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RESEARCH ARTICLE

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In vitro investigation on cinnabar dissolution

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Abstract To study the effects of different chemical factors in the gastrointestinal tract, i.e. pH, proteins, amino acids, ionic strength and Na_2S, on the dissolution of cinnabar. The content of the total mercury in various dissolutions of cinnabar was analyzed by UV/VIS Spectrophotometer. Laser Particle Size Analyzer measured the particle distributions in the dissolution of cinnabar. The chemical species of dissolved substance of cinnabar in the presence of Na_2S were determined using ESI-MS. The results indicate that the solubility of cinnabar could be increased significantly in the presence of Na_2S/S^0, and strong acidic pH, respectively. While the influence of thiol amino acid on promoting dissolution remains relatively low. Cinnabar did not dissolve in the form of nanoparticle. It is postulated that cinnabar could be dissolved in the gastrointestinal tract in various forms of sulfur-containing mercury complexes.

Keywords cinnabar, mercuric sulfide, dissolution, mercuric polysulfide

Cinnabar is an important constituent of Chinese traditional medicine used as a tranquilizer for more than two thousands years. The molecular mechanism of its pharmacological effects has not been clarified yet. Recently, the toxicity of cinnabar earned increasing attention [1,2]. The active components related to either pharmacological or toxicological action of cinnabar still remain unclear. It is necessary to investigate the potential of the dissolution and absorption mechanism of cinnabar in the gastrointestinal tract.

Cinnabar is a naturally occurring mercury sulfide. Its major component is α-mercury sulphide (α-HgS) [3], almost insoluble in water (solubility product $\lg K_{sp} = -52.03$). However, animal experiments suggested that [4,5], oral cinnabar could be absorbed via the gastrointestinal tract, and the amount of mercury absorbed was much greater than theoretical values calculated by the solubility product. Previous research on the dissolution of processed cinnabar showed that cinnabar could dissolve in artificial gastric juice in the form of soluble mercuric salt [6]; however, cinnabar hardly dissolves in artificial intestinal juice. It has also been reported [7] that cinnabar could form complexes with cysteine and glutathione (GSH) and turn into relatively stable coordination complexes *in vivo*. In addition, environmental chemistry researches revealed that mercury could migrate in the form of mercuric polysulfide complexes extensively in nature [8−10]. The results above provided a new explication for the study on the dissolution of cinnabar.

For further research on cinnabar dissolution upon oral administration, it is necessary to compare chemical factors in the gastrointestinal tract to postulate the dissolving forms of cinnabar *in vivo*. Therefore, we investigated the solubility of cinnabar in different pH, ion strength, amino acids, proteins and sulfide solutions in comparison with that of pure mercury sulphide. The results indicated that dissolved components of cinnabar may include $Hg(SH)^+$, $HgS(OH)^-$, $HgS_2(OH)^-$, $HgS_3(OH)^-$ in intestinal environment and probably Hg_2SOH^+ in gastric environment.

1 Materials and instruments

The particle size distribution of dissolved liquid was analyzed by ZEN3600 Particle Size Distribution Analyzer (Britain Malvern). Concentration of Hg was analyzed by 752 Visible Ultraviolet Spectrometer (Shanghai Precise Instrument). Compositions of dissolved components were analyzed by LC-MS 2010 Mass Spectrometer (Shimadzu).

Cinnabar was purchased from the Chinese Traditional Medicines Division of National Institute of Pharmaceutical and Biological Product Controls. The content of HgS in raw material is more than 97.16% by X-ray fluorescence analysis. Other impurities include 1.10% Si, 0.53% Sb, 0.35% Se, 0.30% Ca, 0.22% Fe and 0.16% Na. Red mercury sulfide ($HgS \geqslant 98.5\%$) was purchased from Guizhou Tongren Chemical Regent Factory, and ignition residues include 0.05% sulfate salt, 0.005% Fe and 0.005% heavy metals calculated on Pb; Free mercury and polyvalent mercuric salt were not

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detected. Hg standard solution (1000 $g \cdot mL^{-1}$) was purchased from State Center for Standard Substance. All the other regents are analytical grade.

2 Methods

2.1 Preparation of dithizone stock solution and working solution

Dithizone stock solution: 0.65 g dithizone was dissolved in 100 mL chloroform and stored in 4°C dark environment. Dithizone working solution: 2.0 mL dithizone stock solution was added into a separating funnel, and then 50 mL 2% ammonia water was added for solvent extraction. Organic solvent was removed, and aqueous sample was filtered and used immediately.

2.2 Dissolution of cinnabar

1.0 g cinnabar (three duplicate samples) was weighed and mixed with 10.0 mL various dissolving solutions, respectively. Subsequently, the mixed solutions were placed under a 37°C-closed condition and shaken at 200 $r \cdot min^{-1}$ for 6 h [11]. And afterward, the supernatant liquids obtained from the mixed solutions were centrifuged at 15,000 $r \cdot min^{-1}$ for 10 min, and then the supernatant was collected once again as extracting solution from cinnabar. Mercury contents were measured according to the method described in Section 2.3.

In order to investigate the influence of pH, ion strength, amino acids, proteins and sulfide solutions on the dissolution of cinnabar, dissolving solutions were prepared as follows: different pH solutions prepared with hydrochloride acid and sulfate acid, different ion strength solutions prepared with sodium chloride, potassium chloride and sodium nitrate, as well as solutions containing different concentrations of bovine serum albumin (BSA), pepsin, trypsin, cysteine, glycine and sodium sulfide (Na_2S).

2.3 Determination of mercury

Mercury content was determined using a slightly modified method as described previously [12], 100 μL testing solution was added into 1.0 mL aqua regia for microwave digestion. Excessive acid was evaporated and then 1.0 mL chelating solution (EDTA 50 $g \cdot L^{-1}$, sodium citrate 50 $g \cdot L^{-1}$, sodium tartrate 50 $g \cdot L^{-1}$), 2.0 mL of 6.0 $mol \cdot L^{-1}$ sodium hydroxide solution and 2.0 mL of dithizone working solution were added. Finally, the solution was diluted into 10.0 mL with de-ionized water. Absorption at 551 nm was measured against a blank solution.

All the experimental data were statistically analyzed using an Origin 6.0 program, and errors were presented as standard deviations (SD). Solute concentration, ion strength and pH were taken as an abscissa; meanwhile, the ratio of dissolving mercury of cinnabar in the dissolving solution to that of cinnabar in the pure water (relative mercury solubility) was taken as an ordinate for plotting.

2.4 Determination of the size distribution in dissolved solutions

Sample solutions were measured for size distribution with Particle Size Distribution Analyzer, after 4 h vacuum degassing.

2.5 Mass spectrometric analysis of dissolving solutions

The dissolving solution of cinnabar in 10 $mmol \cdot L^{-1}$ Na_2S was applied to an ESI-MS, in which mobile phase was water and methanol (1 : 1) at a flow rate of 50 $\mu L \cdot min^{-1}$, N_2 flow rate is 1.5 $L \cdot min^{-1}$, and pipe flow is 0.07 $mL \cdot min^{-1}$.

3 Results

3.1 Influences of pH on dissolution

The experimental results indicated that the amounts of dissolved mercury from cinnabar significantly increased with the decline of pH. This is in accordance with the results of cinnabar dissolution in artificial gastric juice [6]. However, dissolved mercury from cinnabar is obviously larger than that of HgS under $pH < 2$ condition. This is perhaps because impurities contained other forms of mercuric compounds in cinnabar (Fig. 1).

3.2 Influences of proteins and amino acids on dissolution

There are many proteins and amino acids in the digestive tract. These substances, especially sulfhydryls, show a binding capacity to mercury. Therefore, we studied the influences of proteins (BSA, pepsin, and trypsin) and amino acids (cysteine, glycine) on the dissolution of cinnabar. The results showed that cysteine had a slightly higher effect on solubility than glycine, but proteins showed no obvious effect (Fig. 2).

3.3 Influences of ionic strength on dissolution

Although soluble salt in the digestive tract, as electrolyte background, may have an effect on dissolution equilibrium, Fig. 3 shows that the change of the ionic strength of different salts (sodium chloride, potassium chloride and sodium nitrate) could not promote HgS dissolution. In addition, merely at a high concentration, the effect of sodium chloride and potassium chloride on cinnabar dissolution is a little more than sodium nitrate, which indicates that chloride-ion may increase cinnabar dissolution to some extent, but has no significant difference.

3.4 Influences of low-concentration Na_2S solution on dissolution

We hypothesized that cinnabar could form mercuric polysulfide in reaction with S^{2-} produced by intestinal

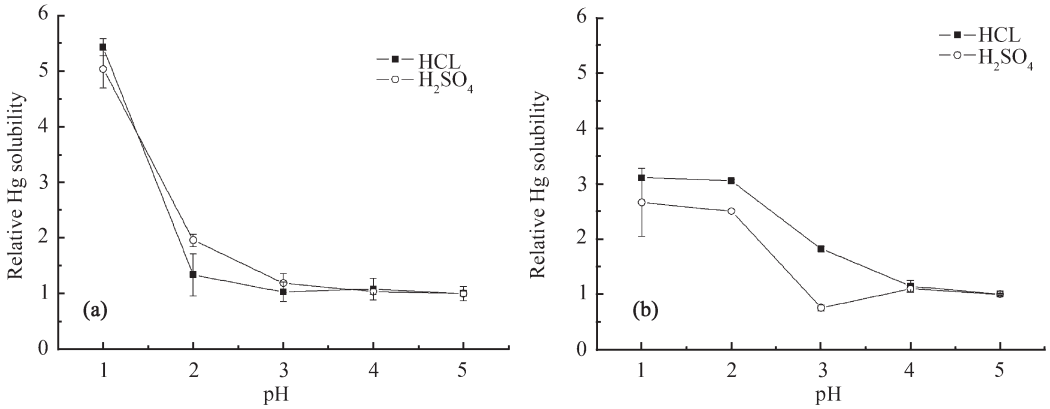

Fig. 1 Solubility curve of cinnabar (a) and HgS (b) in different pH solutions

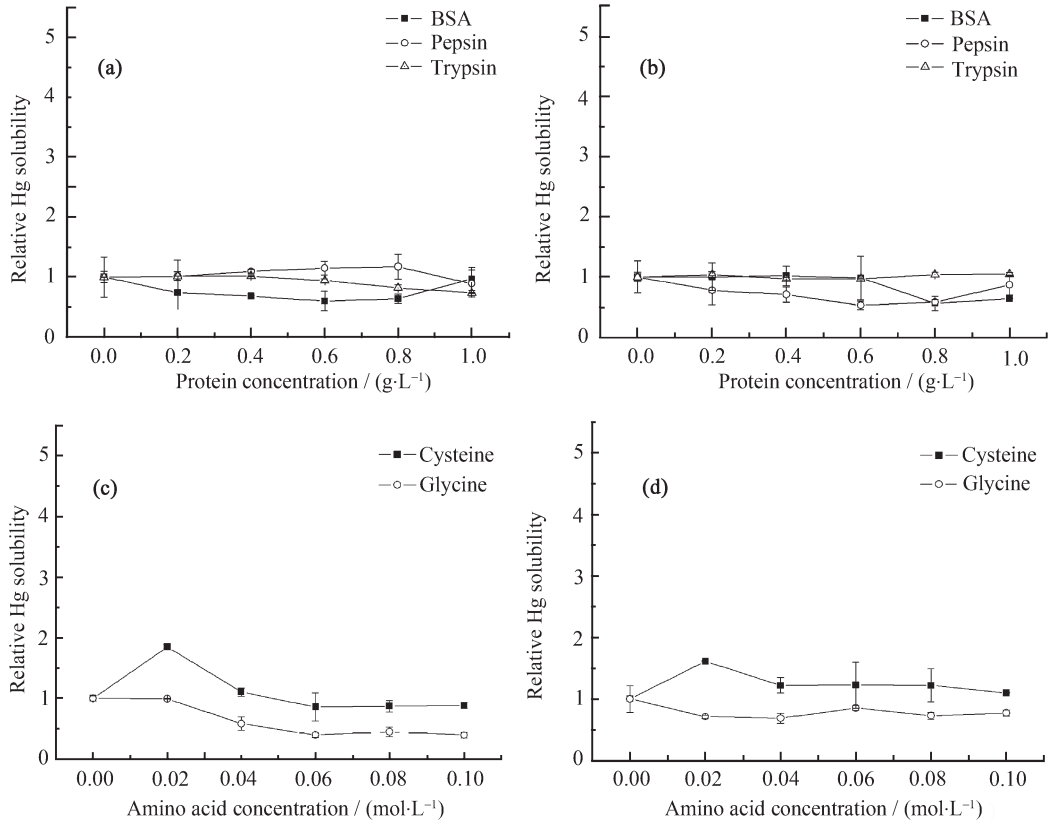

Fig. 2 Solubility curve of cinnabar (a), (c) and HgS (b), (d) in protein or amino acid solutions

bacteria upon oral administration. Therefore, we investigated the solubility of cinnabar in different concentrations of Na_2S solutions. Results in Fig. 4 reveal that the solubility of cinnabar increases with the rise of Na_2S concentration. When the concentration of Na_2S was larger than 0.05 mol·L^{-1}, the solubility of cinnabar was significantly enhanced. The solubility of cinnabar could be further increased in the presence of element sulfur. In 10 mmol·L^{-1} Na_2S solution, the amount of mercury dissolved from cinnabar could become 4–5 times larger than that in pure water, when the ratio of S^0/HgS reached 1 : 3, it suggested a strong effect of solubility enhancement with the element sulfur.

3.5 Identification of dissolved components of cinnabar in Na_2S solution

Dissolved solution of cinnabar in 10 mmol·L^{-1} Na_2S solution was identified with ESI-MS (Fig. 5). Mass spectra peaks of $m/z = 232.9$, 249.0, 279.4 and 312.4 were corresponding to $Hg(SH)^+$($m/z = 233$), $HgS(OH)^-$($m/z = 249$), $HgS_2(OH)^-$ ($m/z = 281$) and $HgS_3(OH)^-$($m/z = 313$).

3.6 Particle size distribution of dissolving solution

In order to clarify whether the dissolved solution of cinnabar is an aqueous solution or colloid, we measured the dissolved

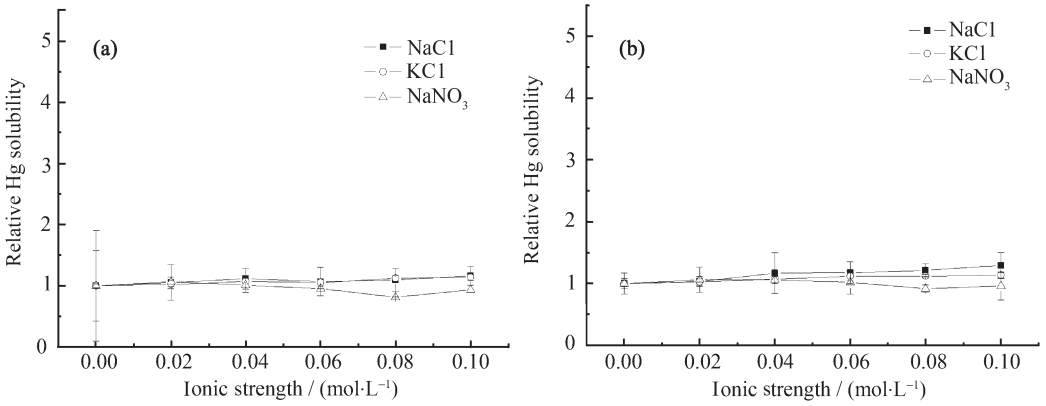

Fig. 3 Solubility curve of cinnabar (a) and HgS (b) in different ionic strength solutions

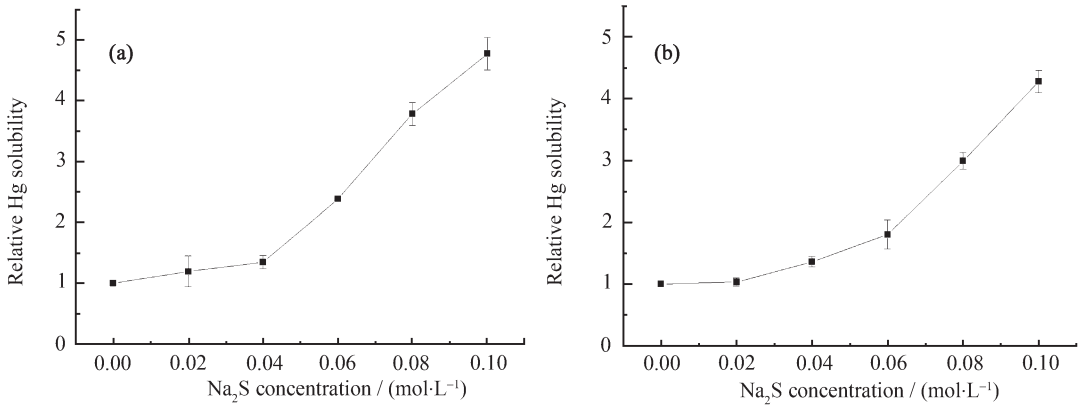

Fig. 4 Solubility curve of cinnabar (a) and HgS (b) in low-concentration Na_2S solution

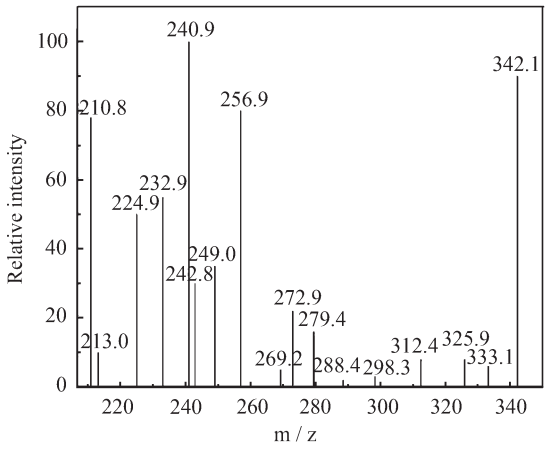

Fig. 5 ESI-MS spectra of cinnabar extractions in 10 mmol · L^{-1} Na_2S solution at 37°C
Note: MS peaks with m/z = 232.9, 249.0, 279.4, 312.4 are corresponding to MPS.

solution of cinnabar for size distribution with Particle Size Distribution Analyzer. The dissolved solution of cinnabar and HgS both contained some particles, the diameters of which were in the range of 0.1−1.0 μm. However, the number of particles was not correlated to the total mercury concentration, indicating that cinnabar did not dissolve in the form of nano-mineral particles.

4 Conclusions

The experimental results above indicate that the dissolution of cinnabar is very little affected by ion strength, protein (BSA, pepsin, trypsin) and nonthiol amino acid (glycine). Thiol amino acid (cysteine) had a little bit effect on dissolution. However, the results suggested that what affects cinnabar dissolution in the gastrointestinal tract could include the following factors.

(1) Low pH in stomach. Above results indicate that the amounts of dissolved mercury from cinnabar increase with the decline of pH. Under the acidic condition, the following chemical reaction of HgS [13] is proposed accounting for the dissolved mercury of cinnabar in acid

$$2HgS+H^{+}+H_2O = Hg_2SOH^{+}+H_2S$$

However, the solubility of cinnabar in hydrochloride acid is a little higher than that of cinnabar in sulphate acid. It is possible that aqueous Cl^- has some capability of coordination with mercury (stable constant of $HgCl_4^{2-}$ is 14.47 [14]), and can become soluble complexes to enhance dissolution, whereas exact forms of coordination complexes require further investigation.

(2) Weak basic and sulfur-rich environment in intestinal tract. The above experimental results indicate that Na_2S and

S^0 could promote the dissolution of cinnabar more effectively than that of thiol amino acid. This may be the reason that the complex stability constant of HS^- and mercury (stable constant of cysteine is 36.8) is larger than that of organic ligand and mercury (stable constant of cysteine is 14.2) [15]. The mass spectra data showed that, cinnabar can form various mercuric complexes containing sulfur, such as $Hg(SH)^+$, $HgS(OH)^-$, $HgS_2(OH)^-$ and $HgS_3(OH)^-$, in the low Na_2S concentration. Conditions for the existence of mercuric polysulfide in the intestine include: weakly basic (pH≈8.4) and abundant H_2S produced by intestinal flora (mainly in the form of HS^-). Therefore, we postulated that mercuric polysulfide complex might be an important form of cinnabar *in vivo*.

In summary, we studied the effects of various chemical factors in the gastrointestinal tract on the dissolution of cinnabar, and found that the potential forms of cinnabar in the intestine may be various mercuric coordination complexes containing sulfur, which have previously been neglected. Further investigation on intestinal absorption and potential pharmacological effects of these proposed dissolved components from cinnabar should be conducted.

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