

ORIGINAL RESEARCH ARTICLE

Glutathione as a novel engineered biomaterial for heavy metal ion quantification and remediation

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ABSTRACT

The prevalence of heavy metal ion contamination is increasing worldwide—posing a growing threat to both ecological and human well-being. In recent years, there have been significant research endeavors focused on the quantitative analysis of these heavy metal ions. There is an increasing demand for cost-effective, sensitive, selective, and rapid methods for detecting them. In the context of functional materials for detection as well as effective diminution of heavy metal ion, glutathione is recognized as well as widely proven for its' robust capacity to form complexes with harmful heavy metal ions, with its solubility in water, enduring action, and convenient accessibility. Consequently, glutathione is increasingly being utilized as a preferred molecular probe in the development of highly sensitive, cost-effective, and easily accessible sensors. Keeping in cue of the increasing use of glutathione, this mini-review provides a summary of the findings from different glutathione-based heavy metal ion detection approaches as documented in recent literature. These approaches are classified according to their respective techniques of signal transduction. The discussion and comparison of their operation and execution, as well as the evaluation of figures of merit such as limit of detection, selectivity, and response time, are presented. Likewise, removal mechanisms along with challenges are also briefed in this mini-review.

Keywords: glutathione; absorption; sensing; heavy metal ion; limit of detection

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1. Introduction

Glutathione, a tripeptide molecule composed of cysteine, glycine, and glutamic acid, has gained attention as a novel engineered biomaterial due to its' unique properties and versatile applications. While traditionally known for its role in cellular defense against oxidative stress and detoxification, researchers have been exploring its potential beyond its biological functions. Overall, glutathione can be utilized as a novel biomaterial in many ways such as biosensors and detection platforms, drug delivery systems and many more. For instance, glutathione's thiol (–SH) group offers a specific binding site for various molecules, including heavy metals, reactive oxygen species, and other analytes^[1,2]. The thiol groups of glutathione can react with disulfide bonds found in the extracellular environment, facilitating the controlled release of therapeutic agents. This mechanism can enhance drug targeting to specific cells or tissues, improving therapeutic efficacy while minimizing side effects. Apart from this, glutathione's antioxidant properties make it a potential candidate for developing materials that combat oxidative stress and inflammation. Incorporating glutathione

into biomaterials, such as hydrogels or coatings, can protect against cellular damage and aid in tissue repair. Meanwhile, glutathione-modified materials can be used in tissue engineering to create scaffolds that support cell growth, proliferation, and differentiation. Notably, glutathione's ability to bind with heavy metal ions has also been exploited for biomedical imaging applications. Glutathione-coated nanoparticles can be used as contrast agents for various imaging modalities, including magnetic resonance imaging (MRI) and fluorescence imaging^[3,4]. Researchers have developed biosensors and detection platforms by immobilizing glutathione onto surfaces, nanoparticles, or other matrices. These sensors can be used to detect pollutants, toxins, or biomolecules in environmental samples, food, and medical diagnostics. Meanwhile, the binding affinity of glutathione to heavy metal ions makes it effective in sequestering and removing these pollutants. Consequently, glutathione has been widely used for heavy metal remediation from contaminated water or soil^[5,6].

Although extensive study has been conducted on many characteristics related to glutathione, it is important to highlight that scholarly literature has not sufficiently addressed the specific aspects of glutathione mediated detection concerning aquatic pollutants. Therefore, there is a pressing requirement for a thorough examination that will furnish readers with a succinct and focused understanding of the current research efforts being undertaken in this field.

The paucity of abundant literature inspires us to compose a succinct review on glutathione. The objective of this mini review is to analyze the sensing capabilities enabled by glutathione in quantification of heavy metal ions in aqueous solution. Furthermore, this work provides a comprehensive description of the procedural aspects involved in the detection of heavy metal ions and other aquatic pollutants using glutathione-conjugates. This study outlines the methodology for the identification of aquatic contaminants, specifically heavy metal ions, by the utilization of glutathione-mediated agents. Furthermore, the identification of potential areas for future research in this nascent discipline is also emphasized.

2. Properties of glutathione

Figure 1 displays the structural configuration of glutathione showing the main constituent amino acids. The unique structural configurations poise glutathione to be embodied with functions of biological as well as chemical engineering domain. In general, glutathione has the chemical formula $C_{10}H_{17}N_3O_6S$ and a molecular weight of about 307.3 g/mol. It consists of three amino acids linked together in a specific sequence: γ -glutamyl-cysteinyl-glycine. These three amino acids are linked by peptide bonds. The peptide bond between cysteine and glycine is of particular importance due to the thiol group ($-SH$) in the cysteine residue. This thiol group is reactive and plays a crucial role in the antioxidant function of glutathione. Again, glutathione exists in two forms: reduced glutathione (GSH) and oxidized glutathione (GSSG). The thiol group in cysteine is responsible for the reducing properties of GSH which accounts for its strong electron-donating ability. The molecular formula of reduced glutathione is $C_{10}H_{17}N_3O_6S$. Its average mass is 307.323 Da and its' monoisotopic mass is 307.083804 Da. Likewise, GSSG is formed when two molecules of reduced glutathione (GSH) are oxidized. This oxidation process involves the donation of electrons from each GSH molecule, leading to the formation of an intermolecular disulfide bond between the sulfur atoms of each oxidized GSH molecule. Apart from GSSG, oxidized forms of glutathione can also comprise disulfides with other thiols to form "mixed disulfides" and more oxidized forms of the thiol group. The molecular formula of oxidized glutathione is $C_{20}H_{32}N_6O_{12}S_2$. Meanwhile, it can also act as a metal chelator—thereby, binding to heavy metals and aiding in their detoxification and removal from the body^[7-10].

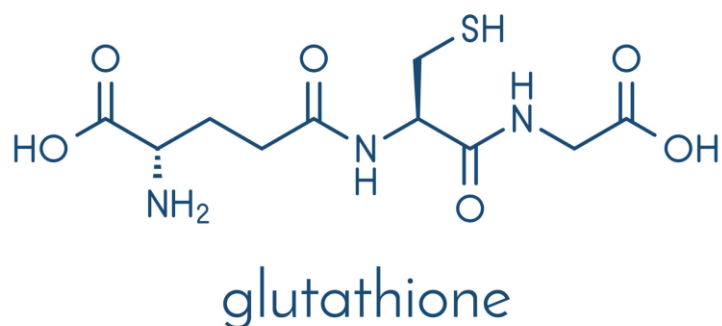


Figure 1. Structure of glutathione.

3. Heavy metal ion proliferation

Proliferation of heavy metals in drinking water or aqueous solutions has no longer been a confined problem. It has now been declared as a global problem. The immediate effects of heavy metal ions can lead to harmful ailments in the living bodies if found above permissible limits as set by the Environmental Protection agency as well as the World Health Organization. **Figure 2** demonstrates the sources as well as bioaccumulation of these heavy metals into our flora and fauna^[11,12].

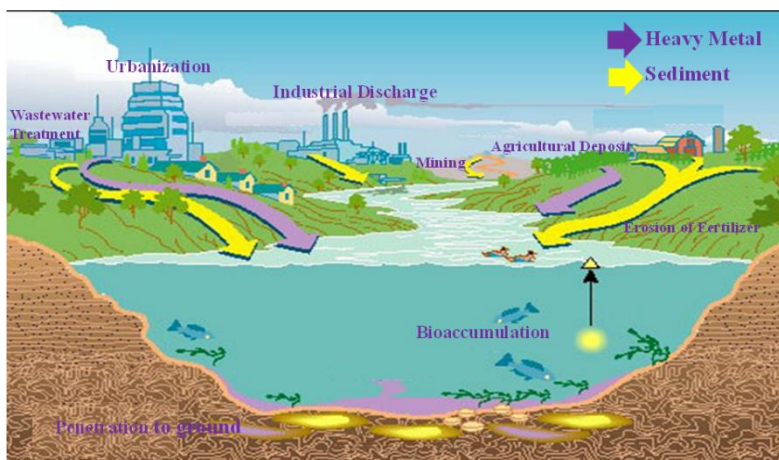


Figure 2. Schematic of heavy metal ion generation and seepage.

As far as the sources of heavy metal ions are concerned, they are mostly anthropogenic, barring a few natural sources such as volcanic eruption or acid rain, etc. The rampant urbanization as well as industrialization has escalated the pollution caused by heavy metal ions. The harmful nature of ubiquitous heavy metal ions such as Pb, As, Hg is of rising concern, in case they are taken above the permissible limit. Notably, the permissible limits set by the world-renowned agencies are kept at parts per billion (ppb) level. Any concentration going beyond the thresholds is declared as toxic. The excess intake of these ions in water may cause several health ailments. Despite growth in remediation measures, these proliferating ions remain a menace to the ecosystem. To curb these, effective identification is the primary task. If the ions are not properly diagnosed, subsequent remediation becomes less effective. As such, proper identification and remediation in the next step are important considering the rising trend of proliferation by heavy metal ions in particular. Accordingly, the next following sections comprehensively deal with these two important issues—assessment and removal of heavy metal ions in the context of glutathione^[13,14].

3.1. Heavy metal detection

Glutathione has a high affinity to bind with heavy metal ions, such as mercury (Hg), cadmium (Cd), lead (Pb), and copper (Cu). This binding occurs through the thiol ($-SH$) group present in the cysteine residue of glutathione. Researchers have systematically exploited this binding affinity to develop detection systems for heavy metal quantification. By immobilizing glutathione onto various surfaces or incorporating it into sensor platforms, scientists have created sensitive and selective detection systems that can quantify heavy metal concentrations in environmental samples (see **Figure 3** for illustration).

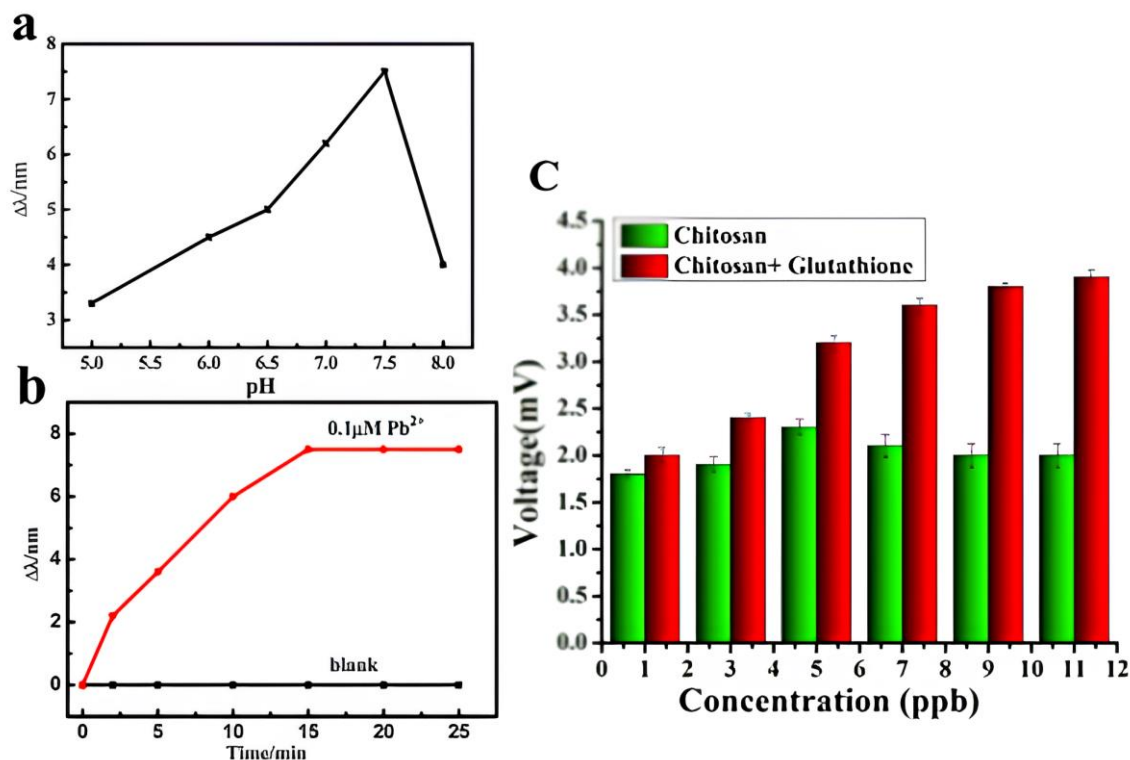


Figure 3. Schematic sensing of Pb (II) *via* glutathione. **a)** wavelength change at optimized pH level; **b)** wavelength response for blank and Pb (II) contaminated sample @glutathione based sensor; **c)** voltage response of chitosan @glutathione at different ppb levels of Pb (II).

The thiol ($-SH$) groups in glutathione have a strong affinity for binding with heavy metal ions, making them useful for designing sensitive and selective sensors. **Figure 4** exemplifies how these groups bind with heavy metal ion such as Pb. Likewise, immobilization of glutathione onto the surface of a sensor substrate, such as an electrode or a nanoparticle, facilitates selective capture heavy metal ions from a sample solution due to the strong metal-thiol coordination bonds formed between the heavy metal ions and the thiol groups of glutathione. As a result, properties such as electrical conductivity, optical properties, or mass get altered which eventually emerge as a discernible signal for proportionate assessment of heavy metal ion. In the same note, glutathione's specificity in binding with heavy metal ions allows for the development of sensors that can distinguish between different types of heavy metal ions. This selectivity is crucial for accurately identifying the presence of specific heavy metals in a complex sample^[7-13].

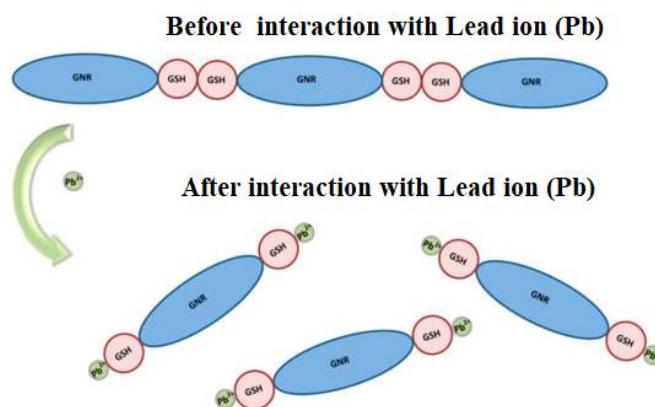


Figure 4. Binding of heavy metal ion with glutathione.

Table 1 enlists the sensing of heavy metal ions *via* glutathione modified matrix.

Table 1. Glutathione based heavy metal assessment schemes.

Interrogation scheme	Sensor base	Heavy metal	Detection limit	References
Colorimetry	AuNPs	Pb (II)	0.1 μ M	[15]
	AgNPs	Pb (II)	1 nM	[16]
	AuNPs	Cd (II)	4.3 pM	[17]
Localized surface plasmon resonance	AuNPs	Pb (II)	50 pM	[18]
	AgNPs	Pb (II)	1 ppb	[19,20]
Fluorescence	AuNPs	Pb (II)	0.1 μ M	[21]
	AgNPs	Pb (II)	0.6 pM (200 ppq)	[22]
Surface-enhanced Raman scattering	AgNPs	As (III)	10.2 nM (0.76 ppb)	[23]
Spectrophotometry	Mn-doped ZnSQDs	Pb (II)	2.2 nM (0.45 μ g/L)	[24]
	Mn-doped ZnSQDs	Pb (II), Cr (II), Hg (II)	0.93 μ M for mixed HMIs	[25]
	AgNPs	Ni (II)	75 μ M	[26]
	Gold Nanostars	Pb (II)	0.5 μ M	[27]
Square Wave Anodic Stripping Voltammetry, SWASV	Magnetic NPs	Cd (II), Pb (II)	1.6 nM (0.182 μ g/L); 0.8 nM (0.172 μ g/L)	[28]
	Glassy-Carbon Electrode	Cd (II)	0.05 nM	[29]
	Carbon Paste Electrode	Cd (II)	8.5 nM	[30]

As evident in **Table 1**, there are reports of lead ion detection *via* colorimetry where glutathione stabilized gold as well as silver nanoparticles had been effectively used^[15–17]. Meanwhile, cadmium had been assessed *via* glutathione cadmium assisted matrix^[17]. Similarly, localized surface plasmon based sensing of lead ion *via* glutathione assisted gold nanoparticles had been conducted^[18–20]. Meanwhile, fluorescent probes for quantifying lead ion level in aqueous solution had been performed where glutathione mediated detection procedure in coordination with gold nanoparticles were executed^[21–23]. In the same note, quantum dots assisted by glutathione enabled effective luminescent/fluorescence probes had been implemented for assessing heavy metal ions^[24–27]. As far as electrochemical detection of heavy metal ions is concerned, researchers had opted for modified

electrodes *via* glutathione mediation for successful quantification of heavy metal ions such as lead as well as cadmium^[28–30].

3.2. Heavy metal remediation

The binding affinity of glutathione to heavy metals has also been investigated for its potential use in remediation processes. Contaminated water bodies and soil often contain elevated levels of heavy metals, which can be harmful to both the environment and human health. Glutathione-modified materials, such as nanoparticles, polymers, or hydrogels, can be employed to adsorb and sequester heavy metals from contaminated matrices. These materials can be designed to have a high surface area and functional groups that enhance heavy metal binding.

The remediation process involves introducing glutathione-modified materials into the contaminated environment. The glutathione molecules bind with the heavy metal ions, forming stable complexes that are then removed from the system, thereby reducing the heavy metal concentrations to safer levels. The choice of material and the design of the remediation strategy depend on factors like the specific heavy metals present, the nature of the contaminated medium, and the desired remediation efficiency. **Table 2** enlists works where glutathione modified matrix were deployed to remove pervasive heavy metal ions such as Hg (II), Pb (II), As (III), etc.^[31–34]. In addition, the radioactive HMIs were also successively removed from contaminated water with the use of specially modified matrix of glutathione. **Figure 5** depicts removal of Cd (II) via metal peptide where glutathione had been used^[35,36].

Table 2. Removal of heavy metal ions via glutathione based matrix.

Process	Matrix	Heavy Metal Ion	Reference
Bacterial phenotypes	glutathione S-transferase	Hg (II)	[31]
Bacterial phenotypes	glutathione S-transferase	Ni (II), Hg (II)	[32]
Metal Peptide complex	Cd-glutathione	Cd (II)	[33]
Chemical adsorption	glutathione conjugated carbon nanotube (CNT) bridged three-dimensional (3D) porous graphene oxide membrane	As (III), As(V), and Pb (II)	[34]
Magnetic separation	Glu@MNPs	Th (IV)	[35]
Magnetic separation	glutathione@magnetite	Ur (VI)	[36]

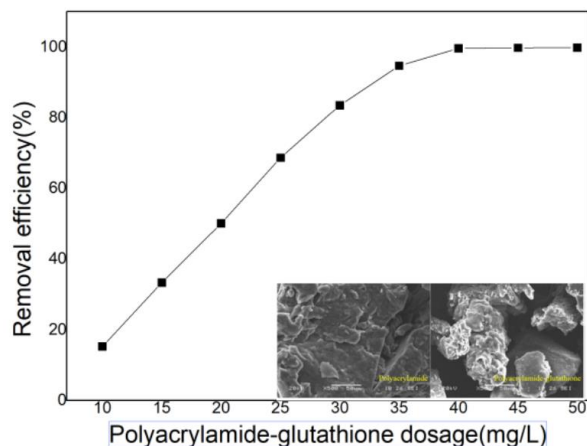


Figure 5. Schematic of removal of Cd (II) via glutathione. Inset shows the surface morphology related to Glutathione composites.

4. Binding of HMI to glutathione

Glutathione is a tripeptide composed of three amino acids: glutamate, cysteine, and glycine. It plays a crucial role in cellular defense against oxidative stress and detoxification processes. One of its important functions is to bind to heavy metal ions, aiding in their detoxification and removal from the body.

Heavy metal ions such as mercury, lead, cadmium, and arsenic can be harmful to living organisms when they accumulate in tissues. Glutathione binds to these heavy metal ions through thiol ($-SH$) groups present in its cysteine residue. The binding process involves the formation of coordination bonds between the metal ions and the sulfur atoms in the thiol groups of glutathione. Apart from this, there are other avenues where glutathione assists in other potential applications. They are as follows.

Detoxification: Glutathione helps in the detoxification of heavy metal ions by forming complexes that are less toxic and more water-soluble. This allows the body to excrete these complexes through urine or bile, reducing the potential harm caused by heavy metals.

Reduction of Oxidative Stress: Heavy metal ions can induce oxidative stress by generating reactive oxygen species (ROS) in cells. Glutathione, as an antioxidant, helps counteract this stress by scavenging ROS and maintaining a reduced cellular environment.

Preventing Cellular Damage: The binding of heavy metal ions to glutathione can prevent these ions from interacting with cellular components and causing damage. This protection can help maintain the proper functioning of cells and tissues.

Transport and Sequestration: Glutathione can also aid in the transport and sequestration of heavy metal ions. It can help move heavy metal ions from sensitive cellular compartments to less harmful ones, reducing their impact on critical cellular processes.

It's important to note that while glutathione plays a significant role in binding heavy metal ions and aiding in detoxification, excessive exposure to heavy metals can overwhelm the detoxification mechanisms and lead to toxicity. Additionally, individual variations in genetics and overall health can influence the effectiveness of glutathione-mediated detoxification.

5. Concluding remarks

Using glutathione as a biomaterial for heavy metal quantification and remediation offers several advantages, including its natural abundance, biocompatibility, and specificity for heavy metal binding. Additionally, the design and synthesis of glutathione-modified materials can be tailored to enhance binding affinity and selectivity for specific heavy metals.

However, there are also challenges to consider. The stability of glutathione-modified materials, their reusability, and the potential impact on non-target species must be carefully evaluated. Furthermore, the scalability and cost-effectiveness of these approaches for large-scale remediation need to be addressed. While glutathione offers promising possibilities as a novel biomaterial, there are challenges to address.

Glutathione's unique properties, particularly its thiol groups and metal-binding capabilities, make it an interesting biomaterial candidate with a range of potential applications. Researchers continue to explore ways to harness these properties effectively while addressing challenges associated with its use. Research in this field is ongoing, and scientists continue to study the mechanisms by which glutathione interacts with heavy metal ions to better understand how to mitigate the harmful effects of heavy metal exposure. Conclusively, glutathione as a

novel engineered biomaterial for heavy metal quantification and remediation holds promise due to its unique properties, but further research and development are necessary to optimize its application and address potential limitations.

Conflict of interest

The author declares no conflict of interest.

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