Role of Selenium dioxide in Spectrophotometric determination of Tetracycline in pure and pharmaceutical formulations

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Abstract

Background: 4-Aminoantipyrine (AAP) is widely used in the pharmaceutical industry, in biochemical experiments and in environmental monitoring. AAP as an aromatic pollutant in the environment poses a great threat to human health. Methods: A spectrophotometric, A Sensitive, simple accurate and inexpensive way has been proposed for the estimation of Tetracycline. The way is based on the oxidation reaction coupling of drug Tetracycline by 4-aminoantipyrren (4AAP) in basic medium, and then reacts by Selenium dioxide (SeO₂) to give a complex colored at temperature room which produce a product having maximum absorption at 553 nm.

Results: The data find that the order of addition, Tetracycline – base - Selenium dioxide (SeO₂) - 4- AAP, gave maximum absorbance and sensitivity. The good base was (0.1N) sodium hydroxide needful for developing the colored product and raise its stability. That complex, at 25°C, gave a better sensitivity and was selected for further utilize in this study. Under optimum experimental conditions obtained via the linearity of the constructed calibration curve was in the range of 1-20 mg. L of Tetracycline drug with the detection limit of 1.12 x 10⁻⁵ mg/L and molar absorptivity was 3.915 x 10³ L/mol.cm. Conclusion: The used way seemed simple, fast, precision and accurate. In adding, it is applicable for the assay of the Tetracycline in study in many amount forms and the data are in best concord with those obtained in previous studies.

Keywords: Pharmaceuticals, Colorimetric, Spectrophotometric, Tetracycline, 4-aminoantipyrine, Selenium dioxide.

Introduction

4-Aminoantipyrine (AAP, structure in the inset of Fig. 1) is a metabolite of amino phenazone and is an aromatic substance with analgesic, antipyretic and anti-inflammatory properties[1, 2]. Though, 4-Aminoantipyrine commonly produces side effects like the risk of agranulocytosis. Even though 4-Aminoantipyrine is scarcely ever administered as an
analgesic because of side effects, as a raw material, it is commonly utilized to produce AAP
derivatives, which have better biological activities [3]. In addition, it is utilized as a reagent
for bio-chemical reactions producing phenols or peroxides and can too be utilized to detect
phenols in the environment Since 4AAP is usually utilized in the pharmaceutical industry,
bio-chemical research and environmental monitoring, AAP has become an environmental
contaminant [4, 5]. Oxidative coupling organic reactions seems to be one of the utmost
popular spectrophotometric techniques for the estimation of more than a few drugs like
methyl dopa , paracetamol, sulphonamide , folic acid, phenylephrine [6][14] and
catecholamine drugs [7-10]. In the present paper, an automated procedures are proposed for
the determination spectrophotometric of Tetracycline via reaction by 4AAP in the presence of
Selenium dioxide (SeO2) in alkaline medium. The reaction product has been
spectrophotometrically measured at 553 nm.

![Fig.1: Chemical structure of 4AAP](image)

**Experimental details**

**Spectrophotometric Determination of Paracetamol drug**

Accurately measured suitable volume of Tetracycline drug was transported from solution
to volumetric flasks 10 ml, that can diluted to gain 2ml Tetracycline drug ,each one
containing 3.0mL of reagent (1%) (4AAP) and 3 mL of SeO2 were added in basic medium,
After 3 min by mixing, to give red color and completing the solution to 10 ml by D.W, the
values of absorbance were measured at 553 nm against the blank reagent.

**Calibration graph and the statistical data**

Under the chosen best conditions, a calibration curve was constructed (Figure 2). The
graph showed that the color system is obeyed law Beer’s in the concentration series of 1 –20
mg/L of Tetracycline drug in a 10 mL of final volume (Table 1) shows the statistical result of
the calibration curve of spectrophotometric estimation of Tetracycline drug. The accuracy,
precision of the method were tested via determining sex replicate of standard drug solution at
three concentration levels. The values of the percentage of relative error (Error %) and (RSD %)
for these replicate measurements of Tetracycline drug were calculated. The detection limit
L.O.D for the proposed way were calculated utilizing the eq (1) \((S/N)\) signal/noise ratio obtained via standard addition quantification and subsequent extrapolation to a signal/noise of 3. \([11, 12]\)

\[
L.O.D = 3 \frac{S.D}{k}
\]  ........................................... (1)

The limits of Quantitation's, LOQ were experimentally calculated as the lowest spiked concentration level of the calibration curve defined as calculated utilizing the eq.(2):\([11, 12]\)

\[
L.O.Q = 10 \frac{S.D}{k}
\]  ........................................... (2)

where S.D is the standard deviation of replicate estimation values in the same conditions and K is the sensitivity, namely the slope of the calibration graph. C conc. of Tetracycline drug (mg.L\(^{-1}\)), X-mean average.

Table 1: Statistical of calibration data for different concentration of Tetracycline drug

| Factors                        | Proposed Process |
|--------------------------------|------------------|
| \(\lambda_{\text{max}} \) (nm) | 553              |
| law Beer’s limit (\(\mu g . ml^{-1}\)) | 1–20             |
| Molar absorptivity (L.mol\(^{-1}\).cm\(^{-1}\)) | \(3.915 \times 10^7\) |
| Sandal's sensitivity (\(\mu g \ cm^{-2}\)) | \(0.113 \times 10^{-6}\) |
| Regression equation            | \((Y = m X + C)\) |
| Slope (m)                      | 0.0881           |
| Intercept (C)                  | 0.1526           |
| Correlation coefficient (\(r^2\)) | 0.9949          |
| % Relative Standard deviation (RSD\%) | 0.449         |
| standard deviation (SD)        | 0.5155           |
| Color                          | Red              |
| limit of Detection L.O.D (\(\mu g . ml^{-1}\)) | \(1.12 \times 10^{-5}\) |
| limit of Quantitation L.O.Q (\(\mu g . ml^{-1}\)) | \(4.03 \times 10^{-2}\) |
Results and Discussion

Effect of volume 4-amenoantpyren

When several volume 4-AAP was added to Tetracycline drug, that found that absorbance rises through increasing volume 4AAP and reached its best value on utilizing 3 mL volume of 4AAP but when the volume of 4AAp increase above 3ml the absorbance decrease at best absorption 553 nm of the Tetracycline drug [13-15] as appear in (Fig.3).

Fig. 3 Best absorption of compound in the found of several volume of 4-amenoantpyren

Effect of the Aspiration order
This factor was studied by preparing a solution with different arrangement (Table 2). The order No. 1: Tetracycline drug—base- SeO$_2$- 4-AAP. Maximum absorbance was selected because it gave highest absorbance and sensitivity [16-18].

### Table 2: Effect of order addition of drug and reagents

| NO. | order addition                                      | Absorbance |
|-----|----------------------------------------------------|------------|
| 1   | Tetracycline drug—base- SeO$_2$- 4-AAP             | 1.8111     |
| 2   | Tetracycline drug- SeO$_2$- base- - 4-AAP          | 1.6211     |
| 3   | Tetracycline drug—base- 4-AAP- SeO$_2$             | 1.2231     |
| 4   | Tetracycline drug- 4-AAP- base- SeO$_2$-           | 0.5122     |

**Influence of nature of base**

In order to determine the best base, we utilized several kind of bases (NaOH, Na$_2$CO$_3$, NaHCO$_3$; Ba(OH)$_2$, NH$_3$) appear in Figure 4. Because color formation, good, great intensity, sensitivity and the maximum absorbance value are dependent upon kind of base and quantity of base, thus, the good base, NaOH, was utilized as the alkaline agent. The latter give the maximum absorbance completion. [18-21].

![Fig. 4: Effect of the kind of base addition of Tetracycline drug](image)

**Selection of suitable oxidizing agent**
To choose the suitable agent of oxidizing, different oxidants were studied like 0.1gm of 
CuSO₄, (K₂Fe (CN)₆), (KMnO₄), (FeCl₃) and (SeO₂). found (SeO₂) give higher absorbance 
color stability thus that selected to give the best absorbance signal and it was a selection of as 
a suitable agent of oxidant (Fig. 5) for further investigates [8, 22].

![Graph showing the effect of different oxidants](image)

**Fig. 5: Effect of deferent type of oxidant agent**

**Effect the Volume of oxidizing agent SeO₂**

To found the good result, the volume of SeO₂ was studied. many volume of SeO₂ was 
additional to of 4AAP 0.1N NaOH. 3ml of solution SeO₂ additional to Tetracycline drug mix 
was sufficient to develop color to its complete intensity above 3 ml,[23, 24] the absorbance 
value of the  blank was rising  causing a decrease the absorbance of sample was used in 
wholly subsequent investigates (Fig.6).
Fig. 6: absorptions value of oxidant in the found of several volume of oxidant

Kinetic of the Absorption spectra

Fig. 7 appear effect of time on the reaction (Kinetic of the Absorption spectra) and too study stability of the dye color. the high intensity and stability can be found after 3 min. from the start of the reaction and the color complex was stable about 5 hr., next that slowly decay among 6-8 hr. Therefore, 2 min was chosen as a waiting time in this study [7, 25].

Fig. 7: Kinetic Absorption spectra of A (20 mg.L$^{-1}$) of Tetracycline drug treated as described below procedure drug 4 hour.

Analytical Applications

The contents of ten capsule of the Tetracycline drug (250mg, 500mg), were weighed and grounded into a fine powder then dissolved in 50mL of distilled water in an ultrasonic bath
for 10min. The solution was filtered through filter paper and washed with water. The filtrate was diluted to 100mL with water. The proposed way was useful positively to the analysis of some formulations of the pharmaceutical having paracetamol. The data in Table 3 indicate with those found via the official spectrophotometric process utilizing 4AAP reagent [21], have great accuracy and sensitivity. Thus, matching process of the analysis Tetracycline drug is extra positive which exhibit a best sensitivity and great efficiency (Table 3).

**Table 3: Determination of paracetamol in several formulations utilizing the official and proposed method**

| Pharmaceutical preparation | Conc. Of paracetamol (mg L⁻¹) | E% | Rec% |
|----------------------------|-------------------------------|----|-----|
|                            | present | Found          |     |     |
| Tetracycline drug tablets 50 mg (DSI), Iraq | 5   | 4.9911         | -0.1783 | 99.821 |
|                            | 15     | 14.876         | -0.8335 | 99.166 |
|                            | 20     | 19.988         | -0.0610 | 99.939 |
| Tetracycline drug tablets 50 mg (DSI), Iraq | 5   | 5.0112         | 0.223  | 100.22 |
|                            | 15     | 14.766         | 1.584  | 98.415 |
|                            | 20     | 20.122         | 0.606  | 100.606 |
| Tetracycline drug tablets 250 mg | 5   | 4.987          | -0.260 | 99.739 |
|                            | 15     | 14.876         | -0.833 | 99.166 |
|                            | 20     | 20.0998        | -0.4196 | 99.580 |

**Conclusions**

The aim of this method-validation study was to document successful implementation of the method for determination of antibiotic drugs, such as Tetracycline drug, under study in their pharmaceutical preparations for stability and higher sensitivity. Current study showed that maximum absorbance was attained at 553nm by using UV-Visible spectrophotometer. this method could be applied with high satisfaction in the determination of Tetracycline drug in pure and dosage forms with a high accuracy and precision.

**Reference**

[1] M.J. Mnguni, AL (2015). A structural study of 4-aminoantipyrine and six of its Schiff base derivatives. *Acta Crystallogr C 71*: 103-109.
[2] Fiamegos, Y, Stalikas, C, and Pilidis, G (2002). 4-Aminoantipyrine spectrophotometric method of phenol analysis: Study of the reaction products via liquid chromatography with diode-array and mass spectrometric detection. *Analytica Chimica Acta 467*: 105-114.
[3] Cunha, S, *et al.* (2005). Structural studies of 4-aminoantipyrine derivatives. *Journal of Molecular Structure 752*: 32-39.
[4] Aljeboree, AM, and Alshirifi, AN (2019). Determination of Phenylephrine Hydrochloride and Amoxicillin in a Binary Mixture using Derivative Spectrophotometry Methods. *International Journal of Pharmaceutical Quality Assurance* **10**: 168-177.

[5] Smith, MG, and Lemmerer, A (2019). Novel crystal forms of 4-aminoantipyrine and its derivatives: Co-crystallizing a reluctant molecule. *Journal of Molecular Structure* **1175**: 307-313.

[6] Aseel M Aljeboree, AFA (2019). Removal of Antibiotic Tetracycline (TCs) from aqueous solutions by using Titanium dioxide (TiO2) nanoparticles as an alternative material. *Journal of Physics: Conf Series* **1294**: 052059.

[7] Aljeboree, AM, and Alshirifi, AN (2019). Oxidative coupling of Amoxicillin using 4-Aminoantipyrine: Stability and higher sensitivity. *Journal of Physics: Conference Series* **1294**: 052001.

[8] Aljeboree, AM (2020). Spectrophotometric and Colorimetric Determination of Pharmaceutical by Oxidative Coupling Reaction: A Review. *Sys Rev Pharm* **11** (2020) 609-615.

[9] Aseel M Aljeboree, AFA (2018). Spectrophotometric Determination of phenylephrine hydrochloride drug in the existence of 4-Aminoantipyrine: Statistical Study. *International Journal of Pharmaceutical Research* **10**.

[10] Hassan, MJM, Khayoon, WS, and Abdul-Fatah Hasssan, S. Batch and flow injection spectrophotometric methods for the determination of barbituric acid in aqueous samples via oxidative coupling with para-toluidine and sodium periodate. *Analytica Chimica Acta* **554**: 184-189.

[11] Al-Abachi, MQ, Haddi, H, and Abdul-Fatah Hasssan, S. Batch and flow injection spectrophotometric methods for the determination of barbituric acid in aqueous samples via oxidative coupling with 4-aminoantipyrine. *Karbala International Journal of Modern Science* **1**: 135-141.

[12] Aseel Musthaq Aljeboree , ANA (2018 ). Colorimetric Determination of phenylephrine hydrochloride drug Using 4-Aminopyridine: Stability and higher sensitivity J Pharm Sci & Res **10**: 1774-1779.

[13] Faust, SD, and Mikulewicz, EW (1967). Factors influencing the condensation of 4-aminoantipyrine with derivatives of hydroxybenzene[1]*I. A critique. Water Research* **1**: 405-418.

[14] Al-Abachi, MQ, Haddi, H, and Al-Abachi, AM (2005). Spectrophotometric determination of amoxicillin by reaction with N,N-dimethyl-p-phenylenediamine and potassium hexacyanoferrate(III). *Analytica Chimica Acta* **554**: 184-189.

[15] Aljeboree, AM, and Alshirifi, AN (2018). Adsorption of Pharmaceuticals as emerging contaminants from aqueous solutions on to friendly surfaces such as activated carbon: A review. *Journal of Pharmaceutical Sciences and Research* **10**: 2252-2257.

[16] Samadi-Maybodi, A, and Hassani Nejad-Darzi, SK. Simultaneous determination of paracetamol, phenylephrine hydrochloride and chlorpheniramine maleate in pharmaceutical preparations using multivariate calibration 1. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* **75**: 1270-1274.

[17] Salam Hussein Ewaid et al 2021 IOP Conf. Ser.: Earth Environ. Sci. 790 012075
[23] Ahmed Alaa Kandoh and Salwan Ali Abed 2021 IOP Conf. Ser.: Earth Environ. Sci. 790 012034
[24] Ahmed Alaa Kandoh et al 2021 IOP Conf. Ser.: Earth Environ. Sci. 790 012073
[25] Ewaid, S.H.; Abed, S.A.; Al-Ansari, N. Crop Water Requirements and Irrigation Schedules for Some Major Crops in Southern Iraq. Water 2019, 11, 756.
[26] Ewaid, S.H.; Abed, S.A.; Al-Ansari, N. Water Footprint of Wheat in Iraq. Water 2019, 11, 535.
[27] Ewaid, S.H.; Abed, S.A.; Al-Ansari, N. Assessment of Main Cereal Crop Trade Impacts on Water and Land Security in Iraq. Agronomy 2020, 10, 98.
[28] Kareem Abass Al-Hassani, M. (2019). SEROLOGICAL DETECTION OF COXIELLA BURNETII CHRONIC INFECTION- PHASE 1 IN SERUM OF HUMAN AND SHEEP AT AL-QADISIYAH PROVINCE, IRAQ. Al-Qadisiyah Journal Of Pure Science, 24(1), 13 - 20.
[29] Jalil Abed, M. (2019). Synthesis and Characterization and evaluation of biological activities of some new pyrroline compounds. Al-Qadisiyah Journal Of Pure Science, 24(1).
[30] Abdul-Hamza, H. kadhun, & Mohammed, G. J. (2019). The inhibitory effect of some nanoparticles on biofilm formation of Streptococcus agalactiae. Al-Qadisiyah Journal Of Pure Science, 24(2).
[31] Esraa M. Ridhaa, DHS, Aseel M. Aljeboree , Ayad F. Alkaim (2020). Sensitive and Simple Method for the Spectrophotometric Determination of Paracetamol Drug Using Oxidative Coupling. International Journal of Psychosocial Rehabilitation 24: 1475-7192.
[32] Aljeboree, AM, and Alshirifi, AN (2019). Colorimetric determination of Amoxicillin using 4-Aminoantipyrine and the effects of different parameters. Journal of Physics: Conference Series 12: 052067.