

Full Length Research Paper

Study of the self-association of amoxicillin, thiamine and the hetero-association with biologically active compound chlorogenic acid

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The self-association of amoxicillin (AMX) and its hetero-association with biologically active compound, chlorogenic acid (CGA) were investigated at room temperature (295°K). The dimerization constant of amoxicillin and thiamine (THIA) analyzed using the dimer model at the wavelength of 278 and 256 nm were found to be $(5.438 \pm 3.2) \times 10^3 M^{-1}$ and $8.991 \times 10^2 M^{-1}$, respectively. The hetero-association constant of amoxicillin and chlorogenic acid analyzed using Benesi-Hildebrand approach were $1.18 \times 10^3 M^{-1}$. Thermodynamic parameters such as Gibbs free energy, enthalpy and entropy of dimerization reactions for the self-association and hetero-association of the compounds were also investigated using Vant's Hoff equation at the temperature ranges (295 to 305°K). The change of enthalpy calculated for amoxicillin, thiamine and the complexes of amoxicillin-chlorogenic acid are 34.73 ± 2.17 , 54.1 ± 4.585 , and $6.988 \pm 0.493 \text{ kJ.mol}^{-1}$ at the temperature of 305°K, respectively. The values of change in enthalpy and entropy indicate that the hydrophobic interaction play the major role in the interaction between the molecules.

Key words: Amoxicillin, thiamine, chlorogenic acid, UV-Vis spectroscopy, thermodynamic, self-association, hetero-association.

INTRODUCTION

Amoxicillin (AMX) is one of the most commonly prescribed penicillin. It is orally active and acid stable belong to a class of drugs known as penicillinase-susceptible semi synthetic penicillin in which its stability

in acid can be designed for oral use. It is also absorbed more rapidly and completely from the gastrointestinal tract and excreted in an active form in the urine (Brunton et al., 2005; Delgado et al., 1995). Amoxicillin has broad

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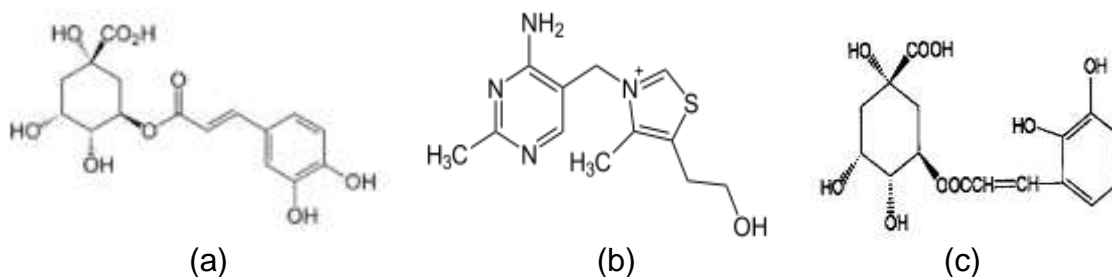


Figure 1. Molecular structure of (a) Amoxicillin (AMX), (b) Thiamine (THIA), (c) Chlorogenic acid (CGA).

spectrum antibacterial activity and it is used for the treatment of various bacterial infections. It is more effective against gram positive than gram negative bacteria (Kaur et al., 2011). The most active against penicillin-resistant *Streptococcus pneumoniae*, *Streptococcus pyogenes* and many strains of *S. pneumoniae* and *Haemophilus influenza*, an effective drug against Lyme disease (Brunton et al., 2005) and important in the treatment of conditions like gonorrhoea, other genital infections, urinary tract infections, *H. influenza*, otitis media, tonsils, throat, larynx, pharynx, joint replacements, dentistry, and lungs ((Brunton et al., 2005; Kaur et al., 2011; Evoy et al., 2003). In addition, amoxicillin acts by inhibiting the synthesis of bacterial cell walls and it is usually considered to be non-genotoxic, a potential to injure genomic DNA possibly via the induction of intracellular reactive oxygen species in mammalian cell (Kaur et al., 2011; Li et al., 2007).

Thiamine (Vitamin B1, THIA) belongs to a group of organosulfur compounds found in a large variety of animal and vegetable products (SCF, 1993; Combs, 1992). It is usually soluble in water with various essential functions such as essential for several biochemical reactions involving co-enzyme of carbohydrate metabolism, induces disease resistance of plants (Finglas, 1993; Gibson et al., 1999; Ahn et al., 2005), and plays a central role in cerebral metabolism which takes energy from food and turning it into energy to brain, nerves and heart. Its metabolism is selective to excess alcohol consumption since the absorption of it is decreased and its excretion is increased by alcohol (SCF, 1993; Fattal-Valevski, 2011). The most common cause of thiamine deficiency in affluent countries is either alcoholism or malnutrition in nonalcoholic patients. Treatment by thiamine supplementation is beneficial for diagnostic and therapeutic purposes (Fattal-Valevski, 2011; Rao et al., 2006).

Similarly, chlorogenic acid (CGA) is a main phenolic natural product isolated from the leaves and fruits of beverage plant such as tea, coffee and wine (Clifford, 1999; Buren et al., 1973; Challis and Bartlett, 1975). The most common chlorogenic acid is 5-O-caffeoylquinic acid (5-CQA), although 3-CQA and 4-CQA isomers have also widespread distribution in plants (Challis and Bartlett,

1975; Pencreac'h et al., 2012). Chlorogenic acid compound plays an important role as pharmacologic properties, such as anti-mutation, antibiotic, anti-hypercholesterolemia, antihypertensive (Svilaas et al., 2004), metal chelation (Wen et al., 2004), in plant metabolism or glucose absorption (Svilaas et al., 2004), antioxidants (Wen et al., 2004; Richelle et al., 2001), as a selective inhibitor for the production of glucose in liver (Schwab, 2001), used in disorders such as obesity, diabetes, and cancer (Wang et al., 2008; Clifford et al., 2010).

There is currently a great interest in the study of the interaction of chlorogenic acid found in various food sources with aromatic drugs since drug-food interaction can affect the drugs pharmacodynamics and pharmacokinetics. Hence, the knowledge of hetero-association interaction of the drugs with chlorogenic acid is important for understanding their binding in biological system. The self-association of chlorogenic acid and its hetero-association with ethidium bromide (EB) (Belay, 2013) and also with five antibiotics drugs (amikacin, ampicillin, ciprofloxacin, erythromycin, and vancomycin) for the application against bacteria has been reported by Hemaiswaryya et al. (2010). On the other hand, as far as our knowledge concerns the self-association of amoxicillin and thiamine and their hetero-association with chlorogenic acid to elucidate structures and the thermodynamic properties of the molecules are not yet investigated. The simplest techniques to study such kind of interactions are UV-Vis spectroscopy (Belay, 2013; Niazi et al., 2006). The technique is highly sensitive, rapid and easily implemented. Therefore, the objective of this work is to study the self-association of amoxicillin and thiamine and hetero-association of amoxicillin with chlorogenic acid (Figure 1).

MATERIALS AND METHODS

Chemicals

The drugs (thiamine, amoxicillin), chlorogenic acid were purchased from Sigma-Aldrich and were used for measurements without any further purification. The drugs and chlorogenic acid were dissolved in distilled water. The UV-Vis spectra were recorded 1 h after the

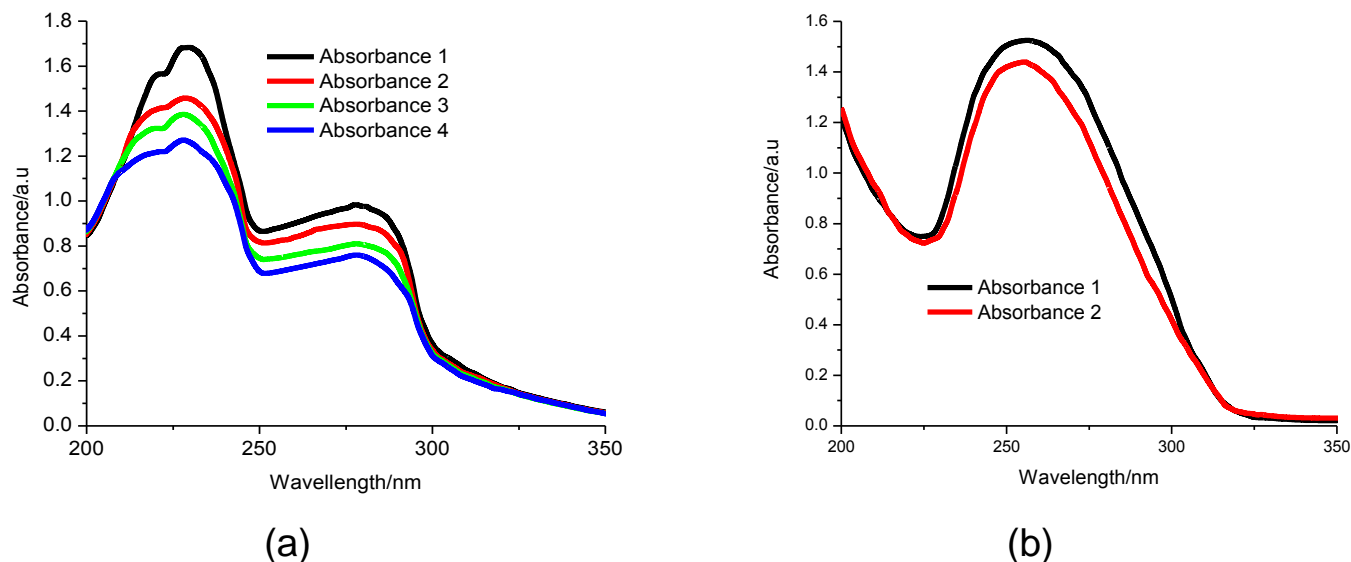


Figure 2. UV-Vis absorption spectrum of (a) Amoxicillin and (b) Thiamine in distilled water for different concentrations.

solution preparation in order to ensure that the equilibrium was reached. The solutions were stored in the dark to avoid photo degradation of the compounds. The UV/Vis spectrophotometer (Cary 50 UV/Vis Spectrophotometer, Varian Australia) with wavelength region (199 to 1100 nm) was used for the electronic absorption measurements of the solution compounds at room temperature (295°K). And also, different apparatus such as digital balance with accuracy of 0.0001 g, measuring cylinders, pipettes, and volumetric flasks, magnetic stirrer with hot plate, beakers and 1 cm size of quartz cuvettes.

Methods of the experiment

The self-association of the compound was studied over the concentration range of $(5.575 - 28.77) \times 10^{-5} M$ and $(1.254 - 13.3) \times 10^{-5} M$ for amoxicillin and thiamine, respectively. The absorbance as a function of concentration was measured at absorption maxima 276 and 256 nm for amoxicillin and thiamine, respectively to obtain the greatest accuracy of detection. For numerical analysis, the molar extinction coefficients and dimerization constant, dimer model equation fitted to the experimental data. Numerical procedure of fitting the experimental data was carried out by non-linear curve fitting based on Levenberg-Marquardt algorithm using origin 6.1 software (Belay, 2013). The molar extinction coefficients and equilibrium constants were used as searching parameters, in order to achieve minimum discrepancy between the experimental data and equations.

Similarly, for studying the complexation of chlorogenic acid with amoxicillin, the constant amoxicillin concentration $5.575 \times 10^{-4} M$ was titrated by chlorogenic acid solution in a concentration range of $(3.28 - 16.93) \times 10^{-4} M$. All the solutions were prepared in distilled water. The Benesi-Hildebrand approach was used for analyzing the numerical values of the association constant and molar extinction coefficient of the complexes between chlorogenic acid and amoxicillin at the maximum wavelength of 283 nm using a linear curve fitting (Benesi, 1949).

The thermodynamic parameters of the self-association amoxicillin and thiamine, and also for the hetero-association of

amoxicillin-chlorogenic acid were studied in the temperature range of 295 to 305°K. These thermodynamic parameters (enthalpy, Gibbs free energy and entropy) were determined using the model of Vant's Hoff's equation by linear curve fitting. For all measurements, the absorbance of the solution was measured in the range of 200 to 500 nm against the corresponding reagent blank. All glassware were thoroughly cleaned, rinsed with distilled water and dried before use.

RESULTS AND DISCUSSION

Self-association of amoxicillin and thiamine

An isobestic point is observed in overlaid spectra when a chromophoric precursor is converted to product with different spectrum. In more complex reactions, the wavelength of isobestic also changes if the molar absorptivity of the precursor changes and the fraction of the precursor converted to multiple product changes (Antonov et al., 1999) Figure 2a and b shows the concentration dependent self-association spectra of amoxicillin and thiamine measured in distilled water at room temperature. Two absorption peaks were observed for amoxicillin at the wavelength 228 and 278 nm. Similarly peak wavelength for thiamine noticed at 256 nm. The quantitative analysis for self-association was carried out using the concentration dependent molar extinction coefficient of the drug molecules at the wavelength of the absorption maximum ($\lambda_{\max} = 278nm$ and $\lambda_{\max} = 256nm$) for amoxicillin and thiamine, respectively. In the graph, Figure 2a and b isobestic points were observed for amoxicillin and thiamine for various concentration shows the existence of the self-association most likely due to hydrogen bonding or hydrophobic interaction between hydroxyl, amine or

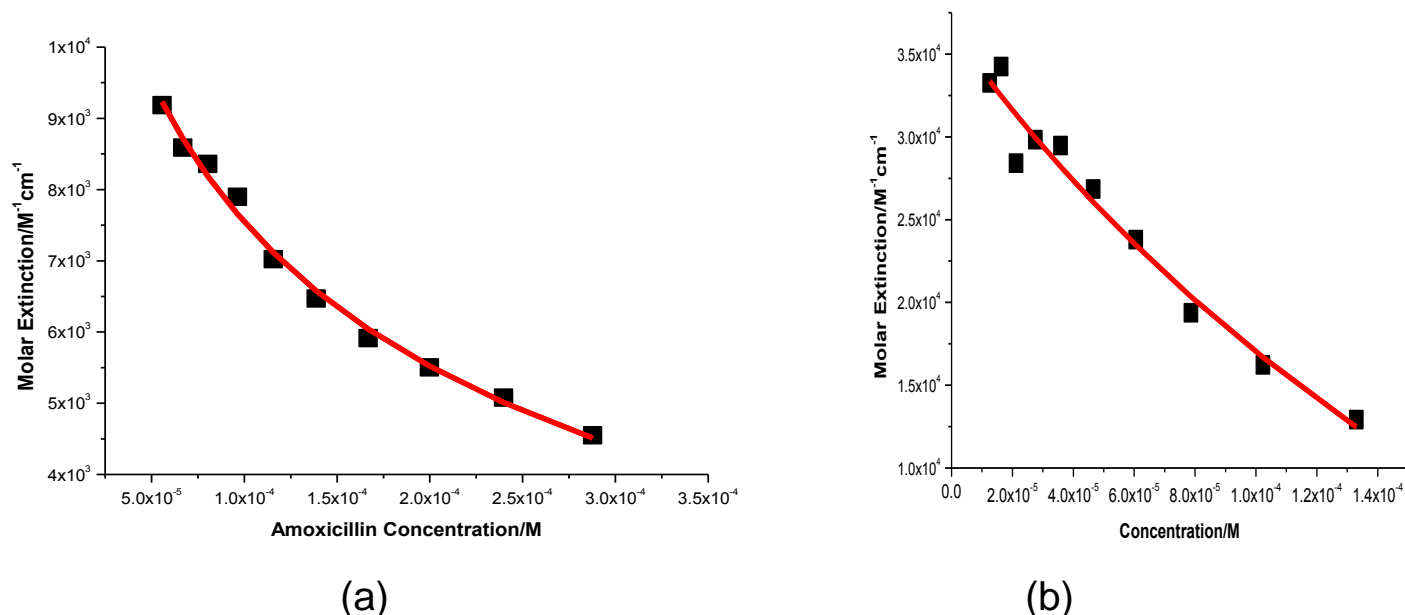


Figure 3. Molar extinction coefficient vs concentration of (a) AMX at Absorption Maxima of 278 nm and (b) THIA at Absorption Maxima of 256 nm.

carboxyl group. A similar situation was reported (Antonov et al., 1999; Belay, 2010) for dyes and other bioactive compounds. The existence of self-association of drug may modify the pharmacokinetic properties of the drugs. Numerical analysis was carried out using dimer model and the equation derived according to the following molecular equilibrium in solutions.



where C_1 and C_2 are monomers and dimers of the drugs, respectively and K_{dc} is the equilibrium dimerization constant. The overall concentration of the dissolved molecules in solution, using the mass conservation law can be written as:

$$[C_0] = [C_1] + 2[C_2], \quad (2)$$

where $[C_2] = K_{dc}[C_1]^2$ and $[C_0]$ is the total concentration. The contribution of the monomer and dimer to the molar extinction coefficient, of the solution is commonly considered to be additive and

$$\varepsilon = \varepsilon_m f_m + \varepsilon_d f_d, \quad (3)$$

where $f_m = \frac{[C_1]}{[C_0]}$, $f_d = \frac{2K_{dc}[C_1]^2}{[C_0]}$, ε_m are molar monomer extinction coefficients, ε_d is molar dimer

extinction coefficients, f_m is equilibrium mole fraction of the molecules in the monomer concentration and f_d is equilibrium mole fraction of the molecules in the dimer concentration. The concentration can be derived from the solution of the mass conservation law of Equation 2 by substituting on the account of Equation 3. Thus, the known dimer model is obtained as follows:

$$\varepsilon = \varepsilon_d + (\varepsilon_d - \varepsilon_m) \frac{1 - \sqrt{8[C_0]K_{dc} + 1}}{4[C_0]K_{dc}}. \quad (4)$$

In the aforementioned Equation 4, there are three unknown parameters ε_m , ε_d and K_{dc} which can be obtained from fitting dimer model equation to the experimental data as shown in Figure 3. Nonlinear curve fitting based on the Levenberg-Marquardt algorithm was used for computing the values of the aforementioned three quantities using origin software. They are serving as search parameters being adjusted in order to achieve the minimum discrepancy between the experimental data and the theoretical value of Equation 4. The values of molar extinction coefficients of the two compounds at the maximum wavelength decrease as the concentration increases as shown in Figure 3. The deviation of the Beer-Lambert's law and depend on concentration, suggest the existence of self-association process of the molecule (Antonov et al., 1999). The calculated association constants K_{dc} obtained for amoxicillin and thiamine at the wavelength of

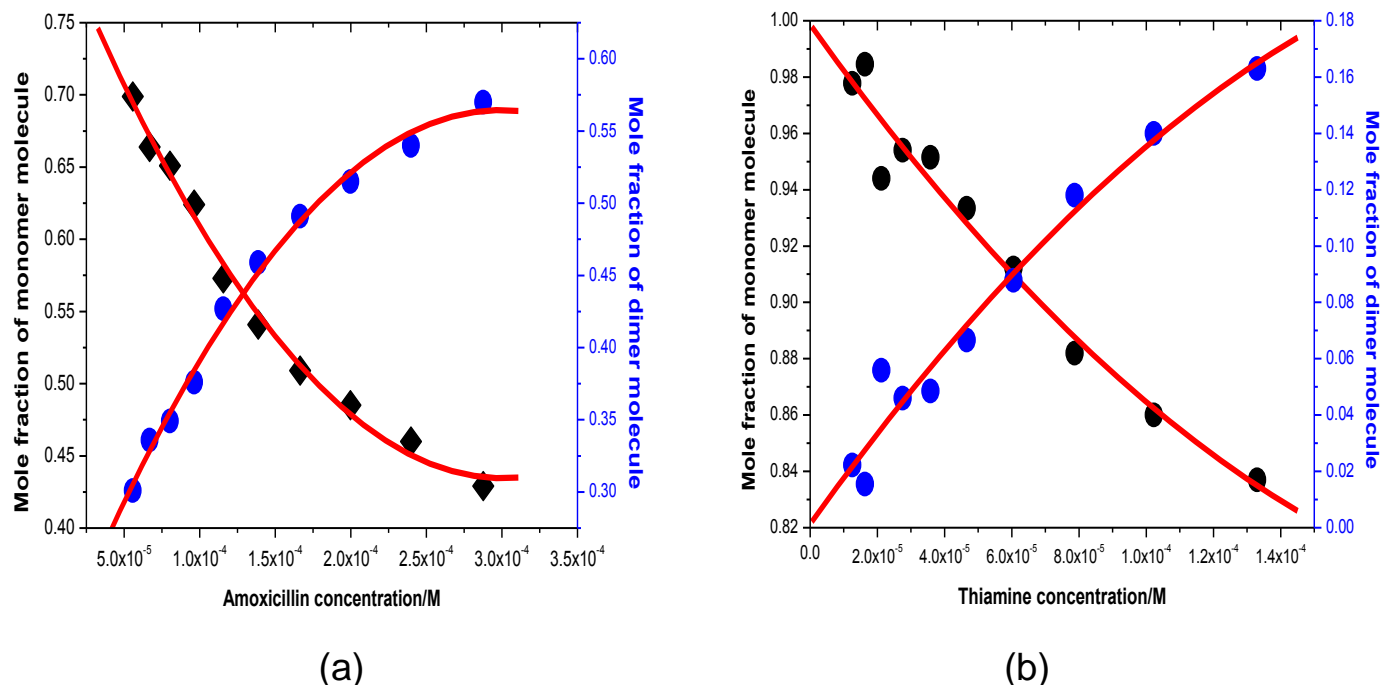


Figure 4. The Mole fraction of monomer and dimer versus total concentration of (a) Amoxicillin under the peak of 278 nm and (b) Thiamine under the peak of 256 nm.

278 and 256 nm are $(5.438 \pm 3.2) \times 10^3 M^{-1}$ and $8.991 \times 10^2 M^{-1}$ respectively.

The obtained values are quite reasonable and comparable with the results obtained by other workers in the UV/Vis region of the spectrum for similar molecules which have hydroxyl, amine and carboxyl functional groups. The dimerization constant for benzoic acid calculated using IR method in CCl_4 solvent for same concentration is $5.89 \times 10^3 M^{-1}$ (Hanrahan and Bruce, 1967). The molar extinction coefficient for Bovine Serum Albumin (BSA) calculated using UV-Vis spectroscopy in the same concentration is $3.66 \times 10^4 L \cdot mol^{-1} \cdot cm^{-1}$ (Jasińska, 2009). In addition, the monomer and dimer extinction coefficients calculated for Merocyanine dye (Wortmann et al., 2003), are also in a good agreement with the monomer and dimer extinction coefficients of amoxicillin. The adsorption equilibrium constant for the water soluble vitamins, ascorbic acid obtained using HPLC Stationary Phase by Matusova et al. (2006) are also in a good agreement with the result obtained for thiamine. Furthermore, the association constant and molar extinction coefficient of octamethylbiphenylene (OMB) obtained using UV/VIS spectroscopy (Kochi et al., 2006) also quite similar with the finding of this study.

Figure 4a and b shows the mole fraction of monomer and dimer versus concentration of the drug molecules under the peak of 278 and 256 nm for amoxicillin and thiamine, respectively. The graphs show increase and

decrease in the mole fraction of dimer and monomer as the concentrations of the drugs are increasing. The results indicated dimerization is favored at high concentration of the drugs.

Complexation of amoxicillin with chlorogenic acid

The quantitative analysis of the complexation of amoxicillin with chlorogenic acid was accomplished using Benesi-Hildebrand approaches (Kochi et al., 2000) under the condition of $[C_0] \gg [D_0]$. A constant amoxicillin solution ($C_{AMX} = [D_0] = 5.575 \times 10^{-5} M$) and different concentrations of chlorogenic acid ($C_{CQA} = [C_0] = 1.69 \times 10^{-3} - 3.28 \times 10^{-5} M$) were used to calculate the equilibrium constant and molar extinction coefficient of the complex formation. Figure 5 shows the effects of CGA concentration on the UV-Vis absorption spectra of amoxicillin solutions. The addition of chlorogenic acid to amoxicillin solutions results in important spectral modification and red band shift was observed. The peak absorbance also decreases as the concentration of chlorogenic acid increases. Moreover, the existences of isobestic points were observed at different wavelength (213, 210, 208, 270, and 323 nm), which indicate the formation of complexes between chlorogenic acid and amoxicillin (Khopkar, 1998). The hetero-association of chlorogenic acid with some aromatic drugs enhance pharmacological activity of drugs

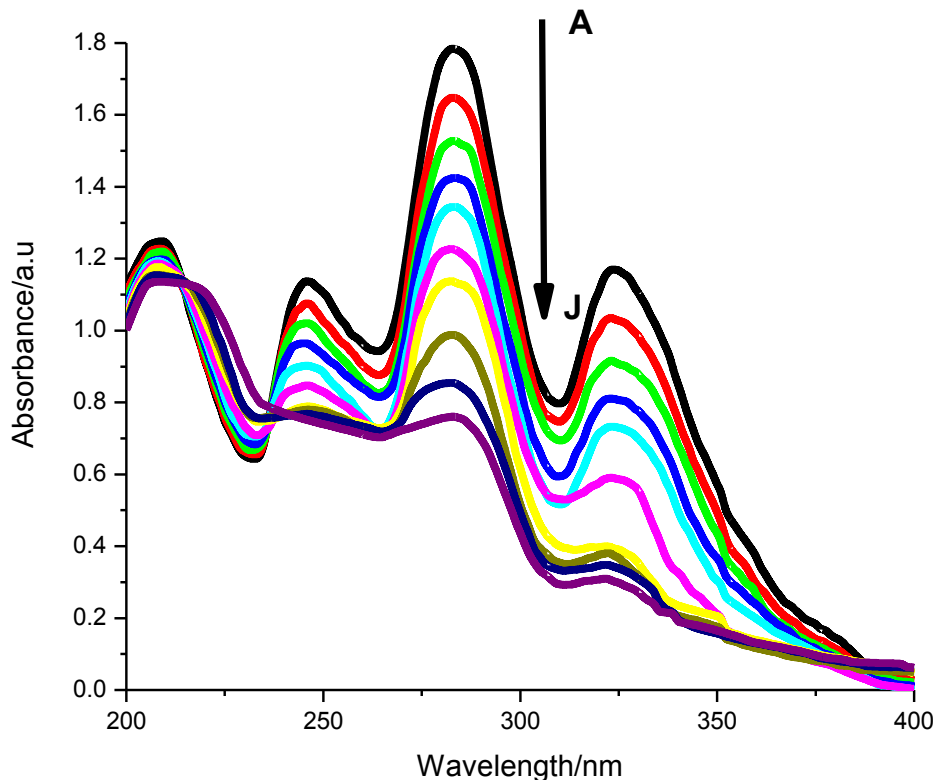


Figure 5. The Absorbance vs. different concentration of Chlorogenic acid A to J ($c= 3.29-169 \times 10^{-5}$ M) and Amoxicillin ($c= 5.57 \times 10^{-5}$ M= constant)

for the applications against some bacteria as reported by (Hemaiswarya et al., 2010). The equilibrium constant for the complex formation K, derived as:



From Equation 9, the equilibrium constant for the complex formation K can be defined as:

$$K = \frac{[CD]}{[C][D]} \tag{10}$$

Where [C], [D], and [CD] are the equilibrium concentration of chlorogenic acid, amoxicillin and association of amoxicillin with chlorogenic acid respectively. The initial concentration of amoxicillin and chlorogenic acid are designated as:

$$[D_0] = [D] + [CD] \tag{11}$$

$$[C_0] = [C] + [CD] \tag{12}$$

Substituting these in the account of Equation 10 gives:

$$K = \frac{[CD]}{([D_0] - [CD])([C_0] - [CD])} \tag{13}$$

For $[C] \gg [D]$, then $[C_0] - [CD] \approx [C_0]$. So, Equation 13 can be written as:

$$K = \frac{[CD]}{([D_0] - [CD])[C_0]} \tag{14}$$

After re-arranging Equation 14, it gives:

$$[CD] = \frac{K[D_0][C_0]}{(1 + K[C_0])} \tag{15}$$

The absorbance (A) for concentration [CD] according to Beers law is:

$$A = [CD]\epsilon l = \epsilon l \frac{K[D_0][C_0]}{(1 + K[C_0])} \tag{16}$$

By re-arranging Equation 16, it can be written as follows:

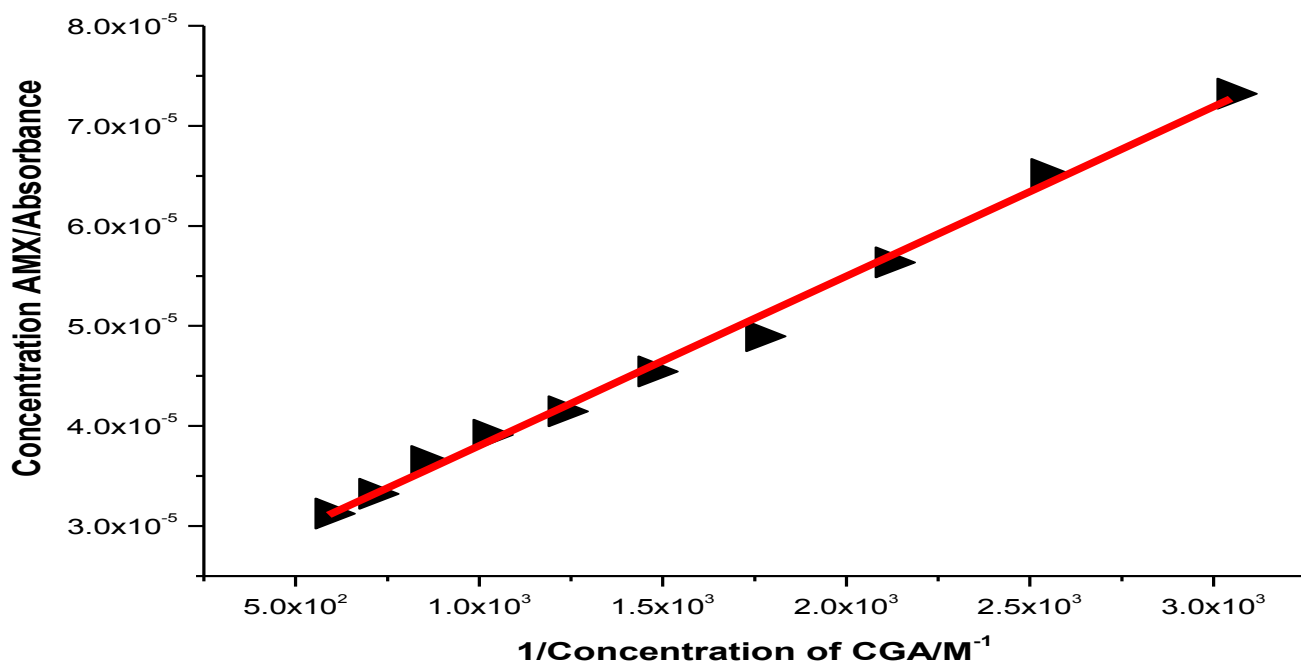


Figure 6. Concentration of AMX/Absorbance versus 1/Concentration of CGA at λ_{max} .

$$A = [CD]\varepsilon l = \varepsilon l \frac{K[D_0][C_0]}{(1 + K[C_0])} \quad (17)$$

When the path length l of the cuvette is 1 cm, the aforementioned Equation 17 can be also written in the form of Benesi-Hildebrand equation as:

$$\frac{[D_0]}{A} = \frac{1}{\varepsilon} + \frac{1}{\varepsilon K[C_0]} \quad (18)$$

The plot of $\frac{[D_0]}{A}$ vs. $\frac{1}{[C_0]}$ gives a straight line with y-intercept $\frac{1}{\varepsilon}$ and slope $\frac{1}{\varepsilon K}$ from Figure 6.

The quantitative analysis of the association of chlorogenic acid with amoxicillin was accomplished by Benesi-Hildebrand equation, under the condition of $[CGA] \gg [AMX]$. The equilibrium constant for the complex formation and molar extinction coefficient calculated by fitting Equation 18 to experimental data (Figure 6) found to be $1.18 \times 10^3 M^{-1}$ and $5 \times 10^4 M^{-1}.cm^{-1}$ respectively. This result is in a good agreement with the result of Belay (2010; 2013) which is $5.52 \times 10^3 M^{-1}$ and $2.4 \times 10^4 M^{-1}.cm^{-1}$ for the equilibrium constant for the complex formation and molar extinction coefficient.

Thermodynamic properties of the self-association of amoxicillin and thiamine, and complexation of amoxicillin with chlorogenic acid

Heating the aqueous solution of amoxicillin, thiamine and amoxicillin-chlorogenic acid complex shows that the absorption spectra of the molecules are strongly dependent on the temperature in the range of 295 to 305°K for the self-association and hetero association, respectively. The equilibrium constants of the drug molecules at the aforementioned temperature were calculated at peak of wavelengths of amoxicillin and thiamine and the complexation between amoxicillin and chlorogenic acid using Equations 3 and 18, respectively.

Figure 7 shows the graph of $\ln K$ versus $f(\frac{1}{T})$. The

magnitude of the enthalpy was estimated from the slope of the approximating line according to Vant's Hoff's equation:

$$\frac{d \ln(K_{dc})}{f(\frac{1}{T})} = -\frac{\Delta H}{R} \quad (5)$$

where ΔH is the molar enthalpy change, $R = 8.31 J.mol^{-1}K^{-1}$ is the universal gas constant and T the temperature in Kelvin. The entropy was derived from Gibbs free energy and enthalpy. The Gibbs free energy and entropy can be expressed as:

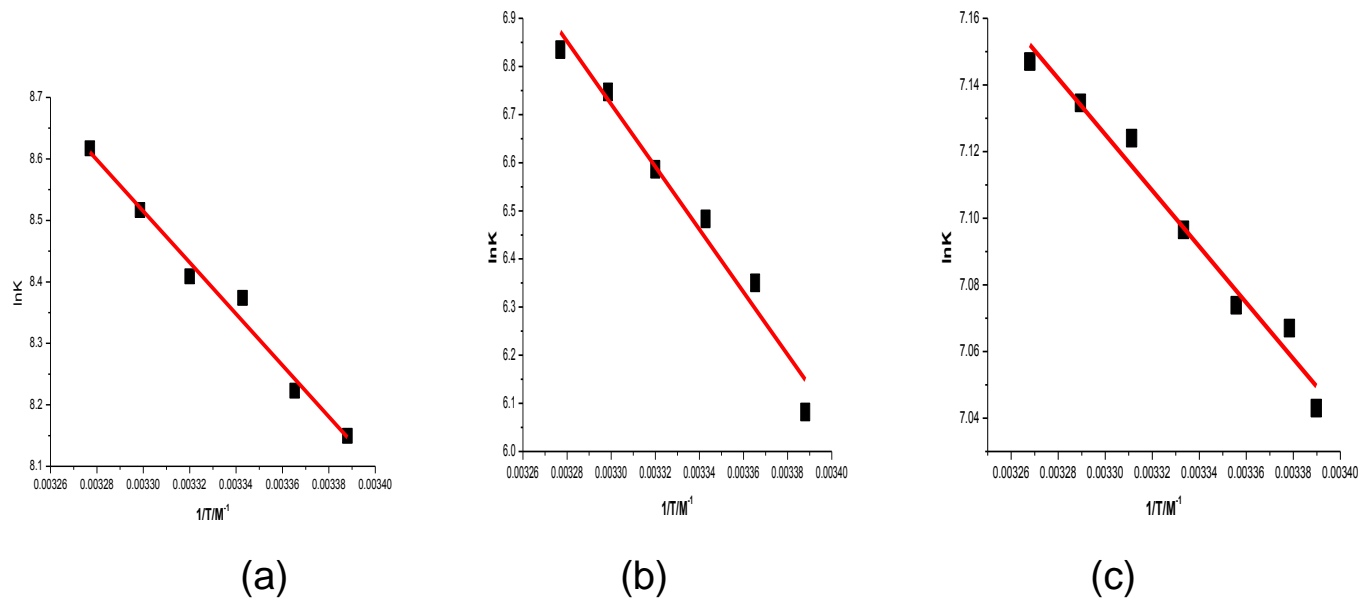


Figure 7. $\ln K_{dc}$ vs. $\frac{1}{T}$ of (a) AMX at Concentration ($5.575 \times 10^{-5} M$) (b) THIA at Concentration ($1.254 \times 10^{-5} M$) (c) AMX-CGA at Concentration ratio of AMX ($5.575 \times 10^{-5} M$) with CGA $3.28 \times 10^{-4} M$.

Table 1. Calculated result of the thermodynamic properties for the self-association of the drug molecules Amoxicillin and Thiamine.

Drug molecules	$\Delta G / kJmol^{-1}$	$\Delta H / kJmol^{-1}$	$\Delta S / kJK^{-1}mol^{-1}$
Amoxicillin	-21.79	34.73 ± 2.17	0.185 ± 0.00723
Thiamine	-17.372	54.1 ± 4.585	$0.234.34 \pm 0.015.26$
AMX-CGA	-18.186	(6.988 ± 0.493)	0.08227

$$\Delta G = -RT \ln(K_{dc}), \quad (6)$$

$$\Delta S = -\frac{\Delta G - \Delta H}{T}. \quad (7)$$

Finally, the Vant's Hoff's equation can be given by;

$$\ln K = \frac{\Delta S}{R} - \frac{\Delta H}{R} \frac{1}{T}. \quad (8)$$

Plots of $\ln K$ versus T^{-1} gives a straight line, whose slope and intercept can be used to determine ΔS and ΔH and Gibb's free energy can be determined at a specific temperature using Equation 7. The calculated value for the Gibbs free energy, enthalpy, and entropy of the drug molecules at a temperature 305°K for the hetero association is obtained as shown in Table 1. The

negative value for the Gibb's free energy indicates that the absorption process of the drugs is continuous. In addition, the positive value of enthalpy shows that the process is endothermic reaction; this indicates that the temperature increases as the equilibrium constant increases. Also, the positive value of entropy confirms the increasing randomness of the solution interface during the absorption process of the drug molecules. The obtained thermodynamic results are similar with the adsorption kinetics of amoxicillin on magnetite and bentonite mixture analyzed by kinetic empirical model obtained by Maichin et al. (2013), the results are $\Delta G = -9.2kJ.mol^{-1}$, $\Delta H = 438.8kJ.mol^{-1}$ and $\Delta S = 1.5kJ.K^{-1}.mol^{-1}$. Generally, the calculated result of the thermodynamic parameters such as ΔG , ΔH and ΔS demonstrated that the amoxicillin absorption was endothermic and spontaneous in nature (Kakavandi et al., 2014). The value for the Gibbs free energy of the thiamine is a good agreement with that previously

reported (Khorsandi et al., 2013) for the interaction between thiamine and dihydroxyanthraquinone dye, which is given by $\Delta G = -19.8 \text{ kJ.mol}^{-1}$ at a temperature of 298°K. The Gibb's free energy of the complexation is also similar with the previously reported thermodynamic results given by $\Delta G = -33.513 \text{ kJ.mol}^{-1}$, $\Delta H = 46.97 \text{ kJ.mol}^{-1}$ and $\Delta S = 0.046 \text{ kJ.mol}^{-1}$ for amoxicillin-albumin complex (Habeeb, 2011). The calculated values of change in enthalpy and entropy indicate that the hydrophobic interaction play the major role in the interaction between the molecules.

Conclusions

This research indicates that amoxicillin and thiamine effectively aggregates in a solution and the amoxicillin forms a complex with biologically active compound chlorogenic acid due to the hydrophobic interaction between the molecules of the drugs. The equilibrium constants, the concentration visibility of monomer and dimer, and thermodynamic properties calculated for amoxicillin, thiamine and the complexes of amoxicillin with chlorogenic acid are interpreting the study of kinetic chemical reaction system of the compounds. Thus, sympathetic, the mechanism of self-association and hetero-association of amoxicillin and thiamine are useful in order to design the advanced and controllable carriers of drugs and food components. In addition, thermodynamic parameters, such as enthalpy, entropy and Gibbs free energy change on dimerization, which are derived from the temperature dependency of the dimerization constant, have given insight into the forces that maintain the dimer and hetero-association structures in the solutions. The values of change in enthalpy and entropy indicated that the hydrophobic interaction play the major role in the interaction between the molecules. Therefore, the investigated results have wider applications in pharmaceutical and food companies in terms of economic and scientific utility.

Conflict of interest

The authors have not declared any conflict of interest.

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