Full Length Research Paper

Determination of self - associated 5- caffeoylquinic acid and its complexation with sodium hydroxide using UV-Vis spectroscopy

Abebe Belay

Department of Physics, Faculty of Science, Addis Ababa University, P. O. Box, 1176, Ethiopia.
Asella Teachers Training College, P. O. Box, 209, Asella, Ethiopia. E-mail: abebebelay96@gmail.com.
Tel: +251 911 71 27 66.

The concentration dependent self - association of 5-caffeoylquinic acid and its complexation with sodium have been determined by UV-Vis spectroscopy. The self - association and hetero - association constants were determined by dimer model and Benesi - Hildebrand equations. The equilibrium constants for the formation of dimer and complexation with sodium at wavelength of 324 nm and 370 were 1813, 13529 M^{-1} respectively. Using the value of dimerzation constant the fraction of monomer and dimer were calculated for every concentration of 5-caffeoylquinic acid. The deviation of molar extinction coefficient from Beer -Lambert's law and existence of isobestic points at different wave-lengths showed the existence of dimerzation and the formation of the complexes in the solution respectively.

Key words: 5-caffeoylquinic acid, UV-Vis spectroscopy, thermodynamic, self-association, hetero-association.

INTRODUCTION

Chlorogenic acid (5-caffeoylquinic acid) is an ester of caffeic acid with quinic acid, has received considerable attentions for its widely distribution and potential biological effects (Cornard et al., 2008; Scalbert et al., 2005). It is naturally occurring in vegetables, cereals, fruits (Cornard et al., 2008; Scalbert et al., 2005; Dillard and German, 2000; Prior, 2003). The compound has attracted the attention of researchers due to health promoting attributes, which include lowering the risks of cardiovascular disease, cancer, diabetes, and other conditions associated with aging. The biological mechanisms behind these effects are protection against free radicals, free radical mediated, inflammation and viral infection (Rice-Evans et al., 1996; Kono et al., 1997; Robinson and Chew, 1999).

On the other hand chlorogenic acid is a multifunctional natural organic acid substance that plays a significant role in binding toxic metals in the natural environment (Cornard et al., 2008). Several papers have reported the complexation of this compound towards different metal ions in aqueous solutions, such as iron (Hynes and Ocoineanainn, 2004), copper, manganese, zinc, aluminum, and lead (Cornard et al., 2008). More-over the

molecular complexation of this compound with Beta-Cyclodextrin (β-CD) (Gornas et al., 2009; Irwin et al., 1999), caffeine (Sondheiemer et al., 1961; D'Amelio et al., 2009), and protein (Suryaprakash and Prakash, 1995) have been reported to design more advanced and controllable carriers of drugs and food components.

Although, from pervious research report it is possible to find the complexation of this compound with other metal ions, polyphenols, and aromatic-hydroxy acid molecules, there is lack of information on the concentration dependent self -association, its thermodynamic properties and the complexation with sodium ions. Usually, investigation of self - associated molecules using UV-Vis spectroscopy 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 (Dearden, 1963). It is known that, the formation of dimer complicate 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 (Marme et al., 2005). Therefore, study of the self – association and hetero -
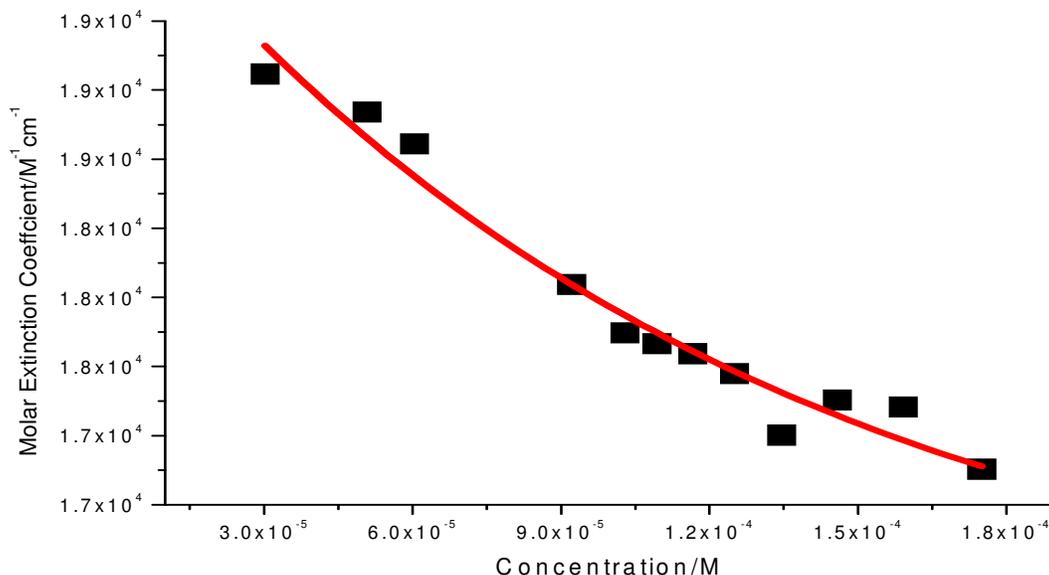
Figure 1. Shows molar extinction coefficient vs concentration of 5-caffeoylquinic acid at absorption maxima of 324 nm.

association of the solute is an important phenomenon. This is accounted in analyzing and interpreting the spectroscopy, photophysics and photochemistry of the system (Dearden, 1963; Marme et al., 2005). Moreover, in order to design a more advanced and controllable carries of drugs or food components it is necessary to know the association mechanisms to control the processes. Therefore, the objectives of this research are determining the concentration dependent self – association and thermodynamic properties of 5-caffeoylquinic acid and investigate the hetero-association of this compound with sodium hydroxide by UV-Vis spectroscopy. The UV-Vis spectrophotometer method is the most suitable for quantitative study of the aggregation properties of the molecules as a function of concentration.

MATERIALS AND METHODS

The compound studied in this work was chlorogenic acid (5-caffeoylquinic acid), bought from Sigma-Aldrich. The absorption spectra were recorded by Perkin -Elmer 19 spectrophotometer with double monochromator, which offers the advantage of a low level of stray light that is significant for the measurement of high value of absorbance using 1 cm quartz cuvette. The spectra were recorded with a spectral band width of 2 nm and scan speed 240 nm min^{-1}. The acquisition of data was performed with 4.3 UVCSS soft ware, using a suitable computer on line with the spectrophotometer. The electronic absorption spectrum of the investigated compound was measured in the UV-Vis spectra range of 200 - 500 nm. The spectra analyzed in this study were obtained by subtracting the spectrum of pure solvent (water) from that of the solution containing chlorogenic acid.

The self - association of the compound was studied over the concentration range of (3.02-17.50) x10^{-5} M in water solutions, since self - association can be detected over a wide range of concentration

and need a careful examination of the changes in the apparent molar extinction coefficients. The absorbance as a function of concentration has been measured at absorption maxima 324 nm to obtain the greatest accuracy of detection. For numerical analysis the (molar extinction coefficients and dimerzation 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. The molar extinction coefficients and equilibrium constants were used as searching parameters, in order to achieve minimum discrepancy between the experimental data and equations. The thermodynamic parameters (enthalpy, Gibb's free energy and entropy) have been determined for the temperature range of 290-328 ^{o}C using Vant's Hoff's equation. The complexations with sodium hydroxide have been studied by Benesi-Hildebrand method (Benesi and Hildebrand, 1949). The association constant and molar extinction coefficient of the complexes calculated from equation of Benesi-Hildebrand (Benesi and Hildebrand, 1949). The UV-Vis spectra were recorded 1 h after the 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.

RESULTS AND DISCUSSION

Self - association of 5- caffeoylquinic acid

Most organic acids are known to form strongly hydrogen bonded dimer and therefore can be found as a combination of monomer and dimer in liquid forms (Hintzes et al., 2001). The concentration dependent self - association of 5-caffeoylquinic acid also expected to be hydrogen bonded due to interaction of hydroxyl and carboxylic groups of the compound. The graph of molar extinction coefficient vs concentration of the compound at the maximum of wavelength (324 nm) indicates increases
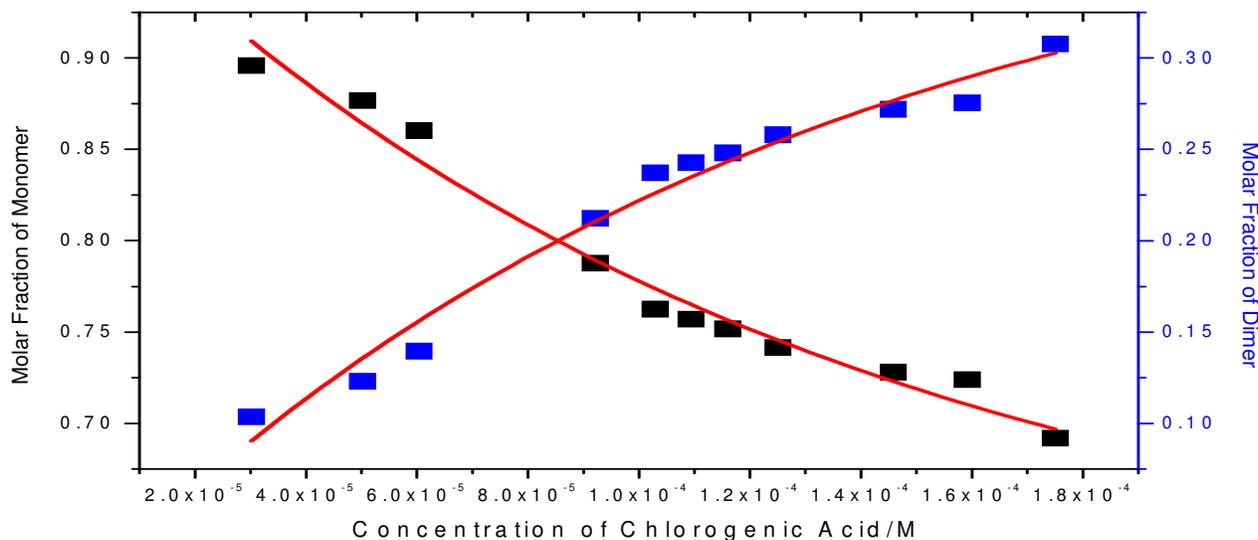
Figure 2. Shows the mole fraction of monomer and dimer versus total concentration of 5- caffeoylquinic acid.

in the concentration range (3.02 - 17.50) x10^{-5} M decreases the extinction coefficient (Figure 1).The deviation of the Beer -Lambert's law and depend on concentration, suggest the existence of self-association process of the molecule. For numerical analysis of the molar extinction coefficients of monomer, dimer and dimerazation constant the known dimer model was derived by considering the following molecular equilibrium in solutions (Bolotin et al., 2006).

$$B_1 + B_1 \xleftrightarrow{\;K_{dB}\;} B_2 \qquad (1)$$

Where, K_{dB} is the equilibrium dimerzation constant, B_1 and B_2 are monomers and dimers of 5-caffeoylquinic acid respectively. The over all concentration of the dissolved molecules in solution is given by the mass conservation law,

$$[B_0] = [B_1] + 2[B_2] \qquad (2)$$

Where, $[B_0]$ is the total concentration of 5-caffeoylquinic acid, $[B_1]$ is the monomer concentration and $[B_2] = K_{dB}[B_1]^2$ is the concentration of the dimers. The contribution of the monomer and dimer to the molar extinction coefficient (ε) of the solution is commonly considered to be additive,

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

Where, ε_m , ε_d are extinction coefficients of 5- caffeoylquinic

acid monomer and dimer respectively, $f_m = [B_1]/[B_0]$ is the equilibrium mole fraction of monomer and $f_d = 2K_{dB}[B_1]^2/[B_0]$ is a mole fraction dimer concentration. The concentration $_{[B_1]}$ can be determined from the solution of the mass conservation law of equation (2). Substituting the solution of equation (2) in to equation (3) the following dimer model was obtained.

$$\varepsilon = \varepsilon_d + (\varepsilon_d - \varepsilon_m) \frac{1 - \sqrt{8[B_0]K_{dB} + 1}}{4[B_0]K_{dB}} \qquad (4)$$

Hence there are three unknown parameters, ε_m , ε_d and K_{dB} in equation (4) which can be obtained from nume-rical analysis of experimental concentration dependence of the molar extinction coefficients of 5-caffeoylquinic acid Figure 1.The values of these three quantities were com-puted by nonlinear regression based on the Lavenberg-Marquardt origin software. They are serving as a search parameters being adjusted in order to achieve the minimum discrepancy between the experimental data and equation (4).

By this method the calculated values of $\varepsilon_m \& \varepsilon_d$, K_{dB} obtained for 5-caffeoylquinic acid at the wavelength 324 nm were 19841.95 $\pm$ 323, 11434.68 $\pm$ 2762 $M^{-1}cm^{-1}$ and 1813.58 $\pm$ 1338 M^{-1} respectively. The calculated dime-rization constant of 5-caffeoylquinic acid is with in the range of previously calculated for organic acid which are known to form strong hydrogen bond. The dimerization constant calculated by Hintzes et al., (2001), for acetic acid and propionic acid at different temperatures are with
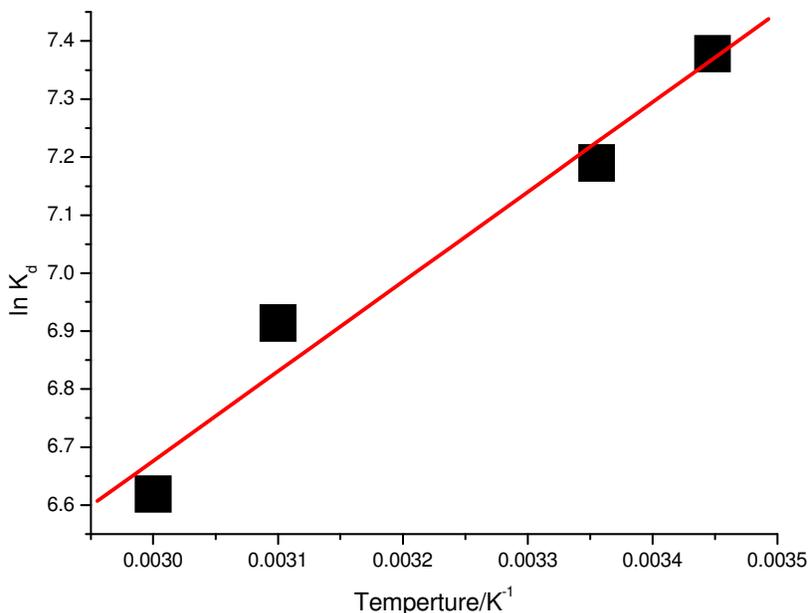
Figure 3. Shows the $\ln k_d$ vs 1/T of caffeoylquinic acid.

in the range of (0.84 to 4.67)x10^3 and (0.46 to 2.63)x10^3 M^{-1} respectively. Similarly the dimerzation constants for acetic acid reported by (Barrow and Yerger, 1954) using FTTR were (1.00 - 2.60) x10^3 M^{-1} for different concentrations. The profile of monomer and dimer concentrations under peak was also determined as a function of total 5-caffeoylquinic acid concentration in the range of (3.02-17.50) x10^{-5} M. Figure 2 shows the mole fraction of monomer and dimer molecules versus concentration of 5-caffeoylquinic acid at the peak of 324 nm. In the above mentioned concentration regions, the formations of dimer molecules are favored by high concentration, in which the mole fraction of monomer and dimer molecules are decrease and increase respectively as it is expected.

Thermodynamic properties of the self-associated 5-caffeoylquinic acid

Heating the aqueous solution of 5-caffeoylquinic acid shows the absorption spectra are strongly depend on the temperature in the range of (290-328 K). As temperature increases, the absorption intensity increases which indicate a dissociation of the molecular associated forms in the solutions. Figure 3 shows the graph of ln K_{dB} versus f (1/T) of 5-caffeoylquinic acid, which is linear indicates the enthalpy, entropy and Gibb's energy of caffeic acid dimerzation are independent of the temperature in the studied range. The magnitude of the enthalpy was estimated from the slope of the approximating line

according to Van't Hoff's equation,

$$\frac{d \ln(K_{dB})}{d\left(\frac{1}{T}\right)} = -\frac{\Delta H}{R} \tag{5}$$

Where, ΔH is the molar enthalpy change, R= 8.31 J mol^{-1} K^{-1}, the universal gas constant and temperature in Kelvin. The entropy was derived from Gibb's free energy and enthalpy. The Gibb free energy and entropy can be expressed as follows;

$$\Delta G = -RT \ln K_{dB} \tag{6}$$

The entropy becomes

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

The calculated values of Gibb's free energy, enthalpy and entropy of 5-caffeoylquinic acid self - association were $\Delta G = -17.90$ kJ mol^{-1}, $\Delta H = -12.89$ kJ mol^{-1} and $\Delta S = 17.00$ J K^{-1}mol^{-1}, respectively. These values are in good agreement with the results recently reported by, (Dearden and Bresnen, 2005) for benzoic acid which is $\Delta G = -18.10$ kJ mol^{-1}, $\Delta H = -12.50$ kJ mol^{-1} and $\Delta S = 18.90$ J K^{-1}mol^{-1}. Moreover, Irwin et al., (1999) also determined thermodynamic parameters of Beta-Cyclodextrin
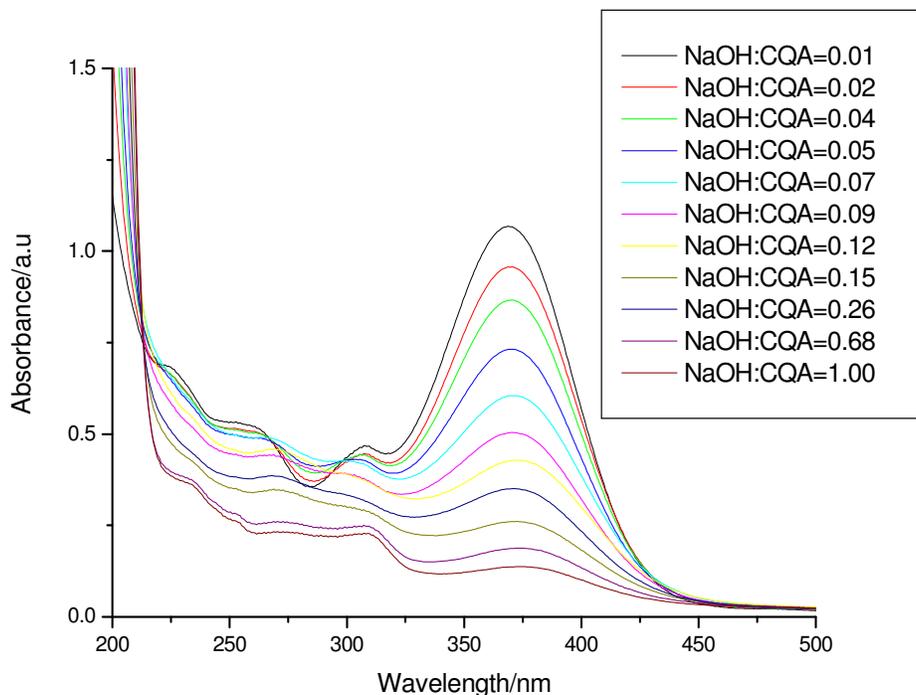
Figure 4. The UV-Vis absorption spectra of 5- caffeoylquinic acid in distilled Water in the presence of NaOH solution for the molar ratio of NaOH: 5- caffeoylquinic acid (CQA) in the range of 0.01 to 1.00.

complexes with chlorogenic acids and the values are similar with $\Delta G = -14.80 \text{ kJ mol}^{-1}$ and $\Delta H = -12.70 \text{ kJ mol}^{-1}$ respectively. From calculated thermodynamic parameters the self-association reaction of 5-caffeoylquinic acid is exothermic reaction result in higher entropy ($\Delta S > 0$) and decrease in enthalpy ($\Delta H < 0$)

Complexation 5-caffeoylquinic acid with Sodium hydroxide

Figure 4 shows the effects of NaOH concentration on the UV-Vis absorption spectra of 5-caffeyloquinic acid solutions. The electronic spectrum of free 5-caffeoylquinic acid in distilled water has two maximum points in the wavelength regions of 200 - 500 nm. The first maximum being at 217 nm with shoulder at 240 nm and the second peak was at 324 nm with shoulder at 296 nm (Belay and Gholap, 2009). The addition of anhydrous of sodium hydroxide to 5-caffeoylquinic acid solutions results in important spectral modification with band shift to higher wavelength (370 nm). Generally there is decrease in relative absorbance as the concentration of NaOH solutions increased. The graph of peak absorbance versus molar ratio of (NaOH: 5-Caffeoylquinic acid) at 370 nm decrease exponentially as the ratio of (NaOH: 5-Caffeoylquinic acid) increases. Moreover, the existences of isobestic points were observed in the absorption spectra,

which indicate the formation of complexes between sodium ions and 5-caffeoylquinic acid.

The analysis of the association of sodium hydroxide with 5-caffeoylquinic acid was accomplished by Benesi-Hildebrand equation, under the condition of [NaOH] >> [5-Caffeoylquinic acid]. The equilibrium constant for the complex formation and molar extinction coefficient calculated at 370 nm were 13529 M^{-1} and 413 $\text{M}^{-1}\text{cm}^{-1}$. The equilibrium constant calculated in this study is in a good agreement with the one reported by (Cornard et al., 2008) for the complexation of this compound with Pb (II).

CONCLUSION

The self - association of 5-caffeoylquinic acid and its complexation with sodium ions have been investigated by UV-Vis spectroscopy. Understanding the mechanism of self - association of 5-caffeoylquinic acid is useful in order to design the advanced and controllable carriers of drugs and food components. On the other hand the results obtained from complexation of the compound with sodium ions are great applications in extraction alkali metals ions selectively from natural environment.

REFERENCES

Barrow GM, Yerger EA (1954). Dimerization equilibrium of acetic acid in

carbon tetrachloride. J. Am. Chem. Soc. 76: 5248-5253.

Belay A, Gholap AV (2009). Characterization and determination of chlorogenic acid (CGA) in coffee beans by UV-Vis spectroscopy. Afr. J. Pure Appl. Chem. 3: 234-240.

Benesi HA, Hildebrand JH (1949). Spectrophotometric investigation of iodine with aromatic hydrocarbons. J. Am. Chem. Soc. 71: 2703-2707.

Bolotin PA, Baranovsky SF, Evstigneev MP (2006). Spectrophotometer investigation of the hetero-association of caffeine and thiazine dye in aqueous solution. Spectrochimica Acta part A 64: 693-697.

Cornard JP, Lapouge C, Dangleterre L, All-Bdelot C (2008). Complexation of lead (II) by chlorogenic acid: Experimental and theoretical study. J. Phys. Chem. A 112: 12475-12484.

D'Amelio N, Fontanive L, Uggeri F, Suggi-Liverani F, Navarini L (2009). NMR Reinvestigation of the caffeine-chlorogenate complex in aqueous solution and in coffee brews. Food Biophysics DOI 10.1007/s11483-009-9130-y.

Dearden JC, Bresnen GM (2005). Thermodynamic of water-octane and water cyclohexane partitioning of some aromatic compounds. Int. J. Mol. Sci. 6: 119-129.

Dearden JG (1963). Investigation of the self-association of phenols and anilines Ultraviolet spectroscopy. Canadian J. Chem. 41: 2683-2691.

Dillard CJ, German JB (2000). Photochemical: nutraceutical and human health. J. Agric. Food Chem. 80: 1744-1756.

Gornas P, Neunert G, Baczynski K, Polewski K (2009). Beta-Cyclodextrin with chlorogenic and caffeic acids from coffee brew: spectroscopic, thermodynamic and molecular modeling study. Food Chem. 114: 190-196.

Hintzes PE, Alisio S, Vaida V (2001). Electronic spectroscopy of organic dimer. Chem. Phys. Lett. 343: 159-165.

Hynes MJ, Ocoineanainn M (2004). The kinetic and mechanism of reaction of iron (II) with caffeic acid, chlorogenic acid, sinapic acid, ferulic acid and naringin. J. Inorganic Biochem. 98: 1457-1464.

Irwin P, Brouillette J, Giampa A, Hicks K, Gehring A, Tu S (1999). Cyclomaltoheptaose (Beta-cyclodextrin) inclusion complex formation with chlorogenic acid:hydration enthalpy, the solvent entropy effect and enthalpy entropy compension. Carbohydrate Res. 322: 67-76.

Kono Y, Kobayashi K, Tagawa S, Adachi K, Ueda A, Sawa Y, Shibate H (1997). Antioxidant activity of polyphenolics in diet, rate constant reaction of chlorogenic acid and caffeic acid with reactive species of oxygen and nitrogen. BioChim. BioPhys. Acta. 1335: 335-342.

Marme N, Habl G, Knemeyer JP (2005). Aggregation behaviour of the red-absorbing oxazine derivatives MR 121: a new method for determination of pure dimer spectra. Chem. Phys. Lett. 408: 221-225.

Prior RL (2003). Fruits and vegetables in the preventation cellular oxidative damage. Am. J. Clin. Nutr. 78: 5705-5785.

Rice-Evans CA, Miller NJ, Paganga G (1996). Structure antioxidant activity relationship of flavonoids and phenolic acids. Free Radical Biol. Med. 20: 933-956.

Robinso JWE, Chew SA (1999). Irreversible inhibition of human immune deficiency virus type-1 integrase by dicaffeoylquinic acids. J. Virol. 73: 3309-3316.

Scalbert A, Johnson IT, Saltmarsh M (2005). Polyphenols: antioxidants and beyond. Am. J. Clin. Nutr. 81: 2155-2175.

Sondheiemer E, Covitz F, Marquisee D (1961). Association of naturally occurring compounds the chlorogenic acid, caffeine complex. Arch. Biophy. Biochem. 93: 63-71.

Suryaprakash P, Prakash V (1995). Interaction of 3-o-caffeoyl D-quinic acid with multi sub unit protein helianthin. J. Bio. Sci. 20: 531-549.