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海洋浮游植物对近海真光层中微量金属的吸附、  
吸收、生物富集及形态分布的影响研究

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吸收、生物富集及形态分布的影响研究

**Effect of marine phytoplankton on metal sorption,  
bioaccumulation, and species distribution in the euphotic  
layer of the coastal seawater**

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## 摘要

研究海洋浮游植物、微量金属及其耦合作用引发光化学过程的机制；建立海水中微量金属锑的价态检测新技术；探讨近海浮游植物在氮磷富营养化状况下对金属吸附、吸收、生物富集和形态分布的影响；探究近海真光层中营养盐、浮游植物、重金属多元体系中光致羟自由基的形成，即光诱导微量金属价态转化的可能机制。取得了以下主要成果：

### 1. 建立锑价态分析、有效避免 Sb(III)氧化的新方法

锑的形态分析是痕量元素形态分析的热点和难点，因为锑被美国环境保护规划署、欧共体和德国列为污染控制优先污染物和环境激素类重金属，Sb(III)极易被氧化；锑在环境、生物和地球化学样品中主要以 Sb(III)和 Sb(V)存在；Sb(III)和 Sb(V)的毒理特性和生物、生理行为有显著差异。

海水中锑含量处于痕量水平，低于测定方法的检出限，且存在大量的干扰物质，形态分析前必须进行预富集，提高测试液中锑的浓度，将锑与干扰组分分离，降低方法的检出限，提高分析结果的精密度和准确度。但最近研究表明 Sb(III)在数小时内即可被氧化为 Sb(V)。在 Sb(V)共存条件下，Sb(III)可被流动注射-氢化物发生-原子吸收光谱法(FI-HG-AAS)选择性测定。

以高比表面积、高吸附容量的纳米 TiO<sub>2</sub> 为固相吸附剂(50mg)，在 pH=3.0 条件下，可快速(15 min)富集 500mL 水样中痕量 Sb(III) 和 Sb(V)，以 10 mL 5mol/L HCl 溶液洗脱；用流动注射-氢化物发生原子吸收分光光度法直接测定洗脱液中 Sb(III)浓度，用 0.5gKI 和 0.2g 硫脲将另一份洗脱液中 Sb(V)还原为 Sb(III)，测定洗脱液中锑的总浓度，差减法得 Sb(V)浓度。本法对 Sb(III) 和 Sb(V)的检出限 ( $3\sigma$ , n=11)分别为 0.05 ng.mL<sup>-1</sup>、0.06 ng.mL<sup>-1</sup>，对试样的富集倍数 50 倍，降低了干扰组成对锑测定的影响；在酸性(pH=3.0)和强酸性(5 mol.L<sup>-1</sup>HCl)条件下对锑进行富集和洗脱，对水样中 Sb(III)潜在氧化剂的分离去除，有效避免了 Sb(III)的氧化。本法用于海水中锑的形态分析，结果满意，为开展海藻-过渡金属-光三元体系对锑的形态转化研究提供检测方法支撑。

### 2. 海藻、过渡金属二元体系对 Sb(III)的光氧化作用

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研究海藻(硅藻、红藻、绿藻)、过渡金属 (Fe(III)、Cu(II)、Mn(II))、海藻与过渡金属二元体系,在高压汞灯光照下对 Sb(III)的光氧化作用,应用氢化物发生法-原子吸收光谱法,通过控制试样酸度选择性测定 Sb(III),计算 Sb(III)光氧化转化率及转化平衡时间。

实验结果首次确认海藻对海水中锑的光化学行为的影响,上述三种体系均对海水中 Sb(III)具有光氧化作用;海藻种类不同,对 Sb(III)光氧化能力不同;在藻单一体系中,海藻浓度越高, Sb(III)的光氧化转化率越高,转化平衡时间越短;过渡金属的光致氧化还原过程诱导了锑的价态转化;经藻与过渡金属离子所构成的二元体系对 Sb(III)的光氧化作用后 Sb(V)/Sb(III)的比值,6种海藻 (*Tetraselmis levis*, *Chlorella autotrophica*, *Nannochloropsis sp.*, *Tetraselmis subcordiformis*, *Phaeodactylum tricornutum*, and *Porphyridium purpureum*)介于 1.07~5.48,只有 *Dunaliella salina* 为 0.92。海藻-过渡金属二元体系对锑在海水真光层中的分布产生重大影响,其中海藻为主要贡献者,两者的联合作用呈协同或拮抗效应。研究结果为表层海水中 Sb(III)和 Sb(V)共存、Sb(V)浓度大于 Sb(III)的分布原因、锑的生物地球化学循环提供了解释,为锑的危险评价提供有效的背景数据。

### 3. 海藻-过渡金属-光三元体系对海水真光层中铬形态分布影响研究

以海洋绿藻、硅藻、红藻和过渡金属(铁、锰、铜)构建海藻-光二元体系、过渡金属-海藻-过渡金属-光三元体系,对比分析不同海藻、不同过渡金属、海藻与过渡金属耦合引发光化学过程。海藻本身对 Cr(VI)的光还原活性首次被证实。海藻浓度的提高、海藻与过渡金属的耦合作用有利于光还原转化率的提高。不同的海藻拥有不同的光还原转化率。通过三元体系的光还原转化,5种绿藻(*Tetraselmis levis*, *Chlorella autotrophica*, *Dunaliella salina*, *Nannochloropsis sp.*, *Tetraselmis subcordiformis*)的光还原转化 Cr(VI)/ Cr (III) 比值为 1.45~2.16,硅藻 (*Phaeodactylum tricornutum*)和红藻(*Porphyridium purpureum*)则分别仅为 0.48 和 0.71。海洋中浮游植物和过渡金属对海洋真光层中铬的形态分布影响极大。实验结果为进一步研究 Cr(VI)和 Cr (III)在生物地球化学循环中分布提供基础,同时也为海洋真光层中铬的危险评价研究提供重要信息。

### 4. 氮磷富营养化、重金属污染对海洋硅藻光致产生羟基自由基的影响研究

海水真光层中高化学活性的羟基自由基对微量元素的生物地球化学循环,即

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微量元素的形态、生物可给性、形态迁移和转化等产生显著影响，据已有研究结果提出的预测值同海水真光层实际值有很大差距。

首次提出海洋浮游植物是羟基自由基重要的“missing source”，并以 *Dunaliella salina* 作为海洋浮游植物的模式藻，以苯甲酸钠为探针，应用 HPLC 法检测羟基自由基( $\cdot\text{OH}$ )，首次证实海洋浮游植物可光致产生羟基自由基，具有光化学活性。以 *Dunaliella salina* 作为海洋硅藻的模式藻，以苯甲酸钠为探针，应用高效液相色谱法检测羟基自由基( $\cdot\text{OH}$ )。首次证实海洋浮游植物可光致产生羟基自由基，具有光化学活性。近海生物经常暴露于重金属污染和氮磷富营养化条件下。实验研究重金属污染( $5.0 \mu\text{g/L Pb(II)}$  和  $0.1 \mu\text{g/L}$  甲基汞)、氮磷营养盐浓度、光照时间、海藻细胞密度对光致产生羟基自由基的影响。海水中羟基自由基的产生量随海藻细胞密度和光照时间增加而明显增大。无论光化学体系是否含海藻，海水中  $\text{Pb(II)}$ 、 $\text{Pb(II)}$ 和甲基汞共存均可显著提高海水中的羟基自由基的产量；仅加入甲基汞，则降低海水中羟基自由基的产量。羟基自由基的产量与海藻细胞表面的碱性功能团数量、单位细胞中叶绿素含量呈正相关性。营养盐添加对羟基自由基产量的影响主要来自硝酸盐的光解作用及其对海藻光化学活性的影响。

#### 5. 氮磷营养盐对杜氏藻吸附、吸收、生物富集铁，以及海水中铁形态分布的影响研究

探讨氮磷营养盐添加，在长时间培养海洋硅藻条件下，对铁生物地球化学循环的影响，即海洋硅藻 *Thalassiosira weissflogii* 和 *Skeletonema costatum* 分别培养 4 天和 6 天，对铁的吸收、吸附、生物富集及水体中铁配合物粒径分布的影响。

实验采用微量元素清洗剂洗脱吸附在细胞表面的铁(吸附)，从而与细胞体内的铁(吸收)分离；应用物理形态分离技术( $0.4\mu\text{m}$  滤膜抽滤，切向流超滤)将水体中铁配合物进行截留，了解铁配合物在颗粒( $>0.4 \mu\text{m}$ )，胶体( $0.4\mu\text{m}$ - $100 \text{ kDa}$ ， $100$ - $50 \text{ kDa}$ ， $50$ - $30 \text{ kDa}$ ， $30$ - $10 \text{ kDa}$ ， $10$ - $3 \text{ kDa}$ )，和可溶态( $<3 \text{ kDa}$ )等 7 个粒径间的分布，ICP-MS 法检测铁浓度。*T. weissflogii* 对铁的生物富集因子(BCF)随 N 浓度(6 到  $64 \mu\text{mol/L}$ )与 P 浓度( $2 \mu\text{mol/L}$  到  $10 \mu\text{mol/L}$ )增加而增加。*S. costatum* 对铁的 BCF 随 N 浓度(16 到  $64 \mu\text{mol/L}$ )增加而增加；基本上随磷浓度( $2 \mu\text{mol/L}$  到  $10 \mu\text{mol/L}$ )增加而减少。氮浓度对硅藻吸附、吸收铁的影响无种间差异性；磷浓度对硅藻吸附铁的影响无种间差异，但对铁的吸收、生物富集因子的影响却呈种间

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差异性。*T. weissflogii* 和 *S. costatum* 于氮、磷浓度为 32、2  $\mu\text{mol L}^{-1}$ ，氮磷比为 16(Redfield ratio)时，对铁的吸收、吸附值即对水体中铁的摄取和需求最低；在高氮区，硅藻对铁的摄取大于低氮区，此为高氮量低叶绿素量(high-nitrate, low-chlorophyll)原因之一。除在高磷( $10\mu\text{mol L}^{-1}$ )条件下，*S. costatum* 对铁的总摄取量、总吸附量、总吸收量均大于 *T. weissflogii*，藻种差异对海水中铁的消耗影响显著。水体中可溶态( $<3\text{ kDa}$ )铁含量与 *T. weissflogii* 对铁的吸收量存在正相关。

#### 6. 氮浓度对海洋浮游植物吸附、吸收和生物富集重金属的影响

海洋浮游植物在微量金属的生物地球化学循环中起着极其重要的作用，营养盐浓度及其比例的改变对浮游植物摄取(uptake，同时包含吸收和吸附)微量金属产生显著影响，但已有研究结果未能对吸收、吸附行为进行区分，未对吸收和吸附影响机制进行探讨。微量金属被海洋浮游植物吸收、吸附后其生物地球化学行为不同，即分布、迁移、转化、归宿、循环和收支平衡情况不同；前者主要通过食物链传递，部分经消化后排泄进入水体；而后者可进一步为生物体所吸收，也可解吸进入水体；且只有得出生物体对微量金属的吸收量，才能得出生物浓缩系数 BCF 这一重要参数。

近海生物经常暴露于重金属污染和富营养化条件下。*Dunaliella salina* 属单细胞、无细胞壁、海洋微藻，课题以此为海洋生物模型，研究在营养盐变化(磷浓度为  $0.5\mu\text{mol L}^{-1}$ ，氮浓度分别为 6, 8, 12.5,  $55\mu\text{mol L}^{-1}$ )和重金属(Fe、Mn、Zn、Cu)污染的环境中，经 10 天的曝露期后，近海浮游生物对 Fe、Mn、Zn、Cu 的细胞外吸附、细胞内吸收和生物富集的影响。营养盐浓度的变化会明显影响海藻对重金属的吸收、吸附及生物富集。当 N:P=16 时，海藻对所有的金属(Fe、Mn、Zn、Cu)的吸收值和生物富集因子都达到最高。对金属的吸附，在 N:P=110 时达到最高值，在 N:P=25 时最低。高营养环境下，*Dunaliella salina* 对金属吸附能力的顺序(Fe>>Mn>Zn>Cu)未受营养盐变化影响，但绝对吸附量受到的影响明显。氮浓度对金属吸附和吸收的影响不同。

**关键词：浮游植物；光；营养盐；微量金属；**

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## Abstract

1. A novel method for prevention of the oxidation of Sb(III) during sample pretreatment, preconcentration of Sb(III) and Sb(V) with nanometer size titanium dioxide (rutile) and speciation analysis of antimony, has been developed. Antimony(III) could be selectively determined by flow injection-hydride generation-atomic absorption spectrometry, coexisting with Sb(V). Trace Sb(III) and Sb(V) were all adsorbed onto 50 mg TiO<sub>2</sub> from 500 ml solution at pH 3.0 within 15 min, then eluted by 10 ml of 5 mol/l HCl solution. One eluent was directly used for the analysis of Sb(III); to the other eluent was added 0.5g KI and 0.2g thiourea to reduce Sb(V) to Sb(III), then the mixture was used for the determination of total antimony. The antimony(V) content is the mathematical difference of the two concentrations. Detection limits (based on 3  $\sigma$  of the blank determinations,  $n = 11$ ) of 0.05 ng/ml for Sb(III) and 0.06 ng/ml for Sb(V), were obtained.

2. The photo-oxidation of Sb(III) to Sb(V) by marine microalgae (diatom, green and red algae) with or without the presence of transition metals (Fe(III), Cu(II) and Mn(II)). The influence of marine phytoplankton on the photochemistry of antimony was confirmed for the first time. The conversion ratio of Sb(III) to Sb(V) increased with increasing algae concentration and irradiation time. Different species of marine phytoplankton were found to have different photo-oxidizing abilities. The photochemical redox of transition metals could induce the species transformation of antimony. After photo-induced oxidation by marine phytoplankton and transition metals, the ratio of Sb(V) to Sb(III) was in the range of 1.07–5.48 for six algae (*Tetraselmis levis*, *Chlorella autotrophica*, *Nannochloropsis* sp., *Tetraselmis subcordiformis*, *Phaeodactylum tricorutum*, and *Porphyridium purpureum*), and only 0.92 for *Dunaliella salina*. The distribution of antimony in the sunlit surface seawater was greatly affected by combined effects of marine phytoplankton (main contributor) and transition metals; both synergistic and antagonistic effects were observed. The results provided further insights into the distribution of Sb(III) and Sb(V) and the biogeochemical cycle of antimony, and have significant implications for the risk assessment of antimony in the sunlit surface seawater.

3. The photo-reduction of Cr(VI) to Cr(III) by marine phytoplankton (diatoms, red and green algae), with or without the presence of transition metals (Fe(III), Cu(II) and Mn(II)) was studied. The direct influence of marine phytoplankton on the photochemical reduction of



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Cr(VI) was confirmed for the first time, and two kinds of mechanism were suggested to be responsible for the species transformation: (a) Cr(VI) in excited state could be reduced by the electron donor in its ground state via photo produced electrons; and (b) the solvated electrons reduce the  $\text{CrO}_4^{2-}$  anions in their ground state. The conversion ratio of Cr(VI) to Cr(III) increased with increasing algae concentration and irradiation time. Different species of marine phytoplankton were found to have different photo-reducing abilities. The photochemical redox of transition metals could induce the species transformation of chromium. After photo-reduction by marine phytoplankton and transition metals, the ratio of Cr(VI) to Cr(III) was in the range of 1.45~2.16 for five green algae (*Tetraselmis levis*, *Chlorella autotrophica*, *Dunaliella salina*, *Nannochloropsis sp.*, and *Tetraselmis subcordiformis*), and only 0.48 for *Phaeodactylum tricornutum* (diatom) and 0.71 for *Porphyridium purpureum* (red alga). The species distribution of chromium in the sunlit surface seawater was greatly affected by combined effects of marine phytoplankton (main contributor) and transition metals; both synergistic and antagonistic effects were observed. The results provided further insights into the species distribution and the biogeochemical cycle of chromium, and have significant implications for the risk assessment of chromium in the sunlit surface seawater.

4. A marine microalga *Dunaliella salina* was used as a model organism in this study. Hydroxyl radical ( $\cdot\text{OH}$ ) was determined by HPLC using sodium benzoate as a probe. The photochemical activity of marine alga on the formation of hydroxyl radicals was confirmed for the first time. Coastal organisms are often exposed to both metal pollution and macronutrient enrichment. The influences of the algal concentration, exposure time, macronutrients (nitrate and phosphate) additions and metal pollution ( $5.0\mu\text{g/L}$  Pb(II) and  $0.1\mu\text{g/L}$  methylmercury) on the photoproduction of hydroxyl radical were examined. The photoproduction of  $\cdot\text{OH}$  was increased with the increasing of algae concentration and exposure time. The photoproduction of  $\cdot\text{OH}$  in seawater with or without *D. salina* could be increased greatly for the addition of Pb(II), Pb(II) and methylmercury, but was decreased by only addition of methylmercury. The photoproduction of  $\cdot\text{OH}$  was positive correlation with the content of basic function group on cell's surface and chlorophyll a per cell. The influence of macronutrient addition on the photoproduction of  $\cdot\text{OH}$  was from the photolysis of nitrate and the effects on the photochemical activity of *D. salina*.

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5. The influences of macronutrient enrichment on iron absorption (intracellular uptake), iron adsorption (cellular surface uptake), and the bioaccumulation factor (BCF) of iron by coastal diatoms were examined. In addition, the distribution of different iron size fractions in seawater in relation to two diatom species exposed to nutrient enrichment over an extended period (6 days for *Thalassiosira weissflogii*, and 4 days for *Skeletonema costatum*), was investigated. Iron concentrations were measured by inductively coupled plasma mass spectrometry of seven size fractions: particles ( $>0.4 \mu\text{m}$ ), colloidal particles ( $0.4\mu\text{m}$ -100 kDa, 100-50 kDa, 50-30 kDa, 30-10 kDa, 10-3 kDa), and soluble species ( $<3 \text{ kDa}$ ). Both the bioaccumulation of iron in the diatoms and the iron species distribution in the water were affected by the diatom species present, as well as the nitrate (N) and phosphate (P) concentration. The BCF of iron by *T. weissflogii* increased with increasing N (from 16 to  $64 \mu\text{mol L}^{-1}$ ) and P (from 2 to  $10 \mu\text{mol L}^{-1}$ ) concentration. Whereas, the BCF of iron by *S. costatum* increased markedly with increasing N concentration (from 16 to  $64 \mu\text{mol L}^{-1}$ ), but decreased with increasing P concentration (from 2 to  $10 \mu\text{mol L}^{-1}$ ). Non-alga-specific influences of N addition on iron absorption, iron adsorption and the BCFs of iron were observed; but the influence of P addition on iron adsorption was non-alga-specific, although it was alga-specific on iron absorption and the BCFs of iron. The addition of P could affect the iron internalization strategy and this was confirmed for the first time. When the ratio of N and P was 16 ( $32:2 \mu\text{mol/L}$  for N:P), both iron absorption and iron adsorption per cell of *T. weissflogii* and *S. costatum*, were at minimum. The iron uptake by *T. weissflogii* and *S. costatum* under a high-N regime was higher than under a low-N regime. Except in the case of the high-P regime ( $10 \mu\text{mol L}^{-1}$ ), total uptake, total adsorption, and total absorption by cells of *S. costatum* were more than those for *T. weissflogii*, i.e., the influence of the diatom species involved in the depletion of iron in seawater was obvious. The content of soluble iron species in seawater was positively correlated with the amount of iron absorption by *T. weissflogii*, but not with *S. costatum*.

6. Coastal organisms are often exposed to both metal pollution and macronutrient enrichment. *Dunaliella salina*, a uni-cell, cell-wall-less green marine microalga, was used as a model marine organism. The influences of major nutrients additions on the absorption (intracellular uptake), adsorption (cellular surface uptake), and bioconcentration of trace metals (Fe, Mn, Zn, and Cu) in *Dunaliella salina* over 10 days were examined. When N:P ratio was 16 (8,  $0.5\mu\text{mol/L}$  for nitrate,

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phosphate), both metal absorption and bioconcentration factor were the most. The maximum and minimum metal adsorptions were obtained, respectively, at N:P ratio of 110 (55, 0.5  $\mu\text{mol/L}$  for nitrate, phosphate) and 25 (12.5, 0.5  $\mu\text{mol/L}$  for nitrate, phosphate). The difference in ambient nitrate concentration did not affect the order of metal adsorption of *Dunaliella salina* ( $\text{Fe} \gg \text{Zn} > \text{Mn} > \text{Cu}$ ), but the absolute adsorption quantities were greatly affected. The influence of nitrate concentration on metal absorption and adsorption was different.

**Keywords: phytoplankton; sunlight; trace metal; macronutrient;**

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## Speciation of Antimony by Preconcentration of Sb(III) and Sb(V) in Water Samples onto Nanometer-size Titanium Dioxide and Selective Determination by Flow Injection-Hydride Generation-Atomic Absorption Spectrometry

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A novel method for prevention of the oxidation of Sb(III) during sample pretreatment, preconcentration of Sb(III) and Sb(V) with nanometer size titanium dioxide (rutile) and speciation analysis of antimony, has been developed. Antimony(III) could be selectively determined by flow injection-hydride generation-atomic absorption spectrometry, coexisting with Sb(V). Trace Sb(III) and Sb(V) were all adsorbed onto 50 mg TiO<sub>2</sub> from 500 ml solution at pH 3.0 within 15 min, then eluted by 10 ml of 5 mol/l HCl solution. One eluent was directly used for the analysis of Sb(III); to the other eluent was added 0.5 g KI and 0.2 g thiourea to reduce Sb(V) to Sb(III), then the mixture was used for the determination of total antimony. The antimony(V) content is the mathematical difference of the two concentrations. Detection limits (based on 3 $\sigma$  of the blank determinations,  $n = 11$ ) of 0.05 ng/ml for Sb(III) and 0.06 ng/ml for Sb(V), were obtained.

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### Introduction

Antimony is ubiquitously present in the environment. Its toxicity has been demonstrated; on the contrary, it has no essential function in animals or people. Antimony exists in various oxidation states (0, III, V), but Sb(V) and Sb(III) are predominant in environmental, biological and geochemical samples.<sup>1</sup> The toxicological and physiological behaviors of antimony depend on its oxidation state.<sup>2</sup> Antimony(III) shows a high affinity for red blood cells and sulfhydryl groups of cell, while erythrocytes are almost impermeable to Sb(V).<sup>3</sup> Generally Sb(III) compounds are claimed to exert a 10-times higher toxicity than Sb(V) compounds.<sup>4</sup> So, the species transformation of antimony would greatly affect its toxicity in the environment. Considering the potentially harmful nature of antimony, the US Environmental Protection Agency,<sup>5</sup> EU<sup>6</sup> and German Research Council<sup>7</sup> listed antimony and its compounds as priority pollutants.

For a better understanding of the fate of Sb in natural water and for a sound risk assessment, speciation data for Sb are urgently required. Typical concentrations of total dissolved antimony are usually less than 1.0  $\mu\text{g/l}$  in non-polluted waters.<sup>1</sup> Because of the extremely low concentrations of inorganic forms of Sb in water, a preliminary concentration is usually necessary. Recently, preconcentration techniques such as solvent extraction,<sup>8</sup> solid phase extraction,<sup>9</sup> precipitation and coprecipitation<sup>10,11</sup> are applied to the speciation of antimony, but the crucial requirement for preserving the Sb species information has not so far been thoroughly investigated nor

considered by most researchers. A recent study<sup>3</sup> revealed that Sb(III) is easily oxidized within some hours to Sb(V), raising the question whether or not the concentrations of Sb(V) found as the major Sb species in extracts of various matrices have been overestimated owing to oxidation of Sb(III) to Sb(V) during sample manipulation. On the other hand, sample preparation is often the bottleneck of an analytical procedure because it is a manual process which makes it slow, work-intensive and a frequent source of errors and contamination, particularly when large numbers of samples are involved.

Flow injection-hydride generation-atomic absorption spectroscopy (HG-AAS) as the detection system for the selective determination of antimony species, with low detection limit and low cost is adopted widely in developing countries.<sup>12</sup> The use of masking agents in hydride generation techniques (HG) for the determination of antimony is one of the most important methods for the control of liquid phase interferences generated by concomitants, mainly transition metals and elements forming the same hydride. The ideal masking agents should be able to produce appropriate chemical modifications in the HG step, reducing interferences and maintain the analytical sensitivity of the technique. L-Cysteine and thiourea are the most popular and effective masking agents in the HG determination of antimony.<sup>13</sup> Thiourea and KI were applied to eliminate interferences from Ag<sup>+</sup>, Cd<sup>2+</sup>, Co<sup>2+</sup>, Cr<sup>3+</sup>, Cu<sup>2+</sup>, Fe<sup>2+</sup>, Fe<sup>3+</sup>, Ni<sup>2+</sup> and Zn<sup>2+</sup> at concentrations of the order of 1 - 100  $\mu\text{g/ml}$ .<sup>14</sup> The prereduction of Sb(V) to its trivalent stable before hydride generation is mandatory when the total content has to be determined. L-Cysteine is particularly attractive for prereducing Sb(V) owing to the low acid concentrations required in the hydride generation.<sup>15</sup> Potassium iodide, alone<sup>15-17</sup> or mixed with thiourea,<sup>18</sup> is the reagent most

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frequently used for previous reduction of Sb(V). The prereluction could be completed rapidly with KI in high acid concentration. Because the instability of KI solutions has been reported,<sup>19</sup> solid KI should be added directly so as to avoid instability. Due to above reasons, solid KI with additional thiourea could be used to (a) prereluct of Sb(V) to Sb(III) in high concentrations of hydrochloric acid; (b) avoid iodide oxidation; (c) mask the interferences in the HG determination of antimony.

Nanometer size TiO<sub>2</sub> could adsorb selectively metal ions, and has a very high adsorption capacity. It has been adopted to study the adsorption behavior of selenium<sup>20</sup> and tungsten.<sup>21</sup> In this paper, studies are reported of the application of nanometer size TiO<sub>2</sub> to the preconcentration of antimony and elimination matrix interferences. Precautions were also taken to avoid the oxidation of Sb(III) during the preconcentration and analysis process of antimony.

## Experimental

### Apparatus

A Model GBC 932AA atomic absorption spectrometer (GBC Co., Australia) with an antimony hollow-cathode lamp operated at 10.0 mA was used for all the determinations of antimony. A slit of 0.2 nm was selected in order to isolate the 217.6 nm antimony absorption line. An open-ended, T-shaped quartz tube (17.5 cm length, 1.4 cm inner diameter and 7 cm tubing inlet) was used for hydride atomization. The atomizer was located above the burner slot, aligned in the optical pass of the hollow cathode lamp and heated with an electrothermal wire. The flow rate of nitrogen was 110 ml/min. The injection volume of sample, KBH<sub>4</sub> solution, and carrier solution 1% (v/v) HCl were 2.5, 1.5, and 5.5 ml. All signals were processed in the peak high mode. A Model WHG-102A2 FI manifold (Peking Huanshi Co., China) was used for the stibine (SbH<sub>3</sub>) generation. The pH values were measured with a Model 320-S pH meter (Shanghai Mettler Toledo Co., China) supplied with a combined electrode. A Model HJ-3 magnetic stirrer (Jiangsu, China), a Model TD4 centrifugal machine (Hunan, China) and Milli-Q-purified water apparatus (USA) were used for the test.

### Reagents

Standard stock solutions of Sb(III) and Sb(V) were prepared, by dissolving appropriate amount of SbCl<sub>3</sub> and SbCl<sub>5</sub> (Merck Co.) in 20% HCl solution. All stock solutions were stored in the dark at 4°C. Working standard solutions of lower concentrations were prepared daily by appropriate dilution. A 1.5% (w/v) KBH<sub>4</sub> (Shanghai Experiment Reagent Co., China) solution was prepared daily from borohydride powder by dissolving 1.5 g of KBH<sub>4</sub> in 0.3% (w/v) NaOH. Nanometer TiO<sub>2</sub> (diameter 20–40 nm) was provided by the laboratory of Inorganic Chemistry, Department of Chemistry, Wuhan University, and the synthesis method and character of the nanometer TiO<sub>2</sub> were described previously in Ref. 22. All other chemicals were of analysis grade from Shanghai Experiment Reagent Co., China. Double deionized water of 18 M cm<sup>-1</sup> specific resistivity, obtained in a Milli-Q plus Millipore system, was used to prepare all the solutions and to rinse the previously cleaned laboratory material. In order to avoid the adsorption of antimony and contamination, Teflon vessels and 0.45 μm Nuclepore membrane filter (Teflon) were used; these were pre-cleaned in concentrated hydrochloric acid for 24 h.

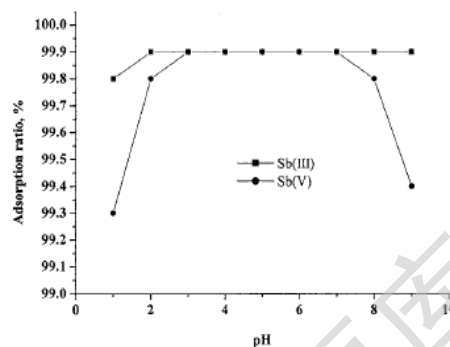


Fig. 1 Effect of pH on the adsorption ratio of Sb(III)/Sb(V) (3 μg/ml, 100 ml) on TiO<sub>2</sub> (50 mg).

### Procedure

After the pH was adjusted to 3.0, a sample solution of 500 ml was added to Teflon beaker containing 50 mg TiO<sub>2</sub>; the mixture was stirred for 15 min and then centrifuged. Trace Sb(III) and Sb(V) were all adsorbed onto TiO<sub>2</sub>, both Sb(III) and Sb(V) were then eluted by 10 ml of 5.0 mol/l hydrochloric acid solution. One eluent was directly used for the analysis of Sb(III); to the other eluent was added 0.5 g KI and 0.2 g thiourea to reduce Sb(V) to Sb(III), then the mixture was used for the determination of total antimony. The prereluction time used was 15 min. Antimony(V) content is the mathematical difference of the two concentrations.

## Results and Discussion

### Effect of pH on the preconcentration of Sb(III)/Sb(V)

Figure 1 indicates the effect of pH on the adsorption ratio by TiO<sub>2</sub> for 100 ml standard solutions of 3 μg/ml Sb(III) and Sb(V). It can be seen from this figure that the ability of nanometer-size TiO<sub>2</sub> to adsorb Sb(III) and Sb(V) was so high that the adsorption ratio of Sb(III)/Sb(V) was over 99.0% at different pH values varying from 1.0 to 9.0. The optimum adsorption ratio could be obtained for pH values from 3.0 to 7.0, where the adsorption ratio of TiO<sub>2</sub> for both Sb(III) and Sb(V) was over 99.9%. Because the oxidation of Sb(III) was found to be inversely proportional to the H<sup>+</sup> concentrations,<sup>23</sup> high acid concentration could be adopted to avoid the oxidation of Sb(III) during the preconcentration of antimony. Trace Sb(III) and Sb(V) in water could be enriched with TiO<sub>2</sub> from the solution at pH 3.0. The subsequent preconcentrations of Sb(III) and Sb(V) were carried out at pH 3.0.

### Adsorption equilibration time and capacity of TiO<sub>2</sub> to Sb(III)/Sb(V)

Because rapid adsorption is of great importance in avoiding the oxidation of Sb(III) and analytical application, especially in speciation analysis, a study of the adsorption equilibration time was performed. The 100 ml solutions containing 50 mg TiO<sub>2</sub> and 3 μg/ml Sb(III)/Sb(V) were stirred from 5 min to 40 min, then the adsorption ratio was determined for different adsorptive times. The adsorption equilibration times were 15 min for both Sb(III) and Sb(V).

In order to determine the adsorption capacity of Sb(III) or Sb(V) by nanometer TiO<sub>2</sub>, we added 50 mg TiO<sub>2</sub> to 100 ml solutions of Sb(III) or Sb(V) at different concentrations, varying from 0.1 to 30.0 μg/ml. A linear relationship between the



Table 1 Elution data (%) for Sb(III) or Sb(V) adsorbed on TiO<sub>2</sub> with different concentrations of HCl

|         | HCl/mol l <sup>-1</sup> |      |      |      |      |
|---------|-------------------------|------|------|------|------|
|         | 3.0                     | 4.0  | 5.0  | 6.0  | 7.0  |
| Sb(III) | 32.9                    | 41.2 | 96.1 | 96.3 | 96.4 |
| Sb(V)   | 49.2                    | 96.9 | 97.4 | 97.5 | 98.0 |

amount adsorbed by TiO<sub>2</sub> and the concentration of Sb(III) and Sb(V) in sample solution was obtained over the entire range of the concentration of antimony, *i.e.*, the saturated adsorptive capacity of TiO<sub>2</sub> could not be obtained under 30.0 µg/ml of Sb(III) or Sb(V). Thus, nanometer TiO<sub>2</sub> has a high adsorption capacity for Sb(III) and Sb(V) and the saturated adsorptive capacity of TiO<sub>2</sub> is greater than 29.6 µg/mg for Sb(III) and greater than 29.9 µg/mg for Sb(V), respectively.

#### Minimum acceptable mass of TiO<sub>2</sub> for the adsorption

In order to get the acceptable mass of TiO<sub>2</sub> for the adsorption of antimony, we added 10, 30, 50, 80, and 100 mg of TiO<sub>2</sub> to 100 ml solutions containing 3.0 µg/ml Sb(III) or Sb(V). These mixtures were stirred for 15 min, then centrifuged. The adsorption ratio of Sb(III) and Sb(V) was improved with the increase of the dosage of TiO<sub>2</sub> from 10 to 50 mg, but when the mass of TiO<sub>2</sub> exceeds 50 mg, the adsorption ratio remained at 99.9% for Sb(III) and for Sb(V). Because of high adsorption capacity, 50 mg of TiO<sub>2</sub> is enough for the adsorption of antimony.

#### Effect of preconcentration factor on Sb(III)/Sb(V) adsorption

A 50-mg aliquot of TiO<sub>2</sub> was added to (a) 100 ml solutions containing 1.0, 1.5, 2.0, 3.0, 5.0, 10, 20, and 30.0 µg/ml of Sb(III) or Sb(V), respectively, and the same amount was also added to (b) 3.0 µg/ml Sb(III) or Sb(V) solution of which volume increased from 100 ml to 1000 ml. Then the adsorption ratio of Sb(III) or Sb(V) was determined. Based on the results of experiments (a) and (b), one can conclude that the adsorption ratio of Sb(III) or Sb(V) rested on the pH value, the concentration of antimony and the solution volume. When the concentration of antimony and the volume of sample were no more than 5.0 µg/ml and 500 ml, respectively, the adsorption ratio of Sb(III) or Sb(V) could be over 99.0%, *i.e.*, the constant and quantitative recovery of antimony species could be obtained, and the ratio is enough for the preconcentration of antimony in samples.

#### Elution

Sb(III) or Sb(V), adsorbed on TiO<sub>2</sub> from 500 ml solutions containing 1 ng/ml Sb(III) or Sb(V), was eluted with 8 ml HCl solution as the eluent. Table 1 presents the elution data (%) for Sb(III) or Sb(V) with different concentrations of the eluent. As could be seen, 8 ml of 5 mol/l hydrochloric acid solution was an appropriate eluent for the determination of Sb(III) or Sb(V). Using a higher concentration of hydrochloric acid solution as the eluent, the elution data would be enhanced a little, but such use would also incur more cost of analysis, and would produce pollution for the manipulator and instrument due to the solution's volatility.

#### Evaluation of interference

The effects of 20 foreign ions on the preconcentration and determination of 1.0 ng/ml Sb(III) and 1.0 ng/ml Sb(V) in 500

Table 2 Analytical results of Sb(III)/Sb(V) in environmental water samples (ng ml<sup>-1</sup>)

| Sample              | Sb added |       | Sb found |       | Recovery, % |       |
|---------------------|----------|-------|----------|-------|-------------|-------|
|                     | Sb(III)  | Sb(V) | Sb(III)  | Sb(V) | Sb(III)     | Sb(V) |
| Zhangpu seawater    | 0.00     | 0.00  | 0.29     | 0.68  | —           | —     |
| Jiulong river water | 2.00     | 2.00  | 2.23     | 2.65  | 97.0        | 98.5  |
| Hotspring water     | 0.00     | 0.00  | 0.53     | 2.02  | —           | —     |
|                     | 2.00     | 2.00  | 2.49     | 3.98  | 98.0        | 98.0  |
|                     | 0.00     | 0.00  | 0.74     | 4.55  | —           | —     |
|                     | 2.00     | 2.00  | 2.65     | 6.64  | 95.5        | 104.5 |

ml solution were tested. The results indicated that no interferences were observed when the concentration (mg/ml) of NaCl (32.3), KCl (1.2), Ca(NO<sub>3</sub>)<sub>2</sub> (1.2), MgCl<sub>2</sub> (0.04) (the concentrations of all such salts added to the sample solution are the same in seawater),<sup>24</sup> Cu<sup>2+</sup> (0.2), Fe<sup>2+</sup> (0.2), Fe<sup>3+</sup> (0.2), Ni<sup>2+</sup> (4), Co<sup>2+</sup> (1.6), Zn<sup>2+</sup> (0.4), Mn<sup>2+</sup> (0.2), Ag<sup>+</sup> (0.2), As(III) (0.5), Sn(II) (0.2), Bi(III) (0.2), Hg(II) (0.2), HCO<sub>3</sub><sup>-</sup> (1.0), HPO<sub>4</sub><sup>2-</sup> (1.0), SO<sub>4</sub><sup>2-</sup> (8.1), Cl<sup>-</sup> (20.0) were in the solution.

#### Method performance

According to the above-mentioned procedure, a 500-ml of solution containing 1 ng/ml Sb(III) and 1 ng/ml Sb(V) was measured. Detection limits (based on 3σ of the blank determinations, *n* = 11) of 0.05 ng/ml for Sb(III) and 0.06 ng/ml for Sb(V), were obtained. The relative standard deviation was 2.1% for Sb(III) and 2.4% for Sb(V) at 1.0 ng/ml level. To study the accuracy of the method, we analyzed a synthetic water sample with 0.2 ng/ml Sb(III) and 0.3 ng/ml Sb(V). The contents obtained in synthetic water sample were 0.200 ± 0.004 ng/ml for Sb(III), and 0.298 ± 0.007 ng/ml for Sb(V). The results demonstrated that: (a) the concentrations of Sb(III) and Sb(V) in synthetic water sample each agreed well with the added concentration; (b) this method gave a concentration enhancement of 50 for 500 ml sample volume, while obtaining low enough detection limits for antimony species in natural water samples; (c) this method avoided the oxidation of Sb(III) during the preliminary concentration and analysis process.

The procedure including sample pretreatment, preconcentration and determination of antimony could avoid the oxidation of Sb(III) for two reasons: (a) High acid concentration was adopted, *i.e.*, Sb(III) was preconcentrated on pH 3.0, eluted by 5 mol/l hydrochloric acid solution and determined at this acidity; Sb(III) is present as Sb(OH)<sub>2</sub><sup>+</sup> in acidic media and as Sb(OH)<sub>4</sub><sup>-</sup> in basic media;<sup>25</sup> the formation of significant amounts of Sb(OH)<sub>4</sub><sup>-</sup> is needed for the oxidation by hydrogen peroxide<sup>26</sup> or iodate;<sup>27</sup> oxidation of Sb(III) by H<sub>2</sub>O<sub>2</sub> was found to be inversely proportional to the H<sup>+</sup> concentrations;<sup>23</sup> the kinetics of exchange of antimony between Sb(III) and Sb(V) in HCl solution is rather slow;<sup>25</sup> (b) The preconcentration with nanometer size TiO<sub>2</sub> led not only to an increase in antimony concentration, but also provided an advantage of rapid (15 min) elimination or decrease, to a great extent, of the oxidants of Sb(III), such as iron and manganese complexes, that could rapidly oxidize Sb(III) to Sb(V).<sup>28,29</sup>

#### Analysis of real samples

Sb(III) and Sb(V) contents were determined by the proposed procedure in water samples collected at sites in Fujian Province, Zhangzhou City, China. Analytical results of samples are listed in Table 2. Because of no standard reference material of Sb(III) and Sb(V), the precision and accuracy of method were tested by

spiking the samples. As can be seen in Table 2, satisfactory results were obtained by the usage of this approach, showing a recovery of 95.5–98.0% for Sb(III) and 98.0–104.5% for Sb(V). Our results proved that the antimony species information in water samples could be preserved. No systematic error could be seen in the added-found method for antimony speciation in samples. The efficiency of the preconcentration and detection system was high.

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