



Original article

Influence of Zn(II) Ion on the Autoxidation of Pyrogallol and Gallic Acid in Weakly Acidic Aqueous Solutions

Aleksandar M. Veselinović, Goran M. Nikolić

University of Niš, Faculty of Medicine, Department of Chemistry, Niš, Serbia

SUMMARY

Pyrogallol-type phenolic compounds are widespread in nature and may have significant impact on human health. As Zn(II) ion was proved to be capable of enhancing some biological activities of pyrogallol-type natural phenolic compounds, we decided to study its influence on the autoxidation of pyrogallol and gallic acid in weakly acidic aqueous solutions.

UV-Vis spectrophotometric measurements showed that autoxidation of pyrogallol was initiated by the influence of Zn(II) ions at pH 5.5 and pH 6.5. The differences in UV-Vis spectra of the first autoxidation products resolved by the application of multivariate curve resolution - alternating least squares (MCR-ALS) method indicated that pH change also changed the mechanism of autoxidation process. Formation of stable Zn(II) ion spin stabilized free radical obtained during the autoxidation of pyrogallol at pH 6.5 was confirmed by using electron spin resonance (ESR) spectroscopy and its structure was determined. Both UV-Vis spectrophotometric and ESR spectroscopic measurements did not give any evidence that gallic acid autoxidation was initiated by the influence of Zn(II) ions in weakly acidic aqueous solutions.

The results of this study may be used for explaining possible differences in the Zn(II) ion influence on various biological activities of pyrogallol-type phenolic compounds containing simple pyrogallol moiety and the ones containing gallate moiety as a part of the molecule.

Key words: autoxidation, pyrogallol, zinc, MCR-ALS, ESR

Corresponding author:

Aleksandar M. Veselinović

e-mail: aveselinovic@medfak.ni.ac.rs

INTRODUCTION

Polyphenolic compounds are a class of molecules widespread in nature with significant impact on human health (1). Pyrogallol-type phenolic compounds have three adjacent (vicinal) hydroxyl groups on the same benzene ring which enables their easy oxidation or autoxidation and also allows metal ion chelation, both characteristics contributing significantly to the wide range of biological activities (2).

Zinc is an essential biometal, however, contrasting some other cationic micronutrients (for example Fe(II) and Cu(II) ions) Zn(II) ion does not give any redox reactions under physiological conditions (3). However, its importance for the activity of antioxidant enzymes like superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) has been widely recognized (4). There are also numerous reports about physicochemical interactions of Zn(II) ion with various natural phenolic compounds, mostly flavonoids (5-7), and more important, *in vitro* and animal experiments demonstrated that Zn(II) ion may enhance the biological activities of various phenolic compounds including pyrogallol-type phenolic compounds (8-11). Because literature data about interactions of Zn(II) ion with pyrogallol and pyrogallol-type phenolic compounds are lacking (12), we decided to study the influence of Zn(II) ion on the autoxidation of pyrogallol and gallic acid, a naturally occurring pyrogallol-type simple phenolic acid whose antioxidant properties and health implications are the subjects of very intensive research (13).

MATERIAL AND METHODS

All the chemicals used in this study were of analytical (p.a.) grade. Pyrogallol (1,2,3-trihydroxybenzene, PG) and gallic acid (3,4,5-trihydroxybenzoic acid, GA)

were purchased from Sigma Aldrich (Germany) and were used without additional purification because their purity was proved to be satisfactory by high-performance liquid chromatography (HPLC). Deionized, air saturated water was used and 0.6 mmol dm⁻³ solutions of phenolic compounds were prepared just before the use by dissolving exactly weighted amount of substances. Acetate buffer (0.2 mol dm⁻³, pH 5.5 or 6.5) containing 0.2 mol dm⁻³ Zn(CH₃COO)₂ was used for the study of Zn(II) ion influence on the autoxidation of PG and GA.

The autoxidation processes of phenolic compounds were initiated by mixing PG or GA solution with equal volume of appropriate buffer solution. For recording UV-Vis spectra Evolution 60 UV-Vis spectrophotometer (Thermo Scientific, USA) with 1.0 cm quartz cells was employed. UV-Vis spectra of mixtures were recorded immediately after mixing and after that at regular time intervals. Multivariate curve resolution - alternating least squares (MCR-ALS) method (14, 15) was used for the analysis of spectrophotometric data in the cases where UV-Vis spectra changed with time. Electron spin resonance (ESR) measurements were carried out at room temperature on an X-band Bruker ESR 300E spectrometer (Bruker, Germany) by using quartz flat cell. The ESR spectrometer settings were as follows: microwave frequency - 9.64 GHz, microwave power - 2.0 mW, modulation frequency - 100 kHz, modulation amplitude - 0.1 G (0.01 mT). ESR spectral parameters were obtained by performing computer simulation of ESR spectra with SimFonia software (version 1.25, Bruker, Germany).

RESULTS AND DISCUSSION

Figure 1 shows the UV-Vis spectra recorded during the autoxidation of PG in aqueous solution at pH 5.5 and 6.5 in the presence of Zn(II) ions.

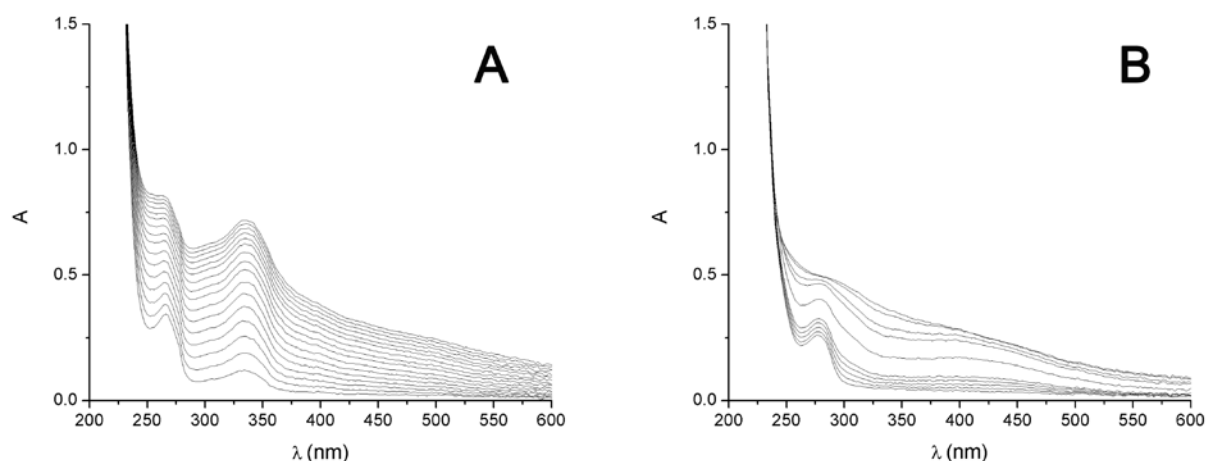


Figure 1: UV-Vis spectra of pyrogallol aqueous solution in the presence of Zn(II) ions ($C(\text{PG}) = 300 \mu\text{mol dm}^{-3}$, $C(\text{Zn(II)}) = 0.1 \text{ mol dm}^{-3}$) at pH 5.5 (A) and pH 6.5 (B). Spectra were recorded at 5-minute time intervals.

At both pH values, a continuous increase of absorbance in the whole wavelength range investigated was observed but no isobestic points appeared. In the UV-Vis spectra recorded during the autoxidation process at pH 5.5, two absorption maxima evolved with time, one at 265 nm and one at 335 nm. On the other hand, at pH 6.5 one clear absorbance maximum at 280 nm and one very broad maximum at around 400 nm appeared. It is necessary to note that significant changes in the UV-Vis spectrum of PG indicating the autoxidation process did not occur at these pH values in the absence of Zn(II) ions for the period of few hours, while autoxidation was observed in pure PG aqueous solutions at pH 7.4 or higher (15). However, the study of Zn(II) ion influence on the PG autoxidation in aqueous solution at pH values higher than 6.5 by UV-Vis spectrophotometry was not possible due to the precipitate formation.

Further analysis of obtained UV-Vis spectral data was performed by using MCR-ALS method. The first step was construction of matrices $D_{\text{PG-Zn-5.5}}$ and $D_{\text{PG-Zn-6.5}}$. The next step was determination of unique values by using singular value decomposition (SVD). Table 1 displays the unique values obtained by SVD for autoxidation processes at both pH values. Data for unique values indicate that the processes follow a simple mechanism with two absorbing species and for this reason matrices $D_{\text{PG-Zn-5.5}}$ and $D_{\text{PG-Zn-6.5}}$ have two significant eigenvalues.

Table 1: Rank analysis of $D_{\text{PG-Zn-5.5}}$ and $D_{\text{PG-Zn-6.5}}$ matrices. Only the first five singular values are shown.

Factors	pH 5.5	pH 6.5
1	32.2334	25.9414
2	5.29112	3.42239
3	0.505954	0.38108
4	0.30671	0.303772
5	0.166221	0.184692

Forward evolving factor analysis (EFA) for all processes indicated that changes in the spectral data from the start of autoxidation can be explained by two factors. Further, the presence of two factors from the start of the reaction may be associated with the presence of two absorbing species in the autoxidation of pyrogallol. The same information is obtained by using backward EFA analysis of matrices $D_{\text{PG-Zn-5.5}}$, $D_{\text{PG-Zn-6.5}}$.

Experimental data were further analyzed by using soft MCR-ALS method with the application of following constraints: non-negativity (concentration and spectra) and uniformity (the concentration profile). The application of MCR-ALS method indicated the existence of two absorbing species in both autoxidation processes. Percentage for the Lack-of-fit (LOF%) for both processes was less than 1%.

Table 2 shows the assessment of the quality of resolution by using the standard deviation of residues vs. experimental data, LOF% and explained variance.

Table 2: The quality of resolution estimated by using the standard deviation of residues compared to experimental data, LOF% and explained variance.

	pH 5.5	pH 6.5
SdRex	0.011476	0.012237
LOF% (PCA)	0.1924	1.2886×10^{-6}
LOF% (exp)	1.9418	2.0434
r^2	99.9623	99.9582

SdRex - standard deviation of residues compared to experimental data

LOF% – lack-of-fit

r^2 – explained variance

Figure 2 shows the MCR-ALS resolved pure spectra of individual absorbing species appearing in solution during the PG autoxidation in the presence of Zn(II) ions and their kinetic profiles.

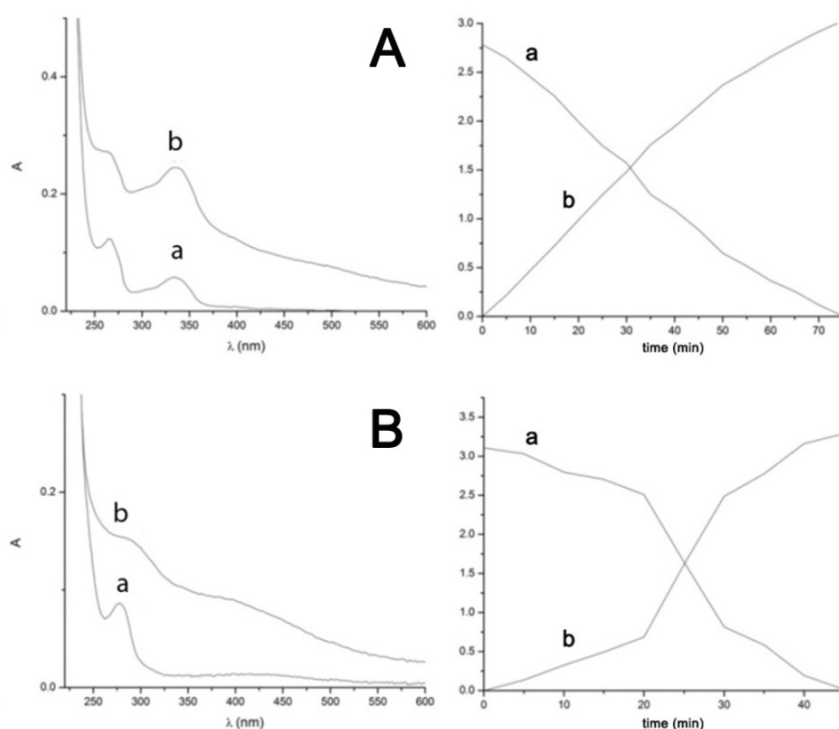


Figure 2: MCR-ALS resolved individual spectra of absorbing species (left side) and their kinetic profiles (right side) for the PG autoxidation in the presence of Zn(II) ions at pH 5.5 (A) and pH 6.5 (B).

Both at pH 5.5 and pH 6.5 two absorbing species in equilibrium were identified in the initial stage of PG autoxidation in the presence of Zn(II) ions. However, dissimilar UV-Vis spectra of the first autoxidation products of PG (compound b) in both parts of Figure 2 at two different pH values indicate different mechanisms of autoxidation processes although in both cases extended π electron delocalization can be assumed. The UV-Vis spectrum of autoxidation product obtained at pH 5.5 with clearly resolved absorption maxima indicates possible formation of quinone, while very broad absorption maxima in the UV-Vis spectrum of autoxidation product obtained at pH 6.5 indicate possible polymer-type product formation because this spectrum is somewhat similar to the UV-Vis spectrum of humic acids (16). Kinetic profiles of absorbing species at two different pH values showed that PG autoxidation in

the presence of Zn(II) ions proceeds at faster rate at higher pH value (6.5 in this case) which is in accordance with the results obtained in the study of PG autoxidation in weakly alkaline aqueous solutions (15).

It is well-known from the literature that Zn(II) ion, as well as many other divalent diamagnetic metal ions, may stabilize the semiquinone-type free radicals obtained during the oxidation or autoxidation of catechol (1,2-dihydroxybenzene) and various catechol-type phenolic compounds (17, 18). Since pyrogallol also contains vicinal hydroxyl groups on the same benzene ring in its structure, the spin stabilization effect of Zn(II) ion may be expected for free radicals formed during the PG autoxidation and thus we performed ESR measurements in our systems. Figure 3 shows the ESR spectrum obtained at the initial stage of PG autoxidation in the presence of Zn(II) ions at pH 6.5.

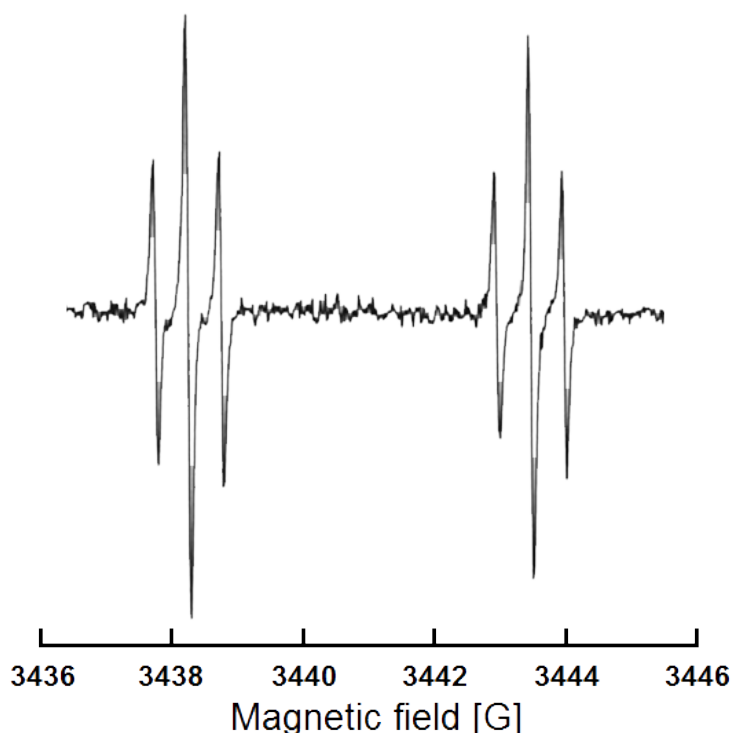


Figure 3: Experimental ESR spectrum of PG in aqueous solution at pH 6.5 in the presence of Zn(II) ions recorded 20 min after mixing PG and Zn-acetate buffer solutions.

Symmetric appearance of the experimental ESR spectrum (two triplets) indicates that only one type of free radical where unpaired electron interacts with two groups of protons (two equivalents and one separate proton) was detected under our

experimental conditions. Hyperfine coupling constant values extracted by the computer simulation of experimental ESR spectrum have values $a(1H) = 5.18$ G and $a(2H) = 0.51$ G. Obtained hyperfine coupling constant values are much smaller

than the corresponding values for the free pyrogallol radical in aqueous solution ($a(1H) = 5.66$ G and $a(2H) G = 0.99$) (19), and that can be explained by the reduced spin density on benzene ring protons due to the interaction of Zn(II) ions with hydroxyl groups. Similar changes of hyperfine coupling constants of

benzene ring protons upon spin stabilization have already been observed for the *ortho*-benzosemiquinone anion radical obtained by the autoxidation of catechol (17). Figure 4 shows the mechanism of formation and the structure of detected free radical.

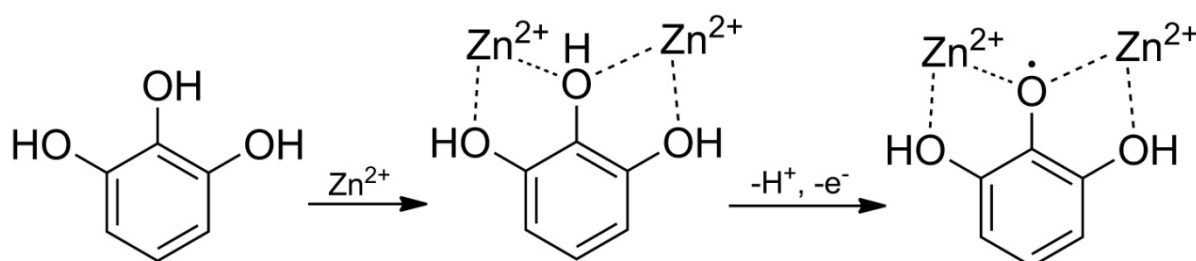


Figure 4: The mechanism of formation and the structure of free radical detected by ESR spectroscopy during the PG autoxidation in aqueous solution at pH 6.5 in the presence of Zn(II) ions.

No other types of free radicals were detected after prolonged (up to two hours) PG autoxidation at pH 6.5 in the presence of Zn(II) ions and only slow ESR signal intensity decrease was observed.

Figure 5 shows the UV spectra recorded during the autoxidation of GA in aqueous solution at pH 5.5 in the presence of Zn(II) ions.

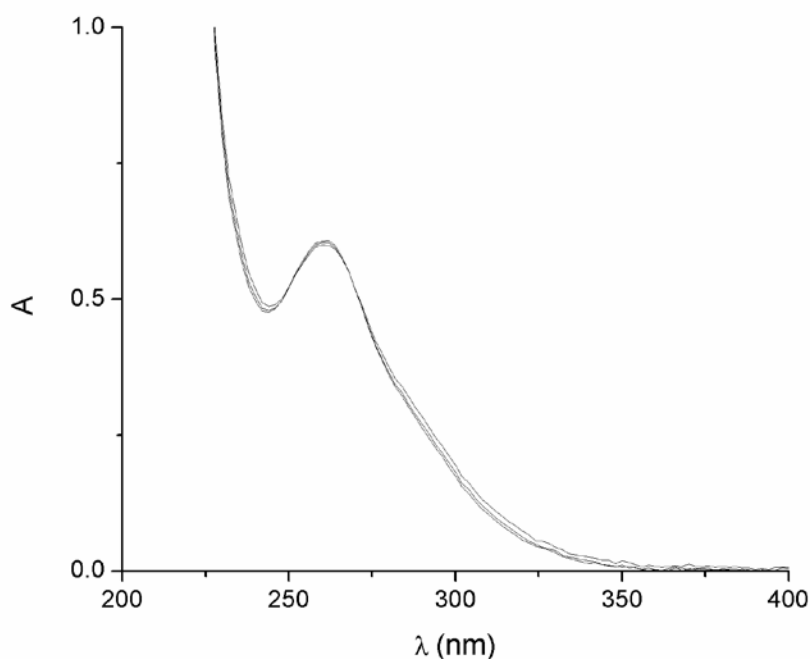


Figure 5: UV spectra of GA aqueous solution at pH 5.5 in the presence of Zn(II) ions ($C(GA) = 300 \mu\text{mol dm}^{-3}$, $C(\text{Zn(II)}) = 0.1 \text{ mol dm}^{-3}$) recorded at 5 hours time intervals for the period of 2 days.

UV-Vis spectra shown in Figure 5 did not give any evidence that GA autoxidation occurred at these particular conditions. Besides the insignificant changes of GA UV spectra in the presence of Zn(II) ions with time, it is also important that these spectra are almost the same as the UV spectrum of free GA in aqueous solution. Such behavior of GA in the presence of Zn(II) ions was very different in comparison to the behavior of GA in the presence of Fe(II) ions in aqueous solution at pH 5.0 where significant spectral changes indicating GA-Fe(II) complexation have been observed (20). The reason for such a different behavior of GA is possibly the very nature of Zn(II) ion which, unlike the Fe(II) ion, is not redox active.

Our earlier studies of GA autoxidation in aqueous solutions indicated that autoxidation was achieved at pH 8.5 and higher (21). However, after mixing GA aqueous solution with Zn(II)-acetate buffer at pH 6.5 precipitate formation quickly occurred and we were not able to use UV-Vis spectrophotometry for obtaining reliable spectral data about this system. ESR measurements indicated that no stable free radical formation occurred under these conditions.

CONCLUSION

UV-Vis spectrophotometry and ESR spectroscopy have been applied for studying interactions of Zn(II)

ion with pyrogallol and gallic acid in weakly acidic aqueous solutions. Autoxidation of pyrogallol was initiated by the influence of Zn(II) ions at pH 5.5 and pH 6.5, but difference in UV-Vis spectra of the first autoxidation products resolved by the application of MCR-ALS method indicated that pH change also changed the mechanism of autoxidation process. ESR spectroscopy revealed the formation of stable spin stabilized radical at pH 6.5. On the other side, both UV-Vis spectrophotometry and ESR spectroscopy did not give any evidence that gallic acid autoxidation was initiated by the influence of Zn(II) ions in weakly acidic aqueous solutions.

The results of this study may be used for explaining possible differences in the Zn(II) ion influence on the various biological activities of pyrogallol-type phenolic compounds containing simple pyrogallol moiety in larger molecule (for example myricetin) and the ones containing gallate moiety as a part of the molecule (for example epigallocatechin gallate).

Acknowledgment

This work was financially supported by the Ministry of Education and Science of the Republic of Serbia under the project no. 172044.

References

1. Scalbert A, Manach C, Morand C, Rémésy C, Jiménez L. Dietary polyphenols and the prevention of diseases. *Crit Rev Food Sci Nutr* 2005; 45(4):287-306.
<http://dx.doi.org/10.1080/1040869059096>
2. Quideau S, Deffieux D, Douat-Casassus C, Pouységou L. Plant polyphenols: Chemical properties, biological activities, and synthesis. *Angew Chem Int Ed* 2011; 50(3): 586-621.
<http://dx.doi.org/10.1002/anie.201000044>
3. Maret W. Zinc biochemistry: from a single zinc enzyme to a key element of life. *Adv Nutr* 2013; 4(1): 82-91.
<http://dx.doi.org/10.3945/an.112.003038>
4. Tang Q, Su S, Fang M. Zinc supplement modulates oxidative stress and antioxidant values in rats with severe acute pancreatitis. *Biol Trace Elem Res* 2014; 159(1-3): 320-4.
<http://dx.doi.org/10.1007/s12011-014-9971-1>
5. Le Nest G, Caille O, Woudstra M, Roche S, Guerlesquin F, Lexa D. Zn-polyphenol chelation: complexes with quercetin, (+)-catechin, and derivatives: I optical and NMR studies. *Inorg Chim Acta* 2004; 357(3): 775-84.
<http://dx.doi.org/10.1016/j.ica.2003.09.014>
6. Lapouge C, Dangleterre L, Cornard J-P. Spectroscopic and theoretical studies of the Zn(II) chelation with hydroxyflavones. *J Phys Chem A* 2006; 110(45): 12494-500.
<http://dx.doi.org/10.1021/jp064362q>

7. Wei Y, Guo M. Zinc-binding sites on selected flavonoids. *Biol Trace Elem Res* 2014; 161(2): 223-30.
<http://dx.doi.org/10.1007/s12011-014-0099-0>
8. McCarthy TJ, Zeelie JJ, Krause DJ. The antimicrobial action of zinc ion/ antioxidant combinations. *J Clin Pharm Ther* 1992; 17(1): 51-4.
<http://dx.doi.org/10.1111/j.1365-2710.1992.tb01265.x>
9. Kagaya N, Kawase M, Maeda H, Tagawa Y, Nagashima H, Ohmori H, Yagi K. Enhancing effect of zinc on hepatoprotectivity of epigallocatechingallate in isolated rat hepatocytes. *Biol Pharm Bull* 2002; 25(9): 1156-60.
<http://dx.doi.org/10.1248/bpb.25.1156>
10. Chen X, Yu H, Shen S, Yin J. Role of Zn²⁺ in epigallocatechingallate affecting the growth of PC-3 cells. *J Trace Elem Med Biol* 2007; 21(2): 125-31
<http://dx.doi.org/10.1016/j.jtemb.2006.12.007>
11. Dabbagh-Bazarbachi H, Clergeaud G, Quesada IM, Ortiz M, O'Sullivan CK, Fernández-Larrea JB. Zinc ionophore activity of quercetin and epigallocatechin-gallate: From Hepa 1-6 cells to a liposome model. *J Agric Food Chem* 2014; 62(32): 8085-93.
<http://dx.doi.org/10.1021/jf5014633>
12. Bors W, Michel C, Stettmaier K. Electron paramagnetic resonance studies of radical species of proanthocyanidins and gallate esters. *Arch Biochem Biophys* 2000; 374(2): 347-55.
<http://dx.doi.org/10.1006/abbi.1999.1606>
13. Thompson MA, Collins PB (eds), *Handbook on gallic acid: Natural occurrences, antioxidant properties and health implications*. Nova Science Publishers Inc., Hauppauge (USA), 2013. ISBN: 978-1-62618-922-5
14. Tauler R. Multivariate curve resolution applied to second order data. *Chemom Intell Lab Sys* 1995; 30(1): 133-46.
[http://dx.doi.org/10.1016/0169-7439\(95\)00047-X](http://dx.doi.org/10.1016/0169-7439(95)00047-X)
15. Veselinović AM, Nikolić RS, Nikolić GM. Application of multivariate curve resolution-alternating least squares (MCR-ALS) for resolving pyrogallol autoxidation in weakly alkaline aqueous solutions. *Cent Eur J Chem* 2012; 10(6): 1942-8.
<http://dx.doi.org/10.2478/s11532-012-0125-z>
16. Veselinović AM, BojićALj, Purenović MM, Nikolić GM, Anđelković TD, Dačić SD, Bojić DV. Investigation of the parameter influences of UV/H₂O₂ process on humic acid degradation. *Hem Ind* 2010; 64(4): 265-73.
<http://dx.doi.org/10.2298/HEMIND100315036V>
17. Eaton DR. Complexing of metal ions with semiquinones. An electron spin resonance study. *Inorg Chem* 1964; 3(9): 1268-71.
<http://dx.doi.org/10.1021/ic50019a016>
18. Lebedev AL, Ivanova MV, Timoshin AA, Ruuge EK. Effect of group II metal cations on catecholate oxidation. *Chem Phys Chem* 2007; 8(12): 1863-9.
<http://dx.doi.org/10.1002/cphc.200700296>
19. Carrington A, Smith ICP. An electron spin resonance study of proton transfer equilibria involving the pyrogallolsemiquinone radical. *Mol Phys* 1964; 8(2): 101-5.
<http://dx.doi.org/10.1080/00268976400100131>
20. Lu L, Li Y, Lu X. Kinetic study of the complexation of gallic acid with Fe(II). *Spectrochim Acta Part A* 2009; 74(3): 829-34.
<http://dx.doi.org/10.1016/j.saa.2009.08.025>
21. Nikolić GM, Veselinović AM, Nikolić RS, Mitić SS. Spectroscopic study of Mg(II) ion influence on the autoxidation of gallic acid in weakly alkaline aqueous solutions. *Russ J Phys Chem A* 2011; 85(13) 2270-3.
<http://dx.doi.org/10.1134/S0036024411130176>

Uticaj Zn(II) jona na autooksidaciju pirogalola i galne kiseline u slabo kiselim vodenim rastvorima

Aleksandar M. Veselinović i Goran M. Nikolić

Univerzitet u Nišu, Medicinski fakultet, Katedra za hemiju, Srbija

SAŽETAK

Fenolna jedinjenja sa strukturom pirogalola su široko rasprostranjena u prirodi i mogu da imaju značajan uticaj na ljudsko zdravlje. Kako je pokazano da Zn(II) jon ima sposobnost da pojačava neke biološke aktivnosti prirodnih fenolnih jedinjenja sa strukturom pirogalola, odlučili smo da ispitamo njegov uticaj na autooksidaciju pirogalola i galne kiseline u slabo kiselim vodenim rastvorima.

UV-Vis spektrofotometrijska merenja su pokazala da prisustvo Zn(II) jona inicira autooksidaciju piroglola na pH 5,5 i pH 6,5. Razlike u UV-Vis spektrima koji su dobijeni hemometrijskom obradom spektrofotometrijskih podataka metodom MCR-ALS za prve proizvode autooksidacije ukazuju da promena pH vrednosti dovodi i do promene mehanizma autooksidacije. Formiranje slobodnog radikala, koji je stabilisan prisustvom Zn(II) jona, potvrđeno je metodom elektronske spinske rezonance (ESR) i određena je njegova struktura. Obe primenjene metode nisu pokazale da prisustvo Zn(II) jona inicira autooksidaciju galne kiseline u slabo kiselim vodenim rastvorima.

Rezultati ovog ispitivanja mogu se koristiti za objašnjenje mogućih razlika u uticaju Zn(II) jona na biološku aktivnost fenolnih jedinjenja pirogalolske strukture koja imaju samo jednostavne strukturne jedinice pirogalola i onih koje u molekulu sadrže ostatke galne kiseline.

Ključne reči: autooksidacija, pirogalol, cink, MCR-ALS, ESR