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KINETIC–SPECTROPHOTOMETRIC APPROACH TO THE AMPICILLIN HYDROLYTIC DEGRADATION APPLIED FOR THE HISTIDINE DETERMINATION

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Abstract. *The objective of this research was to develop a kinetic-spectrophotometric method for the determination of microquantities of L-histidine in pure form and dietary supplements. The method was based on the kinetics of ampicillin degradation by Ni(II) ion as a catalyst in the presence of L-histidine in a strongly alkaline medium. The rate of this reaction was monitored spectrophotometrically by measuring the increase in absorbance at 265 nm as a function of time. The same approach was used for the investigation of the reaction rate in the absence of histidine. A differential variant of the tangent method was used to process the kinetic data. Beer's law was obeyed in the interval of histidine concentration from 1.24 μg/ml to 11.63 μg/ml with the relative standard deviation ranging from 8.1% to 0.7%. The detection limit of 0.46 μg/ml was estimated based on the $3S_0$ criterion. The interference effects of some metal ions, anions, and other molecules on the reaction rate were studied to assess method selectivity. Herein described method was applied for the quantification of histidine in dietary supplements. The point hypothesis test confirmed that there was no significant difference between the proposed and the reference method.*

Key words: *ampicillin, hydrolytic degradation, Ni(II), L-histidine, activator.*

1. INTRODUCTION

Ampicillin (α-aminobenzylpenicillin, Amp) is a semisynthetic β-lactam antibiotic and it behaves essentially as an aliphatic amino acid. As an amphoteric compound, Amp exists in solution in three different forms: cationic (XH_2^+, $pK_1 = 2.55$) in acid, zwitterionic ($HX^{\pm}$, $pK_2 = 7.14$) in neutral, and anionic (X^-) in alkaline medium (Scheme 1). Hou and

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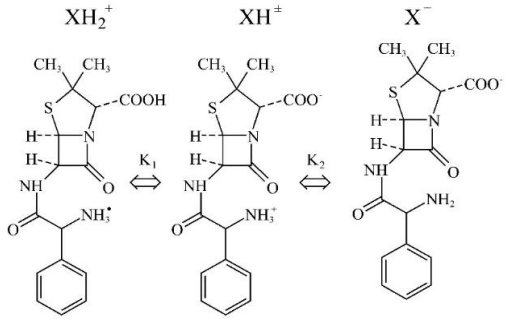
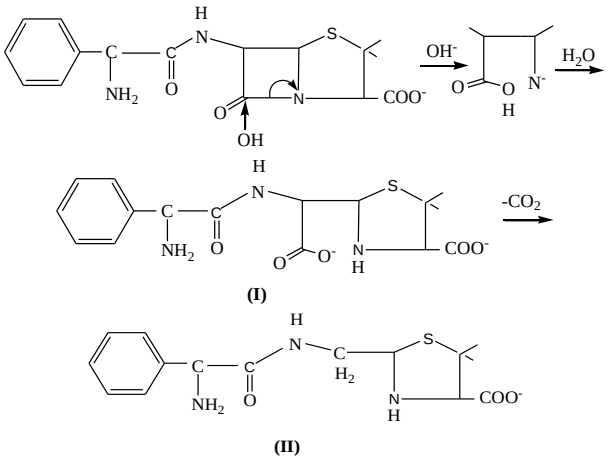
Poole (1969) presented the results of the Amp degradation kinetics in solution. The data obtained by these authors suggested that in the basic medium only one type of reaction was responsible for the reaction rate. Namely, the reaction of hydroxyl ion with the anionic form of ampicillin was followed by the formation of both α-aminobenzylpenicilloic acid (Scheme 2-I) and α-aminobenzylpenilloic acid (Scheme 2-II). In the basic solution (pH = 9.78 at 35 °C) only a decarboxylated product (α-aminobenzylpenilloic acid) was formed. Typical benzyl absorption maxima of Amp at 268 nm, 262 nm, and 257 nm decreased with increasing pH and then completely disappeared in the strong alkaline medium forming an absorption band from 250 to 295 nm. Robinson-Fuentes et al. (1997) investigated the degradation pathways of Amp in an alkaline solution (pH = 12 at 37 °C) by HPLC and NMR methods. The results of that research confirmed that penicilloic acid is the first and penilloic acid is the second product of Amp degradation. The kinetic method for Amp quantification based on the catalytic effect of Ni(II) was reported by Rašić Mišić et al. (2013). Summarizing results regarding the selectivity of our method it was observed that the small amount of L-histidine additionally increased the reaction rate of antibiotic degradation. In general, the rate of catalyzed reaction may be increased due to the presence of a particular substance (activator) in which case it is called an activated reaction.



Scheme 1 Dissociation of Amp zwitterion



Scheme 2 Ampicillin degradation in alkaline solution: α-aminobenzylpenicilloic acid (I) and α-aminobenzylpenilloic acid (II)

L-histidine [2-amino-3(imidazol-4-yl) propanoic acid] (His) is an essential component in many proteins. It is a polyprotic amino acid molecule with three dissociation constants: pKa_1=1.77 (carboxyl group), pKa_2=5.97 (imidazole nitrogen), and pKa_3=9.18 (amino nitrogen). His plays a crucial role in metal transport in biological systems and serves as a ligand for incorporation into enzymes and proteins. Metabolism of His in the body produces histamine which is involved in inflammatory and allergic responses. It helps in the reparation of damaged tissues and the protection of nerve cells. Supplements of His may be used to treat eczema (McKee and McKee, 1996), metabolic syndrome (Feng et al., 2013), diarrhea caused by cholera infection (Rabbani et al., 2005), rheumatoid arthritis (Gerber et al., 1976) and anemia caused by kidney chronic diseases (Reeves et al., 1977).

Literature review brings many analytical procedures for His determination in pure form or in pharmaceutical formulations, as well as in biological samples: colorimetry (Newman and Turnbull, 1960, Jiao et al., 2018, Razavi and Khajehsharifi, 2021), spectrophotometry (Patel Vandana et al., 2009), spectrofluorimetry (Ambrose et al., 1969, Gerber, 1970, Alevridis et al., 2020), chemiluminescence (Zhu et al., 2002, Kiba et al., 2006, Hun, 20159, voltammetry (Jaselskis, 1958, Moreira and Fogg, 1991, Farias et al., 2008), microbiology (Horn et al., 1948), kinetic spectrophotometry (Mitić et al., 2004), potentiometry (Staden and Holo, 2007, Abbaspour et al., 2004) and cation-exchange chromatography (Stampina, 2021).

This study aimed to 1) evaluate optimal kinetic-spectrophotometric conditions for histidine determination based on its effect on ampicillin hydrolytic degradation in the presence of Ni(II), 2) establish reaction rate equations, and 3) estimate analytical figures of merit for the proposed method.

2. Material and Methods

2.1. Apparatus

The reaction rate of the Amp degradation with and without His was monitored by readings of the absorbance on an Agilent 8453 UV/Vis spectrophotometer with a 1-cm optical length cell. Measurements of pH were carried out using Hanna instruments pH meter. Deionized water was produced using MicroMed high purity water system, TKA Wasseraufbereitungssysteme GmbH. The solutions were refrigerated but thermo-stated at 25 ± 0.1 °C prior to use in Julabo MP 5A Open Bath Circulations.

2.2. Reagents

All solutions were prepared with deionized water. The stock solution of Amp ($1.00 \cdot 10^{-3}$ mol/l) was prepared by dissolving the required, accurately weighed, amount of ampicillin-trihydrate (Dolber, Switzerland, Ph.). The His stock solution ($1.00 \cdot 10^{-3}$ mol/l) was prepared by dissolving the required, accurately weighed, amount of L-histidine (Merck, Germany, pro biochemistry). The stock solutions of nickel-chloride ($1.00 \cdot 10^{-1}$ mol/l), potassium-chloride (1.5 mol/l), and sodium-hydroxide (1 mol/l) were obtained by dissolving accurately measured amounts of salts and base of analytical grade purchased from Merck, Germany. All the glassware used was cleaned in the aqueous solution of HCl (1:1) and then thoroughly rinsed with tap, distilled, and finally with deionized water.

2.3. General procedure

The reactions were carried out in the reaction-mixture vessel with four compartments (so-called Budarin's vessel). For the catalytic reaction, the solution of Ni(II) was placed in the first compartment, Amp in the second, NaOH and KCl in the third, and water was added in the fourth compartment up to the predetermined total volume of 10 ml that was held constant during the experiment. In the case of an activated reaction, reagents were pipetted in the same way and His was added along with water into the fourth compartment of the vessel. After thermo-stating the vessel at 25 ± 0.1°C for 5 min, the reaction was initiated by vigorous mixing of the content and transferred into the cuvette. Absorbance (A) was measured at 265 nm spectrophotometrically every 30 s, for 5 min, starting 60 s, after the reactants were mixed. A differential variant of the tangent method was used for the processing of the kinetic data because a linear correlation existed between absorbance and time. The rate of the reaction ($v = \mathrm{tg}\alpha$) was obtained by measuring the slope (dA/dt) of the linear part of the kinetic curve (absorbance-time plot). The calibration curve was constructed by plotting the rate of reaction (tgα) *vs.* concentration (c) of His. The unknown amount of this amino acid was calculated based on the experimentally obtained reaction rate value and its substitution into the linear regression equation (Eq. 4).

2.3.1. Procedure for oral powder and capsules

The appropriate amount of food supplement powder (OstroVit, Poland) was dissolved in deionized water and further used instead of the His stock solution. The content of ten capsules of L-histidine as a dietary supplement (Douglas laboratories, USA) was emptied and homogenized. An amount of resulting powder, equivalent to the mass of one capsule (500 mg of L-histidine), was transferred into the 500 mL volumetric flask, dissolved with deionized water, and sonicated for 10 min. Excipients present in the capsules were magnesium stearate and silicon dioxide. Due to their insolubility in water, the obtained suspension was filtrated (Whatman No. 42). The appropriate aliquots of powder solution and capsules' filtrate were directly used for determining the quantity of histidine covering the concentration range of the calibration curve. The procedure for the comparative method has been described in Yugoslav pharmacopeia 2000.

3. RESULTS AND DISCUSSION

3.1. Absorption spectra

In order to study the impact of histidine on the catalytic degradation of Amp in the presence of Ni(II), the absorption spectra changes with time were recorded within the wavelength interval of 190 – 900 nm (Fig. 1).

The absorption band whose intensity increased with time occurred in the 250-295 nm wavelength interval (Fig. 1A), This absorption band was caused by α-aminobenzylpenilloic acid as a product of Amp hydrolytic degradation (Scheme 2) (Hou and Poole, 1969). A significantly faster reaction rate in the presence of His was observed (Fig 1B). These changes in obtained spectra indicated that His was an activator of hydrolytic degradation of Amp in the presence of Ni(II) as the catalyst. Activating effect of His was confirmed by analyzing the kinetic curves that showed first-order dependence of the signal on His concentration (Fig 2). The rates of catalytic (1) and activated (2) reactions were monitored spectrophotometrically at 265 nm because the biggest changes in absorbance with time appeared at this wavelength.

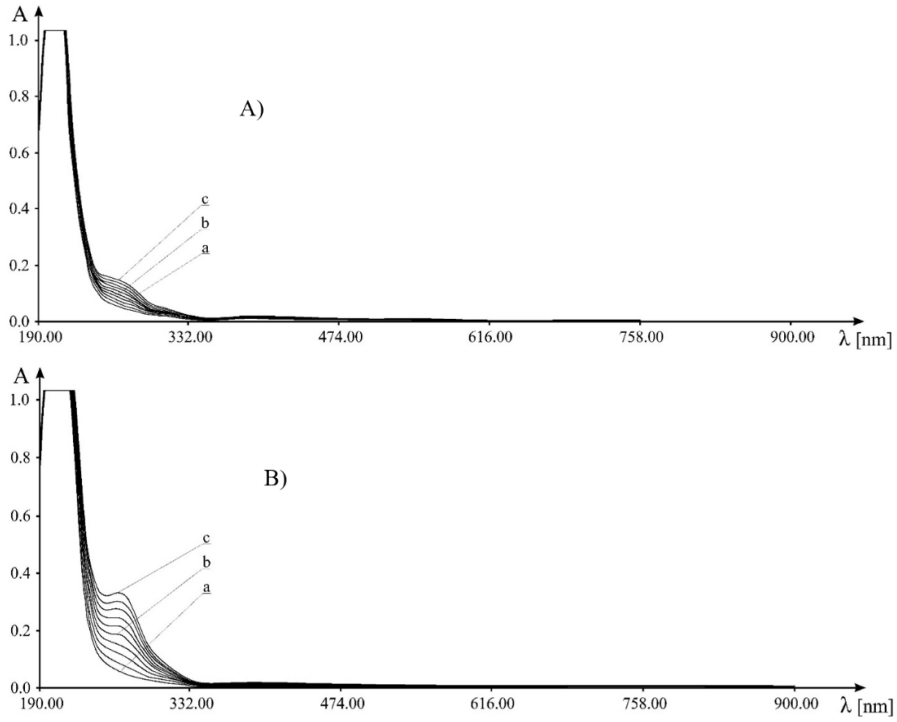


Fig 1 The changes of the absorption spectra of the ampicillin degradation in the absence (A) and the presence of His (B) after 1 min (a), 6 min (b), and 12 min (c). Initial reactants concentrations in solutions: $c(Ni^{2+})$ = $1 \cdot 10^{-4}$ mol/l; c(His) = $1 \cdot 10^{-4}$ mol/l; c(Amp) = $1 \cdot 10^{-4}$ mol/l; c(NaOH) = $3 \cdot 10^{-2}$ mol/l; c(KCl) = $4.5 \cdot 10^{-2}$ mol/l; t = 25.0 ± 0.1 ºC

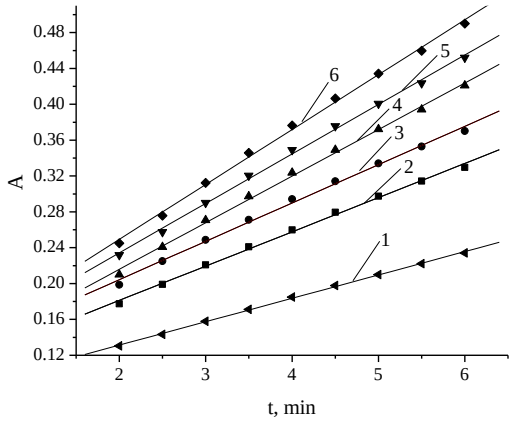


Fig. 2 The change of the absorbance of the solution as a function of time for different concentrations of His (μg/ml): 1) 0.00; 2) 1.5; 3) 3.10; 4) 6.20; 5) 7.75; and 6) 10.85. Intial rectants concentrations in solution: $c(Ni^{2+})$ = $1.0 \cdot 10^{-4}$ mol/l; c(NaOH) = $3.5 \cdot 10^{-2}$ mol/l; c (KCl) = $7.5 \cdot 10^{-2}$ mol/l; c(Amp) = $8 \cdot 10^{-5}$ mol/l; t = 25.0 ± 0.1°C, λ = 265 nm

3.2. Kinetic studies

Preliminary experiments were performed to optimize conditions for the determination of the lowest possible concentration of His. Therefore, the impact of the concentration of each reactant on catalytic (1) and activated (2) reaction rates was studied. The chosen concentration values of variables were maintained constant throughout the experiment.

The effect of the NaOH amount was investigated in the range of $(0.1\text{-}5.0)\cdot10^{-2}$ mol/l (Fig. 3).

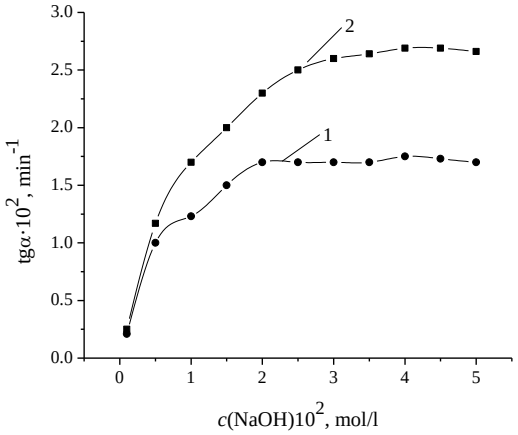


Fig. 3 Dependence of the catalytic (1) and activated (2) reaction rate on the NaOH concentration at t = 25.0 ± 0.1 ºC. Initial reactants concentrations: $c(Ni^{2+}) = 1\cdot10^{-4}$ mol/l; c(Amp) = $5\cdot10^{-5}$ mol/l; c(KCl) = $1.5\cdot10^{-2}$ mol/l; c(His) = 1.55 μg/ml; t = 25.0 ± 0.1 ºC

Based on the displayed plot, both reaction rates significantly increased from $(0.1\text{-}2.0)\cdot10^{-2}$ mol/l NaOH. In the interval $(2.0\text{-}5.0)\cdot10^{-2}$ mol/l catalytic reaction rate became constant because hydrolytic degradation of Amp was completed under the given conditions. The rate of reaction (2) increased with new increments of NaOH up to the concentration of $3.0\cdot10^{-2}$ mol/l and further, it became constant, as well. Both reactions are zero-order with respect to the NaOH concentration in the interval of $(3.0\text{-}5.0)\cdot10^{-2}$ mol/l. The NaOH concentration of $3.5\cdot10^{-2}$ mol/l was selected as the optimal value.

In order to regulate the ionic strength of the reaction mixture KCl was added. The correlations between the tgα and KCl concentration for both reactions are presented in Fig. 4.

The graph shows that both reaction rates decreased with the increase of this electrolyte concentration in the whole examined interval of $(1.5\text{-}15)\cdot10^{-2}$ mol/l. The increment of this compound increased the ionic strength of the solution on one side and decreased the ionic activity (especially of the Ni^{2+} ions) on the other side, which led to a decrease in the reaction rates. Both reactions are of negative first-order concerning the KCl concentration in the whole examined interval. The value of $7.5\cdot10^{-2}$ mol/l was selected as optimal.

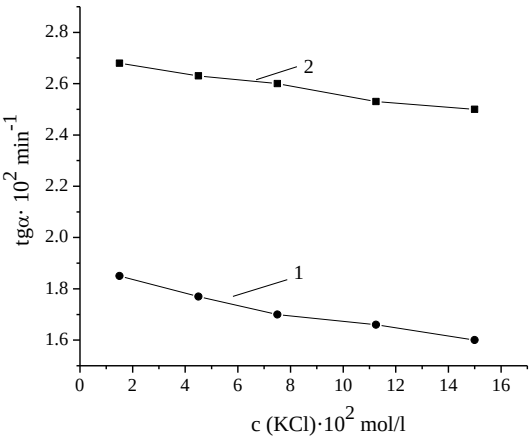


Fig. 4 Dependence of catalytic (1) and activated (2) reaction rate on the KCl concentration. Initial reactants concentrations in solution: $c(Ni^{2+})$ = $1 \cdot 10^{-4}$ mol/l; c(Amp) = $5 \cdot 10^{-5}$ mol/l; c(NaOH) = $3.5 \cdot 10^{-2}$ mol/l; c(His) = 1.55 μg/l; t = 25.0 ± 0.1 ºC.

The dependence of tgα on Ni(II) concentration in the interval of $(0.1\text{-}2.0) \cdot 10^{-4}$ mol/l is shown in Fig. 5.

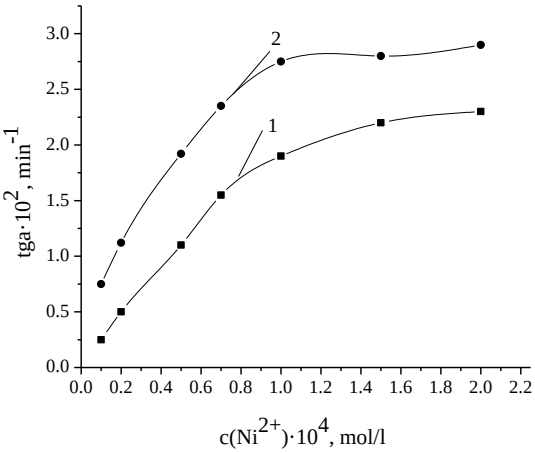


Fig. 5 Dependence of the catalytic (1) and activated (2) reaction rate on the Ni(II) ion concentration. Initial reactants concentrations in solution: c(KCl) = $7.5 \cdot 10^{-2}$ mol/l; c(Amp) = $5 \cdot 10^{-5}$ mol/l; c(NaOH) = $3.5 \cdot 10^{-2}$ mol/l; c(His) = 1.55 μg/ml; t = 25.0 ± 0.1 ºC.

Both reactions are of the first order to the Ni(II) concentration in the interval of $(0.1 - 1.0) \cdot 10^{-4}$ mol/l. The increase of metallic ion concentration up to $2.0 \cdot 10^{-4}$ mol/l showed a very small effect on the reaction rates. For further measurements, the Ni(II) concentration of $1.0 \cdot 10^{-4}$ mol/l was selected.

The correlation between tgα and Amp concentration was displayed in Fig. 6. It could be seen that both reactions were of positive first order concerning the antibiotic concentration in the whole inspected interval $(0.1\text{-}1.0)\cdot10^{-4}$ mol/l. The selected value for further work was $8.0\cdot10^{-5}$ mol/l because the greatest difference between the reaction rates occurred at this concentration.

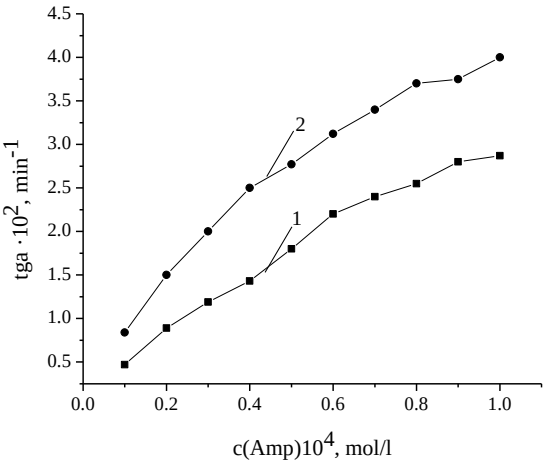


Fig. 6 Dependence of the catalytic (1) and activated (2) reaction rate on the Amp concentration. Initial reactants concentrations in solution: c(KCl) = $7.5\cdot10^{-2}$ mol/l; $c(Ni^{2+}) = 1\cdot10^{-4}$ mol/l; c(NaOH) = $3.5\cdot10^{-2}$ mol/l; c(His) = 1.55 μg/ml; t = 25.0 ± 0.1 ºC

Based on the previously presented dependences, the following kinetic equations for the investigated processes were deduced. The rates of the catalytic (1) and activated (2) reactions are:

$$rate = k_1 \cdot c_{Ni^{2+}} \cdot c_{KCl}^{-1} \cdot c_{Amp} \tag{1}$$

$$rate = k_2 \cdot c_{Ni^{2+}} \cdot c_{KCl}^{-1} \cdot c_{Amp} \cdot c_{\mathrm{His}} \tag{2}$$

where k_1 and k_2 are the rate constants of the catalytic and activated reactions, respectively. These equations were valid for the following concentration intervals of reactants: Ni^{2+} = $(0.1\text{-}1.0)\cdot10^{-4}$ mol/l, NaOH = $(3.0\text{-}5.0)\cdot10^{-2}$ mol/l, KCl = $(1.5\text{-}15)\cdot10^{-2}$ mol/l and Amp $(0.1\text{-}1.0)\cdot10^{-4}$ mol/l. Under the chosen optimal concentrations of Ni^{2+}, KCl, and Amp previous equation for the activated reaction rate may be written as:

$$rate = k_2^{'} \cdot c_{\mathrm{His}} \tag{3}$$

where $k_2^{'}$ is a conditional reaction rate constant.

3.3. Validation of the proposed method

3.3.1. Linearity

For the evaluation of linearity, His concentration was varied in the interval from 1.24 to 11.63 μg/ml (Fig. 7) under the previously chosen optimal reaction conditions. For the determination of His content in the investigated interval the least squares' equation ($y = bx + a$, where *b* and *a* are its slope and intercept, respectively) was applied:

$$tg\alpha \cdot 10^2 = (3.3748 \pm 0.0227) + (0.2679 \pm 0.0032) \cdot c(\text{His}); \quad r = 0.9991;\ S_0 = 0.042;\ n = 15 \tag{4}$$

where *c*(His) is the concentration of histidine expressed in μg/ml, S_0 is the residual standard deviation and *r* is the correlation coefficient.

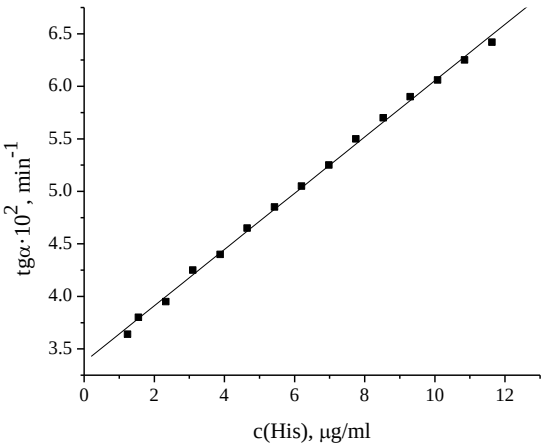


Fig. 7 Dependence of the reaction rate on the concentration of His. Initial reactants concentrations in solution: c(Ni^{2+}) = $1.0 \cdot 10^{-4}$ mol/l; c(NaOH) = $3.5 \cdot 10^{-2}$ mol/l; c(KCl) = $7.5 \cdot 10^{-2}$ mol/l; c(Amp) = $8 \cdot 10^{-5}$ mol/l; t = 25.0 ± 0.1 ºC

3.3.2. The limit of detection (LOD) and the limit of quantification (LOQ)

The minimum concentration of His that can be detected by this method was estimated at 0.46 μg/ml, using the formula LOD = $3 \cdot S_0/b$. LOQ was obtained at 1.52 μg/ml using the equation LOQ = $10 \cdot S_0/b$ (Perez-Bendito and Silva, 1988, Ermer, 2001).

3.3.3. Accuracy and precision

Accuracy and precision were determined for four concentrations from the calibration graph based on five measurements for every chosen value (Table 1). Relative standard deviation values were in the interval of 8.1-0.7% for the concentration range of 1.24 – 11.63 μg/ml. The highest RSD was determined for the first concentration value since it was between the LOD and LOQ of the method.

Table 1 Accuracy and precision of His determination

His concentration (μg/ml)		RSD[b] (%)	$\frac{\bar{x}-\mu}{\mu}\cdot 100$ (%)
Taken μ	Determined $\bar{x}$ ± SD[a]		
1.24	1.11 ± 0.09	8.1	–10.5
1.55	1.58 ± 0.11	6.9	+1.9
6.20	6.25 ± 0.20	3.2	+0.8
11.63	11.47 ± 0.08	0.70	–1.4

a) Mean and standard deviation of five measurements at a confidence level of 95%,
b) Relative standard deviation, c) bias of the method

3.3.4. Selectivity of the method

To assess the selectivity of the method the effect of a variety of common ions and organic molecules on the reaction rate was examined. The tolerance limits for the studied species on the His quantification of $3\cdot10^{-5}$ mol/l (4.65 μg/ml) were given in Table 2. The maximum level of tolerance, according to the 2s criterion, was the one causing an error in the concentration less than twice the standard deviation (Kirkbright, 1966). Li^+ and urea when present in 1000-fold excess, glucose in 100-fold excess, Ba^{2+}, Pb^{2+}, Sn^{2+}, As^{3+}, nicotinic acid, and CH_3COO^- in 10-fold excess, as well as salicylic acid, sorbitol, mannitol, Arg, Met, Ser, Lys, Mg^{2+}, Zn^{2+}, Ca^{2+}, Cd^{2+}, F^-, $C_2O_4^{2-}$, SO_4^{2-} in 1:1 M ratio did not interfere with the method. Lactose and citric acid showed weak, while Al^{3+}, Fe^{3+}, $Co^{2+,}$ and Cu^{2+} showed a strong inhibitory effect. Amino acids such as Ala, Phe, Trp, Asn as well as Hg^{2+}, As^{5+}, NO_3^- and HPO_4^{2-}increased the reaction rate.

Table 2 Tolerance level of foreign ions and molecules on determining His concentration

Tolerance level c_x/c_{His}	ion/molecule (x)
10^3	Li^+, urea
10^2	glucose
10	Ba^{2+}, Pb^{2+}, Sn^{2+}, As^{3+}, nicotinic acid, CH_3COO^-
1	Salicylic acid, sorbitol, mannitol, Arg, Met, Ser, Lys, Mg^{2+}, Zn^{2+}, Ca^{2+}, Cd^{2+}, F^-, $C_2O_4^{2-}$, SO_4^{2-}
Interfere at 1:1	Lactose, citric acid, Al^{3+}, Fe^{3+}, Co^{2+}, Cu^{2+}, Ala, Phe, Trp, Asn, Hg^{2+}, As^{5+}, NO_3^-, HPO_4^{2-}

3.3.5. Applicability of the Method

The proposed method was applied to the quantification of the histidine amount in selected commercially available dietary supplements. The obtained results based on five measurements for each product were statistically compared to the results obtained by the official potentiometric titration method using a point hypothesis test (Table 3). The values of the F-test and Student's t-test at a 95% confidence level did not exceed the critical F- and t-values, confirming no significant difference between the performance of the proposed and the official method.

Table 3 Accuracy and precision of His determination in dietary supplements

Concentration of His (μg/ml)			RSD (%)	$\frac{\bar{x}-\mu}{\mu}\cdot 100$ (%)	Recovery (kinetic method) (%)	F-test[c/]	t-test[c/]
Taken μ	Determined by kinetic method $\bar{x}$ ± SD	Determined by official method $\bar{x}$ ± SD					
6.20[a/]	5.98 ± 0.35	6.11 ± 0.28	5.85	-3.55	96.45	1.56	0.65
6.20[b/]	6.03 ± 0.25	6.17 ± 0.22	4.98	-2.74	97.25	2.53	0.76

[a/]L-Histidine, capsules, [b/] L-Histidine, oral powder, [c/]Theoretical values for F-test (ν_1=4, ν_2=4) and t-test (ν=8) for a confidence level of 95% are 6.39 and 2.306, respectively.

3.4. Comparison with other methods

The comparison of the analytical characteristics of the proposed method with other methods for His determination is given in Table 4. Methods 1 to 5 were proposed for His determination in pharmaceutical formulations. FIA method reported by Zhu et al. (2002) is characterized by the widest linearity interval (1.0×10^{-6} mol/l – 1.0×10^{-3} mol/l), while HPLC methods by Shieh et al. (2007) and spectrophotometric method by Patel Vandana et al. (2009) have the narrowest linearity ranges, 50 – 150 μg/ml and 33 μg/15 ml – 77 μg/15 ml, respectively. The kinetic-spectrophotometric method reported by Mitić et al. (2004) as well as the method described in this paper has linearity ranges of one order of magnitude, 0.15 – 1.56 μg/ml and 1.24-11.63 μg/ml, respectively. The reaction between His and *o*-phthalaldehyde was used for the first time for the development of the fluorometric method for His determination by Ambrose et al. (1969). Recently reported methods for His determination in human urine and serum are based on the same reaction monitored fluorometrically with zone fluidics (Alevridis et al., 2020) and with the combination of ion exchange chromatography with post-column derivatization (IEC-PCD) (Stampina et al., 2021).

Table 4 The comparison of the proposed method with other methods

Method no.	Indicator reaction	Comments, conditions	References
1. FIA	His enhancement of electrogenerated chemiluminescence of luminol	Linearity: 1.0×10^{-6} mol/l – 1.0×10^{-3} mol/l r = 0.992 LOD = 5.6×10^{-7} mol/l Recovery = 97.8%; Pharmaceutical injection	Zhu et al., 2002
2. Kinetic-spectrophotometric	Inhibitor effect of His on the catalytic activity of Co(II) in the reaction of purpurin oxidation by H_2O_2	Reaction time: 5 min λ = 540 nm at 22°C ± 0.1 °C Linearity: His = 0.15 – 1.56 μg/ml LOD = 0.09 μg/ml Bias (pharmaceutical preparation): -12.1%	Mitic et al., 2004
3. HPLC	Purospher STAR RP_{18} endcapped column, mobile phase of 50 mM, phosphate buffer with 3 mM sodium octanosulfonate : acetonitrile (95 : 5), UV detection at 210 nm	Linearity: His = 50 – 150 μg/ml RSD = 1.24 – 0.88% LOD = 2.2 μg/ml LOQ = 7.3 μg/ml Recovery (pharmaceutical tablets) = 98.20 – 101.02%	Shieh et al., 2007

4.	Spectrophotometric (colorimetric)	His + diazotized sulfanilic acid in NaOH (1M)	Reaction time: 6 min λ = 405 nm Linearity: 33 μg/15 ml – 77 μg/15 ml (2.2 μg/ml – 4.5 μg/ml) Recovery (%) = 102.72 RSD (pharmaceutical formulation) = 0.442%	Patel Vandana et al., 2009
5.	HPLC with HILIC column technique	HILIC technique with silica column; His eluted with the mobile phase KH_2PO_4 : acetonitrile (30:70) without derivatization	λ = 200 nm; 30°C ± 2°C LOD: 0.042 μg/ml LOQ = 0.14 μg/ml RSD = 3.9% at LOQ level RSD = < 1% in drug Linearity LOQ level to 150% with respect to analyte concentration	Bhandare et al., 2010
6.	Kinetic-spectrophotometric	The catalytic effect of His on ampicillin degradation in the presence of Ni(II) in strong alkaline solution	Reaction time: 6 min λ = 265 nm; Linearity: 1.24-11.63 μg/ml LOD = 0.46 μg/ml LOQ = 1.52 μg/ml RSD (capsules) = 5.85% RSD (oral powder) = 4.98%	This paper
7.	Colorimetric	His-triggered self-cleavage of DNA duplex-induced gold nanoparticle (AuNP) aggregation	Reaction time: 6 min; A_{625}/A_{523} Linearity: 0 – 400 nM; RSD (serum) < 3.6 % LOD = 3.6 nM	Jiao et al., 2018
8.	Fluorometric	His + *o*-phtalaldehyde; zone fluidics	Reaction time: 120 s; λ_{ex} = 360 nm; λ_{em} = 440 nm Linearity: 125 – 2000 nmol/l LOD = 31 nmol/l LOQ = 93 nmol/l Recovery (human urine) = 87.6 – 95.4%	Alevridis et al., 2020
9.	Colorimetric paper-based sensor	Synthesis (79 h at 40 – 550 °C) and characterization (XRD, SEM, FT-IR) of SBA-15 white powder. Indicator displacement assay: Murexide-Cu^{2+} (orange) + His → $[Cu(His)_2]^{2+}$ + Murexide (magenta)	Linearity = 1 – 90 μM LOD = 0.5 μM RSD (urine) = 2.32-1.87% (5 – 20 μM) Recovery (urine) = 99.74 – 102%	Razavi and Khajehsharifi, 2021
10.	IEC-PCD	His + *o*-phthaldehyde complex	Linearity: 0.5 – 25 μmol/l LOD = 0.160 μmol/l Recovery (human urine and serum) = 89 – 114% RSD (urine) = 5.4% RSD (serum) = 6.9%	Stampina et al., 2021

The colorimetric method by Razavi and Khajehsharifi (2021) gave the widest linearity range (1-90 μM) for His quantification. The disadvantage of this method is the too long time required for the synthesis of SBA-15 powder and the set of expensive analytical tools for its characterization. For the colorimetric detection of His based on the target-triggered self-cleavage of DNA induced by gold nanoparticles, the authors themselves said that despite several advantages of the sensor, there are some challenges that need to be overcome, such as sensitivity for example.

4. CONCLUSION

To the best of our knowledge, no results have been reported on Amp degradation in strong alkaline medium in the presence of Ni(II) ion as the catalyst and His as an activator. Spectral analysis of this antibiotic degradation in the presence and absence of His showed the UV absorption band in the 250-295 nm wavelength interval (Fig. 1). According to the results reported by Hou and Poole (1969) and Robinson-Fuentes et al. (1997) this band is due to the α-aminobenzylpenilloic acid (decarboxylated product of Amp degradation) absorption (Scheme 2). The significant increase of the absorbance with time occurred in the presence of His. The absorption maxima in the visible region were not detected. Ni(II) ions did not precipitate with OH^- ions because metal ions formed stable water-soluble hydroxo complexes (Gamsjager et al., 2005).

The activating effect was assigned to His by analyzing the shapes of kinetic curves (Fig. 2) obtained in its presence and absence. The increase of the reaction rate with respect to that of the catalyzed reaction is a result of the presence of this amino acid, i.e., the higher its concentration the greater the effect it has (Perez-Bendito and Silva, 1988).

The above-mentioned influence of His was used for the development of a new, simple, inexpensive, and efficient kinetic-spectrophotometric method for this amino acid determination in the solution. The impact of each reactant (NaOH, KCl, $Ni^{2+,}$ and Amp) concentration on the catalytic and activated reaction rate was studied and the chosen concentration values of these variables were maintained constant throughout the experiment. A differential variation of the tangent method was used to process the kinetic data. The kinetic equations for both reaction rates were deduced based on the obtained dependencies.

The following figures of merit for this method are linearity interval (1.24 to 11.63 μg/ml), RSD range (8.1–0.7%), LOD (0.46 μg/ml), and LOQ (1.52 μg/ml). After the selectivity testing, the method was easily and successfully applied for the quantification of His in the dietary supplements.

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KINETIČKO-SPEKTROFOTOMETRIJSKI PRISTUP HIDROLITIČKOJ DEGRADACIJI AMPICILINA PRIMENJEN ZA ODREĐIVANJE HISTIDINA

Cilj ovog istraživanja je bio razvoj kinetičko-spektrofotometrijske metode za određivanje mikrokoličina L-histidina u čistom obliku i dijetetskim suplementima. Metoda je zasnovana na reakciji degradacije ampicilina koja je katalizovana Ni(II) jonom u prisustvu L-histidina u jako baznoj sredini. Brzina reakcije praćena je spektrofotometrijski merenjem povećanja apsorbance na 265 nm sa vremenom. Isti pristup je korišćen za praćenje brzine reakcije u odsustvu histidina. Diferencijalna varijanta tangensne metode je upotrebljena za obradu kinetičkih podataka. Metoda je zadovoljavala Beer-ov zakon u intervalu koncentracija od 1,24 μg/ml do 11,63 μg/ml sa relativnom standardnom devijacijom od 8,1% do 0,7%. Detekcioni limit od 0,46 μgcm^{-3} je procenjen korišćenjem 3S_0 kriterijuma. Selektivnost metode je ispitana na osnovu uticaja pojedinih metalnih jona, anjona i organskih molekula na brzinu reakcije. Opisana metoda je primenjena za kvantitativno određivanje histidina u dijetetskim suplementima. Primenom t-testa potvrđeno je da nema značajne razlike između rezultata predložene i referentne metode.

Ključne reči: *ampicilin, hidrolitička degradacija, Ni(II), L-histidin, aktivator.*