Oxidative coupling of Amoxicillin using 4-Aminoantipyrine: Stability and higher sensitivity.

Aseel M Aljeboree¹ and Abbas Noor Alshirifi²

¹College of science for women-Chemistry Department/ university of Babylon -Iraq
²College of science -Chemistry Department/ university of Babylon -Iraq

Email : annenayad@gmail.com

Abstract. Amoxicillin (AMX) is a semi-synthetic β-lactam antibiotic belonging to the group of penicillins. When ingested, it is partially (about 80–90%) excreted as chemically unchanged via faeces and urine into the domestic sewage and thence discharged to the wastewater treatment plants, which subsequently, removing this substance from the aqueous solution is important. Therefore, the aim of current study was to determine the effects of different optimum conditions of the oxidative coupling calorimetric methods for the quantitative determinations of Amoxicillin. Methods: Amoxicillin was determined via using colorimetric spectrophotometric method in the alkali medium and adding 4-aminoantipyrine to give a panic-colored chromophore which is spectrophotometrically estimated at 509 nm, at room temperature in the presence of ferricyanide ion. Beer’s law is observed in a concentration range 5-100 mg/L and linearity($r^2=0.9994$) according to the proposed conditions. Results: The best concentration of 4AAP was 0.3g/ml and that of the oxidant was 0.3g/ml at the same order. Conclusion: the best results of reagent 4AAP is 0.3g/ml to give high stability indeed highly more absorptivity to enhance measurements and reduce error.

Keywords: Spectrophotometry, Amoxicillin, 4- aminoantipyrine, Oxidative coupling, Potassium ferricyanide, colorimetric.

Introduction

Amoxicillin compound is consists of d-4-hydroxyphenylglycine side and 6-aminopenicillanic acid and is a semi-synthetic [1]. Many of (AMX) derivative compounds such as amino penicillin(figure 1) are attracting the attention of scientists due to their properties as medicine for the treatment of bacterial infections. Also, the synthesis of the peptidoglycan at the height of bacterial cell wall happen by interfering of AMX as inhibitor.

The antibiotics activity of β-lactam are employed to treat of bacterial infections produced by Gram-negative and Gram-positive organisms for examples pneumonia, bronchitis, also, infection of nose, skin and ears [1]. Many preparations methods are available for the synthesis of this drug including pure (capsules, injection or powder as oral suspension), or as mixture with other ingredients for instance amoxicillin/ clavulanate tablets[1]. Most of the (AMX) is chemically unchanged which extracted by urine and faeces in to final stage in domestic sewage then treated wast water treatment plants (WWTPs) [2, 3]. The bioactivity of (AMX) may be related to some side-effects on human beings,
which is attributed to their ability as antibiotics such other β-lactam antibiotics, this happen when (AMX), many traditional WWTPs methods are available to treat waste water but there is no method to remove such compounds, pre-treatment processes and costly equipments.[4-6], Subsequently, an effective methods have to employ to remove AMX from aqueous solution.

Nowadays, Many analytical methods are employed to determine (AMX), for examples spectrophotometry [7, 8], UV–Vis spectroscopy [2] chromatography liquid chromatography with tandem mass spectrometry [9], HPLC, atomic absorption spectrophotometry [10], advanced oxidation processes (heterogeneous Fenton reaction, biodegradation, adsorption, UV, UV/H₂O₂, UV/H₂O₂/TiO₂ processes, etc.), membrane filtration and coagulation/flocculation/sedimentation [10], Also, modified carbon electrode and indium tin oxide electrode, Au nanoparticles (NPs) [10], electrochemical method and surface Plasmon resonance (SPR), MWCNTs/FeCr₂O₄ hybrid film [10], Fluorimetric and electrochemical detection techniques [10].

Table 1: Structure and physicochemical properties of Amoxicillin.

| Molecular structure C₁₆H₁₉N₃O₅S |  |
|----------------------------------|--|
| Solubility (mg/L)               | 3430 |
| Formula weight                  | 365.4 |
| f.p                             | 403.3°C |
| Log KOW                         | 0.87 |
| m.p                             | 194°C |
| b.p                             | 743.2°C at 760 mm Hg |
| pKa                             | 7.4(amine), 9.6(phenol) and 2.4(carboxyl) |

Figure 1: The chemical structure of amoxicillin [10].

Material and Methods

Preparation of reagents and samples

Freshly prepared aqueous solution of the pure drugs;

Amoxicillin (AMX) standard solutions (100mg L⁻¹) was prepared by dissolving 0.05gm of AMX in distilled water, the solution was made up to 500mL with distilled water.

4-Aminoantipyrine (4-AAP) and Potassium ferricyanide, in different series was prepared by dissolving (0.02, 0.05, 0.1, 0.3, 0.5, 0.7 and 0.9gm/100mL) and (0.02, 0.05, 0.1, 0.3 and 0.5gm/100mL) of distilled water in a volumetric flask of 100mL at the same order.
Preparation of Calibration Curve

Aliquots of standard samples containing different concentrations (5–100mgL⁻¹) of AMX drug were prepared by simple dilution with distilled water (D.W) of the stock solution (100mg.L⁻¹).

Fresh stock solution was prepared daily by mixing 2 mL of AMX with 2 mL of potassium ferricyanide, and 1mL sodium hydroxyl solution in 10 mL volumetric flask. Then 2 mL of 4-AAP was added to the mixture followed by determining absorbance by UV-vis spectrophotometer as shown in figure 2.

![Figure 2: Calibration curve of Amoxicillin](image)

Results and discussion

Absorption spectra

The colorimetric detection of AMX was employed by interacting between amoxicillin with 4-AAP in the presence of potassium ferricyanide when amoxicillin was initially mixed with oxidizing agent in an alkaline medium and then with 4AAP reagent, panic-colored complex was formed which absorb light in the visible range (590 nm) [10, 11] as shown in Figure 3.
Optimum reaction conditions.

Reaction conditions of the complex reaction formation were optimized to achieve the maximum absorbance, highest selectivity, and sensitivity via exploring preliminary experiments.

Effect of the concentration of reagent

The effects of varied amounts of reagent 4AAP were studied. The highest absorbance was achieved with 0.3gm/100ml of 4AAP reagent solutions (Figure 4a). However, further addition of the reagent concentration did not affect on complex formation (Figure 4b). As a result, solution concentration of 0.3gm/100ml was chosen for further investigations.\(^{12,13}\).
Effect of potassium ferricyanide concentration

Time results showed that the reaction between Amoxicillin and the reagent 4AAP depended on the oxidation process with potassium ferricyanide in basic medium \[11, 14\] . Due to the fact that the better results and minimum blank value were achieved at the concentration of 0.3gm/100 ml, it was considered as optimum concentration \[14\] as shown in (Figure 5a), but, as shown in Figure 5b, after 0.3g/100mL the absorbance had a negative behavior or constant.
Figure 5a: Absorption spectra in the various concentrations of oxidant.

Figure 5b: Absorptions values in the various concentrations of oxidant.

Effect of time on the stability of colored complex

The optimum reaction time and Stability of the colored dye were also studied. Figure 6 shows that complete color intensity was attained after 5 minutes of mixing the complex and the absorbance
remained stable for at least 3h. After that, the complex slowly decayed between 4-5 hours. Thus, 5 minutes was selected as a waiting time in this study.

Figure 6: Kinetic absorption spectra of (100 mgL\(^{-1}\)) of amoxicillin treated for 3 hours.

Conclusion

In this study, the proposed method was simple with higher stability and sensitivity. Maximum absorbance was attained at 509nm by using UV-Visible spectrophotometer. Also, 4AAP appeared to have a very important role on the stability and increasing the sensitivity until reaching equilibrium. However, increasing concentration of 4AAP had a negative behavior and found coupling stability still at least 3 hrs.

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