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# Influence of Traditional Chinese Medicines on Bioaccessibility and Speciation of Arsenic in Realgar and Mercury in Cinnabar

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Influence of traditional Chinese medicines on the bioaccessibility and speciation of arsenic in realgar and mercury in cinnabar was observed using *in vitro* digestion dialysis method. Inductively coupled plasma-mass spectrometry (ICP-MS) and its hyphenation with high-performance liquid chromatography were used to determine the contents of arsenic, mercury and their species, respectively. Here, traditional Chinese medicines including *Radix glycyrrhizae*, *Vigna radiata* and *Folium isatidis* had a positive influence on arsenic bioaccessibility. *Radix glycyrrhizae* and *Vigna radiata* improved the content of As(III) and reduced As(V) in gastric phase, while both of them reduced the content of As(III) in intestinal phase. In addition, *Radix glycyrrhizae*, *Vigna radiata* and *Rhizoma smilacis glabrae* had significant effects on bioaccessibility of mercury in cinnabar. In cinnabar and cinnabar-traditional Chinese medicines mixtures, $Hg^{2+}$ is the only specie. The results indicate that $Hg^{2+}$ doesn't transform to $CH_3Hg^+$ of higher toxicity.

**Keywords: Bioaccessibility, Speciation, Arsenic, Mercury, Mineral Medicines, Realgar and Cinnabar.**

## INTRODUCTION

Mineral medicines, such as realgar (90 % as $As_4S_4$) and cinnabar (96 % as HgS) (Fig. 1), have been used in TCMs for thousands of years[1,2]. Realgar has long been used as a therapeutic agent to treat some diseases[3,4], especially leukemia therapy[4], while cinnabar produces sedative effects on palpitation, epilepsy and insomnia[3]. However, realgar leads to carcinogenic effect, genetic toxicity, and even damages liver and kidney[5,6]. And cinnabar has been pointed to cause neurotoxicity, hepatotoxicity and nephrotoxicity[7-9]. It must be aware of their toxic effects due to the high contents of arsenic and mercury. The estimated human therapeutic dose of realgar and cinnabar used are limited to 0.05-0.1 and 0.1-0.5 g per day[3], respectively. Only a small part of them will be bioavailable. Bioavailability refers to the proportion of the ingested contaminants in matrix that can reach the systemic circulation. Bioaccessibility which is considered as the maximum amount of bioavailability refers to the release of contaminants from matrix into digestive juice in the gastrointestinal tract[10]. Additionally, bioavailability strongly depends on element speciation. Thus, it is essential to study the bioaccessibility and speciation of As and Hg in mineral medicines. *In vitro* digestion method simulating the gastrointestinal tract is a valid method to quantify the bioavailability. It has been applied in many fields, such as environment, food and drug safety evaluation[11-15].

Traditional Chinese medicines (TCMs) including *Radix glycyrrhizae*, *Vigna radiata, Folium isatidi* and *Rhizoma smilacis glabrae* have been claimed to play important roles in reducing the uptake of heavy metals[16-18]. It is attributed to that active ingredients interact with As or Hg, forming indissolvable or larger polarity macromolecular substances which are hard to be absorbed by the gastrointestinal tract.

The aim of this study is to determine the bioaccessibility As in realgar and Hg in cinnabar by ICP-MS and analyze their species by HPLC-ICP-MS as well as observe the effects of TCMs on the bioaccessibility and speciation of As in realgar and Hg in cinnabar, respectively.

## EXPERIMENTAL

The dried traditional Chinese medicines were powdered using a planetary ball-mill (QM-SB, Nanjing University Instrument Factory, China) at the speed of 500 rpm. An *in vitro* digestion method (simulating gastric and intestinal phase digestion) based on PBET procedure[19] with some modifications[13,20] was performed. The gastric solution was prepared by adjusting 1 L of ultrapure water to pH 1.5 with 12N HCl and adding 1.6 g of pepsin. Samples were weighed in 50 mL

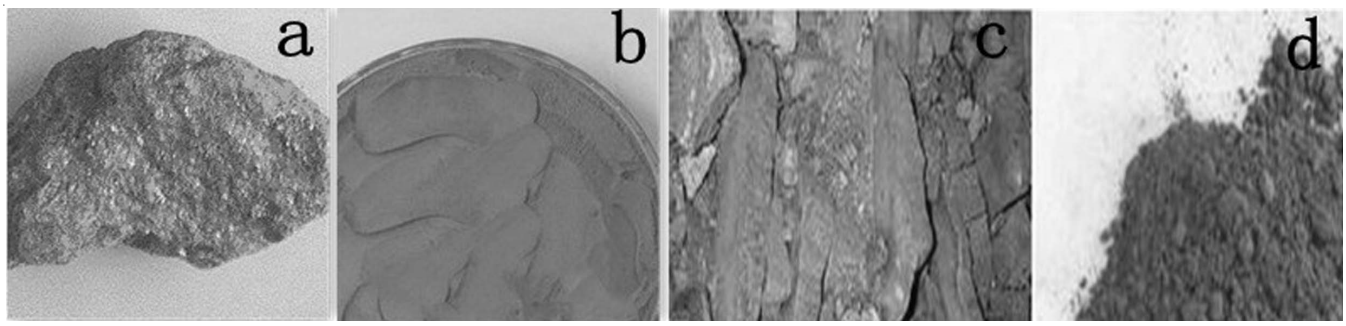


Fig. 1. Realgar and cinnabar (a and c: mineral realgar and cinnabar; b and d: processed realgar and cinnabar)

centrifugal tubes in which the *in vitro* digestion method was conducted under the conditions as described in Fig. 2. The exact amount of $NaHCO_3$ in the dialysis bag was calculated from the neutralization of gastric digestive juice. The centrifugal tubes were oscillated in a constant temperature bath oscillator (THZ-82, Changzhou Guohua Co., Ltd., China)

To observe the influence of TCMs on the bioaccessibility and speciation of As in realgar and Hg in cinnabar, TCMs was added into mineral medicines, respectively.

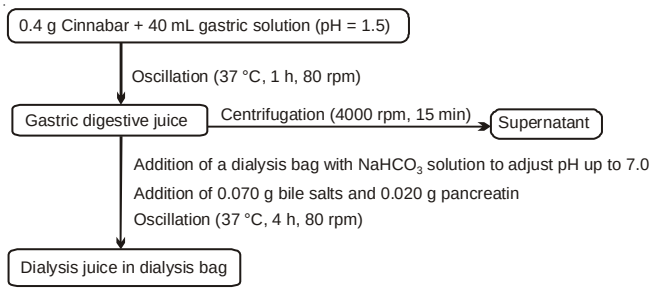


Fig. 2. Procedure of *in vitro* digestion dialysis method

The total content of As was obtained by acid digestion of realgar. For this, 0.2 g of realgar with a mixture of 4 mL of $HNO_3$ and 2 mL of $H_2O_2$ was added in the PTFE vessels into position on the Multiwave 3000 XF100 (Anton Parr, Graz, Austria). The final solutions were determined by ICP-MS.The determination of soluble As after the treatment of *in vitro* digestion method was performed by dynamic reaction cell (DRC)-ICP-MS which used $CH_4$ as the reaction gas which could efficiently remove the interference of $ArCl^+$.

The total and soluble content of Hg was performed by ICP-MS. For this, 0.2 g of cinnabar with a mixture of 3 mL of HCl, 1 mL of $HNO_3$ and 1 mL of $H_2O_2$ was added in the PTFE vessels. Additionally, 0.15 % of L-cysteine (w/v) in 2 % $HNO_3$ solution was added in the standard solutions and final extract solution to inhibit the memory effect of Hg.

As speciation analysis was carried out using HPLC (a Perkin-Elmer Sciex 200) coupled with ICP-MS. The supernatants after the treatment of *in vitro* digestion method were filtered through 0.45 μm filters, extracts were diluted (1 % v/v $HNO_3$) and analyzed using instrumental and chromatographic conditions as presented in Tables 1 and 2, respectively. Under these conditions, As(III), DMA, MMA and As(V) whose respective retention time was 1.92, 2.49, 3.25 and 6.20 min were separated well in 8 min. The typical chromatogram of As speciation was presented in Fig. 3(A). The speciation analysis of mercury was carried out using HPLC-ICP-MS. The supernatant and dialysis juice after the treatment of *in vitro* digestion method were filtered through 0.45 μm filters, prior to performing dilutions (1 % v/v $HNO_3$) and analysis using instrumental and chromatographic conditions as summarized in Tables 1 and 2, respectively. Under these conditions, $CH_3Hg^+$ and $Hg^{2+}$ whose respective retention time was 1.59 and 0.90 min were separated well in Fig. 3(B).

TABLE-1
INSTRUMENT CONDITIONS FOR ICP-MS

| Operation parameters | Operating conditions |
|---|---|
| RF power (W) | 1100 |
| Plasma gas flow rate (L $min^{-1}$) | 15 |
| Auxiliary gas flow rate (L $min^{-1}$) | 1.2 |
| Nebulizer gas flow rate (L $min^{-1}$) | 0.90 |
| Scanning mode | Peak hoping |
| Replicates | 3 |

TABLE-2
CHROMATOGRAPHY CONDITIONS

| Operation parameters | Operating conditions | |
|---|---|---|
| | As | Hg |
| Pump | PE series 200 | PE series 200 |
| Injector | Rheodyne 7225i | Rheodyne 7225i |
| Injection volume (μL) | 20 | 20 |
| Column temperature (°C) | Ambient | Ambient |
| Column | Hamilton PRP-X100 anion exchange column (150 × 4.6 mm, 10 μm) | $C_{18}$ column (100 × 4.6 mm, 5 μm) |
| Mobile phase | 15 mM $(NH_4)_2HPO_4$ (pH 6) + $CH_3OH$ = 99 + 1 | 0.02 M $CH_4COONH_4$, 0.05 % L-cysteine (pH 3) |
| Flow rate (mL $min^{-1}$) | 1.0 | 1.4 |

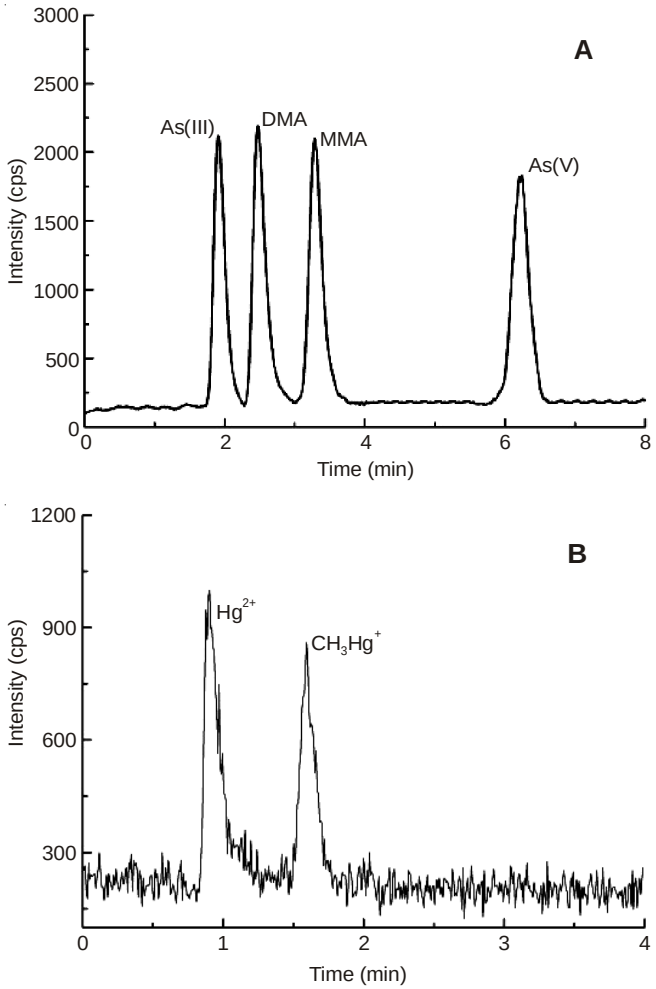


Fig. 3. Typical chromatogram. A: a standard solution of As species at 10 mg $L^{-1}$, B: a standard solution of Hg species at 2 mg $L^{-1}$

## RESULTS AND DISCUSSION

**Analytical performance:** The method detection limits were determined as 3 standard deviation (SD) of the 11 consecutive measurements of the reagent blanks multiplied by the dilution factor used for sample preparation. They obtained for As by ICP-MS and DRC-ICP-MS were 0.024 and 0.021 μg $L^{-1}$ with relative standard deviations (RSDs) of 0.89 and 1.01 %, respectively. The accuracy and precision in determining total As were verified using the certified reference material GBW07427. The value measured of 10.1 μg $g^{-1}$ was in good agreement with certified value of 10.6 ± 0.8 μg $g^{-1}$. The average recovery for soluble As was 100.2 %. In addition, detection limits obtained for As species by HPLC-ICP-MS were 0.21, 0.05, 0.09 and 0.15 μg $L^{-1}$, for As(III), DMA, MMA and As(V), with RSDs of 1.31, 0.65, 0.79 and 0.32 %, respectively.

The method detection limit obtained for Hg by ICP-MS was 0.037 with RSD of 2.92 %. The accuracy and precision in analyzing Hg were verified using GBW07427. The value measured of 0.057 ± 0.001 μg·$g^{-1}$ was close to the certified value of 0.052 ± 0.006 μg·$g^{-1}$.

**Influence of TCMs on bioaccessibility of arsenic in realgar and mercury in cinnabar:** To observe the influence of TCMs on bioaccessibility of arsenic in realgar and mercury in cinnabar, TCMs was added into realgar and cinnabar, respectively. The details were presented in Fig. 4. Bioaccessibility was calculated as:

$$\text{Bioaccessibility (\%)} = \frac{M_s}{M_t} \times 100$$

where $M_s$ is the soluble content of As or Hg (mg $kg^{-1}$); $M_t$ is the total content of As or Hg (mg $kg^{-1}$). Influence of TCMs on bioaccessibility of arsenic in realgar were displayed in Fig. 4(A).

In gastric phase, *Radix glycyrrhizae* (RG) had an enhancing effect on As bioaccessibility. *Vigna radiata* (VR) had a slight positive influence on As bioaccessibility which is probably attributed to the formation of soluble As complexes from the interaction of As and protein present in *Vigna radiata*. For *Folium isatidis* (FI), improved As bioaccessibility in gastric phase. The results indicate that As bioaccessibility uptake may be attributed to the formation of soluble As complexes with lower toxicity. However, As bioaccessibility in intestinal phase didn't vary greatly compared with the control.

The influence of TCMs on bioaccessibility of Hg in cinnabar was exhibited in Fig. 4(B). *Radix glycyrrhizae* apparently enhanced Hg bioaccessibility in gastric phase, while reduced bioaccessibility in intestinal phase. It is probable that *Radix glycyrrhizae* change the increasing level of soluble Hg which

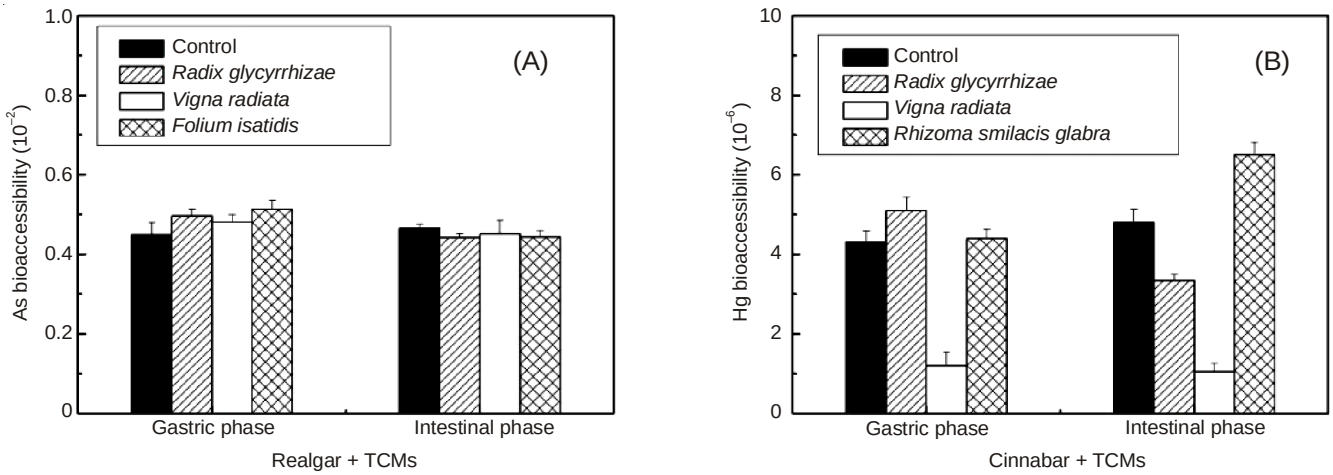


Fig. 4. Influence of TCMs on bioaccessibility of As in realgar and Hg in cinnabar. control: without TCMs

is due to the addition of pancreatin and bile salts. These final results seem to be ascribed to the combination of Hg and active ingredients, glycyrrhizinin, glycyrrhetinic acid, glycyrrhiza polysaccharide, *etc*. *Vigna radiata* inhibited As bioaccessibility in gastrointestinal phase. Obviously, it was different from the influence of *Radix glycyrrhizae* on Hg bioaccessibility. Thus, it is probably due to its physical absorption on Hg. For *Rhizoma smilacis glabrae* (RSG), it didn't make a significant difference in gastrointestinal phase. It demonstrates that *Rhizoma smilacis glabrae* is likely to play physiological treatment.

**Influence of TCMs on speciation of arsenic in realgar and mercury in cinnabar:** As speciation changes in realgar after adding TCMs were shown in Fig. 5. When *Radix glycyrrhizae* and *Vigna radiata* were added to realgar, As species were still As(III) and As(V), inorganic arsenic in gastric phase. *Radix glycyrrhizae* and *Vigna radiata* improved the content of As(III), but reduced As(V). It is worth noting that there was a significant difference in intestinal phase. The content of As(V) increased, but the content of As(III) decreased from 15 to 6.1% and 1.5 % after the addition of *Radix glycyrrhizae* and *Vigna radiata*, respectively. It is well known that As(III) has much higher toxicity compared with other arsenic species. In addition, As complexes weren't detected. The reason is that these complexes couldn't be separated by selected mobile phase.

Fig. 6 presented the Hg species in cinnabar. Only $Hg^{2+}$ was found in cinnabar in the gastrointestinal phase. $CH_3Hg^+$ wasn't detected in the presence of TCMs. It was observed that $Hg^{2+}$ wasn't transformed to $CH_3Hg^+$ whose toxicity is much higher than $Hg^{2+}$, after adding *Radix glycyrrhizae*. Similar to this, there was no significant difference, when *Vigna radiata* and *Rhizoma smilacis glabrae* were added to the cinnabar. The results suggest that $Hg^{2+}$ is the only form in all samples and there is no transformation of Hg speciation.

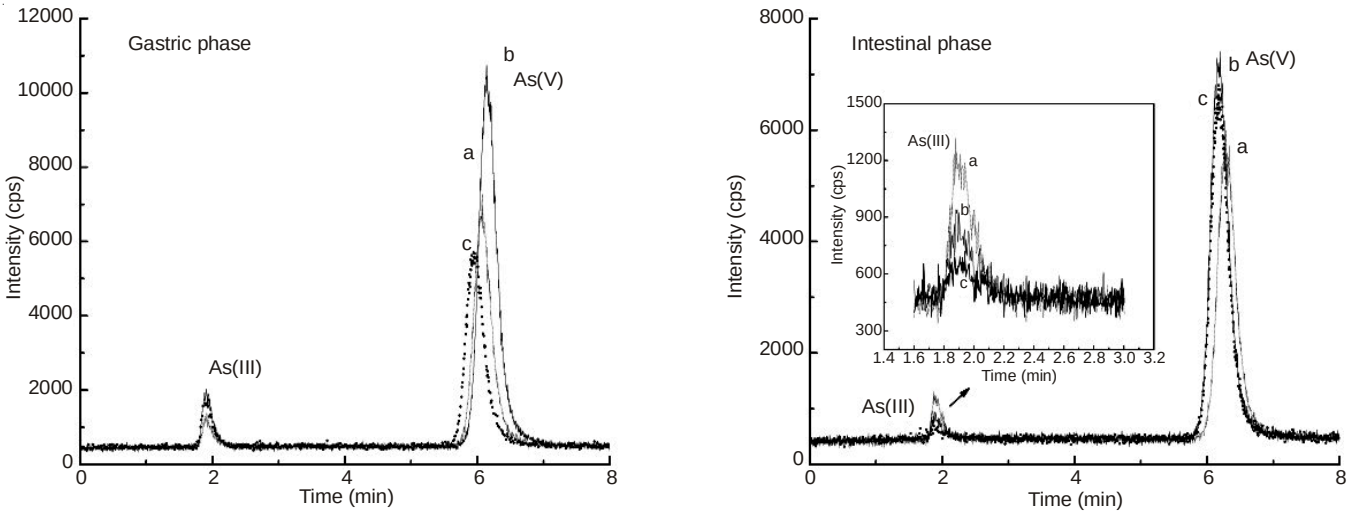


Fig. 5. Effect of *Radix glycyrrhizae* and *Vigna radiata* on As speciation in realgar in gastric and intestinal phase. a: realgar, b: realgar + *Radix glycyrrhizae*, c: realgar + *Vignaradiata*

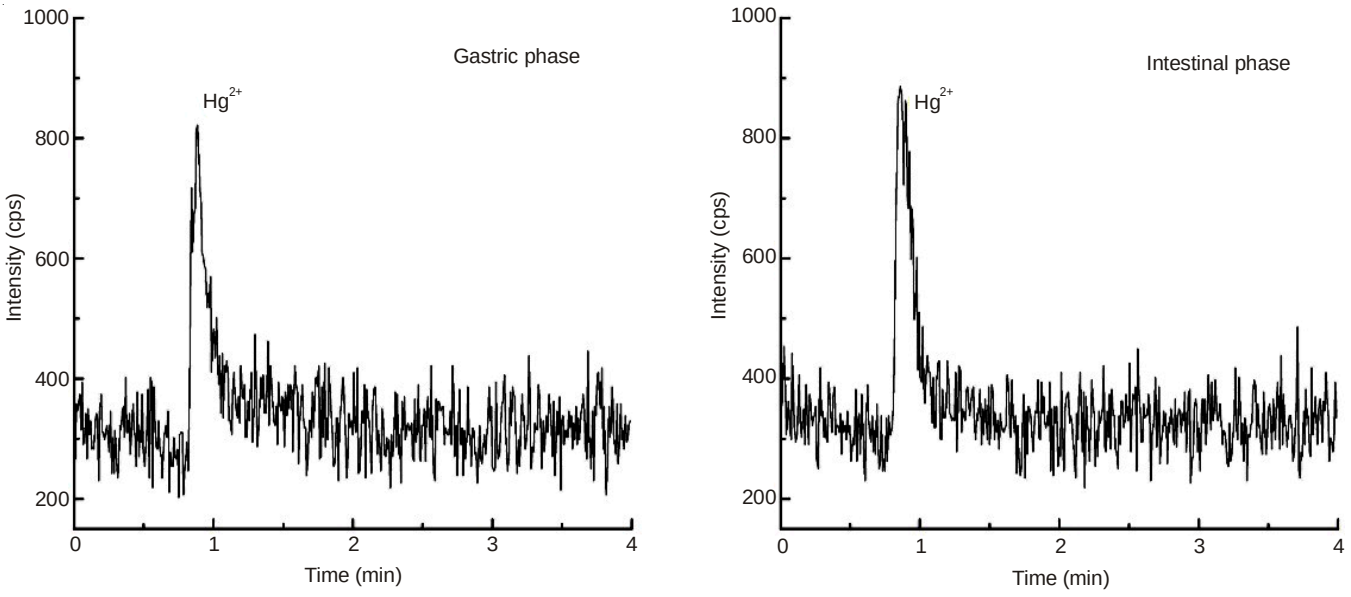


Fig. 6. Hg speciation in cinnabar

## Conclusion

In this work, TCMs had effects on bioaccessibility and speciation of As in realgar. As bioaccessibility was improved after addition of TCMs. *Radix glycyrrhizae* and *Vigna radiata* improved the content of As(III) and reduced As(V) in gastric phase, while both of them reduced the content of As(III) in intestinal phase.

Significant influence on Hg bioaccessiblity was shown in the presence of TCMs. It has been speculated that mechanisms of *Vigna radiata, Radix glycyrrhizae* and *Rhizoma smilacis glabrae* anti-mercurialism are mainly due to physical adsorption, chemical treatment and physiological reaction, respectively. In cinnabar and cinnabar-TCMs mixtures, $Hg^{2+}$ is the only form and there is no transformation of Hg speciation.

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