

Short communication

# Reaction of Hydroxyurea with Iron(III): Formation of Mono- and Bis(hydroxyureato)-iron(III) Complexes

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## Abstract

Complex formation reaction between iron(III) and hydroxyurea in acidic aqueous and dimethylformamide solutions were studied by UV/VIS spectroscopy, cyclic voltammetry and EPR spectroscopy. Under conditions of an excess of hydroxyurea over iron(III), mono- and bis(hydroxyureato)iron(III) complexes are formed. The complexes quickly decompose in aqueous solutions, but are relatively stable in dimethylformamide. Equilibrium quotients for the formation of the mono- and bis- complexes in acidic aqueous solution at 25 °C and  $I = 2.0 \text{ mol dm}^{-3}$  (maintained by  $\text{NaClO}_4$ ) are found to be 0.91 and 0.15, respectively. The kinetic results suggest that the formation of bis complex is much faster process. The cyclic voltammogram exhibits one well-defined quasi-reversible cathodic peak at +0.29 V in dimethylformamide. The redox potential was shifted to less positive values by 140 mV compared to the solvated iron(III) ion due to electron donating effect of ligand molecule. EPR spectra of the radical formed in the solution show presence of the radical species  $\text{H}_2\text{N-CO-NHO}^\bullet$  suggesting the same degradation pathway found in the mono(hydroxyureato)iron(III) complex.

**Keywords:** Hydroxyurea; Mono- and Bis(hydroxyureato)iron(III) complex; Complex stability; Hydroxyurea radical

## 1. Introduction

Hydroxyurea,  $\text{H}_2\text{NC(O)NHOH}$ , has been a compound of scientific and clinical interest for over century. This small molecule, shows many biological activities and are widely used in therapy of several human diseases, including melanoma, chronic myelogenous leukemia, ovarian cancer, psoriasis, polycythemia vera, and sickle cell anemia.<sup>1</sup> In addition, hydroxyurea has been investigated as adjunctive therapy for HIV infection.<sup>2</sup>

Hydroxyurea inhibits DNA synthesis by inhibiting ribonucleotide reductase activity, but does not affect RNA or protein synthesis.<sup>3</sup> The enzyme catalyses the *de novo* biosynthesis of deoxyribonucleotides required for DNA synthesis by reducing the corresponding ribonucleotides.<sup>4</sup> Ribonucleotide reductase is the rate limiting enzyme of DNA synthesis and shows an increase in the activity associated with proliferation and malignant transformation. It was therefore considered to be a target for antitumor and anti-HIV therapy.<sup>5</sup>

In addition to tyrosyl free radical, protein R2, a small subunit of ribonucleotide reductase, contains a

dinuclear  $\mu$ -oxo-bridged iron(III) centre which appear to be essential for the enzyme activity. Iron acts as a cofactor for generation and stabilization of the free tyrosyl radical.<sup>6</sup> The molecular mechanisms describing hydroxyurea action are not fully understood. Inhibition of the enzyme can be caused by scavenging of a free tyrosyl radical or iron sequestering. In active *E. coli* protein R2, hydroxyurea scavenges the tyrosyl radical without affecting the iron center.<sup>7,8</sup> In contrast, hydroxyurea reacts with both the iron center and the radical in mouse protein R2, followed by the release of iron from the protein in the iron(II) form.<sup>9</sup>

It is known that hydroxyurea form a complex with iron(III).<sup>10</sup> Some reports in the literature suggest that only hydroxyureas that are capable of complexing metals show antitumour activity.<sup>11</sup> Thermodynamic and kinetic information relating to the complexation of iron(III) by hydroxyurea are of utmost importance for the understanding of the molecular basis of hydroxyurea action. It has been recently proposed that this reaction may serve as a simple model for testing the assumed hydroxyurea like activity for chemical substances.<sup>12</sup>

In the present work, the formation of the bis(hydroxyureato)iron(III) complex has been investigated with goal to complete description of the iron(III)-hydroxyurea reaction pattern.

## 2. Experimental

### 2.1. Chemicals

Iron(III) perchlorate and sodium perchlorate were purchased from Aldrich. Iron(III) stock solution was prepared and standardized as described previously.<sup>13</sup> Stock solution of  $\text{NaClO}_4$  was standardized by titration of protons released on a cation exchange resin column. Hydroxyurea was purchased from Sigma and their reagents solutions were prepared by dissolving the solid immediately before the measurements were made. Hydrogen ion concentration in the stock solution was determined as described previously.<sup>10</sup> In all experiments where the solvent was water  $\text{NaClO}_4$  was added to produce final ionic strength of 2.0 M. Water used in the experiments was doubly distilled from alkaline  $\text{KMnO}_4$  in a glass apparatus. All other chemicals were of analytical grade and were used without further purification.

### 2.2. Methods

The interaction of iron(III) with hydroxyurea in aqueous solutions was investigated using a Dionex stopped-flow apparatus linked with a Harrick rapid-scan monochromator equipped with a thermostated cell compartment. The UV/visible spectra were recorded on an Agilent 8453 UV-Visible diode-array spectrophotometer. All experiments were performed at  $25 \pm 0.1$  °C. The total concentrations of hydrogen ion in the experiments were calculated by summation of added  $\text{HClO}_4$  and the proton released by the hydrolysis of iron(III) species present in the solutions. The pseudo first order conditions were ensured by holding one reactant in excess over the other. The calculations were done as described earlier.<sup>14</sup>

Voltammetry experiments were performed using an EG&G Princeton Applied Research Model 273A potentiostat. The three-electrode system was composed of a platinum working electrode ( $\varnothing = 2$  mm, EG&G/PAR), a platinum auxiliary electrode and an Ag/AgCl reference electrode, which was separated from the sample solution by a Vycor glass frit. The studied complexes were generated in situ by addition of an appropriate amount of the iron(III) perchlorate stock solution to a solution of hydroxyurea in dimethylformamide containing 0.1 M lithium perchlorate as supporting electrolyte. The ligand concentration was kept at  $5 \times 10^{-2}$  M, and the metal ion was varied from  $5 \times 10^{-4}$  to  $2.5 \times 10^{-3}$  M.

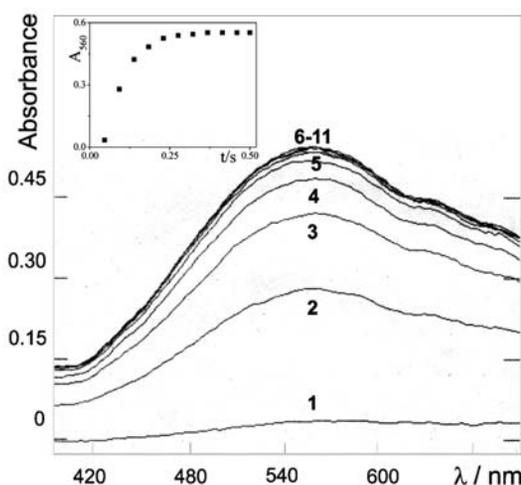
To provide a reproducible active surface and improve the sensitivity and resolution of voltammetric peaks, the working electrode was polished prior to each electro-

chemical measurement with 0.5  $\mu\text{m}$  alumina powder on a polishing cloth. Then, it was thoroughly rinsed with methanol and doubly-distilled water, and gently dried with tissue paper. All the solutions examined using electrochemical techniques were degassed with solvent-saturated nitrogen for five minutes. The samples were blanketed with nitrogen during acquisition. All measurements were performed with positive feedback iR compensation and were carried out at room temperature.

The EPR measurements were carried out using a Bruker ELEXSYS E500 EPR spectrometer. The spectra were recorded immediately after mixing of iron(III) with hydroxyurea in dimethylformamide at 25 °C with microwave frequency 9.85 GHz and microwave power of 2 mW.

## 3. Results and Discussion

The first step in the interaction of iron(III) with hydroxyurea was described in a previous paper.<sup>12</sup> When an aqueous iron(III) solution is mixed with an molar excess of hydroxyurea, a blue colored complex is formed which quickly decomposes. Therefore, the rapid-scan stopped-flow technique was used to record the spectra of the complex formed. The spectra obtained in 0.5 seconds (Fig. 1) show similar shape as in case of mono(hydroxyurea-

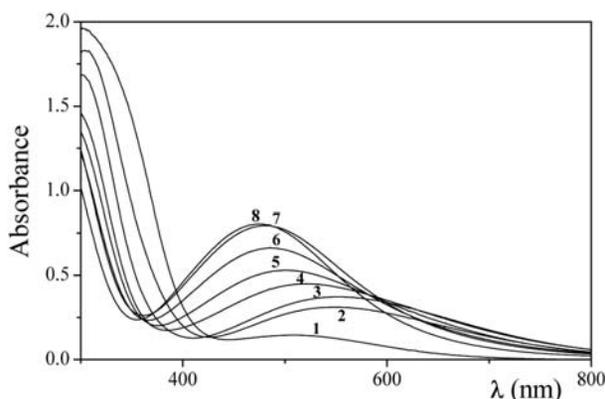


**Fig. 1:** Visible spectra recorded after mixing solution of hydroxyurea ( $1.15 \times 10^{-1}$  M) with iron(III) ( $1.15 \times 10^{-3}$  M). The last spectrum was taken after 0.5 seconds. Conditions:  $c_{\text{H}^+} = 4 \times 10^{-2}$ ,  $I = 2.0$  M ( $\text{NaClO}_4$ ),  $T = 25$  °C. Inset: The absorbance at 560 nm versus time dependence for the same reaction.

to)iron(III) complex ( $\lambda_{\text{max}} = 560$  nm) but with much higher absorptivity than one would expect from molar absorptivity of mono complex ( $\epsilon = 400$   $\text{M}^{-1} \text{cm}^{-1}$ ). It suggests formation of higher complex species such as bis(hydroxyureato)iron(III). Results of spectrophotometric titrations at two  $\text{H}^+$  ion concentration are depicted in

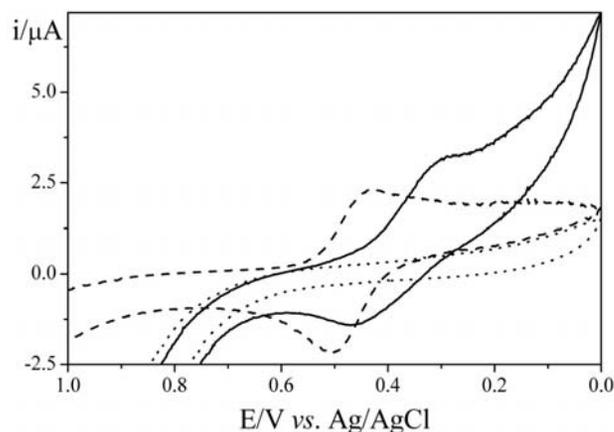


Due to the complex instability in water, the complex formation was studied in dimethylformamide by UV-visible spectroscopy. The changes in absorbance at the wavelength of maximum absorption were recorded for different ligand to metal ratios (Fig. 4). Spectral changes accompanying the increase of hydroxyurea in solution show that the absorption peak at 580 nm increases with the amount of ligand added. As the ligand concentration increase further, the absorption maxima have been shifted to shorter wavelengths ( $\lambda_{\max} = 480$  nm). With increasing hydroxyurea concentration, the change of initial blue colour to red-violete was noticed indicating the formation of bis-complex species.



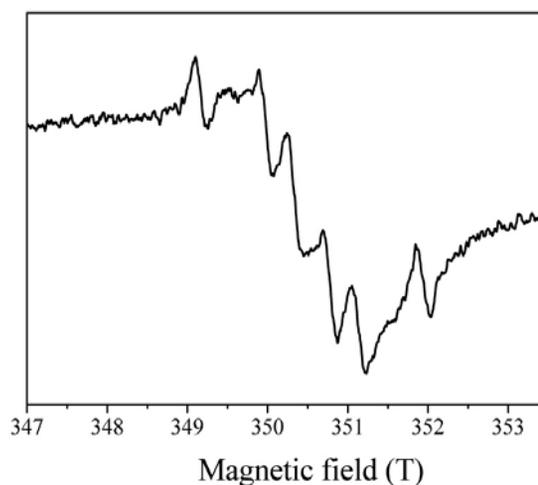
**Fig. 4:** Absorbance spectra of the iron(III) complex with hydroxyurea in dimethylformamide at various ligand concentrations. Conditions:  $c_{\text{Fe}} = 5 \times 10^{-4}$  M,  $c_{\text{HU}} = 0$  (1),  $2.5 \times 10^{-4}$  (2),  $5 \times 10^{-4}$  (3),  $2.5 \times 10^{-3}$  (4),  $5 \times 10^{-3}$  (5),  $1 \times 10^{-2}$  (6),  $1.5 \times 10^{-2}$  (7) and  $3 \times 10^{-2}$  M (8),  $T = 25$  °C.

The electrochemical studies of a solution containing iron(III) and excess of hydroxyurea were carried out in dimethylformamide, in which the complex was stable on the timescale of the cyclic voltammetry measurements. The voltammograms were recorded in the range +1 to 0 V at 100 mV/s scan rate. The cyclic voltammograms of hydroxyurea, its complex with iron(III) and free iron(III) species are shown in Fig. 5. The cyclic voltammogram of hydroxyurea shows that the free ligand is electrochemically inactive in the potential range investigated. The voltammogram of the iron(III) in noncomplex form exhibits reversible one-electron reduction wave at +0.43 mV. The complex was immediately formed after addition of a iron(III) to the ligand solution and one well-defined quasi-reversible cathodic peak was observed at +0.29 V. As a result of complex formation, the redox potential was shifted to less positive values by 140 mV compared to the solvated iron(III) ion due to electron donating effect of ligand molecule. The reduction peak found for the mono(hydroxyureato)iron(III) complex was at +0.34 V.<sup>17</sup> Due to the increased electron density at the iron centre, reduction at a less positive potential would be expected for bis(hydroxyureato)iron(III) complex.



**Fig. 5:** Cyclic voltammograms of hydroxyurea (dotted line), its complex with iron(III) for  $c_{\text{L}}/c_{\text{Fe}} = 50:1$  (solid line) and free iron(III) species (dashed line). Conditions:  $c_{\text{HU}} = 5 \times 10^{-2}$  M, dimethylformamide and 0.1 M  $\text{LiClO}_4$ ; Pt working electrode at 100  $\text{mVs}^{-1}$  scan rate, potential expressed with respect to Ag/AgCl.

The formation of the free radical was monitored by EPR spectroscopy. The room temperature EPR analysis under conditions of an excess of hydroxyurea over iron(III) in dimethylformamide solution revealed a distinct six-line resonance pattern characteristic of the aminocarbonylaminoxyl radical (Fig. 6). The spectral parameters were:  $a_{\text{N}} = 8.09$  and  $a_{\text{H}}^{\text{NH}} = 11.69$  gauss, the  $g$ -value was 2.0087. The spectrum parameters were identical with reported data of the radical generated when iron(III) was presented in a molar excess over hydroxyurea.<sup>17</sup> The spectrum is also identical with the reported spectra of the radical produced by the oxidation of hydroxyurea with excess hydrogen peroxide or oxyhaemoglobin.<sup>18–20</sup> The radical was formed immediately after the reaction was started as a result of the electron transfer from hydroxyurea to



**Fig. 6:** EPR spectrum recorded under conditions of an excess of hydroxyurea over iron(III) in dimethylformamide solution at room temperature with microwave frequency 9.85 GHz and microwave power of 2 mW. Conditions:  $c_{\text{Fe}} = 1 \times 10^{-3}$  M,  $c_{\text{HU}} = 5 \times 10^{-2}$  M.

iron(III). The radical species that would include iron and hydroxyurea was not found. This observation further proves the proposed mechanism of the decomposition of mono(hydroxyureato)-iron(III) complex that we have described previously.

## 4. Conclusions

Under conditions of an excess of hydroxyurea over iron(III), mono- and bis(hydroxyureato)iron(III) complexes are formed. The obtained values for the equilibrium quotients and molar absorptivities of the two complex species are:  $K_1 = 0.91 \pm 0.05$ ,  $K_2 = 0.15 \pm 0.015$ ,  $\epsilon_1 = 590 \pm 30 \text{ M}^{-1}\text{cm}^{-1}$  and  $\epsilon_2 = 1000 \pm 40 \text{ M}^{-1}\text{cm}^{-1}$ . The complexes quickly after the formation undergoes redox processes in which they decomposes forming the aminocarbonylaminooxyl radical.

## 5. Acknowledgements

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## Povzetek

Nastanek kompleksov železa(III) s hidroksiureo v vodnih in v dimetilsulfoksidnih raztopinah smo proučevali z UV/VIS spektroskopijo, ciklično voltometrijo in EPR spektroskopijo. Pri prebitku hidroksiuree nad železom(III) nastanejo mono- in bis(hidroksiureato)železovi(III) kompleksi, ki v vodnih raztopinah hitro razpadejo, so pa bolj stabilni v DM-SO kot topilu. Študirali smo ravnotežje in kinetiko nastanka kompleksov. Ciklična voltometrija v dimetilformamidu daje kvazi-reverzibilen katodni pik pri +0.29 V, ki je in pomaknjen za 140 mV proti manj pozitivnim vrednostim v primerjavi z solvatiranim železovim(III) iono zaradi elektron-donorskega vpliva ligandov. S pomočjo EPR spektrov, ki kažejo na prisotnost radikala  $\text{H}_2\text{N}-\text{CO}-\text{NHO}^\bullet$ , smo predpostavili mehanizem razpada kompleksov.