SPECTROPHOTOMETRIC MONITORING OF THE OXIDATION OF THIOBENZAMIDE AT VARYING OXIDANT CONCENTRATIONS

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Abstract: The metabolism of substances with a thioamide group involves complex oxidation-reduction reactions, which is an important focus in modern pharmaceutical chemistry, so the thioamides act as key components in biologically active substances. Thioamides, with substituents that are aromatic, aliphatic, or heterocyclic, display a wide range of biological activities and include effects such as antimycobacterial, antibacterial, antifungal, antiviral, anticancer and anti-inflammatory properties. Understanding how thioamides behave during oxidation, is important for uncovering their metabolic pathways and for the design of new therapeutic medicaments. This study aim is the oxidation of thiobenzamide, and monitoring the oxidation flow were CrO_3 is used as the oxidizing agent under various conditions. The oxidation products of thiobenzamide were obtained according the literature data and all of the products were used in further oxidation reactions. Experiments were conducted to optimize the reaction parameters and to examine how oxidant concentration affect the reaction pathway. The oxidation reactions were carried out in different mediums, acidic media (KCl/HCl), neutral and alkaline phosphate buffer (pH 7 to 10). To understand how the concentration of oxidant impact the reaction flow, systematically was varied the substrate and oxidant molar ratios at 1:1, 1:5, and 1:10. The progress of the oxidation reaction was monitored directly from the reaction mixture using UV-Vis spectrophotometry. The results show a clear impact of pH and oxidation agent concentration on the reaction flow and the products of the reaction and they also highlight differences in the oxidation pathways under different medium as an acidic, neutral, or basic conditions. By changing the molar ratios of the oxidizing agent and the substrate, the best results were obtained at a molar ratio of 1:10, where majority of the substrate reacted and the best results are achieved at room temperature. Due to the low yield and rapid further reaction with thiobenzamide, some of the products cannot be isolated and detected and the composition of the reaction mixture changes over time. The products obtained during the reaction are stable under the specified experimental conditions. This study offers new insights into how thioamides behave during oxidation reaction with CrO_3 and highlights the usefulness of spectroscopic and chromatographic techniques for tracking changes in thioamides. The findings enhance our understanding of chemistry of thioamides and provide useful information for designing and evaluating biologically active compounds with antimycobacterial activity.
Keywords: oxidation, thioamide, UV-vis spectroscopy, pH.

1. INTRODUCTION

One third of the total global population is infected with Mycobacterium tuberculosis, and 95% of those suffering from tuberculosis come from developing countries. Of these, 75% belong to the most economically productive age group — individuals between 15 and 50 years of age(Keshavjee & Farmer, 2012; Schön et al., 2017). Patients who are not fully cured may expel bacteria that are resistant to the prescribed therapy (Keshavjee & Farmer, 2012; Schön et al., 2017). so far, the most effective medications for the treatment of tuberculosis infections are ethionamide (ETA), rifampicin (prothionamide – PTA), and isoniazid (INH) (Samet & Ristovska, 2016; Vannelli et al., 2002). A common feature of these compounds is the presence of a thioamide or carboxamide fragment in their structures. Using various bacterial media, the minimum inhibitory concentration of substances containing thioamide and carboxamide groups has been determined (Yan & Xu, 2019), providing important insights into the differences in antibacterial and mutagenic activity among these biologically active substances. The differences in antibacterial activity are attributed to the markedly high nucleophilicity of the sulfur atom in the thioamide group compared to the oxygen atom in the amide group. Most reported natural thioamides are of bacterial origin, except for the plant-derived cycasthioamide (Mahanta et al., 2019).

The confirmed antimycobacterial, antibacterial, antifungal, antiviral, anticancer, anti-inflammatory, photosynthesis-inhibitory and antialgal activity of unsubstituted and N-substituted thioamides (where the substituent is an aromatic, aliphatic or heterocyclic structural fragment) (Waisser et al., 1990), is a reason for their continuous study in many laboratories worldwide.

Due to the presence of the thioamide group in the composition of some drugs or some constitutive units of nucleic acids, thiobenzamides are particularly interesting as suitable and simple models for in vitro studies (Koga et al., 2004) of interactions between biologically active substances and living organisms (Lakshmanan et al., 2011). From a pharmacological point of view, it is particularly important to understand the modes of their metabolic degradation, in order to prevent the products of oxidative biotransformation from behaving as potential toxins(Waisser et al.,

1990). This complex redox process has stimulated interest in studying the oxidation reaction of thiobenzamide as a model compound, which is the subject of this research paper.

To monitor the flow of the oxidation of thiobenzamide, it is necessary to synthesize its oxidation products. In the first phase of the work, the putative oxidation products were synthesized unambiguously according to known and modified methods: benzamide, thiobenzamide-S-oxide and 3,5-diphenyl-1,2,4-thiadiazole (SAMET & PAVLOVA, 2025). The obtained substances were identified using spectroscopic methods. The oxidation was carried out in buffer solutions with different pH values (potassium chloride buffer with pH=1 and 2; acetate buffer with pH=3, 4 and 5; phosphate buffer with pH=8, 10 and 11; and buffer with pH=13) (Rasaiah, 1973; Reilly et al., 2002). The flow of the oxidation reaction was qualitatively monitored using spectrophotometric and chromatographic methods The results of the research within the framework of this research paper represent a contribution to the study of the oxidation reactions of thioamides and the application of spectrophotometric and chromatographic methods for qualitative and quantitative monitoring of the flow of the oxidation of thiobenzamide and other oxidizing agents.

2. METHODS AND MATERIALS

Synthesis of oxidation products of thiobenzamide

The Oxidation products of thiobenzamide Benzoyl chloride (Lorriman & Rae, 1961), Benzamide (Lorriman & Rae, 1961), 3,5-difenil-1,2,4-tiadiazol(Cronyn & Nakagawa, 2002; Xie et al., 2016), tiobenzamide-S-oksid (Cashman, 2024; Cashman & Hanzlik, 2002) are obtained according the literature data. All of the products were used in further oxidation reactions.

Used Solutions: CrO_3 solution (1mM) and thiobenzamide solution (0.01 M).

Used Buffers: KCl/HCl buffer ;acetate buffer; phosphate buffers.

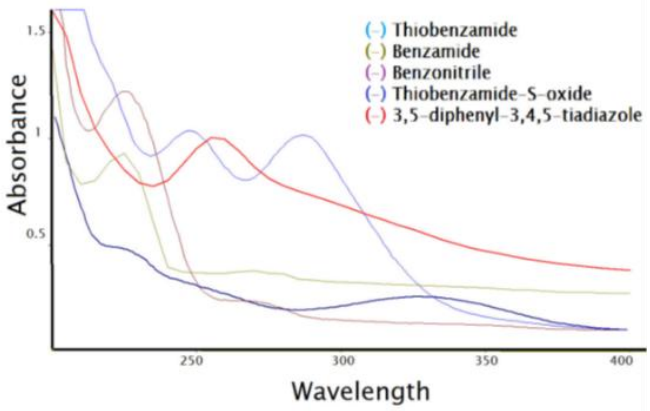
Spectrophotometric monitoring of the flow of oxidation of thiobenzamide with CrO_3

The flow of oxidation of thiobenzamide with CrO_3 was monitored with a Varian UV-vis spectrophotometer model Cary 50. A kinetic method was created for spectrophotometry of the samples in the wavelength range from 200 to 600 nm relative to a blank sample of an appropriate buffer. The scanning was repeated every 15 seconds for 120 minutes. Samples with different molar ratios of substrate and oxidizing agent (1:1, 1:5 and 1:10) were prepared by direct injection into a quartz microcuvette (volume 1.5 ml) for spectrophotometry. The acidity of the reaction medium was varied by performing the oxidation in an appropriate buffer, under identical experimental conditions. The identification of the oxidation products was performed by comparing the UV spectra of the standard substances.

3. RESULTS AND DISCUSSION

Monitoring the flow of oxidation of thiobenzamide in acidic medium

Figure 1. UV spectra of aqueous solutions of thiobenzamide, benzamide, benzonitrile, thiobenzamide-S-oxide and 3,5-diphenyl-1,2,4- thiadiazole, with up to 2.5% acetonitrile content (Samet & Ristovska, 2016).



Source: Author research

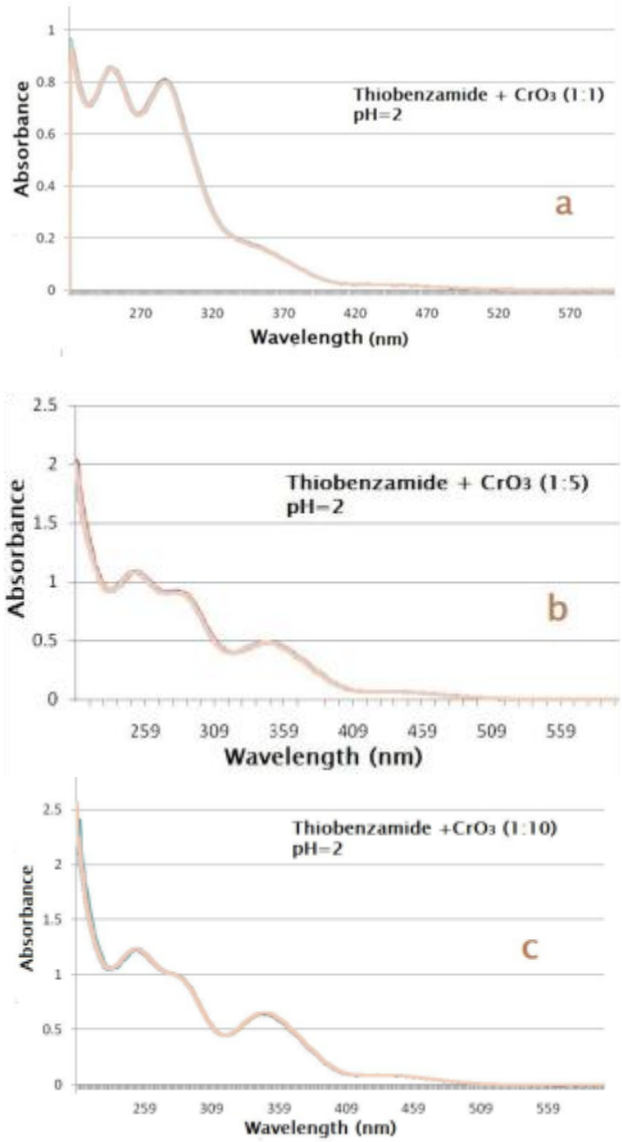
The oxidation of thiobenzamide was monitored spectrophotometrically due to the ultraviolet absorption of thiobenzamide and its oxidation products: benzamide, benzonitrile, thiobenzamide-S-oxide, and 3,5-diphenyl-1,2,4-thiadiazole. As shown in Figure 1, thiobenzamide exhibits two absorption maxima at wavelengths of 248 nm and 287 nm. The characteristic UV spectrum of benzamide shows one absorption maximum at 226 nm and a small

shoulder at 271 nm. The heterocyclic compound 3,5-diphenyl-1,2,4-thiadiazole is characterized by a single absorption maximum at 256 nm. The absorption maximum of benzonitrile is found at 225 nm. Only the absorption maximum of thiobenzamide-S-oxide is shifted to higher wavelengths. At a wavelength of 334 nm, thiobenzamide-S-oxide shows an absorption maximum, whereas thiobenzamide exhibits almost no absorption in this region, allowing for both qualitative monitoring of the oxidation process and quantitative determination of thiobenzamide-S-oxide. The oxidation of thiobenzamide and substrate consumption are observed through the decrease in the intensity of the strong absorption band originating from the thioamide chromophore group, forming the first oxidation product which, in all analysed cases, is thiobenzamide-S-oxide.

Spectrophotometric monitoring of thiobenzamide oxidation with CrO_3 in 1:1, 1:5, and 1:10 ratios in strongly acidic,neutral and basic media.

In a strongly acidic medium at pH values of 2 (KCl/HCl buffer), thiobenzamide is protonated, and the oxidation with equimolar amounts of CrO_3 occurs instantaneously, with thiobenzamide-S-oxide as the only oxidation product.

Figure 2. Spectrophotometric monitoring of thiobenzamide oxidation with an (a) equimolar amount, (b) fivefold excess and (c) tenfold excess of CrO_3 in strongly acidic medium at pH = 2.



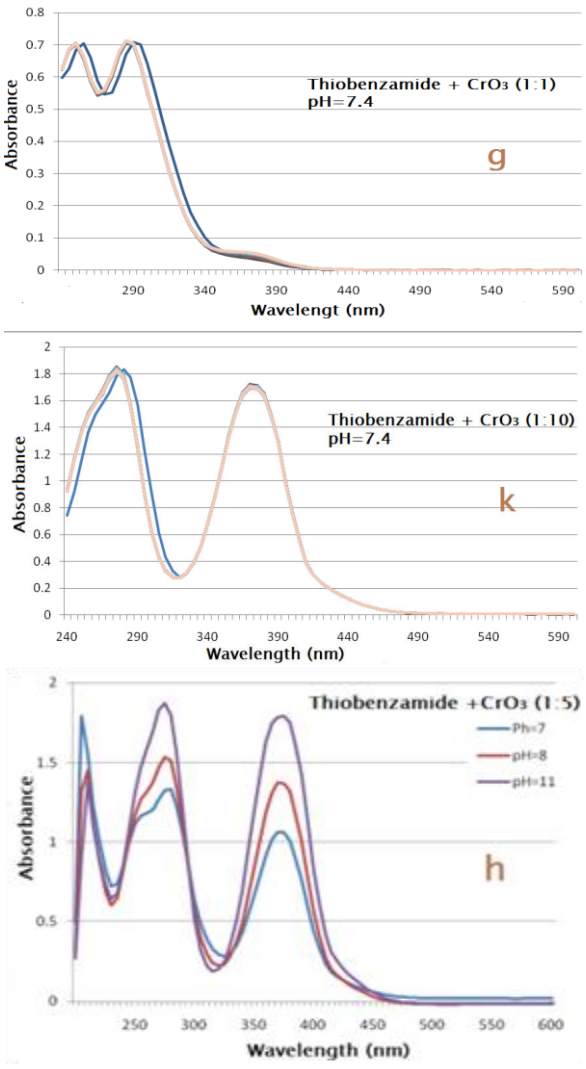
Source: Author research

At these pH values, thiobenzamide-S-oxide is also protonated, and its absorption maximum appears at a wavelength of 347 nm. Upon extended reaction time, there are no significant changes in the concentrations of the substrate and the oxidation product, as shown in Figure 2a.

Based on the absorbance values of thiobenzamide-S-oxide, it can be concluded that a higher concentration of this product is obtained with a tenfold excess of the oxidizing agent (Figures 2b and 2c). Excess oxidant under strongly acidic conditions leads to further oxidation of the substrate and the formed product, resulting in the formation of the cyclic dimer 3,5-diphenyl-1,2,4-thiadiazole, which was identified using thin-layer chromatography and contributes to increased absorbance values around 250 nm. TLC also detected the presence of benzamide in the reaction mixture, likely formed because of acid hydrolysis of thiobenzamide or oxidation of thiobenzamide under vigorous conditions (Cronyn & Nakagawa, 2002; Xie et al., 2016).

The formation of the oxidation product thiobenzamide-S-oxide is instantaneous, and with continued reaction time at room temperature, no significant changes in the concentrations of these organic substances are observed. By comparing the results obtained from the UV spectra and thin-layer chromatography with those obtained for standard substances, the unreacted substrate thiobenzamide and the oxidation products thiobenzamide-S-oxide and the heterocyclic compound 3,5-diphenyl-1,2,4-thiadiazole were identified.

Figure 3. Spectrophotometric monitoring of the flow of thiobenzamide oxidation with an (g) equimolar amount a (k) tenfold excess of CrO_3 in a neutral medium at pH = 7.4; with a (h) fivefold excess of CrO_3 at pH = 7.4, pH = 8.4, and pH = 10.9.



Source: Author research

As can be seen from Figure 3g, where the flow of the oxidation of thiobenzamide with an equimolar amount of Cr (VI) in a neutral phosphate buffer medium (at pH = 7.4) was monitored spectrophotometrically, this reaction, even under conditions of highly diluted solutions (substrate concentration on the order of 10^{-5} M), has an "instant" character. Namely, the first oxidation product, thiobenzamide-S-oxide, is formed upon the very mixing of the reactants, and its absorption maximum is shifted to higher wavelength values (370 nm), unlike its protonated form, which has a maximum at 340 nm. The reaction was monitored over a period of two hours, during which a dynamic equilibrium was established in the reaction mixture between the substrate — thiobenzamide — and its oxidation product thiobenzamide-S-oxide.

According to the results from the spectrophotometric and chromatographic monitoring of the flow of thiobenzamide oxidation with a fivefold and tenfold molar excess of CrO_3 in a neutral phosphate buffer medium (pH = 7.4), the formed thiobenzamide-S-oxide converts into benzonitrile and benzamide (Figure 3k and 3h). These data unequivocally indicate that the presence of excess oxidizing agent promotes the decomposition of thiobenzamide-S-oxide and is the cause for the formation of mixtures with varying proportions of benzamide and benzonitrile depending on the pH of the reaction medium. Buffer solutions are solutions that maintain an approximately constant pH, and they are resistant to reaching especially the pH 7.3, important for many biochemical processes in living organisms When the pH is higher than pH = 6 then the saliva is saturated with phosphate ions, it takes a role of buffer (SAMET et al., 2025). That's the reason of monitoring the reaction flow at the pH value of 7.3.

The detected presence of benzamide in the oxidation reaction of thiobenzamide with a standard CrO_3 solution in a neutral and slightly alkaline medium is due to the desulfurization reaction of thiobenzamide. The proposed mechanism of oxidative sulphur elimination consists of steps in which thiobenzamide-S-oxide and thiobenzamide-S, S-dioxide are formed. According to literature data (Dodge et al., 2006), thiobenzamide-S, S-dioxide is an unstable intermediate that instantly transforms, which is why its presence in the reaction mixture during the oxidation of thiobenzamide with Cr (VI) was not detected. After the sulfur elimination, thiobenzamide-S, S-dioxide transforms into benzamide. Upon prolonged influence of the excess Cr (VI) on the substrate in a neutral and slightly alkaline medium (pH = 7–11), thiobenzamide-S-oxide is formed instantly, and its concentration in the reaction mixture increases at higher pH values (Figure 3h). Under these conditions, the formed thiobenzamide-S-oxide transforms into benzonitrile, which is consistent with literature data (Cashman, 2024; Cashman & Hanzlik, 2002). Namely, when thiobenzamide-S-oxide is treated with a base, benzonitrile is slowly formed, and the reaction is first-order with respect to hydroxide.

4. CONCLUSION

The products of the oxidation of thiobenzamide with chromium (VI) is like the oxidation products obtained with other oxidizing agents. Upon completion of the reaction between equimolar amounts of thiobenzamide and CrO_3 in the region of low concentrations, the only product formed is thiobenzamide-S-oxide, regardless of the pH value of the medium. By changing the molar ratios of the oxidizing agent and the substrate, we determined that the best results were obtained at a molar ratio of 10:1, where majority of the substrate reacted and the highest amount of thiobenzamide-S-oxide was formed. The best results are achieved when the experiments are conducted at room temperature. Due to the low yield and rapid further oxidation and/or reaction with thiobenzamide, some of the products cannot be isolated and detected (thiobenzamide-S, S-dioxide). The composition of the reaction mixture changes over time; thiobenzamide is oxidized to thiobenzamide-S-oxide, which then, under slightly acidic conditions, transforms into the heterocyclic compound 3,5-diphenyl-1,2,4-thiadiazole and benzamide as the main product, while in neutral and slightly basic conditions, benzonitrile and benzamide are obtained. The products obtained during the degradation of thiobenzamide-S-oxide are stable under the specified experimental conditions.

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