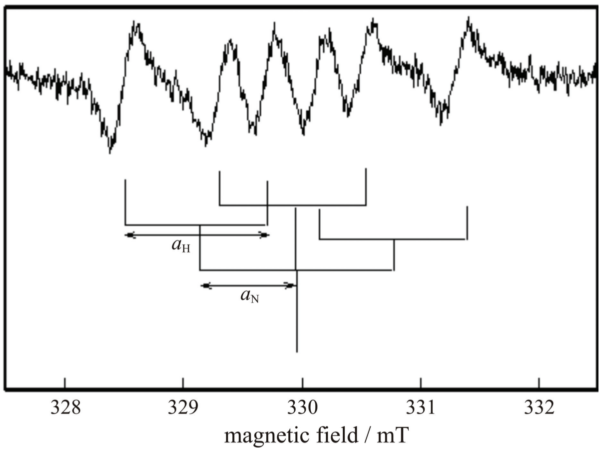


Figure 4. EPR spectrum of the aminocarbonylaminooxyl radical ($H_2N{-}CO{-}NHO^{•}$). Conditions: [HU] = 0.1 mol dm^{-3}, [Fe(CN)$_6^{3-}$] = 0.01 mol dm^{-3}, [HClO$_4$] = 0.01 mol dm^{-3}, at room temperature with f = 9.28 GHz and 10 mW.

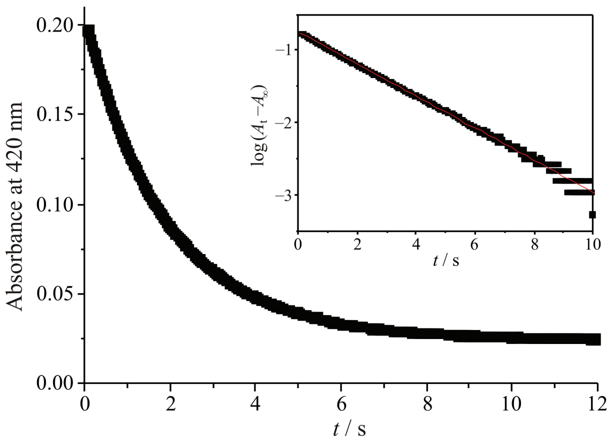


Figure 5. Plot of absorbance vs. time. Conditions: [HU] = 15 mmol dm^{-3}, [K$_3$Fe(CN)$_6$] = 0.5 mmol dm^{-3}, I = 2 mol dm^{-3} (NaClO$_4$), p[H$^+$] = 8.3 (HEPES buffer), l = 0.4 cm, θ = (25.0 ± 0.1) °C.

ting the expression $A_t = \Delta A \exp(-k_{obs}t) + A_\infty$ to the absorbance *vs*. time data. A_t and A_∞ are the absorbance at time t and infinite time, respectively, ΔA is the total absorbance change, and the observed rate constant k_{obs} is the calculated pseudo-first order rate constant (the pseudo-first order confirmed by the inset in Figure 5 which at the same time predict first order in respect with concentration of Fe(CN)$_6^{3-}$).

In order to confirm the first-order rate in respect with hexacyanoferrate(III) concentration, two series of kinetic measurements were carried out (in 0.1 mol dm^{-3} MES at p[H$^+$] = 6.8, and in 0.01 mol dm^{-3} NaOH at p[H$^+$] = 12.0), wherein only the initial concentration of K$_3$Fe(CN)$_6$ was varied. Although as much as two Fe(CN)$_6^{3-}$ ions per HU are required by the overall stoichiometry of the reaction, the single–exponential decay function was successfully fitted to the measured absorbances *vs*. time data. That fact, as well as the observed independence of the pseudo-first order rate constant on the initial Fe(CN)$_6^{3-}$ concentration clearly demonstrate the first order dependence of the redox reaction with respect to concentration of this reactant (Figure 6).

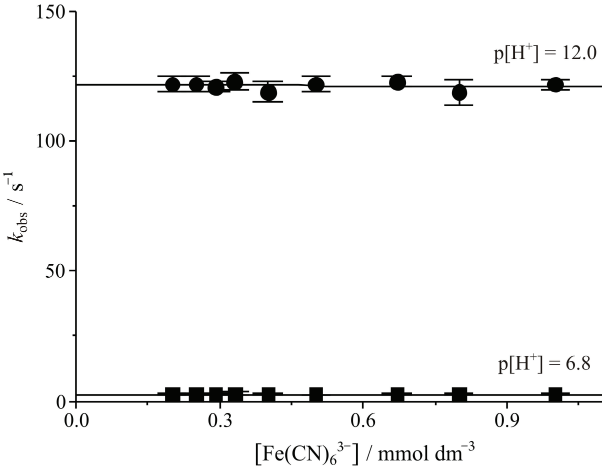


Figure 6. Plots of k_{obs} *vs.* initial [K$_3$Fe(CN)$_6$] for redox reaction between Fe(CN)$_6^{3-}$ and HU at two p[H$^+$]. Conditions: [HU] = 0.02 mol dm^{-3}, I = 2 mol dm^{-3} (NaClO$_4$), θ = (25 ± 0.2) °C.

Variation of the initial concentrations of HU in excess over hexacyanoferrate(III) was used to study effect of HU on the reaction rate. Measurements were performed at p[H$^+$] = 6.8 (in 0.1 mol dm^{-3} MES) and at p[H$^+$] = 13.0 (in 0.1 mol dm^{-3} NaOH). The HU concentration was varied within the range 0.01–0.05 mol dm^{-3}, at the constant 0.5 mmol dm^{-3} initial concentration of hexacyanoferrate(III), and at 2 mol dm^{-3} ionic strength. The variation of the observed rate constants with the initial concentration of HU is presented in Figure 7.

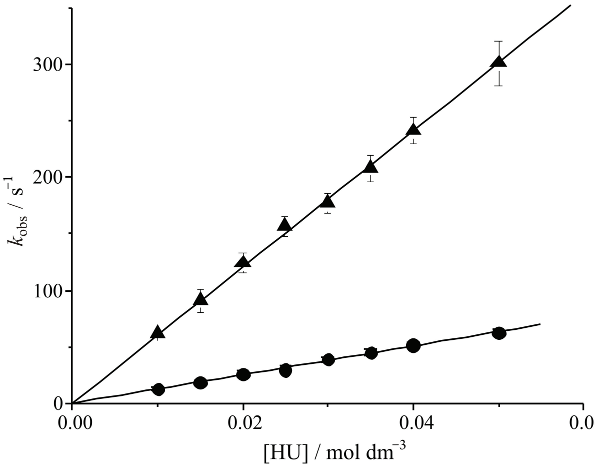


Figure 7. Dependence of the observed pseudo-first order rate constants (***k***$_{obs}$) on the concentration of HU. Conditions: ***I*** = 2 mol dm^{-3} (NaClO$_4$), θ = (25 ± 0.1) °C, (circles) [K$_3$Fe(CN)$_6$]= 0.5 mmol dm^{-3}, p[H$^+$] = 6.8, k_{obs} multiplied by 1000; (triangles) [K$_3$Fe(CN)$_6$] = 1 mmol dm^{-3}, p[H$^+$] = 13.0.

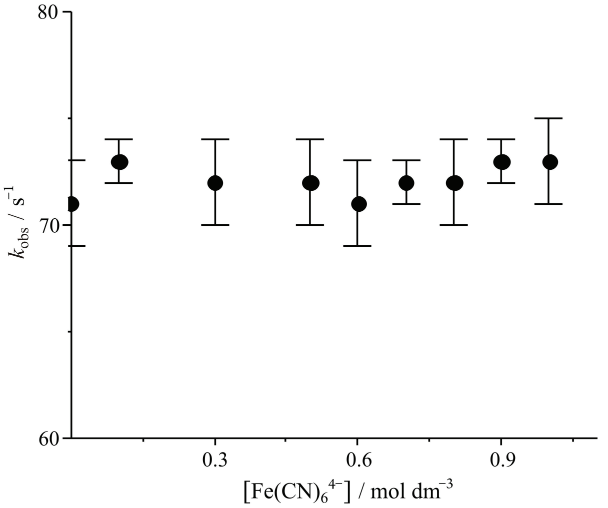


Figure 8. Dependence of k_{obs} on the initially added hexacyanoferrate(II) concentration. Conditions: $[K_3Fe(CN)_6]$ = 0.5 mmol dm^{-3}, [HU] = 15 mmol dm^{-3}, [NaOH] = 10 mmol dm^{-3}, I = 2 mol dm^{-3} ($NaClO_4$), θ = (25 ± 0.2) °C.

A linear relationship between k_{obs} and concentration of HU with the zero intercept shown in Figure 7 indicates the first order dependence in respect with the HU concentration, and clearly show dependence of the apparent second-order rate constants on $p[H^+]$ as well as the "irreversibility" of the studied reaction under the experimental conditions used.

In addition, a possible effect of hexacyanoferrate(II) ions as the reaction product, on the rate of redox reaction was also studied. The variation of initially added hexacyanoferrate(II) to the reaction solutions within the concentration range 0.5–1 mmol dm^{-3}, at fixed concentrations of $Fe(CN)_6^{3-}$, HU, and NaOH, and at constant ionic strength I = 2 mol dm^{-3} ($NaClO_4$), causes no effect on the rate of the redox reaction (Figure 8), additionally ruling out "reversibility" of the rate determining electron-transfer step.

The effect of $[H^+]$ on the rate of redox reaction between $Fe(CN)_6^{3-}$ and HU was investigated within the $p[H^+]$ range 6.8−13.2, keeping all the other reaction variables constant. Within the investigated range, the single-exponential function was satisfactory fitted to the collected absorbance *vs.* time data. The reaction rate initially increases upon increasing $p[H^+]$ and then tends to attain a limiting value. A sigmoid shape of the observed dependence (Figure 9) closely resembles that of a weak acid speciation profile as expected for HU within that pH range.

The overall rate equation that accounts for the experimental findings within the $p[H^+]$ range 6.8–13.2, is of the form given in (2).

$$\frac{k_{obs}}{[\mathrm{HU}]_0} = \frac{a[\mathrm{H}^+]+b}{[\mathrm{H}^+]+c} \qquad (2)$$

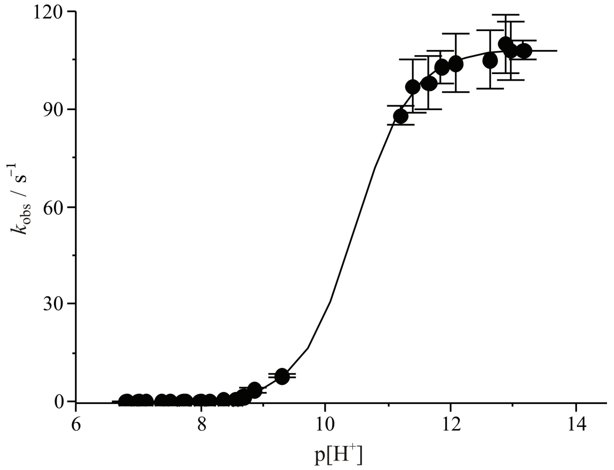


Figure 9. Dependence of k_{obs} on $p[H^+]$. Conditions: $[K_3Fe(CN)_6]$ = 0.5 mmol dm^{-3}, [HU] = 15 mmol dm^{-3}, I = 2 mol dm^{-3} ($NaClO_4$), θ = (25 ± 0.2) °C.

A non-linear regression fit of (2) to the experimental data gives the following results: a = 0.3(1) mol^{-1} dm^3 s^{-1}, b/c = 7.2(3) mol^{-1} dm^3 s^{-1} and $c = 10^{-(10.69\pm0.05)}$ mol dm^{-3}.

The rate-determining step of the hexacyanoferrate(III) oxidations of many organic substrates that follow first order with respect to the oxidant and reductant concentrations is the electron transfer of the first electron from reductant to the oxidant, wherein a free radical is formed. The radical generates another hexacyanoferrate(II) in the following fast step, since oxidations of free radicals by hexacyanoferrate(III) are usually diffusion-controlled steps. As mentioned above and generally accepted for one-electron oxidants, the rate determining-step is "irreversible". Based on the above presented, we could propose the following reaction mechanism for the oxidation of HU by hexacyanoferrate(III):

$$\mathrm{HU} + \mathrm{Fe(CN)}_6^{\,3-} \xrightarrow{k_1} \mathrm{Fe(CN)}_6^{\,4-} + \mathrm{U}^{\bullet} + \mathrm{H}^+ \qquad (3)$$

$$\mathrm{U}^- + \mathrm{Fe(CN)}_6^{\,3-} \xrightarrow{k_2} \mathrm{Fe(CN)}_6^{\,4-} + \mathrm{U}^{\bullet} \qquad (4)$$

$$\mathrm{U}^{\bullet} + \mathrm{Fe(CN)}_6^{\,3-} \xrightarrow{\text{fast}} \mathrm{Fe(CN)}_6^{\,4-} + \text{products} \qquad (5)$$

From the proposed mechanistic model, the following rate law can be derived:

$$R = -\frac{\mathrm{d}\left[\mathrm{Fe}(CN)_6^{\,3-}\right]}{\mathrm{d}t} = \frac{2k_1[\mathrm{H}^+] + 2k_2K_\mathrm{a}}{[\mathrm{H}^+]}[\mathrm{HU}]\left[\mathrm{Fe}(CN)_6^{\,3-}\right] \qquad (6)$$

By relating Eq. (6) to Eq. (2), the following values of the microscopic rate constants for oxidation of HU with $Fe(CN)_6^{3-}$ in 2 mol dm^{-3} sodium perchlorate are calculated: $k_1 = 0.15(5)$ mol^{-1} dm^3 s^{-1}, $k_2 = 3.6(2) \times 10^3$ mol^{-1} dm^3 s^{-1}. Obviously, c from Eq. (5) corresponds to ionization constant of HU, giving the calculated pK_a value in good agreement with the value obtained from our potentiometric experiments.

The effect of temperature on the rate of redox reaction was investigated by measuring the rate of reaction in 2 mol dm^{-3} $NaClO_4$ over the temperature range 10–40 °C at p[H$^+$] ≅ 8.1 (0.1 mol dm^{-3} HEPES) and p[H$^+$] ≅ 10.8 (buffered by half neutralized 0.015 mol dm^{-3} HU) while keeping constant initial total concentrations of $Fe(CN)_6^{3-}$ and HU. Kinetics of redox reaction was not studied in NaOH medium, as the reaction would be too fast for the stopped-flow instrument used. Temperatures above 40 °C were not employed because of possible decomposition of HU. The single-exponential function was satisfactory fitted to the obtained experimental data. Since the proton catalyzed path could be neglected at the p[H$^+$] values used, Eq. (6) reduces to a simpler expression: $R = 2k_2K_a(\mathrm{HU})[\mathrm{HU}][\mathrm{Fe(CN)_6^{3-}}]/([\mathrm{H^+}]+K_a(\mathrm{HU}))$. Therefore, to obtain the activation parameters, calculated values of k_{obs} were graphically treated according to the modified Eyring equation.

$$\ln\frac{k_{\mathrm{obs}}\left(\left[\mathrm{H^+}\right]+K_{\mathrm{a}}\left(\mathrm{HU}\right)\right)h}{kT\left[\mathrm{HU}\right]} = \frac{\Delta S^{\#}+\Delta_{\mathrm{r}}S_{\mathrm{a}}^{\mathrm{o}}\left(\mathrm{HU}\right)}{R}-\frac{\Delta H^{\#}+\Delta_{\mathrm{r}}H_{\mathrm{a}}^{\mathrm{o}}\left(\mathrm{HU}\right)}{R}\frac{1}{T}$$

The proton concentration in the solution at various temperatures was calculated by use of thermodynamic parameters for ionization of HEPES ($\Delta_r H^0 = 32.2 \pm 0.5$ kJ mol^{-1}, $\Delta_r S^0 = -49 \pm 3$ J K^{-1} mol^{-1}) and/or for ionization of HU ($\Delta H^0 = 37.8 \pm 0.8$ kJ mol^{-1}, $\Delta S^0 = -81 \pm 3$ J K^{-1} mol^{-1}) determined by the pH-titrations at the same experimental conditions used throughout the kinetic measurements. From the simultaneous treatment of the data at both acidities, the activation parameters, $\Delta H^{\neq} = 27(4)$ kJ mol^{-1} and $\Delta S^{\neq} = -76(15)$ J K^{-1} mol^{-1}, were calculated from *intercept* and *slope* of Figure 10 combined with the thermodynamic parameters for ionization of HU. Large standard deviations can be explained by a low accuracy of the calculated proton concentrations brought in by using thermodynamic parameters for ionization of HEPES and HU that were determined independently from the kinetic study. This could cause the observed systematic discrepancy between the data at two different acidities.

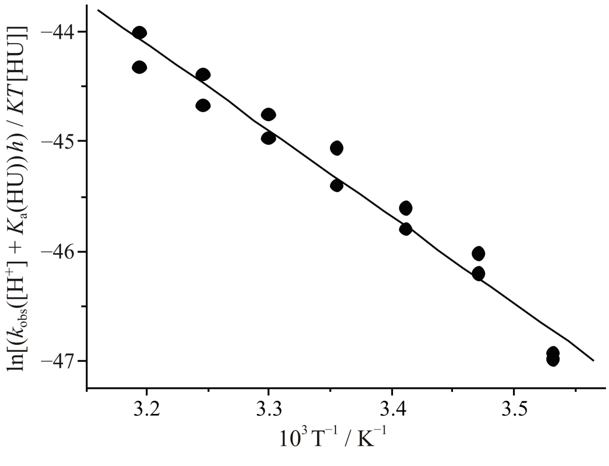


Figure 10. Eyring plot for redox reaction between $K_3Fe(CN)_6$ and HU. Conditions: [$K_3Fe(CN)_6$] = 0.5 mmol dm^{-3}, [HU] = 15 mmol dm^{-3}, I = 2 mol dm^{-3} ($NaClO_4$).

The salt effect

The effect of ionic strength on the reaction kinetics was investigated by varying concentrations of added neutral salts, sodium perchlorate, sodium nitrate or tetramethylammonium nitrate (the latter perchlorate is not soluble enough). Ionic strength of the reaction medium was varied within the range 0.04–2 mol dm^{-3}, while keeping constant concentrations of $Fe(CN)_6^{3-}$ (0.5 mmol dm^{-3}), HU (15 mmol dm^{-3}), and OH$^-$ (10 mmol dm^{-3} NaOH or $(CH_3)_4NOH$).

A distinct kinetic behavior was observed depending on the choice of the supporting electrolyte. In experiments in which the ionic strength was maintained using sodium cations ($NaClO_4$ or $NaNO_3$ salts) the reaction rate increases upon increasing the concentration of salt. The functional dependence of calculated pseudo-first order rate constants on the ionic strength is accurately accounted for by the Guggenheim's modification of Debye-Hückel equation of the form,

$$\log k_{obs} = \log k_0 + 1.02 z_A z_B \left(\frac{I^{1/2}}{1+I^{1/2}}\right),$$

in which k_{obs} is the observed rate constant at the ionic strength I, k_0 is the rate constant in the absence of added salt, while z_A and z_B are formal charges of the reactants.

A linear regression fit to the data in (Figure 11) gives the slope and the intercept of 2.09(2) and 0.81(1), respectively. The obtained positive slope confirms the expected same sign of charges of the reacting species, but the slope value that is ca. one unit lower than expected from the charges of U$^-$ and $Fe(CN)_6^{3-}$, suggests that the rate-determining step involves $NaFe(CN)_6^{2-}$ rather than $Fe(CN)_6^{3-}$. Ion pairing of $Fe(CN)_6^{3-}$ with alkali cations is well described in literature.[34,35] According to these results, the steps (7)–(10) also must be taken into account.

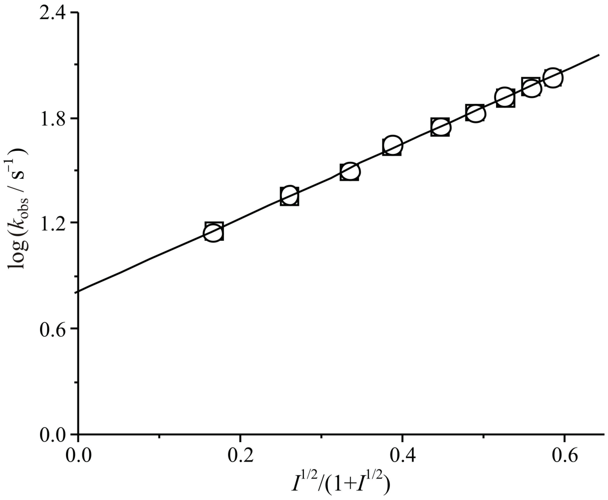


Figure 11. Dependence of log k_{obs} on $I^{1/2}/(1+I^{1/2})$. Conditions: $[Na_3Fe(CN)_6]$ = 0.5 mmol dm^{-3}, [HU] = 15 mmol dm^{-3}, p[H$^+$] = 12.0, θ = (25 ± 0.1) °C. Ionic strength maintained with $NaClO_4$ (squares) and $NaNO_3$ (circles).

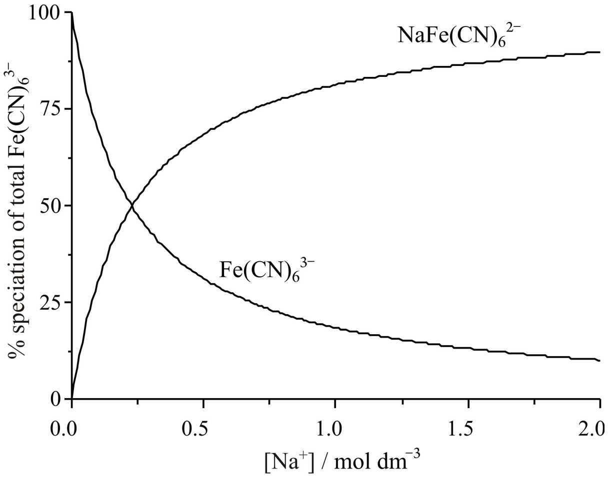


Figure 12. Distribution diagram of 5 mmol dm^{-3} hexacyanoferrate(III) species as function of [Na$^+$] at 25 °C.

$$Na^+ + Fe(CN)_6^{3-} \underset{}{\overset{K_{NaFe}}{\rightleftarrows}} NaFe(CN)_6^{2-} \qquad (7)$$

$$HU + NaFe(CN)_6^{2-} \overset{k_3}{\rightleftarrows} NaFe(CN)_6^{3-} + U^{\bullet} + H^+ \qquad (8)$$

$$U^- + NaFe(CN)_6^{2-} \overset{k_4}{\rightleftarrows} NaFe(CN)_6^{3-} + U^{\bullet} \qquad (9)$$

$$U^{\bullet} + NaFe(CN)_6^{2-} \overset{\text{fast}}{\rightleftarrows} NaFe(CN)_6^{3-} + \text{products} \qquad (10)$$

This leads to the following rate expression:

$$\frac{\mathrm{d}\left[Fe(CN)_6^{3-}\right]_{tot}}{\mathrm{d}t} = 2\frac{\left(k_1\left[H^+\right]+k_2K_a+\left[Na^+\right]K_{NaFe}\left(k_3\left[H^+\right]+k_4K_a\right)\right)}{\left(\left[H^+\right]+K_a\right)\left(1+\left[Na^+\right]K_{NaFe}\right)}\left[Fe(CN)_6^{3-}\right]_{tot}\left[HU\right]_{tot} \qquad (11)$$

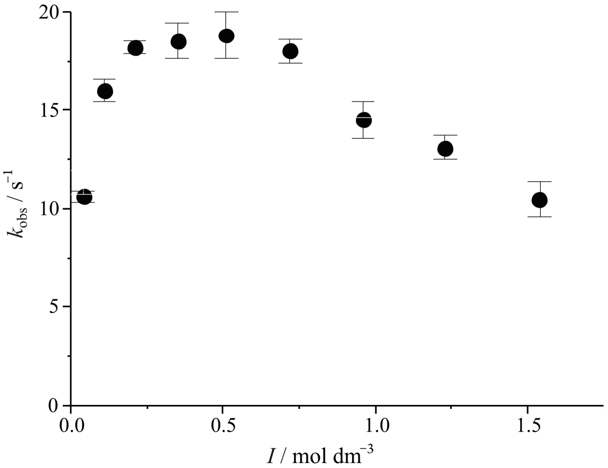


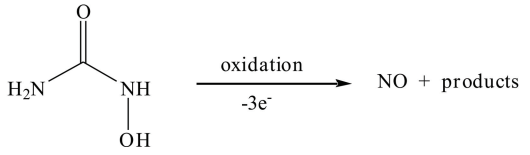
Figure 13. Dependence of the observed pseudo-first order rate constants (k_{obs}) for redox reaction between $[(CH_3)_4N]_3[Fe(CN)_6]$ and HU on the ionic strength at θ = (25 ± 0.1) °C, p[H$^+$] = 12.0, $[Fe(CN)_6^{3-}]$ = 0.5 mmol dm^{-3}, [HU] = 15 mmol dm^{-3}. Ionic strength maintained by $[(CH_3)_4N]NO_3$.

where $[Fe(CN)_6^{3-}]_{tot} = [Fe(CN)_6^{3-}] + [NaFe(CN)_6^{2-}]$ and $[HU]_{tot} = [HU] + [U^-]$. No values for k_3 and k_4 can be calculated, unless value of the K_{NaFe} association constant is known. Using the literature data for association constants of hexacyanoferrate(III) with sodium ions (log K_{NaFe} = 0.7),[36] distribution of species under our experimental conditions can be calculated. Figure 12 clearly shows that at 2 mol dm^{-3} ionic strength the concentration of $Fe(CN)_6^{3-}$ is negligible compared to $NaFe(CN)_6^{2-}$, suggesting that the calculated second-order rate constants in fact correspond to the values of k_3 and k_4. In turn, it is clear that the obtained activation parameters, $\Delta H^{\neq}$ = 27 kJ mol^{-1} and $\Delta S^{\neq}$ = −76 J K^{-1} mol^{-1} are not related to the reaction path defined by Eq. (4) but rather by Eq. (9).

In the experiments in which the ionic strength was varied using tetramethylammonium nitrate, rate constants increases at low concentration of electrolyte, attains its maximum between 0.2 and 0.7 mol dm^{-3} ionic strength, and then falls again. Dependence of k_{obs} on ionic strength is shown in Figure 13. In this case however, the extended Debye-Hückel equation obviously fails to provide the linear correlation. However, the obtained dependence almost match the reported dependence of the standard reduction potential of $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ couple on the $(CH_3)_4N^+$ ion concentration. Since the rate of the redox reaction is independent of the anion used for maintaining the ionic strength (Figure 12), the observed effect must be related to the nature of cation in the supporting electrolyte. The initial increase in the rate constant upon the increase of $[(CH_3)_4N]NO_3$ is probably related to the greater extent of binding of cation to $Fe(CN)_6^{4-}$ than to $Fe(CN)_6^{3-}$ ($K_{(CH_3)_4NFe(CN)_6^{3-}} = 21$ mol^{-1} dm^3 and $K_{(CH_3)_4NFe(CN)_6^{2-}} = 1.8$ mol^{-1} dm^3)[38] that sharply rises the reduction potential.[37,39] The sharp reversal of the effect, as suggested by the authors, may indicate a specific salting-out effect exerted by this organic cation in terms of "structure promoting" influence on the solvent water. In our case however, the reversal of the effect could be related to the preferential formation of associate between the organic cation and anion that could increase the reduction potential of the reductant.

DISCUSSION

In this study we investigated the mechanism of oxidation of hydroxyurea by hexacyanoferrate(III) over a broad p[H$^+$] span. The stoichiometry coefficients in the redox reaction were found for HU and $Fe(CN)_6^{3-}$ to be 1 and 2, respectively. Recently, we reported that oxovanadium(V) ion is capable of oxidizing HU in acidic medium, and stoichiometric coefficients of reactants in this oxidation reaction were also found to be 1 and 2 for HU and V^V, respectively.[16] These results indicates that neither VO_2^+ nor $Fe(CN)_6^{3-}$ ions can oxidize HU to NO in which the oxidation state of nitrogen is +1, and the conversion of hydroxyurea to NO requires a three-electron oxidation (Eq. 12).

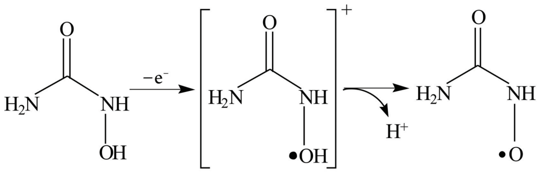


(12)

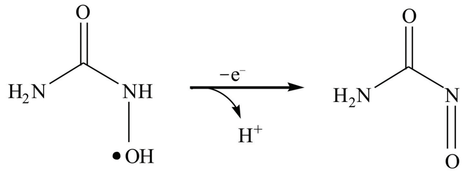
Indeed, the formation of NO could not be proven, either directly through the FT-IR spectrum of the gaseous products, or indirectly, via formation of nitrite or nitrate ions which are stable oxidative decomposition products of NO.[40]

It was shown that redox reactions of HU with various metal ions proceeds via formation of aminocarbonyloaminoxyl free radical that is subsequently transformed into the products. Our EPR measurements reveal that in oxidation of HU by $Fe(CN)_6^{3-}$ the very same free radical is formed, as the obtained spectrum parameters are in excellent agreement with the previously published EPR spectra of that free radical produced during the oxidation of HU either with Cu(II) and H_2O_2,[31] Fe^{3+} [32,33] or VO_2^+.[16] The fact that the same free radical intermediate and reaction products are formed when HU is oxidized at drastically different acidities and with entirely different types of oxidizing agents (one- *versus* two-electron acceptors, coordinating *versus* non-coordinating oxidant) suggests that neither the nature of the oxidizing agent nor the acidity of medium plays a major role in the reaction mechanism of the HU oxidation up to the N_2O oxidation state. This finding points to the potential physiological relevance of our results by providing possible insight into *in vivo* situation (*vide infra*).

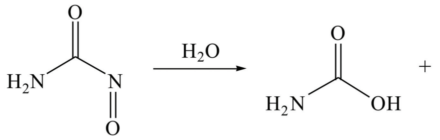
The formation of CO_2, NH_3, N_2O and $Fe(CN)_6^{4-}$ can be rationalized by the reaction mechanism similar to the one already proposed for oxidation of HU by H_2O_2 in neutral medium[8,14,41] (though in oxidation of HU by H_2O_2 the formation of NO was observed), and by Fe(III)[33] and V(V) in acidic medium.[16] Focusing only on HU, and for the sake of simplicity ignoring the full stoichiometry of each step, the oxidation of HU may be depicted by Eqs. (13) – (17).



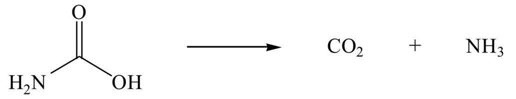
(13)



(14)



(15)



(16)

$$HNO + HNO \longrightarrow N_2O + H_2O \qquad (17)$$

A single-electron acceptor in steps (13) and (14) is the iron ion in $Fe(CN)_6^{3-}$. The radical produced in reaction (13) undergoes further oxidation in reaction (14), forming C-nitroso-formamide that hydrolyzes to carbamic acid and HNO. Carbamic acid decomposes yielding carbon dioxide and ammonia, while HNO is short-lived in aqueous solution, rapidly dimerizing ($k = 2–8 \times 10^9$ mol^{-1} dm^3 s^{-1} [42] and more recently published $k = 8 \times 10^6$ mol^{-1} dm^3 s^{-1} [43]) and dehydrating to nitrous oxide.

A lack of evidence for the NO formation in our reaction, contrary to the oxidation of HU with hydrogen peroxide but in line with the oxidation by Fe^{3+} [32] and VO_2^+ [16] ions, could be explained either by a higher reduction potential of the H_2O_2 ($E^0_{H_2O_2,H^+/H_2O}$ = +1.78 V)[44] than $Fe(CN)_6^{3-}$ ($E^0_{Fe(CN)_6^{3-}/Fe(CN)_6^{4-}}$ = + 0.41 V), Fe^{3+} ($E^0_{Fe^{3+}/Fe^{2+}}$ = + 0.77 V) and VO_2^+ ($E^0_{VO_2^+,H^+/VO^{2+}}$ = +1.00 V) couples, or by a much faster dimerization of HNO than its oxidation with those ions. The former explanation would therefore indicate a value for the standard reduction potential of NO/HNO couple under our experimental condition larger than +0.4 V, but a rather wide range reported for $E^0_{NO,H^+/HNO}$ (from as low as −1.6 V up to +0.4 V)[45] makes any conclusion regarding the formal reduction potential of the NO/HNO couple rather uncertain. Nevertheless, a quite low upper-limit value of reported $E^0_{NO,H^+/HNO}$ might be seen as in favor of the kinetic explanation for a lack of NO detection in our reaction, particularly considering the values of the reduction potentials for Fe^{3+} and VO_2^+ ions. This may also explain why under *in vivo* condition HU can act as an NO donating drug,[5,6] since the dimerization reaction cannot compete with the oxidation of HNO when the concentration of formed HNO may be very low.

The obtained kinetic results clearly confirm the expected outer-sphere electron transfer mechanism for oxidation of hydroxyurea by $Fe(CN)_6^{3-}$, characterized by two parallel oxidation pathways involving HU and U^- in the reactions with two $Fe(CN)_6^{3-}$ ions. The results also demonstrate that the $NaFe(CN)_6^{2-}$ ion-associate acts as the oxidant species rather than $Fe(CN)_6^{3-}$ ion. In a similar study of oxidation of ascorbic acid by $Fe(CN)_6^{3-}$ in acidic media Leal et *al.*[46] found that among other alkali ions the $NaFe(CN)_6^{2-}$ ions are two orders of magnitude more reactive than $Fe(CN)_6^{3-}$. The acceleration stresses an important role played by binding alkali metal ions as to bridge the cyanide ligands of the negatively charged iron complex with the negatively charged substrate.[47] The same argument is used to explain the mechanism of electron transfer between hexacyanoferrate(III) and hexacyanoferrate(II) either in solution or at electrodes. A relevant contribution to the role played by alkali metal cations can also be found in Jain and Nandel's work on the oxidation of sulfite ions with hexacyanoferrate(III).[48]

The rate determining step of the redox reaction is therefore formation of the free radical by the outer sphere one-electron transfer from either HU or U^- to $NaFe(CN)_6^{2-}$. The apparent rate of electron transfer to $NaFe(CN)_6^{2-}$ from U^- in 2 molar $NaClO_4$ at 25 °C is more than four orders of magnitude faster than the transfer from HU (k_3/k_4) = 3.6 × 10^3 mol^{-1} dm^3 s^{-1}/0.15 mol^{-1} dm^3 s^{-1} = 2.4×10^4), but in fact the difference is probably even larger. According to the standard Marcus-Hush model[49] outer-sphere electron transfer process consist of three steps of which the first and the third step are diffusion controlled and the second step involving irreversible electron transfer is the rate determining. The first step is formation of an ion-pair or outer-sphere complex, the second step is the electron-transfer, and the third one the dissociation of the product ion-pair. Under conditions where k_{obs} depends linearly on substrate concentrations, as found in this study, the measured second-order rate constant is equal to the product of the ion-pair (oxidant-substrate) formation constant and the electron-transfer rate constant, *i.e.* $k = k_{et}K_{OS}$. Assuming that the electron transfer occurs within the outer-sphere associates of two redox reactants, the rate constants for electron transfer could be calculated from the following expressions: $k_3^{e.t} = k_3 / K_{os}(HU\cdots NaFe(CN)_6^{2-})$ and $k_4^{e.t.} = k_4 / K_{os}(U^-\cdots NaFe(CN)_6^{2-})$. By use of the Fuoss equation[50] we calculated association constants $K_{os.}(HU\cdots NaFe(CN)_6^{2-})$ and $K_{os.}(U^-\cdots NaFe(CN)_6^{2-})$ in 2 mol dm^{-3} $NaClO_4$ as 1.2 mol^{-1} dm^3 and 0.7 mol^{-1} dm^3, respectively. These two values are in accordance with the observed liner dependence of k_{obs} *vs.* the hydroxyurea concentration up to 0.05 mol dm^{-3} (Figure 7). The calculated ratio $k_4^{e.t.}/k_3^{e.t.}$ = 3.66 × 10^4 corresponds to a difference in the free energies of activation for these two paths at 298 K of 26 kJ mol^{-1}, but extrapolation of k_4 to zero ionic strength ($K_{os.}(U^-\cdots NaFe(CN)_6^{2-})$ = 0.18 mol^{-1} dm^3) reduces the ratio to 2.87 × 10^3 and in turn to ca. 0.4 V lower reduction potential of U^- compared to that of HU.

The electron affinity of HU has been investigated by E. Wold *et al.*[51] who reported the formal reduction potential value of −0.552 V. This value appears to be much too low, for it would predict close to the diffusion controlled rate of oxidation of HU with ferricyanide. Therefore, it seemed worth to estimate the formal reduction potential for hydroxyurea from our kinetic data.

Assuming no primary salt effect of on the rate of redox reaction that involve neutral species and close values of K_{os} for the formation of associates between $NaFe(CN)_6^{3-}$ and various neutral species, it is possible to estimate the standard reduction potential for the $HU^{+\bullet}$/HU couple by comparing the obtained value of rate constant for oxidation of molecular form of hydroxyurea with the analogous values for oxidation of series of diazine derivatives.[52] A plot of logarithm of the second order rate constant for oxidation of diazines with $Fe(CN)_6^{3-}$ against the standard reduction potential of radicals formed from those compounds yields an excellent linear relationship (r = 0.9998) characterized by the slope equal to −10.6 ± 0.2 and the intercept equal to 8.1 ± 0.1. Combining the linear function with the obtained value of rate constant for oxidation of HU (k_3), the formal reduction potential for $HU^{+\bullet}$/HU couple can be estimated as +0.84 V. Furthermore, relating this value to the above predicted shift of the reduction potential caused by the deprotonation of HU, the formal reduction potential for $U^\bullet/U^-$ couple can be estimated as *ca.* +0.44 V.

The latter value can be checked by repeating calculation for U^- in an analogous way as the above carried out for HU. We have performed such a calculation using the data for reduction of ferricyanide with two monoanions for which reliable kinetic parameters and the reduction potentials are available from the literature: ascorbate monoanion (AH^-)[53,54] and SO_2^-.[55,56] A reasonable assumption of comparable values of K_{os} for all three monovalent anions with ferricyanide should allow calculation of the formal reduction potential of the $U^•/U^-$ couple from the assumed linear function for oxidation of AH^- and SO_2^- with ferricyanide, viz. $\log (k / \text{mol}^{-1} \text{dm}^3 \text{s}^{-1}) = -4.68 E^0 + 5.27$. Insertion of the rate constant for oxidation of U^- with ferricyanide projected for $I = 0.5$ mol dm^{-3} (the ionic strength used in study of ascorbate) into that equation results in calculation of the reduction potential $E^0 = +0.47$ V. This value is in better than expected agreement with the above prediction, considering the approximations applied and the fact that the data for SO_2^- were obtained at 0.1 mol dm^{-3} ionic strength and for which the prediction for 0.5 mol dm^{-3} ionic strength could not be done.

The obtained formal reduction potentials for two forms of hydroxyurea appear to be rather high considering that the oxidation of HU with hexacyanoferrate(III) is found to be an "irreversible" process. The observed absence of any effect of $Fe(CN)_6^{4-}$ concentration on rate of the HU oxidation eliminates application of the steady state approximation on the radical and eventual "reversibility" of the rate determining step. This indicates that the driving force for oxidation is thermodynamics of one of the reactions following the rate determining formation of aminocarbonyloaminoxyl free radical. From EPR spectrum of the free radical it is obvious that under the experimental condition used throughout this study the proton dissociation constant of free radical is large enough that the deprotonation of radical can be anticipated as one of the thermodynamic driving forces. In addition, it can be assumed that the formal reduction potential of the formed radical comparing to the potential of ferricyanide (ca. +0.4 V) is low enough to ensure that oxidation of radical with the second hexacyanoferrate(III) ion to C-nitroso-formamide (Eq. 14) is extremely fast and "irreversible" making also this reaction a driving force for the overall redox process. A similar behaviour was reported for ascorbate monoanion, where the formed ascorbate free radical was found to be four orders of magnitude more active for ferricyanide than ascorbate itself.[57]

The dominating reaction path involving hydroxamate anion is characterized by a relatively low apparent activation enthalpy, $\Delta H^{\neq}_{\text{app.}} = 27(4)$ kJ mol^{-1} making favorable the rate of electron transfer. Since the reaction enthalpy for the formation of $NaFe(CN)_6^{2-}$ could be estimated close to 2 kJ mol^{-1}, judged from the data reported for the formation of $KFe(CN)_6^{2-}$,[34] the activation enthalpy can be estimated as $\Delta H^{\neq} \leqq 25$ kJ mol^{-1}. Therefore, relative slowness of the studied redox reaction is mainly caused by a negative apparent activation entropy found for this process, $\Delta S^{\neq}_{\text{app.}} = -76(15)$ J K^{-1} mol^{-1}. A negative entropy is indicative of the formation of a highly ordered transition state, as a consequence of the pre-equilibrium formation of $U^- \cdots Na^+ \cdots Fe(CN)_6^{3-}$ outer-sphere associate. Activation entropy for the rate determining step in which an electron is transferred from U^- to ferricyanide can be estimated as $\Delta S^{\neq} = -109$ J K^{-1} mol^{-1} from the reaction entropy for the formation of $NaFe(CN)_6^{2-}$ species, which can be roughly projected from the analogous value for $KFe(CN)_6^{2-}$ that was found to be +33 J K^{-1} mol^{-1}.[34]

Acknowledgements. The authors gratefully acknowledge financial support from Croatian Ministry of Science, grant number 0006-0061247-0009. We thank Dr. Tomislav Biljan (Faculty of Natural Sciences, University of Zagreb) for performing FT IR measurements.

REFERENCES AND NOTES

1. S. Nyholm, L. Thelander, and A. Graeslund, *Biochemistry* **32** (1993) 11569–11574.
2. B. Zhou, S. Mi, X. Mo, J. Shih, J. Tsai, E. Hu, M. Hsu, K. Kay, and Y. Yen, *Anticancer Res.* **22** (2002) 1369–1377.
3. S. Charache, M. L. Terrin, R. D. Moore, G. J. Dover, F. B. Barton, S. V. Eckert, R. P. McMahon, and D. R. Bonds, *New Engl. J. Med.* **332** (1995) 1317–1322.
4. R. P. Ferguson, A. Arun, C. Carter, S. D. Walker, and O. Castro, *Am. J. Hematolo.* **70** (2002) 326–330.
5. C. J. Marmion, T. Murphy, K. B. Nolan, and J. R. Docherty, *Chem. Commun.* (2000) 1153–1154.
6. J. W. Rupon, S. R. Domingo, S. V. Smith, B. K. Gummadi, H. Shields, S. K. Ballas, S. B. King, and D. B. Kim-Shapiro, *Biophys. Chem.* **84** (2000) 1–11.
7. J. Huang, S. B. Hadimani, J. W. Rupon, S. K. Ballas, D. B. Kim-Shapiro, and S. B. King, *Biochemistry* **41** (2002) 2466–2474.
8. N. S. Kwon, D. J. Stuehr, and C. F. Nathan, *J. Exp. Med.* **174** (1991) 761–767.
9. R. E. Glover, E. D. Ivy, E. P. Orringer, H. Maeda, and R. P. Mason, *Mol. Pharmacol.* **55** (1999) 1006–1010.
10. M. T. Gladwin, J. H. Shelhamer, F. P. Ognibene, M. E. Pease-Fye, J. S. Nichols, B. Link, D. B. Patel, M. A. Jankowski, L. K. Pannell, A. N. Schechter, and G. P. Rodgers, *Brit. J. Haematol.* **116** (2002) 436–444.
11. V. P. Cokic, R. D. Smith, B. B. Beleslin-Cokic, J. M. Njoroge, J. L. Miller, M. T. Gladwin, and A. N. Schechter, *J. Cliv. Invest.* **111** (2003) 231–239.
12. R. Bundy, N. Marczin, A. H. Chester, and M. Yacoub, *Am. J. Physiol.-Heart Circ. Physiol.* **277** (1999) 1799–1807.
13. J. Huang, E. M. Sommers, D. B. Kim-Shapiro, and S. B. King, *J. Am. Chem. Soc.* **124** (2002) 3473–3480.
14. S. B. King, *Free Radical Biol. .Med.* **37** (2004) 737–744.
15. A. Bedrica, M. Biruš, N. Kujundžić, and M. Pribanić, *Croat. Chem. Acta* **61** (1980) 21–31.
16. M. Gabričević, E. Bešić, M Biruš, A. Zahl, and R. van Eldik, *J. Inorg. Biochem.* **100** (2006) 1606–1613.
17. I. Vinković-Vrček, M. Biruš, and M. Buehl, *Inorg. Chem.* **46** (2007) 1488–1501.

18. J. M. Leal, B. Garcia, and P. L. Domingo, *Coord. Chem. Rev.* **173** (1998) 79−131.
19. B. S. Thyagarajan, *Chem. Rev.* **58** (1958) 439−460.
20. D. E. Khoshtariya, A. M. Kjaer, T. A. Marasgishvili, and J. Ulstrup, *J. Phys. Chem.* **96** (1992) 4154 – 4156.
21. P. Gans and B. O'Sullivan, *Talanta* **5**1 (2000) 33−37.
22. A. E. Martell and R. J. Motekaitis, in *Determination and Use of Stability Constants*, VCH Weinheim, 1988, Chapter 1, pp. 7−19.
23. P. Gans, A. Sabatini, and A. Vacca, *J. Chem. Soc., Dalton Trans.* (1985) 1195−1200.
24. P. L. Domingo, B. García, and J. M. Leal, *Can. J. Chem.* **68** (1990) 228−235.
25. Hereafter, figures in parentheses are single standard deviations of the parameters expressed in terms of the last significant digit.
26. I. Vinković-Vrček, I. Kos, T. Weitner, and M. Biruš, *J. Phys. Chem. A*, **112** (2008)11756−11768.
27. A. I. Vogel, *A Text-Book of Quantitative Inorganic Analysis - Theory and Practice*. 5th Ed.; Longman: London, **1955**.
28. K. Nakamoto, *Infrared and Raman Spectra of Inorganic and Coordination Compounds: Theory and Applications in Inorganic Chemistry,* 5th. Ed. ed.; John Wiley and Sons: New York, 1997.
29. K. Grasshof, K. Kremling, and M. Ehrhardt, (Ed.), *Methods of Seawater Analysis*, Wiley-VCH, Weinheim, 1999, pp. 188.
30. D. F. Boltz, *Colorimetric Determination of Nonmetals*, Interscience Publishers, New York, 1958.
31. G. Lassmann and B. Liermann, *Free Radic. Biol. Med.* **6** (1989) 241 – 244.
32. N. Kujundžić, B. Nigović, and K. Sanković, *Z. Anorg. Allg. Chem*. **630** (2004) 2749−2753.
33. B. Nigović, B. N. Kujundžić, and K. Sanković, *Eur. J. Med. Chem.* **40** (2005) 51−55.
34. A. W. Eaton, P. George, and G. I. H. Hanania, *J. Phys. Chem*. **7** (1967) 2016−2021.
35. G. R. Wilkins, *Kinetics and Mechanisms of Reactions of Transition Metal Complexes,* VCH: Weineim, 1991.
36. S. Motomizu and T. Takayanagi, *J. Chromatogr. A* **853** (1999) 63−69.
37. G. I. H. Hanania, D. H. Irvine, W. A. Eaton, and P. George, *J. Phys. Chem.* **71** (1967) 2022−2030.
38. D. W. Larsen and A. C. Wahl, *Inorg. Chem.* **4** (1965) 1281−1286.
39. S. A. Kostina, V. Kotov, and G. A. Tsirlina, *Mendeleev Commun*. (2001) 85−124.
40. D. A. Wink, J. F. Darbyshire, R. W. Nims, J. E. Saavedra, and P. C. Ford, *Chem. Res. Toxicol.* **6** (1993) 23−27.
41. Y. Xu, C. D. Mull, C. L. Bonifant, G. Yasaki, E. C. Palmer, H. Shields, S. K. Ballas, D. B. Kim-Shapiro, and S. B. King, *J. Org. Chem*. **63** (1998) 6452−6453.
42. F. T. Bonner and M. N. Hughes, *Comments Inorg. Chem*. **7** (1988) 215−234.
43. V. Shafirovich and S. V. Lymar, *Proc. Natl. Acad. Sci. USA* **99** (2002) 7340−7345.
44. Hereafter all the reduction potentials are expressesd in V vs. NHE.
45. M. D. Bartberger, W. Liu, E. Ford, K.M. Miranda, C. Switzer, J. M. Fukuto, P. J. Farmer, D. A. Wink, and K. N. Houk, *Proc. Natl. Acad. Sci. USA* **99** (2002) 10958−10963.
46. J. M. Leal, P. L. Domingo, B. Garcia, and S. Ibeas, *J. Chem. Soc., Faraday Trans.* **89** (1993) 3571−3577.
47. M. J. Pregel and E. Buncel, *J. Am. Chem. Soc.* **115** (1993) 10−14.
48. D. V. S. Jain and F. S. J. Nandel, *Chem. Soc. Dalton* (1977) 949−951.
49. R. A. *Marcus, Annu. Rev. Phys. Chem.* **15** (1964) 155−196.
50. R. D. Cannon, *Electron Transfer Reactions*, Butterworths, London 1980.
51. E. Wold, O. Kaalhus, E. S. Johansen, and A. T. Ekse, *Int. J. Radiat. Biol.* **38** (1980) 599−611.
52. C. R. Dennis, A. J. Van Wyk, S. S. Basson, and J. G. Leipoldt, *Inorg. Chem*. **26** (1987) 270−272.
53. B. Bänsch, P. Martinez, J. Zuluaga, D. Uribe, and R. van Eldik, *Z. Phys. Chem.* **170** (1991) 59−71.
54. N. H. Williams and J. K. Yandell, *Aust. J. Chem.* **35** (1982) 1133−1144.
55. S. G. Mayhew, *Eur. J. Biochem.* **85** (1978) 535−547.
56. C. W. J. Scaife and R. G. Wilkins, *Inorg. Chem.* **19** (1980) 3244−3247.
57. T. Iyanagi, I. Yamazaki, and K. F. Anan, *Biochim. Biophys. Acta* **806** (1985) 255−261.

SAŽETAK

Kinetika i mehanizam oksidacije hidroksiuree s heksacijanoferatovim(III) ionima u vodenoj otopini

Ana Budimir, Erim Bešić i Mladen Biruš

Farmaceutsko biokemijski fakultet, Sveučilište u Zagrebu, A. Kovačića 1, 10000 Zagreb, Hrvatska

U neutralnim i lužnatim vodenim otopinama, hidroksiurea (HU) uspješno reducira $Fe(CN)_6^{3-}$ u $Fe(CN)_6^{4-}$ elektronskim prijenosom koji uključuje stvaranje i postupno nestajanje slobodnog radikala, $U^{\bullet}$ ($U^{\bullet} \equiv H_2N{-}C({=}O)N(H)O^{\bullet}$). EPR spektar $U^{\bullet}$ u vodi upućuje da je nespareni elektron uglavnom smješten na hidroksamatnom kisiku hidroksilne skupine. Spektrofotometrijski podaci u vidljivom području pokazuju da je HU dvo-elektronski reducens. Stehiometrija istraživane reakcije je : $2\ Fe(CN)_6^{3-} + NH_2CONHOH + ½2H_2O \rightleftarrows 2\ Fe(CN)_6^{4-} + CO_2 + NH_3 + ½N_2O + 2H^+$. Izostanak stvaranja NO je vjerojatno posljedica brze dimerizacije HNO u usporedbi s brzinom njegove oksidacije, koja je spora zbog niskog redukcijskog potencijala redoks para $Fe(CN)_6^{3-} / Fe(CN)_6^{4-}$.

Kinetika oksidacije HU s $Fe(CN)_6^{3-}$ je istražena tehnikom zaustavljenog toka (stopped-flow), određivanjem ovisnosti o koncentraciji H^+, HU, $Fe(CN)_6^{3-}$ i $Fe(CN)_6^{4}$, te o ionskoj jakosti i temperaturi. Kinetički rezultati pokazuju da se oksidacija HU s $Fe(CN)_6^{3-}$ odvija prijenosom elektrona u vanjskoj sferi ovog posljednjeg (outer-sphere electron transfer). Učinak ionske jakosti na brzinu reakcije pokazuje da je reakcijska vrsta $NaFe(CN)_6^{2-}$ a ne $Fe(CN)_6^{3-}$ ion. Brzina redoks reakcije je prvog reda u odnosu na HU i $Fe(CN)_6^{3-}$, a ovisnost o koncentraciji H^+ iona pokazuje da je reaktivnost U^- oko 10000 puta veća nego HU. Formalni redukcijski potencijali za parove $U^{\bullet}/U^-$ i $HU^{\bullet}/HU$ su procijenjeni iz kinetičkih mjerenja na +0,47 V odnosno +0,84 V.