Determination of Streptomycin in a Pharmaceutical Sample Based on Its Degradation by Hydrogen Peroxide in the Presence of Copper(II)

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The kinetics of streptomycin degradation by hydrogen peroxide at pH 7.4 was investigated. The reaction was catalyzed by traces of Cu(II) ions, and it was followed spectrophotometrically at 322 nm by applying the initial-rate method. The kinetic parameters of the reaction are reported, and a rate equation is suggested. From the dependence of the relative rate constants on the temperature, the activation energy was calculated to be 57.5 kJ/mol. Based on this reaction, a kinetic method for streptomycin determination is proposed. The calibration graph is linear in the ranges 1.94 – 15.48 µg/cm³ and 0.15 – 1.94 µg/cm³. The influence of foreign ions and molecules on the accuracy of the determinations was investigated. The proposed kinetic method has high selectivity and good sensitivity, and enabled to determine streptomycin in pharmaceutical samples.

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Introduction

Streptomycin is a water-soluble aminoglycoside antibiotic drug that is active against numerous gram-negative and gram-positive bacteria. Because of its serious nephrotoxicity and ototoxicity, there is an increasing tendency to reserve the use of streptomycin only for the treatment of tuberculosis in conjunction with other drugs, like isonicotinic acid hydrazide (isoniazid). However, it remains one of the agents of choice for treating certain bacterial infections, such as brucellosis and tularemia.¹ It is marketed as a sulfate salt of streptomycin, which is more stable than the free base.

Streptomycin has been determined by many analytical techniques, like the continuous-flow chemiluminometric method by oxidation with *N*-bromosuccinimide,² the spectrophotometric method with iodine and wool fast blue BL,³ spectrofluorometric determination using 9,10-phenanthraquinone,⁴ HPLC bioanalysis,⁵ and capillary electrophoresis.⁶ On the other hand, kinetic methods of drug analysis are simple and low-cost alternatives. There have been a few studies in which kinetic methods of analysis were applied for the determination of organic molecules, some of which include drugs.⁷ However, there is no data that streptomycin was determined by this method.

The interaction of Cu(II) with H₂O₂ has been investigated in the past as a producer of reactive oxygen species (·OH and O₂)⁸ in Cu-Fenton-like reactions. This mixture of Cu(II)/H₂O₂ is a Fenton-like reagent, and may be used for the oxidation and degradation of organic compounds, owing to its high oxidant power and simplicity, analogous to the Fenton reagent.⁹ Reactive

oxygen species, including superoxide O₂^{·-}, hydrogen peroxide (H₂O₂) and hydroxyl radicals (OH·) are also generated by a number of pathways in the human body.¹⁰ Most of the OH· radicals generated *in vivo* come from the metal-catalyzed decomposition of hydrogen peroxide according to a Fenton-type equation.

In the present work, the kinetics of streptomycin degradation by hydrogen peroxide in the presence of Cu(II) ions at physiologically pH 7.4 was studied. The kinetic method that was applied for the determination of streptomycin in a pharmaceutical sample with high selectivity is also described.

Experimental

Apparatus

The reaction rate of the degradation of streptomycin by H₂O₂ was followed spectrophotometrically. The dependence of the absorbance (*A*) on time (*t*) was measured by a Perkin-Elmer Lambda 15 UV/VIS spectrophotometer, connected to a thermocirculating bath. A Hanna Instruments pH-meter was used to measure the pH values of the solution. Sigma buffers, pH 7.00 ± 0.01 and pH 4.00 ± 0.01, were used to calibrate the pH meter. The solutions were thermostated at 25 ± 0.1 °C before the beginning of the reaction.

Reagents

Pure streptomycin sulfate, (C₂₁H₃₉N₇O₁₂)₂·3H₂SO₄, was kindly provided by Zdravlje-Leskovac (Serbia and Montenegro). A stock solution of streptomycin (1.00 × 10⁻³ mol/dm³) was freshly prepared by dissolving the required amount of streptomycin sulfate in deionized water; it was stable for 24 h. A solution of Cu(II) (1.00 × 10⁻⁴ mol/dm³) was prepared by dissolving CuCl₂·2H₂O (Merck) in deionized water. A

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Fig. 1 Absorption spectra of: 1) aqueous solution of streptomycin sulfate, 2) streptomycin sulfate-phosphate buffer, 3) streptomycin sulfate-Cu(II), 4) streptomycin sulfate-Cu(II)-phosphate buffer, 5) Cu(II)-H₂O₂-phosphate buffer, 6) streptomycin sulfate-phosphate buffer-H₂O₂, 7) streptomycin sulfate-Cu(II)-H₂O₂-phosphate buffer 3, 5 and 7 min after mixing; pH, 7.4; *t*, 25 ± 0.1°C. Concentrations of the reagents in mixtures were: *C*_{Streptomycin}, 6.67 × 10⁻⁶ mol/dm³; *C*_{Cu(II)}, 2.67 × 10⁻⁵ mol/dm³; *C*_{H₂O₂}, 2.00 × 10⁻² mol/dm³.

hydrogen peroxide solution (1.00 mol/dm³) was prepared just before use from a 35% commercial reagent (Merck). The phosphate buffer, pH 7.4, was obtained by mixing solutions of KH₂PO₄ (0.5 mol/dm³) and Na₂HPO₄ (0.5 mol/dm³) in a volume ratio of 2:7 respectively, and the ionic strength of the buffer was calculated by using the equation $I = [\text{KH}_2\text{PO}_4] + 3[\text{Na}_2\text{HPO}_4]$ as 0.1 mol/dm³.

All of the glassware used was washed with aqueous HCl (1:1), and then rinsed with distilled and deionized water.

Streptomycin sulfate vials containing 1 g of streptomycin were from Galenika-Zemun (Serbia and Montenegro).

General procedure

Procedures for calibration curve. A volume of 0.3 cm³ H₂O₂ solution was stored in one compartment of a special vessel (Budarin's vessel); 4 cm³ of a Cu(II) solution was placed in the second compartment, 1 cm³ of the buffer solution in the third, and a different volume of the streptomycin sulfate stock solution in the fourth compartment. Deionized water was added up to the total volume of the reaction mixture of 15 cm³.

The vessel was thermostated at 25 ± 0.1°C and the reaction was initiated by mixing. The reaction solution was put into a spectrophotometer cell with a path-length of 10 mm, and the absorbance at 322 nm was measured every 30 s over a period of 2 – 7 min after mixing.

Procedures for analysis of dosage forms. The contents of five vials were dissolved in deionized water and quantitatively transferred into a 250 cm³ measuring flask. Deionized water was added to the final volume. Then, 1 cm³ of this solution was transferred into a 250 cm³ measuring flask to obtain the final concentration of streptomycin, 80.00 µg/cm³. Aliquots of this drug solution were transferred into vessels covering the concentration range listed in Table 4, and proceeded as described for pure samples. The amount of streptomycin in drug solution was calculated using the calibration curve.

Results and Discussion

The absorption spectra (Fig. 1) were recorded 2 min after

Fig. 2 Correlation between the reaction rate and the pH. Concentrations in measured solutions: *C*_{Streptomycin}, 1.00 × 10⁻⁵ mol/dm³; *C*_{H₂O₂}, 1.33 × 10⁻² mol/dm³; *C*_{Cu(II)}, 3.47 × 10⁻⁵ mol/dm³; *t*, 25°C.

mixing the reagents. No characteristic absorption maximum was seen on the spectrum of streptomycin sulfate-Cu(II) (Fig. 1, curve 3) or streptomycin sulfate-phosphate buffer (Fig. 1, curve 2). Streptomycin sulfate-Cu(II)-phosphate buffer spectra have the maximum at 240 nm (curve 4, Fig. 1), which corresponds to the existence of the Cu(II)-phosphate complex.⁸ After adding of hydrogen peroxide in this solution, an absorption maximum at 322 nm appeared and increased with time (Fig. 1, curve 7). There was no reaction without Cu(II) ions, because the spectrum of the mixture of streptomycin sulfate-phosphate buffer-hydrogen peroxide (Fig. 1, curve 6) has no maximum at 322 nm.

In alkaline solutions, the fast decomposition of H₂O₂ is mainly the result of a bimolecular reaction,¹¹ which proceeds between HOOH and HOO⁻:

The Cu(II) ion has a strong catalytic action on this reaction by a chain reaction mechanism,¹² producing a notable amount of hydroxyl radicals (HO[·]), which react with the molecules of streptomycin:

This degradation in the phosphate buffer (pH 7.4) gave rise to the formation of a yellow-color product, having an absorption maximum at 322 nm. The degradation product was not identified.

Kinetic studies

The initial-rate method was used to determine partial orders.¹³ The reaction was followed up spectrophotometrically, and the rate of change of absorbance at 322 nm was measured. The initial rates of the reaction were determined by measuring the slopes of the initial tangents to the absorbance-time curves, dA/dt .

Effects of the reaction variables

The influences of the temperature, pH, hydrogen peroxide and Cu(II) concentration on the reaction of the degradation of the streptomycin were studied.

The effect of the temperature on the reaction rate was followed in the range 295 – 310 K. The reaction rate was increased with increasing temperature. A linear relationship

Fig. 3 Correlation between the reaction rate and the Cu(II) concentration. Concentrations in measured solutions: $C_{\text{Streptomycin}}$, $1.00 \times 10^{-5} \text{ mol/dm}^3$; $C_{\text{H}_2\text{O}_2}$, $1.33 \times 10^{-2} \text{ mol/dm}^3$; pH, 7.4; t , 25°C .

Fig. 4 Dependence of the reaction rate on the H_2O_2 concentration. Concentrations in measured solutions: $C_{\text{Streptomycin}}$, $1.00 \times 10^{-5} \text{ mol/dm}^3$; $C_{\text{Cu(II)}}$, $2.67 \times 10^{-5} \text{ mol/dm}^3$; pH, 7.4; t , 25°C .

between the logarithm of the relative rate constant (k_r) and the reciprocal of the absolute temperature was found in the range 295–301 K, and thus an optimum reaction temperature of 298 K was selected. At a higher temperature (304 K), the rate of the reaction became too fast and not suitable for following during the period of 2–7 min after mixing.

The influence of the pH is presented in Fig. 2. A complete reaction between streptomycin, hydrogen peroxide and Cu(II) took place only at pH 7.4. The influence of the medium alkalinity was investigated between pH 6.0 and pH 9.0 with different buffer systems. The phosphate buffer was used for the pH range between 6.0 and 7.8; tris(hydroxymethyl)aminomethane-HCl buffer for pH 8.0–9.0, and bicarbonate buffer for pH 9.0. The rate of the reaction had a maximum value at pH 7.4; therefore, the phosphate buffer with pH 7.4 was chosen for all subsequent experiments.

Keeping all other experimental parameters constant, the Cu(II) dependence of the system was studied in the range $1.67 \times 10^{-5} \text{ mol/dm}^3$ to $3.67 \times 10^{-5} \text{ mol/dm}^3$. It was observed that Cu(II) ions had catalytic activity up to $2.67 \times 10^{-5} \text{ mol/dm}^3$. Higher concentrations of Cu(II) yielded lower slope values, probably due to complex formation between Cu(II) and streptomycin, $\text{Cu}_2(\text{St})\text{Cl}_4$.¹⁴ The reaction was *pseudo*-first order with respect to the Cu(II) concentration in the concentration range from $1.67 \times 10^{-5} \text{ mol/dm}^3$ to $2.67 \times 10^{-5} \text{ mol/dm}^3$, and negative *pseudo*-first order at concentrations higher than $2.67 \times$

Table 1 Constant k_r at three temperature

T/K	$k_r \times 10^{-9} \pm \text{SD}^a / \text{dm}^3 \text{ mol}^{-1} \text{ min}^{-1}$
295	8.78 ± 0.09
298	10.96 ± 0.14
301	13.97 ± 0.18

a. SD, standard deviation of the 3 different streptomycin concentrations.

10^{-5} mol/dm^3 , attaining the maximum value for $2.67 \times 10^{-5} \text{ mol/dm}^3$. Figure 3 shows the correlation between the reaction rate and the Cu(II) concentration.

The dependence on the H_2O_2 concentration is presented in Fig. 4. The reaction is *pseudo*-first order with respect to the H_2O_2 concentration. The H_2O_2 concentration of $2.00 \times 10^{-2} \text{ mol/dm}^3$ was selected, because at concentrations higher than $2.67 \times 10^{-2} \text{ mol/dm}^3$ the rate of the reaction became too fast, and was therefore not suitable for kinetic determinations.

The absorbance-time curves for solutions containing different amounts of streptomycin were recorded under selected and constant reaction concentrations of $C_{\text{Cu(II)}} = 2.67 \times 10^{-5} \text{ mol/dm}^3$, $C_{\text{H}_2\text{O}_2} = 2.00 \times 10^{-2} \text{ mol/dm}^3$ and pH 7.4. They thus showed pseudo-zero-order dependencies with respect to Cu(II) and H_2O_2 . Under these experimental conditions the rate of the reaction was found to be ρ (streptomycin) dependent, and the slope indicates a *pseudo*-first-order reaction with respect to streptomycin. The proposed kinetic equation for the catalytic concentration range of Cu(II) from $1.67 \times 10^{-5} \text{ mol/dm}^3$ to $2.67 \times 10^{-5} \text{ mol/dm}^3$ is

$$\text{d}A/\text{d}t = k_r C_{\text{Streptomycin}} \quad (3)$$

where k_r is the relative rate constant.

Based on kinetic Eq. (3), the relative rate constant, k_r , was calculated at three temperatures for different concentrations of streptomycin (Table 1).

The activation energy of the reaction was calculated through the slope of the linear-regression equation between $\log(k)$ and $1/T$ according to Arrhenius equation as 57.5 kJ/mol.

Calibration graph

Under the selected reaction conditions (pH 7.4; $V_{\text{buffer}} = 1 \text{ cm}^3$; $C_{\text{Cu(II)}} = 2.67 \times 10^{-5} \text{ mol/dm}^3$; $C_{\text{H}_2\text{O}_2} = 2 \times 10^{-2} \text{ mol/dm}^3$; $t = 25 \pm 0.1^\circ\text{C}$) the streptomycin concentration was varied from 0.15 $\mu\text{g/cm}^3$ to 15.48 $\mu\text{g/cm}^3$. The linear dependencies were established between the slopes of the absorbance-time curves and the streptomycin concentration.

The analytical and statistical data for the calibration graphs estimated by the linear-regression method with the standard deviation of the fit and correlation coefficient r are given in Table 2. The limit of detection was estimated according to IUPAC¹⁵ as $3S_b/m$ for a confidence level of 99.86%, where S_b is the standard deviation of the blank, and m is the analytical sensitivity. The relative standard deviation, calculated in the range of the calibration graph from 1.94 $\mu\text{g/cm}^3$ to 11.62 $\mu\text{g/cm}^3$ of streptomycin, was up to 1.59%.

Effect of foreign substances

In order to test the applicability of the method, the effect of foreign ions and organic molecules on the determination of $6.67 \times 10^{-6} \text{ mol/dm}^3$ of streptomycin was investigated. The tolerance limit for foreign substances was estimated as the concentration of the substance that gives up to a 3% relative error in the

Table 2 Analytical and statistical analysis of the calibration graphs for the determination of streptomycin

	Range of calibration/ $\mu\text{g cm}^{-3}$	
	1.94 – 15.48	0.15 – 1.94
Number of points	8	11
Limit of detection/ $\mu\text{g cm}^{-3}$	—	0.02
Slope $\times 10^2/\text{cm}^3 \mu\text{g}^{-1} \text{min}^{-1}$	0.4912	0.4860
Intercept $\times 10^2/\text{min}^{-1}$	1.4095	1.3888
Correlation coefficient	0.9992	0.9998
Standard error of the slope, $\times 10^2$	0.0079	0.0036
Standard error of the intercept, $\times 10^2$	0.0773	0.0041
Standard deviation of the fit, $\times 10^2$	0.0991	0.0070

Table 3 Tolerance limits of foreign molecules and ions ($C_{\text{foreign}}/C_{\text{Streptomycin}}$)

Tolerance level ($C_{\text{foreign}}/C_{\text{Streptomycin}}$)	Ion/molecule added
10^2	Lactose, fructose, glucose, starch, amino acids, Ca^{2+} , Mg^{2+} , NH_4^+
10	Zn^{2+} , Al^{3+} , CH_3COO^- , SO_4^{2-} , SO_3^{2-} , citric acid
Interference	Fe^{2+} , Mn^{2+} , Co^{2+} , NO_3^- , $\text{C}_2\text{O}_4^{2-}$

determination of streptomycin. The results are summarized in Table 3.

Regarding the selectivity, as determined by this criterion for a streptomycin concentration of $6.67 \times 10^{-6} \text{ mol/dm}^3$ up to a molar ratio of 100:1, the usual ingredients of powdery drugs (lactose, fructose, glucose, starch), amino acids (arginine, tyrosine, methionine, asparagine, histidine, serine), Ca^{2+} , Mg^{2+} and NH_4^+ ions had practically no influence on the streptomycin determination by this method. Ions Zn^{2+} , Al^{3+} , CH_3COO^- , SO_4^{2-} , SO_3^{2-} , and citric acid interfere only when present in approximately 10-fold (molar concentrations) excesses. Positive interference was found for NO_3^- and $\text{C}_2\text{O}_4^{2-}$ anions. More severe interference was observed for Fe^{2+} , Mn^{2+} and Co^{2+} ions, because they also act as catalysts in the oxidation reaction of the Fenton and Fenton-like type.¹⁶ If their concentrations are larger, the rate of the reaction will increase.

Determination of streptomycin in dosage forms

The proposed method has been directly applied to the determination of streptomycin in streptomycin sulfate vials, as described in procedures for the analysis of dosage forms. The values obtained by the proposed kinetic method were compared with those obtained by the official method, which includes a microbiological assay of antibiotics.¹⁷ The accuracy and precision of the determination were calculated (Table 4).

Conclusion

The proposed kinetic method of streptomycin determination is

Table 4 Determination of streptomycin in streptomycin sulfate vials by the kinetic and official methods

Official method ¹⁷ / $\mu\text{g cm}^{-3}$	Proposed method $\bar{x} \pm \text{SD}/\mu\text{g cm}^{-3}$	RSD, %	Recovery, %
9.71	9.82 ± 0.18	1.83	101.13
8.00	8.23 ± 0.30	3.65	102.90
2.67	2.80 ± 0.26	9.30	104.80

$\bar{x}$, Mean value of 5 replicates; SD, standard deviation of the mean; RSD, relative standard deviation of the mean.

simple, sensitive and accurate. In addition, it is not susceptible to interference from common tablet components.

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