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Multifunctional Bismuth-Based Materials for Heavy Metal Detection and Antibiosis

Yiyan Song and Jin Chen

Abstract

The increasing complexity of environmental contamination has boosted the search of multifunctional nanomaterials that are produced in a green and economical manner. The bismuth-based materials have been long regarded as safe materials used in cosmetics as well as biomedical aspects. Particularly, as one of the most important bismuth oxyhalides, bismuth oxychloride (BiOCl), due to its intrinsic properties including high surface area, superior photocatalytic/electrochemical performance, and good biocompatibility, possesses enormous potential for environmental applications. In this chapter, we mainly introduced bismuth-based materials as typified by BiOCl as ecofriendly multifunctional materials for the purposes of heavy metal detection as well as antibiosis.

Keywords: bismuth, heavy metal detection, electrochemical stripping analysis, antibiosis

1. Introduction

As one of the heavy metal elements in the periodic table, bismuth has attracted considerable interests for industrial and biotechnological applications. Cosmetic and pharmaceutical uses of bismuth compounds can be dated back over two centuries ago. Bismuth materials including metal bismuth as well as its compounds have been long regarded as green materials due to their eco-friendliness. Owing to their biocompatibility, extensive research of bismuth has been conducted in diverse fields. For example, the layered structure of bismuth oxychloride (BiOCl) enabling it to give pearl-like coloring was used in cosmetics. Electrodes decorated with bismuth-based materials such as Bi(NO₃)₃ and Bi₂O₃ have been used for the electrochemical stripping analysis of heavy metals as an alternative method to replace toxic mercury-based ones [1–3].
In particular, owing to its large surface area, extraordinary electronic transport properties and high electrocatalytic activities, BiOCl has been extensively studied especially for industrial purpose such as photocatalytic/electrochemical materials [4, 5]. Notably, it was reported that bismuth subsalicylate (BSS), the active ingredient of an antacid drug with the trademark of Pepto-Bismol that was approved for sale over a century in North America, is hydrolyzed into BiOCl in the stomach [6–9], suggesting its organism safety for clinical practice. Meanwhile, because of their therapeutic efficacy to deal with gastrointestinal disorders and microbial infections, bismuth-based conjugates such as colloidal bismuth subcitrate have been explored in the pharmaceutical industry.

2. The morphology of bismuth-based materials

Despite the extraordinary photo/electro-chemical properties, the layer structure of bismuth-related material typified by BiOCl may limit its practical applications. Therefore, in an effort to obtain BiOCl-based material of defined morphology, mesoporous silica materials including Korea Advanced Institute of Science and Technology-6 (KIT-6) have been tested as structural support to produce BiOCl-KIT-6 composites. As shown in Figure 1A, scanning electron microscopic image (SEM) of BiOCl has revealed its layer assembly, which was composed of microspheres with a diameter of about 3 μm. By comparison, BiOCl-KIT-6 composites exhibited a relatively large form distributing in a size range of tens of micrometers (Figure 1B) with a raspberry-like display of bunched balls. It was found that both BiOCl and BiOCl-KIT-6 composites were composed of nanoplates of several nanometers in thickness, aligning radially and tightly to form hierarchical microspheres. X-ray diffraction (XRD) analysis was performed to study the crystallographic structure of the BiOCl-KIT-6 composites. As shown in Figure 2, the well-crystallized phase of BiOCl-KIT-6 composite agreed well with that of the tetragonal BiOCl (JCPDS Card No.06-0249). The peaks located at 24.0°, 34.8°, and 36.5° correspond to (002), (012), and (003) crystalline planes of the BiOCl structure, respectively, representing the characteristics of lamellar structures. The crystallite size (average size of the

Figure 1. SEM images of (A) BiOCl (Adapted from Ref. [10]) and (B) BiOCl-KIT-6.
coherent scattering region) for the BiOCl component was found to be 17.8 nm, calculated according to the Scherrer formula [10].

3. Bismuth-based sensor for the determination of heavy metals

The method to achieve bismuth-based electrode mainly included in situ plating and ex situ plating. The in situ plating involves the addition of Bi³⁺ into the sample solution. The bismuth film was formed on the surface of the electrode through the deposition during analyzing process. This method is limited as the assay pH of the sample solution has to be kept acidic. The ex situ plating includes plating the bismuth film on the electrode prior to the analysis of the sample solution. As such, preforming bismuth film on the electrode surface is not influenced by the assay conditions, ex situ method is relatively versatile for the real analysis though additional cautions and preparations are needed compared with in situ one. It is worth mentioning that bismuth-based electrode prepared by ex situ method can be reused. After the measurement, the bismuth-based electrode can be reactivated by holding the electrode at an adequate potential, which is more negative than the oxidation potential of bismuth while more positive than the oxidation stripping potential of analyzed metal ions. Therefore, after the reactivation, the bismuth-based electrode can be regenerated without any possible interference of deposited metals.

The application of bismuth-based electrode for heavy metal detection dated back to 2000 when Wang and coauthors tried to plate thin bismuth films on the carbon electrode as an alternative to mercury-based electrode [1]. The main advantage of bismuth-based material modified electrode is its nontoxicity to the environment as well as the biosafety for the operational
personnel. Meanwhile, the bismuth-based sensors provide detection sensitivity and accuracy comparable to that of mercury-based ones [11], which are attractive for practical applications. Recently, bismuth-based sensors, exhibiting improved separation ability of intermetallic compounds, compared with mercury-based ones, have been used to evaluate heavy metals such as Cd$^{2+}$ and Pb$^{2+}$. These toxic heavy metals originating from severe environmental conditions including mining area, smelting works, and sewage plant [12] are harmful for the public health. Nowadays, electrochemical methods owing to their portability, ultrasensitivity, readiness, and low cost have become useful and efficient for the trace determination of heavy metals [13–17]. Among them, anodic stripping voltammetry (ASV), which involves an effective preconcentration step to form metals on the electrode surface by reducing metal ions in the assay solution, followed by a sensitive stripping analysis where metals were reoxidized contributing to a remarkable signal-to-noise ratio, has been intensively employed in this aspect during the past decade (Figure 3). The amplified stripping signal resulting from bismuth-based electrodes reflects the ability of bismuth to form “fused” multicomponent alloys with targeted heavy metals [18]. Bismuth-based sensors thus hold potentials for the assays of heavy metal with applications ranging from continuous remote sensing to disposable chips. Since the bismuth-based electrode was applied for the determination of heavy metals in 2000 [1], many reports related to various bismuth-based material modified electrodes have been devoted to this area. In 2008, Xu and coworkers used a Nafion-coated bismuth electrode to achieve the simultaneous determination of three heavy metals including Pb$^{2+}$, Cd$^{2+}$, and Zn$^{2+}$ in vegetables using differential pulse anodic stripping voltammetry [19]. In 2014, Sosa and coworkers analyzed Cd$^{2+}$ and Pb$^{2+}$ in groundwater based on sputtered bismuth screen-printed electrode [20]. In 2015, Cerovac and coworkers used the bismuth-oxychloride particle-multiwalled carbon nanotube composite-modified glassy carbon electrode to detect trace-level lead and cadmium in sediment pore water [21]. And it is also readily possible to evaluate the heavy metal amounts of human blood sample. For example, Song and coworkers determined Cd$^{2+}$ in blood samples based on BiOCl-KIT-6 modified glassy carbon electrode [11]. During the past decade, many studies have introduced a number of trace heavy metal detection methods based on bismuth-based electrode. Some typical bismuth-based sensors as well as the resulting analytical performance are summarized in Table 1.

Nevertheless, in comparison with mercury-based electrode, bismuth-based one often works well in a relatively narrow potential window, particularly being in a more negative anodic range due to the fact that bismuth is more easily oxidized than mercury. Meanwhile, the
The cathodic limit of bismuth-based electrode posed by hydrogen reduction is close to that of mercury-based one. Therefore, the accessible potential window of bismuth-based electrode is narrower than that made of mercury. Additionally, it was found that the potential window of bismuth-based electrode was strongly affected by the pH values of the sample solution. The relationship of operational potential windows of bismuth-based electrode and assay pH is shown in Table 2 [22]. Moreover, it was observed that the constructed BiOCl-KIT-6/GCE possessed a wide potential window ranging from −1.22 to −0.25 V versus SCE by recording the CV spectra of electrode in 0.1 M acetate buffer (pH 4.5) (Figure 4). The wide potential window of BiOCl-KIT-6/GCE may permit the simultaneous determination of Zn$^{2+}$ (stripped at −1.15 V), Pb$^{2+}$ (stripped at −0.58 V) and Cd$^{2+}$ (stripped at −0.83 V). It should be noted that the stripping analysis of heavy metals is limited if oxidation potentials of analyzed metals are close to or more positive than that of bismuth such as Cu, Sn, and Sb.

In the aspect of heavy metal measurement, many earlier studies have focused on the design of bismuth-based sensors as well as the detection principle. However, their applications to environmental, food, and clinical samples have just started. For example, by the use of anodic stripping voltammetry, cadmium and lead at trace levels in environmental samples [23, 24], food products [25, 26] and biological fluids [11] have been determined. Using square-wave

### Table 1.
Bismuth-based electrodes for determination of Pb (II) and Cd (II) using anodic stripping voltammetry.

<table>
<thead>
<tr>
<th>Sensor</th>
<th>Method</th>
<th>Deposit. potent. (v)</th>
<th>Deposit. time (s)</th>
<th>Linear range (μg/L)</th>
<th>LOD(μg/L)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bi-CNT SPE</td>
<td>SWASV</td>
<td>−1.4</td>
<td>300</td>
<td>2–100</td>
<td>2–100</td>
<td>1.3</td>
</tr>
<tr>
<td>NCBFE</td>
<td>DPASV</td>
<td>−1.4</td>
<td>180</td>
<td>4–36</td>
<td>4–36</td>
<td>0.17</td>
</tr>
<tr>
<td>Bi/GCE</td>
<td>SWASV</td>
<td>−1.2</td>
<td>600</td>
<td>5–60</td>
<td>5–60</td>
<td>0.8</td>
</tr>
<tr>
<td>Bi$_2$SPE</td>
<td>DPASV</td>
<td>−1.3</td>
<td>360</td>
<td>0.5–20</td>
<td>0.3–12</td>
<td>0.16</td>
</tr>
<tr>
<td>BiOCl/MWCNT-GCE</td>
<td>SWASV</td>
<td>−1.2</td>
<td>120</td>
<td>5–50</td>
<td>5–50</td>
<td>0.57</td>
</tr>
<tr>
<td>BiOCl-KIT-6/GCE</td>
<td>SWASV</td>
<td>−1.3</td>
<td>120</td>
<td>0.2–300</td>
<td>0.2–300</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Bi-CNT SPE, bismuth-modified carbon nanotube modified screen-printed electrode; NCBFE, Nafion-coated bismuth film electrode; Bi/GCE: bismuth nanoparticles modified GCE; Bi$_2$SPE, sputtered bismuth screen-printed electrode; MWCNT, multiwalled carbon nanotube; SWASV, square wave anodic stripping voltammetry; DPASV, differential pulse anodic stripping voltammetry.

### Table 2.
Operational potential of bismuth-based materials plated on carbon-paste electrodes at different pH (Data taken from Ref. [22]).

<table>
<thead>
<tr>
<th>Medium</th>
<th>pH</th>
<th>Anodic limit (V)</th>
<th>Cathodic limit (V)</th>
<th>Potential window (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 M HClO$_4$</td>
<td>1.00</td>
<td>−0.05</td>
<td>−1.05</td>
<td>1.10</td>
</tr>
<tr>
<td>0.2 M HAc-NaAc</td>
<td>4.24</td>
<td>−0.25</td>
<td>−1.25</td>
<td>1.00</td>
</tr>
<tr>
<td>0.1 M NaOH</td>
<td>12.17</td>
<td>−0.55</td>
<td>−1.55</td>
<td>1.00</td>
</tr>
</tbody>
</table>
Figure 4. Cyclic voltammetry (CV) analysis of BiOCl-KIT-6/GCE in acetate buffer solution (0.1 M, pH 4.5) (adapted from Ref. [10]).

Figure 5. (A) Square-wave anodic stripping voltammetric (SWASV) responses of peak current on the concentration of Cd$^{2+}$ using BiOCl-KIT-6/GCE in acetate buffer solution (pH 4.5) at the deposition potential of −1.3 V and deposition time of 120 s. (B) Calibration curves of Cd$^{2+}$ over a concentration range of 0.5–300 μg/L (adapted from Ref. [10]).
anodic stripping voltammetry (SWASV), the constructed BiOCl-KIT-6/GCE applied to the determination of cadmium-spiked human blood samples resulted in a calibration curve of $\text{Cd}^{2+}$ with 2 linear ranges from 0.5 to 10 and from 10 to 300 μg/L (Figure 5), respectively, and a detection limit of 65 ng/L. As shown in Figure 6, a good agreement between the detection methods of SWASV and inductively coupled plasma mass spectrometry (ICP-MS) was observed [11]. Moreover, the BiOCl-KIT-6/GCE could be repeatedly used for at least 30 continuous times in one day and at least 10 continuous days with a marginal reduction of stripping peak current of 6.8 and 3.6%, respectively.

Despite considerable progress achieved during the past decade, most of the earlier studies have mainly explored various bismuth-based materials towards the sensor fabrication. Therefore, practical concerns still remain to be addressed to fulfill the demands of environmental protection and so on. Further works to utilize the constructed sensor under harsh operational conditions and to simply the assay procedures are still needed in the future.

4. Bismuth-based materials for biomedical use

Bismuth is a heavy metal element located close to lead and tin elements in the periodic table of elements, and thus shares similar physicochemical properties with these elements. But different from lead and tin, bismuth is usually regarded as nontoxic and biologically safe. Therefore, bismuth as well as its derivate has been extensively explored in biomedical applications. For example, bismuth salts are valuable for synthesis of various bismuth-based complexes as the
active ingredients for pharmaceutical uses. The first report on bismuth-containing medicine in 1786 revealed its efficacy in the treatment of dyspepsia. And since then, there is a growing number of bismuth complexes which were explored to deal with gastrointestinal disorders and microbial infections including syphilis, diarrhea, gastritis, and colitis, which point out desirable therapeutic effects yet low toxicity after the intake of drug molecules.

In the laboratory, the antimicrobial activities of bismuth-based materials can be evaluated by recording the growth curve of model bacteria such as Gram-positive \textit{Staphylococcus aureus} and Gram-negative \textit{Escherichia coli} in the presence of materials. As shown in Figure 7, the growth curves of \textit{S. aureus} cells in the presence and absence of 20 μg/mL BiOCl-KIT-6 were observed. There was an apparent decrease in the growth curve when adding BiOCl-KIT-6 composite, suggesting that BiOCl-KIT-6 composite can be used as an antimicrobial agent against \textit{S. aureus}. The underlying antimicrobial mechanism of bismuth-based materials can be further studied using radical-sensitive fluorescent dyes. The generation of free radicals has been considered...
as one of the causes of metal particle-induced cytotoxicity in bacteria [29]. Therefore, the cytotoxicity of BiOCl-KIT-6 composite may be linked to the formation of reactive oxygen species (ROS). Cellular accumulation of ROS can be detected using the fluorogenic dye DCFDA based on the oxidation of the non-fluorescent 2',7'-dichlorodihydrofluorescein (DCFH) that reacts with $\text{H}_2\text{O}_2$, $\text{O}_2^-$, and $\text{ONOO}^-$ into the green fluorescent dichlorofluorescein (DCF), which provides a general indication of ROS levels. As shown in Figure 8, S. aureus incubated with BiOCl-KIT-6 showed more green fluorescence compared with the untreated group (negative control), indicating the formation of ROS detrimental to the cell growth (Figure 7).

H. pylori has been considered to play a role in the development of chronic gastritis, peptic ulcers, and even gastric cancer, and thus bismuth-based drugs are usually prescribed once H. pylori infection is diagnosed. Based on the clinical survey, H. pylori is susceptible to several antibiotics such as clarithromycin, amoxicillin, metronidazole, tetracycline, and rifabutin and exhibits resistance to many other antibiotics like bacitracin, vancomycin, trimethoprim, polymyxins, and nalidixic acid. Therefore, it remains challenging to cure H. pylori infection. In an effort to minimize the antibiotic resistance, bismuth complex has been utilized to treat gastrointestinal disorders and ulcers with pronounced inhibition activity against H. pylori [9]. Some commercial medicines with bismuth-containing ingredients are summarized in Table 3.
Basically, the bismuth-containing pharmaceuticals have two functions—antiulcer and antibacterial. The antiulcer activity of bismuth complex is explained by the precipitation of bismuth around the ulcer point to form a glycoprotein-bismuth complex as a protective coating promoting the healing of the lesion. The underlying molecular mechanisms of bismuth compounds to deal with *H. pylori* are not fully elucidated. Some preliminary reports have indicated that the biological targets of bismuth compounds are closely related to several *H. pylori*-related proteins [8].

Over the years, to produce a number of bismuth complexes for biomedical uses, the synthesis methods have also historically evolved, which mainly include hydrothermal, solvothermal, and microwave synthesis. Hydrothermal synthesis is a method to synthesize single crystals from high temperature aqueous solutions at high vapor pressures. Chen et al. synthesized bismuth-amino-acid coordination polymers (BACPs) with different sizes by heating the aqueous reacting solution of Bi(NO$_3$)$_3$·5H$_2$O and asparagine at different temperatures [30]. The prepared BACPs synthesized in a facile green hydrothermal method exhibited unique biological activities and potential applications in biomedicine. He et al. prepared low-toxicity bismuth asparagine coordination polymer spheres (BACP-2) with sphere-like microstructures of an average 800 nm in diameter through a hydrothermal reaction at 80°C overnight [31]. Solvothermal synthesis is usually carried out in organic media to produce chemicals of refined crystallinity and morphology through a combination of sol-gel and hydrothermal routes. Andrews and coworkers have developed two new bis-carboxylate Bi(III) complexes including PhBi(α-MeOC$_6$H$_4$CO$_2$)(bipy)-0.5 EtOH and PhBi(C$_9$H$_{11}$N$_2$O$_3$CO$_2$)$_2$·6H$_2$O, for anti-Leishmanial through a refluxing reaction in ethanol for 10-12 h [32]. Li et al. reported a nine-coordinate Bi(III) complex, (Bi(H$_2$L)(NO$_3$)$_2$)NO$_3$ (Figure 9) that was prepared in methanol [33]. Microwave synthesis is an efficient and environment-friendly method to accomplish various inorganic syntheses to achieve products in high yields within shorter reaction times. The coordination behaviors of bismuth (III) compounds with benzothiazolone were investigated by Mahajan et al. and the reaction takes few minutes under 700 W inside a microwave oven [34].

Several bismuth derivatives have revealed antibacterial and antifungal activities. For example, (Bi(H$_2$L)(NO$_3$)$_2$)NO$_3$ shows higher antibacterial properties with the minimum inhibitory concentration (MIC) of 10.66 μM against Gram-positive bacteria *B. cereus* and Gram-negative bacteria *S. typhimurium* than the control of antibiotics chloramphenicol (MIC of 96.71 μM against *B. cereus* and *S. typhimurium*) and kanamycin sulfate (MIC of 107.3 μM against *B. cereus*, *S. typhimurium*).

### Table 3. Bismuth-containing drugs for the treatment of gastrointestinal diseases.

<table>
<thead>
<tr>
<th>Bismuth complex</th>
<th>Name of pharmaceuticals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bismuth potassium citrate</td>
<td>Livzon Dele, Bielomatik</td>
</tr>
<tr>
<td>Bismuth subcitrate</td>
<td>De-nol, Tripotassium dicitratobismuthate</td>
</tr>
<tr>
<td>Bismuth citrate</td>
<td>Ranitidine</td>
</tr>
<tr>
<td>Colloidal bismuth pectin</td>
<td>Colloidal bismuth pectin capsules</td>
</tr>
<tr>
<td>Bismuth aluminate</td>
<td>Debitai, Compound bismuth aluminate tablets</td>
</tr>
</tbody>
</table>
53.64 μM against *S. typhimurium*, respectively [33]. Aqueous colloidal bismuth oxide nanoparticles (Bi$_2$O$_3$ NP) with fungicidal activity against *C. albicans* and antibiofilm capabilities were synthesized by Rene and coworkers [35]. Colloidal Bi$_2$O$_3$ NP displayed better antifungal activity against *C. albicans* growth (colony size is reduced by 85%) and more complete inhibition of biofilm formation than those obtained by chlorhexidine, nystatin, and terbinafine, demonstrating the candidate role of Bi$_2$O$_3$ NP as a fungicidal component of oral antiseptic. Several bis-carboxylate phenylbismuth (III) and tris-carboxylate bismuth (III) complexes reported by Andrews and coworkers showed significant antimicrobial activity against the promastigotes of *Leishmania major* [32].

It should be noted that although bismuth complexes have been widely applied for medical practice against *H. pylori* infection in peptic ulcer, some side effects have been reported including disturbances of taste, dizziness, abdominal pain, and mild diarrhea in some patients, and a few cases of parageusia and glossitis. But so far no statistically identified difference in the incidences of side effects was found among the standard triple therapy, bismuth pectin quadruple and sequential therapies, demonstrating the biosafety of bismuth complexes [36, 37]. According to the statistical analysis, 99% of ingested bismuth is in essence not absorbed by human body but is excreted in the feces. The bismuth encephalopathy may therefore result from the long-term ingestion of overdosed bismuth complexes, attributed to the chronically neurologic dysfunction of bismuth. Although bismuth toxicity is usually difficult to occur, it is necessary to control the dosage of bismuth compounds.

**Figure 9.** (A) Structure of (Bi(H$_2$L)(NO$_3$)$_2$)$_2$NO$_3$ with atomic numbering scheme. (B) Polyhedra showing distorted geometry around the bismuth atom. H$_2$L, 2,6-diaceatapyridine bis(‘N-methylthiosemicarbazone) (adapted from Ref. [33]).
5. Conclusion and outlook

Because of the nontoxicity of bismuth (III) salts, there is growing interest to explore various bismuth (III) salts for diverse applications including stripping analysis of heavy metals as well as antimicrobial agents. The bismuth-based sensors for the heavy metals are extensively studied in an effort to replace the mercury-based methods. The precise control of morphology and surface properties of bismuth-based materials on the modified electrode will greatly determine the sensitivity and accuracy of the constructed sensors. On the other hand, bismuth-based compounds as the inorganic pharmaceutical molecules may offer advantages over organic ones for biomedical use. As the dosage is the major cause of neurotoxicity of bismuth compounds, it is of importance to monitor the in vivo efficacy of bismuth-based pharmaceuticals. Along this direction, some cargo molecules of ordered structure such as metal-organic frameworks (MOFs) for the controlled release of bismuth hold potentials to improve the curative effect.

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Author details

Yiyan Song¹ and Jin Chen¹,²*
*Address all correspondence to: okachen30@gmail.com
1 School of Public Health, Nanjing Medical University, Nanjing, China
2 The Key Laboratory of Modern Toxicology, Ministry of Education, School of Public Health, Nanjing Medical University, Nanjing, China

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