Protocatechuic acid-metal-nicotine complexation study for chelation of smoking-related poisoning

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Nephropathy in tobacco smoker is postulated to be caused by nicotine (Nic) and catalyzed by various heavy metals (M(II) = Ni(II), Pb(II), and Cd(II)) contained in commercial cigarettes. Once absorbed through the lungs, these compounds will eventually be ended in the kidney, and thus, trigger various diseases. Metal chelation therapy is known as one of the efficient treatments to reduce the level of toxic metal in intoxicated patients. Here the interaction tendency of Nic and heavy metals to form complexes was investigated at a physiological condition (310.15 K and 0.15 mol·dm -3 NaCl). The stability constant of the metal-Nic complex obtained in this study shows the possible binding between these two compounds. For the simultaneous removal of heavy metals and Nic, protocatechuic acid (PCA) was used since this antioxidant is also known to be deposited in the kidneys. Thus, the interaction between PCA, Nic, and heavy metals is possible. In this work, stability constant of NiPCANic, NiPCA2Nic, PbPCANic, CdPCANic, and CdPCA2Nic were determined. Species distribution diagram of the ternary system showed that MPCANic complexes could be dominantly formed in pH 7.4 system when an excess amount of antioxidant was added. This result suggests the possible usage of antioxidants for the simultaneous removal of Nic and heavy metals via the metal chelation principle.

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1. Introduction

People with tobacco smoking habits are prone to experience kidney disorders known as nephropathy. Formerly nicotine (Nic) in tobacco was thought to be the sole cause of nephropathy, but this only became a contemporary theory. Many studies have proven that, during smoking, not only Nic but also heavy metals enter the body and then be deposited in kidneys — thus aggravating nephropathy and triggering vascular endothelium damage or other health problems. Nic is an active compound that affects the nervous systems and causes addiction in many cases of tobacco addicts [1,2]. For many tobacco addicts, a sudden cessation of Nic supply (during Nic withdrawal attempt) can cause nausea, sore throat, headache, restlessness, insomnia, constipation, and tingling, thus known as Nic withdrawal syndrome. The peak of these symptoms is perceived between two to three days after withdrawal, which is a critical phase where addicts often gave up [3,4]. In the end, the accumulation of Nic and heavy metals in the kidneys is still an issue for tobacco addicts.

The content of heavy metals in one cigarette varies from 0.5–1.5 μg for Cd, around 1.2 μg for Pb, and 0.078–5 μg for Ni [3–8]. Many studies show that heavy metal from tobacco smoke accumulated in some crucial organs such as kidneys, lungs, brain, and blood cells [4,7–9]. Nic, through its chelating action, is reportedly able to bind to some heavy metals, thus reduce the toxicity of heavy metals [10]. However, along with the consumption of Nic from tobacco smoking, heavy metals will also enter the body and create an endless detox-retoxi cycle.

As a breakthrough to this endless cycle, we proposed the usage of an antioxidant, namely protocatechuic acid (PCA: 3,4-dihydroxybenzoic acid), for heavy metals and Nic intoxication.

PCA is an antioxidant commonly found in fruits, nuts, vegetables, and various herbal medicines. A study by Ma et al. shows that PCA can be readily absorbed into humans’ and animals’ body through metabolism and tends to accumulate in the kidneys after administration [11]. Various studies reported the massive usage and benefit of PCA as a potent antioxidant, antimicrobial, anti-inflammatory agent, as well as protective against heavy metal toxicity [12–15]. Nevertheless, PCA is known for its potential therapeutic activity against numerous diseases.
As mentioned, several studies have shown that the kidney is one of the organs where Nic, PCA, and heavy metals built up \[1,2,9,11\]. Thus, it is expected that these accumulated substances will interact with each other.

In this work, the interaction of metal-ligand complex formation between PCA and Nic as organic ligands and heavy metals was studied. Here we reported that complex formation between PCA, divalent heavy metals (M(II) = Ni(II), Pb(II), and Cd(II)) and Nic are possible due to the presence of active binding sites. Specifically, Nic possesses nitrogen active metal-binding sites in its pyridine and pyrrolidine ring, while PCA possesses three oxygen binding sites in the form of carboxyl and hydroxyl groups. The complex formation constants of binary system of M(II)-PCA or M(II)-Nic, and ternary system of PCA-M(II)-Nic were investigated in this work, with the aim to prove the chelating ability between the investigated substances which can lead to heavy metal and Nic detoxification. The knowledge of the interaction of these compounds in binary and ternary systems will be the foundation for the study of their complex interaction with other coexisting compounds in human blood plasma.

2. Materials and methods

2.1. Materials and solutions

All chemicals used were of analytical grade and were used without any pretreatment. The solutions were prepared daily in distilled deionized water with 18.3 MΩ any pretreatment. The solutions were prepared daily in distilled deionized water with 18.3 MΩ.

2.2. pH-potentiometric titration

The pH-potentiometric titration was done according to the method described by Chandra et al. \[16\]. The solutions were prepared in a 50 mL volumetric flask with composition as listed in Table 1. Prior to titration, each mixture was kept in 100 mL double-walled equilibrium cell at maintained temperature 310.15 K for at least 15 min to reach an equilibrium temperature. Afterward, each solution was titrated against \(-0.1 \text{ mol·dm}^{-3}\) standardized carbonate-free NaOH. The complexation studies were done in aqueous solution ionic strength 0.15 mol·dm\(^{-3}\) NaCl to mimic the human body fluid. The titrations were performed on 888-Titrando autotitrator equipped with an Ecolab Electro Plus pH glass electrode, 805 Dosimat, 802 stirring rod, and 804 Ti stand. For the setting of titration parameter and data acquisition, the autotitrator was connected to a computer equipped with Tiamo 2.3 software. For each solution mixture, at least three repetitions of pH-potentiometry titration were done.

2.3. Data analysis

Hyperquad2008 program was used to process the obtained titration curves. This program uses a non-linear least-square algorithm to fit the proposed composition of complex species with the titration curve from pH-potentiometric titration and signified the best-fitted model by its sigma value \[17\]. For each fitting refinement, at least 100 points were used. From here, the protonation constants of the ligand (L), and stability constant of the metal-ligand in both binary and ternary system can be determined and expressed as the overall formation constant \((\log \beta)\). The higher value of \(\log \beta\) indicated more excellent stability of the metal-ligand complex formed \[18\]. Once obtained, \(\log \beta\) values of various metal-complex species were introduced to the HySS2009 program to simulate and illustrate the distribution of every possible species in the system at different pH \[19\].

3. Result and discussion

The titration curve of the system containing Ni(II) is presented in Fig. 1. Here, the representative curve includes titration curves of PCA, Nic, PCA/Nic + Ni(II) at the metal-ligand ratio of 1:1, and PCA + Nic + Ni(II) at ratio 1:1:1. In both of the PCA + Ni(II) and Nic + Ni(II) systems, the curves shifted to lower pH compared to the respective PCA and Nic only. This shifting indicates that the formation of M(II)–ligand complex occurred when the ligand released its proton; thus, the buffer region was shifted to lower pH values.

3.1. Protonation constant

The protonation constant of the ligands of Nic and PCA are presented as stepwise dissociation constant \((pK_a)\) in Table 2. The sequence of dissociation of Nic is shown in Fig. 2. The first acid dissociation \((pK_a1)\) of Nic is 3.01, which occurs at the ammine site of its pyridine ring. The second dissociation \((pK_a2)\) has a value of 7.85, which accounts for amine in the pyrrolidine ring. The obtained \(pK_a\) is comparable to the previously reported data. However, slight differences can be observed due to the variance in the experimental condition used (temperature and electrolyte) \[20–22\].

Re-determination the \(pK_a\) value from PCA, in this study, yields an excellent agreement with our previous study \[16\]. The \(pK_a\) values for PCA are presented in Table 2, where these values sequentially refer to dissociation at the carboxylic site and the two hydroxyl sites of benzenediol ring.

Table 1

<table>
<thead>
<tr>
<th>Systems</th>
<th>[M]: [L] ratio</th>
<th>[M] (mol·dm(^{-3}))</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>-</td>
<td>1·10(^{-3})</td>
<td>Determination of protonation constant</td>
</tr>
<tr>
<td>M:L</td>
<td>1:1</td>
<td>1·10(^{-3})</td>
<td>Determination of metal-ligand complexes in the binary system</td>
</tr>
<tr>
<td></td>
<td>1.2</td>
<td>5·10(^{-4})</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:3</td>
<td>4·10(^{-4})</td>
<td></td>
</tr>
<tr>
<td>M:1Nic;</td>
<td>1:1:1</td>
<td>1·10(^{-3})</td>
<td>Determination of metal-ligand complexes in the ternary system</td>
</tr>
<tr>
<td>LNic</td>
<td>1:2:2</td>
<td>1·10(^{-3})</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Potentiometric titration curve represented by Ni(II) for the metal.
3.2. Stability of the binary and ternary system

From the Hyperquad2008 program, the stability constants of M(II)-PCA and M(II)-Nic binary complexes along with ternary complex PCA-M(II)-Nic were obtained as overall formation constant (log β) and is presented in Table 3. Overall, the system containing Ni and Pb have comparable stability constant value disregards of the binding ligands, while the one containing Cd showed the lowest stability constant. For the binary complexes of Nic, 1 to 1 metal-ligand complex can be observed with log β 3.83, 3.82, and 3.40 for NiNic, PbNic, and CdNic, respectively. So far, there have been no studies reported the stability constant of Ni with Pb(II) or Cd(II) ions; only Fazary et al. has reported the stability constant of Ni(II) with monoprotonated nicotine NicH (log β[ NicH ] = −2.64) [20]. The NiNic stability constant obtained in our work is significantly higher than the previously reported NiNicH. This is due to the high electronegativity of Nic, which is fully deprotonated so that it can attract and bind the metal cations more strongly. Compared to the stability of Ni(II) and pyridine binding, it seems that the binding mechanism of M(II)-Nic may not be the sole contribution of amine site in pyridine ring because the stability of Ni(II)-pyridine complex is considerably smaller (log β[ Nic-pyridine ] = 1.88) than the one obtained in this work [23]. Thus, it suggests that both amines in pyridine and pyrrolidine account for the formation of NiNic complex.

The stability constants of M(II)-PCA and PCA-M(II)-Nic have the same trend Ni > Pb > Cd. The sequence of complex stability can be evaluated from the effect of the ionic radius. As suggested by Irving-Williams, a larger ionic radius of metal demotes the stability of the complexes. Among the investigated divalent metals, Ni(II) has a smaller ionic radius of 83 pm. Meanwhile, Cd(II) and Pb(II) have a larger ionic radius of 95 and 119 pm, respectively [24]. Following this theory, the interaction of the ligand with Ni(II) produces complexes with the highest stability. According to Pearson hard-soft acid-base (HSAB) principles, metal, as well as the ligand, can be categorized based on their criteria (ionic radius, polarizability, electronegativity, affinity, etc.). PCA with R− and R-COO− groups, is a hard base, while Nic with the N-based binding groups is a borderline base [25]. The divalent metal Pb(II) and Ni(II) are borderline acids, and Cd(II) is a soft ligand. Ligand tends to form a stable complex with metals that have similar criteria. The results obtained in this study are in a good accordance with this HSAB principle, where borderline acid Ni(II) and Pb(II) form a more stable complex with the ligands compared to soft acid Cd(II).

3.3. Effect of PCA amount on detoxification performance at physiological pH

Once obtained, the log β of the complexes was introduced to the HySS2009 program, and the speciation diagram in the ternary system was generated and presented in Fig. 3A-C. In every system, free metal ions can be observed at acidic pH. The complexes of NiNic, PbNic, and CdNic started to form at pH ~3 and reach their respective peak at pH 5.78 (77.09%), 5.98 (82.18%), and 6.32 (79.09%). With further increasing pH, MPCANic complexes started to form. In the Ni-containing system, ~10–11% of NiPCANic and ~87–89% of NiPCANic occurred at pH higher than 6.5 and 7.5, respectively. Similarly, ~91–93% of PbPCANic and ~76–78% of CdPCANic can be obtained at neutral to basic pH (pH > 8). As observed in Fig. 3, the metals tend to form complexes with Nic at more acidic pH and form complexes with PCA in the binary and ternary form at neutral to basic pH.

The speciation diagram also can be used to elucidate the composition of the complexes in the system at a certain pH. Since the goal of this study is to utilize antioxidants such as PCA to reduce Nic and heavy metals poisoning, it is necessary to find out what complexes particularly formed at biological pH. As a representative, the system containing Ni(II) was used. For the system with [M]:[LPCANC]:[LNicC] ratio 1:1:1, it can be observed that at pH 7.4, 0.26% of Ni occurred in its free form, while 11.58% in Ni-Nic, 7.37% in Ni-PCA, 0.96% in Ni-PCA2, 80.02% in NiPCANic, and 0.68% in NiPCANic form.

The UV–vis spectra of Ni(II)/PCA/Nic are shown in Fig. 4. Prominent peak at 283 nm and small hump around 300-390 nm can be observed in Ni-Nic systems. While for the Ni-PCA system, broad spectra between 276 and 360 nm occurred from the UV–Vis reading. In the ternary system of NiPCANic system, the sharp peak hyperchromically pronounced
at 284 nm and small hump can be observed around 353–393 nm, which suggests the binding of Ni and Nic in the ternary system. Besides the typical NiNic spectra, the Ni-PCA-Nic system showed a characteristic broad shoulder peak of Ni-PCA, which bathochromically shifted to 300–352 nm. These UV–Vis spectra indicate the binding of Ni$^{2+}$ to PCA and Nic in the ternary system.

When the amount of PCA was increased, different composition of complexes can be found in the system. As pictured in Fig. 3D, 0.01% of Ni occurred in free form, 0.30% in NiNic, 6.00% in NiPCA, 2.38% in NiPCA$_2$, 70.75% in NiPCANic, and 20.56% in NiPCA$_2$Nic form. This result suggests that by increasing the PCA amount in the system, the free Ni and NiNic can be reduced from 11.84% to 0.31%.

In this work, the species distribution diagram of the system containing all studied heavy metals along with ligands Nic and PCA were simulated. The 1:1:1:1:1 and 1:1:1:5:1 ratio of [M$_{Ni}$]:[M$_{Pb}$]:[M$_{Cd}$]:[L$_{PCA}$]:[L$_{Nic}$] were used to understand the tendencies of the complex formation in the system with excess amount of antioxidant. These speciation diagrams were generated based on Nic in the system (Fig. 5). At concentration 3:1:1 of [M$_{Ni}$+Pb+Cd]:[L$_{PCA}$]:[L$_{Nic}$], 0.18% of Nic occurred in NicH$_2$ and NicH form, 81.70% bind to Ni, Pb, or Cd ions, and 18.11% forming a ternary complex of M-PCA-Nic (Fig. 5A). When an excess amount of PCA added, as can be observed in Fig. 5B, the composition in the system is as follow: 0.41% NiH$_2$ and NiH, 4.06% MNic, 87.93% MPCANic, and 7.60% MPCA$_2$Nic.
suggest that the addition of an excess amount of antioxidant PCA can reduce >95% of the free Nic and MNIC in the system.

The addition of PCA also can reduce the formation of free Nic and MNIC at a broader pH spectrum. At five times the molar ratio of PCA, MPCANiC complexes started to form at pH higher than 6 and reach optimum reduction at pH > 7.3, which depresses the occurrence of free Nic and MNIC to <5%. While with the system containing one molar ratio of PCA, the earliest MPCANiC formation occurred at pH 6.5 and reached its peaks at the same pH (pH > 7.31), with merely 18% of MPCANiC formed. The increased availability of PCA might not be the sole reason for the formation of MPCANiC and MPCA2NiC. Several studies postulated the molecular interaction of tertiary amines and various carboxylic acids [26–28]. The log β values of MPCA, which lie between 6.62 and 7.99, suggest that PCA act as a bidentate ligand by chelating the divalent metal ions with the deprotonated catecholate sites to form five-membered ring complex. The log β values of MPCA also found to be greater than the stability constant of Ni2+ and carboxylate site (0.67), which confirm that the binding did not occur in the carboxylate site of PCA [29]. Thus, it is possible that the free carboxylic acid site of PCA may interact with one of the amine sites, either in pyridine or pyrrolidine ring of Nic and later synergistically binds the heavy metals and promote the formation of MPCANiC and MPCA2NiC complexes.

The intake of non-nutrient antioxidants (or total phenolic compounds) consumed by elderly individuals was 2196 mg/person/day [30]. This normal daily intake is significantly greater than five times the dosage of PCA used in this study (which is 77 mg). The five times increase of PCA dosage is chosen with consideration to investigate the minimum dosage that needed to give enough therapeutic effect in the human body. Particularly for this work, the consideration is based on the simultaneous reduction of heavy metals and Nic.

4. Conclusion

The complex formation constant of MPCA, MNiC, MPCANiC, and MPCA2NiC were determined in this work. Disregard of the ligand (PCA or Nic), Pb and Ni were able to form stronger complexes compared to Cd. Similarly, in the ternary system, NiPCANiC had the largest stability constant value (log β 11.19) followed by PbPCANiC and CdPCANiC had the smallest stability constant (log β 9.73). Species distribution diagram of the ternary system showed that optimum NiNiC, PbNiC, and CdNiC formation occurred between pH 5.78 to 6.32. In a system containing a combination of all metal ions, Nic, and PCA, administration of PCA at excess amount can reduce the content of free metals, free Nic, and MNIC complexes up to 95%. This suggests the possible usage of antioxidant PCA for the simultaneous binding of Nic and heavy metals via ternary metal-ligand complex formation.

CRediT authorship contribution statement

Vania Bundjaja: Investigation, Formal analysis. Shella Permatasari Santosoto: Methodology. Writing - original draft. Writing - review & editing. Alchris Woo Go: Supervision, Resources. Ronald Wijaya: Visualization. Chi Thanh Truong: Writing - review & editing. Maria Yuliana: Validation, Writing - review & editing. Felicia Edi Soetedjo: Supervision, Writing - review & editing. Yi-Hsue Ju: Supervision, Writing - review & editing. Artik Elisa Angkawijaya: Conceptualization, Funding acquisition, Methodology, Formal analysis, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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