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SYNTHESIS AND EVALUATION OF SCHIFF BASE COMPOUNDS AS COPPER AND NICKEL CHELATORS AND RADICAL SCAVENGERS

SÍNTESE E AVALIAÇÃO DE COMPOSTOS DE BASE DE SCHIFF COMO QUELANTES DE COBRE E NÍQUEL E SEQUESTRADORES DE RADICAIS

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[1 2 3 4 5 6] Centro Universitário Norte do Espírito Santo da Universidade Federal do Espírito Santo, Rodovia BR 101 Norte, Km. 60, Bairro Litorâneo, CEP 29932-540, São Mateus. [1] vivian.silveira@ufes.br [2] kelex10@gmail.com [3] martinsflucas@gmail.com [4] christiane.nogueira@ufes.br [5] ana.o.costa@ufes.br [6] ana.n.mendes@ufes.br

RESUMO

Existem diversos processos que envolvem reações oxidativas no cotidiano, porém alguns deles são indesejáveis como a deterioração de biocombustíveis, fazendo com que os antioxidantes assumam um papel de grande importância em diversas áreas industriais. Neste cenário três compostos de base de Schiff foram sintetizados, caracterizados (N,N'-Bis(o-vanilina) etilenodiamina - Ovalen, N,N'-Bis(vanilina) etilenodiamina - Valen e N,N'-Bis(4-hidroxisalicilideno) etilenodiamina - Dhben) e avaliados quanto ao potencial antioxidante na atuação como sequestradores de radicais e quelantes de metais de transição. Suas constantes de estabilidade com os íons Ni(II) e Cu(II) foram calculadas e sua capacidade antioxidante foi determinada por um ensaio de atividade quelante e pelo método DPPH. O Dhben mostrou uma maior capacidade de quelar íons cobre do que íons níquel em baixas concentrações da base de Schiff. Ovalen também é um ótimo agente quelante, sendo melhor com níquel do que com cobre. Os resultados concordam com as constantes de ligação obtidas 9.09×10^2 L mol^{-1} para [Cu-dhben] e 9.91×10^2 L mol^{-1} para [Ni-ovalen]. No ensaio DPPH, foi observado que dhben e ovalen foram capazes de diminuir a concentração de DPPH, no entanto, dhben poderia atingir o IC_{50} mesmo em baixas concentrações. O dhben e o ovalen são bons agentes quelantes de cobre e níquel, e dhben é o melhor agente antioxidante.

ABSTRACT

There are several processes involving oxidative reactions every day, however some of them are undesirable, such as the deterioration of biofuels. Therefore, making antioxidants take on a very important role in many industrial areas. In this scenario, three Schiff base compounds were synthetized, characterized (N,N'-Bis(o-vanillin)ethylenediamine – Ovalen, N,N'-Bis(vanillin)ethylenediamine - Valen and N,N'-Bis(4-hydroxysalicylidene)ethylenediamine dhben) and evaluated for antioxidant potential acting as radical scavengers and as chelators of transition metals. Their stability constants with Ni (II) and Cu (II) ions were calculated and their antioxidative capacity were determined by a chelating activity assay and a DPPH method. Antioxidant dhben shows a greater ability in chelating copper ions than nickel ions in low concentrations. Antioxidant ovalen is also a great chelating agent, being better with nickel than with copper. The results agree with the obtained binding constants 9.09×10^2 L mol^{-1} for [Cu-dhben] and 9.91×10^2 L mol^{-1} for [Ni-ovalen]. In DPPH assay, it was observed that dhben and ovalen were capable to decrease DPPH absorbance concentration, however dhben could reach the IC_{50} even at low concentrations. Both dhben and ovalen are good copper and nickel chelating agents, and dhben is the best tested antioxidant agent.

INTRODUCTION

Several processes involving oxidative reactions have a great importance in daily life, such as corrosion, fermentation process, breathing and fuel combustion. However, some of them are undesirable, such as fuel tank and other engine or vehicle parts corrosion, biodiesel deterioration, oils and fats auto-oxidation processes and biomolecules oxidative damage (Singh, et al., 2012; Dunn, 2002; Kowalska, et al., 2014; Parsons, 2018). The oxidative reaction rate is influenced by microbial growth, moisture, temperature, ionizing radiation, presence of the metal and feedstock used (Luo, et al., 2017).

Many researchers investigated the effects of oxidation inhibitors on numerous substrates (Nimse & Pal, 2015). Bioactive antioxidant compounds denote an ability to maintain their cell structure and function by effectively clearing free radicals. This way, they inhibit lipid peroxidation reactions, and avoid other oxidative damages (Wang, et al., 2018). The synthetic antioxidants can prevent the biodiesel oxidation by the same principles (Suzuki, et al., 2016).

Antioxidants can remove or inactivate free radicals, as well they chelate transition metals that are oxidative reaction catalysts. Synthetic antioxidants, as tert-butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT), and some natural antioxidants (e.g., tocopherols), have been used to inactivate free radicals through the donation of hydrogen to these molecules, stopping the chain reaction (Zhang, et al., 2015).

Antioxidants, such as ethylenediamine tetraacetic acid (EDTA), citric acid and their salts, phosphoric acid, phosphates or amino acids, remove the substrate metal ions, mainly copper and iron (which are lipid oxidation catalysts), and act as chelating agents. An unshared pair of electrons in their molecular structure promotes the complexation (Almaroai, et al., 2013).

Copper is an essential metal found in plants and microorganisms and plays important roles in the biological system. Moreover, copper causes many cellular processes damage, that may lead to diseases and even to the cell death (Rombel-Bryzek, Rajfur, & Zhuk, 2017).

Nickel is nutritionally essential for many animal species, microorganisms and plants, and it is also present in alloys and nickel-plated items. Nevertheless, the excessive exposure to it leads to adverse effects on human health due to its oxidative potential (Kwon, et al., 2013).

Besides that, Cu and Ni are often found in many parts of diesel engine such as fuel tank, filter and pump, and even small concentrations of metal contaminants influence the oxidation stability of biodiesel. The high processing temperature and the biofuel exposure to air also leads to its fuel characteristics deterioration, what affects its stability and marketability (Jain & Sharma, 2011).

BHT is the most used antioxidant, recognized as safe to use in high-fat food, pharmaceuticals and petroleum products (Yehye, et al., 2015). Also, is applied to increase biodiesel oxidative stability (Karavalakis & Stournas, 2010). EDTA is ordinarily used to increase shelf life of food containing lipids and biodiesel fuel due to its chelating properties (Santos, et al., 2012).

Since antioxidants are very important in many industrial areas, there were prepared three Schiff base compounds that showed antioxidant activity and can act as radical scavengers or metal

chelators. Herein, these compounds were synthesized, characterized and investigated for their ability to bind Ni(II) and Cu(II) in the presence of magnesium metal and stability constants determination. Their ability to promote inactivation of free radicals was determined by DPPH method.

## EXPERIMENTAL

1. REAGENTS AND EQUIPMENT

The reagents 2,4-dihydroxybenzaldehyde (98%), nickel(II) acetate (98%), copper(II)perchlorate hexahydrate (98%), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ortho-vanillin and vanillin were purchased from Aldrich Chemical Co. Ethylenediamine and all solvents used are from Merck Chemical Co.

The elemental analysis was carried out in a Perkin–Elmer 2400 CNH Elemental Analyzer performed at the Analytical Center of Universidade de São Paulo. Infrared spectra were recorded in AgilentCary 630 FTIR instrument, in the range 4000–400 cm^{-1}, while UV-vis spectra were recorded on a double beam Q898UVDB equipment from Quimis at Universidade Federal do Espírito Santo. The ^{1}H NMR experiments were performed on an NMR spectrometer (Varian VNMRS 11.75 Tesla) at 500 MHz, using CDCl$_3$ or DMSO-d$_6$ as solvent at NMR Multi-User Laboratory of Universidade Federal Fluminense.

2. SCHIFF BASE SYNTHESIS

The aldehyde reactants (16.70 mmol), ethylenediamine (8.35 mmol) and ethanol 96% (10 mL) were placed in a round bottomed flask. The pH was adjusted to 5.5 and the mixture was stirred for 1 hour at ice bath, producing a yellow solid, according to the procedure for Schiff base compounds preparation (Viswanathamurthi, et al., 1998). The compound was filtered off, washed with ethanol and dried over fused CaCl$_2$ in a desiccator. (Scheme 1).

Scheme 1. Synthesis of the Schiff-base compounds.

Comp.	R$_1$	R$_2$	R$_3$
1 (ovalen)	OH	OCH$_3$	H
2 (valen)	H	OCH$_3$	OH
3 (dhben)	OH	H	OH
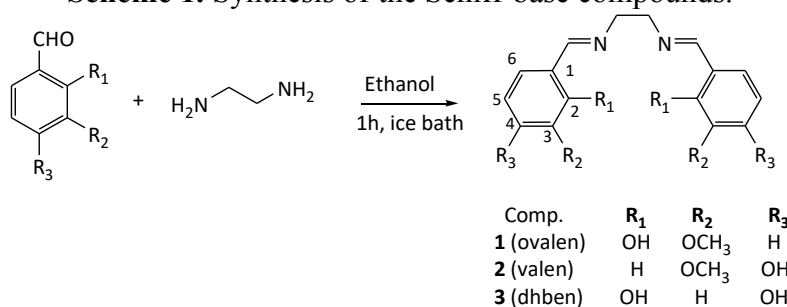
Compound 1: *N,N'*-Bis(*o*-vanillin)ethylenediamine (Ovalen), yield 72%: ν$_{max}$ 1650, 1590, 1508, 1330, 1200 cm^{-1}; ^{1}H NMR (CDCl$_3$, 500 MHz) δ 13.57 (2H, br s, O*H*), 8.33 (2H, s, N=C*H*), 6.90 (2H, dd, *J* = 7.7, 1.5 Hz, C*H*-6), 6.84 (2H, dd, *J* = 7.7, 1.5 Hz, C*H*-4), 6.77 (2H, t, *J* = 7.5 Hz, C*H*-5), 3.96 (4H, s, NC*H$_2$*), 3.88 (6H, s, OC*H$_3$*). Anal. Calc. (C$_{18}$H$_{20}$O$_4$N$_2$): C 65.84%, H 6.14%, N 8.53%. Found: C 66.32%, H 6.09%, N 8.41 %.

Compound 2: *N,N'*-Bis(vanillin)ethylenediamine (Valen), yield 64%: ν$_{max}$ 1610, 1230, 1150 cm^{-1}. ^{1}H NMR (*d$_6$*-DMSO, 500 MHz) δ 8.18 (2H, s, N=C*H*), 7.30 (2H, d, *J* = 2.5 Hz, C*H*-2), 7.09 (2H, dd, *J* = 13.5, 2.5 Hz, C*H*-6), 6.80 (2H, d, *J* = 13.5 Hz, C*H*-5), 3.78 (10H, s, NC*H$_2$*, OC*H$_3$*). Anal. Calc. (C$_{18}$H$_{20}$O$_4$N$_2$): C 65.84%, H 6.14%, N 8.53%. Found: C 64.54%, H 6.20%, N 8.73 %.

Compound 3: *N,N'*-Bis(4-hydroxysalicylidene)ethylenediamine (Dhben), yield 55%: ν_{max} 1634, 1475, 1350, 1236, 1165 cm^{-1}. ^{1}H NMR (*d*$_6$-DMSO, 500 MHz) δ 8.35 (2H, s, N=C*H*), 7.15 (2H, d, *J* = 14.0 Hz, C*H*-6), 6.25 (2H, dd, *J* = 14.0, 4.0 Hz, C*H*-5), 6.15 (2H, d, *J* = 14.0 Hz, C*H*-3), 3.78 (4H, s, NC*H*$_2$). Anal. Calc. (C$_{16}$H$_{16}$O$_4$N$_2$): C 63.99%, H 5.37%, N 9.33%. Found: C 62.71%, H 5.32%, N 9.38 %.

3. STABILITY CONSTANT DETERMINATION

UV-Visible measurements were used to calculate the association constants and extinction molar coefficient of complexes with Schiff bases compounds and Cu(II) or Ni(II) dissolved in methanol (Belay, 2010). The acetate and perchlorate anions acted as counter-ion, outside the coordination sphere. Copper and nickel titration solution (0.4 mmol L^{-1}) were performed with the synthesized ligands solutions, ranging from 0.4 to 2.0 mmol L^{-1}. The complexes gave an absorption peak at 698 nm for [Cu-valen], 586 nm for [Cu-ovalen], 573 nm for [Cu-dhben], 392 nm for [Ni-valen], 455 nm for [Ni-ovalen] and 540 nm for [Ni-dhben]. These absorption peaks were used for analytical measurements. Corresponding spectra were recorded at room temperature, 10 min after each Schiff base compound addition. The experiment was carried out in triplicate.

4. ANTIOXIDANT CAPACITY ASSAY

Our research group determined the chelating activity of valen, ovalen and dhben compounds by Cu(II) or Ni(II) ions based on method described in literature, with modifications (Lopez-Martínez, et al., 2017). In this experiment, 1 cm of magnesium ribbon was cleaned with steel wool and weighed. Samples of each Schiff base at concentrations 6, 10 and 15 mmol L^{-1} were incubated per 5, 10 and 15 minutes, with and without magnesium and with or without Cu(II) or Ni(II) ions (6 mmol L^{-1}). Samples absorbance were measured at the complex formation wavelengths. The absorption peak for each complex were: 698 nm for [Cu-valen], 586 nm for [Cu-ovalen], 573 nm for [Cu-dhben], 392 nm for [Ni-valen], 455 nm for [Ni-ovalen] and 540 nm for [Ni-dhben]. Samples with Schiff base and metal ions were adopted as positive control and the antioxidant efficacy were verified through magnesium oxidation inhibition in the presence of the metals.

5. DPPH ASSAY

The modified spectrophotometric method uses 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical at its specific absorbance wavelength (515 nm). Absorbance decreases when the radical is reduced by antioxidants. DPPH· radical scavenging activity was examined using the described method in literature (Zang, et al., 2017). The assay was realized mixing 65 μM DPPH concentration with different Schiff base compounds, at different concentrations, dissolved in methanol, ranging from 0.2 to 6.2 mmol L^{-1} and incubated for 60 min in the dark.

RESULTS AND DISCUSSION

1. CHARACTERIZATION OF THE SCHIFF BASE COMPOUNDS

Infrared spectra of the Schiff base compounds showed the expected characteristic imine ν(C=N) band situated in the range from 1610 to 1650 cm^{-1}. Another band in the region 1200-1236 cm^{-1} has been assigned to phenolic C-O stretching, according with bis(o-vanilin)ethylenediimine synthesis (Silverstein, Webster & Kiemle, 2006).

^{1}H NMR spectra of Schiff base compounds **1-3** were well resolved and chemical-shift assignments were based on the analysis of the multiplicity patterns of proton resonances. The ^{1}H NMR spectra were compared with others spectra in the literature, which confirmed the desired products formation (Costes, Laussac & Nicodème, 2002; Sacarescu, et al., 2005). An important assignment for these class of compounds are the protons from the imine and methylene group, confirming the ovalen ^{1}H NMR spectra singlets at δ 8.33 and 3.96 ppm; in valen δ 8.18 and 3.78 ppm; in dhben δ 8.35 and 3.78 ppm, respectively.

Electronic spectra of the three compounds were registered in methanol. In the valen spectra, two bands were observed at 360 and 407 nm ($\varepsilon = 9.7 \times 10^2$ mol^{-1}L cm^{-1}and 4.2×10^2 mol^{-1}L cm^{-1}, respectively). In ovalen compound, there were two intense bands at 274 and 331 nm ($\varepsilon = 1.6 \times 10^3$ mol^{-1}L cm^{-1} and 3.8×10^3 mol^{-1}L cm^{-1}, respectively). Similarly, dhben compound exhibited an absorption band with maximum at 387 nm ($\varepsilon = 2.0 \times 10^3$). These bands can be attributed to internal ligand transitions ($\pi \rightarrow \pi*$) (Silverstein, Webster & Kiemle, 2006).

After elucidated the compounds structure by spectroscopic methods, stability constants of compounds were determined. The ability to bind nickel and copper in the presence of magnesium metal and to promote inactivation of free radicals by DPPH method was investigated.

2. BINDING CONSTANT DETERMINATION

The binding constant between the three Schiff base compounds, as electron donor systems (D), and Cu(II) and Ni(II), as electron acceptors (A), were determined using the Benesi-HildeBrand and modified Benesi-Hildebrand equation, respectively, in conjunction with the nonlinear fit analysis in Origin program.

Binding constant (K^{AD}) and molar extinction coefficient (ε^{AD}) for Cu (II)-D complex have been determined using the Benesi-Hildebrand equation (Eqn 1) (Wang, et al., 2017).

$$\frac{[A]_o}{Abs} = \frac{1}{K^{AD}\varepsilon^{AD}} \cdot \frac{1}{[D]_o} + \frac{1}{\varepsilon^{AD}} \qquad (1)$$

Where Abs is the absorbance of the complex at a maximum of its charge transfer band, λ_{CT}. K^{AD} and ε^{AD} are respectively the binding (association) constant, and the molar extinction coefficient of the complex AD.

The Benesi-Hildebrand equation has been modified when complex is not the only absorbing specie in the mixture, as it is the case of Ni(II)-D complex (Khouba, Benabdallah & Maschke, 2014). This equation is known as Ketelaar equation (Eqn 2) and is used to consider a significant absorbance of each component at λ_{CT} of the resulting complexes.

$$\frac{[A]_o}{A^a} = \frac{1}{K^{AD}\varepsilon^a} \cdot \frac{1}{[D]_o} + \frac{1}{\varepsilon^a} \qquad (2)$$

Where A^a (Eqn 3) and ε^a (Eqn 4) represent the apparent absorbance and the apparent extinction coefficient of the resulting complex AD, respectively.

$$A^a = A - A^A - A^D \qquad (3)$$
$$\varepsilon^a = \varepsilon^{AD} - \varepsilon^A - \varepsilon^D \qquad (4)$$

Where A represents the absorbance of the mixture at λ_{CT};

$A^A = \varepsilon^A [A]_0 l$: initial absorbance of the acceptor in the absence of the donor;

$A^D = \varepsilon^D [D]_0 l$: initial absorbance of the donor in the absence of the acceptor;

ε^A and ε^D represent the molar extinction coefficients of the acceptor and the donor respectively, at λ_{CT}, and l is the optical path length. The initial concentrations of A and D in the mixture are $[A]_0$ and $[D]_0$, respectively.

Both acceptor and donor absorb at a maximum of the wavelength λ_{CT} of the complex AD. The absorbance of the mixture (A) can be written as Eqn 5.

$$A = \varepsilon^A [A]l + \varepsilon^D [D]l + \varepsilon^{AD} [AD]l \qquad (5)$$

Where [AD] represents the concentration of the complex in the mixture and is expressed by the Eqn 6.

$$[AD] = \frac{K^{AD}[A]_0[D]_0}{1 + K^{AD}[D]_0} \qquad (6)$$

The Eqn 7 is obtained in conjunction with Eqns 5 and 6.

$$\frac{A - A_D}{A_A} = \frac{\frac{1}{K} + \gamma[D]_0}{\frac{1}{K} + [D]_0} \qquad (7)$$

Where $\gamma = (\varepsilon^{AD} - \varepsilon^D)/\varepsilon^A$. Thus, Eq. 7 becomes into Eqn 8's form.

$$y = \frac{a + bx}{a + x} \qquad (8)$$

The fitting parameters a and b can be expressed as Eqns 9 and 10, respectively.

$$a = \frac{1}{K^{AD}} \qquad (9)$$

$$b = \gamma = \frac{(\varepsilon^{AD} - \varepsilon^D)}{\varepsilon^A} \qquad (10)$$

Where $y = (A - A^D)/A^A$ (the reduced absorbance at λ_{CT}) and $x = [D]_0$ (the initial concentration of the donor). Binding constant K^{AD} and molar extinction coefficient ε^{AD} can be calculated from the parameters a and b, obtained by the nonlinear curve fit and Eqns 9 and 10.

The Eqns 1 and 2 are valid under the condition $[D]_0 > [A]_0$ for complexes of the type 1:1 (one donor to one acceptor), the variation of $[A]_0/A^\alpha$ against $1/[D]_0$ leads to a linear relationship, with a slope defined by $1/(K^{AD}\varepsilon^A)$ and the intercept $1/\varepsilon^A$. A rough estimation of ε^{AD} and K^{AD} complexes constants are deduced and used for nonlinear fit analysis (Wang, et al., 2017; Khouba, Benabdallah & Maschke, 2014).

3. ANALYSIS OF THE OBTAINED UV-VISIBLE DATA

The spectrophotometric data of methanolic solutions of the starting reagents Cu(II), Ni(II), valen, ovalen, dhben, and their resulting complexes [Cu-valen], [Cu-ovalen], [Cu-dhben], [Ni-valen], [Ni-ovalen], [Ni-dhben] are summarized in Table 1. The obtained UV-Visible data were used to estimate the values of the formation constants and the molar extinction coefficients (K^{AD} and ε^{AD}) by Eqns 1 or 2.

Table 1. Wavelengths λ_{max} and molar extinction coefficient ε of starting reagents and resulting complexes, in methanol.

Compounds	λ_{max} (nm)	ε (mol^{-1}L cm^{-1})
[Cu(ClO$_4$)$_2$]	847	3.2 x 10^2
[Ni(CH$_3$COO)$_2$]	**415**[a]	8.4 x 10^2
Valen	360; 407	9.7 x10^2; 4.2 x 10^2
Ovalen	274; 331	1.6 x 10^3; 3.8 x 10^3
Dhben	387	2.0 x 10^3
[Cu-valen]	698	64
[Cu-ovalen]	586	2.2 x 10^2
[Cu-dhben]	573	3.0 x 10^2
[Ni-valen]	**392**[b]	
[Ni-ovalen]	**455**[b]	
[Ni-dhben]	**540**[b]	

[a] The visible band of nickel (II) in methanol.
[b] Charge transfer bands of the Ni-Schiff base complexes.

The metal and Schiff base compounds activities can be observed by the UV-visible data shown in Figure 1 and 2, with an imine (2.0 mmol L^{-1}) and metal concentration of 0.4 mmol L^{-1}.
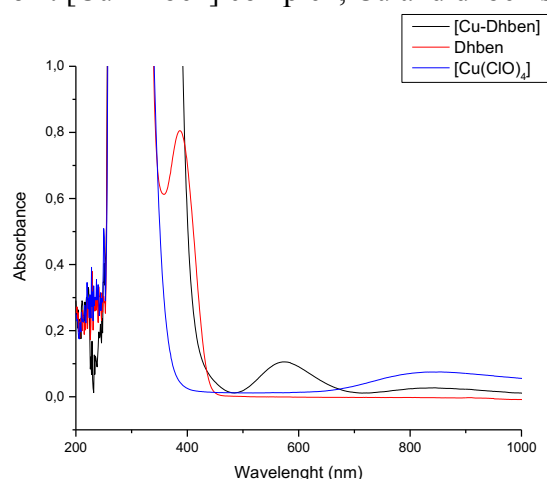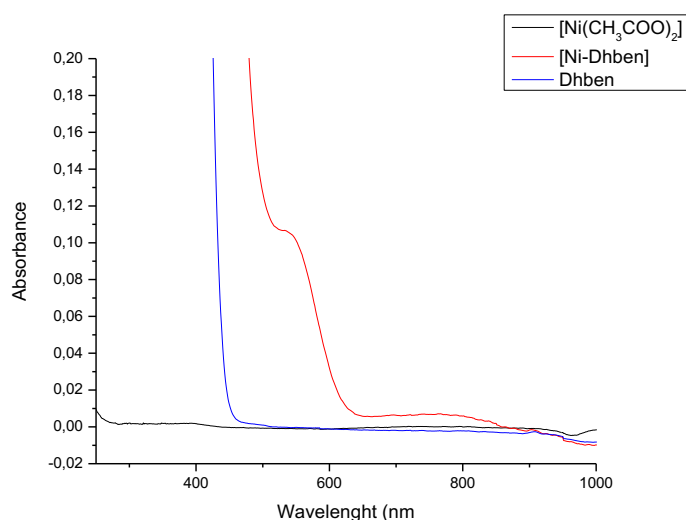
Figure 1. [Cu-Dhben] complex, Cu and dhben spectra.

Figure 2. Dhben-Ni complex, Ni and dhben spectra.

Cu-complexes data were applied in Eqn 1 to estimate the binding constants K^{AD} and the molar extinction coefficient ε^{AD} values, whereas they absorb in different regions than Cu(II). The plots, for each complex, lead to a straight line with a good correlation factor ($R^2 > 0,98$) (Figure 3 a-c).

Figure 3. Plots $[Cu]_0/Abs$ against $1/[Imine]_0$ according to the Benesi-Hildebrand Eqn 1, for (a) [Valen-Cu], (b) [Ovalen-Cu] and (c) [Dhben-Cu] complexes. The experimental data are shown by symbols, whereas continuous lines represent the theoretical linear fits. The absorption peak at 698 nm for [Cu-valen], 586 nm for [Cu-ovalen], 573 nm for [Cu-dhben] were used for the analytical measurements.
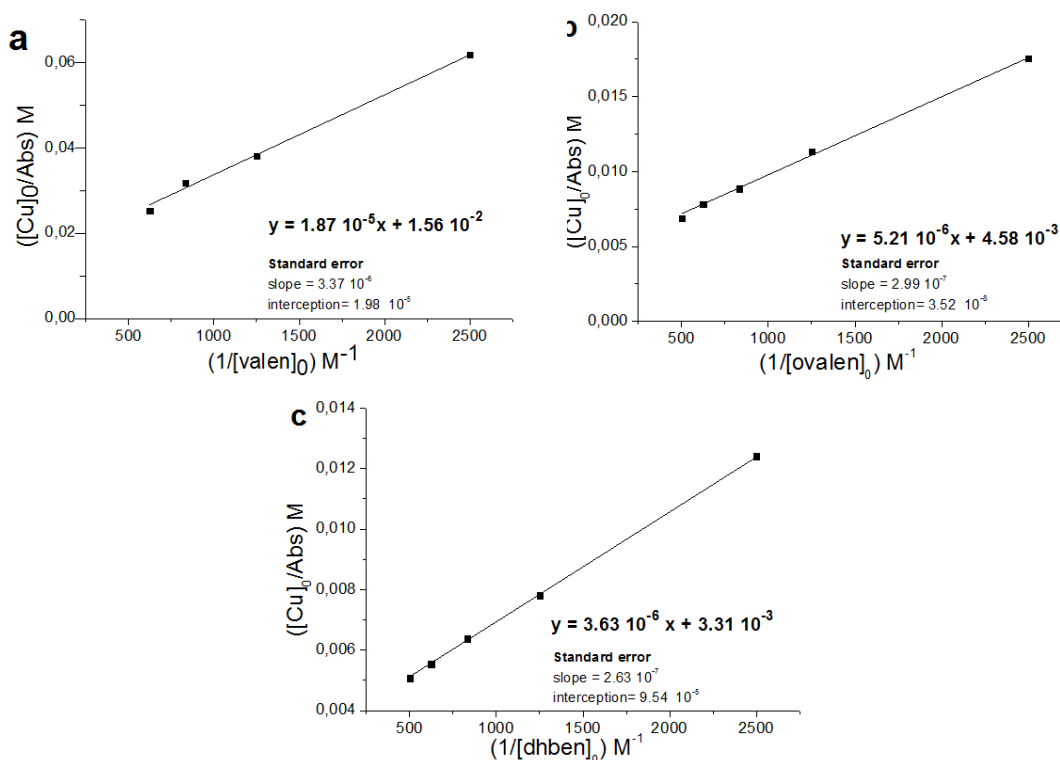

Ni-complexes absorb in the same region that the Ni(II) and for this reason the Ketelaar equation (Eqn 2) was employed to estimate the binding constants K^{AD} and the molar extinction coefficients ε^{AD}. The plot for each complex also leads to a straight line with a good correlation factor ($R^2 > 0,98$), as shown in Figure 4 a-c.

Figure 4. Plots $[Ni]_0$/Abs against $1/[Imine]_0$ according to the Ketelaar Eqn 2, for (a) [Ni-Valen], (b) [Ni-Ovalen] and (c) [Ni-Dhben] complexes. The experimental data are shown by symbols, whereas continuous lines represent the theoretical linear fits. The absorption peak at 392 nm for [Ni-valen], 455 nm for [Ni-ovalen] and 540 nm for [Ni-dhben] were used for the analytical measurements.
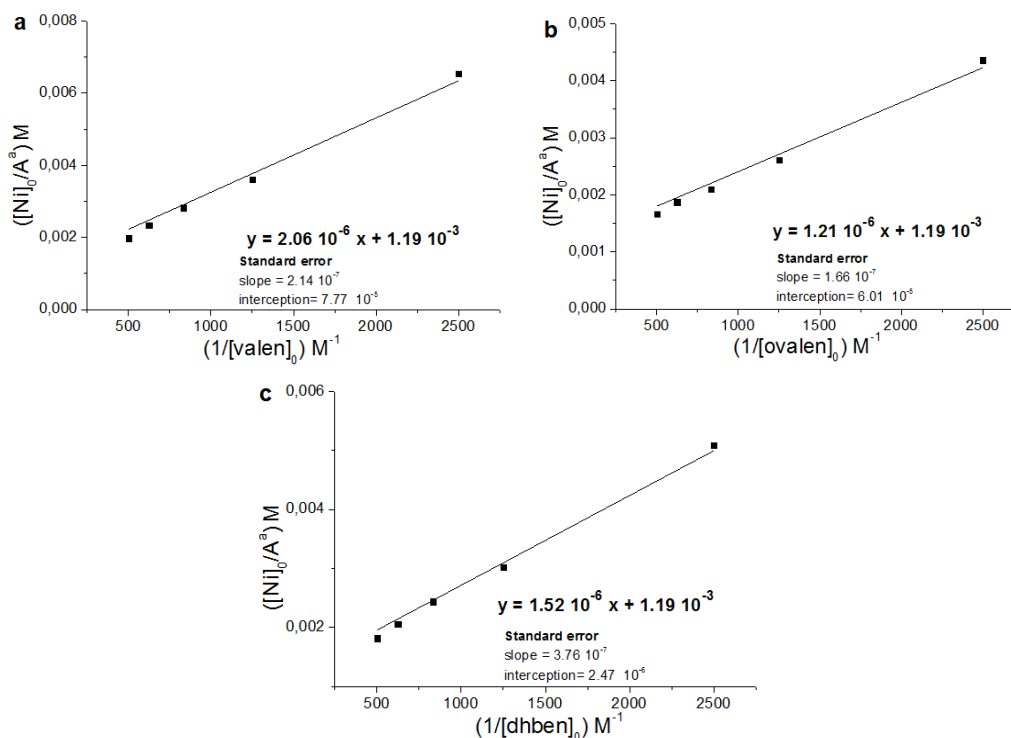
The K^{AD} values related to the Ni e Cu complexes derived from valen, ovalen and dhben were obtained from the intercept of the line with the ordinate and from the gradient. The results are summarized in Table 2.

Table 2. Values of the fitting parameters *a* and *b*, calculated formation constants K^{AD} and molar extinction coefficients ε^{AD} of the iminic complexes in methanol.

Complexes	Fitting parameters (a)	(b)	ε^{AD} (mol^{-1}L cm^{-1})	K^{AD} (L mol^{-1})	Log K
[Cu-valen]	$1.87\ 10^{-5}$	0.0157	63.8	8.38×10^2	$2{,}92 \pm 0.01$
[Cu-ovalen]	$5.21\ 10^{-6}$	0.0046	218	8.80×10^2	2.94 ± 0.01
[Cu-dhben]	$3.63\ 10^{-6}$	0.0033	303	9.09×10^2	2.96 ± 0.01
[Ni-valen]	$2.06\ 10^{-6}$	0.0012		5.83×10^2	2.76 ± 0.03
[Ni-ovalen]	$1.21\ 10^{-6}$	0.0012		9.91×10^2	3.00 ± 0.02
[Ni-dhben]	$1.52\ 10^{-6}$	0.0012		7.90×10^2	2.90 ± 0.01

The calculated values of K^{AD} constants (Table 2) related to copper complexes derived from valen, ovalen and dhben are respectively 8.38×10^2, 8.80×10^2 and 9.09×10^2 L mol^{-1}. From these results, the order of stability of copper complexes can be established as: [Cu-dhben] > [Cu-ovalen] > [Cu-valen]. For nickel complexes derivate from valen, ovalen and dhben, the K^{AD} constants are respectively 5.83×10^2, 9.91×10^2 and 7.90×10^2 with the order of stability corresponding to: [Ni-ovalen] > [Ni-dhben] > [Ni-valen].

Among copper complexes, dhben seems to lead to a stable complex, which can be explained by the high electronic density of the hydroxyls position and iminic clusters, increasing the nucleophilicity of the ligand, consolidating the ligand-copper links and stabilizing the complex. The [Cu-ovalen] complex seems to be stable than its analogue [Cu-valen]. It can be also explained by the hydroxyls position and iminic clusters.

For nickel complexes, ovalen seems to lead to a stable complex, being followed by dhben. This can be explained by the same reason of their stable copper complexes.

4. ANTIOXIDANT CAPACITY ASSAY

The chelating activity of valen, ovalen and dhben compounds by Cu(II) or Ni(II) ions were determined spectrophotometrically in presence of magnesium. It was observed the wavelength (λ_{max}) of each compound with and without about 1 cm of magnesium and with or without Cu(II) or Ni(II) methanol solution (6 mmol L^{-1}) to verify if there was an absorption band decrease. The antioxidant capacity was determined using the Eqn 11 (Ramalho & Jorge, 2005).

$$Antioxidant\ capacity(\%) = \frac{[A_a]}{[A_0]}.100 \qquad (11)$$

Where A_a is the maximum compound absorbance at its wavelength in the presence of magnesium; and A_0 is the positive control absorbance, which is taken at the maximum in absence of magnesium wavelength. The calculated antioxidant capacity values are shown in Figure 5 and 6.

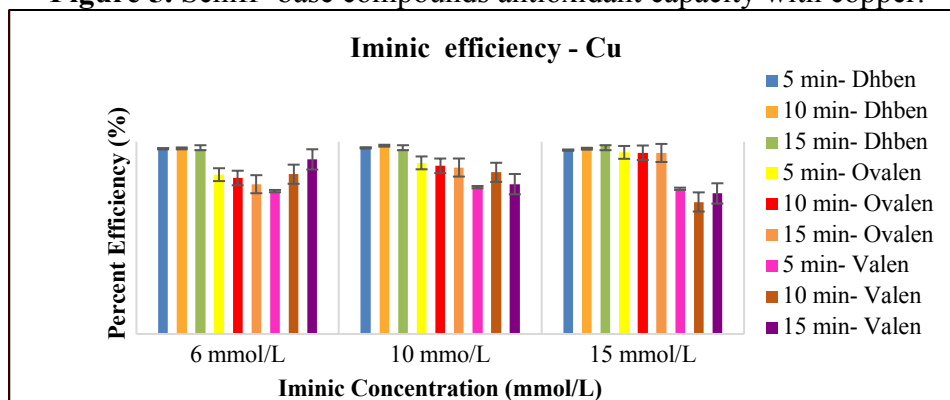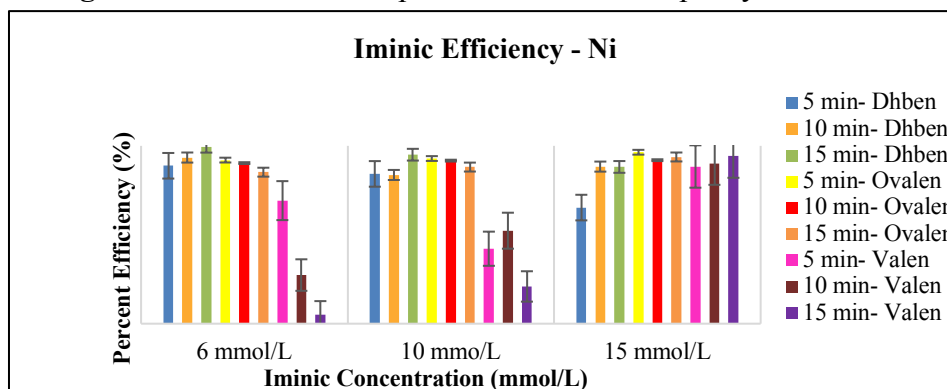**Figure 5.** Schiff- base compounds antioxidant capacity with copper.

Figure 6. Schiff- base compounds antioxidant capacity with nickel.

Spectrophotometric data revealed that dhben and ovalen are better Cu(II) and Ni(II) chelating agents than valen, because the metal ions cannot be released from the ligands causing magnesium oxidation. The decreased absorbance would be expected if the Cu(II) or Ni(II) were released from Schiff base compounds.

The chelating activity of ovalen in the presence of nickel ions is better than in copper ions, while dhben is better in the presence of copper ions. However, the ovalen chelating activity is better in the presence of copper ions even in lower compound concentration.

5. Dpph assay

The Schiff base antioxidant activities were measured in terms of their radical scavenging ability by UV–vis spectrophotometer using the stable DPPH (Zang, et al., 2017; Bernini, et al., 2018). The decrease in absorbance at 515 nm was converted into percentage by using the Eqn 12.

$$Antioxidant\ Activity\ (AA)[\%] = 100\left[1 - \frac{Abs_{sample}}{Abs_{control}}\right] \qquad (12)$$

The DPPH scavenging activity of the different Schiff base compounds concentration are shown in Figure 7 and 8.

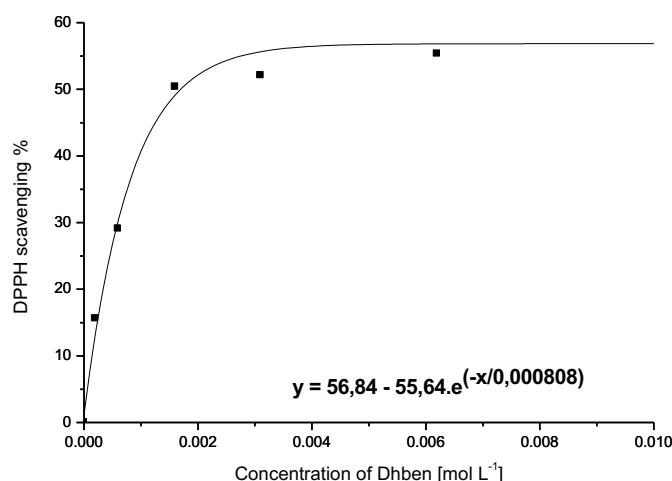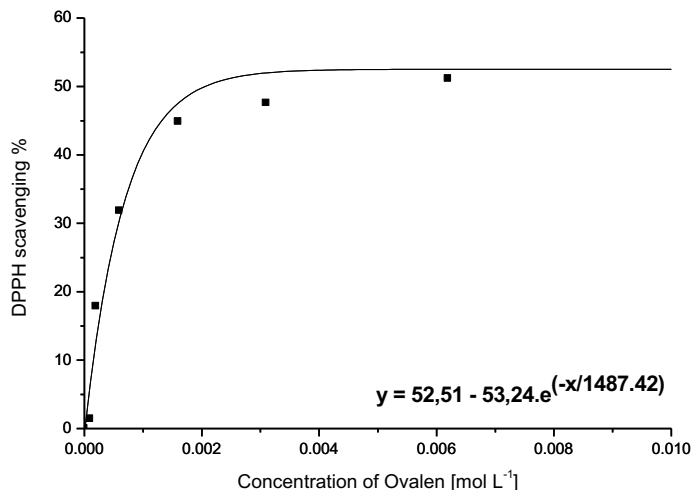**Figure 7.** DPPH scavenging activity of dhben.

$y = 56,84 - 55,64.e^{(-x/0,000808)}$

Figure 8. DPPH scavenging activity of ovalen.

$y = 52,51 - 53,24.e^{(-x/1487.42)}$

The valen compound does not show antioxidant activity, because the DPPH radical was not scavenged even at high concentration of Schiff base compound.

The decrease in DPPH absorbance at 515 nm was observed for both dhben and ovalen concentration increasing. The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a substance in inhibiting other. The dhben IC_{50} is $1.79 \ 10^{-3}$ mol L^{-1} and for ovalen it is $2.12 \ 10^{-3}$ mol L^{-1}. Therefore, the free radical scavenging activity of the dhben was greater than ovalen activity.

CONCLUSION

The Schiff base compounds valen, ovalen and dhben were characterized and their binding constant with nickel and copper metal were determined. Through the binding constant, in general, it was realized that the tested Schiff bases have better affinity with copper than with nickel.

The dhben has greater ability in chelating copper ions than nickel ions in low concentrations and ovalen is also a great chelating agent, being better with nickel than with copper. The results agree with the obtained binding constants 9.09×10^2 L mol^{-1} for [Cu-dhben] and 9.91×10^2 L mol^{-1} for [Ni-ovalen].

In DPPH assay, dhben and ovalen were capable to decrease DPPH absorbance concentration. However, dhben could reach the IC_{50} in low concentrations, and ovalen could not reach the IC_{50} according to the obtained fitting. Therefore, dhben presented a better performance than ovalen.

In general, the valen did not have a good performance in any analysis. Both dhben and ovalen are good Cu(II) and Ni(II) chelating agents, but when it is about free radicals, dhben is the best agent. So dhben compound can do both efficiently – scavenge free radicals and chelate metals. For example, TBHQ, BHA and BHT are effective antioxidant industrial in trapping free radicals but not as good at chelating metals.

Further studies with this compound used as antioxidant in biodiesel should be conducted. Antioxidant compounds are added in biodiesel to increase its oxidative stability because biodiesel has thermal and microbial decomposition and low oxidative stability.

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