

Analytical and Biological Implications of Complex Combinations of Hydroxyurea with Iron (II)

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Hydroxyurea (Hy) in aqueous solutions has several tautomeric forms due to the formation of a >N-OH bond. The azometinic group >C=N- is present in all these tautomeric forms. The structural forms influence the hydrophil-lipophil character of Hy and it determines the complexing of the Fe(II) ions, thus explaining the appearance of anemia, due to the treatment with hydroxyurea. The paper presents a spectrophotometric absorption method in UV through which Fe(II) is determined quantitatively using Hy as reagent, as well as the complexing percentage of these ions from the organism following the administration of the medicine. The complex formed by Hy with the Fe(II) ions is stable in water for a pH = 7.4-7.6 with a maximum of absorption at 267nm and molar absorptivity of $\epsilon = 3.4559 \cdot 10^4 \cdot \text{mol}^{-1} \cdot \text{L} \cdot \text{cm}^{-1}$. The combination rate has been established using the isomolar series method at 1:1 (M/L). The calculated value of the stability constant is $1.26 \cdot 10^6$. The Lambert-Beer law is observed in the concentration interval 10-60 μg Fe(II), in a final volume of 10 mL. The correlation coefficient (r^2) is 0.9999, the standard deviation (SD) = 0.0114 ($n = 6$). The detection limit (LD) is 2.71 $\mu\text{g/mL}$ and the quantification limit (LQ) is 9.04 $\mu\text{g/mL}$. Under these conditions, the complexing reaction of Fe(II) is interfered by the cations of Fe(III), Co(II). The proposed method has been applied successfully in order to determine Fe(II) from pharmaceutical products and the Hy-Fe(II) complex present in the urine of the patients that underwent treatment with Hydrea of 500mg for more than ten years.

Key words: Hy, Hy-Fe(II) complex, spectrophotometric determination

Hydroxyurea (fig. 1) inhibits the enzyme ribonucleotide reductase, responsible for the conversion of the ribonucleotides in deoxyribonucleotides, blocking the AND synthesis and determining the stopping of the cellular division [1-3]. For this reason, it is used in the treatment of numerous types of cancer. Hy reduces the plasmatic clearance of ferum, as well as its use by the erythrocytes being the cause of some severe effects such as anemia. The pharmacological effects are determined by the structural modifications from inside the molecules due to the solvent [4]. In contact with water, Hy has several tautomeric forms in equilibrium (fig. 2) in which the solvent acts as a catalyser on the >N-H bond resulting in the formation of the >N-OH bond. The IR spectra have a strong absorbance at 1650 cm^{-1} . due to the presence of the >C=N-bond (the same as for the Schiff bases and bis bases used as reagents for determining some cations)[5]. Studies on these tautomeric forms have been carried out through NMR, HPLC and quantitative determinations [6-8].

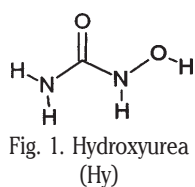


Fig. 1. Hydroxyurea (Hy)

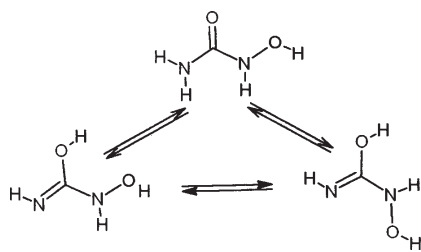


Fig. 2. Conformational and Tautomeric System of Hydroxyureas (Hy)

Different structural forms can prevail "in vivo" depending on the polarity of the medium and the prevailing structure influences the transport, the passing through membranes, the partition and the interaction with the ions [9].

Based on these tautomeric forms in aqueous solutions, an "in vitro" study has been carried out regarding the complexing capacity of Hy with Fe(II). The formed complex at pH 7.4-7.6 is extremely stable and it has a maximum of absorption at 267nm.

The results of the study have been materialized through a determination method of Fe(II) from pharmaceutical products, but also of the Hy-Fe(II) complex present in the urine of the patients that underwent treatment with Hydrea of 500mg for more than ten years. The presence of the complex in the urine of the patients explains the anemia as a side effect.

Experimental part

Materials and methods

- Stock solution Fe(II) 0.1 mg/mL: 0.07021g $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ is dissolved in 100 mL distilled water, then from this, standard solutions are prepared through dilution, with concentrations ranging between 10-60 $\mu\text{g/mL}$; (fresh solutions);
- Reagent solution Fe(II) 30 $\mu\text{g/mL}$ in distilled water (fresh solutions);
- Standard solution of Fe(II) 10^{-4}M , $5 \cdot 10^{-4}\text{M}$, 10^{-5}M ;
- Reagent solution Hy 10^{-4}M , $5 \cdot 10^{-4}\text{M}$, 10^{-3}M in distilled water (it is used after 24 h);
- Reagent solution Hy 40 $\mu\text{g/mL}$ in distilled water (it is used after 24 h);
- Buffer solution of phosphate: KH_2PO_4 - Na_2HPO_4 0.2 M, (pH= 7-8.6);
- Pharmaceutical product: FERRO-FOLGAMMA capsules;
- Biological fluid: 24 h urine sample;
- UV-VIS Hewlett-Packard 8453 Spectrophotometer.

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General method for the quantitative determination of Fe(II)

An aliquot of a sample solution containing 10-60 μg Fe(II) is transferred into a series of 10 mL calibrated flasks. A volume of 1 mL of buffer solution with pH 7.6, followed by 1 mL reagent solution Hy 40 μg and distilled water are added to it. After 15 min, the extinction of the complex is read at 267 nm against a witness solution.

Quantitative determination of Fe(II) from pharmaceutical products (gelatins capsules): an amount ranging between 0.30 and 0.40 g is measured from the content of a capsule. It is brought to a calibrated flask of 50 mL with distilled water. 1 mL of sample solution is diluted with distilled water up to 10 mL. 1 mL of diluted solution is processed according to the general method for the determination of Fe(II).

Method for the determination of hydroxyurea and hydroxyurea -Fe(II) complex eliminated by biological fluid in 24h: 1 mL of a biological fluid sample collected in 24 h is diluted with saline solution up to 10 mL. 1 mL solution

Fe(II) 30 μg is treated with 1 mL of buffer solution with pH = 7.6, 1 mL diluted biological fluid sample and distilled water are used to obtain 10 mL and then the solution is stirred well. After 15 min the extinction of the complex is read at 267 nm against a witness (the obtained absorbance value corresponds to the total hydroxyurea: the free hydroxyurea and the hydroxyurea from Hy-Fe(II) complex which is excreted). 1 mL solution Fe(II) 30 μg is treated with 1 mL of buffer solution with pH = 7.6, 1 mL reagent solution Hy 40 μg and distilled water are used to obtain 10 mL and then the solution is stirred well. After 15 min the extinction of the complex is read at 267 nm against a witness (the obtained absorbance corresponds to free hydroxyurea which is excreted).

Results and discussions

Study of the reaction of hydroxyurea with Fe(II)

An analytic study has been carried out on the complexing reaction of Fe(II) by the hydroxyurea, in order to establish the best working conditions:

a) Hydroxyurea and the Fe(II) form a complex stable in water that has a maximum peak at 267 nm (fig. 3).

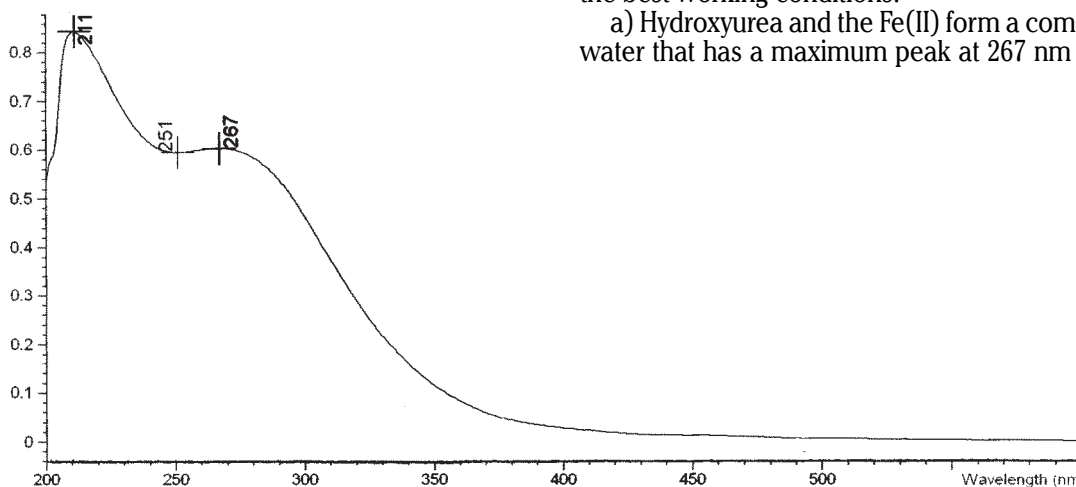


Fig. 3. Hy-Fe(II) complex

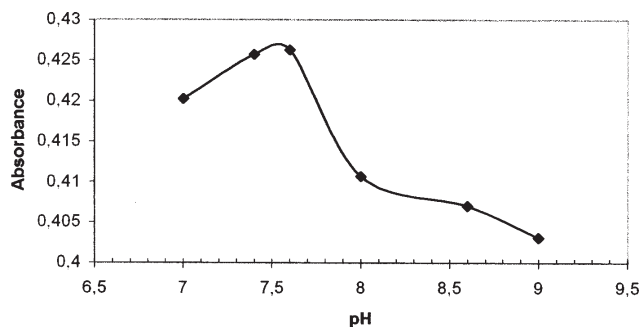


Fig.4. Influence of the pH

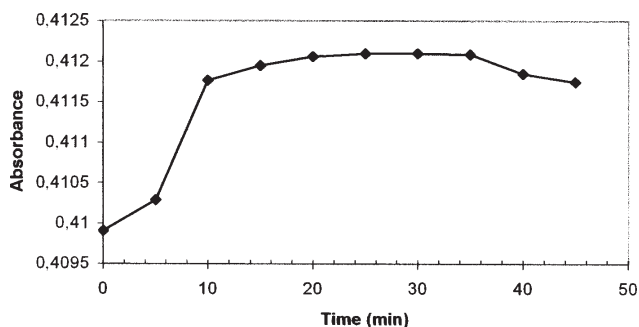


Fig. 5. Stability in time

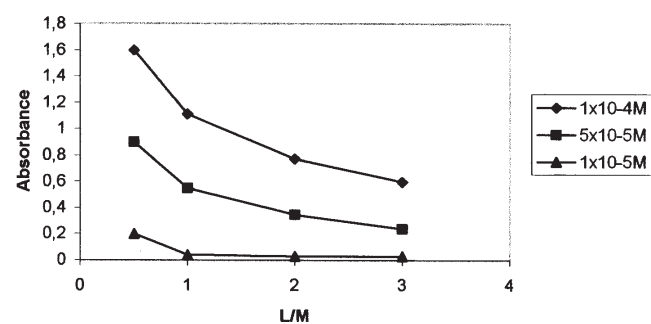


Fig. 6. Combining ratio

b) The complexing reaction of Fe(II) is influenced by the value of the pH; in order to follow the variation of the absorbance according to the pH of the medium, buffer solutions are used with pH = 7.0-8.6 and a standard solution of 30 $\mu\text{g}/\text{mL}$ Fe(II). From figure 4, it results that the best pH for the quantitative determination of the hydroxyurea -Fe(II) complex is 7.4 -7.6.

c) The stability in time of the hydroxyurea -Fe(II) complex has been studied. The results are illustrated in figure 5. From the graphic results that 15 min after the Hy reagent is added, the absorbance of the complex is maximum and the value of the absorbance is maintained for at least 30 min.

d) The combining ratio, illustrated in figure 6, is established through the method of isomolar series and it is 1:1 (L/M).

Table 1
DETERMINATION OF Fe(II) FROM CAPSULES

Pharmaceutical product	Certified value of Fe (II) (mg/capsule)	Obtained Value (mg/capsule)	Recovery %	RSD %	
FERRO FOLGAMMA R.P.SCHERER GmbH, GERMANY	37 ± 2.775	37 ± 0.0137	100.20	0.034	n = 5

Table 2
DETERMINATION OF THE HYDROXYUREA - Fe(II) COMPLEX

Eliminated hydroxyurea	Obtained value %	RSD %	Eliminated hydroxyurea - Fe(II) complex	Obtained Value %	RSD %
	80.186 ± 0.32	3.167 (n = 5)		19.446 ± 2.764	11.44 (n = 5)

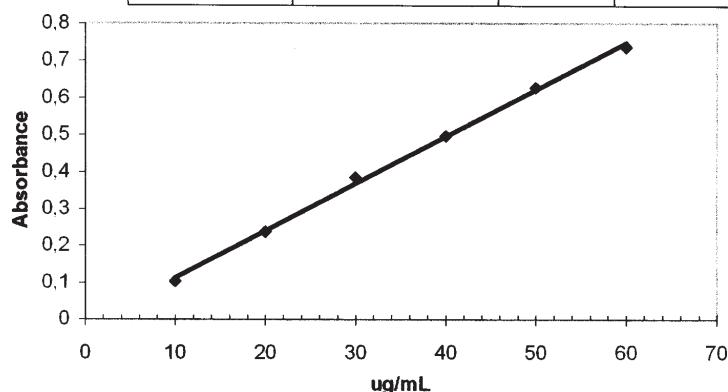


Fig. 7. Calibration curve

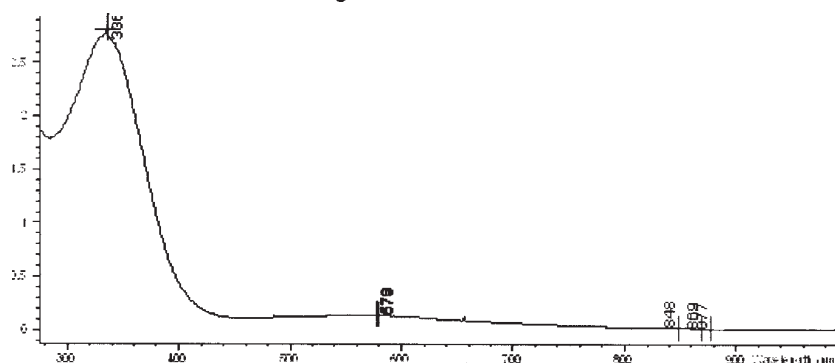


Fig. 8. Hy - Fe(III) complex

e) The stability constant is determined through the dissociation degree method of Harvel and Manning [10] according to the relations:

$$K_i = \alpha^2 / C / 1 - \alpha \quad (1)$$

$$\alpha = Am - As / Am \quad (2)$$

α = dissociation degree;

Am = Maximum absorbance (0.13800);

As = Equilibrium absorbance (0.10366);

C = Concentration of the solutions of Fe(II) and hydroxyurea ($10^{-5}M$).

$\alpha = 0.2488$;

$K_i = 8.24 \cdot 10^{-7}$;

$K_s = 1.21 \cdot 10^6$

$\epsilon = 3.4559 \cdot 10^4 \text{ mol}^{-1} \cdot L \cdot \text{cm}^{-1}$.

f) Calibration curve: the concentration range in which the Lambert-Beer law is observed is 10-60 $\mu\text{g Fe(II)}$ (fig. 7); the correlation coefficient $r^2 = 0.9999$; the slope of the straight line is 0.0129; the intercept is - 0.01363; the

standard deviation $SD = 0.0114$; the detection limit $LD = \text{standard deviation} \times 3 / \text{slope of the straight line} = 2.71 \mu\text{g/mL}$; the quantification limit $LQ = \text{standard deviation} \times 10 / \text{slope of the straight line} = 9.04 \mu\text{g/mL}$

g) Interferents: under the same reaction conditions, Hy forms complex combinations with other ions: Fe(III) with maximum absorption at 386 nm and 578 nm, but also Co(II) with $\lambda_{\text{max}} = 297 \text{ nm}$ and 430 nm (fig. 8) [11].

Under different reaction conditions, the hydroxyurea complexes the Al (III) and Mg (II) ions, these reactions being used for the quantitative determination [12].

N-hydroxyurea can form a bidentate complex combination with Zn(II) that strongly inhibits the metalloenzyme [13].

The volatile residual solvents from the pharmaceutical products determine the degradation of the hydroxyurea in urea and lactic acid which interferes with its determination [14,15].

Results of the determination of Fe(II) from capsules

The established method has been applied in order to determine Fe(II) from capsules. The concentration of Fe(II)/cp, in mg is calculated following the formula:

$\text{mg Fe(II)/cp} = (A + 0.0136) \times V \times \text{Gm} / 0.0129 \times a$;

A = value of the absorbance of the sample; V = volume where it can be found the sample (500mL); Gm = average weight of 20 capsules (0.56g).

The obtained results are registered in table 1.

Composition of capsules: Fe(II) 37 mg; folic acid, 5 mg; cyanocobalamin, 0.01mg; E172; ethylvaniline; sorbitol solution 70%; glycerol 85%.

The statistical analysis of the results shows a significant accuracy and precision for the proposed method (RSD 0.034%).

Results of the determination of the hydroxyurea - Fe(II) complex from a biological fluid sample collected in 24 h

1 g hydroxyurea has been administered daily for 6 months to a patient who underwent this treatment for 12 years. This patient is eliminating an average of 2000 mL of biological fluid a day.

From the calibration curve it was determined the hydroxyurea total quantity and the quantity of free hydroxyurea ($\mu\text{g/mL}$), which is eliminated through the biological fluid in 24 h. By subtraction we will obtain the quantity of the eliminated Hy ($\mu\text{g/mL}$) which will be reported to those 2 L of the biological fluid and to the daily administered drug quantity.

The obtained result was in concordance with the literature data [1].

From the eliminated Hy the Hy-Fe (II) complex proportion is determined.

The obtained results are registered in table 2.

Conclusions

Hydroxyurea in the presence of water has tautomeric forms in which the $>\text{N-OH}$ bond and the $>\text{C=N}$ -azometinic group appear, contributing to the formation of the complex with Fe(II), having extremely important biological implications.

Following the study carried out on the complexing reaction of Fe(II) with hydroxyurea, the work parameters have been established: the best pH, the combining ratio, the stability in time of the complex, the stability constant, the molar extinction coefficient, the detection limit, the interferences.

The obtained results recommend the use of the hydroxyurea for the spectrophotometric quantitative determination in UV of Fe(II).

The proposed method has been successfully applied to determine Fe(II) from soft gelatin capsules. The statistical analysis of the results shows a significant accuracy and precision for the proposed method.

The proposed method was applied to determine the Hy which is 80 % excreted, through the biological fluid, according to literature data. From the excreted Hy, the Hy-Fe (II) complex presence is determined for the first time.

The presence of the Hy-Fe(II) complex in the biological fluid of the patients in a percentage of 20% explains the appearance of anemia as a side effect of the treatment with hydroxyurea for a long period of time.

References

1. Med Ex 2006, Ed. Medic Art, **4**, 2006, p. 19
2. MCCLARTY, G.A., CHAN, A.K., CHAY, B. K., WRIGHT, J.A., J. Biol. Chem., **265**, 1990, p. 7539
3. NYHOLM, S., THELANDER, L., GRÄSLUND A., Biochemistry, **32**, 1993, p. 11569
4. GLOVER, R.E., IVY, E.D., ORRINGER, E.P., MAEDA, H., MASON, R. P., Mol. Pharmacol. 1999, **B**, p. 1006
5. TANTARU, G., DORNEANU, V., STAN, M., Journal of Pharmaceutical and Biomedical Analysis, **27**, 2002, p. 827
6. JAMES, H., NAHAVANDI, M., WYCHE, M. Q., TAYLOR, R. E., Journal of Chromatography, **831**, Issue 1-2, p. 42
7. IKAVA, M., SCHOPER, T.D., DOLLARD, C.A., SASNER, J.J., Journal of Agricultural and Food Chemistry, **51**, 7, 2003, p. 1811
8. IGAMU, E. W., ROA, P. D., KAPSOMBUT, P., AGUINAGA, M.D.P., TURNER, E. A., Journal of Chromatography, B: Biomedical Applications, **709**, 1, 1998, p. 119
9. PARKER, G., LEMKE, T., Journal of Medicinal Chemistry, **20**, 9, 1977, p. 1221
10. Journal Chem, Ed. 69, 1992, p. 833
11. NIGOVIĆ, B., KUJUNDŽIĆ, N., SANKOVIĆ, K., European Journal of Medicinal Chemistry, **40**, 1, 2005, p. 51
12. EL-KOSASY, A. M., Journal of AOAC International, **86**, 1, 2003, p. 15
13. TEMPERINI, C., INNOCENTI, A., SCOZZAFANA, A., SUPURAN, C., Bioorganic and Medicinal Chemistry Letters, **16**, 16, 2006, p. 4316
14. WANG, X., JIANG, T., YUAN, J.P., CHENG C.G., LIU, J.H., Analytical and Biomedical Chemistry, **386**, 6, 2006, p. 1082
15. MUGUETA, P., ALEGRE, C., MANOL, E., VARA, J. I., Analytical Biochemistry, **357**, 1, 2006, p. 147

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