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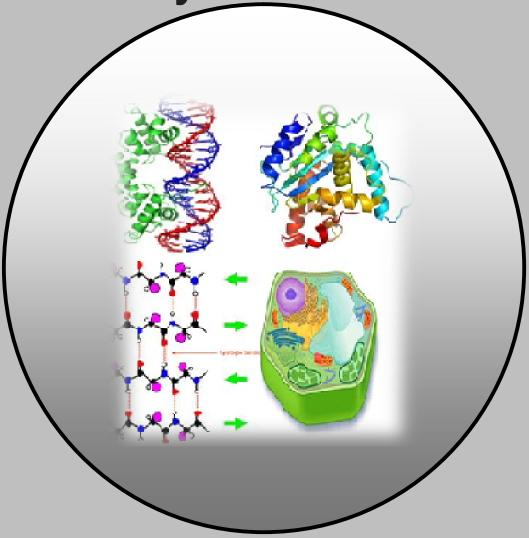

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RESEARCH PAPER

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Determination the Optical Transition Probabilities and Number Densities of Ethidium Bromide (EB) in Hetero-Association by Integrated Absorption Coefficient Techniques

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ABSTRACT

In this research, the optical transition probabilities (molar decadeic absorption coefficient, integrated absorption cross-section, transition dipole moment, oscillator strength and full width at half maxima of the spectra) and number densities of EB in hetero-association (EB-CA) were calculated by integrated absorption coefficient techniques in the wave number regions 17680-25406 cm^{-1} at room temperature (298 K).The optical transition probabilities of EB calculated at different pH values show linear relation (R^2=0.99) with pH value of the medium. On the other hand, exponentially decreases in the optical transition probabilities of EB monomers in hetero-association complex (EB-CA) were observed as caffeine concentrations increase. The relative molar ratio of free and bound EB calculated by integrated absorption coefficient techniques at concentrations of EB and caffeine (50 μM and 2.53 mM) are 15.46 and 84.54 %. In addition, the hetero-association of EB-CA was analyzed using modfied Benesi-Hildebrand model and the determined association constant is found to be 41 M^{-1}.

Key words: Ethidium Bromide, caffeine, caffeic acid, transition probabilities, pH value, hetero-association.

INTRODUCTION

Ethidium Bromide (EB) is a typical mutagenic intercalating dye for DNA and RNA (Tettet et al., 1999; Severini and Morgan, 1991).

It is widely used in various biophysical applications as a model for DNA intercalating ligands (Tragonos et al., 1991; Zdunek et al., 2000). The conformational changes in the DNA macromolecules caused by intercalation of Ethidium Bromide are lead to inhibition of DNA replication and transcription processes in the cell (Graves and Velea, 2000; Eliot, 1963).

In cell systems, it has been shown that caffeine is capable of reducing the toxicity of a typical DNA intercalator, Ethidium, Bromide. The studies conducted previously by (Traganos et al., 1991; Larsen et al., 1996; Piosik et al., 2011; Baranovsk et al., 2009) showed that caffeine forms complex with aromatic molecules, which effectively lowers the concentrations of free Ethidium Bromide and hereby reduces the biological activity of this drug, that means it assumed (Larsen et al., 1996) that caffeine acts as an interceptor of biologically active aromatic molecules which bind to DNA by intercalation. The hetero-association of caffeine with Ethidium Bromide has been investigated using various mathematical models and analytical procedures to interpret the experimental results (Weller et al., 1984; Kaps et al., 1993; Chen and Shiao, 1994; Baxter et al., 1996; Larsen et al., 1996). However most of the proposed models of molecular hetero-association only cover limited sets of condition and are not applicable for the general case. In this research, integrated absorption coefficient techniques developed previously by (Belay et al., 2010) have been implemented to study the hetero-associations of caffeine with EB, which is simple, fast and inexpensive. Moreover, the techniques are more powerful in measuring the intensity of absorption light when there are line broadening and spectral shift due to temperature and pressure variations, or the concentrations of solute in solution high or a reaction field exists due to solute and solvent (Bayliss, 1950; MacRae, 1957; Mataga et al., 1956; Onsager, 1936; Ooshika, 1954; Burnett and Lovell, 1970; Vigasin, 2004). The techniques included characterization of the optical transition probabilities and determine the number densities in hetero-association complex. The molecular transition probabilities (transition dipole moment, oscillator strength and integrated absorption cross-section) of compounds are important in order characterize the electron transition probabilities and interpret the absorption spectra of the compound and the complex. In addition, the determined optical transition parameters are also useful for direct experimentally application in the emission, absorption and dispersion and in providing stringent test of atomic and molecular structure calculation in theoretical works.

Therefore the objectives of this research is to determine the optical transition probabilities of EB and its number densities in hetero-association complex with caffeine using integrated absorption coefficient techniques.

MATERIAL AND METHODS

All the chemicals (Caffeine and Ethidium Bromide) shown in fig 1a-b were purchased from Sigma-Aldrich and used for measurements without any further purification. For electronic absorption measurement of EB and its complexation with caffeine different laboratory apparatus, a 1 cm quartz cuvette and double UV-Vis-NIR spectrometer, Perkin Elmer Lambda 19

(Perkin Elmer, D-7770 Ueberlingen, Germany) with wavelength regions of 170-3200 nm were used. The instrument was operated by 4.3 UVCSS soft ware. Scanning speed 240 nm per min and slit width 2 nm was used. Data acquisition performed by computer interfaced with spectrometer.

The optical transition probabilities (transition dipole moment, oscillator strength and integrated absorption cross-section) for pure compound EB were studied at various pH values of the medium but its complex with caffeine at pH 6.0. The optical transition probabilities of caffeine have already been reported in our previous works (Belay et al., 2008; Belay, 2010). For the hetero-association of EB-CA, the solution of [EB] =$5x10^{-5}$ M= constant were titrated by caffeine of concentration ranges of $5.0x10^{-4}$ to $2.53x10^{-2}$ M. The optical transition probabilities were measured by integrating the absorption coefficient and molar decadic absorption coefficients in the wave number regions of (17680-25406) cm^{-1}.UsuallytheUV-Vis spectrometer measures the concentration in terms of absorbance versus wavelength; this was recalculated into absorption coefficient or molar decadic absorption coefficient versus wave number using Origin 6.1 software**.** In addition the Origin 6.1 software was used for data analysis such as changing the ASCII file to the spectra; measurement of height and line width of the spectra; and to draw graphs.

RESULTS AND DISCUSSION

Optical Transition Probabilities of EB

Molar decadeic absorption coefficient (ε_{480})

Fig 2 shows the UV-Vis absorption spectrum of EB in the wavelength region of 200-600 nm at room temperature. The absorption spectrum of EB has one sharp peakin the ultra violet regions ($\lambda_{\max} = 285nm$) with shoulder around 300 nm and the other peak available in the visible regions ($\lambda_{\max} = 480nm$). The peak in the visible regions is due to $\pi \rightarrow \pi^*$ electronic transitions of chromophores groups (Larsen et al., 1996; Bakhkshiev, 1961; Bayliss and McRae, 1954; Firth et al., 1983).

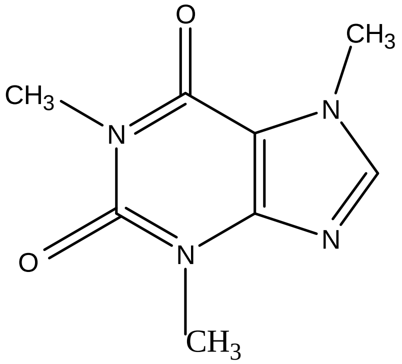

Fig. 1a Chemical Structure of caffeine.

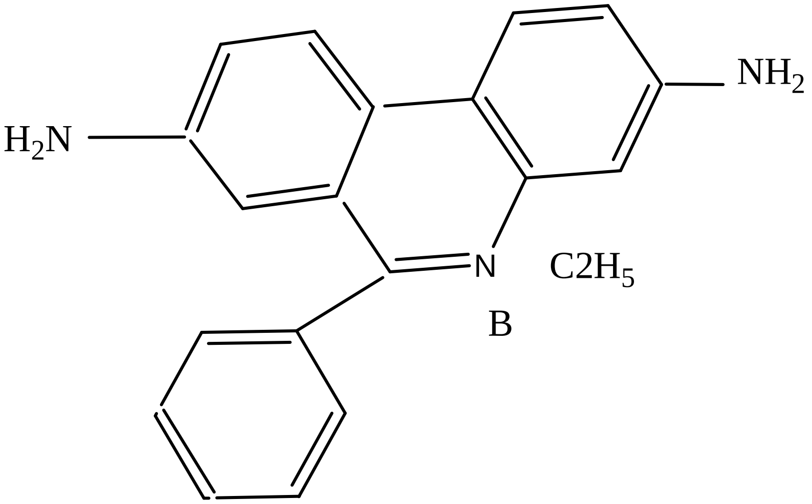

Fig. 1b Chemical structure of ethidium bromide.

The absorption spectrum of this peak depends on the solvent environment of the chromophores (Larsen et al., 1996) and these effects were observed on the absorption spectra of EB at various pH values. Fig 3 shows the super impose spectra of EB in the wavelength region of 300-600 nm at room temperature. The dot shows the absorption spectrum of EB at pH 6.68and the line shows the spectrum of EB at pH 6.00. The absorption spectrum of EB at pH value of 6.68 has higher intensity strength at peak but smaller line width as compared to the other. In addition, the over lapped spectra have two isobestic points ($\lambda = 431$and 523 nm).
From UV-Vis absorption spectrum, the molar decadic absorption coefficient (ε_{480}) of EB were calculated at various pH values. Fig 4 shows the calculated ε_{480} versus pH values which is linear with (R^2=0.99). The result indicates the dependence of absorption intensity of EB on the pH value of the solution. Previously many coworkers have studied the hetero-association complex of EB with caffeine at various pH values by (Piosik et al., 2010; Barnovsky et al., 2009; Garbett et al., 2004; Pohl et al., 1972), however, the corresponding molar decadic absorption coefficient are rarely available. The calculated ($\varepsilon_{480} = 5400$ M cm^{-1}) in this research at pH 7.00 is quite similar with the result previously reported by (Pohl et al., 1972; Garbett et al., 2004) in the same pH value.

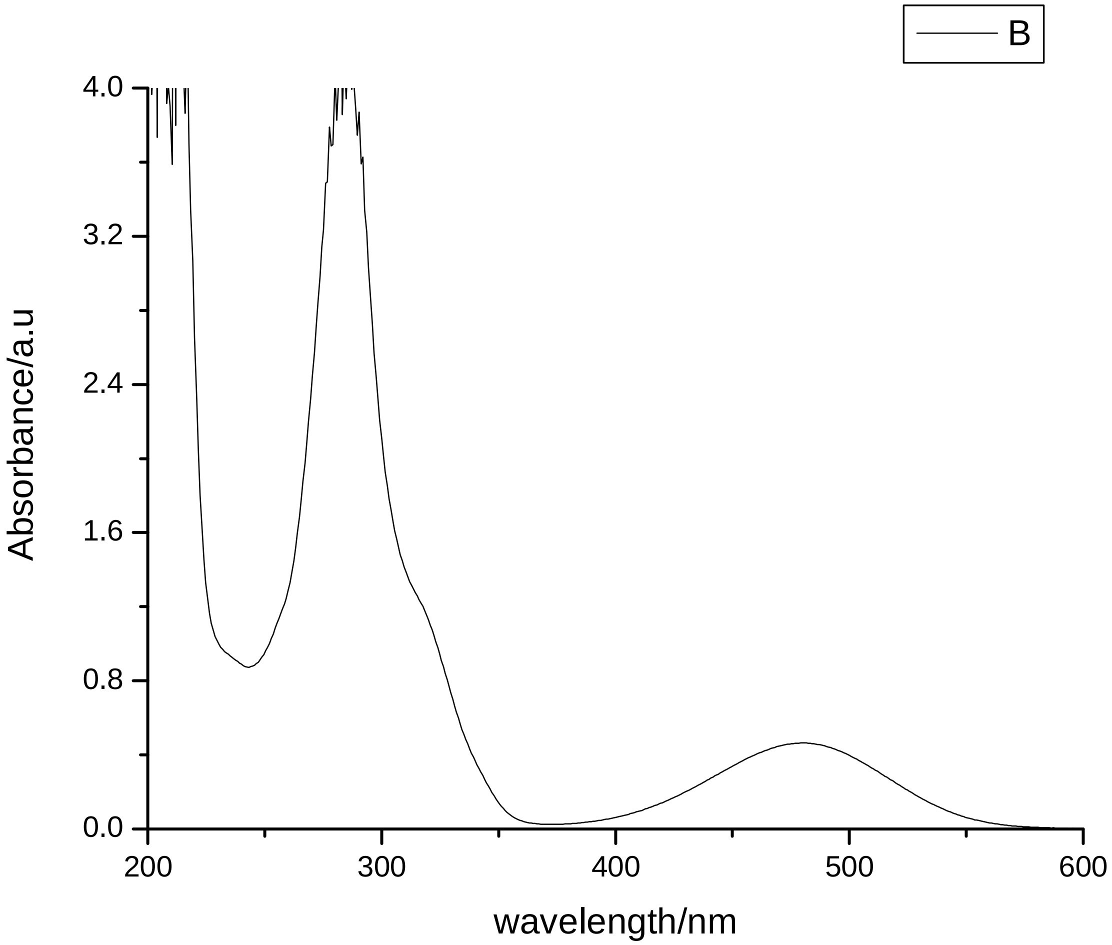

Fig 2. shows the UV-Vis absorption Spectrum of EB in water solution in the wavelength regions of 200-600 nm.

Integrated Absorption Cross-Section

For intensity of incident wave propagating a distance l into the absorbing medium, the Beer-Lamberts Law expressed by

$$P_\lambda = P_\lambda^{\,0} 10^{-a_\lambda l} \qquad (1)$$

Where P_λ and $P_\lambda^{\,0}$ are the transmitted and incident light intensities, respectively, and a_λ is the absorption coefficient. From equation (1), absorption coefficient can be expressed as

$$a_\lambda = \frac{1}{l}\log\left(\frac{P_\lambda^{\,0}}{P_\lambda}\right) \qquad (2)$$

The absorption cross-section σ_λ related to the absorption coefficient a_λ at a single frequency for N number of molecules per unit volume expressed by the following relation (Rao, 1975; Thorne, 1988, Belay, 2010).

$$a_\lambda = N\sigma_\lambda \qquad (3)$$

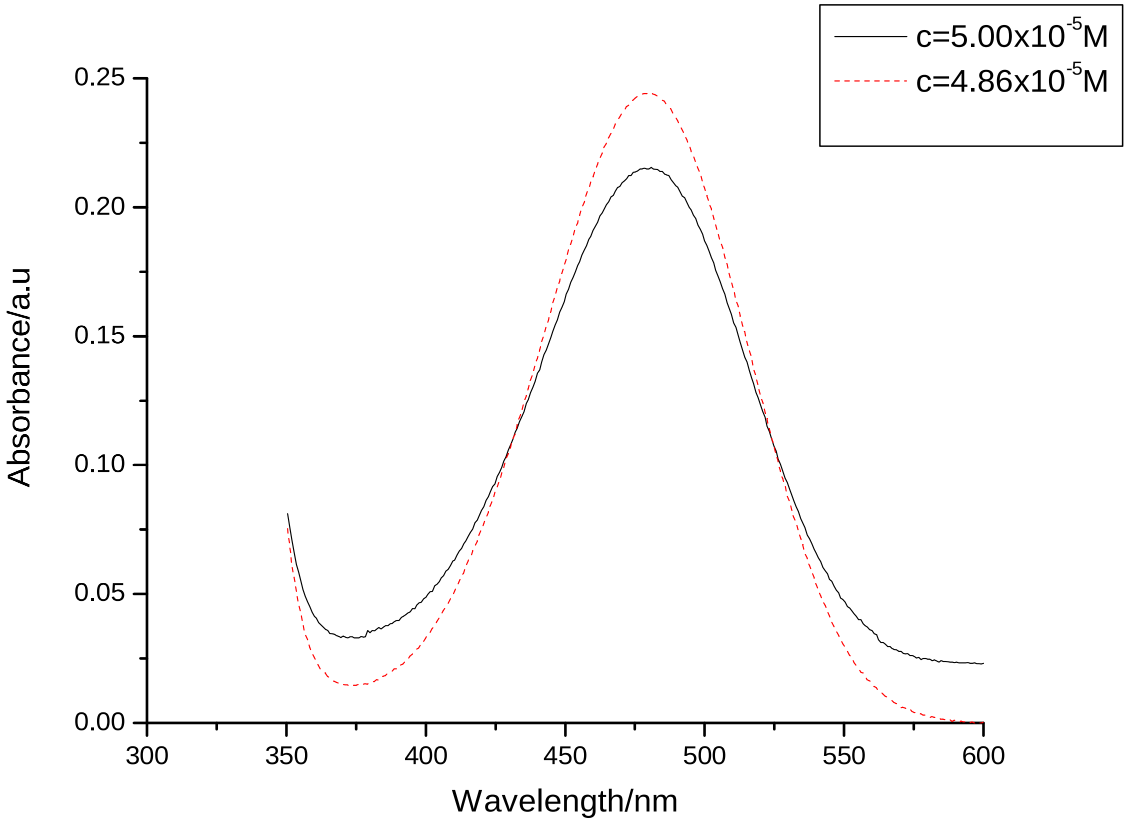

Fig 3. Shows the overlapped spectra of EB at different pH values. The line shows EB spectrum at pH 6.00 and dot at pH 6.68.

However, in a UV-Vis spectrometer, the absorption of molecules in a liquid occurs over a certain range of frequencies rather that at a single frequency. Therefore, absorption coefficient measured at any single frequency may not express the true intensity of the molecular transition. Integrated absorption coefficient which is the sum of absorption coefficients for all frequencies in the band is preferable in such cases; the techniques are useful for different applications, since it is independent of the line function which may vary by parameters like pressure, temperature, concentration of the solute and solute-solvent interaction (Bayliss, 1950; MacRae, 1957; Mataga et al., 1956; Onsager, 1936; Ooshika, 1954; Burnett and Lovell, 1970; Vigasin, 2004).

In addition, the techniques are very important in the absence of a high-resolution spectrometer. Therefore, in liquids and solutions where the above effects are observed, the true integrated absorption intensity of a band should be defined by the following equation (Rao, 1975; Thorne, 1988; Belay, 2010; Belay et al., 2009).

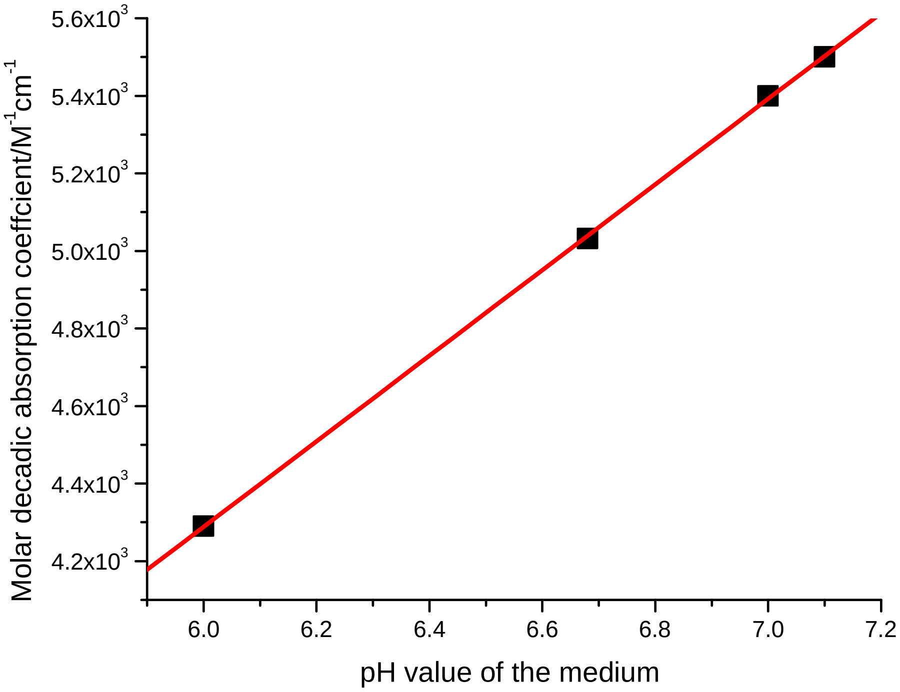

Fig 4. Shows Molar decadic absorption coefficients vs pH values of the medium.

$$a_t = \int a_\lambda d\nu \qquad (4)$$

Substituting equation (2) into (4), the integrated absorption coefficient was expressed as

$$a_t = \frac{1}{l}\int \log\left(\frac{P_\lambda^0}{P_\lambda}\right)d\nu \ (5)$$

The integral being measured over the limit of the absorption band. From the integrated absorption coefficient having no line shape dependence, the integrated absorption cross-section was calculated using the following equation (Milonni and Eberly, 1988; Belay, 2010; Belay et al., 2009).

$$\sigma_t = \frac{1}{Nl}\int \frac{1}{l}\log\frac{P_\lambda^0}{P_\lambda} d\nu \ (6)$$

Where σ_t are the integrated absorption cross-section, a_t the integrated absorption coefficient, and N is the number density.

For calculating the integrated absorption cross-section of EB at various pH values, the absorbance's versus wavelength of EB was recalculated in to absorption coefficient versus wave number using Origin 6.1 software. From area under the spectrum, integrated absorption cross-section of EB was calculated in the wave number regions of 17680-25406 cm^{-1}. The calculated integrated absorption cross-section from independent measurements of EB is(33.65±0.68) $\times10^{-15}$, (31.84±0.38) $\times10^{-15}$ cm $molecule^{-1}$ for pH 6.68 and 6.00 respectively. On the other hand, the peak absorption cross-section of EB at the center was calculated using equation (3). The calculated absorption cross-section at peak was (8.36±0.01) $\times10^{-18}$and (7.13±0.01) $\times10^{-18}cm^{2}molecule^{-1}$. The peak absorption cross section variations between (pH 6.68 and 6.00) is 14.71 %, which is higher as compared with the variation observed in integrated absorption cross-section(5.38 %).The results indicate the powerful methods of integrated absorption cross-section in measuring the true intensity of the molecules where broadening and shifting spectral line of the molecules existed. The peak absorption cross-sections calculated for EB in this research is quite similar with the results calculated by (Cheng et al., 2006) for photo absorption of NH_3, NH_2D, NHD_2 and ND_3, in the spectral region of 140-220 nm at 298 K using synchrotron radiation. In addition, the integrated absorption cross-section obtained in this research slightly agrees with one reported by (Canosa-Marttin et al., 1987) for NO_3 at room temperature. The resemblance in the peak absorptions of (Cheng et al., 2006) and our results confirms that peak's of EB in the visible region is due to electronic transition of the amino functional groups available on the compound.

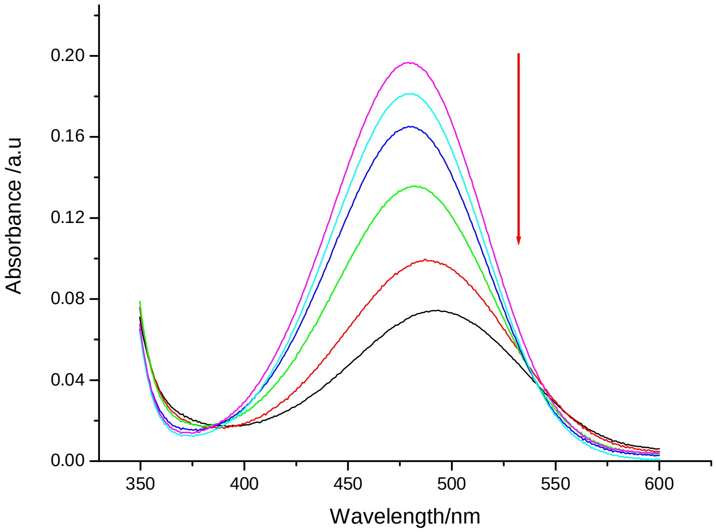

Fig. 5 Electronic absorption spectra of aqueous solution of EB (pH 6.00, T=298 K): As concentration of Caffeine increase ($5.1x10^{-4}$-$2.53x10^{-2}$) the absorption spectra EB at (λ_{max}= 480 nm) decrease.

Transition Dipole Moment

The Transition dipole moment is the electric dipole moment associated with the transition between the two states. It is a complex vector quantity that includes the phase factors associated with the two states. Its direction gives the polarization of the transition, which determines how the system will interact with an electromagnetic wave of a given polarization and while the square of the magnitude gives the strength of the interaction due to the distribution of charge within the system.

The transition dipole moment for the $k \rightarrow m$ transition is given by the relevant off-diagonal element of the dipole matrix, which can be calculated from an integral taken over the product of the wave functions of the initial and final states of the transition,

$$\vec{\mu}_{mk} = \int \psi_m{}^* \vec{\mu} \psi_k d^3 r = \langle \psi_m | \vec{\mu} | \psi_k \rangle \tag{7}$$

The rate of transition probability that any molecule will make to excited sate, m as a result of absorption under the perturbing effect of the electric field radiation related to transition dipole moment by the following equation

$$\frac{d}{dt}(c_m{}^* \; c_m) = \frac{\pi \rho(\omega)}{3\varepsilon_0 \hbar^2} |\mu_{xmk}|^2 \tag{8}$$

In order to compare the theoretical expressions with the experimentally measurable quantities consider the following. If $\frac{d}{dt}(c_m{}^* \; c_m)$ is the rate of probability for a single molecule changes as a result of absorption of radiation under perturbing effect of electric field radiation then $\frac{d}{dt}(c_m{}^* \; c_m) N dl$ is the number of molecules excited in a layer dl with energy absorption $h\nu_{mk}$, so the loss in intensity becomes (Barrow, 1962; Banwell, 1972).

$$-dI = \frac{d}{dt}(c_m{}^* \; c_m) N h \nu_{mk} dl \tag{9}$$

Further, the loss in intensity of light when light passes through material whose concentration, $c = \frac{N}{N_a}$ and molar decadic absorption coefficient, ε given by

$$-dI = \varepsilon(\nu) \frac{N}{N_a} I_0 \ln(10) dl \tag{10}$$

From comparison of equations (9), and (10) we can express rate of probability equation as follows,

$$\frac{d}{dt}(c_m{}^* \; c_m) = \frac{\varepsilon(\nu) c \rho \ln(10)}{N a h \nu}, \tag{11}$$

If the energy density is assumed to be constant throughout the bands the total rate of probability for the entire absorption band is obtained by integrating over the entire frequency range. The total intensity of the band is obtained by measuring ε in the region of absorption and usually determined by integrating the area under the graph.

So the integrated absorption coefficient due to transition can be expressed as (Michale, 1999; Liptay, 1969; Barrow, 1962). Therefore, the transition dipole moment, which is purely theoretical expression, related to the molar decadic absorption coefficient, experimentally measurable quantities by the following equation (Liptay, 1969; Michale, 1999).

$$\frac{1}{3}S|\mu_{fi}|^2 = \int \frac{\varepsilon(\nu)}{\nu} d\nu \qquad (12)$$

Where $S=2.9352\times10^{60}C^{-2}mol^{-1}$, μ_{fi} transition dipole moment and ε molar decadic absorption coefficient.

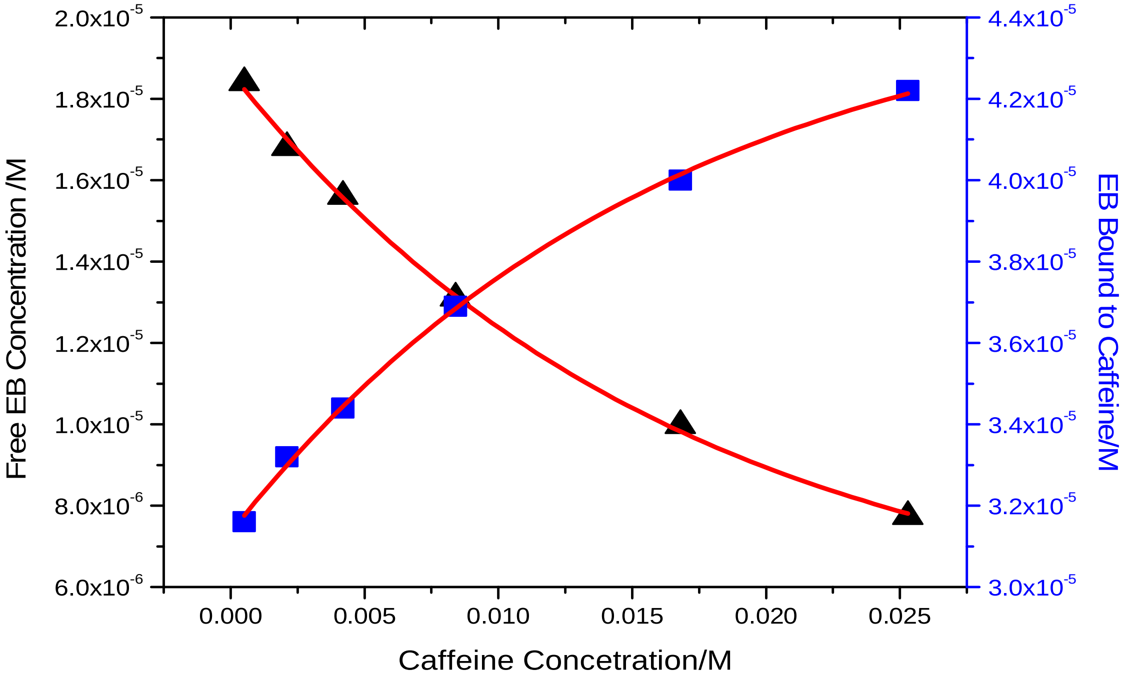

Fig. 6 shows the concentrations of free EB and EB bound to caffeine vs concentration of caffeine from IACT.

Using equation (12) the transition dipole moment of EB in the wave number regions of (17680-25406) cm^{-1} were calculated and good linear correlation coefficient (R^2=0.99) were obtained between the transition dipole moment of the compound and pH values. The decreases in transition dipole moment of the molecule EB as the pH values decrease may be attributed either the decreases of the already existed molecular dipole or the already existed dipole not to be reoriented to electric field.

The transition dipole moment EB calculated at pH value of 6.68 and 6.00 are (9.86±0.33) $\times 10^{-30}$, (9.57±0.43) $\times 10^{-30}$ respectively with the discrepancy variation between the two are 2.94 %. Although, there are no theoretical as well as experimental research reports on the transition dipole moment of EB, however, the obtained results have similarity with the result reported by (Belay et al., 2008) for caffeine compound in different solvents.

Oscillator Strength

The oscillator strength is considered to be the other useful parameter providing the intensity of transition; it expresses the relative strength of electron transition. It is one of the most fundamental quantities in analytical spectroscopy. In practice, it determines the sensitivity of a given atomic resonance line and needs to be accurately known if one needs to relate the magnitude of the absorption signal to its concentration. Oscillator strength can be determined directly through absolute emission, absorption or dispersion measurement. In this research, the oscillator strength of EB was calculated by absorption measurements. Oscillator strength related to the molar decadic absorption coefficient by the following equation (Forsman and Clark, 1973; Georgakopoulous et al., 2004; Radwan, 2007)

$$f=4.32\times10^{-9}\int \varepsilon d\nu \qquad (13)$$

Where ε is molar decadic absorption coefficient carrying the unit L mol^{-1} cm^{-1} and wave number ν in cm^{-1}.

Furthermore, an equation relating integrated absorption coefficient with number density and oscillator strength for the Gaussian shaped spectrum will be given by Thorne (1988).

$$\int a \ d\nu=2.65\times10^{-6}Nf \quad (14)$$

Where N is number density in molecules cm^{-3}, a in m^{-1} and ν in Hz; f is oscillator strength of the transition molecule. By equation (13), the oscillator strength of EB was calculated at its pH values of 6.68 and 6.00 in the wave number region of (17680-25406) cm^{-1}. The calculated oscillator strength of EBare0.087±0.002 and0.083±0.002 respectively, the results show the relative strength of the electronic transition of EB decreases as pH value decreases which is consistent with previously results. The observed discrepancy variations between the two are 4.60 % which higher than the transition dipole moment but smaller than the integrated absorption cross section.

Furthermore, the experimentally calculated oscillator strength results in this research are within the range of the results previously reported for NH_3 which has a similar chemical structure with amino groups available on the phenanthrene compound. The experimentally calculated oscillator strength previously by (Cheng et al., 2006) for NH_3, NH_2D, NHD_2 and ND_3 are 0.080, 0.088, 0.081 and 0.082 using synchrotron radiation. In addition, the results are also similar with the results reported for $NH_3$by (Burton et al., 1993; Zeis et al., 1977), 0.080 and Watanabe (1954), 0.088.

Furthermore, the result is also similar with the result obtained by (Chantranupong et al., 1991), 0.087 using quantum mechanically calculation.

Full Width at Half Maxima (FWHM)

The other basic parameters describing any individual absorption band is a band width at half maximal intensity, defined as (Antonov and Nedltcheva, 2000)

$$\Delta\nu_{1/2}=\nu_1-\nu_2 \qquad (15)$$

It measures the anti-bonding character of the excited state. The anti-bonding character is a function of inter nuclear distance and in some cases the change of the band width can indicate the change of this distance. The band width at half maximum of EB has an inverse relation with pH values, the results show the decrease in peak intensity compensated by line broadening of the spectrum.

The Optical Transition Probabilities and Association Constant of EB in Hetero-Association Complex Optical transition probabilities

Fig 5 shows the EB spectra in water solutions with different concentration of caffeine. Titrating EB with caffeine leads to a hypochromic effect and a bathochromic shift of the absorption band by 14 nm relative to the peak in the monomer band of the dye (480 nm). The observed red shifts of the absorption spectrum in the presence of caffeine are characteristics in the solvent environment associated with intercalator. The complexation between caffeine and EB results in the replacement of water molecules solvating the intercalator by more hydrophobic caffeine molecule (Larsen et al., 2004). At the same time there are two isobestic points were observed at wavelength of 384 and 543 nm as shown in fig 5. The spectral variation and the existence of the isobestic points are indicative of the interaction between the EB and caffeine molecules Bolotin et al., 2006; Baranovsk et al., 2009; Baranovsk et al., 2007).

The relative peak shifts and isobestic points observed in this research (pH 6.00) is also different from the result obtained by (Baranovsk et al., 2009) (pH 6.86) due the sensitivity of EB chromophores on solvent environment. Relatively small peak shift and the isobestic point observed at longer wavelength (543 nm) as compared with (Baranovsk et al., 2009) at (497 nm). On the other hand, the isobestic point reported in this research is almost similar with the result reported by (Pioski et al., 2010) in which, EB dissolved in distilled water but CA solution prepared in buffer with pH 6.00 or different in protocol from the present study.

Table 1. The calculated transition probabilities of EB monomer at various caffeine concentrations in hetero-association complex.

Caffeine Concentration/M	$\varepsilon_{\lambda=480nm} / M^{-1}cm$	μ_{fi} / Cm	ω_{FWHM} / cm^{-1}	f
$2.53\mathrm{x}10^{-2}$	2.77	$0.25\mathrm{x}10^{-30}$	4167 4304	$5.81\mathrm{x}10^{-5}$
$1.68\mathrm{x}10^{-2}$	5.95	$0.35\mathrm{x}10^{-30}$	4030 4153	$1.12\mathrm{x}10^{-4}$
$8.4\mathrm{x}10^{-3}$	16.66	$0.57\ \mathrm{x}10^{-30}$	3899 3980	$2.95\mathrm{x}10^{-4}$
$4.2\mathrm{x}10^{-3}$	38.09	$0.88\ \mathrm{x}10^{-30}$	3797 3877	$7.00\mathrm{x}10^{-4}$
$2.1\mathrm{x}10^{-3}$	85.71	$1.29\ \mathrm{x}10^{-30}$	3726 3848	$1.50\mathrm{x}10^{-3}$
$5.1\mathrm{x}10^{-4}$	392	$2.75\ \mathrm{x}10^{-30}$	3768 3848	$6.82\mathrm{x}10^{-3}$

Table 1 shows the calculated optical transition probabilities of EB monomers titrated by caffeine concentrations. The transition probabilities of EB monomers are decrease as caffeine concentration increases. The graph of experimental data best fit the second order exponential decay ($y = y_0 + A_1 e^{(-\frac{x}{t_1})} + A_2 e^{(-\frac{x}{t_2})}$). In the mention concentrations range, the percentage of decreasing in $\varepsilon_{\max}, \mu_{fi}$ and f as caffeine concentrations increasing are 98 %, 90.9 %, 84.5 % respectively. On the other hand, the corresponding FWHM increasing by 7 %, the effects of this line broadening are due the solute solvent interaction.

The concentrations of free and bound EB to caffeine calculated at various caffeine concentrations using Beer-Lambert's law and integrated absorption techniques. In both techniques, as caffeine concentrations increase, the concentrations of free EB decrease while the concentrations of EB bound to caffeine increase. For example the relative molar ratios of the free and bound EB calculated by Beer-lambert's law at concentration of EB and caffeine (50 μM and 253 mM) are33.4 and 66.6 % respectively. On the other hand, the corresponding free and bound EB calculated by integrated absorption coefficient techniques (fig 6) are15.46 and 84.54 % respectively. The relative molar ratio of free and bound EB calculated by integrated techniques or equation (14) are quite similar with the results of (Larsen et al., 1996) obtained using time resolved fluorescence technique. The percentage of free and bound EB calculated by (Larsen et al., 1996) at concentrations EB and caffeine (30 μM and 44 mM) are 16 and 84 %respectively. The similarities of the two techniques show the powerfulness of integrated absorption techniques to determine the number densities of hetero-association complex of the compound.

Hetero-association constants

The hetero-association of EB-CA was analyzed using modified Benesi-Hildebrand model (Bolotin et al., 2006; Baranovsk et al., 2009; Baranovsk et al., 2007). For quantitative analysis, numerical procedures of fitting the model to experimental data was carried out by non-linear curve fitting based on Levenberg Marquardt algorithm using origin 6.1 software. The hetero-association constant was obtained at the minimum discrepancy between the experimental data and theoretical expression the obtained result is $K = 41 \pm 2M^{-1}$. The calculated association constant for the complexation of EB-CA in this research is almost similar with the result obtained previously by (Pioski et al., 2010), $K = 60.2 \pm 23M^{-1}$, which is different from the present study, both in experimental protocol and theoretical model . In addition, the results are also comparable with (Baranovsk et al., 2009)which is $K = 71 \pm 8M^{-1}$ in which the model implemented are similar but different pH medium. From the obtained results the differences in the experimental protocol and the pH values has an effect on the qualitative and quantitative spectra of the complex, in which more dominance observed on the pH values. The hetero-complexation of EB-CA presumably stabilized as results of dispersive and hydrophobic interactions of the cyclic chromophores of these molecules.

CONCLUSION

Optical transition probabilities and number densities of EB in hetero-association were determined by integrated absorption coefficient techniques. The determined optical transition probabilities have great applications both in experimental and theoretical work for elucidation of the molecular structures. On the other hand, the integrated absorption coefficient techniques are simple, fast and most convenient methods to investigate the hetero-association of some bioactive compound with DNA intercalator.

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