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Oxidative Coupling-Base Reaction for the Indirect Spectrophotometric Determination of Paracetamol in Drug Formulations

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Abstract: A simple, rapid, and specific spectrophotometric method was developed for the determination of paracetamol in pure form and drug formulations. The method depended on the hydrolysis of paracetamol to p-aminophenol, which, oxidized under the effect of dissolved oxygen to benzoquinoneimine. In the alkaline medium, benzoquinoneimine reacts with Methyldopa to produce a high stabile indophenol dye. The absorbance was measured at 580 nm, and the molar absorptivity was found to be 7.75 x 10-5 L/(mol cm). Paracetamol was determined in pharmaceutical products in the 10–100 µg.mL-1 concentration range with a detection limit of 1.5µg.mL-1 PAP. The developed method can be applied to the determination of both paracetamol and p-aminophenol in the presence of each other without prior separation. The proposed method is successfully employed for the determination of paracetamol in various synthetic mixtures and pharmaceutical preparations. Our obtained results were statistically compared with those given by the similar methods and the procedures evaluated as regards to both figures of merit and ease of applicability.

Keywords: Paracetamol, Spectrophotometric, Methyldopa, Oxidative–Coupling, Determination

Introduction

Paracetamol (N-(4-hydroxy-phenyl) acetamide) is a mild pain reliever and a fever reducer medication (ABDURRAHMAN, 2016). It has been in general use for more than 60 years; however, the exact mechanism of action is still not fully understood. It is highly recommended for adults and children 12 years and over in dose 500 to 1000 mg every four hours as necessary. It's moreover prescribed for youthful children in lower measurements. It's unsafe to require more than the recommended dosage as you'll harm your liver, which may be irreversible and lethal (Soller, Ho, & Lightwood, 2015; Yoon, Babar, Choudhary, Kutner, & Pyrsopoulos, 2016). Paracetamol available under a variety of brands and, it is often combined with other medications such as cold medications. It is ordinarily utilized either by mouth or rectally but is additionally accessible by infusion into a vein (Alabere, Lucky, Ogundare, Akinwunmi, & Abimbola, 2020; Németh, Jankovics, Németh-Palotás, & Kőszegi-Szalai, 2008). P-aminophenol is a fundamental material for the synthesis of paracetamol. It is also an intermediate molecular for the hydrolysis of paracetamol within the human body (Almandil et al., 2019; Chu, Jiang, Tian, & Ye, 2008; Riggin, Schmidt, & Kissinger, 1975). Furthermore, under poor storage conditions, paracetamol drug formulations may degrade to form harmful compounds for the human organism such, as p-aminophenol (Abdolkader, 2011; Song & Chen, 2001).

Numerous analytical approached have been investigated to determine the paracetamol in pure form, drug formulations and, biological fluids such as chromatography (Abdelaleem, Naguib, Hassan, & Ali, 2015; Belal, Awad, & Clark, 2009; Shaikh & Devkhile, 2008), flow injection (Calatayud, Pascual Marti, & Vives, 1986; Frangu, Pravcová, Šilarová, Arbneshi, & Sýs, 2019; Tzanavaras & Themelis, 2007), voltammetry (Goyal, Gupta, & Chatterjee, 2010), and spectrophotometry (Hoang, Ly, Tho, & Minh Thi Nguyen, 2014). Most of the spectrophotometric determination methods depended on hydrolysis of paracetamol into p-aminophenol in an acid medium. Then, the coupling-base reaction applies to form a colored dye. Oxidative coupling reactions may be considered as a type among the most important organic reactions which have wide applications in analytical chemistry. Oxidative coupling reactions depend on the coupling of two organic compounds in the presence of an oxidizing agent under suitable conditions. Several coupling agents such as p-xylenol (Filik & Tavman, 2007) 1,2-naphthoquinone-4-sulfonate (Shalash, Salhin, Saad, & Rahman, 2012), o-cresol (Al-Abachi, Al-Safi, & Al-Ward, 2015), 8-hydroxyquinoline (Bouhsain, Garrigues, Morales-Rubio, & de la Guardia, 1996; Filik, Hayvali,

& Kilic, 2005), m-cresol, phenol,resorcinol and sodium iodylbenzoate (Wiener, 1978) have been used for the indirect determination of paracetamol. However, Some of these approaches are not simple for routine analysis or used toxic materials. In the present study, we provide a sensitive spectrophotometric method for the simultaneous determination of paracetamol and p-aminophenol in drug formulations by using dissolved O_2 as oxidant regent.

Experimental

Instruments and reagents: (the type of UV-visible used) UV–Visible spectrophotometer was utilized for the spectral and absorbance measurements by using 1-cm silica match cells. Reagents preparation: All chemicals were of analytical grade, and pure distilled water was used throughout this work.

Paracetamol stock solution in a concentration of 1000 μg.mL^{-1} was prepared directly by dissolving 0.25 g of pure paracetamol that powder obtained from the state company for drug industries and medical appliance (S.D.I.), Samara-Iraq) in 10 mL of ethanol and diluted to 250 mL with distilled water. Others working solutions were freshly prepared by subsequent dilutions from the stock solution.

The Stock Solution of Hydrolyzed Paracetamol (100 μg.mL^{-1})

Exactly, 150 mL of 100 μg.mL^{-1} paracetamol stock solution and 20 ml of 4M hydrochloric acid were mixed into a 250 mL round bottom flask. Then, the mixture refluxed for 60 min. to hydrolyze almost all the paracetamol to p-aminophenol. After that, 16.6 mL of this solution transferred to a 100 mL volumetric flask and completed to the mark with distilled water to get a concentration of 100 μg.mL^{-1}

Methyldopa (MD) solution in a concentration of 1.0 x 10^{-3} M was prepared by dissolving 0.2111g of the reagent in distilled water and then diluted by it to 100 ml in a volumetric flask.

Preparation of Drugs Formula Samples

Tablets

10 tablets of paracetamol contain drugs were weighed, finely, and dried. Then, accurately weighed mass of the powder that, equivalent to 0.25 of paracetamol was moved into a 100 mL volumetric flask and dissolved in 10 mL of ethanol. After that, the volume was completed with distilled water and filtered through filter paper. Finally, 15mL of this solution proceed with the procedure for the stock solution of hydrolyzed paracetamol.

Syrup and Ampule

Equivalent to 0.25g paracetamol of syrup or ampule samples was measured and transferred to 100 mL volumetric flask. The volume was completed with distilled water. After that, 15mL of this solution proceed with the procedure for the stock solution of hydrolyzed paracetamol.

General Assay Procedure

Different amounts of the standard solution of hydrolyzed paracetamol (HP) transfer into a series of 20 mL volumetric flasks to cover the range of concentration between 0.1 to 10 μg.mL^{-1} in the final volume. Then, add 2 mL of Methyldopa (MD) solution in a concentration of 1.0 x 10^{-3}M. Add 1ml of 1M sodium hydroxide solution. Shake the flasks well and allow them to stand for 10 minutes. After that, dilute to the mark with distilled water and measured the absorbance of produce dye against blank solution at 580 nm.

Results and Discussion

In the presence of dissolved atmospheric oxygen and base medium, the hydrolyzed paracetamol (p-aminophenol) reacts with MD solution to form red color that gives a maximum absorbance at 580 nm. Figure 1 shows the absorption spectra of the produce dye and reagent blank. When the same experiment was repeated for the same system but under a nitrogen atmosphere and after removing the dissolved oxygen, there are no colored

compound was observed. The hypothetical mechanism for the acid hydrolysis of paracetamol and the reaction of p-aminophenol with MD in an alkaline medium. The hydrolyzed p-aminophenol is oxidized by the effect of dissolved atmospheric oxygen to form an active benzoquinone imine which, reacts at one of the active positions at methyldopa due to the electrophilic attack to produce a green indophenol dye as a product of the oxidation coupling reaction.

Indophenol dye stability. It has been mentioned in the literature that some indophenol dyes are unstable (Dubos, 1929; Scheiner, 1976) where the dye absorbance showed a continual decrease with time after it maximized, and thus, several stabilizing agents such as Cu(II) was added in order to stabilize the produce dye (Cekic, Filik, & Apak, 2005). However, our formed dye shows high stability, and their absorbance measurements reached the maximum after a few minutes, and then it shows high stability for more than 2h, as shown in Figure 2.

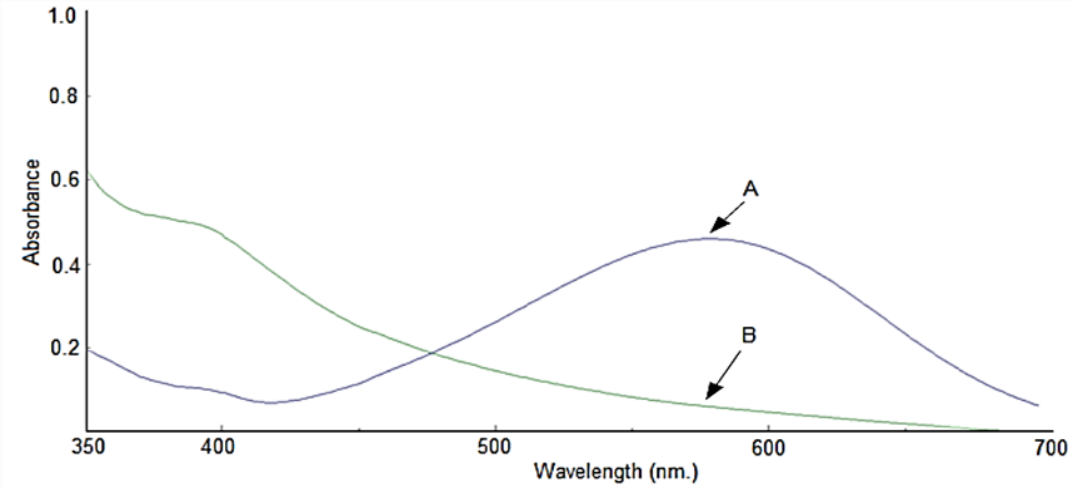


Figure 1. Absorption Spectra of (A) 100 ppm of Hydrolyzed Paracetamol with 2ml of $1x10^{-3}$ MD and 1mL of 4M Sodium Hydroxide in Final Volume 20 ml versus Blank, and (B) 2ml of $1x10^{-3}$ MD and 1L of 4M Sodium Hydroxide in Final Volume 20 mL versus Distilled Water

Effect of temperature. The general assay procedure was examined at different temperatures. As shown in Figure 2, the absorbance dye increased with increasing the temperature until 35C°. After that, the absorbance decrease with increased temperature especially, at degrees higher than 50C°. The absorbance remains constant at room temperature for a stable time to carry out all measurements; thus, the room temperature was recommended for all measurements.

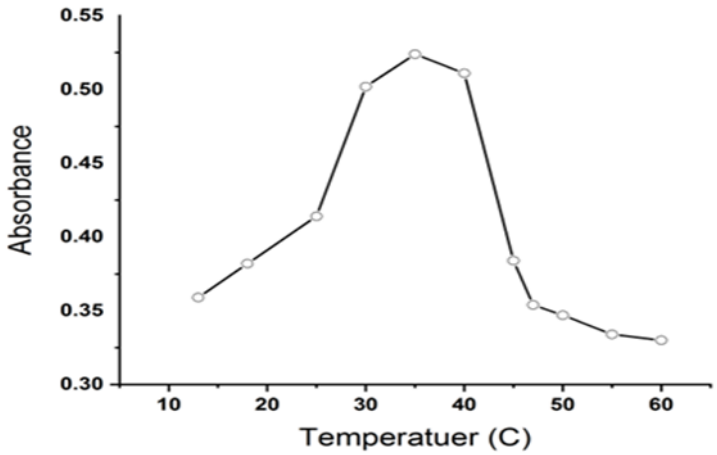


Figure 2. The Effect of Temperature on the Produce Dye Intensity

Order of additions. Our development method contains only two additions after hydrolysis of the paracetamol, the addition of MD stander solution, and the base addition. It was observed that the order addition of reagents was not important.

Effect of the base. The effect of base type and concentration on the proposed oxidative coupling reaction was examined by adding various bases (weak and strong). It found that weak acid generally give weak absorbance measurements compare to strong especially sodium hydroxide that the higher absorbance measurements; thus, it recommended in subsequent experiments. In order to obtain high sensitivity, various volume of 4M NaOH were studied. It found that 1mL 4M NaOH give higher absorbance for that it recommended in all subsequent experiments.

Table 1. The Effect of NaOH Concentration the Proposed Oxidative Coupling

mL of 4M NaOH	Absorbance of 100 µg.mL^{-1}
0.25	0.390
0.50	0.398
0.75	0.408
1.00	0.416
1.50	0.392
2.00	0.335
2.50	0.219
3.50	0.201

The effect of Methyldopa solution concentration. It was inspected by adding different volumes of fixed concentration (1.0 x 10^{-3}) Methyldopa solution and the results in table (1) show that 2 mL of the reagent give the maximum color intensity for produce dye; thus, it depended for all following measurements.

Table 2. The Effect of MD Concentration on the Dye Intensity

mL of MD (1 x 10^{-3})	Absorbance of 100 µg.ml^{-1}
1.0	0.318
2.0	0.424
2.5	0.421
3.0	0.417
3.5	0.395
4.0	0.354

Calibration graph (Standard curve).the instrumental response to paracetamol concertation in a sample has been predicted by a calibration curve. At optimal conditions, Figure 3 shows the linearity of the calibration curve within concentrations (10-100) µg.mL^{-1} of an analyte. A negative draft from linearity was observed within the concertation higher than 100 µg.mL^{-1}. The molar absorptivity of produced dye, calculated at 580 nm was 7.733 x 10^{-5}.

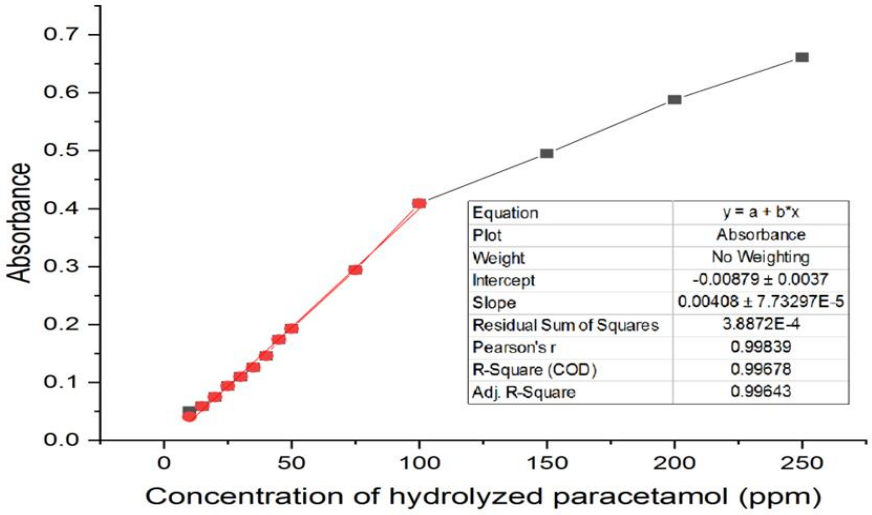


Figure 3. Calibration Graph for Paracetamol based on Proposed Procedure

The stoichiometry and the mechanism of the reaction: Under the optimum condition, the reaction between hydrolyzed paracetamol and methyldopa was investigated by Jod's method. As shown in figure 4, the

equivalence between the reactants was 1:1. Best on these results, we suggest a mechanism in which paracetamol converts to p-aminophenol as a result of the acid hydrolysis of acetamide group to amine. Then, in an alkaline medium, p-aminophenol is oxidized by the effect of dissolved atmospheric oxygen to form an active benzoquinone imine which, reacts at one of the active positions at methyldopa due to the electrophilic attack to produce a green indophenol dye as a product of the oxidation coupling reaction. Scheme 1 below shows the possible mechanism for the reaction hydrolysis of paracetamol in acid medium and explain the reaction between hydrolyzed paracetamol and methyldopa.

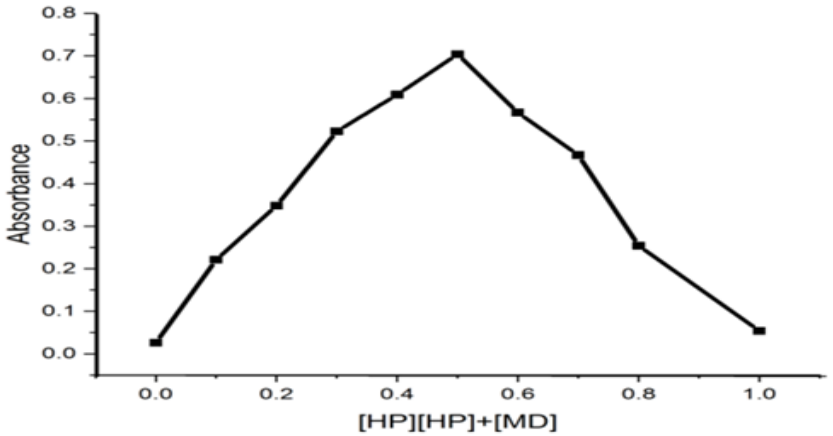
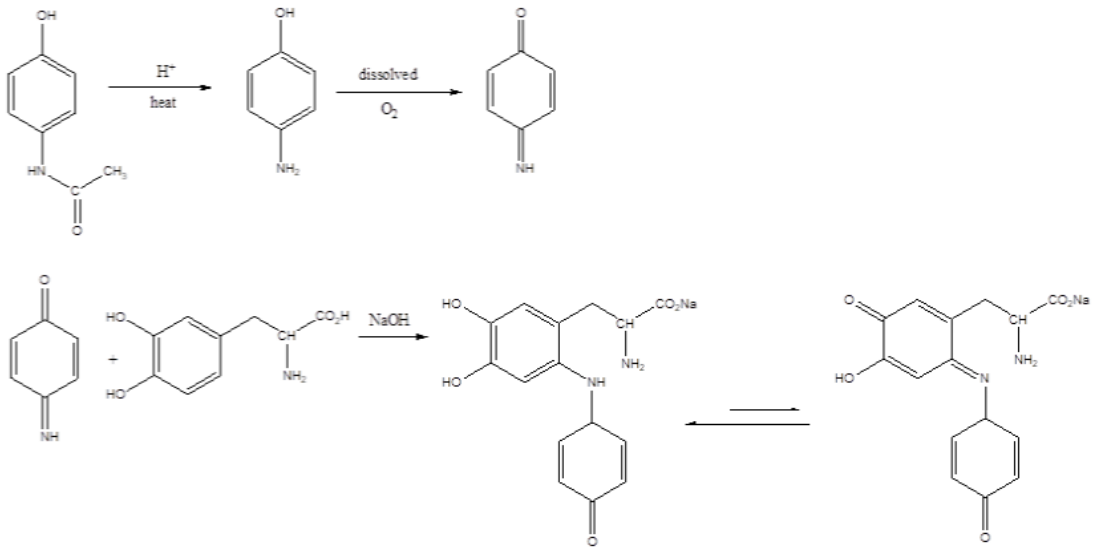


Figure 4. The Stoichiometry Ratio between Hydrolyzed Paracetamol and Methyldopa Investigate by Jod's Method

The effect of interferences. the effect of interferences was studied by adding 2 mL of 3 x 10^{-3} M various types of commons interferences for pharmaceutical preparations such as lactose, starch, glucose, and Arabic acacia to the medium of the reaction. The result indicated that the presence of these compounds was considered not to interfere.



Scheme 1. The Possible Mechanism for the Reaction Hydrolysis of Paracetamol in Acid Medium and Explain the Reaction between Hydrolyzed Paracetamol and Methyldopa

The effect of surfactants. the effect of surfactants was investigated by adding 2.5 mL of 1% various types of commons surfactants (cationic, anionic and neutral) to the alkaline medium of the reaction. The result indicated that the presence of surfactants was considered to interfere with the reaction and deceased the dye intensity much.

Evaluation Analytical Data. The proposed method for indirect determination of paracetamol has been validated base on the international conference on harmonization guidelines (ICH) (Phillips, Ebbutt, France, & Morgan, 2000). First, The linearity has been checked on the calibration carve results which, show linear plots

(n= 10) with perfect correlation coefficients. The linearity was obtained in the concentration ranges of 10–100 µg.mL-1 for paracetamol with linearity equations y=0.0408x+0.0037 and R2=0.99678. Under the optimum, the repeatability of the proposed method was estimated by performing 5 repeat measurements for 50 µg.mL-1 of paracetamol. Then, the mean and standard deviation have been calculated to give values 49.904 and ± 0.25 respectively and that indicates the high repeatability of our proposed method. The precision and accuracy of the developed method were investigated by recovery study by adding standard paracetamol solution at three different concentration levels within the range of linearity. Through recovery studies, the results show that the method is validated statistically is successfully applied for the determination of paracetamol in pure and dosage forms with percent recoveries ranged from 98.9% to 101.1%.

Conclusion

A highly simple, accurate and sensitive indirect spectrophotometric method has been proposed for the determination of the amounts of paracetamol in various drugs formals. The methods carry out based on the oxidative coupling reaction of analyte with Methyldopa in the presence of dissolve oxygen. The proposed method does not require temperature control or solvent extraction steps.

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