Interaction of gallic acid with trypsin analyzed by spectroscopy

Hao Song a, Chaoyin Chen a,*, Shenglan Zhao b,**, Feng Ge a, Diqiu Liu a, Dandan Shi a, Tiancai Zhang a

a Faculty of Life Science, Kunming University of Science and Technology, Kunming 650500, People’s Republic of China
b Faculty of Traditional Chinese Medicine, Yunnan University of Traditional Chinese Medicine, Kunming 650200, People’s Republic of China

1. Introduction

In recent years, there has been much attention to the interaction of small molecules with proteinases [1–3], which is related to understanding the medicine, chemistry, food science, toxicology, and biology [4]. Trypsin (EC 3.4.21.4) is an important protease in the human digestive system and plays an important role in many physiological processes [5]. Therefore, studies on the interactions of trypsin with small molecules have great significance in understanding the inhibition mechanisms of the small molecules [6]. The structure of trypsin is shown in Fig. 1A. Fruits, health drinks, and functional beverages are the main sources of the small molecules entering the human body, which influence the trypsin activity in vivo [7,8]. Several reports about interactions between trypsin and several proteinase inhibitors have been published, such as cinnamic acid [9], methotrexate [10], caffeine, and theophylline [11]. However, there was no report on the interactions between polyphenol and trypsin.
Gallic acid (3,4,5-trihydroxybenzoic acid, GA) is one of the natural occurring polyphenolic compounds in food, biological, pharmaceutical, chemical, and other fields [12]. The structure of the GA is shown in Fig. 1B. Reports confirmed the restoration of lysosomal damage, myocardial damage, and lipid peroxidation induced by isoproterenol during GA treatment [13]. GA shows cytotoxicity against cancer cells without damaging normal cells [14]. GA can be used to treat diabetes and albuminuria, and also exhibits a variety of pharmacologic properties such as antioxidant, antitumor, antiinflammatory, antibacterial, and antiviral activities [15–22]. However, there has not been enough attention paid to the interaction of GA with trypsin by spectroscopy. The use of fluorescence quenching is simple and sensitive. Therefore, it is an important method to study the interaction between small molecules and proteinases [23–25]. Fluorescence spectroscopy can not only explain the result of the experiment, but also obtain dynamic information and special structure [26,27]. In this paper, the effects of GA on the structure and activity of trypsin and the interaction mechanism between GA and trypsin were studied by UV-vis absorption spectroscopy, fluorescence spectroscopy, synchronous fluorescence spectroscopy and resonance light scattering (RLS). The data provided information on the influence of the binding constant, binding distance, binding force, and thermodynamic aspects in the binding process.

2. Materials and methods

2.1. Apparatus

All the fluorescence spectra were recorded on an LS-50 fluorescence spectrophotometer (Perkinelmer Corp., London, UK) equipped with 3 cm quartz cell. UV-vis absorption spectra were measured at room temperature with a Ultrospec 2100 pro spectrophotometer (General Electric Company, London, UK) equipped with 3 cm quartz cell. The widths of the excitation and the emission slits of trypsin were 5.0 nm and 5.0 nm, respectively.

2.2. Reagents

The trypsin (Sigma Aldrich, New York, USA) was obtained from the USA and the GA (Guizhou Dida Biological Technology Co., Ltd, Guiyang, China) was purchased from Guizhou Dida Biological Technology Co., Ltd. The trypsin solution (4 × 10^{-5} mol/L) was prepared in pH 7.40 phosphate buffer. GA solution was prepared in deionized water. Other reagents were analytical grade and deionized water was used throughout. All solution was stored at 4°C in a refrigerator.

2.3. Fluorescence measurements

The trypsin (2 mL, 4 × 10^{-5} mol/L) solution and 2 mL different concentration of GA were added into a 10 mL volumetric flask, and phosphate buffer was added to dilute the mixture to the scale mark. The fluorescence intensities of all solutions were measured (excitation at 280 nm and emission wavelengths of 300–500 nm) with a LS-50 fluorescence spectrophotometer at three temperatures (25°C, 31°C, 37°C). Synchronous fluorescence spectra were recorded with λex = 280 nm. The D-value (ΔD) was 15 nm and 60 nm, respectively.

2.4. UV-vis absorbance measurement

The UV-vis absorbance spectra of GA and trypsin were recorded at 298 K. Trypsin of fixed concentration in the absence and presence of GA was added to the test tube.

2.5. Study of trypsin activity

The catalysis activity of trypsin was determined by the modified Lowry assay. Specific methods of operation were 0.5% casein, GA of different concentrations, and 4 × 10^{-5} mol/L trypsin solution preheated for 5 minutes. GA (2 mL) and casein (2 mL) solutions were added to 2 mL trypsin solution. The reaction kept for 10 minutes at 40°C (accurate to seconds). An amount of 4 mL 0.4 mol/L Trichlooacetic acid was added to the mixture to end the reaction. They were then kept for 20 minutes at 40°C. The precipitate was removed by filtration. Amounts of 1 mL supernatant, 5 mL 0.4 mol/L Na2CO3, and 1 mL Folin-phenol reagent were mixed and colored for 20 minutes at 40°C. The absorbance of the mixture was measured at a wavelength of 680 nm. No GA-mixed trypsin was taken as the blank group. Trichloroacetic acid solution was added to the trypsin solution to make the trypsin inactive. Then, the casein solution was added to the mixture as the blank group.
control group. Each experiment group had a control group. The inhibition rate was calculated as follows:

\[
\text{Inhibition rate (\%)} = \frac{A - a}{A} \times 100
\]  

(1)

where A and a were the absorbance of the blank group and the experimental group, respectively.

2.6. Molecular modeling study

The 3D structure of trypsin (PDB ID: 4IBG) was downloaded from Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). AutoDock Vina 1.1.2 was used for all docking calculations. The AutoDockTools 1.5.6 package (http://mglttools.scripps.edu) was employed to generate the docking input files. The search grid of trypsin was identified as center_x: -1.793, center_y: -14.925, and center_z: -16.941 with dimensions size_x: 16, size_y: 16, and size_z: 16. The value of exhaustiveness was set to 20. For Vina docking, the default parameters were used if it was not mentioned. The best-scoring pose as judged by the Vina docking score was chosen and visually analyzed using PyMOL (http://www.pymol.org/).

3. Results and discussion

3.1. UV-vis absorption spectra

UV-vis absorption is a simple and effective method to research the change of structure and the formation of a complex [28]. The change of microenvironment around the chromophore in trypsin can result in the change of absorption spectra about protein. So, the UV-vis absorption spectra of protein can be used for studying the changes of protein structure in solution. The absorption spectral changes of trypsin in the presence and absence of drugs are shown in Fig. 2. There was an absorption peak at 290 nm, which was related to the aromatic amino acid absorption. With the addition of GA, the peaks of trypsin increased and a red shift was observed. The results indicated the formation of a new complex between GA and trypsin. Fig. 2 shows different spectra between trypsin, GA, and GA-trypsin. If no interaction happened between trypsin and GA, the spectra of trypsin and (gallic acid + trypsin)-gallic acid should be the same. The results illustrated that there was a change in the conformation of trypsin and the microenvironment around the tryptophan residues when GA was mixed. GA influenced the functional groups and exposed the chromophore in the trypsin molecules to the surfaces of molecules.

3.2. Fluorescence quenching of trypsin

The method of fluorescence can reveal some information about binding between small molecules and proteins [29]. Due to the fluorescent properties of tryptophan and tyrosine in trypsin, tryptophan, and tyrosine can be regarded as probes to detect the conformation change of trypsin. Tryptophan and tyrosine residues are excited when the excitation wavelength is at about 280 nm. The fluorescence intensity and position are related to the microenvironment of these residues [30].

Fluorescence quenching is a phenomenon of interaction between fluorescent substances and other molecules [31]. The mechanisms of fluorescence quenching include dynamic quenching and static quenching [32].

The influence of GA on fluorescence intensity of trypsin was investigated. The GA is nonfluorescent. The trypsin fluorescence peak was at about 350 nm. An obvious decrease of trypsin fluorescence intensity was observed when GA was added into the solution as shown in Fig. 3A, B, and C. The quenching efficiencies were 80.75%, 80.04%, and 79.48%, respectively. The data of fluorescence were analyzed by the Stern-Volmer equation:

\[
\frac{F_0}{F} = 1 + K_{sv}[Q] = 1 + K_{sv}[Q]
\]

(2)

where \(F_0\) and \(F\) are the trypsin fluorescence intensities before and after GA is added into solution, respectively, \(K_{sv}\) is the quenching rate constant of trypsin [33], \(r_0\) is the fluorescent life of trypsin in the presence of quencher and taken as \(10^{-8}\) [34], \(K_{sv}\) is the Stern-Volmer quenching constant, and \([Q]\) is the concentration of GA. \(K_{sv}\) and \(K_{sv}\) can be determined by mapping for \(F_0/F\) and concentration of GA.

Fig. 4 shows the Stern-Volmer plots of the fluorescence quenching of trypsin by GA. Good linearity appeared in the curves. Linear Stern-Volmer plots may reveal the occurrence of a single type of quenching [35]. For dynamic quenching, the maximum scatter collision quenching rate constant of various quenchers is \(2.0 \times 10^{10} \text{L/mol/s} \) [36]. In this paper, \(K_{sv}\) and \(K_{sv}\) values at different temperatures are shown in Table 1. In this table, \(K_{sv}\) was negatively correlated with temperature, which revealed that the quenching mechanism between trypsin and GA was due to the formation of a GA-trypsin complex. In addition, all the values of \(K_{sv}\) are greater than the maximum collisional quenching constant of various quenchers for biological macromolecules (\(2.0 \times 10^{10} \text{L/mol/s}\)). Therefore, these results indicated that the quenching mechanism of trypsin with GA was initiated by the formation of a GA-trypsin complex.

3.3. Binding constants

For the static quenching interaction, the binding constant (\(K_b\)) between trypsin and GA can be obtained from the Lineweaver-Burk equation [37]:

![Fig. 2 – The UV absorption spectra of gallic acid (GA) bound to trypsin at pH 7.4. \(C_{GA} = 1 \times 10^{-5} \text{mol/L}\), \(C_{trypsin} = 1.5 \times 10^{-5} \text{mol/L}\).]
where $K_A$ is the binding constant, which can be calculated from the slope and intercept of the curve from $1/F_0 - F$ vs. $1/|Q|$, as shown in Fig. 5. The $K_A$ values at different temperatures are shown in Table 2. The binding constant decreased with the increasing temperature, which indicated the formation of a low stability complex.

### 3.4. Thermodynamic parameters and binding forces

The interaction forces between biomolecules and small molecules include hydrogen bonds, electrostatic interactions, van der Waals interactions, hydrophobic forces, etc. [38]. Thermodynamic parameters are major evidence for certifying the binding force. If the effect of temperature is small in the reaction, the enthalpy change ($\Delta H$) can be regarded as a constant. The values of enthalpy change ($\Delta H$), entropy change ($\Delta S$) and the free-energy change ($\Delta G$) can be calculated using the Van't Hoff and thermodynamic equations [39]:

$$
\ln K_A = \frac{-\Delta H}{RT} + \frac{\Delta S}{R}
$$

$$
\Delta G = \Delta H - T\Delta S
$$

$K_A$ is the Lineweaver-Burk binding constant at different temperatures. $R$ is the gas constant ($R = 8.314$ J/mol/K). $\Delta H$ and $\Delta S$ can be calculated by the linear relationship between $\ln K_A$ and $1/T$ based on Equation (4). The free energy change ($\Delta G$) can be obtained from Equation (5). The Van’t Hoff curve is shown in Fig. 6. The fitting equation is $\ln K_A = 7.1977 + 795/T$ ($R^2 = 0.999$). The values of $\Delta H$ and $\Delta S$ could be calculated from the intercept and slope of the curve, respectively. The values of $\Delta H$, $\Delta S$, and $\Delta G$ for GA binding to trypsin are presented in Table 3.

There are several noncovalent interaction forces between small molecules and protein macromolecules according to Ross and Subramanian [40], such as hydrogen bonds, van der Waals forces, hydrophobic, and electrostatic interactions [41]. $\Delta G < 0$ means that the binding of GA and trypsin is a process of spontaneous exothermic. $\Delta H < 0$ and $\Delta S > 0$ show that hydrophobic interactions are involved in the binding process. Electrostatic interactions are characterized by $\Delta H \approx 0$ and
In addition, \( \Delta S > 0 \). In addition, \( \Delta H < 0 \) could also indicate van der Waals interactions. As shown in Table 3, it could be inferred that hydrophobic and electrostatic interactions were drawn into the binding process. In most cases, the reaction of small molecules and the proteins is the synergetic effect of a variety of forces. Three hydroxyl groups in GA molecules also indicate that hydrogen bonds may participate in the reaction. Therefore, it could be concluded that hydrogen bonds, van der Waals, hydrophobic, and electrostatic interactions were involved in the binding process of GA and trypsin.

### 3.5. Energy transfer between trypsin and GA

Fluorescence resonance energy transfer is an important technology, which has wide applications for interaction analysis between biological macromolecules and small molecules, cellular physiology, and immunity analysis [42]. According to Förster’s theory [43], the nonradiative energy transfer may occur if the compound met the following conditions: (1) the donor emits fluorescence; (2) there is enough overlap between the fluorescence emission spectrum of the donor and absorption spectrum of the receptor; and (3) the maximum distance between the donor and the receptor is \(< 7 \text{ nm} \). The binding distance between the emission fluorescence groups can be determined between protein molecules and small molecules by the Förster theory.

The overlap between UV-vis absorption spectrum of GA and the fluorescence emission spectrum of trypsin is shown in Fig. 7. The efficiency of energy transfer \( E \) can be described by the Förster nonradiative energy transfer theory in the following equation:

\[
E = \frac{R_0^6}{R_0^6 + r^6} = 1 - \frac{F}{F_0}
\]

where \( R_0 \) is the Förster critical distance when the transfer efficiency is 50%, and \( r \) is the distance between the donor and the receptor. \( F \) and \( F_0 \) are the fluorescence intensities of trypsin in the presence and absence of GA, respectively:

\[
R_0^6 = 8.8 \times 10^{-25} K^2 N^{-4} \Phi
\]

In Equation (7), \( K^2 \) is the spatial orientation factor of the dipole related to the random distribution of the donor and the receptor. \( N \) is the refractive index of the medium. \( \Phi \) is the fluorescence quantum yield of the donor and \( J \) is the overlap integral between the fluorescence emission spectra of the donor and the absorption spectra of the receptor, which can be calculated by the equation:

\[
J = \frac{\sum F(\lambda) \epsilon(\lambda) \lambda^4 d\lambda}{\sum F(\lambda) d\lambda}
\]

### Table 1 – Stern-Volmer quenching constants for the gallic acid (GA)-trypsin system at different temperatures.

| T (°C) | Equation | \( R^2 \) | \( K_{sv} \) (10⁵ L/mol) | \( K_q \) (10¹³ L/mol/s) |
|--------|----------|------------|--------------------------|--------------------------|
| Trypsin + GA | 25°C | \( F_0/F = 1.0165 + 0.2008 \times 10^5 [Q] \) | 0.9906 | 2.008 | 0.0030 + 0.0158 \times 10^5 [Q] | 0.9906 |
| | 31°C | \( F_0/F = 1.0413 + 0.1603 \times 10^5 [Q] \) | 0.9927 | 1.603 | 0.0035 + 0.0202 \times 10^5 [Q] | 0.9927 |
| | 37°C | \( F_0/F = 1.0743 + 0.1269 \times 10^5 [Q] \) | 0.9976 | 0.1269 | 0.0045 + 0.0245 \times 10^5 [Q] | 0.9976 |

### Table 2 – The binding constants between gallic acid (GA) and trypsin by Lineweaver-Burk equation.

| T (°C) | Lineweaver-Burk equation | \( K_A \) (10⁴ L/mol) | \( R^2 \) |
|--------|--------------------------|------------------------|------------|
| GA + trypsin | 25°C | \( Y = 0.0003 + 0.003 \times 10^5 [Q] \) | 1.9371 | 0.9969 |
| | 31°C | \( Y = 0.0035 + 0.020 \times 10^5 [Q] \) | 1.8192 | 0.9949 |
| | 37°C | \( Y = 0.0045 + 0.024 \times 10^5 [Q] \) | 1.7465 | 0.9970 |
where $F(\lambda)$ is the donor fluorescence intensity at the wavelength of $\lambda$, and $r$ is the molar absorption coefficient of the receptor at the wavelength of $\lambda$.

In the above equations, $K^2 = 2/3$, $N = 1.336$, and $\Phi = 0.118$. According to Equations (6–8), $I$, $R_0$, $E$ and $r$ were calculated and are shown in Table 4. The binding distance was $<7$ nm and $0.5 < r < 2.0$ $R_0$. The results showed that the nonradiative energy transfer occurred between GA and trypsin.

### 3.6. Characteristics of the RLS spectra

RLS is a new analysis to determine the scattering signal by the fluorescence spectrophotometer. The RLS analysis is simple, rapid, and highly sensitive. Therefore, it becomes more popular in life sciences, environmental science, nanomaterial analysis, and testing [44,45]. The RLS spectra of trypsin, GA, and GA-trypsin complex synchronously scanned from 200 nm to 600 nm with $\Delta \lambda = 0$ nm are recorded. The results are shown in Fig. 8. An obviously increased RLS was found when a little GA was added to trypsin solution. It was guessed from the results that trypsin might interact with GA in solution, forming a new GA-trypsin complex that could be expected to be an aggregate [46]. The size of GA-trypsin particles may be larger than those of trypsin, and the increased light scattering signal appeared under the given conditions.

### 3.7. Conformation investigation

Synchronous fluorescence spectroscopy is a common method to provide information about the conformational changes near fluorophore functional groups [47]. Synchronous fluorescence spectroscopy is a useful method and tool to investigate the environment of amino acid residues and the influence of drug on the protein conformation. When the $\Delta \lambda$ values between excitation wavelength and emission wavelength are set at 15 nm or 60 nm, the characteristic information of tryptophan and tyrosine residues are given by the synchronous fluorescence.

The influence of GA on trypsin synchronous fluorescence spectra is shown in Fig. 9. The synchronous fluorescence spectra gave the characteristic information of tyrosine (Fig. 9A) and tryptophan (Fig. 9B), respectively. The red shift indicated that the tryptophan and tyrosine residues in the nonpolar environment moved to a more polar environment. Intensities decreased of tyrosine and tryptophan residues and the red shift of peaks were observed when GA was gradually added into the trypsin solution. It can be concluded that GA may bind to trypsin. Tryptophan and tyrosine residues are sensitive to the microenvironment and show the change of trypsin tertiary structure by the synchronous spectra shift.

### 3.8. Effect on trypsin activity

In order to investigate the influence on the enzymatic activity of trypsin, the inhibition effects of GA on the activity of trypsin are shown in Fig. 10. With casein as a substrate, the absorbance of trypsin in the presence of GA was determined. $IC_{50}$ can be calculated from the fitting equation:

$$Y = -0.0155x^3 + 0.4925x^2 - 2.0931x + 7.1922$$

(9)

where $x$ is the concentration of GA, and $Y$ is the inhibition rate. $IC_{50}$ was $3.9 \times 10^{-6}$ mol/L obtained from Equation (9). The results indicated that the inhibition rate of GA to trypsin was increased by the addition of GA, which was in accordance with previous reports.
In order to confirm the inhibition mode, the Lineweaver-Burk plots of trypsin in the absence and presence of GA were drawn (Fig. 11). When GA was added, $K_m$ was 0.283 mmol/L, while without GA, $K_m$ was 0.211 mmol/L. From the above enzymatic activity experiment data, the results indicated that GA could interact with trypsin by the formation of a trypsin-GA complex, resulting in inhibition of trypsin activity.

3.9. **Computational modeling of the trypsin-GA**

The molecular modeling method was originally performed in drug design and widely used to provide visible binding information between small molecules and proteins in recent years [48]. As shown in Fig. 12, GA fitted into the binding pocket of trypsin and produced a high density of van der Waals contacts with the residues. Importantly, one of the hydroxyl groups forms a hydrogen bond with residue Gln192, and the other two hydroxyl groups form hydrogen bonds with residue Ser195. Additionally, two hydrogen bonds were observed between the carboxy group and side chain of residues Ser190 and Gly219. From Fig. 12B, we could see that there were several tryptophan and tyrosine residues around GA and their microenvironment may be changed because of the addition of exogenous substance. All in all, the above molecular simulation gives us a rational explanation for the excellent activity of GA against trypsin, which provided valuable information for further study of trypsin inhibitors.

4. **Conclusion**

This paper shows spectroscopic studies on the interactions between trypsin and GA. The fluorescence spectroscopy results revealed that GA could bind to trypsin by a static quenching to form a new compound. Hydrogen bonds, van der Waals, hydrophobic, and electrostatic interactions were involved in the formation process of the GA-trypsin complex.
The structural change of trypsin was shown by using UV-vis absorption spectra, synchronous fluorescence spectra, and RLS spectroscopy, when GA was added to the trypsin solution. The tryptophan and tyrosine residue microenvironments were changed by GA. The results indicated that GA was a strong quencher and inhibitor. The molecular docking results agreed well with the experiment data and offered more specific evidence for explaining the interaction mechanism. Finally, these results provided a new method to probe the inhibition effect of digestive enzymes induced by other small molecules and further advanced the interaction mechanism between the small molecules and proteinases.

Conflicts of interest

All authors declare no conflicts of interest.

Acknowledgments

We gratefully acknowledge financial support from the Fund for Science and Technology Project of Yunnan Province (2009E8081, 2011AB006) and Supporting Plan Issue of the Ministry of Science and Technology (2011BAD46B00).

REFERENCES

[1] Rufino FPS, Pedroso V, Araujo JN, Franca AF, Rabelo LM, Migliolo I, Kiyota S, Santos EA, Franco OL, Oliveira AS. Inhibitory effects of a Kunitz-type inhibitor from Pithecellobium dumosum (Benth) seeds against insect-pests’ digestive enzymes. Plant Physiol Biochem 2013;63:70–6.

[2] Rawdkuen S, Benjakul S, Visessanguan W, Lanier TC. Cysteine protease inhibitor from chicken plasma: fractionation, characterization and autolysis inhibition of fish myofibrillar proteins. Food Chem 2007;101:1647–57.

[3] Maltas E. Binding interactions of niclosamide with serum proteins. J Food Drug Anal 2014;22:549–55.

[4] Goncalves R, Mateus N, Planet J, Laguerre M, de Freitas V. Mechanisms of tannin-induced trypsin inhibition: a molecular approach. Langmuir 2011;27:13122–9.

[5] Chen JM, Montier T, Feree C. Molecular pathology and evolutionary and physiological implications of pancreatitis-associated cationic trypsinogen mutations. Hum Genet 2001;109:245–52.

[6] Silva Jr FP, De-Simone SG. S1 subsite in snake venom thrombin-like enzymes: can S1 subsite lipophilicity be used to sort binding affinities of trypsin-like enzymes to small-molecule inhibitors. Bioorg Med Chem 2004;12:2571–87.

[7] Zuo Y, Chen H, Deng Y. Simultaneous determination of catechins, caffeic and gallic acids in green, Oolong, black and pu-erh teas using HPLC with a photodiode array detector. Talanta 2002;57:307–16.

[8] Mammela P, Savolainen H, Lindroos L, Kangas J, Vartianen T. Analysis of oak tannins by liquid chromatography-electrospray ionisation mass spectrometry. J Chromatogr A 2000;891:75–83.

[9] Zhang H, Zhou Q, Cao J, Wang Y. Mechanism of cinnamic acid-induced trypsin inhibition: a multi-technique approach. Spectrochim Acta Part A 2013;116:251–7.

[10] Wang Y, Zhang H, Cao J, Zhou Q. J Mol Struct 2013:1051–78.

[11] Wang R, Kang X, Wang R, Wang R, Dou H, Wu J, Song C, Chang J. Comparative study of the binding of trypsin to caffeine and theophylline by spectrofluorimetry. J Luminescence 2013;138:258–66.

[12] Mansouri MT, Naghizadeh B, Ghorbanzadeh B, Farbood Y, Sarkaki A, Bavarasad K. Gallic acid prevents memory deficits and oxidative stress induced by intracerebroventricular injection of streptozotocin in rats. Pharmacol Biochem Behav 2013;111:90–6.

[13] Do QD, Angkawaiya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Imsadji S, Ju YH. Effect of extraction solvent on total phenol content, total flavonoids content, and antioxidant activity of Limnophila aromatica. J Food Drug Anal 2013;22:296–302.

[14] Priscilla DH, Prince P. Cardioprotective effect of gallic acid on cardiac troponin-T, cardiac marker enzymes, lipid peroxidation products and antioxidants in experimentally
induced myocardial infarction in Wistar rats. Chem Biol Interact 2009;179:118–24.

[15] de Mejia EG, Chandra S, Ramirez-Mares MV, Wang W. Catalytic inhibition of human DNA topoisomerase by phenolic compounds in Ardisia compressa extracts and their effect on human colon cancer cells. Food Chem Toxicol 2006;44:1191–203.

[16] Luo JH, Li BL, Li NB, Luo HQ. Sensitive detection of gallic acid based on polyethyleneimine-functionalized graphene modified glassy carbon electrode. Sens Actuators B 2013;186:84–9.

[17] Wang X, Wang J, Yang N. Flow injection chemiluminescent detection of gallic acid in olive fruits. Food Chem 2007;105:340–5.

[18] Chuyisinuan P, Chimnoi N, Techasakul S, Supaphol P. Gallic acid-loaded electrosput poly (L-lactic acid) fiber mats and their release characteristic. Macromol Chem Phys 2009;210:814–22.

[19] Neo YP, Ray S, Jin J, Gizdavic-Nikolaidis M, Nieuwoudt MK, Locatelli C, Filippin-Monteiro FB, Creczynski-Pasa TB. Alkyl modification of natural biopolymer by electrospinning technique: a physicochemical study based on zein–gallic acid system. Food Chem 2013;146:1013–21.

[20] Lu Z, Nie G, Belton PS, Tang H, Zhao B. Structure–activity relationship analysis of antioxidant ability and neuroprotective effect of gallic acid derivatives. Neurochem Int 2006;48:263–74.

[21] Locatelli C, Filippin-Monteiro FB, Creczynski-Pasa TB. Alkyl esters of gallic acid as anticancer agents: A review. Eur J Med Chem 2013;60:233–9.

[22] Punithavathi VR, Prince PSM, Kumar R, Selvakumari J. Catalytic inhibition of human DNA topoisomerase by electrospun poly (L-lactic acid) fiber mats and their release characteristic. Macromol Chem Phys 2009;210:814–22.

[23] Neo YP, Ray S, Jin J, Gizdavic-Nikolaidis M, Nieuwoudt MK, Locatelli C, Filippin-Monteiro FB, Creczynski-Pasa TB. Alkyl modification of natural biopolymer by electrospinning technique: a physicochemical study based on zein–gallic acid system. Food Chem 2013;146:1013–21.

[24] Lu Z, Nie G, Belton PS, Tang H, Zhao B. Structure–activity relationship analysis of antioxidant ability and neuroprotective effect of gallic acid derivatives. Neurochem Int 2006;48:263–74.

[25] Liu D, Quek SY. Encapsulation of food grade antioxidant in polyethyleneimine-functionalized graphene modified glassy carbon electrode. Sens Actuators B 2013;186:84–9.

[26] Ohtaki H, Radnai T. Structure and dynamics of hydrated serum albumins. Spectrochim Acta Part A 2005;61:629–36.

[27] Molina-García L, Santos JLM, Ruiz-Medina A, Llorente-Martinez EJ. Determination of ketoprofen based on its quenching effect in the fluorescence of quantum dots. J Food Drug Anal 2013;21:426–31.

[28] Bi S, Song D, Tian Y, Zou X, Liu Z, Zhang H. Molecular spectroscopic study on the interaction of tetracyclines with serum albumins. Spectrochim Acta Part A 2005;61:629–36.

[29] Ni Y, Su S, Kokot S. Spectrometric studies on the interaction of fluoroquinolones and bovine serum albumin. Spectrochimica Acta Part A 2010;75:547–52.

[30] Zhang HM, Wang YQ, Zhou QH. Investigation of the interactions of quercetin and morin with trypsin. Luminescence 2009;24:355–62.

[31] Szmacinski H, Ray K, Lakowicz JR. Metal-enhanced fluorescence of tryptophan residues in proteins: application toward label-free bioassays. Anal Biochem 2009;385:358–64.

[32] Fraiji LK, Hayes DM, Werner T. Static and dynamic fluorescence quenching experiments for the physical chemistry laboratory. J Chem Educa 1992;69:424.

[33] Gerbanowski A, Malabat C, Rabiller C, Guéguen J. Grating of aliphatic and aromatic probes on rapeseed 2S and 12S proteins: influence on their structural and physicochemical properties. J Agric Food Chem 1999;47:5218–26.

[34] Hegde AH, Sandhya B, Seetharamappa J. Evaluation of binding and thermodynamic characteristics of interactions between a citrus flavonoid hesperitin with protein and effects of metal ions on binding. Mol Biol Rep 2011;38:4921–9.

[35] Li Y, He WY, Liu H, Yao X, Hu Z. Daidzein interaction with human serum albumin studied using optical spectroscopy and molecular modeling methods. J Mol Struct 2007;831:144–50.

[36] Naveenraj S, Raj MR, Anandan S. Binding interaction between serum albumins and perylene-3, 4, 9, 10-tetracarboxylate–A spectroscopic investigation. Dyes Pigm 2012;94:330–7.

[37] Cui F, Zhang Q, Yan Y, Yao X, Qu G, Lu Y. Study of characterization and application on the binding between 5-iodouridine with HSA by spectroscopic and modeling. Carbohydr Polym 2008;73:464–72.

[38] Liang J, Cheng Y, Han H. Study on the interaction between bovine serum albumin and CdTe quantum dots with spectroscopic techniques. J Mol Struct 2008;892:116–20.

[39] Zhao H, Ge M, Zhang Z, Wang W, Wu G. Spectroscopic studies on the interaction between riboflavin and albumins. Spectrochim Acta Part A 2006;65:811–7.

[40] Ross PD, Subramanian S. Thermodynamics of protein association reactions: forces contributing to stability. Biochem 1981;20:3096–102.

[41] Reidenberg MM, Affrime M. Influence of disease on binding of drugs to plasma proteins. Ann N Y Acad Sci 1973;226:115–26.

[42] Haldar B, Mallick A, Chattopadhyay N. Photophysics of pyrene-end-capped poly (ethyleneoxide) in aqueous micellar environments. J Mol Liq 2004;115:113–20.

[43] Förster T. Zwischenmolekulare energiewanderung und fluoreszenz. Annalen der physik 1948;437:55–75 [in German].

[44] Zhi J. Determination of aniline and related mono-aromatic amines in indoor air in selected Canadian residences by a modified thermal desorption GC/MS method. Environ Int 2004;30:135–43.

[45] Collings PJ, Gibbs EJ, Sturr TE, Vafek O, Yee C, Pomerance LA, Pasternack RF. Resonance light scattering and its application in determining the size, shape, and aggregation number for supramolecular assemblies of chromophores. J Phys Chem B 1999;103:8474–81.

[46] Xiao J, Shi J, Cao H, Wu S, Ren F, Xu M. Analysis of binding interaction between puercarin and bovine serum albumin by multi-spectroscopic method. J Pharm Biomed Anal 2007;45:609–15.

[47] Hu YJ, Liu Y, Pi ZB, Qu SS. Interaction of cromolyn sodium with human serum albumin: a fluorescence quenching study. Bioorg Med Chem 2005;13:6609–14.

[48] Lien GS, Chen CS, Chen WY, Huang SH, Cheng KT, Lin CM, Shao SH. Photoproductions of indomethacin exhibit decreased hydroxy radical scavenging and xanthine oxidase inhibition activities. J Food Drug Anal 2013;21:332–6.