STUDY OF THE SELF-ASSOCIATION OF CAFFEINE AND CHLOROGENIC ACID AND THEIR HETERO-ASSOCIATION WITH METHYLENE BLUE USING SPECTROPHOTOMETRIC METHOD

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Abstract. The self-association of caffeine, chlorogenic acid and their hetero-association with Methylene Blue were studied in aqueous solution by UV/Visible spectrophotometry. The dimerization constants of caffeine and chlorogenic acid analyzed using dimer model at the wavelengths of 272.8 and 324 nm were found to be 565.33 and 1027 M^{-1} respectively. The hetero-association constants of methylene blue with caffeine and chlorogenic acid analyzed using Benesi-Hilde brand approach at the wavelength of 664 nm were found to be 8418 and 14542 M^{-1} respectively. Thermodynamic parameters such as Gibbs free energy, enthalpy and entropy of dimerization reactions of the compounds were also investigated using Vant's Hoff equation at the temperature range of 295–301 K for self-association and 295–302 K for hetero-association. The change of enthalpy calculated for caffeine, chlorogenic acid and the complexes of MB-CAF and MB-CGA at the temperature of 301 K are –12.86 ± 0.073, –14.24 ± 0.839, 5.12 ± 0.09, and 3.61 ± 0.012 $kJ \cdot mol^{-1}$, respectively. The obtained thermodynamic parameters indicated that self-and hetero-association of the compounds are mainly electrostatic and hydrophobic interactions played a major role in the binding reactions respectively.

Key words: Caffeine, chlorogenic acid, methylene blue, UV/Vis spectrophotometer, self-association, hetero-association, thermodynamic properties.

INTRODUCTION

Caffeine (Fig. 1a) is probably the most popular drug in the world because we consume it on daily basis in various foods and beverages [35]. Clearly it is an important drug-food substance in our society which deserves consideration. The recommended daily intake of this compound for stimulation is 200–300 mg per

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person per day for adults and 35–40 mg for children [10, 37]. The molecules of caffeine are aggregate in aqueous solution by plane-to-plane stacking [14, 15]. This kind of stacking interactions is usually important in connection with the pharmacological action of caffeine and nucleic acids [28]. Therefore, caffeine is considered to be a potential regulator of biological activity for aromatic dyes and antibiotics *in vivo*, which is definitely of interest for clinical applications [15]. It is also responsible for interactions and complexation with a lot of molecules of interest in food and drug formulation. On the other hand, when caffeine is administered together with aromatic cytotoxic drugs (such as doxorubicin, ethidium bromide, and novantrone) there is a remarkable reduction *in vitro* toxicity of the drugs acting on nuclear DNA [18, 38, 43]. Moreover, the direct complexation between caffeine and some aromatic drugs may also modify the pharmacokinetic properties of the drugs or lead to chemical degradation [39].

Chlorogenic acid (Fig. 1b) and many other polyphenol compounds are extensively used in pharmaceutical and food industries [27]. Chlorogenic acid is used as additive in beverages, cosmetics, tea products and foods as well as drug precursors in drug design [26]. The compound has attracted the attention of researchers due to health promoting attributes, which include lowering the risks of cardiovascular disease, cancer, diabetes, obesity and other conditions associated with aging [20, 30]. Moreover, anti-oxidant properties which are suggested to play an important role in protecting food, cells and any organ from oxidative degeneration [32]. The molecular complexations of chlorogenic acid with sodium hydroxide [5], toxic metal ions [11, 12], caffeine [6, 13], with other aromatic molecules like, protein [42], beta-cyclodextrin [22, 24] and amoxicillin [2] in aqueous solution have been reported by theoretical as well as spectrophotometric techniques to design more advanced and controllable carriers of drugs and food components.

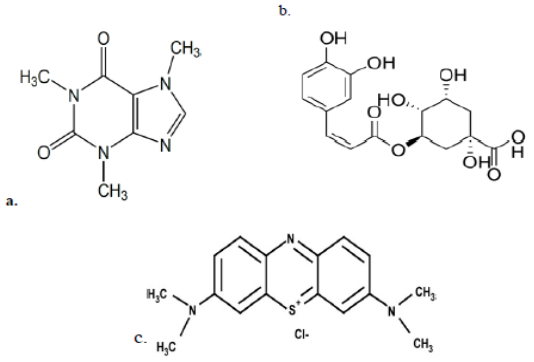

Fig. 1. Chemical structure of a. caffeine, b. chlorogenic acid, and c. methylene blue.

Methylene blue (Fig. 1c) is a heterocyclic aromatic dye, a member of thiazine dyes [33]. It is a remarkable compound in the history of pharmacology and chemotherapeutics [44]. Methylene blue was the first phenothiazine compound developed and it has active biological properties which have been under investigation for over 120 years [44]. It is a phenothiazine derivative, capable to bind with nucleic acids and, in particular, with virus nucleic acids [1]. Sensitivity of MB to different conformational transitions of DNA in cell nuclei allows using it as a label for investigation of structural changes in biopolymer molecules. Methylene blue is an active photosensibilizator and is utilized during photodynamic therapy of malignant cancers [36].

Currently there is a great interest in the study of the interaction of caffeine and chlorogenic acid found in various food sources with aromatic drugs/dyes, since drug-food interaction can affect the drugs pharmacodynamics and pharmacokinetics. Hence, the knowledge of hetero-association interaction of the drugs with biologically active compounds is important for understanding their binding in biological system. The self-association of caffeine and chlorogenic acid (5-CQA) and their hetero-association with aromatic drugs and sodium hydroxide are studied by [5, 15] using NMR and UV/Visible spectrophotometric methods respectively. As far as our knowledge concerns the self-association of caffeine and the hetero-association of methylene blue with caffeine and chlorogenic acid to clarify structures and the thermodynamic properties of the molecules are not investigated yet. Studying the self-association and hetero-association of the compounds using UV/Vis spectrophotometer is simple, fast and inexpensive compared to other methods [6]. Investigations of self-association of caffeine and chlorogenic acid and their hetero-association with methylene blue in water are interesting from the pharmacological point of view, since self- and hetero-associates and competitive binding may influence the activity of drugs.

Mostly, study of self-associated molecules using UV/Vis spectrophotometer is limited due to difficulty in obtaining the spectra of highly concentrated solutions and need of careful examination of changes in the apparent molar extinction coefficients over a wide range of concentrations [16]. It is known that the structure of dimer makes difficult the use of Beer-Lambert's law. Extinction coefficients and shape of the absorption band of the fraction of dimerized molecules are usually unknown that often leads to difficulty in the interpretation of experiment [31]. The technique is highly sensitive, rapid and easily implemented. Therefore, the objective of this work is to study the self-association of caffeine and chlorogenic acid and their hetero-association with methylene blue.

MATERIALS AND METHODS

MATERIALS AND CHEMICALS

The chemicals, caffeine, chlorogenic acid and drug, methylene blue were purchased from Sigma-Aldrich (Germany) and as solvent distilled water (AAU) was used without any further purification. The UV-Vis spectrophotometer, Perkin Elmer Lambda 19 (Perkin Elmer, D-7770 Ueberlingen, Germany) with wavelength ranges of 170–3200 nm, was used for the electronic absorption measurements of the solution compounds at room temperature. The instrument operated by a powerful software package termed UVCSS. It provides a wide range of operating mode for the instrument and it also includes comprehensive data handling and file management capabilities. Scanning speed of 240 nm per min and slit width 2 nm were used during spectral data acquisition. The instrument is a PC-driven spectrometer.

METHODS OF THE EXPERIMENT

The self-association of caffeine and chlorogenic acid was studied over the concentration range of $(5.93–12.80)\times10^{-5}$ and $(6.39–18.12)\times10^{-5}$ in water solutions respectively. The absorbance as a function of concentration was measured at absorption maxima 272.8 nm and 324 nm for caffeine and chlorogenic acid, respectively to obtain the greatest accuracy of detection. For numerical analysis, the molar extinction coefficients and dimerization constants, the known dimer model was used. Numerical procedure of fitting the experimental data was carried out by non-linear curve fitting based on Levenberg-Marquardt algorithm using Origin 8 software. The molar extinction coefficients and equilibrium constants were used as searching parameters, in order to achieve minimum discrepancy between the experimental data and equations.

During the hetero-association studies of MB with CAF and CGA the concentration of MB in the mixed solutions was maintained constant $C_{MB} = 3.82\times10^{-5}$ M. The range of CAF $(15.45–39.84)\times10^{-5}$ M and CGA $(12.23–21.64)\times10^{-5}$ M concentrations were used for MB titration. The visible spectra were recorded 1 hr after the solution preparation in order to ensure that the equilibrium was reached. The hetero-association constants and molar extinction coefficients of the complexes were calculated from equation of Benesi-Hildebrand at 664 nm [8].

Thermodynamic investigations of compounds in aqueous solution were made in a 1.0 cm quartz cuvette in the temperature range of 295–301 K for self-association and 295–302 K for hetero-association studies. The thermodynamic

parameters (change in Gibbs free energy, enthalpy and entropy) have been determined using the model of Vant's Hoff's equation by linear curve fitting to the experimental data [9].

RESULTS AND DISCUSSION

SELF-ASSOCIATION OF CAFFEINE AND CHLOROGENIC ACID IN AQUEOUS SOLUTION

The quantitative analyses of caffeine and chlorogenic acid self-association were carried out using the concentration dependence of molar extinction coefficient of the molecules. The values of molar extinction coefficients at the maximum wavelengths of 272.8 nm and 324 nm are decreased as the concentration increases for caffeine and chlorogenic acid respectively. The deviation from Beer-Lambert's law depends on concentrations and suggests the existence of a self-association process of the molecules [3]. This effect is plotted in Fig. 2(a) and (b), where the points show the experimental data and the lines are the dimer model fitted to the experimental data. The existence of self-association of the compounds may modify the pharmacokinetic properties of the compounds [2].

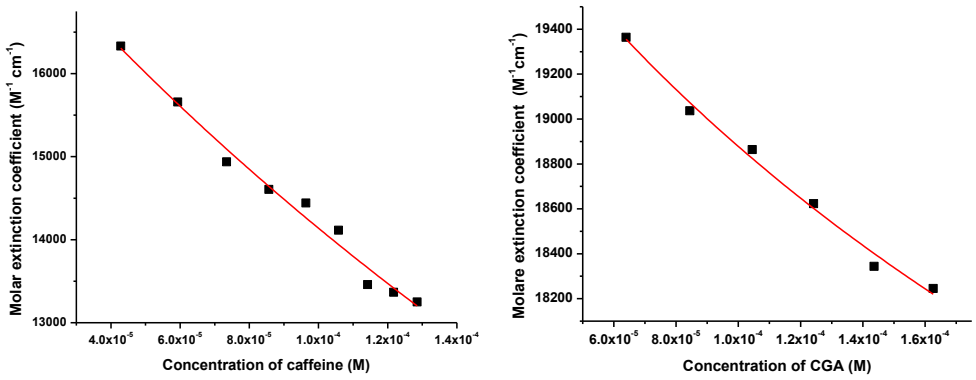

Fig. 2. The molar extinction coefficient *vs.* concentration of (a) caffeine at absorption maxima of 272.8 nm, (b) chlorogenic acid at absorption maxima of 324 nm.

For numerical analysis of the molar extinction coefficient of monomers, dimers and dimerization constants, the known dimer model was derived according to the following molecular equilibrium in solutions [5].

$$E_1 + E_1 \xrightarrow{K_{dE}} E_2 \tag{1}$$

where K_{dE} is an equilibrium constant of molecular dimerization; E_1 and E_2 are monomer and dimer forms of the compounds, respectively. The total concentration of the dissolved molecules in solution, using the mass conservation law, can be written as;

$$[E_0] = [E_1] + 2[E_2] \text{ and } [E_2] = K_{dE}[E_1]^2 \tag{2}$$

where $[E_0]$ is the total concentration of compounds; $[E_1]$ is the monomer concentration; $[E_2]$ is the dimer concentration. According to the model shown in equation (1), the experimental molar extinction coefficient ε can be represented as [5].

$$\varepsilon = \varepsilon_m f_m + \varepsilon_d f_d \tag{3}$$

where ε_m and ε_d are molar extinction coefficients of caffeine and chlorogenic acid monomer and dimer respectively, f_m is the equilibrium mole fraction of the molecules in the monomer form; f_d is the equilibrium mole fraction of the molecules within the dimer.

$$f_m = \frac{[E_1]}{[E_0]} \text{ and } f_d = 2K_{dE}\frac{[E_1]^2}{[E_0]}. \tag{4}$$

The concentration $[E_1]$ can be derived from the solution of the mass conservation law of equation (2) by substituting on the account of equation (3). Thus, the dimer model is obtained as follows:

$$\varepsilon = \varepsilon_d + (\varepsilon_d - \varepsilon_m)\frac{1 - \sqrt{8[E_0]K_{dE} + 1}}{4[E_0]K_{dE}}. \tag{5}$$

In equation (5), there are three unknown parameters ε_m, ε_d and K_{dE} which can be obtained from fitting known dimer model equation to the experimental data as shown in Fig. 2 (a) and (b). The values of three quantities were computed by non-linear curve fitting based on the Levenberg-Marquardt algorithm using origin 8 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 (5). The calculated values for ε_m, ε_d and K_{dE} of the compounds obtained at the maximum wavelength are as shown in Table 1.

Table 1

Calculated results of molar extinction coefficients of monomers and dimers and dimerization equilibrium constants of self-association of CAF and CGA

Compounds	ε_m ($M^{-1}\cdot cm^{-1}$)	ε_d ($M^{-1}\cdot cm^{-1}$)	K_{dE} (M^{-1})
CAF	18270±734	26198±50900	565.33±893
CGA	20500±5150	9580±7680	1030±1360

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 amine and carboxyl functional groups. The dimerization constant calculated and reported for caffeine at room temperature was 11.8 M^{-1} [15] using NMR spectrophotometry for very high concentrations. Also, the values of K_{dE} determined for the same compound in aqueous solution using UV/Vis and FT-IR spectrophotometry were 1.62×10^2 M^{-1} and 1.58×10^2 M^{-1} for low concentrations and high concentrations respectively by [25, 19] and which are in agreement with the results obtained by the present work. The value of molar extinction coefficient determined for caffeine in aqueous solution at maximum wavelength 273 nm was 9.74×10^3 and 9.9×10^3 $M^{-1}\cdot cm^{-1}$ reported by [21, 29]. In addition, the monomer and dimer extinction coefficients, calculated for 5--caffeoylquinic acid and caffeic acid in water solutions [5, 7], are also in a good agreement with the monomer and dimer extinction coefficients of caffeine.

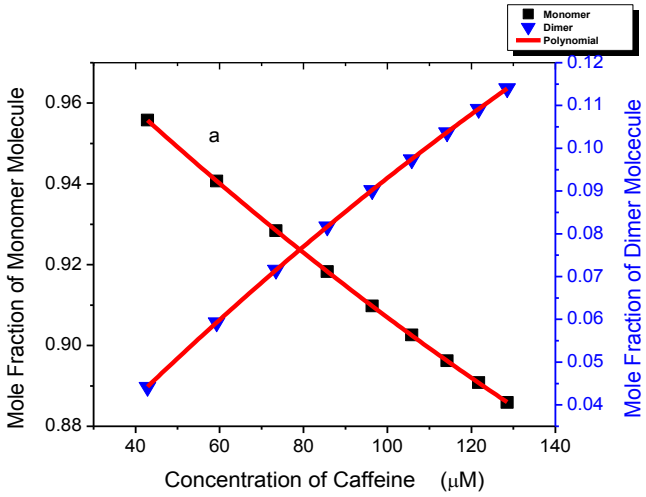

Fig. 3

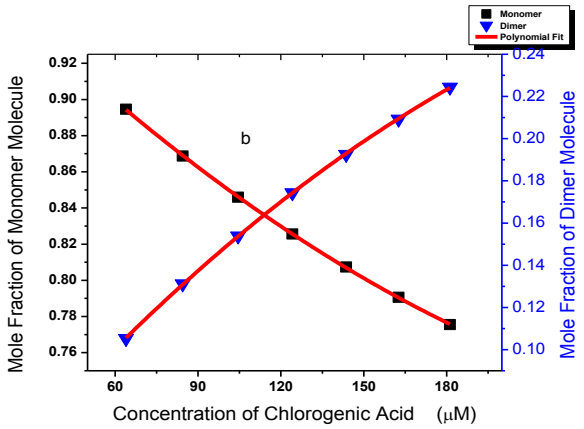

Fig. 3 (continued). The mole fraction of monomer and dimer *versus* total concentration of (a) caffeine under the peak of 272.8 nm, (b) chlorogenic acid under the peak of 324 nm.

The dimerization constant calculated at different temperature ranges previously by [23] were 1.37×10^3, 7.55×10^2, 1.54×10^3 and 1.13×10^3 M^{-1} for acetic, propionic, isobutyric, and butyric acid respectively. The dimerization constants for acetic acid, which are reported by [4] using FTIR, was $(1.00–2.60)\times10^3$ M^{-1} in different concentrations. Also, the dimerization constant for 5-CQA reported by [5] using the same method is in a good agreement with the result obtained in the present work. The molar extinction coefficient for 5-CQA calculated by using the same method but in a different concentration is 1.85×10^4 $M^{-1}\cdot cm^{-1}$ [41]. Furthermore, the molar extinction coefficients of 5-CQA and caffeic acid in aqueous solutions reported by [5, 7] are in a good agreement with the molar extinction coefficients of chlorogenic acid.

Fig. 3(a) and (b) shows the mole fraction of monomer and dimer *versus* concentration of the compounds under the peak of 272.8 and 324 nm for caffeine and chlorogenic acid, respectively. The graphs show increase and decrease in the mole fraction of dimer and monomer as the concentrations of the compounds are increasing. The results indicated that dimerization is favored at high concentrations of the compounds.

THERMODYNAMIC PROPERTIES OF THE SELF-ASSOCIATION OF CAFFEINE AND CHLOROGENIC ACID

Heating of the aqueous solution of caffeine and chlorogenic acid shows that the absorption spectra of the molecules are strongly dependent on the temperature range of 295–301 K. As temperature increases, the absorption intensity increases, this indicates a dissociation of molecular associated forms in solution [9]. The

equilibrium constants of the compounds molecule at the above mentioned temperature were calculated at peak of wavelengths of caffeine and chlorogenic acid using equations (3). Fig. 4(a) and (b) show the graph of $\ln K_{dE}$ *versus* $f(1/T)$, of caffeine and chlorogenic acid. The magnitude of the enthalpy was estimated from the slope of the approximating line according to Vant Hoff's equation:

$$\frac{d\ln(K_{dE})}{f(\frac{1}{T})} = -\frac{\Delta H}{R} \tag{6}$$

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:

$$\Delta G = -RT\ln(K_{dE}) \tag{7}$$

$$\Delta S = \frac{\Delta H - \Delta G}{T} \tag{8}$$

Combining equations (7) and (8), the dependence of the logarithmic equilibrium constant on the temperature is obtained as:

$$\ln(K_{dE}) = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \tag{9}$$

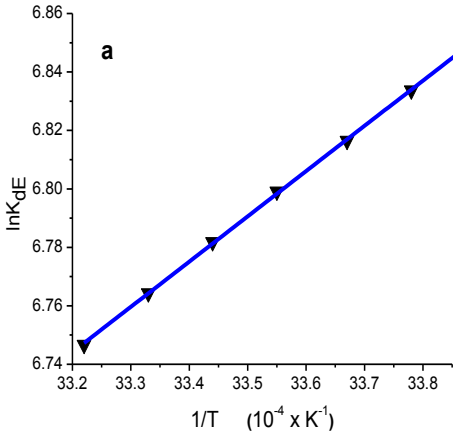

Fig. 4

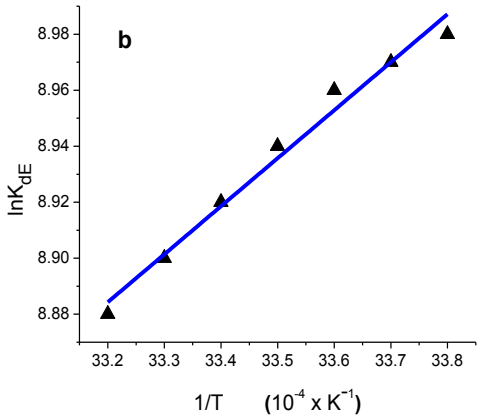

Fig. 4 (continued). ln K_{dE} *vs* 1/*T* of (a) caffeine at concentration 1.217×10^{-4} M; (b) chlorogenic acid at concentration 84.42 μM.

From Fig. 4(a) and (b) slopes and intercepts can be used to determine Δ*H*, Δ*S* and ΔG at specific temperature by using equation (8). The calculated value for the Gibbs free energy, enthalpy, and entropy of the compounds at a temperature 301 K for the self-association is obtained as shown in Table 2. The negative value for the Gibb's free energy indicates that the absorption process of the compounds is continuous. In addition, the negative value of enthalpy shows that the process is exothermic reaction; this indicates that the temperature increases as the equilibrium constant decreases. Also, the positive value of entropy confirms the increasing randomness of the solutions interface during the absorption process of the compounds.

Table 2

Calculated results of the thermodynamic properties of the self-association of CAF and CGA

Compounds	Δ*G* (kJ·mol^{-1})	Δ*H* (kJ·mol^{-1})	Δ*S*(J·K^{-1}·mol^{-1})
CAF	–16.88	–12.86±0.073	13.34±0.24
CGA	–22.22	–14.24±0.839	26.53±2.812

The obtained thermodynamic parameters are in a good agreement with the results recently reported by [5] for 5-CQA which are $\Delta G = -17.9$ kJ·mol^{-1}, $\Delta H = -12.89$ kJ·mol^{-1} and $\Delta S = 17$ J·K^{-1}·mol^{-1}. Moreover, the thermodynamic parameters of the compounds are similar with the previously reported results given by $\Delta G = -18.10$ kJ·mol^{-1}, $\Delta H = -12.50$ kJ·mol^{-1} and $\Delta S = 18.90$ J·K^{-1}·mol^{-1} for benzoic acid [17]. The sign and magnitude of the thermodynamic parameters associated with various individual types of interaction were characterized previously [40]. The calculated values of change in enthalpy and entropy indicate

that an electrostatic interaction plays the major role in the interaction between the molecules of the compounds [34].

HETERO-ASSOCIATION OF METHYLENE BLUE WITH CAFFEINE AND CHLOROGENIC ACID

Fig. 5(a) and (b) show the effects of caffeine and chlorogenic acid concentrations on the UV/Visible absorption spectra of methylene blue solutions. The electronic spectrum of free methylene blue in distilled water was reported by [9]. The addition of CAF and CGA to MB solutions results in decrease of absorption intensity on increasing the concentrations of caffeine and chlorogenic acid. Furthermore, the observed hypo-chromic effect suggests the existence of a hetero-association between MB with CAF and CGA due to hydrophobic interactions of amine group on MB and carbonyl on CAF and carboxyl on CGA [2, 6].

The quantitative analysis of the complexation of MB with CAF and CGA was accomplished by Benesi-Hildebrand equation [8], under the condition of [F] >> [E]. A solution of CAF and CGA, whose concentration ranges are (15.45–39.48)×10^{-5} M^{-1} and (12.23–21.64)×10^{-5} M^{-1} respectively, and MB (3.82 × × 10^{-5} M^{-1} = constant) solution were prepared in distilled water to calculate the equilibrium constants and molar extinction coefficients of the complex formation. According to [7], the equilibrium constant for the complex formation K derived as follows:

$$E + F \xrightarrow{K} EF \tag{10}$$

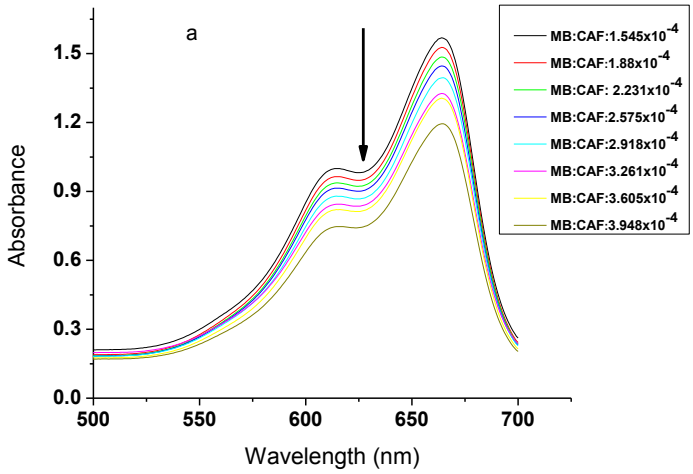

Fig. 5

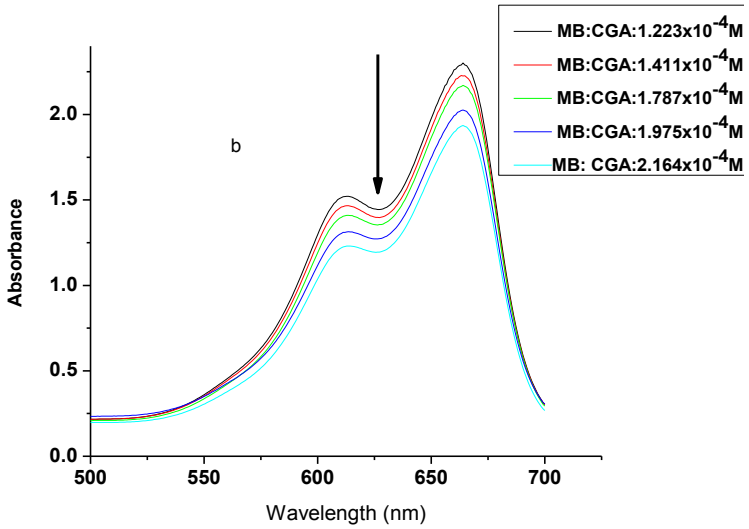

Fig. 5 (continued). Absorbance *versus* different concentrations of (a) caffeine $c = (154.5–394.8) \times 10^{-4}$ M and methylene blue $c = 3.82 \times 10^{-5}$ M, (b) chlorogenic acid $c = (1.223–2.164) \times 10^{-4}$ M, and methylene blue $c = 3.82 \times 10^{-5}$ M.

For equation (10), the equilibrium constant for the complex formation K can be defined as

$$K = \frac{[EF]}{[E][F]} \tag{11}$$

where $[E]$, $[F]$, and $[EF]$ are the equilibrium concentrations of MB, CAF/CGA, and complexation of MB with CAF/CGA respectively, if the initial concentration of CAF/CGA and MB designated as

$$[E_0] = [E] + [EF] \tag{12}$$

$$[F_0] = [F] + [EF] \tag{13}$$

Substituting these in the account of equation (11) above gives

$$K = \frac{[EF]}{([E_0] - [EF])([F_0] - [EF])} \tag{14}$$

For $[F] >> [E]$, it follows that $[F] >> [EF]$; $[F_0] - [EF] \approx [F_0]$. So, equation (14) can be written as

$$K = \frac{[EF]}{([E_0]-[EF])([F_0])} \tag{15}$$

After re-arranging equation (15) it gives;

$$[EF] = \frac{K[E_0][F_0]}{1+K[F_0]} \tag{16}$$

The absorbance (A) for concentration $[EF]$ according to Beer's Law is:

$$A = \varepsilon l[EF] = \frac{\varepsilon lK[E_0][F_0]}{1+K[F_0]} \tag{17}$$

By re-arranging equation (17), it can be written as follows:

$$\frac{[E_0]}{A} = \frac{1+K[F_0]}{\varepsilon lK[F_0]} \tag{18}$$

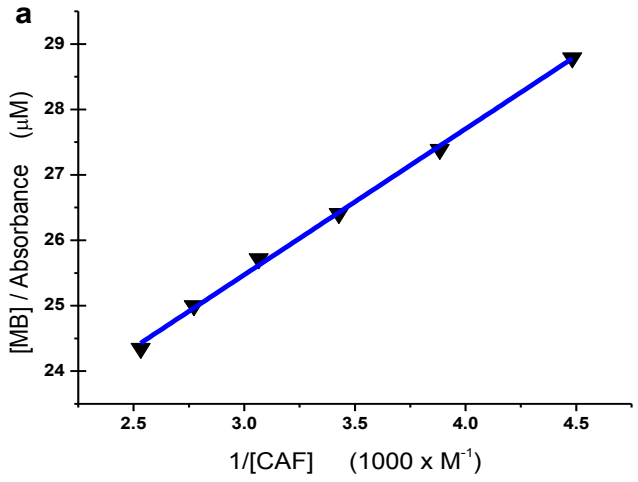

Fig. 6.

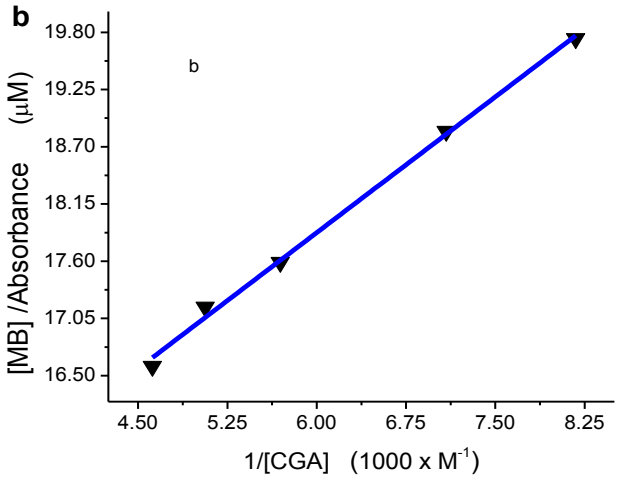

Fig. 6. (continued) Concentration of MB/absorbance *versus* 1/concentration of (a) caffeine at 272.8 nm, (b) chlorogenic acid at 324 nm.

When the path length *l* of the cuvette is 1 cm, the above equation (18) can be also written in the form of Benesi-Hildebrand [8] equation as:

$$\frac{[E_0]}{A} = \frac{1}{\varepsilon} + \left(\frac{1}{[F_0]}\right)\frac{1}{\varepsilon K} \tag{19}$$

The plot of $[E_0]$ /A *vs* $1/[F_0]$ must be linear with the slope $1/\varepsilon K$ and intercept $1/\varepsilon$ as shown in Fig. 6 (a) and (b). The quantitative analysis of the complexation of MB with CAF and CGA was accomplished by Benesi-Hildebrand equation [8], under the condition of $[F] >> [E]$. The equilibrium constants for the complex formation and molar extinction coefficients calculated by linear fitting of equation (19) to experimental data Fig. 6 (a) and (b) were found to be 8.418×10^3 M^{-1}, 5.324×10^4 $M^{-1}\cdot cm^{-1}$, 1.45×10^4 M^{-1}, 7.90×10^4 $M^{-1}\cdot cm^{-1}$, and for MB-CAF and MB-CGA, respectively. From this result we conclude that the hetero-association constants between MB-CAF and MB-CGA are greater than self-association of caffeine and chlorogenic acid. Moreover, the obtained results are in a good agreement with previously reported results by [2, 7], for AMX-CGA and caffeic acid with sodium hydroxide respectively. It can be concluded that the hetero-association of MB with CAF and CGA molecules results in lower effective concentration of the dye in solution, which may account for the modification of its biological activity [9].

THERMODYNAMIC PROPERTIES OF THE HETERO-ASSOCIATION OF METHYLENE BLUE WITH CAFFEINE AND CHLOROGENIC ACID

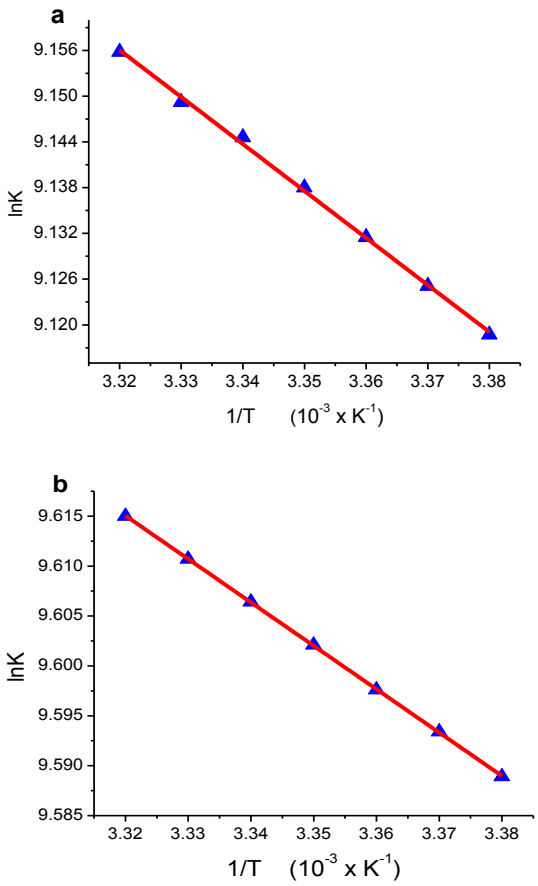

Fig. 7. ln *K vs* 1/*T* of (a) MB-CAF at concentration ratio of MB 38.2 μM with CAF 120.1 μM at 664 nm, (b) MB-CGA at concentration ratio of MB 38.2 μM with CGA (122.3 μM).

Heating an aqueous solution of MB-CAF and MB-CGA complex shows that the absorption spectra of the molecules are strongly dependent on the temperature in the range of 295–302 K. On increasing temperature, the absorption intensity increases indicating dissociation of molecular associated forms in the solutions [5]. The hetero-association constants between MB with CAF and CGA molecules at the aforementioned temperature were calculated at the peak of 664 nm using equation (19). The values of ΔH and ΔS were determined from the slope and intercept of the plots of ln$K$ *versus* 1/T of MB-CAF and MB-CGA as shown in Fig. 7(a) and (b)

and Gibb's free energy (ΔG) can be determined at specific temperature using equation (8).

The calculated values for the Gibbs free energy, enthalpy, and entropy of the complexes of MB-CAF and MB-CGA at the above mentioned temperature ranges are obtained as shown in Table 3. The obtained thermodynamic parameters are in good agreement with the recently reported results by [2] for AMX-CGA which are $\Delta G = -18.186$, 6.988 kJ·mol^{-1}, and 82.27 J·mol^{-1}·K^{-1}.

Table 3

Calculated results of the thermodynamic properties of hetero-association of MB-CAF and MB-CGA

Compounds	ΔG (kJ·mol^{-1})	ΔH (kJ·mol^{-1})	ΔS(J·K^{-}1·mol^{-1})
MB-CAF	–22.89	5.12±0.09	93.07±0.3
MB-CGA	–24.05	3.61±0.012	91.89±0.039

From obtained thermodynamic parameters the hetero-association constant of MB with CAF and CGA increases with the temperature. This suggests that the hetero-association processes are endothermic, and the increasing temperature benefits CAF and CGA binding with MB. Accordingly, the positive values of enthalpy and entropy indicate that hydrophobic interactions played a major role in the reaction between methylene blue with caffeine and chlorogenic acid, whereas the negative sign for change in Gibb's free energy indicates the spontaneity of the binding for these complexes [40].

CONCLUSIONS

The self-association of biologically active compounds, caffeine, chlorogenic acid and their hetero-association with methylene blue (MB), were studied in aqueous solution by a UV/Vis spectrophotometer. The equilibrium constants, the molar extinction coefficients and thermodynamic properties calculated for caffeine, chlorogenic acid and their complexes with methylene blue (MB) are interpreting the study of the kinetic chemical reaction system of the compounds. Hence, understanding, the mechanism of self-association CAF and CGA and their complexation with methylene blue are useful in order to design the advanced and controllable carriers of drugs and food components. In addition, the thermodynamic parameters determined using Van't Hoff equation indicated that binding occurs spontaneously involving an electrostatic and hydrophobic interaction which played a major role in the reaction of self- and hetero-association respectively. Hence, the investigated results have wider applications in pharmaceutical and food companies in terms of cost-effective and scientific utility.

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