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Determination of human exposure to mercury

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Abstract:
Mercury is a toxic heavy metal that poses significant risks to human health. In many industrial and occupational settings, employees are at high risk of mercury exposure due to the nature of their work. Consequently, biomonitoring and routine testing mercury levels in the working environment are crucial to ensure occupational health and prevent adverse health effects.

This master’s thesis reviews the literature on occupational exposure to mercury and its impact on human well-being. The review focuses on the pathways through which mercury enters the body and the transformations it undergoes. Protective strategies and adopted regulations are also investigated. The analytical methods used for detection of mercury in biological samples, such as cold vapor atomic absorption spectroscopy (CV-AAS) and inductively coupled plasma mass spectrometry (ICP-MS), are explored, including comparison of their efficacy.

The primary objective of the experimental part of this research was to validate the use of flow injection mercury system (FIMS) as a method for determining mercury levels in human blood and urine samples. Additionally, ICP-MS was employed for comparative analysis of mercury levels in urine samples. The analytical parameters of FIMS and the potential for selective analysis of two reducing agents, stannous chloride ($SnCl_2$) and sodium tetrahydroborate ($NaBH_4$), were evaluated. This process included calibration, analysis of control materials, optimization of reductant concentration, and calculation of limits of detection (LODs) and quantification (LOQs). Various approaches for the preparation of blood samples were tested. Issues associated with the incompatibility of a particular FIMS setup with the intended goals were identified, and possible solutions were proposed.

The study demonstrates practical value, as it clarifies the prospects for using FIMS in the biomonitoring of mercury.

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1 Literature review

1.1 Introduction

This literature review will explore the significance of mercury level testing, discuss the sources of mercury in different industries and occupational settings, highlight the associated health risks, and examine the analytical methods used for testing.

Traditionally, the determination of mercury in biological samples has been performed using techniques such as inductively coupled plasma mass spectrometry (ICP-MS) and cold-vapor atomic absorption spectroscopy (CV-AAS), which provide high sensitivity and accuracy. However, specialized methods such as the flow injection mercury system (FIMS) and the direct mercury analyzer (DMA-80) have emerged as promising alternatives. It is important to note that these methods are not completely independent but are based on atomic absorption spectrometry (AAS), specifically designed for the determination of mercury.

Despite advances in mercury determination methods, there remains a need for comparative studies to evaluate the effectiveness and reliability of new methods in detecting mercury levels in biological samples. This is especially important in occupational settings where accurate assessment of mercury exposure is paramount to ensuring worker safety and compliance with regulatory standards.

1.2 Mercury and its forms

Mercury exists naturally in the environment in elemental form or is present in inorganic or organic mercury compounds. It is mostly found in the Earth's crust in the form of sulfides. Cinnabar, a red sulfide, is the primary constituent of mercury-rich ores that are extracted from mines, and it may contain as much as 70% mercury.

Mercury is naturally cycled in the biosphere, with a total of 5500 metric tons being released into the atmosphere via degassing from the Earth's crust and the seas. Furthermore, human activities, such as burning of fossil fuels and other industrial emissions, result in the annual release of 2500 metric tons of mercury into the environment. Each year, some 2000 tons of mercury are manufactured for industrial purposes, with just a fraction of it being used in the synthesis of organic mercury compounds. Over the last two decades, there has been a gradual decrease in the global production of mercury for commercial purposes. As a result, the

European Union and the United States have implemented a prohibition on the exportation of mercury.[1]

The primary form of mercury discharged into the atmosphere through natural mechanisms is elemental mercury vapor (Figure 1). It is the most volatile form of mercury, has a vapor pressure of 0.3 Pa at 25 °C and transforms into the vapor phase at typical room temperatures. It is relatively insoluble in water (56 µg/L at 25 °C). Elemental mercury is soluble in lipids and nitric acid, in pentane (2.7 mg/L), and in sulfuric acid upon boiling, but is insoluble in hydrochloric acid.

When exposed to oxygen, metallic mercury undergoes fast oxidation, resulting in the formation of an ionic state. Mercuric salts, including halides, sulphates, and nitrates, are soluble in water. In water solutions, an equilibrium between Hg, Hg^{+}, and Hg^{2+} is observed. The distribution of the three oxidation states is dictated by the redox potential of the solution and the number of molecules capable of forming complexes with the mercuric ions. The mercuric ion Hg^{2+} has the capability to create several stable complexes with naturally abundant molecules or moieties, such as thiol groups. Mercuric mercury may form four distinct complexes with the chloride anion in aqueous solution: $HgCl^{+}$, $HgCl_2$, $HgCl^{-3}$, and $HgCl_4^{2-}$. Mercurous mercury Hg^{+} is unstable when exposed to biological substances.[1]

Organic mercury refers to a group of mercury compounds that contain carbon-hydrogen (C-H) bonds, with methylmercury (CH_3Hg^{+}) being the most well-known and toxic form. Short-chain alkylmercuric compounds react with halogens to produce volatile salts that are particularly toxic when present in large quantities in the air at room temperature.[2]

Methylmercury is formed by the combinations of divalent mercury and mercury sulfide through a natural process known as methylation, primarily occurring in aquatic ecosystems. In these environments, certain microorganisms, notably sulfate-reducing bacteria, catalyze the conversion of inorganic mercury (typically found as Hg^{2+}) into methylmercury. The degree of methylation depends on the oxygen content and the depth of the water system[3]. This transformation sets the stage for bioaccumulation and biomagnification, whereby methylmercury concentrations increase along aquatic food chains, ultimately leading to higher levels in fish and other aquatic organisms.

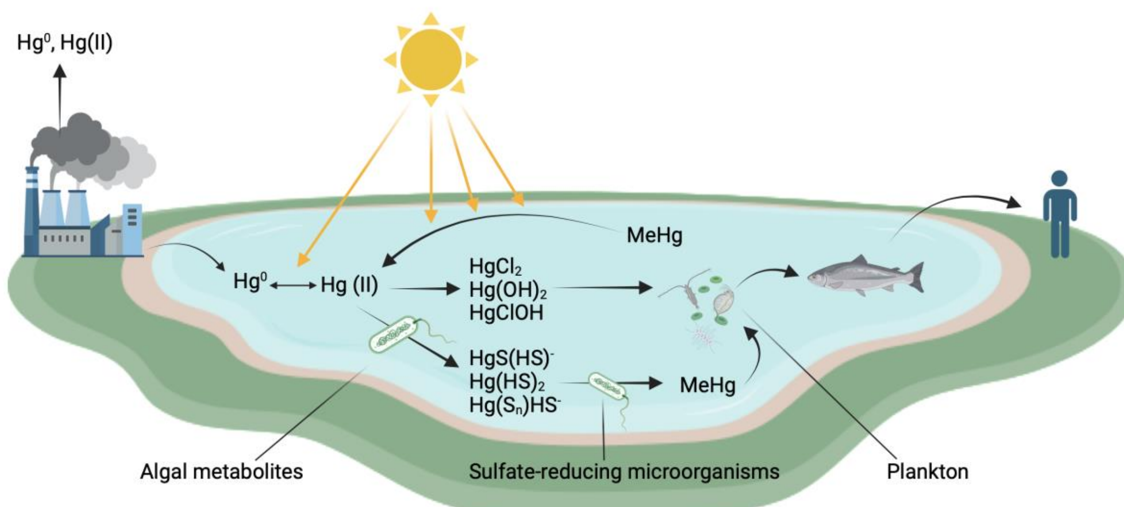


Figure 1. The natural cycle of mercury with the focus on anthropogenic emissions and transformation in the aqueous system leading to methylmercury entering the food chain.

One of the most concerning attributes of methylmercury is its exceptional bioavailability. Due to its high solubility in lipid membranes methylmercury is readily absorbed and assimilated by living organisms, making it a pervasive threat. As it enters the food web, methylmercury exhibits a remarkable propensity to accumulate in the tissues of organisms, particularly in fish. This bioaccumulation phenomenon can result in significantly higher methylmercury concentrations in fish compared to the surrounding water.

1.3 Occupational exposure to mercury

Mercury can be present at workplaces either as a byproduct of manufacturing processes or as an inherent component of certain materials. Some common sources of mercury in industries include gold extraction, chloralkali production, the manufacturing of mercury thermometers and fluorescent bulbs, and waste incineration. In these sectors, mercury can be emitted into the atmosphere or released into wastewater, leading to environmental contamination.

Mining and gold extraction workers are commonly associated with increased mercury exposure since it is often used in small scale gold mining for gold extraction. This especially applies to the artisanal and small-scale gold mining (ASGM), which refers to the process of extracting gold by manual labor and simple technology, and is the primary source of human-caused mercury emissions (Figure 2).[4] This industry is regulated by the Minamata Convention,

which seeks to decrease mercury emissions in countries that have signed the agreement. One requirement of the Minamata Convention is for nations that engage in ASGM to actively monitor and decrease workers' exposure to mercury. Additionally, the Convention requires to develop a public health policy to safeguard the well-being of the miners.[5]

Mercury forms an amalgam with gold, allowing for the separation of gold from other minerals in the ore. Miners and processing workers are exposed to mercury vapors during the amalgamation process handling and mixing mercury with the crushed ore to form an amalgam, which is then heated to vaporize the mercury, leaving behind the gold. Inhalation of mercury vapors is a primary route of exposure for miners. Mercury vapor is readily absorbed through the lungs and can lead to severe health effects. Skin contact with liquid mercury or contaminated materials can also result in intoxication, especially if protective gloves and clothing are not used.[6]

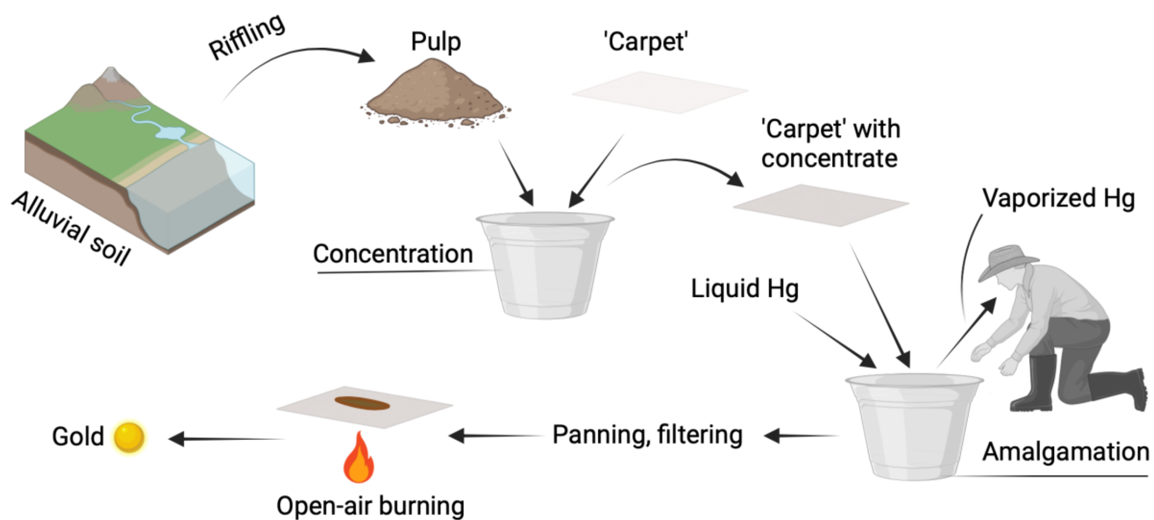


Figure 2. The process of artisanal and small-scale gold mining involving the manual amalgamation with mercury.

Dentists and dental staff (including hygienists, assistants, and laboratory technicians) are also at high risk due to the use of dental amalgams. Dental amalgam is a mixture of metals used to fill cavities caused by tooth decay. It includes silver, tin and copper, but the major constituent of the amalgam making up approximately 50% of the composition, is elemental mercury.[7] In recent years, there has been a growing shift towards alternative filling materials not containing mercury such as composite resins, glass ionomers, and ceramic materials.[8]

However, mercury amalgam continues to be used in dental practices throughout the world due to its low cost, greater durability, and ease of installation. Dental workers are at risk of exposure to mercury vapor and particulate matter during a variety of clinical procedures involving amalgam.[9] Procedures performed at high temperatures, such as drilling and polishing, are especially dangerous because of more intense evaporation.[10] Such procedures put not only the dentist at risk, but the patient as well. Thus, after removal of an amalgam filling, an increase in blood and plasma mercury levels was observed within 3–48 hours, followed by a decline.[7] The spectrum of mercury exposure in dental workers and patients is demonstrated in Figure 3.

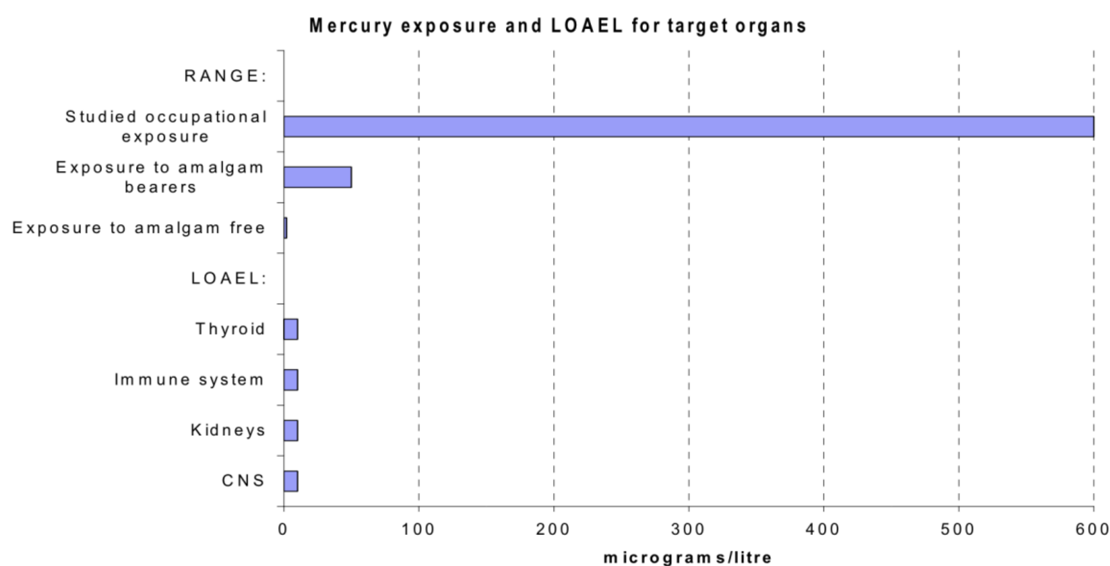


Figure 3. The study examined the spectrum of mercury exposure, defined by the rate of mercury excretion in urine, in individuals bearing amalgam fillings, amalgam-free subjects, and occupationally exposed dental workers. The lowest adverse effect exposure limit (LOAEL) for the central nervous system, thyroid, kidney, and immune system is also shown.[10] Reprinted with the permission from Elsevier under license 5797420760049.

Workers involved in the production of thermometers and barometers may be exposed to mercury, as well as some branches of chemical industry may also be potentially hazardous to employees. Thus, the level of mercury in methyl mercury form in the lake situated near a thermometer factory was reported to be twice as high as in two other lakes in the same region.[11]

A current important consumer of mercury in the European Union is chloralkali production, which contribution of total anthropogenic emissions in Europe was estimated to approximately 15% in the early 2000s.[12] Chloralkali plants are industrial plants that produce chlorine gas (Cl_2) and sodium hydroxide (NaOH) by electrolysis of brine (sodium chloride solution). During this process an electric current pass through a brine solution in an electrolytic cell, decomposing sodium chloride to chlorine gas, sodium hydroxide, and hydrogen gas. Historically, mercury was used as cathode material in the electrolysis process. Plant workers faced significant risks of mercury exposure through inhalation and skin contact, as well as through the consumption of contaminated water and food. In response to growing concerns about environmental pollution and worker health risks, many countries gradually phased out mercury cell technology in chloralkali production in the late 20th and early 21st centuries, replacing it with the membrane cell technology. However, some chloralkali plants in developing countries continue to operate using mercury cell technology due to cost considerations and regulatory gaps.[13] Due to the increased aerial concentration of mercury in the area of the plant, workers are subject to increased exposure, receiving mercury both at the workplace and from the air (Figure 4).

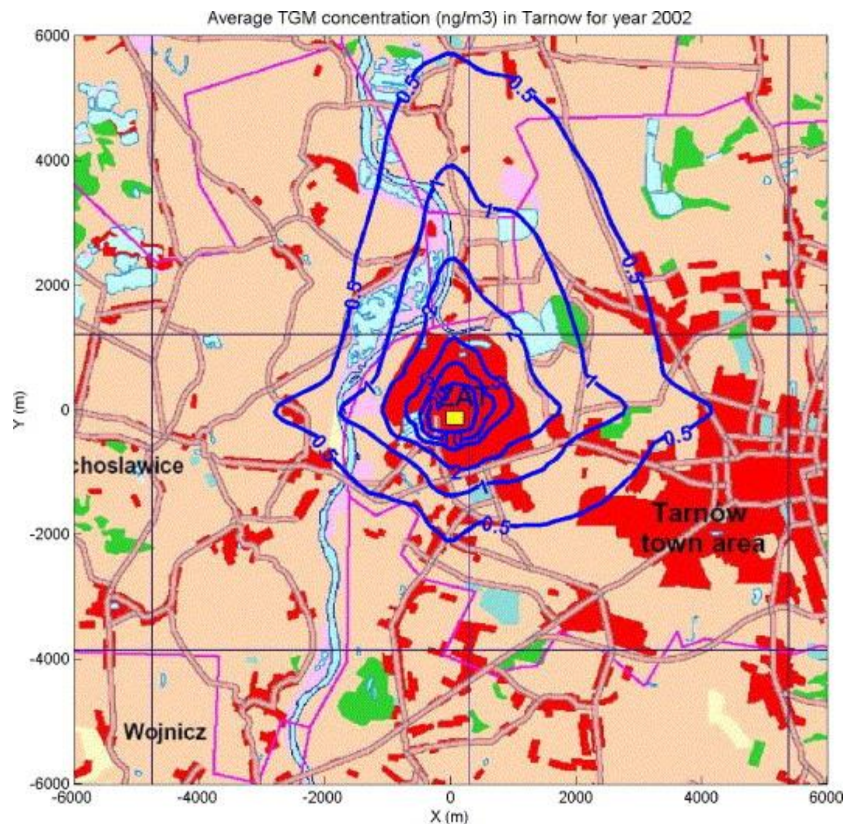


Figure 4. The average annual total gaseous mercury (TGM) concentration (ng/m^3) for the Tarnow plant in Poland in 2002. The yellow square in the center shows the position of the chloralkali plant.[12] Reprinted with the permission from Elsevier under license 5797501203034.

Another industry that exposes the employees to this type of risk is the manufacturing and recycling of compact fluorescent lamps (CFLs). Due to reduced energy consumption, minimized carbon dioxide emissions and 10-fold increase in lifetime, the CFLs are widely used as a replacement option for incandescent bulbs, and the prevalence of their domestic use is constantly growing. Nevertheless, a significant drawback of CFLs is the presence of milligram amounts of mercury in them, since these consist of a phosphor-coated glass tube containing mercury vapor and argon gas. As part of the recycling process, lamps are crushed, which results in the emission of mercury vapor and dust particles. Workers using drum-top crushing machines or fluorescent bulb recycling machines may be exposed to hazardous conditions if bulbs are accidentally shattered outside the machine or if the machine's air filtration system is malfunctioning.[14 15]

The duration and frequency of exposure strongly affect the extent of mercury intoxication. Prolonged or repeated exposure to mercury vapors or dust particles increases the risk of adverse health effects. Workers engaged in continuous or repetitive tasks involving mercury-

containing materials are at higher risk of chronic exposure, highlighting the importance of implementing control measures and monitoring protocols.

Control of mercury exposure in occupational settings is carried out using engineering solutions, use of personal protective equipment, job rotation strategies, and regular monitoring of mercury levels at workplace and in biological samples. The engineering solutions mainly mean effective ventilation systems. Proper airflow helps to dilute and remove mercury vapors and particles from the air, reducing the risk of inhalation. Ventilation strategies including local exhaust systems, general dilution ventilation, gas sensors, data transmission systems, and engineering controls tailored to specific processes and work areas, are widely implemented in modern mines.[16] The engineering control systems may include closed-loop systems, containment enclosures, and mercury capture systems.

Temperature and humidity can also influence mercury vaporization rates and airborne concentrations in the workplace. High temperatures and low humidity levels promote the release of mercury vapors from contaminated surfaces and materials, strengthening exposure risks. Conversely, in cold and humid environment the vaporization and dispersal of mercury is lower, though the potential for condensation and surface contamination might be increased.[3]

The use of personal protective equipment (PPE) minimizes direct contact with mercury and reduce exposure risks (Figure 5). PPEs may include gloves, goggles, respirators, and protective clothing designed to prevent skin contact or inhalation of hazardous substances. Maintaining strict hygiene practices, training, and adherence to PPE protocols are reported as effectively preventing mercury contamination and minimizing exposure risks in the workplace. The recommendation to wash hands and exposed skin thoroughly after handling mercury-containing materials or equipment is usually accepted at enterprises involving high risk of exposure, as well as recommendation to clean contaminated work surfaces and tools regularly using appropriate methods and cleaning agents to prevent cross-contamination and spread of mercury.

Figure 5. An example of PPEs for a workplace with risk of mercury exposure.

An increased demand for safety and well-being of employees led to the development of comprehensive training and education programs highlighting the hazards of mercury exposure and promoting safe work practices among workers. Training cover topics such as the health effects of mercury, proper handling and storage procedures, use of PPE, emergency response protocols, and the importance of regular monitoring and medical surveillance.

Regular monitoring of mercury levels in the workplace environment and in biological samples are now an integral part of the workflow in most of the above industries. Air sampling and analysis techniques, such as atomic absorption spectrometry (AAS) and gas chromatography (GC), are widely used for quantification of airborne mercury concentrations and exposure control measures. Biological monitoring involves measuring mercury levels in blood, urine, or hair samples to assess internal dose levels and identify individuals at risk of overexposure.

Another approach to mitigate the risk of chronic mercury intoxication includes job rotation strategies allowing workers to alternate between tasks with varying levels of exposure. Rotating job assignments can reduce cumulative effect and prevent the development of severe health effects associated with prolonged exposure to mercury.[17]

Without denying all the above, it is worth clarifying that the most optimal strategy for preserving the health of workers is still the substitution of mercury-containing materials with safer alternatives.

1.4 Health effects of mercury exposure

Subsequent health implications can be severe. Mercury vapor is readily absorbed through inhalation and can accumulate in the body over time. Prolonged exposure to elevated levels of mercury can result in neurological disorders, respiratory problems, kidney damage, and even death.[1] Certain groups, such as pregnant women and individuals with pre-existing health conditions, are particularly vulnerable to the harmful effects.

Methylmercury (MeHg) obtained by occupational exposure is readily absorbed (90% absorption rate) and remains in the body for a significant period of time (half-life of about 70 days).[18] At oral administration, MeHg is fully distributed into the bloodstream within a period of 30 hours, resulting in a blood concentration equivalent to about 7% of the administered dosage. Circulating methylmercury mostly accumulates in red blood cells. It attaches to cysteinyl (-SH) residues on the haemoglobin beta chain and then gradually spreads throughout the body, reaching an equilibrium with other tissues in about 4 days. Accumulating in significant quantities, MeHg induces the intoxication of cells and tissues through different cellular and molecular mechanisms, including inhibition of DNA, RNA and protein synthesis, erosion of microtubules, and oxidative stress. It also increases the level of intracellular Ca^{2+}, suppressing the neurotransmitter function.[2] On the cell level, due to the high binding ability to sulfhydryl groups in mitochondria, mercury may disrupt the mitochondrial electron transport chain, produce glutathione depletion, oxidative stress, and raise levels of reactive oxygen species. Additionally, Hg can injure the mitochondrial membrane, leading to mitochondrial growth and cell death signaling.[19]

The circulation of mercury in the human body affects almost all internal organ systems (Figure 6).

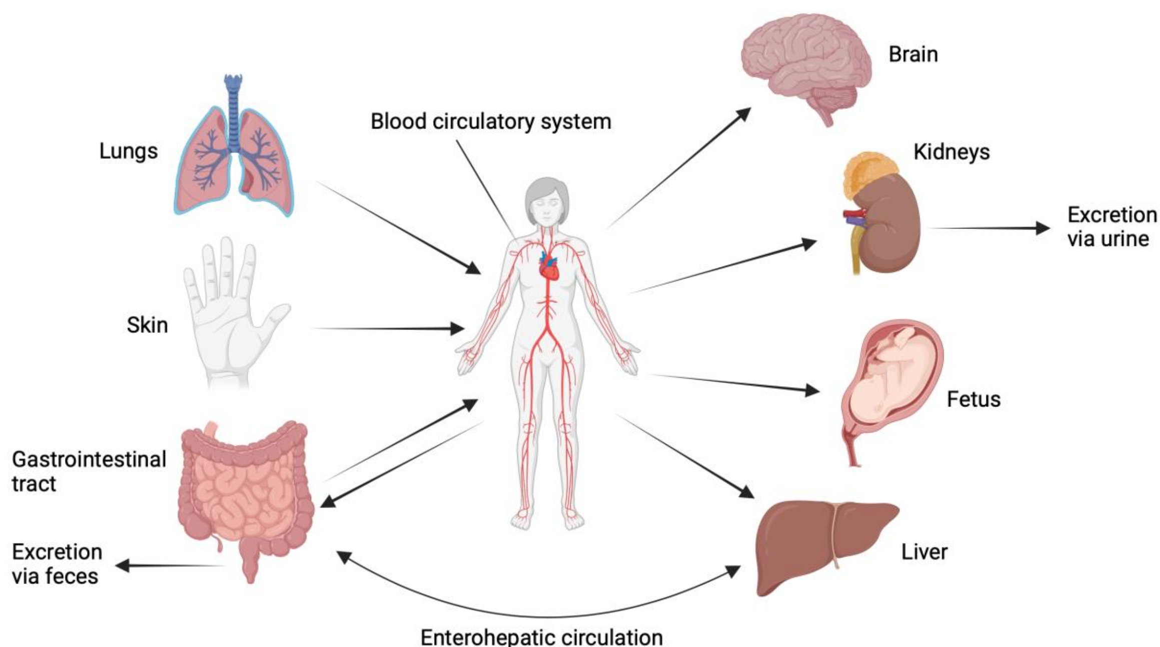


Figure 6. The distribution of methylmercury in the human body.

Within the brain, MeHg experiences gradual demethylation and transforms into inorganic mercury. After prolonged exposure approximately 10% of mercury remains in the brain cells with uneven distribution (the astrocytes and microglia had the highest deposits of Hg, whereas the buildup in neurons is notably lower).[2] Common symptoms of mercury-induced damage in the central nervous system may include peripheral vision loss and paresthesia, often experienced in the hands, feet, and around the mouth. Impaired motor coordination and muscle weakness may be observed as well.[20] Multiple studies suggests that the pathology of Alzheimer's and Parkinson's diseases might be at least partially caused or escalated by inorganic mercury.[21] [22] [23]

Kidney damage caused by mercury is reported to be a reason for dose-related tubular dysfunction and idiosyncratic nephrotic syndrome, which is expressed in increased protein excretion into the urine.[24] Mercury poisoning has been linked to the cause and development of different autoimmune diseases, such as the Morvan syndrome.[25]

MeHg easily passes through the placenta and builds up in the developing fetus at higher levels than in the mother. Thus, neonatal cord blood mercury levels are more than double the level found in maternal blood during parturition. However, mercury levels in infant blood heavily drop within the first 13 weeks of the child's life. After three months, maternal MeHg

concentrations are higher than those of the infant, which is the opposite of the situation during childbirth.[26] [27] Nearly 5% of the whole amount of mercury present in the maternal blood is detected in breast milk.[2]

Apparently, the most massive and shocking case of mercury poisoning was the famous Minamata disease. It appeared because of an environmental disaster that occurred in the city of Minamata, Japan, in the late 1950s. The Chisso Corporation, a chemical company, released industrial wastewater contaminated with methylmercury into Minamata Bay. Methylmercury had accumulated in the body of local fish and shellfish, which then entered the food chain and affected the people who consumed them. It is estimated that thousands of people were affected by this illness during the peak of the outbreak in the 1960s.[28] Minamata disease mostly affects the central nervous system, resulting in sensory disturbances, ataxia, tremors, and memory loss. Severe instances may lead to paralysis, unconsciousness, and death. The impact of Minamata disease extended to multiple generations, as the effects of methylmercury exposure were also observed in unborn children whose mothers were affected.[29]

In 2013, the international community adopted the Minamata Convention on Mercury as a reaction to the worldwide danger of mercury contamination. The primary objective of this convention is to protect human health and the environment from the hazards associated with mercury. The agreement establishes measures for regulating mercury emissions, diminishing mercury use in products and processes, and lobbying for safe handling, storage, and elimination of trash containing mercury.[5]

Another notorious example of mercury poisoning is erethism, or "Mad Hatter's disease", also known as "hatter's shakes" or "hatter's syndrome," which refers to a historical occupational illness that affected hat makers (hatters) in the 18th and 19th centuries, particularly those who were involved in the production of felt hats.[30] The disease was characterized by a range of neurological and psychological symptoms, and it was eventually linked to chronic mercury exposure in the workplace. The primary cause of Mad Hatter's disease was the prolonged and direct exposure of hatters to mercury, specifically using mercuric nitrate in the hat-making process. Hatters used mercuric nitrate to soften and shape animal fur (usually rabbit or hare) into felt, which was then used to make hats. During the hat-making process, hatters were exposed to mercury in the form of vapor.[31] Mercury vapor is colorless, odorless, and tasteless,

making it difficult to detect. Hatters would inhale these vapors and get mercury absorbed through the skin. The chronic exposure to mercury vapor led to various symptoms associated with Mad Hatter's disease. These symptoms included tremors (hence the term "hatter's shakes"), slurred speech, mood swings, irritability, anxiety, and hallucinations. Some hatters also experienced coordination problems and muscle weakness. The term "Mad Hatter" became synonymous with the eccentric character from Lewis Carroll's "Alice's Adventures in Wonderland." With the decline of the hat-making industry in the late 19th and early 20th centuries, cases of Mad Hatter's disease became increasingly rare. The adoption of safer practices and the use of alternative materials eliminated the primary source of mercury exposure for hatters.[30]

1.5 Regulations and guidelines for occupational mercury exposure

In addition to the already mentioned Minamata convention, many others guidance documents have been implemented to limit and regulate the industrial use of mercury.

REACH (Registration, Evaluation, Authorization, and Restriction of Chemicals) regulation adopted in 2006 by the European Chemical Agency (ECHA) requires companies to register substances they manufacture or import, evaluate their hazards, and take appropriate risk management measures.[32] Directive 2004/37/EC on Occupational Exposure Limits and amending Directive (EU) 2017/2398 both establish binding occupational exposure limits (OELs) for chemical agents, setting maximum concentrations of substances in the air that workers may be exposed to during their work activities. It includes mercury among the highly hazardous substances.[33] Regulation (EU) 2017/852 specifically targets mercury, establishing measures for mercury reduction in various sectors of industry. It particularly aims to phase down the use of amalgam containing mercury in dental fillings, promoting the use of alternative materials where feasible. It sets strict requirements for the handling, use and disposal of mercury amalgam.[17] CLP Regulation (EC) 1272/2008 on classification, labeling, and packaging of substances and mixtures classifies dangerous substances including mercury and mercury compounds according to their hazardous properties, providing clear labeling and safe handling requirements across the EU.[34]

All these documents together aim at maximizing the safety of workers in industries with a risk of mercury exposure.

1.6 Existing mercury detection methods

1.6.1 Cold vapor atomic absorption spectroscopy and cold vapor atomic fluorescence spectroscopy

1.6.1.1 Cold vapor atomic absorption spectroscopy

Cold vapor atomic absorption spectroscopy (CV-AAS) works on the principles of atomic absorption spectrometry. In the case of mercury analysis, the sample is first subjected to chemical vapor generation. This involves converting the different forms of mercury in the sample into gaseous elemental mercury (Hg^0). The gaseous mercury is then introduced into an atomic absorption spectrometer, where it is exposed to a monochromatic light source at the resonant wavelength of elemental mercury (Figure 7). Mercury atoms absorb the light at this wavelength, causing a reduction in the transmitted light intensity. The degree of absorption is directly proportional to the concentration of mercury in the sample, which can be quantified based on the reduction in light intensity.

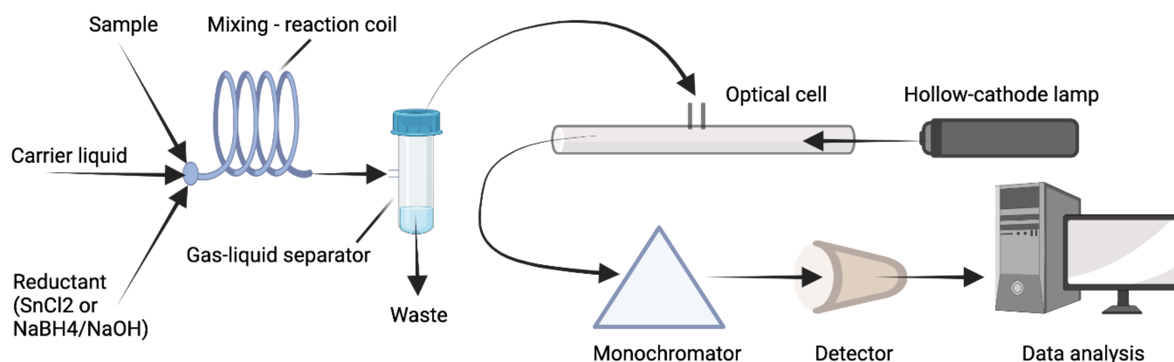


Figure 7. Typical CV-AAS setup for the determination of mercury.

CV-AAS is well-suited for mercury analysis due to its ability to selectively determine mercury at low concentrations. It is particularly effective for the analysis of total mercury in various sample matrices, including biological samples.[35] CV-AAS can achieve low detection limits for mercury, making it suitable for trace-level analysis.[36] [9] [38] [39] A solid advantage is that the technique can differentiate between different forms of mercury, allowing for speciation analysis. Sample preparation is relatively simple, which reduces the risk of contamination and minimizes potential interferences.[40] [41] [42] CV-AAS has been used for decades and has evolved with advancements in instrumentation and methodology.[43] [44] [45] Improvements in automation, detection limits, and sample handling have been made.[46]

Despite its usefulness, CV-AAS may still face challenges related to interferences that can arise from the co-existence of elements with similar absorption wavelengths or from compounds that form stable complexes with mercury. However, the major problem of the method is its' inability to determine mercury species: AAS provides information about the total mercury content only. This limitation is particularly significant when trying to assess the toxicity and environmental behavior of different mercury compounds.[47 48] To perform speciation analysis with AAS, a chromatographic separation step is often required to isolate different mercury species. This adds complexity to the analysis and requires additional equipment and method development.

1.6.1.2 Direct mercury analyzer

Multiple studies are implemented with DMA-80 (direct mercury analyzer), which is a highly specialized instrument designed for the direct determination of mercury in solid, liquid, and gaseous samples. It works on the principle of thermal decomposition and atomic absorption spectrometry, similar to CV-AAS but without the chemical vapor generation step. The heart of the DMA-80 is a high-temperature quartz tube furnace, inside which the sample is subjected to high temperatures (often around 650-900°C) under a controlled environment. The thermal decomposition process volatilizes and converts all mercury compounds (organic or inorganic) into elemental mercury (Hg^0). This step also homogenizes the sample and eliminates potential interferences from other elements or compounds in the sample matrix.

After thermal decomposition, the elemental mercury vapor is carried out of the furnace by a carrier gas (typically oxygen or a mixture of oxygen and nitrogen) to a trapping system. It can involve a gold-coated amalgamation trap, where the mercury atoms are absorbed onto the gold surface, or a sulfur-impregnated activated carbon trap, which captures the mercury by forming mercury sulfide.[49] The trapped mercury is subsequently desorbed from the trapping material by heating the trap. The mercury vapor is then directed into an atomic absorption spectrometer for analysis. The DMA-80 uses atomic absorption spectrometry in a cold vapor configuration and demonstrates high accuracy and precision in mercury analysis, often being considered as a preferred choice in the research related to occupational health.[41 50 51 52 53 45]

1.6.1.3 Flow injection mercury system

Building on the principles of CV-AAS, flow injection mercury system automates and optimizes sample introduction and reagent mixing. In FIMS, the sample is introduced into a carrier stream and transported to a reaction coil where it reacts with reducing agents to form mercury vapor. This vapor is then detected by atomic absorption spectroscopy, providing accurate measurements of mercury concentrations. This system allows for precise and continuous monitoring of mercury levels, improving the accuracy and efficiency of the analysis.[54]

1.6.1.4 Cold vapor generation atomic fluorescence spectroscopy

Cold vapor generation atomic fluorescence spectroscopy (CV-AFS) is a related technique that provides even greater sensitivity for mercury analysis than CV-AAS. It is often preferred for very low-level mercury determination. The key difference is in the detection principle. CV-AAS measures mercury absorption, while CV-AFS measures the fluorescence emitted by mercury atoms after excitation. CV-AFS has proven to be generally more sensitive and capable of measuring mercury at lower concentrations compared to CV-AAS and is preferred when extreme sensitivity is required. [55 42 56 57 58 59 60]

1.6.1.5 Sample preparation techniques for cold vapor atomic absorption spectroscopy and cold vapor atomic fluorescence spectroscopy

The commonly used biological sample preparation techniques for CV-AAS/AFS include digestion in nitric acid or in a mixture of acids,[35 53 37] solvent extraction, or preconcentration techniques.[40 42 58] The typical solvent for blood dilution include ethylenediaminetetraacetic acid (EDTA), propanol or butanol, Triton X-100 and tetramethylammonium hydroxide (TMAH) in various concentrations.[41] In urine analysis, creatinine corrected mercury levels are measured.[51 36]

In search for an optimal combination of high precision, accuracy, fast response time and cost-effectiveness, different instrumental set-ups continue to be tested. Various techniques of vaporization have been reported. To separate different mercury species in the sample, AAS or AFS are often combined with GC. This set-up has repeatedly shown high separation and quantification ability.[56 61 62] Thermal decomposition amalgamation atomic absorption spectrometry (TDA-AAS) is similar to the DMA-80 method mentioned earlier. It involves thermal decomposition to convert mercury compounds into elemental mercury, which is then

measured by AAS. TDA-AAS is effective for total mercury determination in various sample types.[41] Graphite furnace atomic absorption spectrometry (GF-AAS) involves atomizing the sample using a graphite furnace and can be used for both solid and liquid samples. While not specific to mercury, GF-AAS can be employed for mercury determination in biological matrices with lower mercury concentrations.[40] [63] Photochemical vapor generation atomic fluorescence spectrometry (PVG-AFS), involving the generation of volatile mercury species from the sample using photochemical reactions, is reported as a highly sensitive and suitable method for both total mercury and speciation analysis.[64] [60]

1.6.1.6 Applications of CV-AAS and CV-AFS

Both AAS and AFS are widely used in biomonitoring of individuals in industries involving mercury handling. In most cases, monitoring is carried out using blood and urine samples. Blood analysis provide insights into current mercury levels in circulation, while urine indicate the excretion of metabolites from the body. Hair analysis provides a unique advantage for assessing chronic mercury exposure since hair accumulates mercury over time through circulation and entry into the shaft. However, regular hair sampling is uncomfortable for the person being tested. On the other hand, although saliva sampling is simple and non-invasive, the possible release of mercury from dental fillings can make the results difficult to interpret, leading to inaccurate estimates of occupational exposure levels. Thus, saliva mercury testing is rarely used in occupational health monitoring.

Both AAS and AFS are very common methods for monitoring mercury. These have been actively used in recent decades in research and service laboratories, at the industrial enterprises themselves and in the laboratories of regulatory organizations. CV-AAS is also widely used for environmental monitoring and quality control purposes.[13] Table 1 shows some typical sample preparation methods and limit of detection (LOD) values for the determination of mercury in various matrices using CV-AAS.

Table 1. Reported mercury detection methods using CV-AAS

Year	Matrix	Sample preparation	LOD	Ref.
2004	blood	acid digestion, $KMnO_4$[1] oxidation	1.84 µg/L	35
2007	hair, nails, urine	digestion with HNO_3, $HClO_4$, and H_2SO_4	0.01 ng/g	65
2009	cell lines	ethylation	0.008 µM (MeHg), 0.005 µM (InHg)	66
2009	blood, urine	traditional on-line digestion	14–259 nmol/L	47
2010	hair, urine	acid digestion	0.075 µg/L	67
2010	saliva, hair	acid digestion, $KMnO_4$, tetramethyammonium hydroxide (tmah)	0.01 µg/l	68
2010	urine, hair	vortex/washing	3xSD of blank	50
2011	hair	acetone/β-mercaptoethanol washing	0.020 µg/g	69
2011	urine, hair, blood	acid extraction, digestion	0.2 µg/L (blood), 0.01 µg/g (hair)	70
2014	blood, hair	acid extraction, centrifugation	0.05 ng/g	71
2015	urine	not reported	0.2 µg/L	9
2015	urine, hair, blood	oxygenated decomposition	0.014 µg/L	72
2016	blood	freezing, thawing	0.2 µg/L	43
2016	hair	acid digestion	0.04 µg/g	37
2016	urine	digestion (NaOH, L-cysteine)	0.009 mg/L	59
2017	breast milk	pasteurization, freezing, acid digestion	1 µg/kg	73
2017	blood, hair, urine	ethylenediaminetetraacetic acid (EDTA)	not reported	74
2017	hair	acetone rinsing, acid digestion	0.0003-0.1 µg/kg	52
2020	urine, blood	creatinine-adjusting	0.05 µg/L	53
2020	blood	not reported	0.158 µg/L	51

[1] Potassium permanganate

Year	Matrix	Sample preparation	LOD	Ref.
2021	blood, hair, urine	microwave digestion, GC-ECD (MeHg in hair)	0.5 µg/L (blood) 0.1 mg/kg (hair) 0.14 µg/L (urine)	44
2021	blood	acid digestion, $SnCl_2$	0.001 ng/mL	27
2022	brain tumor, hair, blood	EDTA, acid digestion	0.08 µg/L	39
2022	hair, sand, urine	digestion, NH_4OH + HCl	0.0015 ng/g	60
2022	hair, blood	sodium heparin	0.61 µg/L (blood), 0.81 µg/g (hair)	75
2023	blood	cloud point assisted dispersive ionic liquid-micro solid-phase extraction (CPA-DIL-µ-SPE)	0.005–3.60 µg/L	46
2023	urine, hair, blood	oxygenated decomposition	0.09 ng (hair), 0.23 µg/L (blood)	36

A review of research conducted in recent decades in the field of mercury detection in biological samples using CV-AAS shows that LOD for blood decreased from 1.84 µg/L in early 2000s to 0.23 µg/L in 2023. LOD for urine reduced from 0.2 µg/L to 0.14 µg/L for approximately the same period. In hair, the reported LODs have not changed that much, demonstrating the lowest value as 0.05 ng/g. Acid digestion remains the most used sample preparation technique.

Typical sample preparation methods and LOD values for the determination of mercury in various matrices using CV-AFS are given in Table 2.

Table 2. Reported mercury detection methods using CV-AFS

Year	Matrix	Sample preparation	LOD	Reference
2004	hair	acid digestion, SPME ($NaBEt_4$, polydimethylsiloxane)	40 ng/g	76
2011	hair, urine	acid digestion, BrCl, $SnCl_2$	not reported	69
2021	urine	extraction	0.3 µg/L (MeHg), 1.9 µg/L (InHg)	77
2022	hair, nails	acid digestion, BrCl, $SnCl_2$	0.0006 µg/kg	61
2023	hair	acid digestion, ethylation, purge, trap	0.07 ng/g	64

The results of the reported CV-AFS analyses are comparable to those using the CV-AAS technique. The detected mercury concentrations are approximately of the same order of magnitude. For sample preparation, acid digestion and the cold vapor technique using $SnCl_2$ as a reducing agent, are commonly used.

1.6.2 Inductively coupled plasma mass spectrometry

Inductively coupled plasma mass spectrometry (ICP-MS) is an analytical technique used for the detection and quantification of trace elements, including mercury, in various sample matrices, including biological samples like blood, hair, urine, saliva, and sweat. ICP-MS combines two main components: the inductively coupled plasma (ICP) and mass spectrometry. The ICP is a high-temperature ionization source that atomizes and ionizes the sample, breaking it down into its constituent elements. This ionized material is then introduced into the mass spectrometer, where it is separated based on mass-to-charge ratio. By measuring the abundance of ions with specific mass-to-charge ratios, the concentration of mercury in the sample can be determined with high sensitivity and precision.

In the 1990s, a couple of decades after the researchers began to explore the use of inductively coupled plasma as an ionization source for mass spectrometry, the method began to be actively used for mercury testing.[78] At first, ICP-MS became a crucial tool for monitoring mercury in environmental samples, such as water, soil, and sediments. Additionally, its application expanded into the field of clinical and biomedical research for analyzing mercury levels in biological samples, such as blood, hair, urine, saliva, and sweat.[79] This innovation allowed to exceed the limitations of AAS and AFS techniques in terms of sensitivity and selectivity, especially when dealing with complex biological matrices. ICP-MS offered far greater sensitivity, enabling the detection of trace levels of mercury in complex samples.[47 48] Besides high sensitivity, ICP-MS offers excellent precision and accuracy in quantifying mercury isotopes.[80 64] Another major advantage was the ability of ICP-MS to analyze multiple elements simultaneously, especially in fields like environmental science, where various trace elements needed to be monitored.[79 81 82]

1.6.2.1 Inductively coupled plasma mass spectrometry coupled with high-performance liquid chromatography

To accurately separate and quantify different mercury species, chromatographic techniques, such as high-performance liquid chromatography (HPLC), are often coupled with mass spectrometry (Figure 8).

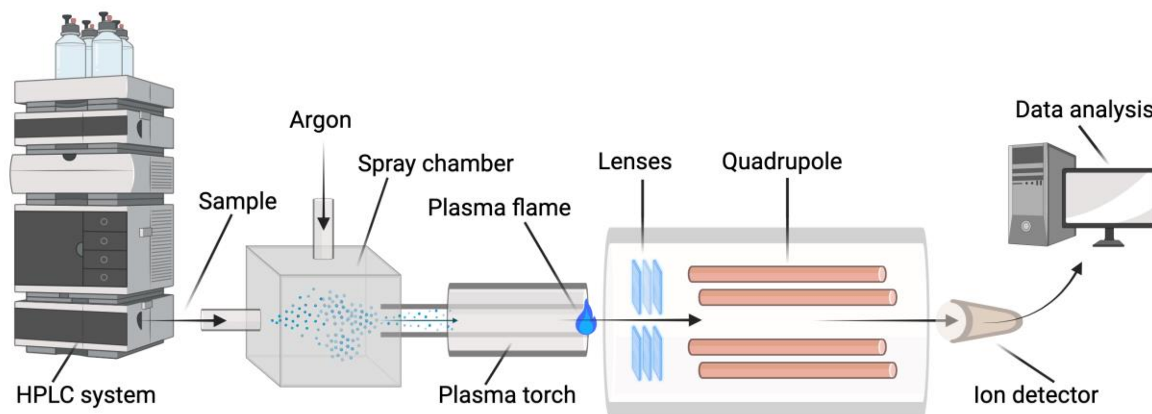


Figure 8. A typical HPLC-ICP-MS setup for determination of mercury

The choice of column, mobile phase, and other chromatographic conditions must be optimized for mercury speciation.

HPLC-ICP-MS is a highly effective technique, combining the high separation power of HPLC with the sensitivity of ICP-MS. It is widely used for the separation and quantification of different mercury species, such as methylmercury and inorganic mercury, in biological samples. Advantages include simultaneous determination of multiple mercury species and improved selectivity.[83] [84] [85]

1.6.2.2 Inductively coupled plasma mass spectrometry coupled with gas chromatography

In recent research, GC-MS is also often used for the analysis of volatile mercury species in biological samples, such as methylmercury in fish or organic mercury compounds in hair, providing information on the specific forms of mercury present. Some studies use gas chromatography electron ionization mass spectrometry (GC-EI-MS) to ionize and detect non-volatile or less volatile compounds, making it suitable for a wider range of mercury species, including inorganic mercury compounds. [86] [87]

The dynamic reaction cell (DRC) is an additional component in the ICP-MS system that reduces spectral interferences by using reaction chemistry to eliminate isobaric interferences (same mass-to-charge ratio) and enhance analytical specificity. In biomonitoring, ICP-DRC-MS is used to improve the accuracy of mercury quantification by mitigating interferences that can affect traditional ICP-MS measurements.[82] It is especially useful in the analysis of complex matrices like biological samples.[88] To extend the capability of ICP-MS, attempts to increase the number of mass analyzers have been made. The triple quadrupole ICP-MS (ICP-QQQ-MS) set-up, using three quadrupoles, was established for targeted and highly sensitive quantification of specific mercury species, making it suitable for trace-level speciation analysis. It showed better results compared to the conventional ICP-MS system with only one quadrupole mass filter.[89] An in-column high-pressure nebulizer introducing the sample into the ICP-MS and enhancing sample transport, was estimated as a technique significantly improving sensitivity of analysis.[90]

1.6.2.3 Sample preparation techniques for inductively coupled plasma mass spectrometry

The development of different techniques of sample preparation for testing mercury levels with ICP-MS has been the target of many studies in recent years. To efficiently extract and analyze the mercury content in blood, urine and hair, convenient procedures such as acid digestion and extraction are widely used. Besides homogenization and removing of organic interferences, in the case of mercury, acid digestion transforms inorganic species like Hg^{2+} (mercuric ions) into a more stable and analyzable form.[91 92 93 55]

In blood sample preparation, researchers often resort to additional agents, for instance ethylenediaminetetraacetic acid, to chelate and stabilize mercury in blood samples, preventing potential losses or changes in mercury speciation and maintain the integrity of the sample.[82] Another agent to prevent the loss or transformation of mercury species, solubilize and stabilize analyte to ensure accurate results, is 2-mercaptoethanol.[85] Titanium dioxide (TiO_2) is often used as an adsorbent to preconcentrate mercury from the sample before ICP-MS analysis. Preconcentration enhances sensitivity, especially for trace-level mercury analysis.[94] Derivatization (converting mercury into a more stable and easily detectable form), typically by adding sodium tetrapropylborate ($NaBPr_4$), demonstrated high efficiency in multiple studies.[95] Less common, but well-proven way to facilitate the analysis of inorganic

mercury species and minimize interference is sodium borohydride ($NaBH_4$) used to reduce inorganic mercury (Hg^{2+}) to elemental mercury (Hg^0) for improved analysis.[96] Enzymatic creatinine determination was tested to normalize mercury concentrations in blood samples to account for variations in urine dilution. It provided a more accurate representation of mercury levels by accounting for differences in blood volume and urine excretion.[79] The combination of TMAH (tetramethylammonium hydroxide), Triton X-100, and ammonium pyrrolidinedithiocarbamate (APDC) efficiently helps to create conditions that promote the formation of complexes between mercury and chelating agents, facilitating extraction and concentration. This method enhances the extraction of mercury species from the sample matrix and promote their stability during subsequent processing and analysis.[97]

In terms of urine sample preparation, application of L-cysteine deserves interest. This agent is used to stabilize and reduce inorganic mercury (Hg^{2+}) in urine samples, converting it into more stable and analyzable species, typically methylmercury. This approach reduces potential losses and changes in mercury species during sample preparation.[85] More conventional preparation techniques, such as spiking, which involves adding a known amount of a mercury standard to the sample, typically result in high recovery and accuracy during the analysis.[88]

A sophisticated technique based on thermal separation with isotope dilution, gas chromatography, and ICP-DRC-MS (TSID-GC-ICP-DRC-MS) appeared to be highly accurate and allowed for precise quantification of mercury species. It is particularly valuable in research and regulatory applications.[96]

1.6.2.4 Applications of inductively coupled plasma mass-spectrometry ICP-MS

The field of applications of ICP-MS for determination of mercury is similar to that of CV-AAS/AFS (Table 3). Many research and service laboratories are equipped with both types of instrumentations, which allows to perform comparative analysis. ICP-MS is the preferred method for analysis of extremely low trace values of mercury. It can also analyze multiple elements simultaneously, which makes it a powerful tool for comprehensive elemental analysis.[79] The Table 3 shows some described in literature sample preparation methods and LOD values for the determination of mercury in organic matrices using ICP-MS.

Table 3. Selected reported studies on mercury using ICP-MS.

Year	Setup	Hg type	Matrix	Sample preparation	LOD	Ref.
2005	direct ICP-MS	total	blood, plasma, urine, hair	acid mineralization/ enzymatic creatinine determination	0.079 µg/L	79
2008	direct ICP-MS	total	blood	TMAH+Triton X-100, APDC	0.40 µg/L	97
2008	direct ICP-MS	total	hair	L-cysteine, 2-mercaptoethanol, EDTA	not reported	6
2009	GC-ICP-MS	org	blood	methyl tert-butyl ether (MTBE), citrate buffer	0.1 µg/L	86
2013	HPLC-ICP-MS	org, in	plasma	mercaptoethanol, L-cysteine, HCl	12 ng/L (InHg) 4 ng/L (MeHg)	98
2014	optical emission spectroscopy ICP-OES	total	postmortem blood, hair, urine	$NaBH_4$+NaOH+HCl to reduce Hg^{2+}	0.013-0.231 µg/L (blood), 0.0048-0.042 µg/L (urine)	48
2017	direct ICP-MS	in	air	Hg vapor badge	6 ng/L	4
Year	Setup	Hg type	Matrix	Sample preparation	LOD	Ref.
2018	direct ICP-MS	total	blood	heparin	0.1 µg/l	93
2019	direct ICP-MS	ions	urine	TiO_2 preconcentration, vapor generation chip	0.75 ng/L	94
2019	GC-ICP-MS	all	blood	isotope dilution	0.16 µg/L (Ihg) 0.13 µg/L (MeHg) 0.4 µg/L (THg)	99
2021	octopole reaction system ORS-ICP-MS	org, in	blood	alkaline solution	0.16 µg/L	100
2021	HPLC-ICP-MS	total	blood	cysteine buffer, HNO_3, heating	6 ng/L	91
2022	ICP-QQQ-MS	total	blood	dilution (water, TMAH, ethanol)	0.17 ug/L	89
2023	direct ICP-MS	in	blood	extraction (NH_4OH, EDTA, Triton X-100)	0.37 µg/L	81
2023	direct ICP-MS	total	blood	sodium heparin	0.05-0.06 µg/L	101

In can be concluded from the research review that LOD and LOQ values are strongly dependent on the type of sample and sample preparation. The most sensitive analysis was demonstrated in 2023 with the LOD of mercury in cord blood as low as 0.05-0.06 µg/L.[101]

Sample preparation methods vary and include solvent extraction, digestion and preconcentration techniques.

1.6.3 Comparison of methodologies

According to the comparative research, the conventional CV-AAS method underestimated the analysis of total Hg by about 69% in blood and 14% in urine compared to ICP-MS.[47] This discrepancy may be attributed to the incomplete oxidation and reduction of methyl Hg species in the CVAAS method. Interestingly, this contradicts the forensic research suggesting that at high concentrations of mercury CV-AAS is more sensitive than ICP-MS for blood samples.[48] For both techniques, sensitivity to inorganic mercury is higher than to organic specie, which suggests that the analytical parameters depend on the chemical form of mercury. This leads to the necessity to use both types of mercury in the preparation of standard solutions for calibration.[102]

2 Experimental part

2.1 Introduction

The Finnish Institute of Occupational Health (FIOH/THL) started an important project to modify the flow injection mercury system (FIMS) equipment with the goal of improving analytical capabilities for mercury determination in biological samples, especially blood analysis. This project began with the purchase of the FIMS apparatus to replace the outdated cold vapor atomic absorption spectrometry (CV-AAS) instrument.

Performance, sensitivity, and analytical capabilities were the main reasons for switching from the CV-AAS to the FIMS device. Still, even with the FIMS device's promising future, first efforts to use it for blood analysis turned up unexpected challenges. After introduction into the FIMS device blood samples started to foam within the tubes and leak to the filter, because of which the instrument was rendered useless for its intended purpose.

An ageing equipment, like the CV-AAS, has major cost consequences for the laboratory. The analysis requires a large amount of manual work. The long analysis time and restricted functionality compromise analytical performance economic efficiency. Laboratory maintenance, repair and replacement of outdated equipment are rather expensive, while downtime and poor performance result in revenue and productivity losses.

The main goal of this experimental work includes four directions in view of the difficulties with the FIMS equipment. First, to evaluate, especially regarding blood samples, the viability and functionality of the FIMS device for mercury analysis. To this end, a thorough assessment of the instrument's capabilities and operating features was carried out using water solutions as surrogate samples.

Secondly, to develop such a ratio of components in a mixture of carrier liquid and reducing agent, that would achieve maximum selectivity for methylmercury and inorganic mercury. This requirement is dictated by the difference in toxicity of two types of mercury, due to which the service laboratory needs to have a method for determining both total mercury and separately methylmercury.

Furthermore, to investigate and refine blood analysis sample preparation methods that would lessen the foaming and leakage problems noted with the FIMS device. Enabling the successful adaption of the FIMS instrument for blood analysis and releasing its full analytical potential required an understanding of the underlying causes of these events and the development of practical solutions.

Finally, as a component of this experimental study, analysis of urine samples by inductively coupled plasma mass spectrometry (ICP-MS) was compared to the FIMS device. This comparison method aimed to assess the FIMS instrument's analytical performance, sensitivity and dependability in respect to a well-known and extensively used analytical method for mercury detection.

The benefits of achieving these goals were expected to be the resolution of technical difficulties with the FIMS equipment, optimization of blood analysis performance, and increase of mercury analytical capabilities in the laboratory environment.

2.2 Analysis of urine samples using ICP-MS for the determination of inorganic mercury

Urine mercury analysis is a commonly used biomonitoring method to evaluate individual and population-level exposure to mercury. ICP-MS is successfully used in the FIOH, consistently demonstrating the excellent sensitivity, accuracy, and handling of complicated matrices, which make it the best option for trace metal analysis. Due to the high efficiency in detecting low concentrations of mercury, ICP-MS can serve as a robust reference method for comparison studies.

FIMS combines flow injection analysis with atomic absorption spectrometry, providing rapid and direct measurement of mercury in samples. While FIMS is known for its quickness and simplicity of use, sensitivity and matrix interferences may be more problematic than with ICP-MS. In urine mercury analysis, the justification for comparing FIMS results with ICP-MS is the need to confirm and validate the reliability of the method. By benchmarking against ICP-MS, which is considered a gold standard, the study aims to evaluate the accuracy, precision, and potential limitations of FIMS.

Several challenges were expected in this comparative study. Matrix effects are a significant concern in urine analysis. Urine is a complex biological matrix containing various organic and inorganic constituents that can interfere with mercury detection and impact accuracy. While ICP-MS is generally well-equipped to handle complex matrices due to its high sensitivity and robustness, FIMS may experience more noticeable interference effects, potentially leading to inaccurate results.

Another challenge is the detection limit and quantification capability of each method. As has been considered in section 1.6. of the literature review, ICP-MS typically offers lower detection limits compared to CV-AAS, allowing for the detection of trace amounts of mercury that might be missed by FIMS. This difference in sensitivity needs to be thoroughly assessed to determine the applicability of each method under varying mercury concentration scenarios. Additionally, practical factors such as the operational efficacy and cost-effectiveness of any approach, should also be considered. While ICP-MS is highly accurate, it is also more expensive and requires sophisticated instrumentation and trained personnel. In high-throughput setting taking place in the Finnish Institute of Occupational Health, FIMS provides a more simple and faster analytical method, although potentially less sensitive.

In conclusion, the comparison of ICP-MS and FIMS for urine mercury analysis aims to establish a comprehensive understanding of their respective strengths and limitations.

2.2.1 Sample preparation and analysis

Urine samples and standard solutions were provided by FIOH and prepared for analysis in accordance with standard protocols for ICP-MS analysis. To guarantee the accuracy and robustness of the analytical results, an internal standard solution was employed as a solvent. The components of the internal standard are indicated in the Table 4.

Table 4. Components of the internal standard solution for ICP-MS analysis.

Component	Volume	Initial concentration
Ga aqueous solution	525 μL	100 ppm
Ge aqueous solution	525 μL	100 ppm
In aqueous solution	53 μL	100 ppm
Pt/Pd aqueous solution	53 μL	100 ppm
Sc aqueous solution	53 μL	1000 ppm
Bi aqueous solution	21 μL	100 ppm
HNO_3	10 mL	65%
MilliQ H_2O	L	■

The internal standard solution was added to samples and calibration standards to correct for changes in sample injection, ionization efficiency, and instrument drift during analysis. Gallium, germanium, indium, platinum and scandium are commonly used as internal standards in ICP-MS due to their isotope stability and compatibility with the analytical matrix. Bismuth and platinum are used in quantification as these metals are close to mercury in atomic mass. Palladium, as a platinum group metal, is added to monitor potential interferences and matrix effects. The preparation of the internal standard was done using plastic 1 L volumetric flasks, 5 mL and 1000 μL laboratory pipettes. All metal solutions are manufactured by Merck Group, 65% HNO_3 is manufactured by ROMIL.

Seven standard solutions with concentration of inorganic mercury Hg^{2+} 0.5 μg/L, 1.0 μg/L, 2.5 μg/L, 5.0 μg/L, 10 μg/L, 20 μg/L, and 40 μg/L were used for calibration. Lypocheck Controls LK, L1 and L2 (Bio-Rad, Hercules, CA, USA) were selected for quality control for their established traceability and consistency in mercury content. The use of controls allowed us to monitor the performance of the analytical method and to detect any potential sources of error or variability in the analysis. The internal controls provided by the laboratory (K_0, $K_{2.5}$ and K_5) were used for additional quality control in line with the protocol used in the FIOH. The standard solutions as well as stock solutions of internal controls and Lypochek were provided by the laboratory. Sixteen urine samples prepared according to Intercomparison programs 66 – 69 for toxicological analyses in biological materials by Institute and Outpatient Clinic for occupational, Social and environmental Medicine of the University of Erlangen-Nuremberg were used for analysis.

For rinsing and carrying, 2% aqueous solution of HNO_3 was prepared by adding 40 mL of concentrated (65%) HNO_3 to certain amount of water in 2 L plastic volumetric flask and then adjusting with water to the mark.

Prior to sample preparation procedure, samples, controls, and standard solutions were handled for one hour at room temperature. This stage reduces temperature fluctuations-related differences in sample matrix composition. By departing from the recommended handling conditions, accuracy of the analysis may be compromised. Due to high concentration of mercury in the Lypochek 2, it was diluted twice to set it into the tolerance range. The samples were prepared in 1-mL disposable glass vials. During the preparation, 250 μL of each stock solution (including Lypocheck 2 twice diluted with water) was pipetted into 4.75 mL of internal standard. After preparation, all vials were vortexed for 30 sec and introduced into the autosampler. The analysis was performed using Thermo Scientific iCAP RQ ICP-MS instrumentation equipped with the Qtegra software, that is intended for ICP-MS analysis data collecting, processing, and instrument control. There were two runs made for each sample, and the whole analysis was repeated five times.

2.2.2 Results

The concentration of mercury was quantified regarding to ^{200}Hg and ^{202}Hg as the most abundant isotopes. The deviation from the mean (RSD) in concentration value measured by those isotopes was calculated. Measured results, calculations and tolerance ranges are given in Table 5.

Table 5. Measured concentrations and tolerance ranges of inorganic mercury in internal controls K_0, $K_{2.5}$ and K_5, Lypocheck Controls and urine samples prepared in according with Intercomparison programs 66 – 69.

Sample List	Run	^{200}Hg	^{201}Hg	^{202}Hg	Average in isotopes	SD	RSD	Average in runs	Tolerance range
	#	μg/L					%	μg/L	μg/L
K_0	1	0,279	0,276	0,280	0,278	0,001	0,307	0,237	-0,088 – 0,265
	2	0,201	0,196	0,189	0,196	0,006	2,901		
$K_{2.5}$	1	2,817	2,662	2,667	2,715	0,076	2,806	2,639	1,6 – 3,4
	2	2,570	2,550	2,570	2,564	0,004	0,151		
K_5	1	5,349	5,376	5,350	5,358	0,005	0,094	5,242	3,25 – 6,75
	2	5,175	5,149	5,053	5,126	0,061	1,191		

Sample List	Run	^{200}Hg	^{201}Hg	^{202}Hg	Average in isotopes	SD	RSD	Average in runs	Tolerance range
	#	μg/L					%	μg/L	μg/L
$K_{2.5} - K_0$								2,403	1,6 – 3,4
$K_5 - K_0$								5,005	3,25 – 6,75
LK	1	0,596	0,470	0,580	0,549	0,024	4,386	0,520	0,221 – 0,481
	2	0,493	0,502	0,476	0,490	0,009	1,843		
L1	1	49,327	50,466	48,088	49,294	0,706	1,432	48,511	31,92 – 59,28
	2	47,421	48,318	47,443	47,727	0,171	0,358		
L2 (1:2)	1	120,91	123,86	119,98	121,589	0,804	0,662	120,74	94,4 – 141,6
	2	118,23	122,80	118,64	119,892	0,865	0,721		
66 2A	1	8,893	6,436	8,137	7,822	0,551	7,040	7,776	4,5 – 8,7
	2	8,684	6,370	8,138	7,731	0,478	6,184		
66 2B	1	32,896	29,550	32,124	31,524	0,688	2,183	31,343	23,3 – 36,5
	2	32,646	29,515	31,324	31,162	0,814	2,613		
66 8A	1	0,485	0,522	0,501	0,502	0,010	1,955	0,481	0,22 – 0,70
	2	0,462	0,481	0,438	0,460	0,014	2,959		
66 8B	1	0,783	0,693	0,854	0,777	0,043	5,575	0,805	0,50 – 0,98
	2	0,858	0,785	0,857	0,833	0,014	1,687		
67 2A	1	5,758	3,971	5,298	5,009	0,378	7,544	5,014	2,8 – 5,2
	2	5,856	3,848	5,352	5,019	0,422	8,401		
67 2B	1	19,655	17,076	19,223	18,651	0,503	2,699	18,225	10,6 – 17,8
	2	18,803	16,358	18,235	17,799	0,504	2,830		
67 8A	1	0,315	0,340	0,310	0,322	0,006	1,802	0,306	0,13 – 0,43
	2	0,321	0,277	0,269	0,289	0,026	9,074		
67 8B	1	1,373	1,341	1,347	1,353	0,014	1,002	1,383	0,77 – 1,55
	2	1,412	1,415	1,410	1,412	0,001	0,079		
68 2A	1	6,213	5,612	5,974	5,933	0,151	2,549	5,955	3,3 – 6,3
	2	6,300	5,590	6,042	5,977	0,171	2,856		
68 2B	1	22,508	19,087	21,734	21,109	0,700	3,318	20,926	12,9 – 21,3
	2	22,485	18,251	21,490	20,742	0,874	4,216		
68 8A	1	0,156	0,111	0,118	0,129	0,020	15,330	0,123	0,04 – 0,16
	2	0,118	0,136	0,101	0,118	0,010	8,582		
68 8B	1	0,397	0,441	0,362	0,400	0,021	5,258	0,397	0,24 – 0,54
	2	0,389	0,382	0,410	0,394	0,011	2,735		
69 2A	1	6,579	4,252	5,954	5,595	0,498	8,899	5,670	3,3 – 6,3
	2	6,608	4,527	6,097	5,744	0,434	7,561		
69 2B	1	33,687	30,977	32,438	32,367	0,743	2,295	32,764	25,2 – 39,0
	2	33,921	31,548	34,014	33,161	0,468	1,411		
69 8A	1	0,263	0,205	0,254	0,241	0,011	4,668	0,234	0,10 – 0,28
	2	0,261	0,201	0,219	0,227	0,022	9,910		
69 8B	1	0,818	0,857	0,890	0,855	0,036	4,185	0,811	0,37 – 0,85
	2	0,759	0,763	0,778	0,767	0,009	1,219		

The deviation from the mean (RSD) in concentration value measured by different isotopes in most cases did not exceed 10%, which is considered acceptable according to the laboratory protocol. In the first run of sample 68 8A the deviation was 15,33% which may be due to technical errors during the analysis. The average measured concentrations were compared to

the tolerance ranges provided by Finnish Institute of Occupational Health (for internal controls K_0, $K_{2.5}$ and K_5), BIO-RAD (manufacturer of Lypocheck Controls) and Institute and Outpatient Clinic for Occupational, Social and Environmental Medicine of the University of Erlangen-Nuremberg (Intercomparison programs 66 – 69, 2020 for toxicological analyses in biological materials). With the exception of one sample (67 2B), the measured concentrations of mercury were neatly set within the tolerance range and were close to the median reference value. This data allows to use the ICP-MS technique as a reference method in validation of other newly adopted method for measuring mercury. In case of successful development of the protocol for the analysis of mercury in biological samples using FIMS technique, the data obtained through ICP-MS method can be used for comparative analysis.

2.3 Analysis of water solutions and biological samples using Flow Injection Mercury System (FIMS) for the determination of methylmercury and inorganic mercury

In the development of a method for analyzing mercury in biological samples using FIMS technique, it was essential to first verify the instrument's functionality. After being out of use for an extended period, the instrument needed a careful assessment to guarantee its functionality. Prolonged periods of time spent stationary may cause several issues such as degradation of lamp components, contamination, or malfunction of the detection system. Therefore, before proceeding with the complex analysis of biological matrices, some measures such as sensitivity test and calibration with water solutions have been taken to confirm that the FIMS instrument was in optimal working condition. LOD and LOQ were calculated. Furthermore, attempts were made to establish the optimal concentration of the reducing agent for the selective determination of methylmercury and inorganic mercury. Tests of urine samples were performed using different reducing agents. Finally, various options for sample preparation of blood samples were tested.

2.3.1 Instrumentation and parameters of analysis

A PerkinElmer flow injection mercury system FIMS 400 (Uberlingen, Germany) equipped with an autosampler PerkinElmer S10 was used for analysis at the specific for mercury wavelength 253.7 nm. General view of FIMS setup is shown in Figure 9.

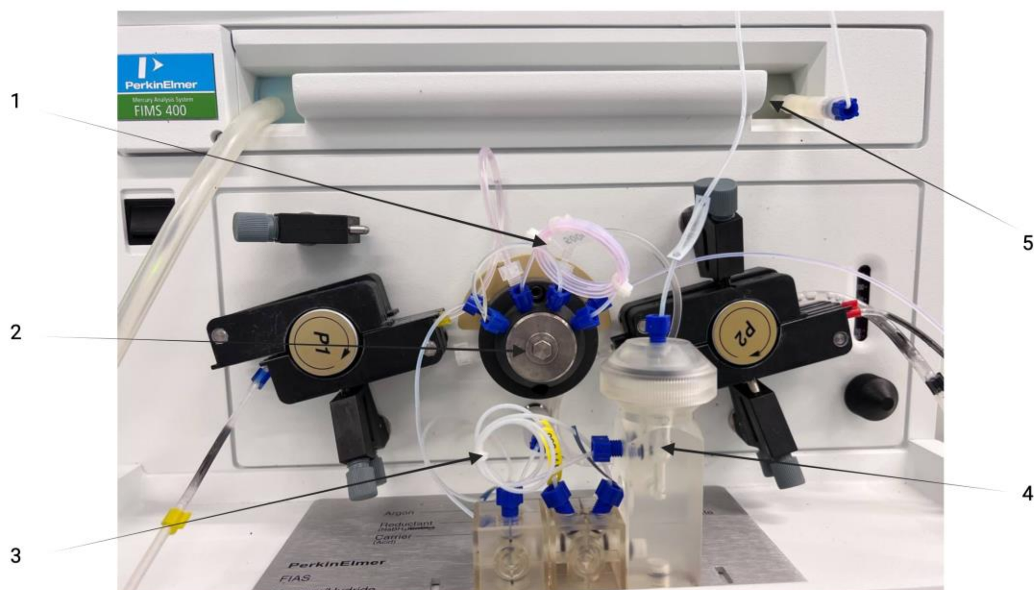


Figure 9. FIMS 400 setup. Pump P2 deliver the carrier and reductant to the mixing coil (1), while the autosampler pump delivers sample solution. After the coil is filled with the precise volume of the sample, the injection valve (2) turns, and the mixture is introduced into the reaction coil (3). In the reactor, the mercury species are converted into elemental mercury vapor, which is separated from the liquid phase in the gas-liquid separator (4) and is directed into the optical cell (5). Pump P1 is responsible for removing the waste.

The carrier solution was 3.0% (v/v) HCl with the flow rate of 10 ml/min. As reducing agents 1.1% $SnCl_2$ in 3.0% HCl and 0.2%[2] $NaBH_4$ in 0.05% NaOH were used, the reductant flow rate was 6 mL/min. Argon gas (Ar) with the flow rate of 50 ml/min was used as a carrier. The speed of pumps #1 and #2 were 100 rpm and 120 rpm respectively. The parameters of the reaction coil were 110 mm x 1.0 mm (i.d.). The sample volume used for analysis was 600 μl. The Syngistix Software for AA Version 3.1.0.1682 was used to set analysis parameters, control the system, and interpret the results. Tubing parameters and configuration are shown in Figure 10.

[2] Starting concentrations are given. The concentrations of carrier liquid and $NaBH_4$ reductant were adjusted during the study.

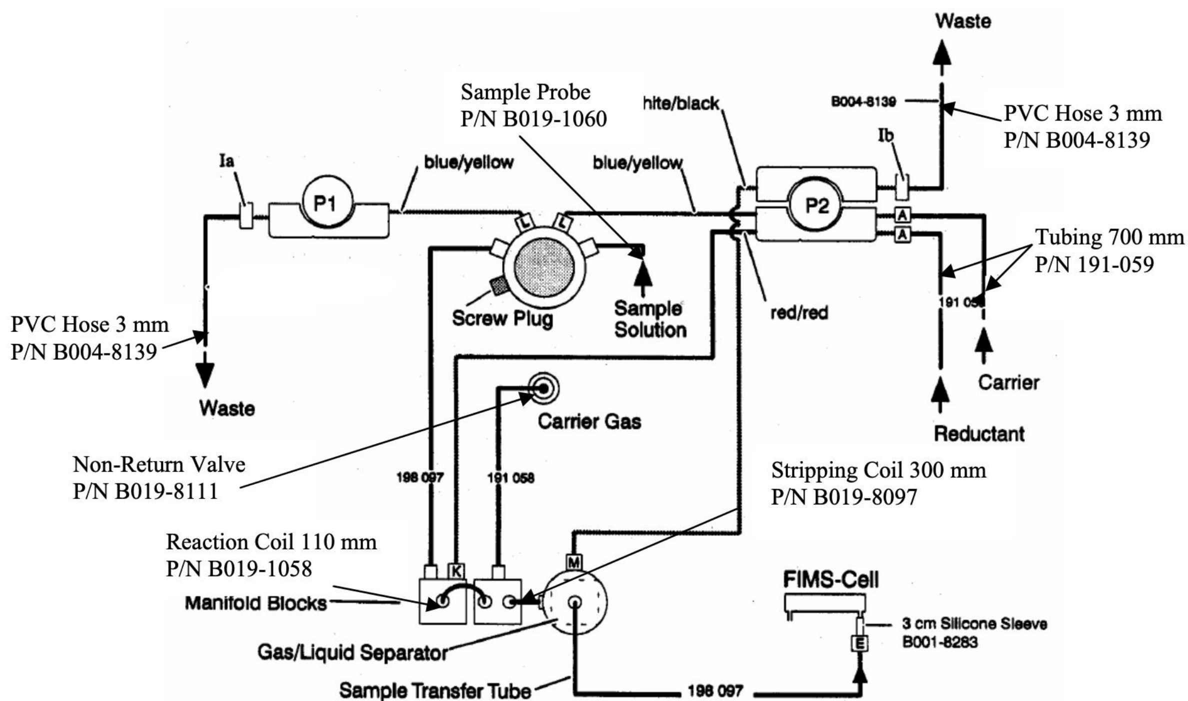


Figure 10. The parameters of tubing at FIMS 400 setup as it given in the laboratory manual.

2.3.2 Preparation of carrier liquid, reducing agent and standard solutions

3% v/v HCl carrier solution was prepared by adding of 30 ml of concentrated 37% v/v HCl (ROMIL-SpA) to 500 ml of H_2O in 1 L glass volumetric flask and then filling the bottle to the mark with H_2O. The carrier liquid was poured into the wide neck plastic bottle and renewed as it was used up. This solution was also used for the most of dilutions during sample preparation. 1.1% w/v stannous chloride reductant was made fresh daily by adding 5.5 g of $SnCl_2 \cdot 2H_2O$ (Merck KGaA, Darmstadt, Germany) to the mixture of 5 mL of H_2O and 15 mL of 37% v/v HCl, sitting during 10 min and then dissolving with water to the mark in 500 mL volumetric flask. Further, the reductant was poured into the wide neck plastic bottle.0.2% w/v sodium borohydride reductant in 0.05% w/v sodium hydroxide was made fresh daily as follows: first, 0.05% w/v NaOH (Merck KGaA) was made based on the proportion by dissolving 2 pellets of NaOH in the appropriate amount of water calculated based on weight of pellets and the target proportion of 0.5 g NaOH per 500 mL of H_2O. Then, 2 g of $NaBH_4$ (Merck KGaA) was dissolved in 500 mL of 0.05% w/v NaOH. Further, the reductant was poured into the wide neck plastic bottle.

5% w/v $KMnO_4$ stabilizing solution was prepared once in the beginning of the work by dissolving 0.5 g of $KMnO_4$ crystals (Sigma-Aldrich) in 10 mL of H_2O and stored in an opaque plastic bottle.

Inorganic mercury solutions:

100 mg/L stock inorganic mercury (InHg) solution was prepared once at the beginning of the work by adding 13.5 mg of $HgCl_2$ (Merck KGaA) to 100 mL of H_2O in 100 mL volumetric flask and stored in refrigerator closed by Parafilm to prevent change in concentration due to vaporization. 10 mg/L intermediate InHg solution was prepared by pipetting 1 mL of 100 mg/L stock InHg solution in 10 mL volumetric flask and filling to the mark with 3% v/v HCl. 0.2 mg/L intermediate InHg solution was prepared by pipetting 1 mL of 10 mg/L intermediate InHg solution in 10 mL volumetric flask and filling to the mark with 3% v/v HCl.1 μg/L, 2 μg/L, 5 μg/L, 10 μg/L, 15 μg/L and 20 μg/L working InHg standard solutions were prepared fresh daily, since the presence of $KMnO_4$ in them significantly reduce their shelf life. Respectively 0.1 mL, 0.2 mL, 0.5 mL, 1 mL, 1.5 mL and 2 mL of 0.2 mg/L intermediate InHg solution were pipetted into 20 mL volumetric flasks and filled to the mark with 3% v/v HCl. One drop of 5% w/v $KMnO_4$ was added to each standard solution to prevent premature unwanted reduction. Solutions were mixed thoroughly.

Organic mercury solutions:

Stock, intermediate and working organic mercury (MeHg) standard solutions were prepared from methylmercury chloride in the same concentrations as InHg standard solutions, using methanol as a solvent. Subsequently, two options were tested and compared:

1. use of pure methanol through all steps of preparation of MeHg standard solution,
2. dissolution of dry CH_3HgCl in pure methanol and use of 3% v/v HCl as a solvent in following dilutions.

Since the comparison of these two methods revealed no differences in the analysis performance, the MeHg standard solutions in 3% v/v HCl were used in further study.

Total mercury solutions:

2 μg/L, 4 μg/L, 10 μg/L, 20 μg/L, 30 μg/L, and 40 μg/L total mercury (TotHg) solutions were prepared by pipetting respectively 0.05 mL, 0.1 mL, 0.25 mL, 0.5 mL, 0.75 mL, and 1.0 mL of

each InHg and MeHg 0.2 mg/L stock solutions to 10 ml volumetric flasks. After that the flasks were filled to the mark with 3% v/v HCl, and one drop of 5% $KMnO_4$ solution was added to each flask.

The choice of the concentrations of TotHg solutions is justified by the need to compare the reducibility of mercury species separately and in combination with each other.

2.3.3 Sensitivity test and calibration

The sensitivity test was carried out according to the instructions given by the manufacturer to evaluate the ability of the instrument to detect and quantify low concentrations of mercury accurately. The test was performed using 5 μg/L standard InHg solution in 11 replicates. The mean absorbance was 0.0812 which corresponds to the parameters provided by manufacturer, namely exceeds the minimum required value 0.0800.

To determine the selectivity of the method, the following sequence of standard solutions was analyzed:

1. InHg solutions: 1 μg/L, 2 μg/L, 5 μg/L, 10 μg/L, 15 μg/L and 20 μg/L,
2. MeHg solutions with the same concentrations,
3. TotHg solutions: 2 μg/L, 4 μg/L, 10 μg/L, 20 μg/L, 30 μg/L, and 40 μg/L.

The preparation procedure for the standard solutions is shown on Figure 11.

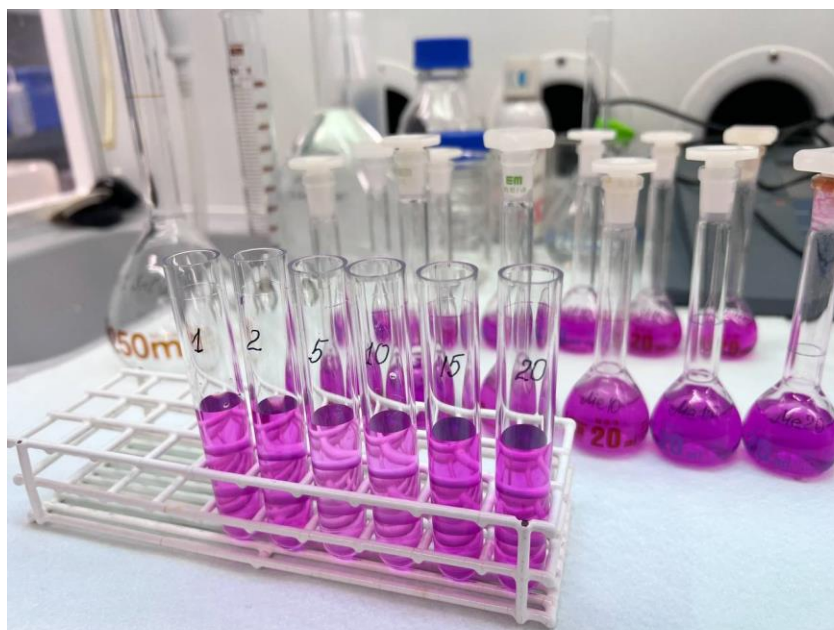


Figure 11. Preparation of InHg, MeHg and TotHg standard solutions to determine selectivity of reducing agents.

The results of the analysis with use $SnCl_2$ as a reducing agent are given in Table 6.

Table 6. Comparative analysis of InHg, MeHg and TotHg using FIMS and $SnCl_2$ reductant.

Conc, μg/L	InHg solutions		MeHg solutions		Total absorbance	Conc, μg/L	TotHg solutions		*Ratio, %
	Absorbance	RSD, %	Absorbance	RSD, %			Absorbance	RSD, %	
1	0.0163	0.271	0.0018	0.229	**0.0180**	2	**0.0191**	0.382	94.6
2	0.0337	0.201	0.0030	0.633	**0.0367**	4	**0.0370**	0.539	99.1
5	0.0848	0.045	0.0085	0.419	**0.0933**	10	**0.1033**	0.239	90.3
10	0.1693	0.256	0.0191	0.716	**0.1884**	20	**0.2012**	0.224	93.7
15	0.2492	0.281	0.0235	0.435	**0.2726**	30	**0.2834**	0.491	96.2
20	0.3241	0.241	0.0312	0.528	**0.3553**	40	**0.3776**	0.372	94.1

Low RSD values demonstrate excellent repeatability of the method. The comparative analysis shows the high selectivity of stannous chloride as a reductant, since the absorbance values of methymercury were an order of magnitude less than those of inorganic mercury. InHg and MeHg solutions were analyzed both separately and in the mixture. The difference in the reducibility of mercury species remained the same. The total absorbance was calculated as sum of the absorbances of separately analyzed InHg and MeHg solutions. The last column *Ratio demonstrates that in all cases the total absorbance was slightly less than the absorbance of TotHg solution.

Since the method can be considered as selective method for determination of inorganic mercury, LOD and LOQ for InHg were calculated as 0.07 μg/L and 0.21 μg/L respectively. The calibration curve and the calculation are given in Figure 1a and Equations 1a-3a in the Appendix.

According to the laboratory procedure protocols adopted by FIOH, the dilution of urine and blood samples during sample preparation for CV-AAS and ICP-MS analysis is done in proportion 1:19. Though the uniform concentration limit of mercury in human is not established, according to most sources, the average expected concentration of mercury in human blood is less than 10 μg/L, and concentration above 20 μg/L is to be considered dangerous. Considering the calculated LOQ 0.21 μg/L, it obviously should be possible to accurately determine the concentration of mercury in human urine and blood diluted 20 times using FIMS.

2.3.4 Analysis of Lypocheck Controls (urine matrix)

To examine possible interference with complex organic matrix, the analysis of Lypocheck Controls was conducted with both reducing agents: 1.1% w/v $SnCl_2$ and 0.2% w/v $NaBH_4$ in 0.05% w/v NaOH. According to the information provided by the manufacturer, the reference concentrations (C_1) of inorganic mercury in Lypocheck L1 and L2 are 45.6 μg/L and 118 μg/L respectively. To adjust concentration so that it falls within the limits of the calibration curve designed for the analysis of human urine (C_2), L1 and L2 were diluted with 3% v/v HCl in proportion given in Table 5. Further, all standard and Lypocheck solutions were diluted with 3% v/v HCl in proportion 1:19 as required by the Laboratory Procedure Manual and as it was done for ICP-MS analysis. The dilution was made in 15-mL plastic disposable vials by adding 500 μL of pre-diluted Lypocheck Control to 9.5 ml of 3% v/v HCl.

Based on measured concentrations, initial concentrations of L1 and L2 were calculated and compared to the tolerance range. The results are also given in Table 7.

Table 7. Dilution scheme and result of analysis of Lypocheck Controls

	C_1, μg/L	$V_{L1/L2}$, mL	$V_{3\%v/v\ HCl}$, mL	Expected C_2, μg/L	Measured C_2, μg/L		$C_{initial}$, μg/L		Tolerance range, μg/L
					$SnCl_2$	$NaBH_4$	$SnCl_2$	$NaBH_4$	
L1	45.6	2.5	7.5	11.4	4.899	9.076	**19.596**	**36.304**	31,92 – 59,28
L2	118	1.25	8.75	14.75	8.066	12.602	**64.528**	**100.816**	94,4 – 141,6

The values of L1 and L2 concentrations, obtained using sodium tetrahydroborate as reducing agent, were set within tolerance range. Stannous chloride demonstrates significantly lesser results, not reaching the lower limit of the tolerance range. Based on the above, it can be suggested that $NaBH_4$ is an effective reducing agent for the detection of mercury in organic matrices, providing the necessary sensitivity and reliability for biomonitoring purposes, whereas $SnCl_2$ is not suitable for the determination of mercury in urine using a FIMS system. The inability of $SnCl_2$ to achieve adequate sensitivity in this context is likely due to its lower efficiency in reducing mercury in complex organic matrices. Urine matrix contains various organic and inorganic components that can interfere with the reduction process, resulting in incomplete conversion of mercury species to detectable form.

Interestingly, previous study has shown that stannous chloride exhibits high selectivity for inorganic mercury in weak acidic solutions. This selectivity makes $SnCl_2$ a potentially valuable reducing agent for the analysis of water, which is in demand in a variety of contexts, including environmental monitoring, industrial wastewater testing, and drinking water safety. In case of successful validation of the method, the high selectivity of $SnCl_2$ for inorganic mercury may be useful for these purposes, providing accurate and reliable measurements of mercury contamination in water samples. To validate the use of 1.1% w/v $SnCl_2$ solution for water analysis, internal control water samples with known concentrations of inorganic mercury should be prepared. By performing testing and calibration, the accuracy and precision of the method for aqueous matrices can be ensured. However, the focus of this study was specifically on developing a method suitable for biomonitoring in the occupational health field, where organic matrices predominate. As water analysis is not required in this laboratory, method validation for aqueous solutions was not included in this research project.

Another significant challenge associated with using the FIMS system is the requirement for separate reaction coils for each reducing agent, as specified in the instrument's manual. This necessitates changing the working manifold each time a different reductant is used, which involves substantial manual work. In a high-throughput service analysis setting, this additional labor is impractical and inefficient, leading to increased time and operational costs. The need for frequent manual adjustments undermines the feasibility of achieving selectivity by using two different reductants in massive service analysis, where rapid and consistent results are paramount.

Given these limitations, sodium borohydride was chosen as the preferred reducing agent for further studying of mercury determination in urine and blood. $NaBH_4$ demonstrated superior sensitivity and reliability in detecting mercury in urine, making it more suitable for biomonitoring applications. Its ability to produce accurate results in the presence of complex organic matrices aligned with our objective of adapting the FIMS method for occupational health monitoring.

2.3.5 Adjusting the concentration of NaBH4, NaOH and HCl

In an effort to improve the selective detection of mercury species using FIMS, sodium borohydride ($NaBH_4$) in aqueous sodium hydroxide (NaOH) was selected as the reducing agent. The need for selective analysis of organic and inorganic mercury is driven by their distinct toxicological profiles. Organic mercury, particularly methylmercury, is more toxic and poses greater health risks than inorganic mercury. Despite this, inorganic mercury remains a concern due to its potential to undergo methylation, converting into the more hazardous organic form. Effective monitoring of both mercury species is necessary for comprehensive health and environmental assessments.

Our objective was to develop a method that allows for the selective detection of MeHg and InHg in a single analytical setting with minimal manual intervention. Ideally, this could be achieved by simply changing one reagent bottle, making the process efficient and user-friendly. The selected reducing agent presumably offers unique advantages in this regard due to its two-component nature, comprising both sodium borohydride and sodium hydroxide. This duality enhances our ability to optimize solution composition to achieve selective detection. Examination of different proportions of $NaBH_4$ and NaOH aimed to determine two optimal configurations: one designed to detect specific type of mercury (presumably inorganic), and the other to determine total mercury. By adjusting the concentrations of both components, the selective reduction capabilities of the reducing agent were exploited, thereby distinguishing mercury species in the sample matrix. At the same time, the change in concentration of NaOH had to be carried out considering its stabilizing role in the solution. In the absence of NaOH, $NaBH_4$ quickly decomposes in water, releasing hydrogen gas and losing its reducing power. If the proportion of NaOH is insufficient, $NaBH_4$ will decompose prematurely, compromising the reduction process. Thus, the stabilizing effect of NaOH is necessary, but it also places restrictions on the possible ratios of $NaBH_4$ and NaOH. The challenge was to find optimal concentrations of these components that will not only maintain the stability of $NaBH_4$, but also allow selective detection of different types of mercury. Therefore, our experimental approach involved systematic change of these concentrations to determine the optimal conditions under which $NaBH_4$ remains stable and effective for selective mercury reduction.

In addition, different concentrations of carrier liquid in the FIMS system were also examined to evaluate its effect on mercury reducibility and overall analytical results.

Preparation procedure:

The initial concentrations were chosen based on the minimum concentrations used for mercury determination described in the literature: 10^{-3} w/v for NaOH and 10^{-4} w/v for $NaBH_4$. Since NaBH4 decomposes rapidly in water, it cannot be prepared in aqueous solutions without a stabilizer. On the other hand, it was impossible to weight such a small amount of dry $NaBH_4$ suitable for direct preparation of $10^{-4}/10^{-3}$ solution. To address this issue, a typical stock solution of 0.2% w/v $NaBH_4$ in 0.05% w/v NaOH was prepared. Then the stock solution was diluted with water to decrease the concentration of NaOH to the target level of 0.001%. This involved careful volumetric dilutions to maintain accuracy.

Further adjustments were made by diluting the resulting solution with 0.001% NaOH solution, which allowed for precise variation of $NaBH_4$ concentration while maintaining the necessary stabilizing environment provided by NaOH.

The step-by-step process for preparing the reductant solution was as follows:

0.05% NaOH: 2 pellets of NaOH were dissolved in the appropriate amount of water calculated based on weight of pellets and the target proportion of 0.5 g NaOH per 1 L of H_2O. Preparation was done in 1 L flask using graduated 500 mL beaker.

0.001% NaOH: 1 L volumetric flask was half-filled with water. 20 mL of 0.05% NaOH was added to the flask, then the flask was filled with water to the mark.

Stock solution 0.2% w/v $NaBH_4$ in 0.05% w/v NaOH: 2 g of $NaBH_4$ was dissolved in small amount of 0.05% w/v NaOH in 100 mL beaker and then immediately (to avoid decomposition of $NaBH_4$) poured into 500 mL volumetric flask, which then was filled with 0.05% NaOH w/v to the mark.

Intermediate solution 0.004% w/v $NaBH_4$ in 0.001% w/v NaOH: 500 mL volumetric flask was half-filled with water. 10 mL of stock solution (0.2% w/v $NaBH_4$ in 0.05% w/v NaOH) was pipetted into the flask, then it was filled with water to the mark.

Further dilutions with 0.001% w/v NaOH were made in proportions given in Table 1a in the Appendix. The choice of proportion range is justified by the results of first few runs. At the beginning, the dilution was carried out by a larger step. When it was discovered that the signal

of InHg was obtained at the concentration of $NaBH_4$ approximately 0.002%, more precise adjusting was done in 0.0001% increments. The target volume of 100 mL of each solution is justified by the volume of plastic bottle shipped with the instrument, and liquid intake design. The dilution was performed in a 100 mL volumetric flask using 50 mL graduated beaker and 5 mL pipette for more accurate measuring.

Standard InHg and MeHg solutions with a concentration of 10 µg/L, and standard TotHg solution 20 µg/L were prepared as described in Section 3.2. These concentrations were chosen as these represent the acceptable limit of Hg concentration in human blood, making it the most relevant and adequate value for biomonitoring purposes.

To ensure the reliability and reproducibility of the results, the entire analysis was repeated ten times. This repetition helps in assessing the consistency of the method and in identifying any potential variability in the measurements. The results of analysis are given in the Table 8. The latest measurements were done without testing of the smallest concentrations, since the target concentration has already been approximately determined. In all cases the analysis was stopped when the ratio of InHg and MeHg reached 2:1.

Table 8. Comparative analysis of InHg, MeHg and TotHg standard solutions using 0.0001% - 0.0024% w/v $NaBH_4$ in 0.001% w/v NaOH as a reductant

	Absorbance (253,7 nm, Hg)										
# of exp.	1	2	3	4	5	6	7	8	9	10	RSD, %
0.0001% NaBH4											
In10	-0,0001	0,0001	0,0001								
Me10	-0,0003	-0,0002	0								
In10+Me10	0,0001	0,0001	0,0001								
0.001% NaBH4											
In10	0,0169	-0,0010	0,0023								
Me10	0,0023	-0,0012	0,0022								
In10+Me10	0,0133	-0,0001	0,0023								
0.0013% NaBH4											
In10	0,0624	0,0159	0,0069								
Me10	0,0065	0,0019	0,0021								
In10+Me10	0,0251	0,0146	0,0078								
0.0014% NaBH4											
In10	0,1307	0,0155	0,0101								
Me10	0,0161	0,0185	0,0026								
In10+Me10	0,0690	0,0437	0,0292								

	Absorbance (253,7 nm, Hg)										
# of exp.	1	2	3	4	5	6	7	8	9	10	RSD, %
0.0015% NaBH4											
In10	0,1351	0,0553	0,0246								
Me10	0,0210	0,0169	0,0055								
In10+Me10	0,0849	0,0838	0,0274								
0.0016% NaBH4											
In10	0,1679	0,0689	0,0733	0,0602	0,0171	0,0133	0,0287	0,0372	0,0492	0,0369	44,45
Me10	0,0355	0,0184	0,0174	0,0703	0,0201	0,0028	0,0070	0,0113	0,0226	0,0100	62,06
In10+Me10	0,1302	0,0919	0,0948	0,1107	0,1240	0,0185	0,0325	0,0400	0,0513	0,0626	67,77
0.0017% NaBH4											
In10	0,1730	0,1205	0,1236	0,1021	0,0512	0,0482	0,0446	0,0532	0,0594	0,0543	9,89
Me10	0,0493	0,0261	0,0294	0,0782	0,0537	0,0104	0,0114	0,0151	0,0169	0,0184	77,78
In10+Me10	0,1906	0,1267	0,1505	0,1914	0,1169	0,0546	0,0412	0,0456	0,0606	0,0692	85,80
0.0018% NaBH4											
In10	0,1774	0,1597	0,1592	0,1242	0,0980	0,0931	0,0514	0,0738	0,0794	0,0704	21,62
Me10	0,0628	0,0442	0,0388	0,0836	0,0680	0,0361	0,0355	0,0249	0,0382	0,0287	39,61
In10+Me10	0,2182	0,2023	0,1960	0,2175	0,2349	0,1525	0,0978	0,0882	0,0982	0,0922	45,39
0.0019% NaBH4											
In10		0,1767	0,1674	0,1525	0,1306	0,1466	0,1108	0,1146	0,1012	0,1121	13,76
Me10		0,0534	0,0436	0,0896	0,0721	0,0155	0,0387	0,0321	0,0347	0,0325	49,69
In10+Me10		0,2244	0,2117	0,2308	0,2514	0,2312	0,1838	0,1216	0,1218	0,1463	31,71
0.0020% NaBH4											
In10		0,1815	0,1706	0,1645	0,1562	0,1639	0,1428	0,1708	0,1729	0,1694	7,03
Me10		0,0608	0,0502	0,0951	0,0841	0,0431	0,0462	0,0566	0,0601	0,0593	24,87
In10+Me10		0,2349	0,2190	0,2371	0,2971	0,2535	0,1988	0,2228	0,2318	0,2499	13,77
0.0021% NaBH4											
In10		0,1826	0,1771	0,1648	0,1649	0,1793	0,1697	0,1720	0,1804	0,1788	3,61
Me10		0,0675	0,0578	0,0997	0,1195	0,0981	0,0791	0,1117	0,0984	0,1056	13,62
In10+Me10		0,2408	0,2334	0,2436	0,2899	0,2801	0,2822	0,2601	0,2725	0,2833	16,10
0.0022% NaBH4											
In10		0,1879	0,1776	0,1666							
Me10		0,0934	0,0631	0,1037							
In10+Me10		0,2914	0,2412	0,2482							
0.0023% NaBH4											
In10			0,1802	0,1692							
Me10			0,1121	0,1096							
In10+Me10			0,2971	0,2549							

In the first four experiments the dilution was performed by adding the appropriate amount of the intermediate solution 0.004% w/v $NaBH_4$ in 0.001% w/v NaOH into 100 mL volumetric flask and then filling in to the mark with 0.001% w/v NaOH. In the last six experiments the addition was done by separately measured amounts of intermediate solution and the solvent. This method can be considered as more accurate, which presumably explains the decrease of the concentration value at which mercury was reduced, and thus increase of the method sensitivity. The RSD in all experiment was relatively high, with the smallest value regarding to InHg results. Thus, the repeatability of the analysis can be called insufficient, which is possibly attributed to the extremely low concentrations of the solutions and increment, resulting in the difficulty of maintaining consistency during the preparation. The reduction of inorganic specie apparently occurs more completely and accurately at these concentrations. The results at lower concentrations are in general less stable than at higher ones. Interestingly, the

absorbance of TotHg sample is more or less equal to the sum of InHg and MeHg absorbances at concentrations above 0.0016% but can be significantly less at smaller concentrations (in some cases even less than absorbance of separate InHg).

The goal of the experiment was to establish the concentration value at which InHg would be completely reduced, and the signal of MeHg would be as small as possible. Considering the last six experiments as a representative sample, the average absorbance values and ratio of MeHg:InHg absorbance values at each concentration of reductant are given in the Table 9.

Table 9. Average absorbance values and InHg:MeHg ratio using $NaBH_4$ in 0.001% NaOH as reductant

C_{NaBH4}, %	0.0016	0.0017	0.0018	0.0019	0.0020	0.0021
	Absorbance (253.7 nm, Hg)					
InHg	0.0304	0.0518	0.0777	0.1193	0.1627	0.1742
MeHg	0.0123	0.0210	0.0386	0.0376	0.0582	0.1021
TotHg	0.0548	0.0830	0.1273	0.1760	0.2423	0.2947
MeHg:InHg, %	40.46	40.54	49.68	31.52	35.77	58.61

The maximum selectivity was observed at 0.0019% and 0.002% $NaBH_4$ in 0.001% NaOH. When the concentration increases above these values, the level of reducibility of MeHg enhances; subsequently, the degrees of reduction of both species equalize and then increase synchronously. However, even at the optimal concentrations, sodium borohydride demonstrates lesser selectivity than stannous chloride.

Further, the impact of the concentration of carrier solution on the selectivity was examined. In 5% HCl, MeHg reduced easier, and signals of InHg and MeHg became almost equal at 0.0016% concentration of reductant. In 7% HCl, this happened even earlier, at 0.0014%. At concentrations of HCl less than 3%, results were quite unstable. Thus, adjusting the concentration of carrier liquid cannot be used to increasing the selectivity.

To examine the impact of NaOH concentration on selectivity, the solutions with concentration 0.005%, 0.01%, 0.05%, 0.1% and 0.5% were prepared. The concentration of $NaBH_4$ should

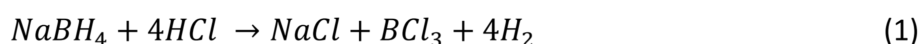
have been consistent 0.002% through the experiment. The preparation process is shown in Figure 3a and described in Appendix. The complexity and multi-step nature of preparation procedure is justified by the necessity of maintaining balance between both components and impossibility of weighting such small amounts of dry sodium hydroborate suitable for direct dilution. However, the analysis revealed no significant difference in the selectivity of the reducing agent at different concentrations of NaOH, while the absorbance of TotHg decreased at concentrations of 0.01% and below.

Overall, experimentation with the concentrations of all components did not allow us to bring the level of selectivity of sodium borohydride to this of stannous chloride. However, the method still demonstrates high efficiency in determination of total mercury. Since the method was considered suitable for the TotHg analysis, LOD and LOQ were calculated as 0.54 μg/L and 1.64 μg/L respectively. The calibration curve and the calculation are given in Figure 2a and Equations 4a-5a in the Appendix.

2.4. Testing of sample preparation for blood

The final objective of this research project was to examine the possibility to adapt the FIMS instrument for the analysis of mercury in blood samples, since previous attempts to do it encountered significant challenges, namely the foaming of blood samples within the reactor. This foaming, probably caused by contact with 3% v/v hydrochloric acid, sodium borohydride and sodium hydroxide, led to leakage and hindered the ability to do the analysis.

Blood contains a high concentration of proteins, such as albumin and globulins, which can act as natural surfactants. One of the possible explanations of foaming is the decrease of the surface tension of the liquid due to hydrogen gas generation during chemical reaction between $NaBH_4$ and HCl (Equation 1). Reaction can be intensified in the presence of catalysts or impurities, generating significant amounts of gas that form bubbles and contribute to foaming.



$$NaBH_4 + 4HCl \rightarrow NaCl + BCl_3 + 4H_2 \quad (1)$$

Lipids with amphiphilic properties and lipid-protein complexes can accumulate at the air-liquid interface. Forming a monolayer at the surface of bubbles, lipids create a more stable interface that can prevent the bubbles from bursting.

NaOH in the reaction mixture can also promote foaming, further altering the properties of blood proteins and other components. An alkaline environment can cause denaturation and unfolding of proteins, increasing their surface activity and tendency to foam.

The FIMS setup described in the literature as successfully used for blood analysis, has additional features included in construction such as inlets for online microwave digestion, online addition of stabilizing 5% $KMnO_4$ solution, and extended reactor design. The apparatus available in the laboratory does not have such options, thus the scope of the research project was limited to testing the sample preparation methods. Moreover, the time-consuming methods such as offline microwave digestion were not considered due to the requirements of high-throughput service analysis. All methods tested have been reported in the literature as being successfully used for CV-AAS and FIMS analysis. All analyzes used blood collected from one person and treated with anticoagulating agent (heparin) according to the laboratory manual. Then the blood was aliquoted into 15 mL plastic tubes and stored in refrigerator at 4^{0} C. Prior to preparation, aliquots were kept for one hour at room temperature.

Looking ahead, it should be acknowledged that our subsequent experiments also could not effectively solve the problem of foaming. The detailed descriptions of these failed attempts are given below.

L-cysteine/octanol/NaCl/NaOH

1% NaCl w/v and 45% w/v NaOH solutions were prepared from dry NaCl and NaOH pellets respectively by dissolving them in appropriate amounts of water. 100 g of L-cysteine hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 100 ml of water. 5 mL of 1% w/v NaCl, 2 mL of L-cysteine solution and 250 μL of 1-octanol were pipetted to 10 mL of 45% w/v NaOH. After that, 2 mL of blood was pipetted to the solution. The probe was vortexed for 1 minute.

0.12% v/v Triton X-100

0.12% v/v Triton X-100 solution was prepared by pipetting 0.6 mL of concentrated Triton X-100 (Sigma-Aldrich) to 500 mL of water, mixing thoroughly, and then diluting with water to the mark in 1 L volumetric flask. Blood was diluted with this solution by a factor of 11 by

pipetting 500 μL of blood to 5 mL of Triton X-100 solution. The probe was vortexed for 1 minute.

Butanol/EDTA/NH_3/Triton X-100

2% w/v EDTA solution was prepared by dissolving 20 g EDTA (Titriplex III solution, Merck KGaA, Netherlands) in 1 L of water. 25 mL of pure butanol, 25 mL of 2% EDTA, 11 mL of 25% ammonia solution, and 0.5 mL of concentrated Triton X-100 solution (all Sigma-Aldrich) were added to water in 1 L volumetric flask and then filled with water to the mark. 250 μL of blood was pipetted to 4.75 mL of the solvent, the probe was vortexed for 1 minute.

10% w/v tetramethylammonium hydroxide

10% w/v TMAH was prepared by diluting 4 mL 25% w/v TMAH (Sigma-Aldrich) with 6 mL of water. 500 μL of 10% w/v TMAH and 500 μL of blood was pipetted to the bottom of disposable glass tube and incubated for an extended period. Different periods of incubation time were tested (30 min, 1 hour, 1.5 hours). After that, 4 mL of 3% v/v HCl was added to the tube. The probe was vortexed for 1 minute.

Antifoaming agent and 5% w/v $KMnO_4$

All of the above methods were also tested with alternate or joint addition of 1 mL of an Antifoam B emulsion (Sigma-Aldrich) and 1 drop of 5% w/v $KMnO_4$. The antifoaming agent is an emulsion containing 30% w/w silicon.

In all cases, it was not possible to collect data for analysis due to leaks through the filter. With a significant decrease in the concentration of the reducing agent, it was possible to carry out analysis; nonetheless, the absorbance values obtained were inadequate.

3 Conclusion

The literature review of this master's thesis explored the various forms and cycles of mercury in nature and in the human body. An overview of the diverse pathways, through which people are occupationally exposed to mercury, shows the importance and relevance of this field of study. Different exposure routes can lead to varying health effects. Understanding these pathways helps to develop specific protective strategies and ensure the safety of workers.

Additionally, the contemporary analytical methods for mercury determination in human blood, urine, and hair, focusing on different approaches of sample preparation and the technological advancements that enhance detection sensitivity and selectivity were discussed. This knowledge also supports the development of more accurate biomonitoring techniques, leading to better detection and management of mercury-related health issues.

In the experimental part of the master's thesis, the effectiveness of two reducing solutions, stannous chloride and sodium borohydride, in a flow injection mercury system (FIMS) for the analysis of mercury in various matrices was examined. The results highlight the strengths and weaknesses of each reductant and pose significant challenges, especially in the context of complex organic matrices and blood sample analysis.

Stannous chloride demonstrated good selectivity and a low limit of detection (LOD) in water solutions, making it effective for inorganic mercury detection in simpler matrices. However, it proved inadequate for analyzing complex organic matrices, as evidenced by its poor performance in Lypocheck controls. The presence of organic substances likely interferes with the reduction process, compromising the reliability of the results. Sodium borohydride exhibited superior sensitivity in urine matrix, showing promise for total mercury analysis in such an environment. However, we were unable to identify a concentration at which $NaBH_4$ could work selectively for specific mercury species. The inability to achieve selective analysis with $NaBH_4$ implies that achieving both total and species-specific mercury determination would require alternating between reducing agents. This necessity is undesirable due to the need to change the reactor manifold and tubing as per the manufacturer's guidelines, complicating the workflow and increasing the potential for errors.

A major challenge encountered was the foaming of blood samples in the reaction coil, leading to leaks and interruptions in the analysis. Offline sample preparation methods failed to resolve this problem, indicating that blood analysis in the current FIMS setup is impractical without significant modifications.

Three potential solutions can be proposed to address the issues identified:
Extending the reactor length. By extending the length of the reactor, the blood will still foam but may not reach the filter, thus potentially not affecting the quality of the analysis. This extension could be 3D printed as a cylinder with threads on both ends. The impact of foaming on the analysis would need to be tested once leaks are prevented.
Combining FIMS with the old CV-AAS instrument. Integrating the FIMS lamp with the existing CV-AAS instrument could enhance sensitivity and accuracy. Unfortunately, this solution does not reduce the manual labor required by the old CV-AAS method but serves as a backup option in case the old instrument fails.
Purchasing a new manifold for FIMS. Acquiring a new manifold with an extended reactor and inlets for online microwave digestion and potassium permanganate ($KMnO4$) digestion could address the foaming issue more comprehensively. This option should be more economical than replacing the entire FIMS instrument and could improve both the efficiency and reliability of the mercury analysis.

In all cases, further study of a new setup with both reductants and selection of the optimal method of blood sample preparation is required.

4 List of abbreviations

Abbreviation	Definition
AAS	Atomic absorption spectroscopy
AFS	Atomic fluorescence spectroscopy
APDC	Ammonium pyrrolidinethiocarbamate
ASGM	Artisanal and small-scale gold mining
CFLs	Compact fluorescence lamps
CLP	Classification, labeling, and packaging
CV-AAS	Cold-vapor atomic absorption spectroscopy
DMA-80	Direct mercury analyzer
DNA	Deoxyribonucleic acid
DRC	Dynamic reaction cell
ECHA	European Chemical Agency
EDTA	Ethylenediaminetetraacetic acid
EI	Electron ionization
EU	European Union
FIMS	Flow injection mercury system
FIOH	Finnish Institute of Occupational Health
GF-AAS	Graphite-furnace atomic absorption spectroscopy
GC	Gas chromatography
HPLC	High performance liquid chromatography
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-QQQ-MS	Inductively coupled triple quadrupole plasma mass spectrometry
InHg	Inorganic mercury
LOAEL	Lowest adverse effect exposure limit
LOD	Limit of detection
LOQ	Limit of quantification
MeHg	Methylmercury
MTBE	Methyl tert-butyl ether
OELs	Occupational exposure limits

PPE	Personal protective equipment
PVG-AFS	Photochemical vapor generation atomic fluorescence spectroscopy
REACH	Registration, evaluation, authorization, and restrictions of chemicals
RNA	Ribonucleic acid
TDA-AAS	Thermal decomposition amalgamation atomic absorption spectroscopy
TGM	Total gaseous mercury
TMAH	Tetramethylammonium hydroxide
TotHg	Total mercury
TSID	Thermal separation with isotope dilution

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6 Appendix

The calibration curve for the determination of inorganic mercury using 1.1% $SnCl_2$ as reducing agent is shown in Figure 1a.

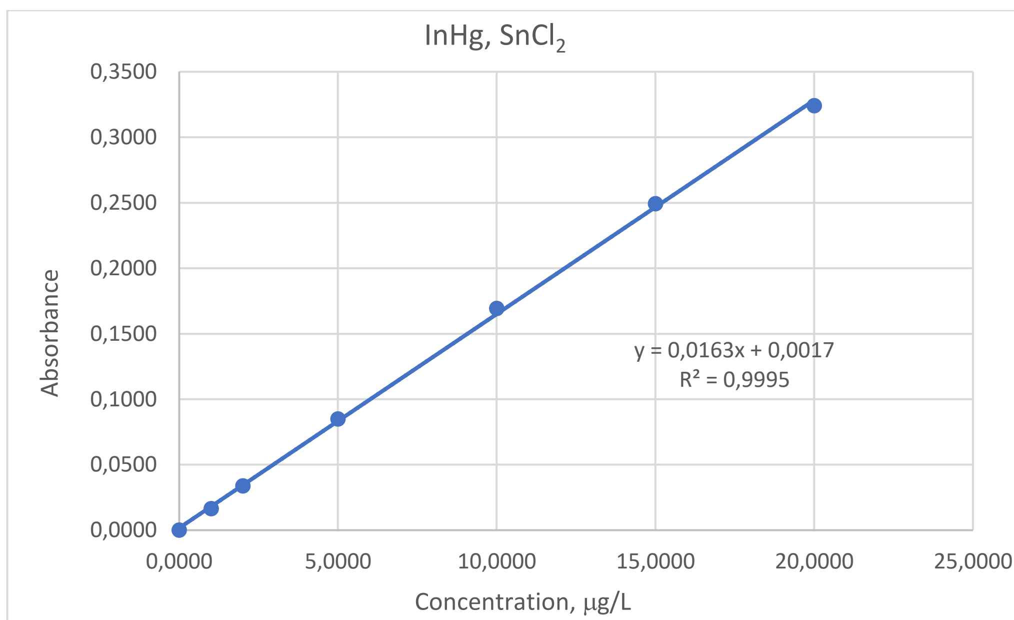

Figure 1a. Calibration curve for the analysis of InHg standard solutions with $SnCl_2$ reductant.

Standard deviation of intercept was calculated using the Equation 1a:

$$SD_y = \frac{SE_y}{\sqrt{n}} = \frac{0{,}0008244\ \mu g/L}{\sqrt{6}} = 0.0003\ \mu g/L \quad (1a)$$

where n refers to the number of measurements, SE_y refers to standard error of intercept calculated using the Regression analysis function in Microsoft Excel as 0.0008244 µg/L.

Limit of detection was calculated using the Equation 2a:

$$LOD = 3.3\frac{SD_y}{a} = 3.3\frac{0.0003\ \mu g/L}{0.0163} = 0.07\ \mu g/L \quad (2a)$$

Limit of quantification was calculated using the Equation 3a:

$$LOQ = 10\frac{SD_y}{a} = 10\frac{0.0003\ \mu g/L}{0.0163} = 0.21\ \mu g/L \quad (3a)$$

Table 1a. The dilution proportions of working reductant solutions $NaBH_4$ in NaOH

Target concentration of $NaBH_4$, %	**0.004% $NaBH_4$ in 0.001% NaOH, ml**	**0.001% NaOH, ml**
0.0001	2.5	97.5
0.001	25	75
0.0013	32.5	67.5
0.0014	35	65
0.0015	37.5	62.5
0.0016	40	60
0.0017	42.5	57.5
0.0018	45	55
0.0019	47.5	52.5
0.0020	50	50
0.0021	52.5	47.5
0.0022	55	45
0.0023	57.5	42.5
0.0024	60	40

The calibration curve for the determination of total mercury using 0.2% $NaBH_4$ in 0.05% NaOH as reducing agent is shown in Figure 2a.

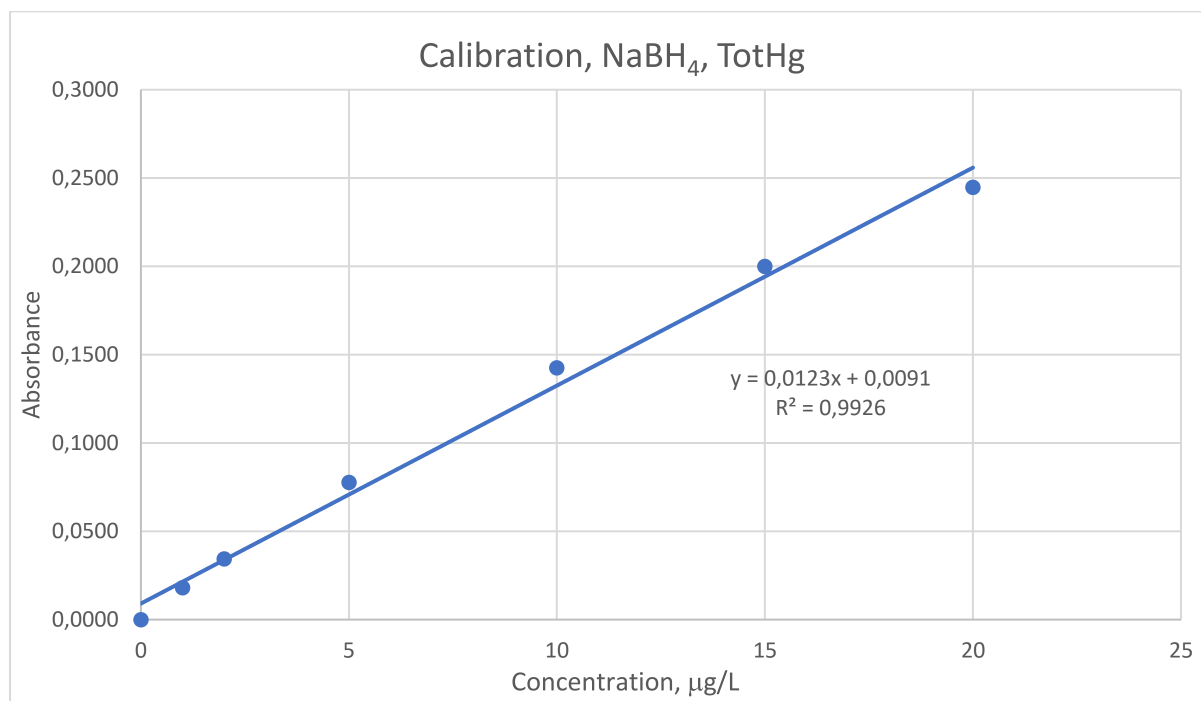

Figure 2a. Calibration curve for the analysis of TotHg standard solutions with $NaBH_4$ in NaOH as reductant.

Standard deviation of intercept was calculated using the Equation 4a:

$$SD_y = \frac{SE_y}{\sqrt{n}} = \frac{0{,}004957\ \mu g/L}{\sqrt{6}} = 0.002\ \mu g/L \qquad (4a)$$

where n refers to the number of measurements, SE_y refers to standard error of intercept calculated using the Regression analysis function in Microsoft Excel as 0,004957 μg/L.

Limit of detection was calculated using the Equation 5a:

$$LOD = 3.3\frac{SD_y}{a} = 3.3\frac{0.00202\ \mu g/L}{0.0123} = 0.54\ \mu g/L \qquad (5a)$$

Limit of quantification was calculated using the Equation 6a:

$$LOQ = 10\frac{SD_y}{a} = 10\frac{0.00202\ \mu g/L}{0.0123} = 1.64\ \mu g/L \qquad (6a)$$

The preparation process for adjusting the concentration of NaOH in reducing solution (Figure 3a).

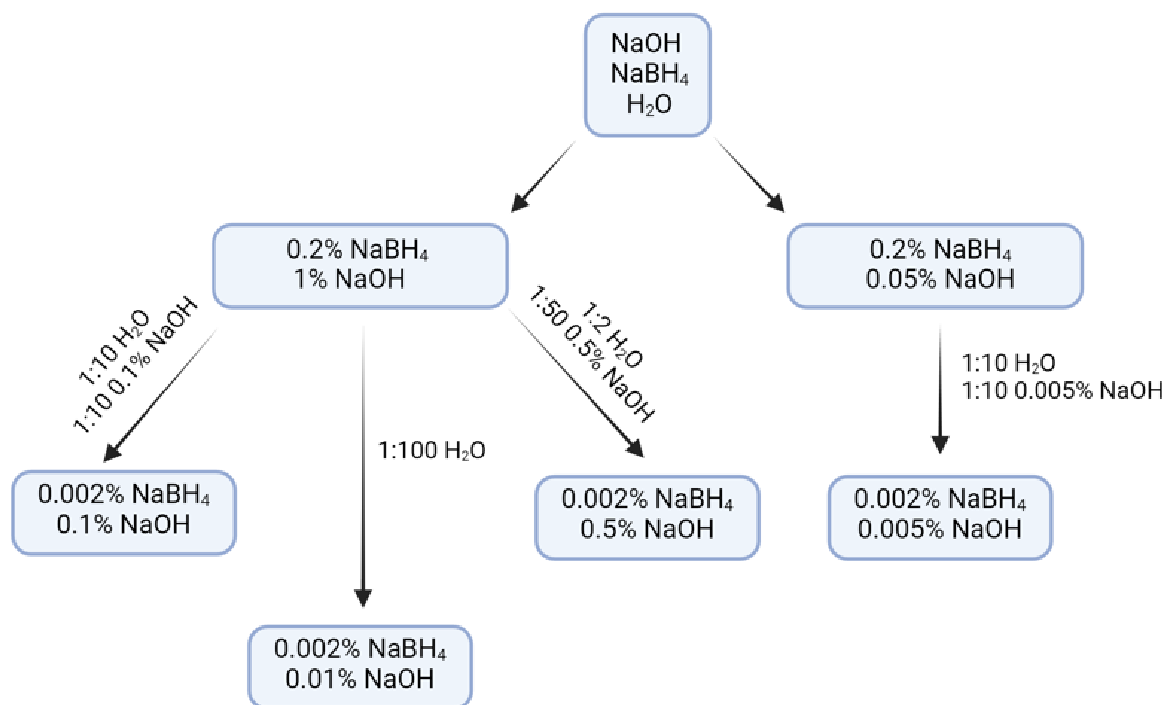

Figure 3a. Preparation scheme for $NaBH_4$ in NaOH solutions.

1% w/v NaOH: 1 pellet of NaOH is weighed and dissolved in an appropriate amount of water based on the proportion 1g of NaOH per 100 ml of water.

0.1% w/v NaOH: 10 mL of 1% w/v NaOH was pipetted into half-filled with water 100 mL volumetric flask and then filled with water to the mark.

0.01% w/v NaOH: 20 mL of 0.05% w/v NaOH was diluted with 80 mL of water.

0.005% w/v NaOH: 10 mL of 0.05% w/v NaOH was diluted with 80 mL of water.

0.001% w/v NaOH: 20 mL of 0.05% w/v NaOH was diluted with water in a 1 L volumetric flask.

0.2% w/v $NaBH_4$ in 1% w/v NaOH: 0.1g of $NaBH_4$ was dissolved in 50 mL 1% w/v NaOH.

0.002% w/v $NaBH_4$ in 0.1% w/v NaOH: 1 mL of 0.2% w/v $NaBH_4$ in 1% w/v NaOH was pipetted into 10 mL of water and then diluted with 0.1% w/v NaOH to the mark in 100 mL volumetric flask.

0.002% w/v $NaBH_4$ in 0.5% w/v NaOH: 1 mL of 0.2% w/v $NaBH_4$ in 1% w/v NaOH was diluted with 0.5% w/v NaOH to the mark in 100 mL volumetric flask.

0.05% w/v NaOH and intermediate solution 0.2% w/v $NaBH_4$ in 0.05% w/v NaOH: as described in Section 2.2.

0.04% w/v $NaBH_4$ in 0.01% w/v NaOH: 10 mL of 0.2% w/v $NaBH_4$ in 0.05% w/v NaOH was diluted with 40 mL of water.

0.002% w/v $NaBH_4$ in 0.01% w/v NaOH: 5 mL of 0.04% w/v $NaBH_4$ in 0.01% w/v NaOH is diluted with water in 100 mL volumetric flask.

0.02% w/v $NaBH_4$ in 0.005% w/v NaOH: 5 mL of 0.2% w/v $NaBH_4$ in 0.05% w/v NaOH is diluted with water in 50 mL volumetric flask.

0.002% w/v $NaBH_4$ in 0.005% w/v NaOH: 10 mL of 0.02% w/v $NaBH_4$ in 0.005% w/v NaOH is diluted with 0.005% w/v NaOH in 100 mL volumetric flask.

This thesis utilized AI language model ChatGPT 3.5 for language correction and editing purposes.

The illustrations are drawn with BioRender.