Nickel(II) interactions with chlorophylls in solution: impact to degradation induced by UV-irradiation

Jelena Zvezdanović[1*], Sanja Petrović[1], Aleksandar Lazarević[1]

1-University of Niš, Faculty of Technology, Bulevar Oslobodjenja 124, 16000 Leskovac, Serbia

ABSTRACT

Chlorophylls and metal substituted complexes of chlorophylls with known photosensitizing abilities can be used in many types of biological and solar energy applications; thus, it is important to analyze their basic properties when they are treated by visible light and/or UV radiation. Nickel(II) can replace central magnesium from chlorophyll (Chl) molecule to form a metal substituted, "central" Ni-Chl complex. So, the possible formation of nickel-chlorophyll complexes in 95% ethanol at 40 °C was studied by UHPLC-DAD chromatography (UltraHigh Performance Liquid Chromatography–Diode Array Detector). Nickel(II)-chlorophylls interactions in the reaction solutions at 40 °C lead not only to the formation of the Ni-related Chls, Ni-Chl complexes but several oxidation and demetalation products of chlorophylls. On the other hand, the influence of nickel(II) – chlorophyll interactions on chlorophylls solutions stability under continual UV-A, -B, and -C irradiation in 95% ethanol, was followed by UV-Vis spectrophotometry. Chlorophylls as well as their reaction solutions with nickel(II) undergo photochemical degradation obeying first-order kinetics. In general, the degradation is "energy-dependent", *i.e.* proportional to the UV photons energy input. Chlorophylls are less stable than their reaction solutions with nickel(II), probably due to the higher stability of Ni-chlorophylls complexes, to all three subranges of UV-irradiation.

Keywords: chlorophyll, nickel, complex, UV-irradiation

---

* Corresponding author: jelite74@yahoo.com

Introduction

Porphyrins and their metal derivatives have attracted significant attention in various areas because of their role in many types of biomolecules such as chlorophylls, as well as in numerous biological and solar energy applications (Ali & van Lier, 1999; Bozja et al., 2003; Er et al., 2015; Kay & Grätzel, 1993; Kay et al., 1994; Kruth et al., 2014; Youssef et al., 2018). These applications are using light or UV irradiation treatments of the porphyrins (as well as chlorophylls) and their metal derivatives leading to the excitation and triplet excited states formation in so-called photosensitizing reactions. Chlorophyll as the major photosynthetic pigment is dihydroporphyrin *i.e*., chlorine, with four pyrrole rings coordinated with magnesium; it also contains a long hydrophobic side chain derived from phytol (Figure 1). Higher plants mostly contain chlorophyll *a* and chlorophyll *b* (Chl*a* and Chl*b*, respectively). Many heavy metals (such as copper and nickel), can easily replace labile-bonded central magnesium to form metal substituted, "central" M-Chl complexes. Detailed consequences of these reactions *in vivo* and general reactions of chlorophylls with metals *in vitro* have been already discussed (Clijsters & van Assche, 1985; Drzewiecka-Matuszek et al., 2005; Grajek et al., 2020; Küpper et al., 1996, 2002, 2006; Luna et al., 1994; Molas, 2002). Nickel is considered to be an essential micronutrient for plants (Eskew et al., 1983), but at higher concentrations, this metal becomes toxic for the majority of plant species (Gajewska et al., 2006; Küpper et al., 2006; McIlveen & Negusanti, 1994; Molas, 2002; Zehetner et al., 2002). Nickel-chlorophylls were taken into account in dye-sensitized solar cells applications (Pratiwi et al., 2018). Also, a nickel-chlorophyll derivative has been already used as a multimodal agent for tumor imaging and photodynamic therapy which uses a light treatment for that purpose (Er et al., 2015). That's why the examination of chlorophylls basic properties (porphyrins in general) and their metal-complexes when they are treated by UV light is common for numerous biological and solar energy applications. The aims of this work were to examine Ni(II) – chlorophyll interactions in a way to determine the possible formation of the corresponding metal complexes (Ni-Chl) in solutions, *in vitro*, and show how Ni(II) – chlorophyll interactions influenced chlorophylls stability against continual UV irradiation in three used subranges, UV-A, UV-B, and UV-C. The results of Ni(II) – chlorophyll interactions were studied by using UV-Vis spectrophotometry and UHPLC chromatography coupled with DAD (UltraHigh Performance Liquid Chromatography – Diode Array Detector) while the UV irradiation effects were studied by UV-Vis spectrophotometry.
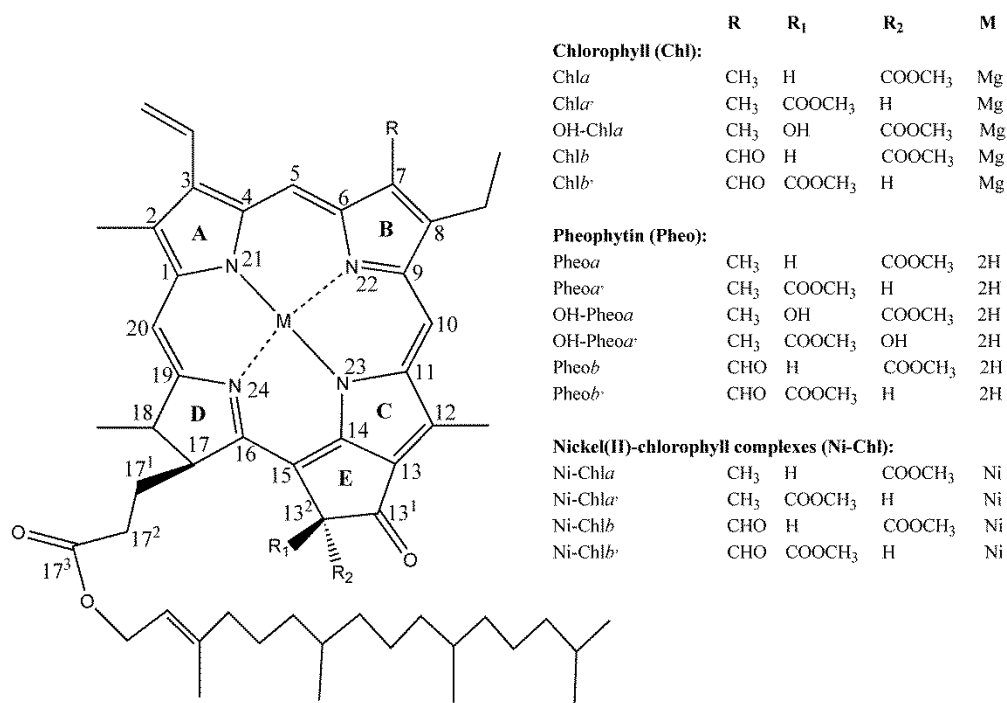
	R	R_1	R_2	M
Chlorophyll (Chl):				
Chl*a*	CH_3	H	$COOCH_3$	Mg
Chl*a'*	CH_3	$COOCH_3$	H	Mg
OH-Chl*a*	CH_3	OH	$COOCH_3$	Mg
Chl*b*	CHO	H	$COOCH_3$	Mg
Chl*b'*	CHO	$COOCH_3$	H	Mg
Pheophytin (Pheo):				
Pheo*a*	CH_3	H	$COOCH_3$	2H
Pheo*a'*	CH_3	$COOCH_3$	H	2H
OH-Pheo*a*	CH_3	OH	$COOCH_3$	2H
OH-Pheo*a'*	CH_3	$COOCH_3$	OH	2H
Pheo*b*	CHO	H	$COOCH_3$	2H
Pheo*b'*	CHO	$COOCH_3$	H	2H
Nickel(II)-chlorophyll complexes (Ni-Chl):				
Ni-Chl*a*	CH_3	H	$COOCH_3$	Ni
Ni-Chl*a'*	CH_3	$COOCH_3$	H	Ni
Ni-Chl*b*	CHO	H	$COOCH_3$	Ni
Ni-Chl*b'*	CHO	$COOCH_3$	H	Ni

Figure 1. Structures of chlorophylls discussed in this paper (Petrović et al., 2017; Zvezdanović et al. 2014).

Experimental

The solvents used in the experiments were HPLC grade. Methanol and acetonitrile used in UHPLC experiments were purchased from Baker, The Netherlands, and Fisher Scientific, UK, respectively. Ethanol and crystalline Ni(II) chloride hexahydrate were purchased from Zorka, Serbia, and Sigma–Aldrich, Germany, respectively.

The experimental procedure described below was performed under "shade" conditions as much as possible, inside vessels and equipment covered with aluminum foil to prevent photooxidation of chlorophylls (Hynninen, 1991).

Chlorophylls isolation

Chlorophylls were extracted from spinach leaves (*Spinacia oleracea* L.) using a modified method proposed by Svec (Svec, 1978, 1991; Zvezdanović et al., 2014). The obtained extract was

a mixture of pigments containing a large amount of various Chl-forms (predominantly Chl*a* and Chl*b*) as well as accessory pigments, carotenoids. The chlorophylls (*i.e.* Chl-fraction) were isolated using a modified procedure of Svec (1978) and Backman & Risch (1991), by open column chromatography with silica gel as the adsorbent and *n*-hexane/acetone mixtures as the eluent, in gradient elution regime (Zvezdanović et al., 2014). The concentration of Chl*a*, Chl*b*, and total carotenoids in 95% ethanol (*v/v*) was determined spectrophotometrically in the chlorophyll fraction (Lichtenthaler, 1987), showing the predominant contribution of Chl*a* (molar ratio Chl*a*:Chl*b* > 5:1) and absence of the carotenoids.

Nickel(II)-chlorophyll reaction solutions

Solid chlorophylls obtained by evaporation of solution in the stream of nitrogen were dissolved in 95% ethanol (*v/v*) and a solution of $NiCl_2$ was then added to adjust the final concentrations of chlorophylls and Ni(II) in the reaction solution - 2×10^{-5} mol/dm^3 and 5×10^{-5} mol/dm^3, respectively. The reaction between Chl molecules and Ni(II) in a molar ratio 1 : 2.5, was performed by heating of reaction solution on the reflux apparatus for 60 min at 40 °C, according to the modified method proposed by Küpper (Küpper et al., 2000; Zvezdanović et al., 2012). The same experiments were done with chlorophylls in 95% ethanol (*v/v*) without Ni(II), as a "control sample" for 60 min of incubation.

UV-irradiation treatment

Continuous irradiation of chlorophylls, as well as Ni(II) – chlorophyll reaction solutions (after 60 min of incubation in 95% ethanol, *v/v*) was performed in a cylindrical photochemical reactor "Rayonnet" with eight symmetrically placed Hg-lamps having emission maxima at 350 nm (UV-A), 300 nm (UV-B) and 254 nm (UV-C). The samples were irradiated in quartz cells (1 × 1 × 4.5 cm) placed on a rotating circular holder. The total measured energy flux (hitting the samples) was 10.3 W/m^2 for 350 nm, 12.0 W/m^2 for 300 nm, and 14.3 W/m^2 for 254 nm, at 10 cm distance from the lamps. The energy flux for UV-A and UV-B irradiation was measured by using Radiometer PRO-UV35 (Probus group) and for UV-C by using Solarmeter SM 8.0 UVC (Solartech Inc.).

UV-Vis spectrophotometry

The spectrophotometric measurements were made on a Varian Cary-100 spectrophotometer. All spectra before and after irradiation were recorded from 300 to 800 nm with 1.0 bandwidth, after different irradiation time periods, t_{irr} enabling kinetics analysis in the manner earlier described in detail (Zvezdanović & Marković, 2008). Data analysis was performed by the Origin 6 software.

Ultra-high performance liquid chromatography-diode array analysis

Liquid chromatography (ultra-high performance chromatography – UHPLC) runs were realized using a Dionex Ultimate 3000 UHPLC+ system equipped with a diode array (DAD) detector. The separations were performed on a Hypersil gold C18 column (50×2.1 mm, 1.9 µm) from the same producer, at a 25 °C temperature. The mobile phase consisted of methanol and acetonitrile with a gradient program at a flow rate of 0.200 ml min^{-1} as reported previously (Petrović et al., 2017). The injection volume was 5 µL.

Absorption UV–Vis spectra were recorded on DAD with a total spectral range between 200 and 800 nm. Complexes of chlorophylls derivatives with nickel were proposed according to their UV-Vis spectra within selected peaks from the corresponding UHPLC chromatograms by comparison with the published ones with similar chlorophyll derivatives; the rest of chlorophyll derivatives were identified by comparison of the results shown in previous work in which are used the same chromatography conditions (Petrović et. al., 2017), as well as their corresponding UV-Vis spectra obtained from UHPLC-DAD signals.

Results and Discussion

Nickel(II)-chlorophyll interactions

Absorption spectra

Absorption spectra of Ni(II)–chlorophyll reaction solutions from the beginning of nickel(II)–chlorophyll interaction at 40 ºC, following increasing time periods, were shown in Figure 2A. The corresponding absorption spectra of chlorophylls (Chl-fraction), Ni(II) – chlorophyll solution and the assumed "central" Ni-Chl complex (after 60 min of incubation) have

shown in Figure 2B. The spectrum of the assumed Ni(II) complex with chlorophyll and chlorophyll derivatives is obtained by subtraction method from the spectra of Ni(II) – chlorophyll solution and the corresponding Chl-fraction. In general, chlorophylls have two major absorption regions in the visible range (400–800 nm), producing "red" (Q_y) and "blue" (Soret or *B*) band (Hoff & Amesz, 1991). The interaction of nickel with the chlorophyll in solution was followed by using the Q_y band as a sensible indicator of central metal in the chlorophyll structure (Svec, 1978). Since for the control experiments significant changes weren't observed after 60 min incubation of Chl-fraction (not shown), the changes detected in the absorption spectra of Ni(II)–chlorophyll solutions could be assigned to the Ni(II) presence and its interactions with chlorophylls in the solution. A hypochromic effect related to the Chl absorption maximum of the Q_y band (665 nm) is observed for Ni(II)–chlorophyll solutions (Figure 2A) during the period of interaction. The formation of the substitution Ni-Chl complex is usually followed by a characteristic hypsochromic ("blue") shift of the Q_y absorption band compared to Chl itself (Boucher & Katz, 1967; Helfrich & Rüdiger, 1992; Küpper et al., 1996; Pilch et al., 2013), has been observed as a shoulder at a shorter wavelength in this work (Figure 2A).
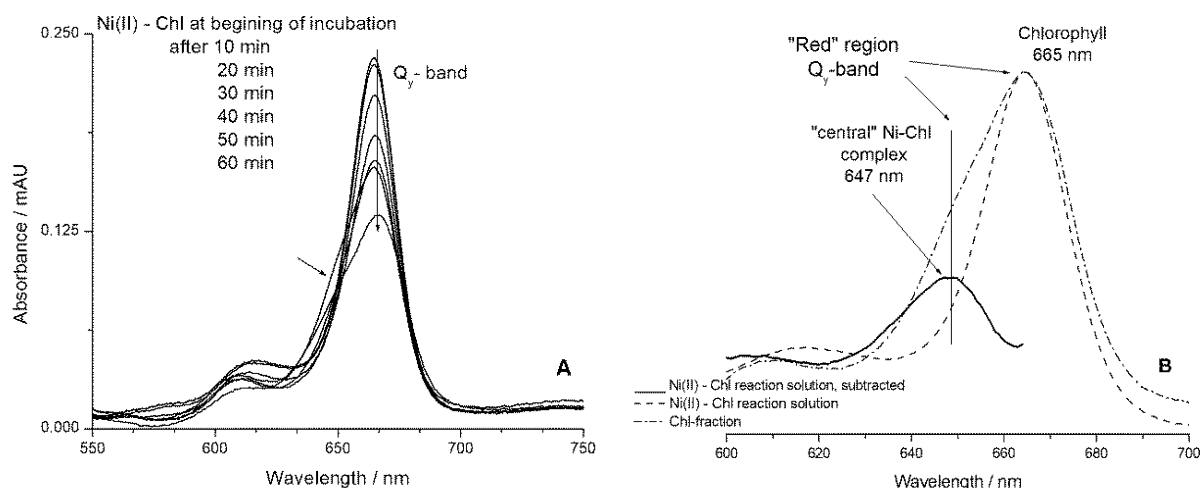

Figure 2. Absorption spectra of Ni(II)–chlorophyll reaction solutions in the "red" spectral region, following increasing periods of incubation, in 95 % ethanol (**A**). Absorption spectra of Chl in the "control" sample, Ni(II) – chlorophyll reaction solution (normalized to Q_y band absorption maximum) and assumed Ni-Chl complex, after 60 min of incubation (**B**).

This effect was clearly detected in the subtracted spectrum of Ni(II)–chlorophyll solution (Q_y band at 647 nm, Figure 2B), assumed to be for Ni-Chl complexes formed after 60 min of incubation. The term Ni-Chl complexes used in this paper refers to the substitution Ni(II) complexes of Chl*a*, Chl*b*, their conformation isomers (Chl*a'* and Chl*b'*), as well as their derivatives such as hydroxychlorophylls and potential oxidation products produced during the incubation treatment (Figure 1).

UHPLC-DAD analysis

Chromatograms of the Chl-fraction, control Chl-fraction, and Ni(II)-treated Chl-fraction (Ni(II) – chlorophyll solution), from the DAD signal at a detection wavelength, $\lambda_{det.} = 420$ nm, were shown in Figures 3A, B, and C, respectively. The main detected Chl-derivatives with their chromatographic ($t_{ret.}$) and UV-Vis spectroscopic parameters using UHPLC-DAD data are listed in Table 1.
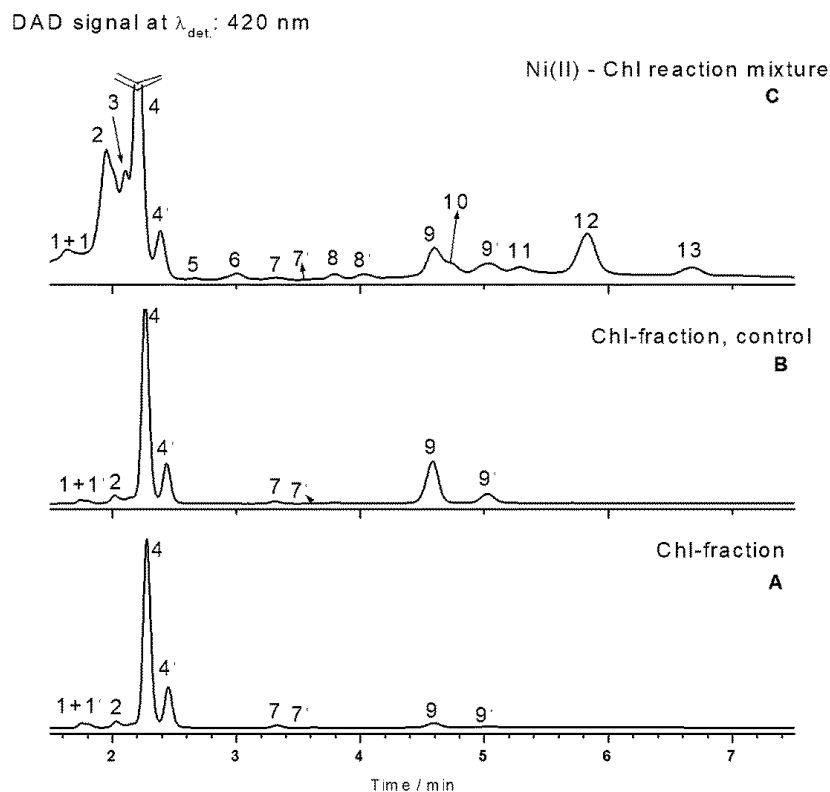

Figure 3. The UHPLC-DAD chromatograms for the Chl-fraction (**A**), "control" Chl-fraction (**B**), and Ni(II) – chlorophyll reaction solution (**C**), in 95 % ethanol after 60 min at 40 °C, recorded at 420 nm.

Chlorophyll fraction proved the presence of chlorophylls basically related to Chl*a* and Chl*b* (compounds 1, 1', 2, 7, 7', 9, 9') assigned as Chl*b*, Chl*b*', OH-Chl*a*, Pheo*b*, Pheo*b*', Pheo*a* and Pheo*a*', with the dominant contribution of Chl*a* and its conformation isomer Chl*a*' (4 and 4'), which is in accordance to UV-Vis spectrophotometric measurements; the corresponding identification was done by using previous work with the same chromatography conditions used and the corresponding absorption UV-Vis spectral data (Figure 3A, Table 1) (Petrović et al., 2017). In the control sample, incubated Chl-fraction without Ni(II) ions, no new compounds, only higher content of Pheo*a* derivatives (as a consequence of central Mg replacing by two hydrogen atoms), was detected after 60 min of incubation (comp. No. 9 and 9' in Figure 3B). In the Ni(II) – chlorophyll reaction solutions, various products, derivatives of Chls as well as Ni-Chls were detected after 60 min (Figure 3C, Table 1). Partial identification of the products was done by using absorption spectral data: Soret and Q_y bands wavelengths and absorption maximums ratios ($A_{Soret.}/A_{Qy}$) in accordance with available literature (Boucher & Katz, 1967; Pilch et al., 2013). Absorption spectra of chlorophyll *a* and its derivatives consisted of two bands, Soret at 430 nm and Q_y at 660 nm, with the corresponding absorbance ratio $A_{Soret.}/A_{Qy}$ between 1.1 and 1.3 in various solvents (Boucher & Katz, 1967; Jeffrey et al., 1996; Milenković et al., 2012); the corresponding spectra for Ni(II) complexes with Chl*a* derivatives consisted from Soret band at ≈423 nm and Q_y band at ≈652 nm, the band ratio ≈0.95 and with Chl*b* derivatives at ≈430 nm, ≈632 nm and 1.93, respectively (Boucher & Katz, 1967). Formation of Ni(II) complexes with Chl*a* and its derivatives in the Ni(II) – chlorophyll reaction solutions was assumed for compounds no. 10, 11, 12, and 13 according to the Soret band positioned at ≈420 nm, Q_y band between 644 and 654 nm (hypsochromic effect) and the absorbance maximums ratio values between 1.10 and 1.28 (Table 1) (Boucher & Katz, 1967; Pilch et al., 2013). Nickel complexes with Chl*b* or its derivatives were assumed for the compounds No. 3 and 5 according to the corresponding absorption maximums at 420 nm and 414 nm, 652 nm and 648 nm, respectively, as well as band ratio at 2.00 (Table 1) (Boucher & Katz, 1967). On the other hand, two more products in the Ni(II)-treated Chl solutions were detected, compounds no. 6 and 8 shown in Table 1. Compounds no. 8 and 8' were assigned as OH-Pheo*a* and OH-Pheo*a*', the hydroxy-pheophytins, without magnesium in the central position of chlorophyll *a* structure and OH group at C-13^2 position (Figure 1). Identification was done by considering the same chromatography conditions and spectral data from the previous

paper (Petrović et al., 2017). Compound no.6 is according to the UV-Vis spectral data certainly a chlorophyll derivative but was not fully identified (Table 1).

Table 1. Peak assignment of chlorophylls (Fig.3) separated by UHPLC-chromatography.

UHPLC-chromatography		Diode Array Detection			Detected in		
Proposed structure	$t_{ret.}$/ min	Absorption maximums in the mobile phase/nm		Absorption maximums ratio	not-treated	control	Ni(II)-treated
		Soret	Q_y	$A_{Soret.}/A_{Qy}$			
1 Chl*b* 1' Chl*b*'	1.75 1.81	466	651	2.90	+	+	+
2 OH-Chl*a*	2.02	430	665	1.41	+	+	+
3 n.i. Chl*b* derivative complex with Ni	2.10	420	652	2.00	-	-	+
4 Chl*a* 4' Chl*a*'	2.27 2.44	431, 411	665	1.10	+	+	+
5 n.i. Chl*b* derivative complex with Ni	2.67	414	648	2.00	-	-	+
6 n.i. Chl derivative	3.01	427	654	7.00	-	-	+
7 Pheo*b* 7' Pheo*b*'	3.33 3.62	435	654	5.50	+	+	+
8 OH-Pheo*a* 8' OH-Pheo*a*'	3.81 4.02	407	666	2.48	-	-	+
9 Pheo*a* 9' Pheo*a*'	4.60 5.05	408	666	2.40	+	+	+
10 n.i. Chl*a* derivative complex with Ni	4.73	423, 400	652	1.10	-	-	+
11 n.i. Chl*a* derivative complex with Ni	5.28	411, 391	644	1.28	-	-	+
12 n.i. Chl*a* derivative complex with Ni	5.8	422, 401	652	1.18	-	-	+
13 n.i. Chl*a* derivative complex with Ni	6.67	422, 401	652	1.20	-	-	+

n.i. - not identified

So, Ni(II) ions and chlorophylls interactions at 40 °C in the water-ethanol reaction solutions lead not only to formation of the Ni-related Chls, but also oxidation and demetalation chlorophyll derivatives such as OH-Chl*a*, OH-Pheo*a* and their conformation isomers, as well production some

new, not identified Chl derivatives. After 60 min, full transformation of Chl*a* and its isomer Chl*a'* to the various products wasn't provided, Ni-Chl complexes formation was not the only reaction that takes place in the conditions described in this paper, at Ni : Chl molar ratio (2.5 : 1).

UV-irradiation of chlorophyll and nickel(II)-chlorophyll reaction solutions

Absorption spectra of chlorophylls (Chl-fraction) and Ni(II) – chlorophyll reaction solutions following increasing periods of continual UV-B irradiation were shown in Figures 4A and B, respectively; spectra of assumed Ni-Chl complexes obtained by subtraction method, were shown in the increment of Figure 4B. The corresponding spectra following increasing periods of continual UV-A and UV-C irradiation, have shown similar spectral behavior (not shown). The effects of UV-A, -B, and -C continuous irradiation on chlorophyll fraction and Ni(II) – chlorophyll reaction solution in 95% ethanol were followed by using UV-Vis spectrophotometric measurements; the changes were recorded at Q_y absorbance maximum values for the Chl and Ni-Chl, 665 and 647 nm, respectively.
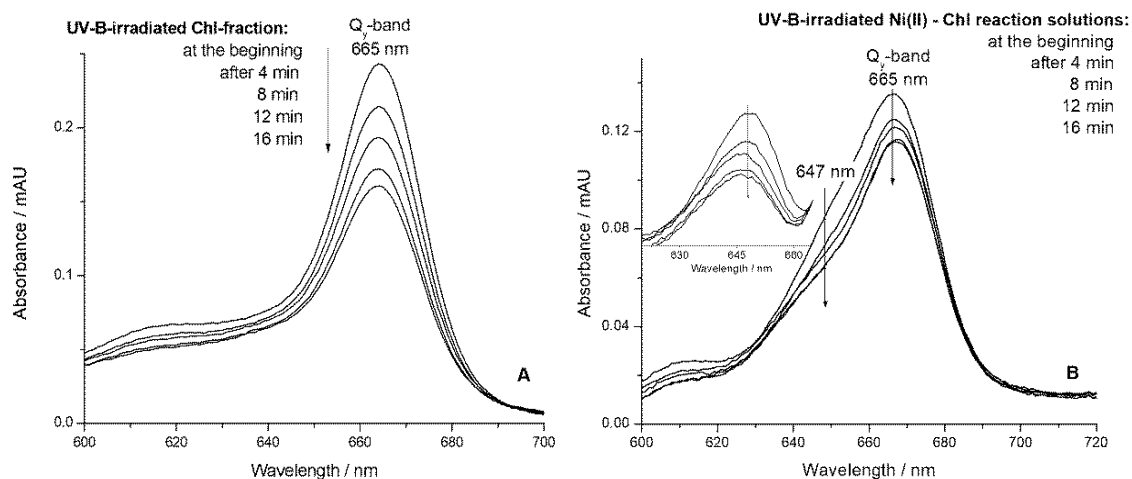

Figure 4. Continual UV-B irradiation induced degradation of chlorophyll fraction and Ni(II) – chlorophyll reaction solutions in 95% ethanol – changes in the absorption spectra during time exposure to UV-B irradiation (**A** and **B**, respectively); the corresponding spectra of assumed Ni-Chl complexes obtained by subtraction method, were shown as the increment of Figure 4B.

Continual UV irradiation of the chlorophylls and Ni(II) – chlorophyll reaction solutions induce a gradual decrease of the absorption in the whole measured spectral range (300-800 nm), *e.g.* a

hypochromic effect has been clearly observed. A gradual decrease of the Q_y-band intensity is shown in Figures 4A, and B for UV-B irradiated samples. Continual UV irradiation clearly results in the irreversible degradation – *bleaching* of chlorophylls in all samples, especially under UV-C irradiation (not shown). Similarly, UV-induced changes of chlorophylls were already detected in various solvents like acetone, *n*-hexane, and methanol-aqueous solutions (Petrović et al., 2017; Zvezdanović & Marković, 2008; Zvezdanović et al., 2009, 2012).

Chlorophylls bleaching kinetics obey a first-order law, as already reported in several papers (Petrović et al., 2017; Santabarbara, 2006; Zvezdanović & Marković, 2008; Zvezdanović et al., 2009, 2012). Nickel(II)-chlorophyll solution also obeys first-order kinetics, and all the plots show linear dependencies with the corresponding *R* values of about 0.98. Speaking about the energy input influence, UV-induced Chls bleaching rates progressively increase from UV-A to UV-C. The calculated degradation rate values of the Ni(II) – chlorophyll reaction solutions bleaching are also in proportion to the energy inputs, as expected (from UV-A to UV-C, Table 2). Calculated degradation rate constants for Chls generally show higher values than the same ones calculated from the experiments with Ni(II) – chlorophyll reaction solutions (Table 2). These differences between Chls and Ni(II) – chlorophyll reaction solutions stability against UV irradiation seem to be related to difference in the stability of Chl*a* and Ni-Chl molecules which are in major present in the samples (results of UV-Vis and UHPLC-DAD experiments). Ni(II) – chlorophyll reaction solutions are more stable compared to chlorophylls themselves, to all three subranges of UV irradiation, as shown in Table 2. For example, UV-A irradiation-induced bleaching rate constants for Ni(II) – chlorophyll reaction solution (calculated by using absorbance values at 665 nm and 647 nm) were 3.4 and 6.4 times lower, respectively, than the same calculated for chlorophyll fraction (at 665 nm, Table 2).

Generally, the stability of "central" metal complexes of chlorophylls in solutions can be explained by theoretical analysis of Falk's "stability factor" (Hynninen, 1991). The factor includes the charge number of the metal ion, the effective radius of the metal ion in Å, and Pauling's electronegativity, and its value was already used in some works (Küpper et al., 2000; Stanley et al., 1991; Zvezdanović & Marković, 2009; Zvezdanović et al., 2012). According to Falk's "stability factor", Ni-Chl complexes are more stable in comparison to the Chls themselves. Metal complexes of porphyrins (chlorophylls) which contain the metals like Ni(II) with d^8 configuration of the

corresponding energy levels have significant metal to porphyrin orbital interaction (*i.e*. metal to ligand $d\pi$ - backbonding), with an increased π-π* energy separation as a result, seen as a "blue" schift in the appropriate spectra (Milgrom, 1997; Petrović et al., 2006; Zvezdanović & Marković, 2008, Zvezdanović et al., 2012), as observed in presented experiments (Figure 2). So, heavy metal - nitrogen bonds in the central position of porphyrin structure seem to play a significant role in the stability of metal complexes to UV irradiation which is reflected as the corresponding higher stability of Ni(II) – chlorophyll reaction solutions (Table 2). While chlorophylls and metallochlorophylls strongly absorb in the visible range of the spectrum, they also considerably absorb in the UV spectral range used in this work (Johnson & Day, 2002). In turn, excited chlorophylls (and the corresponding Ni-Chls) can be relaxed through complex mechanisms which can lead to their degradation (bleaching). Pilch and co-workers explained the significant photostability of Ni-Chl complexes in which a symmetry of a ligand field created in the central spot of the Ni-chlorophyll molecule is very important (Pilch et al., 2013). The central N - Ni(II) bonds, formed *via* the donation of two electrons from each of the sp^2 orbitals of two central nitrogens to an empty $s-d_{x2-y2}$ hybrid centered on Ni(II), have a considerable covalent character having an effect resulting in a similarity of their equilibrium geometries in the ground and the excited states. They lead to very fast and efficient relaxation of excited Ni-Chls by conversion to molecular vibrations and dissipation as heat. The authors concluded that these Ni-substituted pigments can be a fine exemplification of symmetry control over properties of excited states of transition metal complexes (Pilch et al., 2013).

Table 2. Chlorophylls degradation kinetics by continual UV-A, UV-B, and UV-C irradiation treatments in 95% ethanol.

Irradiation treatment	Chlorophylls (at 665 nm)	Ni(II) – chlorophyll reaction solution (at 665 nm)	Ni(II) – chlorophyll reaction solution (at 647 nm)
	Rate constants, k / (1/min)		
UV-A	0.02605	0.00775	0.00405
UV-B	0.02638	0.01023	0.00926
UV-C	0.15063	0.04834	0.04660

Conclusion

Nickel(II)-chlorophylls interactions at 40 °C in the water-ethanol reaction solutions lead not only to the formation of the Ni-related Chls, Ni-Chl complexes (which are predominantly formed) but also several oxidation and demetalation products of chlorophylls. The time of incubation of 60 min and/or metal concentration is not enough to convert all chlorophyll content into Ni-Chl complexes. Continual UV-irradiation of the chlorophyll fraction and Ni(II) – chlorophyll solution results in irreversible bleaching obeying first-order kinetics. The bleaching rate of Chls and their Ni-complexes depends on UV-photon energy input. Nickel(II)-chlorophylls solutions are in general more stable compared to chlorophylls themselves to all three ranges of UV irradiation, due to the higher stability of the corresponding Ni-Chl complexes formed during incubation treatment. In conclusion, due to their stability, nickel complexes of chlorophylls can be the right candidates for applications that use UV light.

Acknowledgment

Conflict-of-Interest Statement

The authors did not declare any conflict of interest.

References

Ali, H., & van Lier, J.E. (1999). Metal complexes as photo- and radiosensitizers. Chemical Reviews, 99 (9), 2379–2450.

Backman, H., & Risch, N. (1991). Preparative Chromatography. In H. Scheer (Ed.), Chlorophylls (pp. 103-114). Boca Raton: CRC Press.

Boucher, L.J., & Katz, J.J. (1967). Aggregation of metallochlorophylls. Journal of the American Chemical Society, 89 (18), 4703-4708.

Bozja, J., Sherrill, J., Michielsen, S., & Stojiljkovic I. (2003). Porphyrin-based, light-activated antimicrobial materials. Journal of Polymer Science: Part A: Polymer Chemistry, 41 (15), 2297–2303.

Clijsters, H., & van Assche, F. (1985). Inhibition of photosynthesis by heavy metals. Photosynthesis Research, 7, 31-40.

Drzewiecka-Matuszek, A., Skalna, A., Karocki, A., Stochel, G., & Fiedor, L. (2005). Effects of heavy central metal on the ground and excited states of chlorophyll. Journal of Biological Inorganic Chemistry, 10 (5), 453-462.

Er, O., Lambrecht, F.Y., Ocakoglu, K., Kayabasi, C., & Gunduz, C. (2015). Primary evaluation of a nickel-chlorophyll derivative as a multimodality agent for tumor imaging and photodynamic therapy. Journal of Radioanalytical and Nuclear Chemistry, 306, 155–163.

Eskew, D.L., Welch, R.M., & Cary, E.E. (1983). Nickel: an essential micronutrient for legumes and possibly all higher plants. Science, 222 (4624), 621–623.

Gajewska, E., Sklodovska, M., Slaba, M., & Mazur, J. (2006). Effect of nickel on antioxidative enzyme activities, proline and chlorophyll contents in wheat shoots. Biologia Plantarum, 50 (4), 653-659.

Grajek, H., Rydzyński, D., Piotrowicz-Cieślak, A., Herman, A., Maciejczyk, M., & Wieczorek, Z. (2020). Cadmium ion-chlorophyll interaction – Examination of spectral properties and structure of the cadmium-chlorophyll complex and their relevance to photosynthesis inhibition. Chemosphere, 261, 127434.

Helfrich, M., & Rüdiger, W. (1992). Various metallopheophorbides as substrates for chlorophyll synthetase. Zeitschrift für Naturforschung C, 47, 231-238.

Hoff, A.J., & Amesz, J. (1991). Visible absorption spectroscopy of chlorophylls. In H. Scheer (Ed.), Chlorophylls (pp. 723-738). Boca Raton: CRC Press.

Hynninen, P.H. (1991). Modifications. In H. Scheer (Ed.), Chlorophylls (pp. 145-209). Boca Raton: CRC Press.

Jeffrey, S.W., Mantoura, R.F.C., & Wright, S.W. (1996). Phytoplankton pigments in oceanography: guidelines to modern methods, UNESCO Publishing.

Johnson, G.A., & Day, T.A. (2002). Enhancement of photosynthesis in *Sorghum bicolor* by ultraviolet radiation. Physiologia Plantarum, 116 (4), 554–562.

Kay, A., & Grätzel, M. (1993). Artificial photosynthesis. 1. Photosensitization of TiO_2 solar cells with chlorophyll derivatives and related natural porphyrins. Journal of Physical Chemistry, 97, 6272-6277.

Kay, A., Humphry-Baker, R., & Grätzel, M. (1994). Artificial photosynthesis. 2. Investigations on the mechanism of photosensitization of nanocrystalline TiO$_2$ solar cells by chlorophyll derivatives. Journal of Physical Chemistry, 98, 952-959.

Kruth, A., Peglow, S., Rockstroh, N., Junge, H., Bruser, V., & Weltmann, K.-D. (2014). Enhancement of photocatalytic activity of dye sensitised anatase layers by application of a plasma-polymerized allylamine encapsulation. Journal of Photochemistry and Photobiology A: Chemistry, 290, 31–37.

Küpper, H., Küpper, F., & Spiller, M. (1996). Environmental relevance of heavy metal-substituted chlorophylls using the example of water plants. Journal of Experimental Botany, 47, 259-266.

Küpper, H., Šetlik, I., Spiller, M., Küpper, F. C., & Prášil O. (2002). Heavy metal-induced inhibition of photosynthesis: targets of in vivo heavy metal chlorophyll formation. Journal of Phycology, 38 (3), 429-441.

Küpper, H., Spiller, M., & Küpper, F.C. (2000). Photometric method for the quantification of chlorophylls and their derivatives in complex mixtures: fitting with Gauss-peak spectra. Analytical Biochemistry, 286 (2), 247-256.

Küpper, H., Küpper, F., & Spiller, M. (2006). [Heavy metal]-Chlorophylls formed in vivo during heavy metal stress and degradation products formed during digestion, extraction and storage of plant material. In B.Grimm, R.J. Porra, W. Rüdiger, H. Scheer (Eds.) Advances in Photosynthesis - Chlorophylls and Bacteriochlorophylls (pp. 67-77). Dordrecht: Kluwer Academic Publishers.

Lichtenthaler, H.K. (1987). Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods in Enzymology, 148, 350-382.

Luna, C.M., González, C.A., & Trippi, V.S. (1994). Oxidative damage caused by an excess of Cu in oat leaves. Plant & Cell Physiology, 35, 11-15.

McIlveen, M.D., & Negusanti, J.J. (1994). Nickel in terrestrial environment. The Science of the Total Environment, 148 (2-3), 109-138.

Milenković, S.M., Zvezdanović, J.B., Anđelković, T.D., & Marković, D.Z. (2012). The identification of chlorophyll and its derivatives in the pigment mixtures: HPLC-chromatography, visible and mass spectroscopy studies. Advanced Technologies, 1 (1), 16-24.

Milgrom, L.R. (1997). The Colours of Life: An Introduction to the Chemistry of Porphyrins and Related Compounds, Oxford: OUP.

Molas, J. (2002). Changes of chloroplast ultrastructure and total chlorophyll concentration in cabbage leaves caused by excess of organic Ni(II) complexes. Environmental and Experimental Botany, 47 (2), 115–126.

Petrović, J., Nikolić G., & Marković, D. (2006). *In vitro* complexes of copper and zinc with chlorophyll. Journal of the Serbian Chemical Society, 71 (5), 501-512.

Petrović, S., Zvezdanović, J., & Marković, D. (2017). Chlorophyll degradation in aqueous mediums induced by light and UV-B irradiation: An UHPLC-ESI-MS study. Radiation Physics and Chemistry, 141, 8-16.

Pilch, M., Dudkowiak, A., Jurzyk, B., Łukasiewicz J., Susz, A., Stochel, G., & Fiedor, L. (2013). Molecular symmetry determines the mechanism of a very efficient ultrafast excitation-to-heat conversion in Ni-substituted chlorophylls. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 1827 (1), 30–37.

Pratiwi, D.D., Nurosyid, F., Kusumandari, Supriyanto, A., & Suryana, R. (2018). Effect of Ni doping into chlorophyll dye on the efficiency of dye-sensitized solar cells (DSSC). AIP Conference Proceedings, 2014, 020066. https://doi.org/10.1063/1.5054470.

Santabarbara, S. (2006). Limited sensitivity of pigment photo-oxidation in isolated thylakoids to singlet excited state quenching in photosystem II antenna. Archives of Biochemistry and Biophysics, 455 (1), 77–88.

Stanley, K.D., de la Vega, R.L., Quirke, J.M.E., Beato, B.D., & Yost R.A. (1991). Comparison of the spectroscopic properties of metalloporphyrins. Chemical Geology, 91 (2), 169- 183.

Svec, W.A. (1978). The isolation, preparation, characterization, and estimation of the chlorophylls and the bacteriochlorophylls. In D. Dolphin (Ed.) The Porphyrins (pp. 341–399). New York: Academic Press.

Svec, W.A. (1991). The distribution and extraction of the chlorophylls. In H. Scheer (Ed.), Chlorophylls (pp. 89-100). Boca Raton: CRC Press.

Youssef, Z., Colombeau, L., Yesmurzayeva, N., Baros, F., Vanderesse, R., Hamieh, T., Toufaily, J., Frochot, C., Roques-Carmes, T., & Acherar, S. (2018). Dye-sensitized nanoparticles for heterogeneous photocatalysis: Cases studies with TiO_2, ZnO, fullerene and graphene for water purification. Dyes and Pigments, 159, 49-71.

Zehetner, A., Scheer, H., Siffel, P., & Vacha, F. (2002). Photosystem II reaction center with altered pigment-composition: reconstitution of a complex containing five chlorophyll *a* per two pheophytin *a* with modified chlorophylls. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 1556 (1), 21– 28.

Zvezdanović, J., & Marković, D. (2008). Bleaching of chlorophylls by UV irradiation *in vitro*: the effects on chlorophyll organization in acetone and *n*-hexane. Journal of the Serbian Chemical Society, 73 (3), 271-282.

Zvezdanović, J., & Marković, D. (2009). Copper, iron, and zinc interactions with chlorophyll in extracts of photosynthetic pigments studied by VIS spectroscopy. Russian Journal of Physical Chemistry A, 83, 1542–1546.

Zvezdanović, J., Cvetić, T., Veljović-Jovanović, S., & Marković, D. (2009). Chlorophyll bleaching by UV-irradiation *in vitro* and *in situ*: absorption and fluorescence studies. Radiation and Physics Chemistry, 78 (1), 25–32.

Zvezdanović, J.B., Petrović, S.M., Marković, D.Z., Anđelković, T.D., & Anđelković, D.H. (2014). Electrospray ionization mass spectrometry combined with ultra high performance liquid chromatography in the analysis of *in vitro* formation of chlorophyll complexes with copper and zinc. Journal of the Serbian Chemical Society, 79 (6), 689-706.

Zvezdanović, J.B., Marković, D.Z., & Milenković, S.M. (2012). Zinc(II) and copper(II) complexes with pheophytin and mesoporphyrin and their stability to UV-B irradiation: VIS spectroscopy studies. Journal of the Serbian Chemical Society, 77 (2), 187–199.