Biomacromol. J., Vol. 4, No. 1, 28-34, July 2018.

Biomacromolecular Journal
www.bmmj.org

Comparing the Interactions and Structural Changes in Milk Carrier Protein of β-Lactoglobulin upon Binding of 5-Fluorouracil and Oxali-palladium

A. Leilabadi-Asl[a], A. Divsalar[b,*], A.A. Saboury[c] and K. Parivar[a]

[a]*Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran*

[b]*Department of Cell & Molecular Biology, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran*

[c]*Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran*

(Received 10 November 2018, Accepted 9 December 2018)

ABSTRACT

β-lactoglobulin (β-LG) is the major protein in the whey of ruminant milk and it can be used as a carrier for anticancer drugs. In this study, the comparison interaction of two chemotherapeutic anti-cancer drugs of 5-Fluorouracil and oxali-palladium with milk carrier protein of β-LG was investigated using fluorescence spectroscopy. The analysis of fluorescence spectra showed that the addition of the 5-Fluorouracil and oxali-palladium to β-LG solution led to a significant reduction in the intrinsic fluorescence spectra of protein and then quenched it. The binding sites and thermodynamic parameters of 5-Fluorouracil and oxali-palladium on the protein were calculated by analyzing of van't Hoff equation at different temperatures. Binding results have represented that there are 2 and 1binding sites on β-LG for binding of 5-Fluorouracil and oxali-palladium at physiological temperature, respectively. According to the results, van der Waals and hydrogen bonding have the main role of the interactions of β-LG with oxali-palladium, while electrostatic interactions have a major role in the interaction of β-LG with 5-Fluorouracil. Finally, regard to the above results, it can be concluded that the anticancer drugs of 5-Fluorouracil and oxali-palladium can bind to the carrier protein of β-LG and changed the structure of it differently.

Keywords: β-lactoglobulin, 5-Fluorouracil, Oxali-palladium, Fluorescence

INTRODUCTION

Protein-drug interaction plays a main role in pharmaceutics of the chemotropic drugs. β-Lactoglobulin (β-LG) is a main whey protein of ruminants and is a small globular protein, with 162 amino acid residues and a molecular weight of 18.3 kDa [1]. β-LG interactions were investigated by amphiphilic and hydrophobic ligands [2]. Combine of anticancer drugs with β-LG protein can be advantageous at the molecular level for the identification of mechanism of action of β-LG and determination of it binding methods and binding sites [3].

Platinum-based chemotherapy drugs widely have used in the treatment of a variety of tumors during the last three decades but the primitive generations had serious toxicities [4]. Therefore, attempts were carried out for developing new platinum-based anticancer drugs with improved activity and reduced toxicity. Oxali-palladium was the new generation of platinum-based drugs with clinical effectiveness and less side effects [5].

5-Fluorouracil (5-FU) introduced as an anti-cancer drug in 1957. Today, 5-FU recognized useful in the cancer chemotherapy, especially with tumors of the head, breast and neck [6-8]. This drug can be injected intravenously and it is also used orally. Previous reports have shown that 5-FU has induced serious side effects including cardio toxicity, chest pain, cognitive impairment and low blood counts [9]. 5-FU widely was used in the treatment of cancer but it had toxicity side effects [10]. The last three decades, platinum-based chemotherapeutic drugs were used in the treatment of a variety of tumors [5]. Oxali-palladium is an anticancer complex based on palladium and is the analogue of oxaliplatin [11]. Oxali-palladium as a new synthetic anticancer drug like a 5-FU have the most serious side effects that they can be reduced by the use of targeted drug delivery systems [12].

In the present study, the interaction between both chemotherapeutic drugs of 5-FU and oxali-palladium

*Corresponding authors. E-mail: divsalar@khu.ac.ir

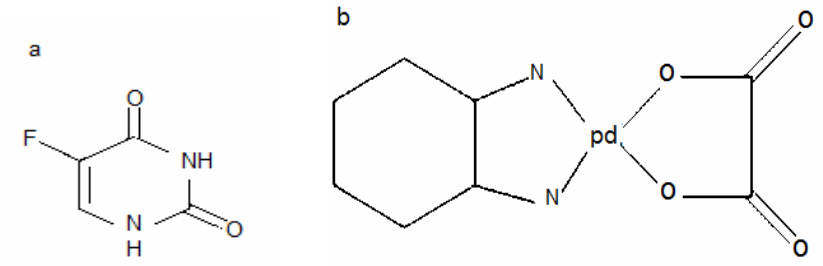

Fig. 1. The molecular structure of a) 5-Fluorouracil and b) Oxali-palladium.

(Fig. 1) with β-LG was studied and compared together by using fluorescence spectroscopy. Also the binding site, the type of interaction force and the binding constants were investigated.

MATERIALS AND METHODS

Materials

β-LG and 5-FU with high purity (>99%) were purchased from Sigma. Oxali-palladium was synthesized according to the previous method [2]. In this study, a NaCl solution, 5 mM, adjusted to pH 7 was used as a solvent.

Fluorescence Studies

Fluorescence studies were conducted by a Hitachi spectrofluorometer model MPF-4 at an excitation wavelength of 295 nm to measure the Trp fluorescence intensity of β-LG (10 μM). The emission spectra of samples in the absence and presence of various concentrations of 5-FU were recorded in the 300-500 nm range at ambient and physiological temperatures (25 and 37 °C). Also, alterations in the emission spectra of protein in the absence and presence of various concentrations of oxali-palladium were recorded in the 300-500 nm range at the same condition to 5-FU. The cuvette width that used for the fluorescence was 1 cm. The excitation and emission slit widths were set at 5 and 10 nm, respectively.

RESULTS AND DISCUSSION

Tryptophan chromophore has the sensitivity to the changes in polarity of its microenvironments. Hence, the intrinsic fluorescence of tryptophan residues is used for the intensity of emission [13]. β-LG in solution was excited at 295 nm (Trp excitation wavelength) and the emission spectra were collected in the wavelength range of 300-500 nm (emission maximum 335.5 nm) at physiological temperature in the absence and presence of different concentrations of 5-FU and oxali-palladium, respectively (Figs. 2a-b). As it is shown in Figs. 2a-b, by adding the both drugs separately into β-LG solution, the intrinsic fluorescence intensity of protein was reduced and quenched the protein intensity regularly.

Maximum fluorescence quenching intensity of β-LG was recorded in versus of different concentrations of 5-FU and oxali-palladium at physiological temperatures (Fig. 2c) that was indicated reduction of the intrinsic fluorescence of β-LG protein. The intrinsic intensity of fluorescence decreased, therefore quenching was occurred using quenchers in the interaction of ligand with protein. Interaction of ligands with a protein in different mechanisms *via* intrinsic fluorophores was caused quenching, which are commonly called dynamic and static quenching [12,15]. Quenching mechanism is explained by the Stern-Volmer equation [16] (Eq. (1)):

$$\frac{F_0}{F} = 1 + K_{SV}[Q] = 1 + k_q \tau_0 [Q] \quad (1)$$

Where F_0 and F are the β-LG fluorescence intensities in the absence and presence of quenchers (5-FU and oxali-palladium), respectively; $[Q]$ is the total concentration of the quenchers and K_{SV} is the Stern-Volmer quenching constant, which is explained by the following equation [17] (Eq. (2)):

$$K_{SV} = k_q \tau_0 \quad (2)$$

Where k_q is the fluorescence quenching rate constant and τ_0

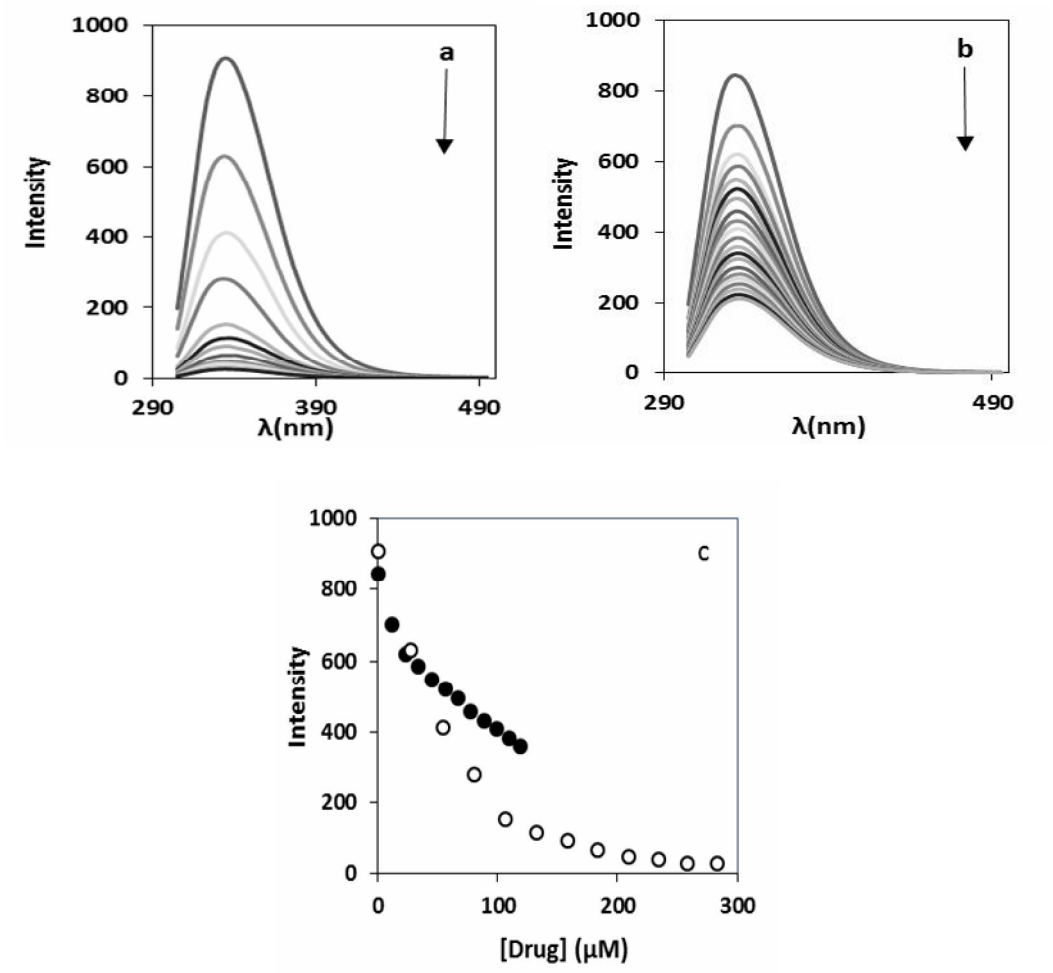

Fig. 2. β-LG fluorescence quenching spectra (10 μM) in the absence and presence of various concentrations of 5-FU (a) and oxali-palladium (b) at physiological temperature. Maximum fluorescence changes of β-LG in the absence and presence of different concentrations of 5-FU (white circle) and oxali-palladium (black circle) at physiological temperatures (c).

is the fluorescence average lifetime of the fluorophore. τ_0 was reported for Trp residues of β-LG at neutral pH as 1.28 ns [18]. The values of k_q for oxali-palladium -β-LG and for 5-FU with β-LG were calculated 3.98 ns^{-1} M^{-1} at 25 °C to 18.59 ns^{-1} M^{-1} at 37 °C and 3.9 ns^{-1} M^{-1} at 25 °C to 8.04 ns^{-1} M^{-1} at 37 °C, respectively.

The Stern Volmer quenching constant was calculated from Fig. 3b results. The results of Fig. 3a showed that the Stern-Volmer plot is non-linear. For more details studied about static and dynamic quenching mechanism, the modified Stern-Volmer equation was used [19]. (Eq. (3)):

$$\frac{F_0}{\Delta F} = \frac{F_0}{F_0 - F} = \frac{1}{faKsv}\frac{1}{[Q]} + \frac{1}{fa} \quad (3)$$

Where f_a is the fraction of the initial fluorescence that for the quencher is achievable. The plot of $F_0/(F_0 - F)$ values *versus* the invers of quencher concentration (1/[Q]) is linear. The values of f_a and K_{sv} were found from the values of intercept and slope, respectively [19,20]. The values of f_a for β-LG in the presence of 5-FU and oxali-palladium at physiological temperature were obtained 1.4 and 0.75, respectively.

Determination of quenching mechanism can be obtained *via* quenching constant of Stern-Volmer curve of β-LG using fluorescence quenching data. When the Stern-Volmer quenching constant *(K_{SV})* between ligand and protein increased with increasing temperature, the quenching mechanism is a type of dynamic mechanism and if K_{SV} decreased with increasing temperature is a type of static mechanism [10,14]. In this study, analysis the values of K_{SV} of the both of the drugs of oxali-palladium (5.1 M^{-1} at 25 °C to 23.8 M^{-1} at 37 °C) and 5-FU (5 M^{-1} at 25 °C to 10.3 M^{-1} at 37 °C) with β-LG increased with increasing the temperature (from 25 to 37 °C). Then, the quenching mechanism showed that dynamic mechanism has a main role in fluorescence quenching of β-LG in the presence of 5-FU and oxali-palladium [21].

Binding Study

To obtain the binding and thermodynamic parameters of the interaction of 5-FU and oxali-palladium with β-LG at physiologic temperature, the plot of log*(F0 - F)/F vs.* log*[Q]* according to the following equation (Eq. (4)) was applied [22]:

$$\log\left[\frac{F_0 - F}{F}\right] = \log K_a + n \log[Q]$$

Where n is the number of sites and K_a is the binding constant [22]. The interaction of 5-FU and oxali-palladium with β-LG was observed one binding site to oxali-palladium and two binding site to 5-FU at physiological temperature. The binding parameters were obtained for the interaction of 5-FU and oxali-palladium with β-LG at physiological temperature respectively that showed in Fig. 4 and Table 1. Beta-lactoglobulin, is one of the most studied ligand-binding food proteins. β-LG had two potential binding sites for lipophilic nutrients. Also, it was indicated that retinol compete for the same site on β-LG [24] and α-tocopherol is the most abundant and biologically active form of E-group hydrophobic vitamins. It has two binding sites on β-LG, that secondary binding site is dependent on the α-tocopherol concentration [23]. In this study, the obtained results have been indicated that oxali-palladium anticancer drug has one binding site on β-LG and 5-FU anticancer drug has two binding site on β-LG. Also, in previous study illustrated that the number of binding site on the βLG protein did not change in competitive studies, therefore the data was indicated the presence of independent binding sites for anticancer drugs of oxali-palladium and 5-FU on the carrier protein of βLG. Previous studies have suggested that presence of multiple binding sites and ligand-dependent binding properties on β-LG can prepare the chance of binding of different ligands simultaneously to this protein [23].

Also, according analysis of binding curves, the binding constants *(K_a)* values were calculated 28 × 10^2 M^{-1} for binding of oxali-palladium and 15 × 10^7 M^{-1} for binding of 5-FU on β-LG.

Thermodynamic Parameters

Due to the dependence of binding constant (K_a) to temperature, the interactions were surveyed through thermodynamic process. Thermodynamic parameters that are temperature dependent, such as enthalpy change *($\Delta H°$)*, entropy change *($\Delta S°$)*, and Gibbs free energy change *($\Delta G°$)* were used in the interaction of β-LG with anticancer drugs of 5-FU and oxali-palladium.[14]. $\Delta G°$ parameter reflects the possibility of reaction, and $\Delta H°$ and $\Delta S°$ are the major evidences to describe the interaction forces. For ligand binding to proteins, there are four important types of interaction forces such as: hydrogen bonds, van der Waals forces, electrostatic, and hydrophobic interactions [2]. According previous reports, if $\Delta H° > 0$, $\Delta S° > 0$, the main force is hydrophobic interaction; if $\Delta H° < 0$, $\Delta S° < 0$, the main forces are van der Waals and hydrogen bond interactions, if $\Delta H° < 0$, $\Delta S° > 0$, the major force is electrostatic effect [25].

The thermodynamic parameters can be estimated from the van't Hoff equation (Eq. (5)) [26]:

$$\ln K_a = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R}$$

Where T is the temperature, and R is the gas constant. Also, the Gibbs free energy change *($\Delta G°$)* is described from the following equation [17] (Eq. (6)):

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ = -RT\ln K_a$$

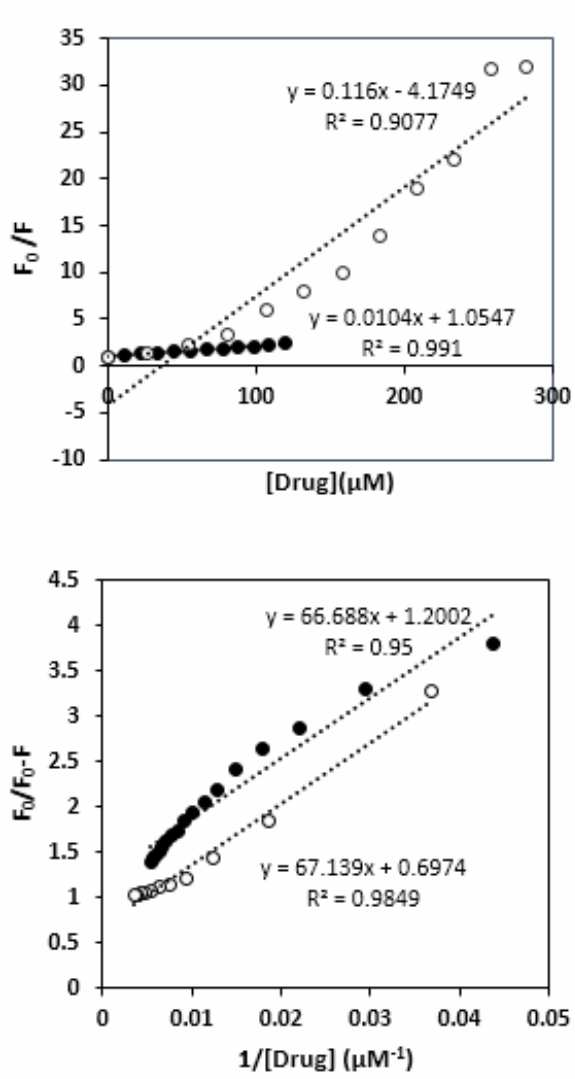

Fig. 3. (a) The Stern-Volmer plot of β-LG interaction with 5-FU (white circle) and oxali-palladium (black circle). (b) The Modified Stern-Volmer plot of β-LG interaction with 5-FU (white circle) and oxali-palladium (black circle) at physiological temperature.

The results were showed that the values of $\Delta H°$ and $\Delta S°$ for 5-FU are -12.5 kJ mol^{-1} and +0.11 J mol^{-1} K^{-1}, and for oxali-palladium are -166.4 kJ mol^{-1} and -0.48 J mol^{-1} at physiological temperature (37 °C). Also, the values of $\Delta G°$ were calculated -48 and -20 kJ mol^{-1}, at physiological temperature (37 °C), respectively.

According to description of the above, the force binding

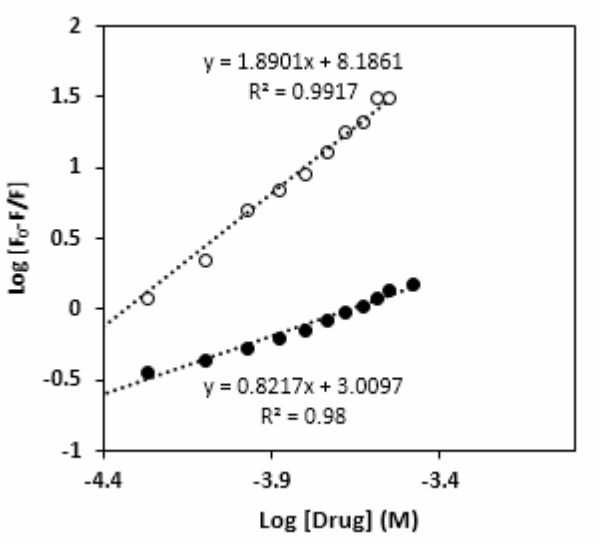

Fig. 4. Calculation of the binding constant *(K)* of each binding site and the number of binding sites (*n*) for 5-FU (white circle) and oxali-palladium (black circle) on β-LG at physiological temperature.

of 5-FU interaction with β-LG is a type of electrostatic interactions while Van der Waals and hydrogen bond interactions have the main role in interaction of oxali-palladium with β-LG (Table 1).

CONCLUSIONS

5-Fluorouracil and oxali-palladium are widely recognized today as effective for cancer chemotherapy in combinational therapy of cancer [27]. Also, according to previous study, 5-FU and oxali-palladium have shown hopeful antitumor activity [4,12]. So, in the present study, fluorescence spectroscopy applied to investigation and comparison of the interactions of milk carrier protein of β-LG with chemotherapeutic drugs of oxali-palladium and 5-FU at physiologic temperature. Results have shown that Van der Waals and hydrogen bond have the main role in interaction of oxali-palladium with β-LG. Whereas, 5-FU, as a hydrophilic drug, can bind on the protein via electrostatic interactions. Also, the number of binding sites on β-LG for binding of oxali-palladium (one binding site) and 5-FU (two binding sites) are different. The obtained

Table 1. Comparison of the Binding Parameters of 5-FU and Oxali-palladium Interactions with β-LG at 37 °C

Component	$K_{SV} \times 10^3$ (M^{-1})	n	K_a (M^{-1})	ΔG^o (kJ mol^{-1})	ΔH^o (kJ mol^{-1})	ΔS^o (J mol^{-1} K^{-1})
Oxali-palladium	23.8	0.88	2.8×10^2	-20	-166.4	-0.48
5-FU	10.3	1.89	15×10^7	-48	-12.5	+0.11

data suggested that the β-LG-5FU and β-LG-oxali-palladium have different interactions and β-LG is a suitable carrier for each of two drugs with different interactions and they can be used in drug design and drug delivery systems.

ACKNOWLEDGEMENTS

The financial support of the Research Council of the Kharazmi University is highly appreciated.

REFERENCES

[1] G. Kontopidis, C. Holt, L. Sawyer, Dairy. Sci. 87 (2004) 785.

[2] B. Ghalandari, A. Divsalar, A.A. Saboury, Spectrochim. Part A: Mol. Biomol. Spectrosc. 118 (2014) 1038.

[3] M. Eslami Moghadam, A. Divsalar, A. Abolhosseini Shahrnoy, A.A. Saboury, Biomol. Structure. Dyn. (2015) 1.

[4] E.Z. Jahromi, A. Divsalar, A.A. Saboury, S. Khaleghizadeh, H. Mansouri-Torshizi, I. Kostova, Iran. Chem. Soc. 13 (2016) 967.

[5] A. Divsalar, M. Razmi, A.A. Saboury, H. Mansouri-Torshizi, F. Ahmad, Cell. Biochem. Biophys. 71 (2015) 1415.

[6] O. Abazari, Z. Shafaei, A. Divsalar, M. Eslami-Moghadam, B. Ghalandari, A.A. Saboury, Biomol. Structure. Dyn. 34 (2016) 1123.

[7] S. Bakkialakshmi, D. Chandrakala, Int. J. Pharm. Pharm. Sci. 4 (2012) 46.

[8] D.B. Longley, D.P. Harkin, P.G. Johnston, Nature. Rev. Cancer. 3 (2003) 330.

[9] H. Kouchakzadeh, S. Hoseini Makarem, S. Shojaosadati, Nanomedicine 3 (2016) 35.

[10] S. Bakkialakshmi, D. Chandrakala, J. Pharm. Sci. Res. 3 (2011) 1326.

[11] S. Shahraki, H. Mansouri-Torshizi, M. Sadeghi, A. Divsalar, A.-A. Saboury, Biol. Macromol. 1 (2015) 242.

[12] A. Leilabadi-Asl, A. Divsalar, A.A. Saboury, K. Parivar, Int. J. Biol. Macromol. (2018).

[13] B. Ghalandari, A. Divsalar, M. Eslami-Moghadam, A.A. Saboury, T. Haertlé, M. Amanlou, Appl. Biochem. Biotechnol. 175 (2015) 974.

[14] K. Abbasi-Tajarag, A. Divsalar, A. Saboury, B. Ghalandari, H. Ghourchian, Biomol. Structure. Dyn. (2016) 1.

[15] A. Saboury, F. Karbassi, Thermochimica Acta 362 (2000) 121.

[16] M.-F. Zhang, Z.-Q. Xu, Y.-S. Ge, F.-L. Jiang, Y. Liu, Photo. Chem. Photo. Biol. 108 (2012) 34.

[17] S. Tabassum, W.M. Al-Asbahy, M. Afzal, F. Arjmand, Photochem. Photo. Biol. 114 (2012) 132.

[18] A. Divsalar, S. Khodabakhshian, Mol. Liquids 206 (2015) 82.

[19] J. Essemine, I. Hasni, R. Carpentier, T. Thomas, H. Tajmir-Riahi, Int. Biol. Macromol. 49 (2011) 201.

[20] M. Eslami Moghadam, M. Saidifar, A. Divsalar, H. Mansouri-Torshizi, A.A. Saboury, H. Farhangian, Biomol. Structure. Dyn. 34 (2016) 206.

[21] J. Ding, L. Yuan, L. Gao, J. Chen, Luminescence 132 (2012) 1987.

[22] R. Rahimi-Vaghar, A. Divsalar, H. Mansouri-Torshizi, Phys. Chem. Res. 1 (2013) 185.

[23] J. Zhang, X. Liu, M. Subirade, P. Zhou, L. Liang, Food Chem. 165 (2014) 256.

[24] L. Liang, V. Tremblay-Hébert, M. Subirade, Food Chem. 126 (2011) 821.

[25] L. Yang, D. Huo, C. Hou, M. Yang, H. Fa, X. Luo,

Spectrochim. Part A: Mol. Biomol. Spectrosc. 78 (2011) 1349.

[26] Y. Sun, S. Wei, C. Yin, L. Liu, C. Hu, Y. Zhao, Bioorganic. Medicinal. Chem. 21 (2011) 3798.

[27] M.-L. Pascu, B.D. Carstocea, A. Staicu, M.A. Ionita, S. Truica, R. Pascu, Laser Florence. Laser Med. World (2001) 52.