# Binding and Precipitation of Germanium(IV) by Four Hydrolyzable Tannins

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The complexation conditions and complex characteristics of 1,2,3,4,6-O-galloyl-β-D-glucose (PGG), a typical hydrolyzable tannin, and $Ge^{4+}$ were studied. Species of the PGG-$Al^{3+}$ complex formed in various water-methanol systems (20% to 100%, v/v) were investigated. The results showed that the amount of PGG and Ge in the precipitate was substantially influenced by the initial Ge/PGG molar ratio. The highest amount of the precipitate was obtained at the initial Ge/PGG molar ratio of 1:2 and the stoichiometric ratio (Ge/PGG molar ratio) of the precipitates gradually decreased from 0.72 to 0.30 concomitant to a decrease in the initial Ge/PGG molar ratio from 1:1 to 1:4. Precipitation experiments of $Ge^{4+}$ by four hydrolyzable tannins from Chinese gallnut (TA), chestnut shell (CT), *Platycarya strobilacea* infructescence (PT), and Valonia (VT) were compared. The results indicated that the amount of the precipitate was substantially influenced by the initial tannin/Ge ratio and pH and was slightly influenced by the reaction time. The highest amount of the precipitate was obtained at the initial tannin/Ge mass ratio of 2.3:1 and pH 7.2. Stability of the TA-Ge, CT-Ge, VT-Ge, and PT-Ge precipitates in several solutions was determined. The TA-Ge precipitates showed relatively high stability in the test solvents compared to that of other tannins.

*Keywords: Hydrolyzable tannins; Germanium; Metal ions; Complexation; Precipitation*

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## INTRODUCTION

Tannins are a type of small molecular weight (500 to 3000 Da) plant metabolite, which are a mixture of gallic acid, low molecular weight gallic acid derivatives, and gallotannins. Due to the structural differences, the authors divided tannins in two parts. One part comprises hydrolyzable tannins that can be fractionated hydrolytically into their components by treatment with hot water or with tannases. The other part comprises condensed tannins that include nonhydrolyzable oligomeric and polymeric proanthocyanidins (Karamali and Teunis 2001).

Chemically, hydrolyzable tannins contain several phenolic hydroxyl groups and can form complexes with various metal ions chelated by six or eight regular octahedron ligands or without a fixed structure (Miyamoto *et al.* 1987). Moreover, tannins are present in every aspect of human society in the form of a special seasoning for the human diet (the astringency of tea and wine) while being used in leather tanning, dyeing, and beer clarifying, and as feed additives. In the medical field, extensive efforts were made to determine that tannins have antioxidant, antibacterial, and anticancer activities and can cure cardiovascular and cerebrovascular diseases (Akiyama *et al.* 2001; Athanasiadou *et al.* 2001; Yuan *et al.* 2003; Hervé *et al.* 2006). Previous studies have demonstrated that tannins combined with metal ions, such as $Al^{3+}$, $Fe^{2+}$, $Fe^{3+}$, $Cu^{2+}$, and $Zn^{2+}$, can reduce the toxicity of metal elements and improve the biocompatibility of metal drugs (Gutierrez and Gehlen 2002; Castro and Blanco 2004; Panhwar *et al.* 2010; Rahim *et al.* 2014). A study reported that natural polyphenols extracted from certain plants have a strong effect on aluminum detoxification and that high molecular weight polyphenols (hydrolyzable tannins) have high affinity and solubility, which are beneficial for detoxification of aluminum in the environment (Zhang *et al.* 2016).

Germanium (Ge) is located in the ⅣA group of the periodic table. Similar to silicon, Ge is an excellent semiconductor, and it is widely used in rectifiers and transistors (Ravi 2011; Coteli *et al.* 2017). Moreover, Ge can be used in infrared light materials, precision instruments, and catalysts (Tao and Bolger 1997). Ge photodiodes were reported as transfer standards in the field of fibre optics in 1990s (Stock 1990). Currently 30% of Ge is being used in fibre optics (US Geological Survey 2019), which is the most important application of Ge (Pevec and Denis 2019). Ge is distributed and dispersed in the earth's crust; hence, Ge is mainly extracted from the metallurgical dust of nonferrous metal ores and power coal ashes (Powell *et al.* 2007). Yunnan Lincang Xinyuan Germanium Industrial Co., Ltd. is the largest germanium supplier in the world. The widely used method of extracting germanium is hydrometallurgical germanium recovery, which involves placing germanium ore into a neutral solution and treatment of the pregnant leach solution with various chemicals followed by recovery of germanium from the solution (Holness 1948; Liang *et al.* 2008). This method produces a waste mixture containing tannin (tannic acid) and acid, which leads to inefficiency and high cost in the germanium extraction industry. Worldwide, about 30% of the total germanium consumed is produced from recycled materials (US Geological Survey 2019). The behavior of tannins in complexation with various metal ions was investigated by Liang *et al*. (2008) to understand the complexation process of germanium by hydrolysable tannins, to reduce tannins use, and to improve the germanium recovery as a byproduct in zinc metallurgy. In our previous work (Zhang *et al*. 2018a), we studied the coordination reactions and the stoichiometry of $Ge^{4+}$-tannins and found that the pH greatly influenced the stoichiometric ratio and conditional associate constants ($\log K$) of germanium-polyphenol complexes and the characteristics of germanium-tannin complexes are strongly influenced by the molecular size of the polyphenol, the metal-polyphenol ratio, the pH, the solvents, and the metal species present in the reaction mixture. Thus, precipitation is a crucial process in recovering germanium from the blend. The choice of the most appropriate technique depends to a large extent on the type of solution used, the concentration of hydrazine in the solution, and other components of the solution. Furthermore, the choice of a suitable tannin and the optimal conditions for the precipitation is a problem that needs an urgent solution.

Four plant-derived hydrolyzable tannins from various plant materials were selected to investigate the binding and precipitation conditions of the tannin-metal complexes. Tannic acid (TA) is extracted from Chinese gallnut, a characteristic forestry resource in China, and belongs to hydrolyzable tannins (Okuda *et al.* 1995). Tannic acid applications have high value in the fields of medicine, organic synthesis, food, agriculture, minerals, *etc.* Chestnut (*Castanea mollissima* Blume) is a type of an essential commercial nut currently growing in the majority of Asian regions. In addition to the edible fruit, chestnut wood and chestnut shells contain tannin that belongs to hydrolyzable tannins. *Platycarya strobilacea* infructescence is one of the raw materials for hydrolyzable tannins in China. Ellagic acid, a crucial pharmaceutical intermediate, is obtained by hydrolysis of tannin extracted from *P. strobilacea* infructescence. Valonia (*Quercus acutissima* Carruth.) is a tree species widely cultivated in western and southwestern Asia. Valonia is rich in hydrolyzable tannins, and it is one of the main sources for industrial tannin production in China. Valonia tannin is a macromolecular polyphenol, which is a combination of lactone-like ellagic acid and gallic acid that form an ester bond (Mämmelä *et al.* 2000).

To identify the mechanism of complexation and precipitation of germanium and hydrolyzable tannins, a structured hydrolyzable tannin, 1,2,3,4,6-O-galloyl-β-D-glucose (PGG, Fig. 1a), was selected to determine the binding conditions and species of the PGG-metal complexes and precipitates (Zhang *et al.* 2018a, 2018b; Liu *et al.* 2018). Four hydrolyzable tannins, TA, CT, VT, and PT, are widely available in China and are produced by the industry. The effects of the initial Ge/tannin molar ratio, pH, and reaction time on the metal-tannin precipitates were investigated. The effect of the solvents on the stability of the Ge-tannin precipitates was also studied.

## EXPERIMENTAL

### Materials

The PGG was obtained from Chendu Biopurify Phytochemicals Ltd. (Chendu, China). The TA was from Zhangjiajie Jiurui Biotechnology Co., Ltd. (Zhangjiajie, China). Chestnut shells were collected from Qinglong County, Hebei Province, China. Valonia was collected from Nanyang City, Henan Province, China. *P. strobilacea* infructescence was collected from Lianyungang, Jiangsu Province, China. Tannins were extracted from chestnut shells, Valonia, and *P. strobilacea* infructescence and purified using a Sephadex LH-20 column according to the method described by Zhang and Lin (2008) to obtain chestnut shell tannins (CT), valonia tannins (VT), and *P. strobilacea* infructescence tannins (PT), respectively. A working solution of PGG (0.36 mM) was prepared in methanol while the solutions of TA (100 mg/mL, pH 3.05, and 10 mg/mL, pH 3.4), CT (10 mg/mL, pH 3.3), PT (10 mg/mL, pH 3.2), and VT (10 mg/mL, pH 3.2) were prepared in water. Germanium ($GeO_2$) was purchased from Acros Organics Company (Belgium, USA). $AlCl_3$ was purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Sephadex LH-20 was purchased from GE Healthcare Bio-Sciences (Uppsala, Sweden). Working solutions of $Ge^{4+}$ (10 mM, pH 6) were prepared as described in a previous study **(**Zhang *et al*. 2018a**)**. The mixture was then diluted with water to a required final concentration. A working solution of $Al^{3+}$ (1 mM) was prepared by dissolving $AlCl_3$ in methanol. The solutions of EDTA-2Na (5-100 mM, pH 4.5) were prepared by dissolving ethylenediaminetetraacetic acid disodium salt (EDTA-2Na) in deionized water.

#### *Equipment*

Ultraviolet-visible (UV-Vis) spectra were recorded at room temperature (25 °C) using an Agilent Cary 8454 spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) equipped with 1.0-cm quartz cells. The pH values were recorded using a pH meter (Mettler-Toledo Inc., Columbus, OH, USA). The high-performance liquid chromatography (HPLC) analyses were conducted with a Shimadzu HPLC system (Shimadzu, Kyoto, Japan) consisting of a binary pump (LC-20AB liquid chromatography), and autosampler (SIL-20AB autoinjector). Analysis of metal contents in the precipitates was performed using an inductively coupled plasma-light emission spectrometer (ICP-OES; Optima 7000DV; PerkinElmer Inc., Waltham, MA, USA). Water was delivered by a Milli-Q-water purification system and used throughout the experiments.

## Methods

### *PGG-germanium precipitation experiment*

The PGG-Ge precipitation experiment was performed as described in the authors' previous work (Zhang *et al*. 2018a). Briefly, 2.5 to 40 μL of 100 mM PGG was mixed with 100 μL of 10 mM $Ge^{4+}$ and diluted with a pH 6.0 buffer solution to 1 mL volume. The pH of the mixture was adjusted to approximately 2 with a small volume of 1 M hydrochloric acid solution. The mixture was vortexed for 10 s and then incubated at 4 °C for 1 h. Precipitate was removed by centrifugation and the amounts of PGG and $Ge^{4+}$ that remained soluble and unbound after sequential pH changes were determined by HPLC and ICP methods, respectively. Based on these data and the initial amounts of PGG and $Ge^{4+}$ in the solution, the amounts of PGG and $Ge^{4+}$ in the precipitate (or PGG/Ge molar ratio) were calculated.

### *PGG-aluminum titration experiment*

The titration experiment was performed according to Davila *et al*. (2012) with slight modifications. Methanol was used as a blank in a spectrometer cell. The stoichiometric ratios of the complex in various solvents ($H_2O$ and 20 to 100% methanol, v/v) were determined by the molar titration method. The initial concentration of 1 mL PGG was kept constant (0.36 mM), and the titration was started with the addition of 10 μL $Al^{3+}$ (1 mM) solution to the cuvette. The spectrum was recorded after 30 s of mixing for each titration step while a total volume of at least 140 μL of a metal solution was added. Analysis of the spectral data by the chemometric methods was performed using the Reactlab Equilibrium software (JPlus Consulting Inc., version 1.1, East Fremantle, Australia), and the conditional stability constant (log $K$) of the metal and phenolic compounds was evaluated at various concentrations of methanol in solution.

### *Tannin-germanium complex experiment*

The UV-Vis absorption spectra of a mixture containing 25 μL of 10 mg/mL TA, CT, PT, or VT solutions, and 2975 μL acetate buffer, pH 6.0, was titrated with 10 mM $Ge^{4+}$ (0, 5, 10, 15, and 20 μL), and the optical density was recorded at the wavelength range from 220 to 400 nm at room temperature. Water was used as a blank in a spectrometer cell.

### *Tannin-germanium precipitation experiment*

To investigate the effect of the initial tannin/Ge ratio on the amount of precipitated germanium, 1 mL of 10 mM $Ge^{4+}$ was mixed with 40 to 200 μL of 10 mg/mL solutions of various tannins. The mixture was vortexed for 10 s and incubated at 4 °C for 1 h. Precipitate was removed by centrifugation at 15,000 rpm for 10 min and the supernatant was collected for analysis. To estimate the effect of reaction time on the amount of precipitated germanium, 1 mL of 10 mM $Ge^{4+}$ solution was mixed with 160 μL of 10 mg/mL solution of a tannin. The mixture was immediately vortexed and incubated at 4 °C for 1, 2, 3, 12, and 24 h. After the reaction, the precipitate was removed by centrifugation, and the supernatant was collected for analysis. To assess the effect of pH on the amount of precipitated germanium, 1 mL of 10 mM $Ge^{4+}$ solution was mixed with 160 μL of 10 mg/mL TA, PT, CT, or VT solutions. The pH of the mixture was adjusted to the defined pH values with a small volume of 1 M hydrochloric acid solution or 1 M sodium hydroxide solution, and the solution was vortexed and incubated at 4 °C for 30 min. Precipitate was removed by centrifugation and the supernatant was collected for analysis. The content of germanium in the supernatant ($M_s$) was determined by the ICP-OES method. The amount of precipitated germanium ($M_p$) was determined as follows,

$$M_p = M_i - M_s \tag{1}$$

where $M_i$ is the initial amount of germanium (μmol) added to the solution.

*Stability of the tannin-germanium precipitate*

To investigate the effect of the solvents on the solubilization of germanium from the tannin-germanium precipitate, 1 mL of 10 mM $Ge^{4+}$ was mixed with 160 μL of 100 mg/mL TA, 10 mg/mL VT, 10 mg/mL CT, or 10 mg/mL PT to obtain four tannin-germanium precipitates. Fresh solutions of $H_2O$, $CH_3COOH$, 50% $CH_3COOH$, or aqueous EDTA-2Na (5 to 100 mM) were added to the precipitates. Then, the solutions with the precipitates were thoroughly mixed and sonicated for 5 min. Finally, the precipitate was removed by centrifugation and the supernatant was collected. The content of germanium in the supernatant was determined by the ICP-OES method and the percentage of germanium solubilized from the precipitate was calculated. The experiments were repeated three times.

## RESULTS AND DISCUSSION

### PGG-Germanium Precipitation

The amounts of precipitated PGG and germanium were substantially influenced by the initial Ge/PGG molar ratio in the solution (Fig. 1b-d). The amount of precipitated PGG gradually increased from 0.96 to 3.28 μmol concomitant to a decrease in the initial Ge/PGG molar ratio from 1:1 to 1:4. Approximately 95% of PGG was precipitated by Ge at the initial Ge/PGG molar ratios of 1:1 and 1:2, whereas the value was decreased to approximately 82% at the initial Ge/PGG molar ratio of 1:4 (Fig. 1b). The amount of precipitated Ge increased from 69.13 to 97.97 μmol concomitant to a gradual decrease in the initial Ge/PGG molar ratio from 1:1 to 1:4, and 69% of $Ge^{4+}$ was precipitated by PGG at the Ge/PGG molar ratio of 1:1; this value reached approximately 98% at the Ge/PGG molar ratio of 1:4 (Fig. 1c). The data indicate that almost all PGG and germanium were precipitated at the Ge/PGG molar ratio of 1:2; the experimental data indicate that the highest amount of precipitate was achieved at the initial Ge/PGG molar ratio of 1:2. Furthermore, the stoichiometric ratio (Ge/PGG molar ratio) of the germanium-PGG precipitate was decreased from 0.7 to 0.3 concomitant to a gradual reduction in the initial Ge/PGG molar ratio from 1:1 to 1:4 (Fig. 1d). The results indicate that germanium-PGG was precipitated with stoichiometric ratios (Ge/PGG molar ratio) of 2:3, 1:2, and 1:3 at the initial Ge/PGG molar ratios of 1:1, 1:2, and 1:4, respectively.

Zhang *et al.* (2018a) found that PGG can react with $Ge^{4+}$ to form soluble GePGG and $Ge_2PGG$ species at pH 6.0 or 4.0. The authors' study demonstrates that an increase in the amount of PGG in the solution resulted in the formation of the polyphenol-metal ion networks with concomitantly decreasing stoichiometric ratios of 2:3, 1:2, and 1:3, and subsequent formation of a precipitate. The results of the present study suggest that a stoichiometric ratio to obtain a PGG-germanium precipitate is strictly defined. Certain studies have found that the ortho-dihydroxyl group on the flavonoid B-ring of condensed tannins can complex with germanium to form a tris-chelate (Zhai *et al.* 2018). Compared with condensed tannins, hydrolyzable tannins have a higher number of ortho-dihydroxyl groups at the same molecular weight; hence, hydrolyzable tannins can complex with a higher number of germanium ions. Zhai *et al.* (2018) reported that an active flavonoid compound rutin (RU) can form a precipitate with germanium at the stoichiometric ratio (RU/Ge molar ratio) of 2:3. Quercetin (QU) can react with germanium to form GeQU and $Ge_3QU_2$ species in the mixtures containing an excess of QU or an excess of Ge (Yao and He 2008). A series of experiments determined that a defined Ge/tannin stoichiometric ratio is required to obtain a germanium-polyphenol precipitate.

**Table 1.** Conditional Formation Constants (log*K*) of the Al-PGG Complexes ($M_nL_m$) in Aqueous MeOH

| | log*K* * | | | | |
|---|---|---|---|---|---|
| | 20% MeOH | 40% MeOH | 60% MeOH | 80% MeOH | 100% MeOH |
| Al + L=AlL | 5.51 ± 0.03 | 5.06 ± 0.04 | | | |
| 2Al + L = $Al_2L$ | 9.46 ± 0.04 | 8.71 ± 0.06 | 8.31 ± 0.03 | 9.10 ± 0.04 | 9.88 ± 0.07 |

* The log*K* values were established using chemometric modeling of spectrophotometric titration data; data are given as means ± SD.

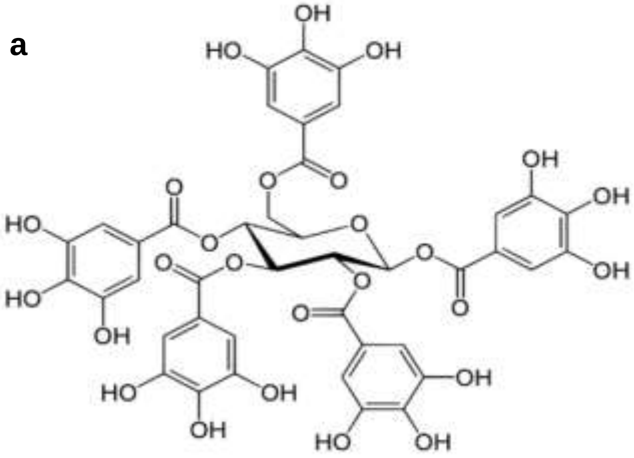


**Fig. 1a.** (a) Structure of 1,2,3,4,6-O-galloyl-β-D-glucose (PGG) Effect of the initial Ge/PGG molar ratio on percentage the of precipitated (b) PGG and (c) $Ge^{4+}$ (d) Effect of the initial Ge/PGG molar ratio on the Ge/PGG molar ratio in the precipitate

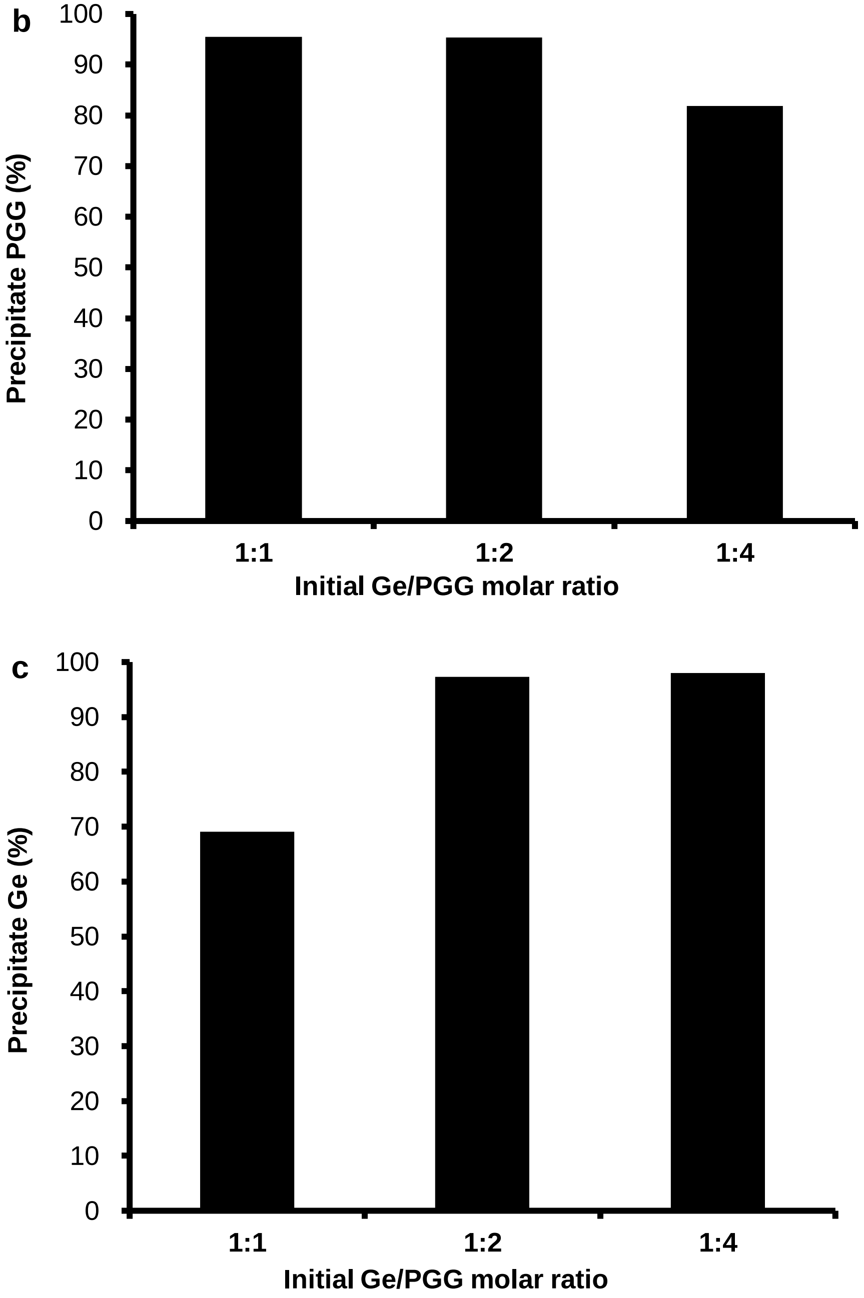


**Fig. 1b, 1c.** (a) Structure of 1,2,3,4,6-O-galloyl-β-D-glucose (PGG) Effect of the initial Ge/PGG molar ratio on percentage the of precipitated (b) PGG and (c) $Ge^{4+}$ (d) Effect of the initial Ge/PGG molar ratio on the Ge/PGG molar ratio in the precipitate

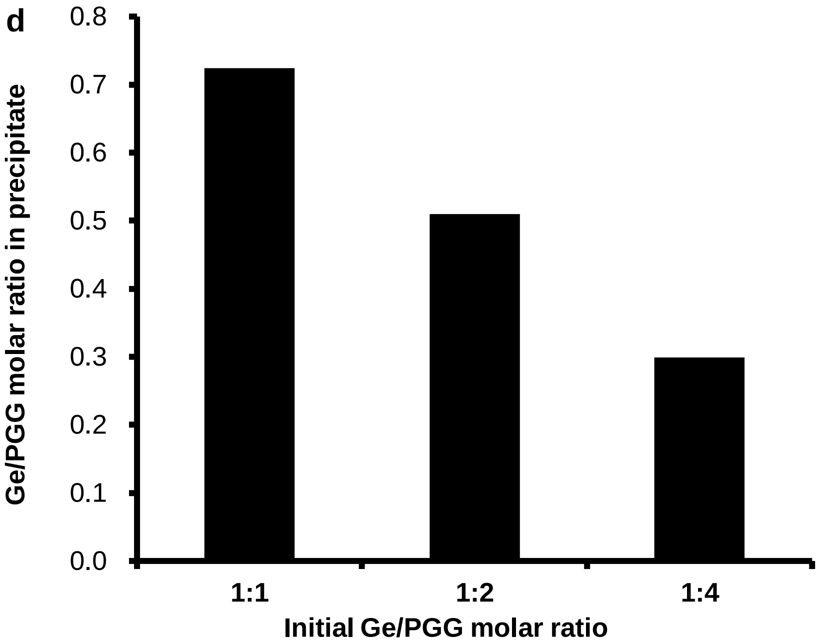
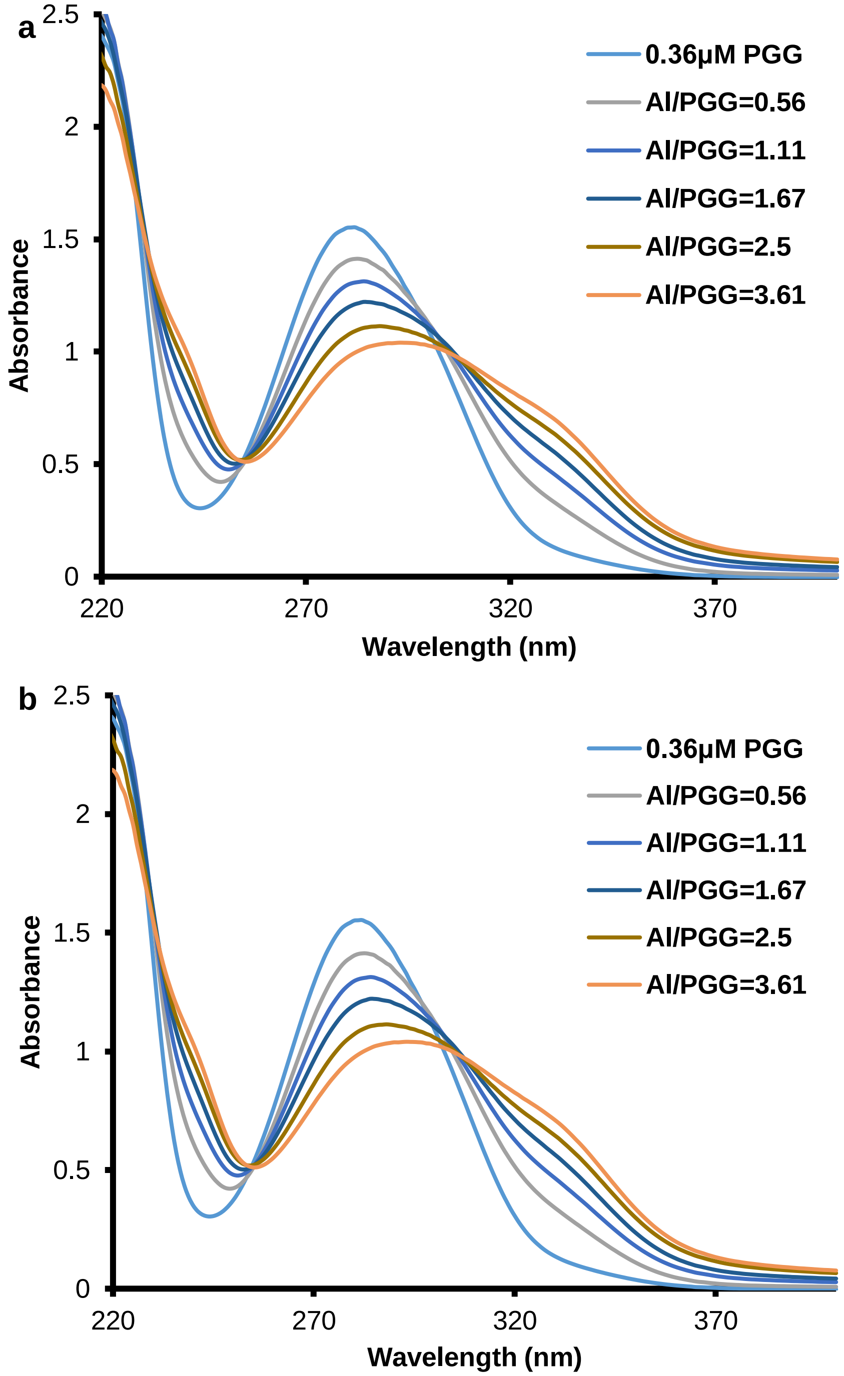


**Fig. 1d.** (a) Structure of 1,2,3,4,6-O-galloyl-β-D-glucose (PGG) Effect of the initial Ge/PGG molar ratio on percentage the of precipitated (b) PGG and (c) $Ge^{4+}$ (d) Effect of the initial Ge/PGG molar ratio on the Ge/PGG molar ratio in the precipitate

## Evaluation of the PGG-aluminum Complex

The $Al^{3+}$-PGG complex species in the aqueous methanol system was assessed by using the chemometric modeling of the spectrophotometric titration data as described by Zhang *et al.* (2018a), and the results are shown in Table 1. The UV-Vis spectra of PGG in the absence and in the presence of various concentrations of $Al^{3+}$ were investigated (Fig. 2a, b, c, d, and e). Similar to previous work by Ohyoshi (1986), Chen and Hagerman (2005), and Liu *et al.* (2018), the PGG solution exhibited a maximum absorbance at 280 nm. Addition of the solution of $Al^{3+}$ produced a distinct redshift in the maximum absorbance to 330 nm, indicating that there was a direct interaction between $Al^{3+}$ and PGG, which was consistent with the observation in the authors' previous work (Zhang *et al.* 2016). Preceding studies have indicated that the results evaluated by this method are relatively reliable and can be used to determine the log *K* values of a metal-ligand complex and the configuration of a complex formed in the reaction (Davila e*t al.* 2012).

According to the chemometric methods, AlPGG and $Al_2PGG$ were formed in 20% and 40% MeOH, while $Al_2PGG$ was the only species formed in 60% to approximately 100% MeOH under the test conditions. The conditional stability constant (log *K*) calculated for $Al_2PGG$ increased from 8.31 ± 0.03 to 9.88 ± 0.07 concomitant to an increase in methanol concentration from 60% to 100% (Table 1), indicating that the binding of $Al^{3+}$ to PGG can be enhanced in the presence of high concentrations of methanol in the reaction. These results are consistent with the experimental results of Davila *et al.* (2012). The predicted spectra are an adequate fit of the experimental data (Fig. S1) and the standard deviations of the log *K* values suggest that this method is relatively reliable.


a
2.5
2
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Absorbance
220
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320
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Wavelength (nm)
0.36μM PGG
Al/PGG=0.56
Al/PGG=1.11
Al/PGG=1.67
Al/PGG=2.5
Al/PGG=3.61
b
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Wavelength (nm)
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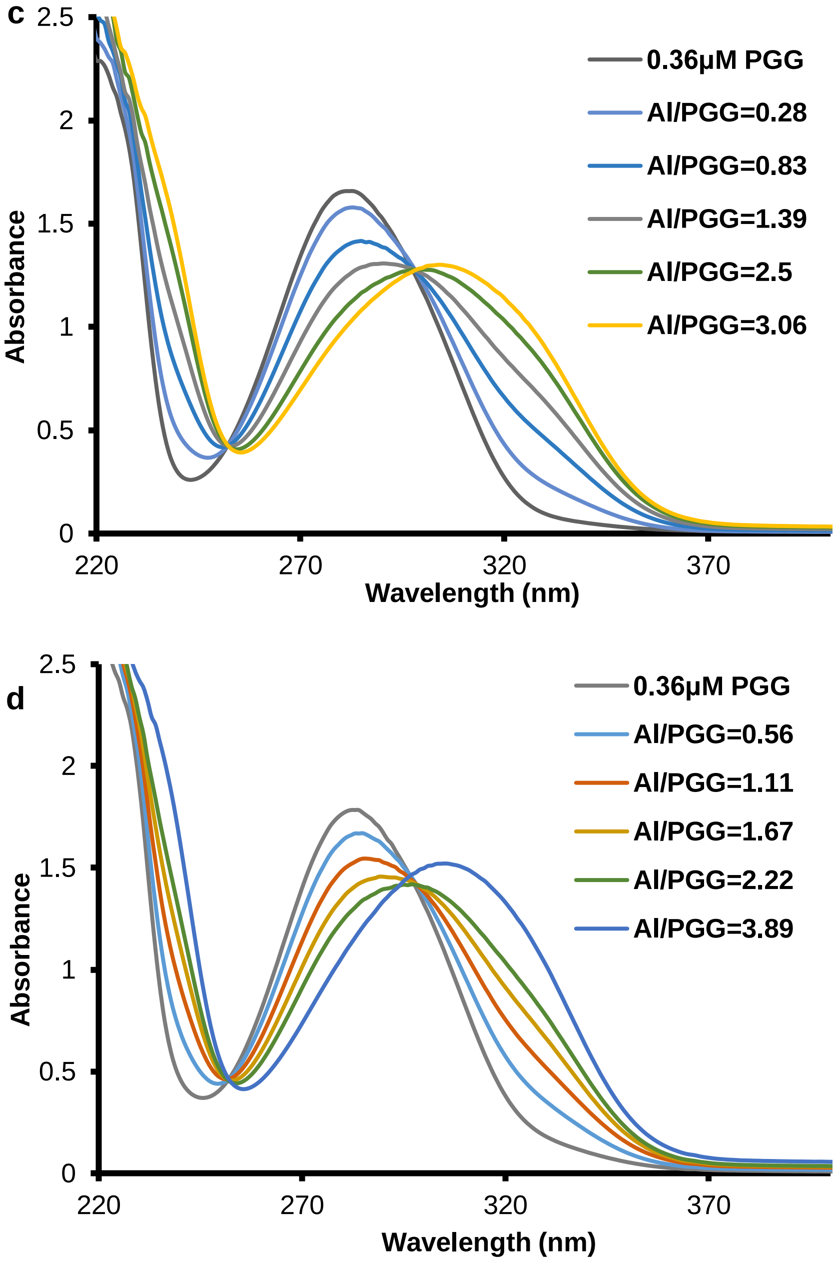
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0.36μM PGG
Al/PGG=0.28
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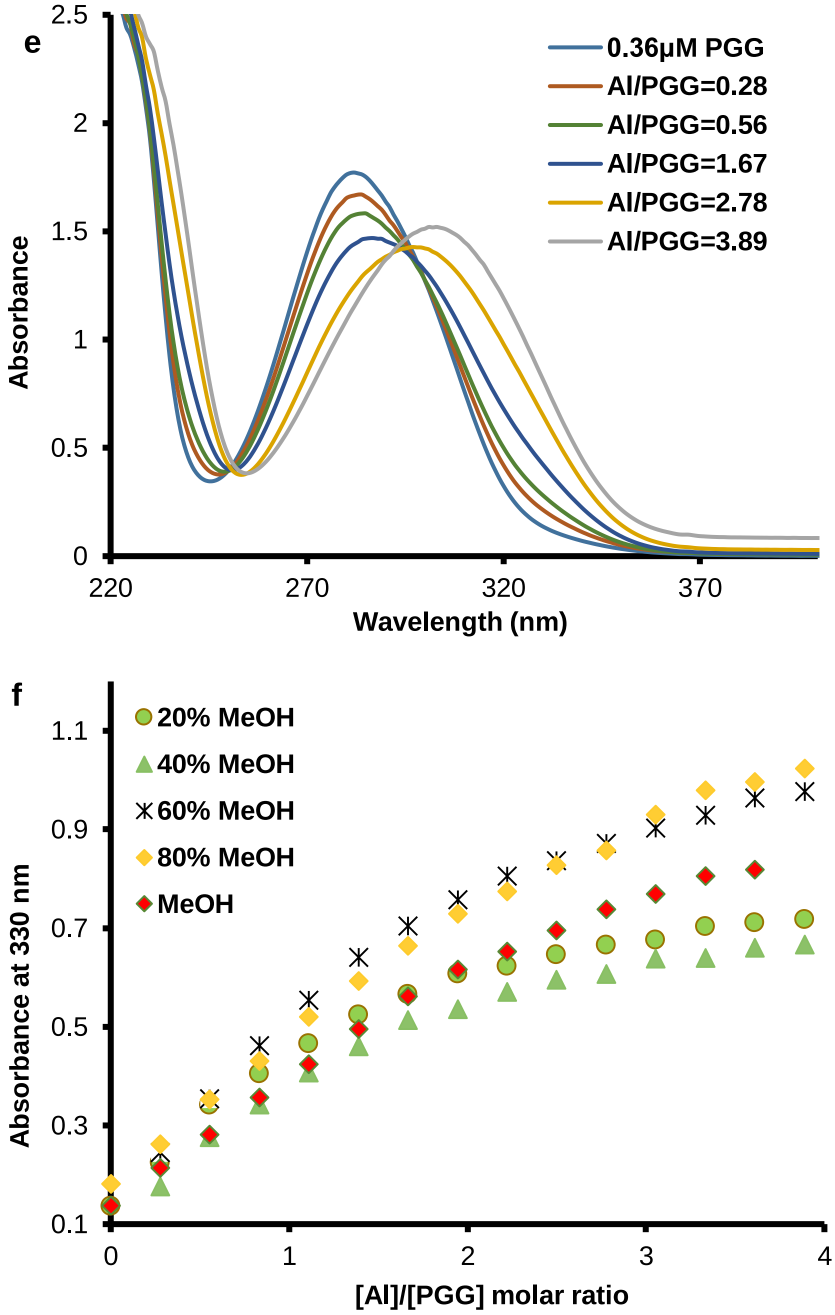


**Fig. 2.** UV-Vis spectral changes of PGG titrated with $Al^{3+}$ solution in: (a) 20% MeOH, (b) 40% MeOH, (c) 60% MeOH, (d) 80% MeOH, and (e) MeOH; (f) Absorbance changes at 330 nm correspond to PGG binding of $Al^{3+}$ in various solvents

Figure 2f shows the concentration patterns of the Al-PGG complexation as a function of the gradient concentration of the MeOH-$H_2O$ system. A good linear correlation between the complex concentration and the addition of $Al^{3+}$ solution from 0 to approximately 1 was observed in the 20% MeOH-$H_2O$ system. Titration of $Al^{3+}$ resulted in a slow increase in the absorbance, indicating that only a single species (AlPGG) was formed at the beginning of titration. An increase in $Al^{3+}$ added into system induced a continual increase in the absorbance until the Al/PGG molar ratio reached approximately

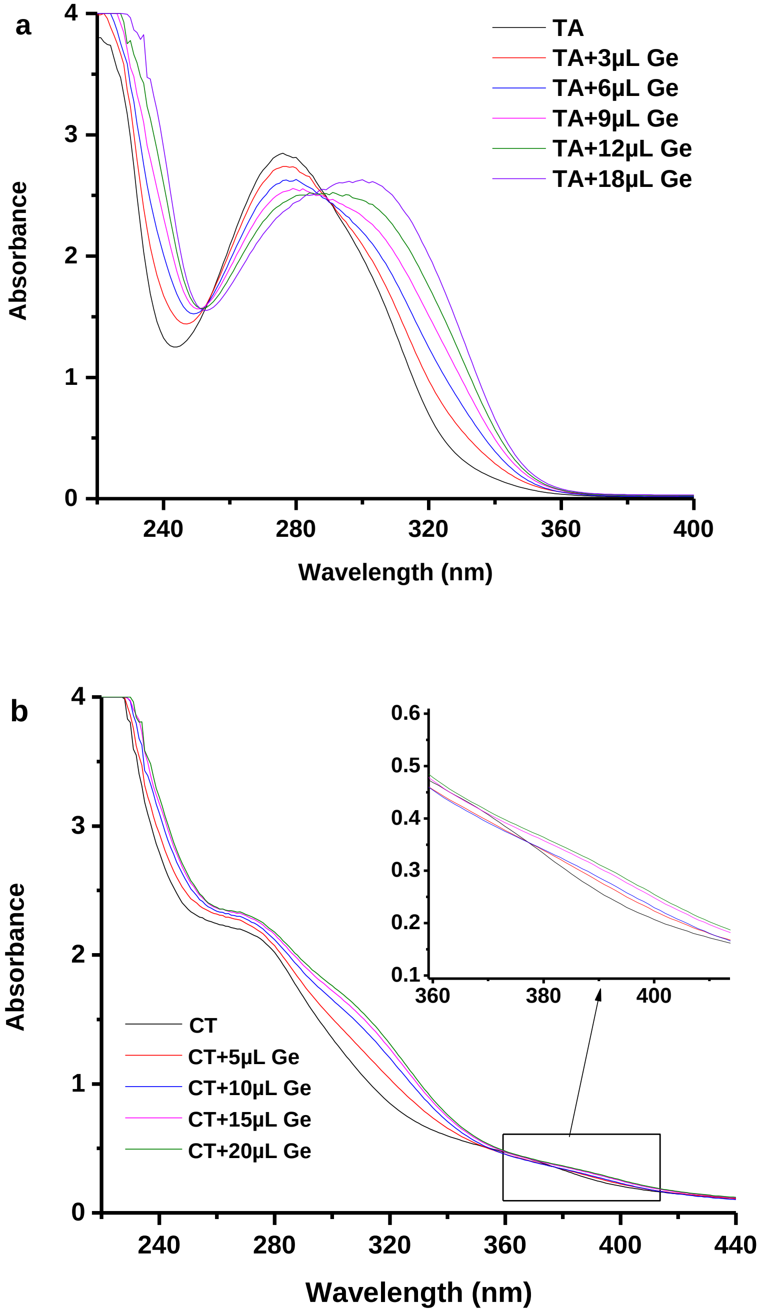
2, indicating production of a new $Al_2PGG$ species. This result confirmed the data of the chemometric modeling study, indicating that AlPGG was the only species formed at the Al/PGG molar ratio < 1 and that addition of $Al^{3+}$ generated the $Al_2PGG$ species. The AlPGG and $Al_2PGG$ species coexisted until the Al/PGG molar ratio reached approximately 3. Similar results were observed in the 40% MeOH-$H_2O$ system, *i.e.*, AlPGG species were formed during the initial titration with subsequent formation of the $Al_2PGG$ species.

The modeling results were confirmed by previous titration experiments. The concentration profiles of PGG and the PGG-aluminum complexes in methanol solutions were predicted by the chemometric modeling method, and the results are shown in Fig. S2. Using software analysis, the concentration patterns suggest that the concentrations of the AlPGG and $Al_2PGG$ complexes in the mixture were 19.64 and 14.39 μM at the end of the titration, respectively, and the total concentrations were approximately equal to the initial concentration of PGG in the solution (35.9 μM) before titration. Moreover, according to the predicted concentration profiles, similar results were observed in the 40% MeOH-$H_2O$ system. In the 60%, 80% MeOH-$H_2O$, and MeOH systems, a good linear correlation was observed at the Al/PGG molar ratio increasing from 0 to approximately 2. Then, the curve tended to plateau, suggesting that $Al_2PGG$ was the only species formed during titration in these systems. The modeling results were in good agreement with the results of the authors' titration experiments. In the MeOH system, the concentrations of the $Al_2PGG$ species and free PGG in the mixture at the end of the titration were calculated as 33.11 and 2.61 μM, respectively, and the sum of the $Al_2PGG$ species and free PGG was approximately equal to the initial PGG concentration in the solution (35.9 μM). Similar results were observed in the 80% and 60% MeOH-$H_2O$ systems.

## Evaluation of the Tannin-germanium Complex

The UV-Vis absorption spectra of $Ge^{4+}$ complexed with TA, CT, VT, or PT solutions are shown in Fig. 3. In the presence of various concentration of $Ge^{4+}$, remarkable spectral modifications were observed; this type of shift of UV absorption spectra is considered evidence for metal chelation by certain organic molecules containing the benzene ring structures (Zeng *et al.* 2019).

The spectrum of the TA solution was characterized by the main UV absorption band at 278 nm with a shoulder at 245 nm (Fig. 3a), which has been reported in a previous work (Cruz *et al.* 2000). Addition of $Ge^{4+}$ resulted in a new band at 310 nm, indicating a direct complexation between TA and $Ge^{4+}$. The structure of TA is similar to that of PGG and has a distinguishing spectral shift at 278 nm or 280 nm after addition of various concentrations of $Ge^{4+}$. Figure 3b shows that the characteristic peak of CT was not clearly visible; the absorbance at approximately 278 nm was increasing and a slight blueshift from 278 nm to approximately 275 nm was induced by addition of $Ge^{4+}$, indicating the complexation between the deprotonated form of CT and $Ge^{4+}$ (Zeng *et al.* 2019). Similar to CT, VT does not have a clearly visible characteristic peak at approximately 278 nm (Fig. 3c); however, there was a decline at 360 nm and a new band at 390 nm. Both VT and PT belong to the ellagic tannin type (Karamali and Teunis 2001); hence, this spectral shift may be due to the complexation between the lactone-like ellagic acid moieties in the VT structure and $Ge^{4+}$ (Fang *et al.* 2006). The PT solution had a strong band at 275 nm with a shoulder at 245 nm (Fig. 3d). The characteristic peak at 275 nm was deceasing and shifted to 278 nm after addition of $Ge^{4+}$; meanwhile, a new band appeared at 310 nm. Moreover, there were slight spectral modifications including a decline at 360 nm and a new band at 390 nm, suggesting that the phenolic hydroxyl groups and lactone-like ellagic acid in PT were complexed with $Ge^{4+}$



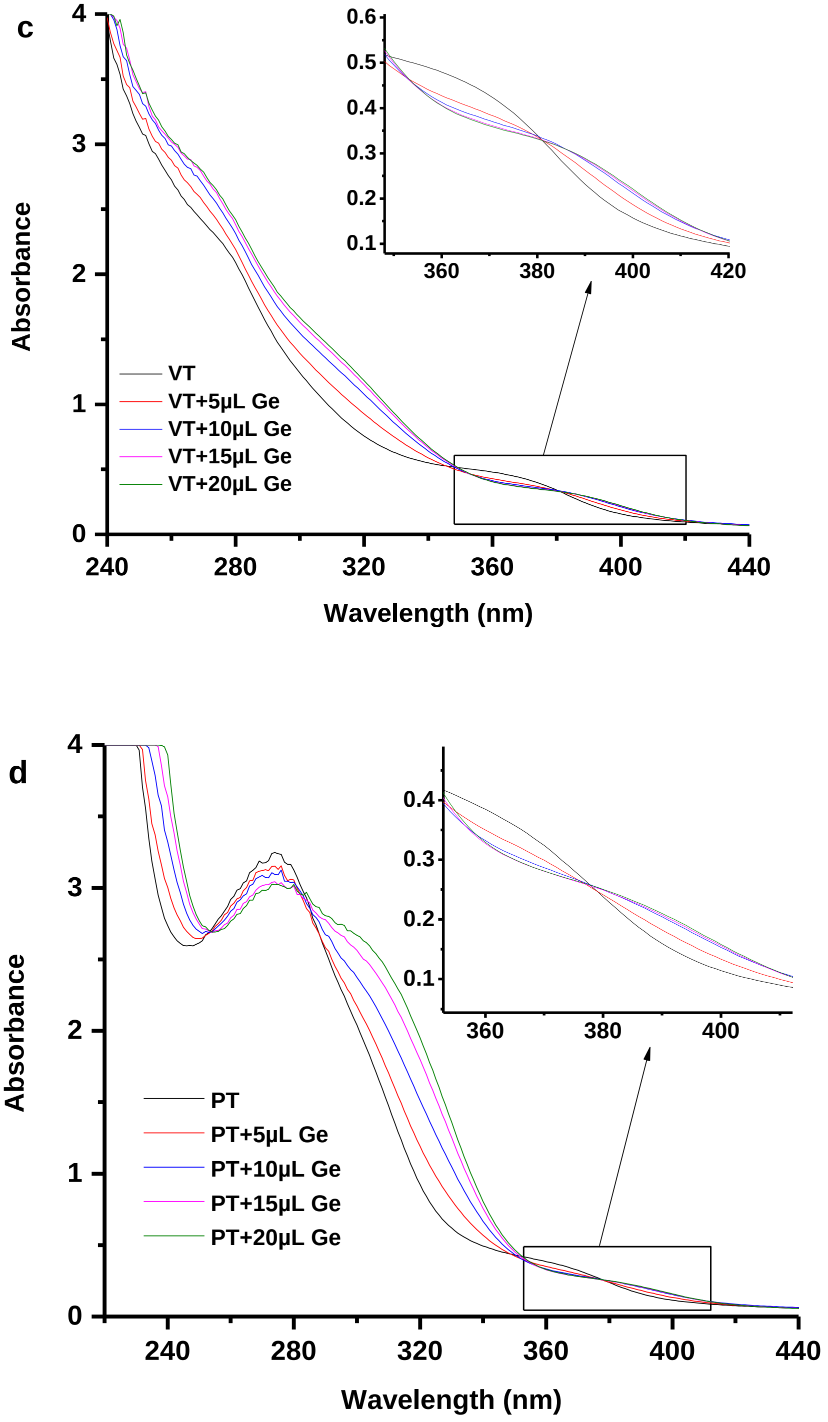


**Fig. 3.** Effects of $Ge^{4+}$ concentrations on the UV-Vis spectra of: (a) TA, (b) CT, (c) VT, and (d) PT

## Tannins and Metal Precipitation

No precipitate was observed when 200 µL (10 mg/mL) TA was mixed with 5 mL (10 mM) $Ge^{4+}$ at pH 3. However, significant precipitation was observed when $Ge^{4+}$ reacted with CT, VT, and PT at the same concentration (supplemental Fig. 3). Thus, CT, VT, and PT were used to determine the conditions for $Ge^{4+}$ precipitation by various hydrolyzable tannins. The percent of precipitated $Ge^{4+}$ was substantially influenced by the initial tannin/Ge ratio in the solution (Fig. 4a). The results indicate that the percent of precipitated $Ge^{4+}$ is gradually increased concomitant to an increase in the initial tannin/Ge mass ratio from 0.5 to 2.5; compared to CT and PT, VT has a stronger ability to precipitate $Ge^{4+}$. The data indicate that 22.6% germanium was precipitated by VT at the tannin/Ge ratio of 2.3, while 19.3% and 17.9% germanium was precipitated by CT and PT, respectively, at the same tannin/Ge mass ratio. The results of evaluation of the tannin-germanium complex indicate that the tannin structure is the most impactful factor of tannin precipitation of germanium. The reaction time has no significant effect on the precipitation of the metal by tannins at the tannin/Ge mass ratio of 2.3 (Fig. 4b); the Ge-tannin precipitation was only slightly enhanced when time was increase from 3 to 12 h.

Ge-tannin precipitates were substantially influenced by the pH values (Fig. 4c); an increase in pH resulted in an increase in metal ions participating in binding to tannins. The percent of precipitated VT was increased from 22.0% to 32.2% concomitant to an increase in pH from 2.4 to 7.2. Higher levels of $Ge^{4+}$ were precipitated by VT at pH 7.2 at the initial tannin/Ge mass ratio > 2.5, indicating that an increase in pH of the solution can reduce the use of raw materials. The highest amount of precipitate of all tested tannins was obtained at pH 7.2 at the initial tannins/Ge mass ratio of approximately 2.3:1, which was in agreement with the results of the PGG-Ge precipitation experiment described above. The highest amount of precipitation was obtained at the initial Ge/PGG molar ratio of 1:2. The authors' results suggest that in addition to pH and the initial tannin/Ge mass ratio in the solution, hydrolyzable tannins from various plants have variable ability to precipitate germanium. Karama (2009) studied the complexes of $Cu^{2+}$, $Zn^{2+}$, and $Fe^{2+}$ with tannins extracted from walnuts, hazelnuts, and almond seeds and determined the best chelator fraction of metal ions by calculating the chelating capacity of individual tannin fractions from various plants. Wang *et al*. (2005) focused on the complexation of metal ions by myrtan tannins and determined the highest complex capacity of metal ions at the optimized pH values. Related experiments showed that specific mass ratio and reaction conditions of metal ions and tannins are necessary and meaningful for optimal precipitation.

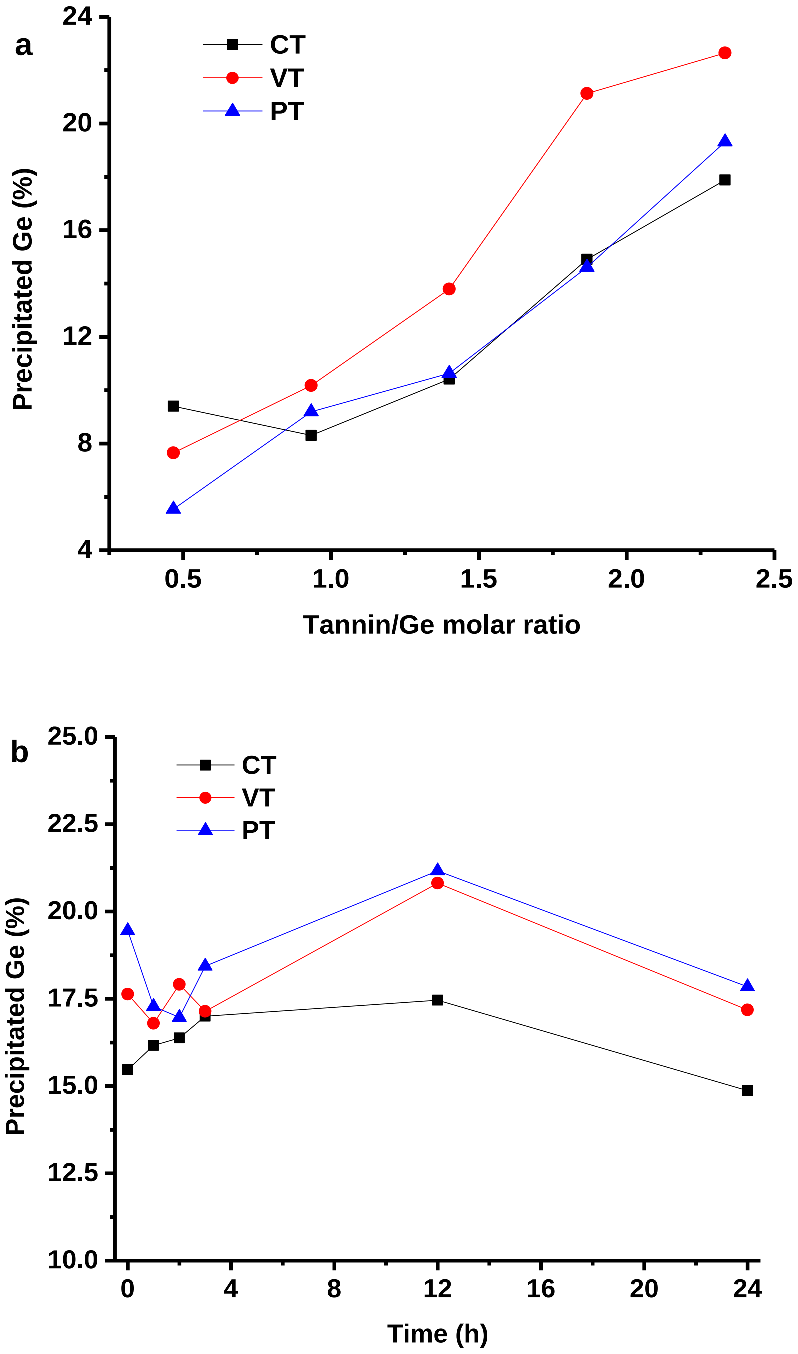


**Fig. 4a & 4b.** Effect of (a) the initial tannin/Ge molar ratio, (b) reaction time, and (c) pH on the amount of precipitated $Ge^{4+}$

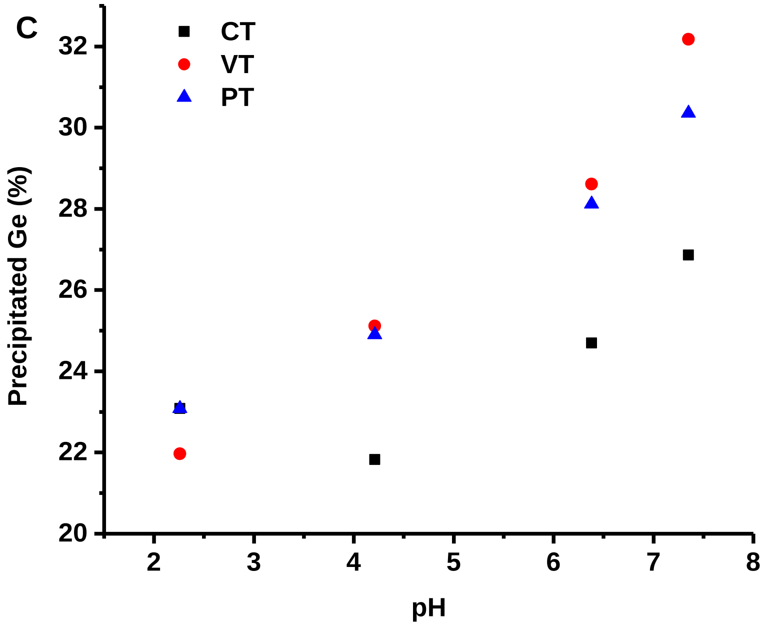
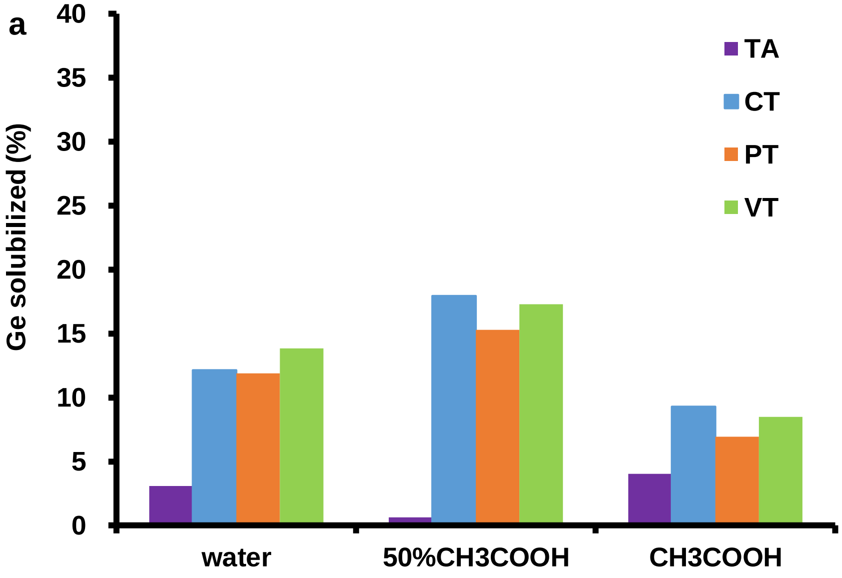


**Fig. 4c.** Effect of (a) the initial tannin/Ge molar ratio, (b) reaction time, and (c) pH on the amount of precipitated $Ge^{4+}$

## Stability of the Tannin-germanium Precipitates

The data of Fig. 5a and b indicate that CT, VT, and PT can form complexes with germanium and stable precipitates under acidic conditions (pH 3 to 4). The results show that the reaction system of a tannin and hydrazine was different in various solutions. In Fig. 5a, a small amount (less than 5%) of germanium was solubilized from the TA-Ge precipitates in the presence of water, 50% $CH_3COOH$, and $CH_3COOH$.



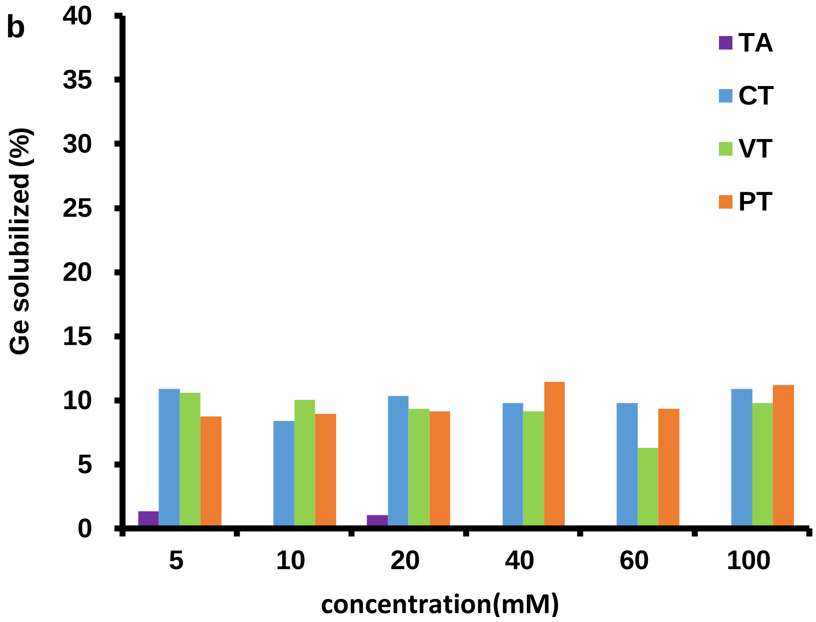


**Fig. 5.** (a) Effect of water, 50% $CH_3COOH$, and $CH_3COOH$ on the solubilization of germanium from the Ge-tannin precipitates; (b) Effect of aqueous EDTA-2Na on the solubilization of germanium from the Ge-tannin precipitates

Germanium solubilization from the CT-Ge precipitates was 17.9% in 50% $CH_3COOH$, 12.1% in water, and 9.3% in $CH_3COOH$. The range of germanium solubilized from the VT-Ge and PT-Ge precipitates was 7.5% to 17.3% and 6.9% to 15.3%, respectively; slight differences between the percentage of germanium solubilized from the CT-Ge, VT-Ge, and PT-Ge precipitates by three test solvents and from the TA-Ge precipitates indicate a relatively high stability in three solutions compared with that observed in other cases. It should be noted that the Ge-tannin precipitates were more stable in pure acetic acid than in water and 50% acetic acid, suggesting that the concentration of $H^+$ in the solution influenced the solubilization of the Ge-tannins precipitates. Considering that acetic acid is a covalent compound, a small amount of $H^+$ can be neglected, and the amount of $H^+$ in the three test solvents decreased in the following order: 50% $CH_3COOH$ > water > $CH_3COOH$, which was confirmed by the authors' experimental results described above (Jencks *et al.* 1966). The data of Fig. 5b indicate that regardless of the concentration of EDTA-2Na, the range of germanium solubilized from the CT-Ge, VT-Ge, and PT-Ge precipitates was 6.3% to 11.2%, indicating that solubilization of germanium from the CT-Ge, VT-Ge, and PT-Ge precipitates was not significantly changed. Germanium was not solubilized from the TA-Ge precipitates in the solvents containing 10, 40, 60, and 100 mM EDTA-2Na, indicating that the TA-Ge precipitates were more stable than the other precipitates used in the authors' experiments. The results indicate that the Ge-tannin precipitates were more stable in 10/40/60/100 mM EDTA-2Na than in three others tested solutions. Solution systems can influence the stability of the Ge-tannin precipitates.

## CONCLUSIONS

1. Four hydrolyzable tannins, TA, CT, VT, and PT, were found to form complexes and stable precipitates with germanium. Tannins structure affect the tannin-metal complex: VT has a stronger germanium precipitate ability compared with TA, CT, and PT at the test condition.
2. The precipitation experiment showed that almost all PGG and germanium were precipitated at the initial Ge/PGG molar ratio of 1:2, and a stoichiometric ratio of 1:2 to obtain a high content of PGG-germanium precipitate was strictly defined.
3. AlPGG and $Al_2$PGG species formed in 20% and 40% MeOH, while only $Al_2$PGG species formed in 60% to 100% MeOH, showing that the solution can affect the PGG-metal complex species.
4. The results indicate that further studies can concentrate on tannins from more distinct plant resources to identify an efficient tannin to enhance the germanium recovery industry.

## ACKNOWLEDGMENTS

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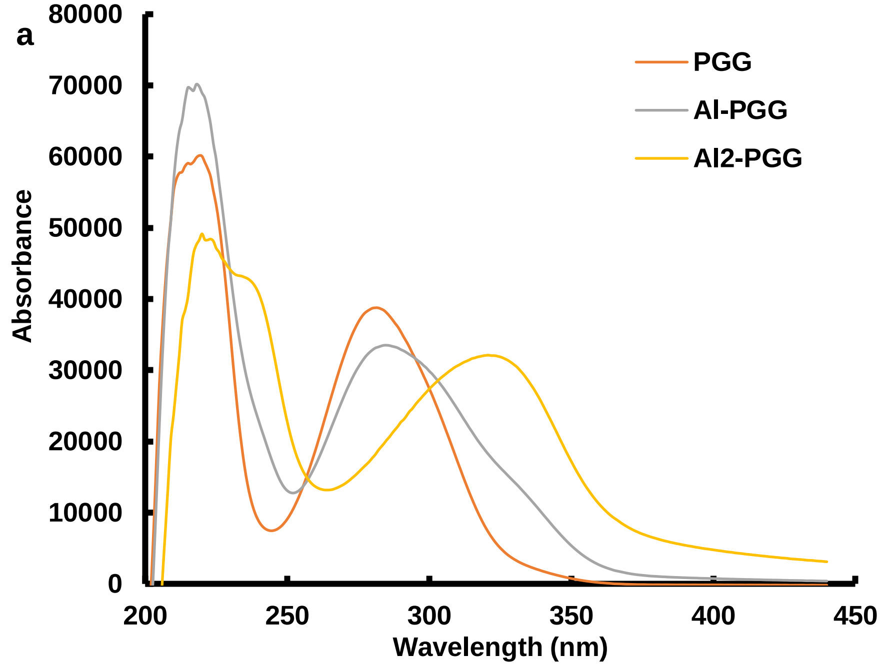
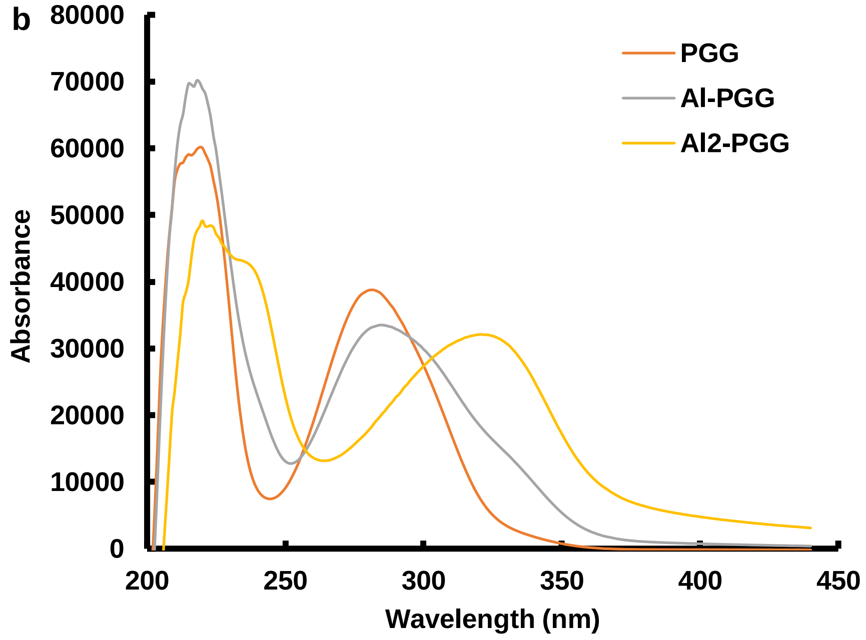
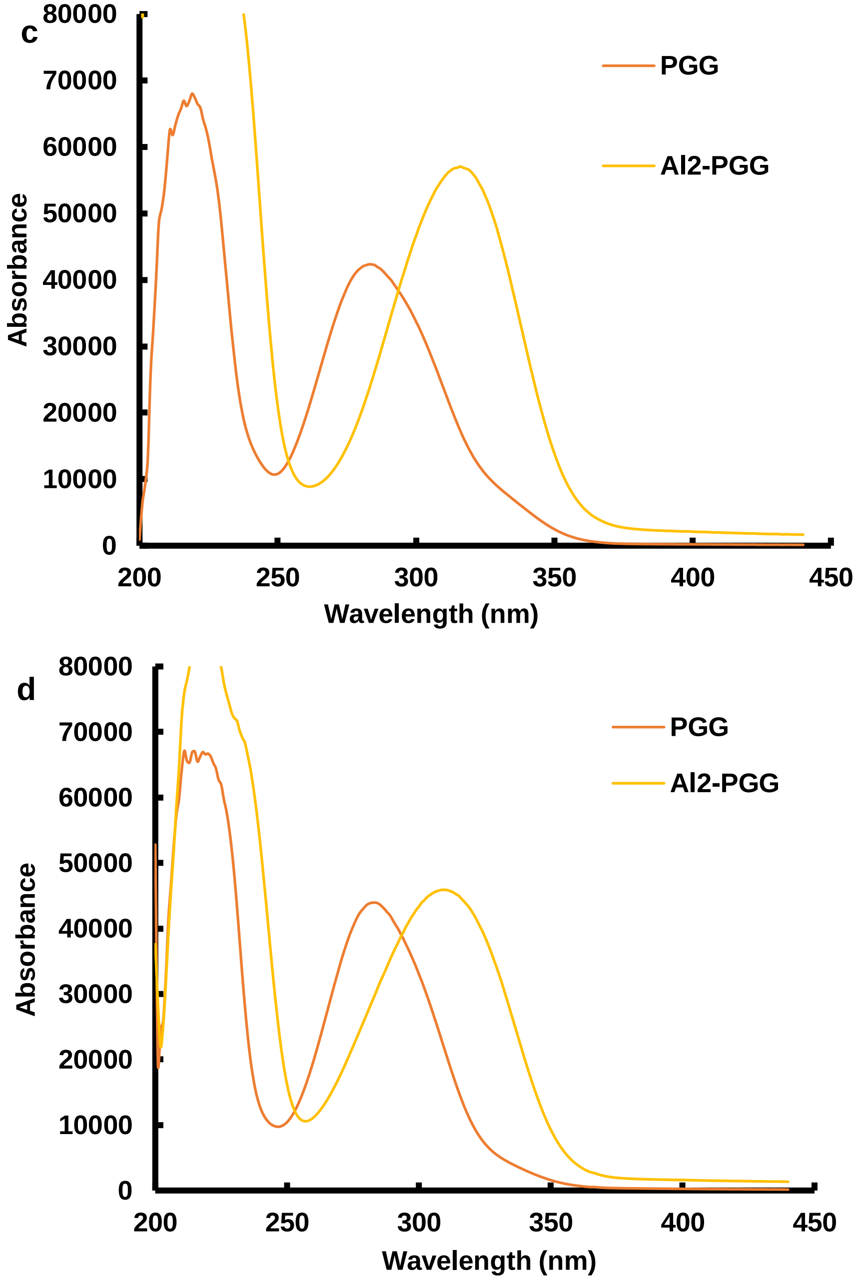
DOI: 10.15376/biores.15.1.1205-1232

## APPENDIX: SUPPLEMENTARY INFORMATION



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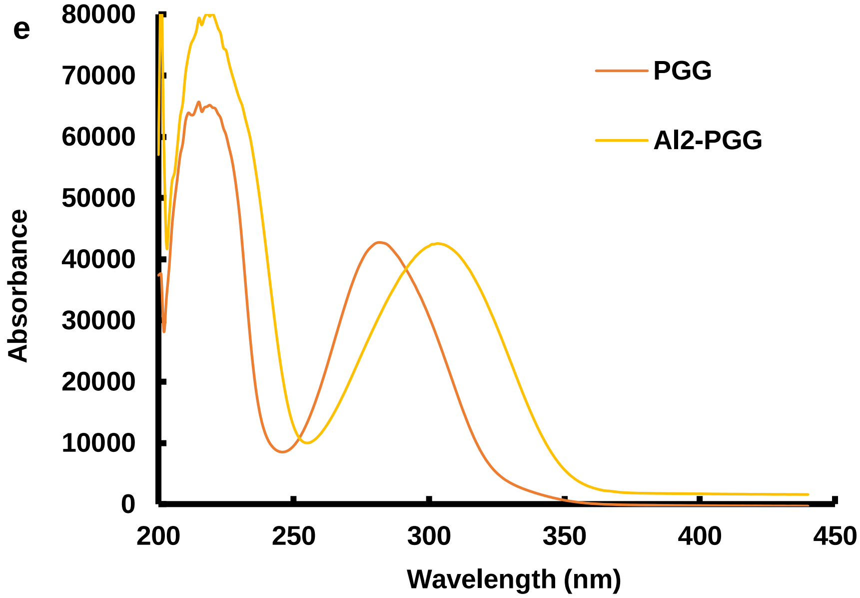
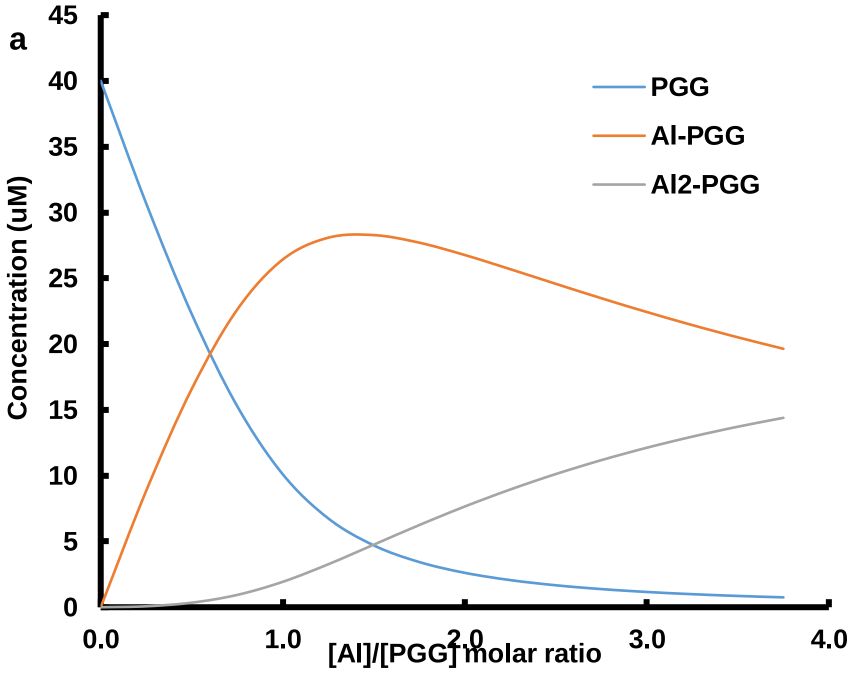
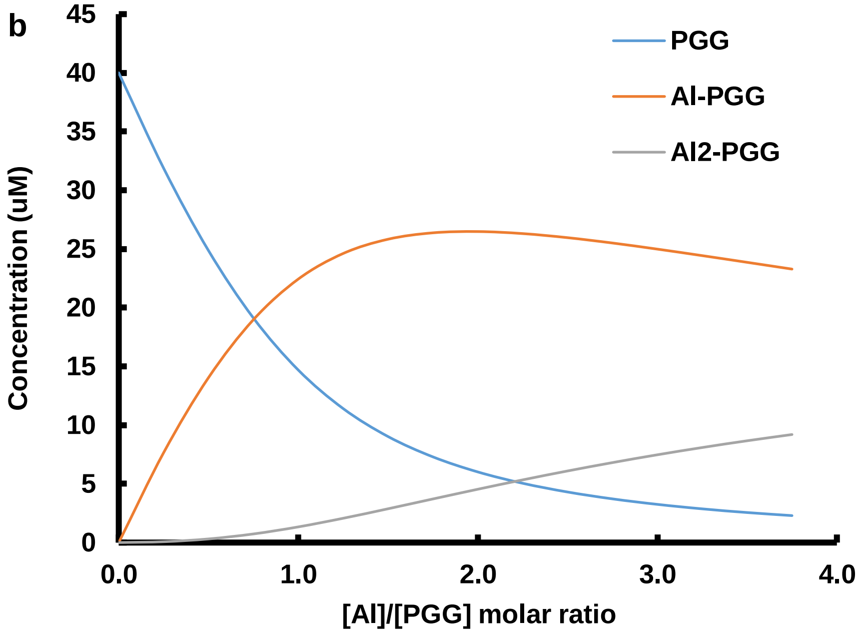


**Fig. S1.** The characteristic spectra of purified PGG and its $Al^{3+}$ complexes produced in (a)20%MeOH, (b) 40%MeOH, (c) 60%MeOH, (d)80%MeOH, and (e)MeOH predicted using the chemometric modeling method

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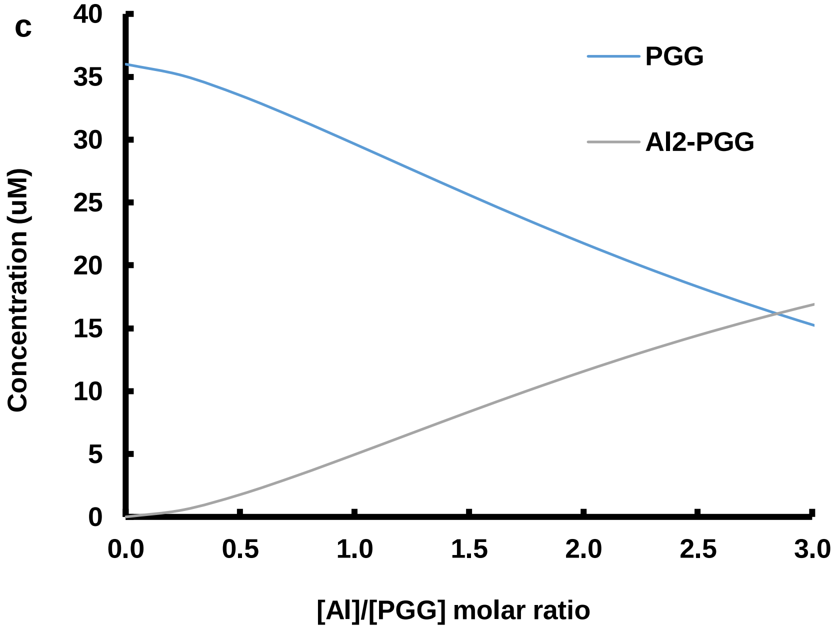
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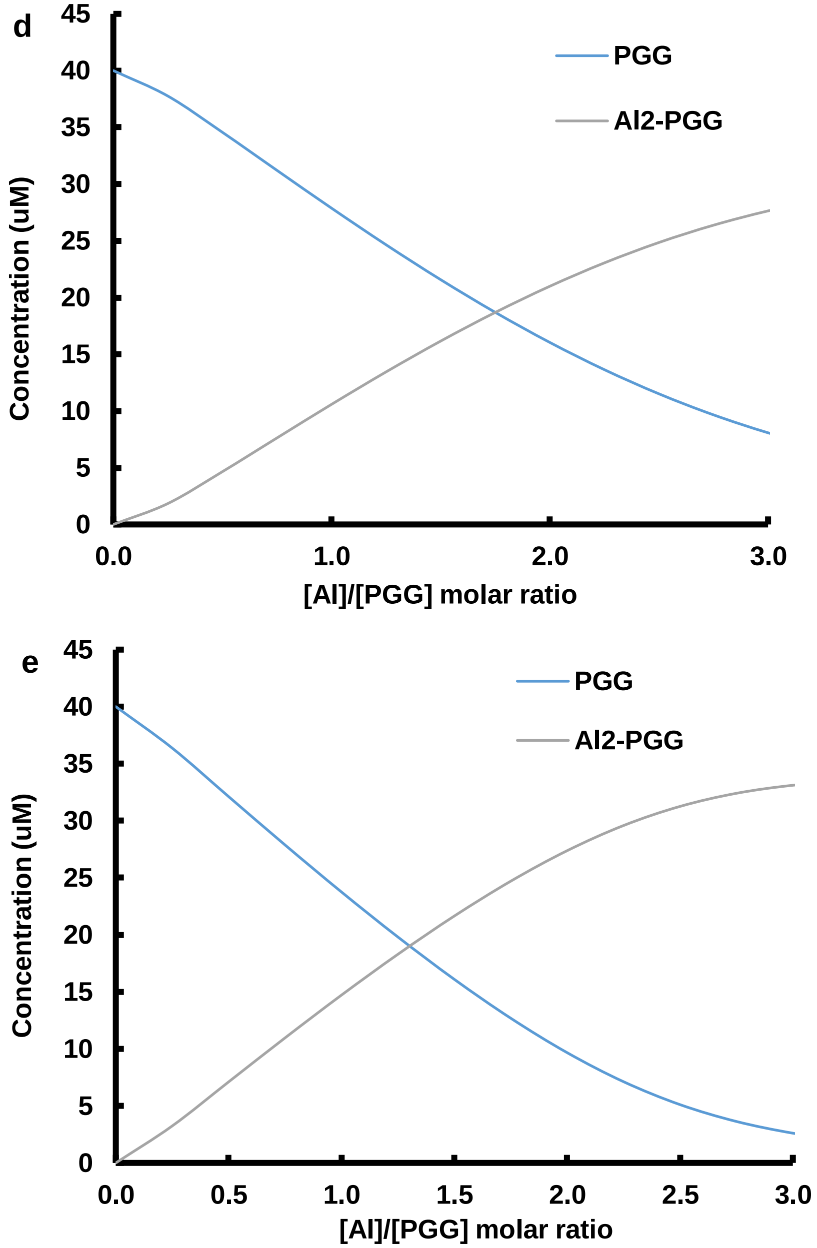

**Fig. S2.** The concentration profiles of PGG and its $Al^{3+}$ complexes in the(a)20%MeOH, (b) 40%MeOH, (c) 60%MeOH, (d)80%MeOH, and (e)MeOH predicted by the chemometric modeling method

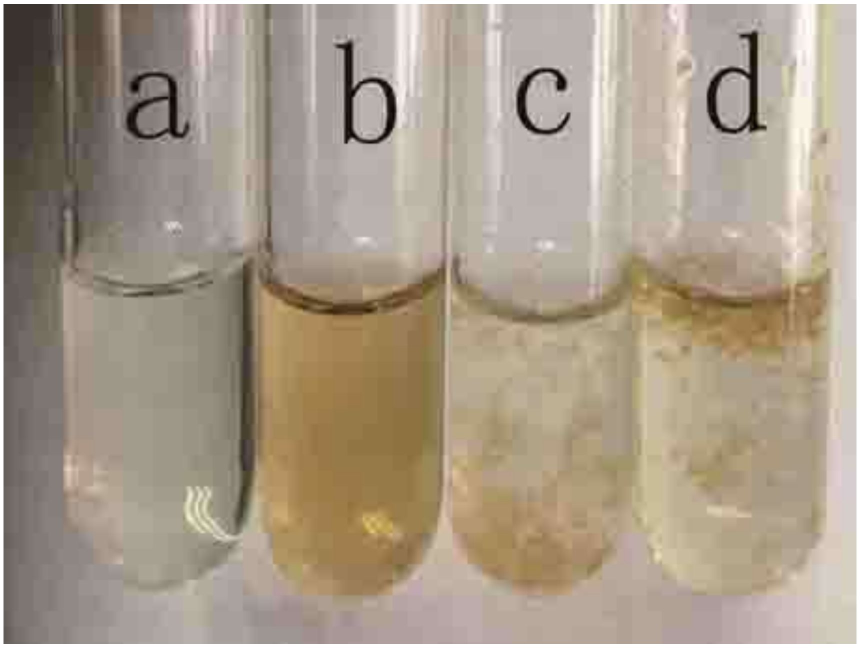

**Fig. S3.** Five milliliters of 10 mM $Ge^{4+}$ mixed with 200 µL of 10 mg/mL (a) TA, (b) CT, (c) PT, and (d) VT