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Development of Nickel Ion Sensor for Urine Analysis for the Rapid Assessment of Human Exposure

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ABSTRACT

Nickel is a widely used metal which is toxic to humans. In particular, workers in the nickel industry are exposed to high levels of nickel. Most dangerous are airborne nickel compounds, which are increasing significantly the prevalence of disease of the respiratory tract in occupationally exposed people. To minimise long-term health effects, a proper and frequent nickel exposure assessment is crucial. Urine has shown to be an accurate matrix to analyse for nickel exposure, with 10 µg L^{-1} nickel exceed normal levels found in urine and a detection range of interest of 5 to 150 µg L^{-1} for exposure assessment. An in depth literature review was conducted to locate all available rapid, cheap and portable nickel detection techniques, with a focus on spectroscopic, bioanalytical and electrochemical methods. It was found that the best technique to detect nickel is adsorptive stripping voltammetry (AdSV), with dimethylglyoxime (DMG) as chelating agent. Environmentally friendly screen-printed sensors were used, based either on carbon, bismuth or gold working electrodes. Factors influencing the stripping performance at the electrodes, including ammonia buffer, bismuth and DMG concentration, deposition time and interference mitigation, were examined. The best performance was achieved using either bare carbon macro-electrodes (BCE) covered with Nafion or bare carbon microband-electrodes (BCME). After a preconcentration time of 90 seconds the limit of detection was determined at 3.0 µg L^{-1} and 0.9 µg L^{-1} for the BCE and BCME, respectively. A good linearity ($R^2 > 0.99$) up to 200 µg L^{-1} nickel was obtained at both electrodes. The applicability of the BCE covered with Nafion and BCME in a direct measurement of nickel in a spiked urine sample was tested. However, no meaningful results were achieved due to organic compounds in urine, which complex with nickel ions. Hence, for a feasible determination it is suggested to use a pretreat method to extract the nickel ions, such as acidification or magnetic nanoparticles.

Keywords: Adsorptive Stripping Voltammetry, Dimethylglyoxime, Microband-Electrode, Nafion, Screen-printed Electrodes.

ABSTRACT (GERMAN)

Nickel ist ein weit verbreitetes Metall, welches bei Menschen Gesundheitsschäden verursachen kann. Besonders betroffen sind Arbeiter in der Nickel Industrie, die teils hohen Nickelkonzentrationen in der Atemluft ausgesetzt sind. Diese berufliche Exposition erhöht vorwiegend die Prävalenz von Krankheiten des respiratorischen Trakts. Um die gesundheitlichen Folgen zu minimieren ist es notwendig in regelmäßigen Abständen die Nickel Exposition zu bestimmen. Da die Nickelkonzentration im Urin eine hohe Korrelation zu jener in Luft besitzt, ist sie besonders dafür geeignet. Die normale Konzentration im Urin liegt bei gesunden Erwachsenen unter 10 µg/L und zur Bestimmung der Exposition am Arbeitsplatz wurde ein Messbereich von 5 bis 150 µg/L gewählt. Als Basis für die weitere Arbeit wurde eine Literaturrecherche über schnelle, billige und portable Nickelsensoren, im Bereich spektroskopischer, bioanalytischer und elektrochemischer Methoden, durchgeführt. Als besonders geeignet stellte sich das Verfahren der „Adsorptive Stripping Voltammetrie (AdSV)“ mit Diacetyldioxim (DMG) als Chelatbildner heraus. Umweltfreundliche Siebdruck-Elektroden, welche entweder aus Kohlenstoff, Bismut oder Gold bestanden, wurden zur Messung benutzt. Faktoren welche die Messung beeinflussen, wie Ammonium Puffer, Bismut und DMG Konzentration, Ablagerungszeit und Methoden zur Interferenz Verringerung, wurden untersucht. Die besten Ergebnisse wurden mit den Kohlenstoff Elektroden (BCE) beschichtet mit Nafion und den Kohlenstoff Microband-Elektroden (BCME) erreicht. Nach 90-sekündiger Anreicherungszeit wurde eine Detektionsgrenze von 3.0 µg/L an der BCE und 0.9 µg/L an der BCME bestimmt. Des Weiteren wurde eine hohe Linearität ($R^2 > 0.99$) an beiden Elektrodentypen und bei Nickelkonzentrationen bis zu 200 µg/L beobachtet. Die Eignung beider Elektrodentypen wurde in einer direkten Messung einer mit Nickel versetzten Urinprobe untersucht. Allerdings wurden keine aussagefähigen Ergebnisse erzielt. Dies lässt sich auf organische Verbindungen im Urin, welche mit Nickel komplexieren, zurückführen. Es wird daher vorgeschlagen die Nickel Ionen zu extrahieren, etwa durch Säuerung der Probe oder mit Hilfe von magnetischen Nanopartikeln.

Schlagwörter: Adsorptive Stripping Voltammetrie, Diacetyldioxim, Microband-Elektrode, Nafion, Siebdruck-Elektroden.

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LIST OF ABBREVIATIONS AND SYMBOLS

AES	Atomic Emission Spectroscopy
AdSV	Adsorptive Stripping Voltammetry
ATSDR	Agency for Toxic Substances and Disease Registry
b.w.	Body Weight
BCE	Bare Carbon Electrode
BCME	Bare Carbon Microband-Electrode
BFE	Bismuth Film Electrode
CE	Counter or Auxiliary Electrode
CI	Confidential Interval
CV	Cyclic Voltammetry
DMG	Dimethylglyoxime
DNA	Deoxyribonucleic Acid
DPV	Differential Pulse Adsorptive Stripping Voltammetry
EA	Environmental Agency
EAAS	Electrothermal Atomic Absorption Spectrometry
EU	European Union
GFE	Gold Film Electrode
GFME	Gold Film Microband-Electrode
HPLC	High Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
ICP	Inductively Coupled Plasma
ID	Isotope Dilution
Ig	Immunoglobulin
IR	Infra Red
IUPAC	International Union of Pure and Applied Chemistry
L	Ligand
LD_{50}	Lethal Dose 50% (Dose were 50% of the specimen die)
LOD	Limit Of Detection
M	Molar concentration in Mole per Litre
MS	Mass Spectrometry

n. a.	Not Applicable
n. s.	Not Specified
N_2	Nitrogen gas
Ni	Nickel
Ni(II)	Ni^{2+}
NIOSH	National Institute for Occupational Safety and Health
NTP	National Toxicology Programme
RE	Reference Electrode
Redox	Reduction-Oxidation
SCE	Saturated Calomel Electrode
SD	Standard Deviation
SIR	Standardised Incidence Rate
SSCE	Silver-Silver Chloride Electrode
SWV	Square-Wave Adsorptive Stripping Voltammetry
WE	Working Electrode
WHO	World Health Organisation

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CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 OUTLINE

Nickel is a widely used metal in industrial applications and is also present in various foods and beverages (ATSDR, 2005). It is toxic and known to cause diseases of the respiratory tract and the central nervous system (Gupta *et al.*, 1997). Furthermore, its classification as “carcinogenic to humans” is in preparation for nickel compounds by the International Agency for Research on Cancer (IARC, 2010). In particular, exposure in the nickel processing industry may be very high. Hence, occupational legal limits of exposure are becoming lower to protect workers (Nickel Institute, 2007a).

The focus of this research is to develop a rapid, cheap, portable and environmentally friendly sensor for the determination of nickel exposure for mine workers. Urine as the method of sampling has been chosen because it is a convenient specimen for relating to nickel exposure from inhalation and ingestion (Sunderman *et al.*, 1986a). Moreover, it is possible to collect samples in a non-invasive and easy way. A literature review was carried out to gain background information and available detection methods currently used or developed for nickel. The results are summarised in chapter 1.

At the beginning a short discussion on background information is presented, including chemical and physical properties as well as occurrence, applications, and fate and behaviour in the environment. The toxicological profile of nickel is then assessed with an evaluation of the human exposure. The focus is on occupational exposure in the nickel industry and occupational limits. All nickel compounds present in air and their influence on the nickel concentration in urine is determined. In the following sections the results of a literature review on all available rapid detection techniques and their properties with a focus on field based tools are discussed, starting with spectrometric methods, and followed by bioanalytical and electroanalytical methods. Furthermore, a short section is included dealing with the most common and referenced lab-based methods, as well as the availability of already purchasable sensors for the determination of nickel in urine. In the final section the aims and objectives of this study are presented.

1.2 NICKEL – BACKGROUND

Nickel is a silver white metal which was first discovered in kupfernickel *(niccolite)* by Axel Fredrik Cronstedt in 1751 (Lide, 2008). It is an element and in place 28 of the periodic table. A picture of various nickel products can be seen in Figure 1.1.

Figure 1.1: Picture of various products made out of solid nickel (Nickel Institute, 2007b).

The most important properties can be seen in Table 1.1. For the discussion of nickel and its compounds it is important to know that nickel occurs in the oxidation states 0, I, II and III. However, the most common form is Ni^{2+} and states I and III are not stable in aqueous solutions and can only exist under special conditions (Bradl *et al.*, 2005). Cobalt (atomic number 27) and copper (atomic number 29) are the two neighbours of nickel in the periodic table. These two elements have quite similar properties compared to nickel, primarily cobalt with an atomic weight of 58.9332 (Lide, 2008). Hence, many nickel detection techniques are particularly influenced by the presence of cobalt and copper. Some further properties of nickel are that it is one of three elements to be ferromagnetic at room temperature, its malleable, and is a fair conductor of electricity and heat (Lide, 2008).

Table 1.1: Properties of nickel.

Symbol	Ni
Atomic Number	28
Atomic Weight	58.6934 g mol^{-1}*
Phase	Solid
Group	Metallic solids
Electron Configuration	2-8-16-2
Oxidation States	0, +1, +2, +3**
Melting Point	1455 °C*
Boiling Point	2913 °C*
Density	8.902 g cm^{-3} (25 °C)*

* Lide (2008) ** Bradl *et al*. *(*2005)

Since nickel can form various compounds these are, when appropriate, summarised in groups with similar properties to simplify the discussion in the different section. These groups are metallic nickel (Ni), soluble nickel compounds (such as nickel chloride ($NiCl_2$), nickel nitrate ($Ni(NO_3)_2$), and nickel sulfate ($NiSO_4$)) and less- or insoluble nickel compounds (such as nickel subsulfide (Ni_3S_2) and nickel oxide (NiO)).

A special case is nickel carbonyl ($Ni(CO)_4$), a colourless liquid-metal compound, which is highly volatilized and readily dissociates in air (Shi, 1994). It is considered to be one of the most hazardous chemicals in the nickel industry. However, it is industrially produced by and for special procedures. Hence, it plays no role in the majority of industrial processes and will not be discussed in much detail in this thesis.

1.2.1 Occurrence and Applications

In order of abundance, nickel ranks 24th in Earth's crust and 5th over all on Earth (Duke, 1980). The average concentration is 0.0086% and 2%, respectively. The typical average concentration in ore deposits is between 1% and 4% (Duke, 1980) and the most important deposits can be found (in order of mining output) in Russia, Indonesia, Canada, Australia, and New Caledonia, all above 100,000 tons in 2009 (Kuck, 2010). A map showing all major nickel deposits worldwide can be seen in Figure 1.2. In Europe nickel mines are found in Albania, Finland, Greece, Macedonia, Norway, Russia and Spain (Kuck, 2009).

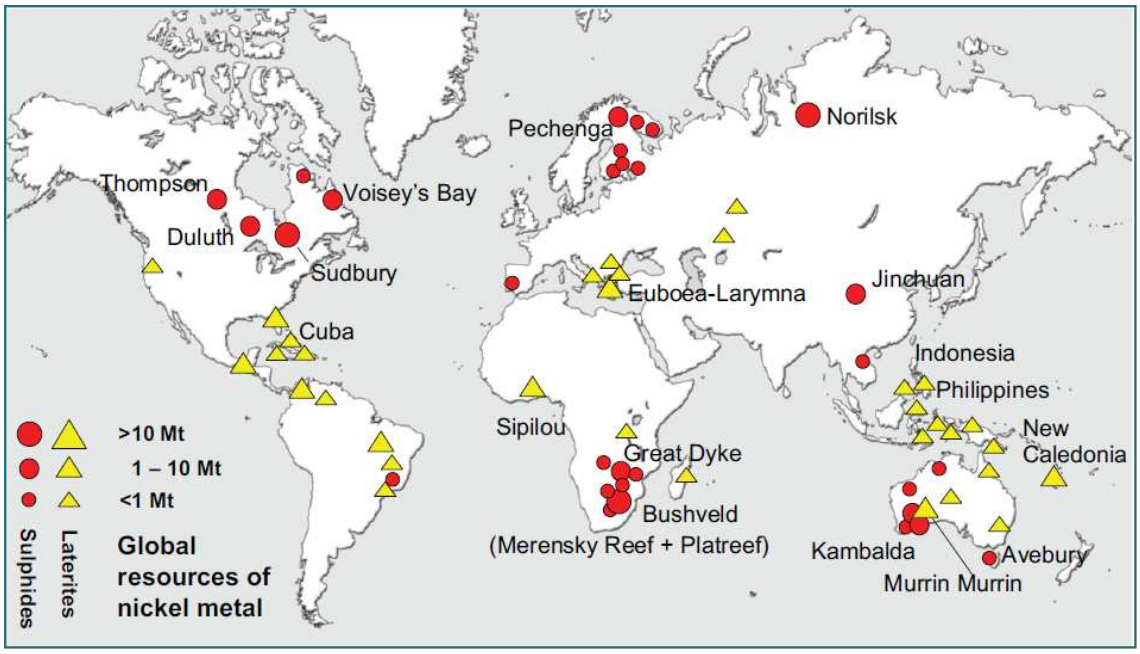


Figure 1.2: World distribution of significant sulfide and laterite nickel deposits (BGS, 2008).

In general there are two different types of nickel ore mines (Warner, 1984). On the one hand there are underground mines where magmatic sulfide ore is raised. The igneous rocks contain high concentrations of iron and magnesia. Various metals, but mainly iron, nickel, copper and cobalt, are collected by the sulphur and form different compounds. The nickel concentrations are in the range from 1% to 3%. On the other hand there are surface mines where oxide ore or garnierites are raised. As before, the igneous rocks contain high concentrations of iron and magnesia. However, no agent is collecting the metals. A process called chemical weathering (occurs under tropical climate conditions) forms compounds containing nickel, iron and cobalt. Since this process is known as laterization, the rocks are often referred to as laterite ore. These rocks only contain 0.2% to 0.3% nickel.

Recovering nickel from ore usually includes crushing, roasting, smelting and a refining step (BGS, 2008). As discussed in the paragraph before on nickel ore composition, the majority of metals found in these industries next to nickel are iron, magnesia, copper and cobalt. In particular, cobalt, due to its similar physical and chemical properties when compared to nickel, can occur in high concentrations. Before the refining step the metal mixture consists of approximately 70% nickel and the rest is mainly cobalt (BGS, 2008).

The reported world consumption of nickel was 1.32 million tons in 2007, after an all time high of 1.40 million tons in 2006 (Kuck, 2009). The field of application is, first of all, in the production of stainless steel and other corrosion-resistant alloys, but nickel is also used in coins, ceramics, magnets, batteries and to give glass a green colour (Lide, 2008). Nickel can be recovered from scrap; however, reports from the United States of America indicate that, due to the recession which started in 2008, less scrap is now collected and the low prices for nickel are making its recycling less attractive (Kuck, 2009).

1.2.2 Fate and Behaviour in the Environment

The nickel cycle starts with the release of nickel particulates, which are adsorbed to particular matters, into the air from sediments and rocks. Particles are distributed by wind and are removed either through washout by rain and rainout or dry deposition or gravitational settling (Schroeder *et al.*, 1987). Afterwards the nickel is deposited in the world's oceans with an amount of 8 to 11 gigagrams (wet deposition), 14 to 17 gigagrams (dry deposition) and 1,411 gigagrams (riverine input) per year (Duce, 1991). The nickel then sinks to the sea bed and is deposited again in the earth's crust.

Since most of the analytical methods provide data about nickel content rather than its specific compounds or species, little is known about its chemical form or chemical and physical transformation (ATSDR, 2005). Nickel from anthropogenic sources is thought to be mainly present as oxide in air, whereas windblown dust may contain mineral species (Schroeder *et al.*, 1987). With OH^-, SO_4^{2-} and HCO_3^-, nickel forms strong complexes in natural water; however, most of the existing complexes are hydrated Ni^{2+} (Rai and Zachara, 1984). The compounds and species found in soil depend largely on the pH value of the soil (Sadiq and Enfield, 1984). In acidic soils the major compounds are Ni^{2+}, $NiHPO_4$ and $NiSO_4$, and in alkaline soils these are Ni^{2+} and $Ni(OH)^+$.

1.3 TOXICOLOGICAL PROFILE

In this report the focus is on human studies. However, studies on animals will be considered when data from human studies are missing or insufficient. Furthermore, within all exposure routes the emphasis will be on nickel inhalation, because this is the major route workers are exposed to at their workplace.

1.3.1 Toxicokinetics

Absorption: Nickel in air is absorbed in the upper and lower respiratory tract. Approximately 20% to 30% of inhaled nickel is absorbed into the bloodstream, the rest either stays in the respiratory tract, is swallowed or expectorated (ATSDR, 2005). The absorption pattern depends on the respiratory area, and the chemical form, size, shape and electrical charge of the nickel compound (ATSDR, 2005). Particle size decreases from the nasopharyngeal area (5 to 30 µm) towards the pulmonary alveoli (<1 µm). Soluble nickel compounds are easily absorbed as nickel ion (Ni^{2+}), whereas insoluble compounds may be phagocytized or are stored in the respiratory tract (Torjussen and Andersen, 1979; Das *et al.*, 2008). According to a study by Patriarca *et al.* (1997) conducted on four human volunteers, 28.7% to 40.1% of ingested stable nickel isotopes were absorbed into the digestive tract. This was based on data from faecal excretion. Four days after administration, up to 49.6% of the absorbed nickel was still not excreted in the urine. Nickel may also be absorbed through skin contact. According to a review by the ATSDR (2005), absorption ability depends strongly on the particular nickel compound. Whereas 55-57% of nickel sulfate penetrates human skin within 24 hours, only 0.23% to 3.5% of nickel chloride penetrates after 155 hours.

Distribution: Rezuke *et al.* (1987) carried out an autopsy study to examine the distribution of nickel in non occupational exposed individuals (six men, four women). The highest concentration in the contaminated organs can be seen in Table 1.2. A study on 15 deceased occupational exposed workers revealed that the average amount of nickel in a lung is 50±150 mg kg^{-1} dry-weight (Svenes and Andersen, 1998) and in general 100 to 150 times higher then in a control group (five men, five women). High nickel levels can also be found in the nasal mucosa of workers exposed to insoluble nickel compounds (Torjussen and Andersen, 1979). Compared to non exposed individuals, higher concentrations of nickel can be found in occupational exposed workers (Torjussen and Andersen, 1979; Angerer and Lehnert, 1990). However, levels are higher in workers exposed to soluble nickel compounds than in workers exposed to insoluble nickel compounds (Torjussen and Andersen 1979). Ingested nickel leads, after 1.5 and 2.5 hours, to a peak in serum (Patriarca *et al.*, 1997).

Table 1.2: Nickel concentration in different human organs (Data taken from Rezuke et al., 1987).

Organ	Mean Ni concentration (µg kg^{-1} dry-weight)
Lungs	173±94
Thyroid	141±83
Adrenals	132±84
Kidneys	62±43
Heart	54±40
Liver	50±31
Whole brain	44±16
Spleen	37±31
Pancreas	34±25

Metabolism: Nickel binds mainly to albumin, but also to L-Histidine, and α2-macroglobulin in human serum to travel around within the body. The low molecular nickel-L-Histidine complex is able to cross cell membranes (Sarkar, 1984). On the other hand nickel forms strong non-exchangeable complexes with specific α2-macroglobulins (Sunderman, 1986a).

Excretion: Absorbed nickel in the respiratory tract is excreted in urine (Torjussen and Andersen, 1979; Hassler *et al.*, 1983; Angerer and Lehnert, 1990). Excretion in faeces can occur through mucociliary clearance via the gastrointestinal tract (Hassler *et al.*, 1983). Higher nickel levels in urine are found in workers exposed to soluble nickel compounds compared to those exposed to insoluble ones (Bernaki *et al.*, 1978; Torjussen and Andersen 1979). Most ingested nickel is not absorbed and excreted in faeces; the nickel which is absorbed is mainly excreted in urine (Patriarca *et al.*, 1997). However, their study showed that four days after ingestion between 18.0% and 49.6% of the absorbed nickel was still not excreted in urine. A minor amount of the absorbed nickel is also excreted through sweat, bile, saliva, hair and mother's milk (Sunderman, 1993). For more detailed informations about excretion see also section 1.6 (Nickel concentration in urine).

1.3.2 Health Effects after Inhalation Exposure

Death: 13 days after a 38 year old man was exposed to 383 mg m^{-3} metallic nickel in air for 90 minutes he died due to an Adult Respiratory Distress Syndrome (Rendall *et al.*, 1994). Of the particles 64.6% had a diameter below 1.4 µm and it was estimated, therefore, that he had inhaled one gramme of nickel. The lethal dose in air after 12 days (during a 16 day period), and 6 hours and 10 minutes exposure per day is 10 mg m^{-3} and 10 mg m^{-3} nickel subsulfite, and 30 mg m^{-3} and 7 mg m^{-3} nickel sulfate for rats and mice, respectively (NTP, 1996a and 1996b). No deaths were observed in the nickel oxide groups, in levels up to 30 mg m^{-3} (NTP, 1996c).

Systemic Toxicity: A 38 old man who had been accidentally exposed to 383 mg m^{-3} metallic nickel in air for 90 minutes showed a well marked tubular necrosis of the kidneys, alveolar wall damage and oedema in the alveolar spaces (Rendall *et al.*, 1994). However, this is just an isolated case. Many other studies have investigated the effects of nickel in air on all kinds of organs and systems. A review by ATSDR (2005) came to the following conclusion: There is no increased mortality among nickel exposed people regarding non-malignant respiratory diseases. However, there is a higher incidence rate of chronic bronchitis and moderate pulmonary fibrosis. Furthermore, human levels of β_2-microglobolin in urine were influenced due to the excretion of nickel from serum by the kidneys. Among nickel exposed workers there is no increased risk of suffering from a cardiovascular disease. Animal studies showed inflammation of the lungs and atrophy of the nasal olfactory epithelium, but no musculoskeletal, hepatic and endocrine effects (NTP, 1996a, 1996b and 1996c). The results from haematological, metabolic and body weight animal studies were influenced by inflammation of the lungs. All animal studies included rats and mice, lasted for 12 days (during a 16 day period), exposure took place via inhalation 6 hours and 10 minutes per day, and nickel subsulfite, nickel sulfate and nickel oxide were used.

Immunological Toxicity: 38 production workers in the nickel industry showed increased levels of some immunoglobulins (IgG, IgA, IgM) and some proteins (α2-macroglobulin, α1-antitrypsin, ceruloplasmin, lysozyme), as well as decreased levels of the

immunoglobulin IgE in serum (Bencko *et al.*, 1986). Levels of exposure and the types of nickel compounds were not evaluated.

Neurological Toxicity: Maximum exposure level (up to 60 mg m^{-3}) for 12 days (during a 16 day period) and 6 hours and 10 minutes per day, to nickel subsulfite, nickel sulfate and nickel oxide in air did not show any changes in the brains of rats and mice, but there was atrophy of the olfactory epithelium (NTP, 1996a, 1996b and 1996c).

Reproductive and Developmental Toxicity: Spontaneous and threatening abortions were reported twice as often from 758 women (232 pregnancies) working in a hydrometallurgy refining plant compared to a control group (Chashschin *et al.*, 1994). Moreover, malformations among offspring from female workers were reported to be three times higher as compared to a control group. The nickel level in air was between 0.13 mg m^{-3} and 0.2 mg m^{-3}, and mostly present in the form of nickel sulfate. However, their study struggles with some confounders, the most important ones being heavy lifting and exposure to heat. A study by Vaktskjold *et al.* (2008) did not find a statistical significant risk for female nickel refinery workers in Norway for an increased risk of spontaneous abortions. However, a weak excess risk is not excluded. Exposure for two years to nickel subsulfite (0.73 and 0.88 mg m^{-3}), nickel sulfate (0.11 and 0.22 mg m^{-3}) or nickel oxide (2 and 3.9 mg m^{-3}) by rats and mice, respectively, did not show any changes in the reproductive organs (NTP, 1996a, 1996b and 1996c).

Carcinogenicity: Several epidemiological studies have already been conducted to investigate the cancer rate among people occupationally exposed to nickel. Two studies which evaluated all kinds of cancer from nickel exposed workers can be seen in Table 1.3. The cohorts were composed of 1155 nickel smelter and refinery workers (Antilla *et al.*, 1998) and 4764 nickel refinery workers (Andersen *et al.*, 1996). All workers were mainly exposed to soluble nickel compounds and had worked for at least three months and one year in the nickel industry, respectively. In both studies a statistically increased incidence rate was found for lung, nasal and nasal cavity cancers. These outcomes are supported by other studies which had their focus mainly on cancer of the respiratory tract (Roberts *et al.*, 1989; Sandström *et al.*, 1989; Muir *et al.*, 1994; Grimsrud *et al.*,

2002). A statistical small increased lung cancer incidence was also observed for workers exposed to insoluble nickel compounds (Antilla *et al.*, 1998). However, the latency is longer compared to workers exposed to soluble nickel compounds and exceeds 20 years.

Table 1.3: Observed and expected numbers and SIRs of selected cancer types. (Data taken from Andersen et al., 1996 and Antilla et al., 1998).

	Antilla *et al.* (1998)				Andersen *et al.* (1996)			
Type of cancer	**Obs**	**Exp**	**SIR**	**95% CI**	**Obs**	**Exp**	**SIR**	**95% CI**
All sites	71	69.5	1.02	0.80±1.28	749	531.2	1.4	1.3-1.5
Stomach	9	5.7	1.57	0.72±2.97	45	48.2	0.9	0.7-1.3
Colon	2	3.2	0.63	0.08±2.27	76°	69.2	1.1	0.9-1.4
Rectum	2	2.6	0.76	0.09±2.74	76°	69.2	1.1	0.9-1.4
Nose, sinuses	**2**	**0.2**	**8.79**	**1.06±31.7***	**32**	**1.8**	**18.0**	**12.3-25.4**
Larynx	1	1.3	0.79	0.02±4.40	11	7.0	1.6	0.8-2.8
Lung	**21**	**15.1**	**1.39**	**0.86±2.13**	**203**	**68.3**	**3.0**	**2.6-3.4**
Pleura	0	0.3	0.00	0.00±13.0	3	1.9	1.6	0.3-4.6
Prostate	6	7.1	0.85	0.31±1.84	129	91.1	1.4	1.2-1.7
Testis	0	0.9	0.00	0.00±4.17	7	7.8	0.9	0.4-1.8
Kidney	3	3.0	1.00	0.21±2.90	19	19.6	1.0	0.6-1.5
Bladder	2	3.5	0.57	0.07±2.04	33	36.2	0.9	0.6-1.3
Skin melanoma	3	2.5	1.22	0.25±5.36	21	17.3	1.2	0.8-1.9
Nervous system	3	2.9	1.02	0.21±2.98	12#	15.7	0.8	0.4-1.3
Haematopoietic tissue		n. s.			34	43.0	0.8	0.6-1.1
Other specified sites		n. s.			95	82.6	1.2	0.9-1.4
Unspecified sites		n. s.			29	21.5	1.4	0.9-1.9

Obs	Observed
Exp	Expected
SIR	Standardised Incidence Rate
CI	Confidential Interval
°	Colon and rectum
*	P < 0.05
#	Brain only

Amongst other evidence, these studies are the reason why the International Agency for Research on Cancer will shortly classify nickel compounds “as carcinogenic to humans” (IARC, 2010). In 1990, metallic nickel and nickel alloys had already been classified as “possibly carcinogenic to humans”.

1.3.3 Health Effects after Oral Exposure

Death: Nickel subsulfate LD_{50} among male and female rats is 325 and 275 mg kg^{-1} b.w., respectively. Whereas for nickel oxide these values are >5000 and >5000 mg kg^{-1} b.w., respectively (Mastromatteo, 1986).

Systemic and Immunological Toxicity: 32 workers in an electroplating plant developed symptoms including diarrhoea, vomiting, nausea, abdominal discomfort, cough and shortness of breath after they drank water contaminated with 1.63 g L^{-1} nickel (Sunderman *et al.*, 1988). The amount of nickel (sulfate and chloride) ingested was estimated to be in the range of 0.5 g to 2.5 g. Elevated levels of blood reticulocytes, serum bilirubin, and urine albumin were observed in seven, three and two workers, respectively. Symptoms disappeared within a few hours and the workers returned to their workplace after eight days. Furthermore, orally ingested nickel sulfate can cause a nickel contact allergy. Two studies (Hindsén *et al.*, 2001; Jensen *et al.*, 2003) revealed that the number of people which had flare-up reactions correlates with the dose of nickel sulphate ingested. In both studies the experiment was carried out one month after a patch test and only nickel sensitive individuals were chosen for the experiment.

Neurological Toxicity: From 32 workers who ingested by accident 0.5 g to 2.5 g of sulfate and chloride nickel in water, seven reported giddiness, six weariness and five headache (Sunderman *et al.*, 1988). Seven hours after ingestion of 50 µg kg^{-1} b.w. nickel sulfate, a 55 year old volunteer developed a left homonymous hemianopsia (Sunderman *et al.*, 1989a). The visual impairment lasted for two hours; however, this effect was not seen in any of the other volunteers, who ingested 12 or 18 µg kg^{-1} b.w. nickel sulfate in water.

Reproductive and Developmental Toxicity: As reviewed by ATSDR (2005), the results regarding animal tests on reproductive toxicity are inconsistent. The studies regarding animal developmental toxicity indicate an increased loss and decreased survival rate of mice (90.6 mg kg^{-1} b.w. per day on gestational days 8–12) and rats (30 mg kg^{-1} b.w. per day for 4 weeks prior to mating until lactation) offspring. Neurodevelopmental toxicity, change in body weights and gross necropsy of abnormalities was observed in offspring from rats (30 mg kg^{-1} b.w. per day for two generations). In all studies, nickel chloride was used.

Carcinogenicity: Female and male mice were exposed to 5 mg L^{-1} nickel acetate in drinking water for a whole lifetime. Compared to the control groups there was no

elevated risk of developing any kind of cancer (Schroeder *et al.*, 1964; Schroeder and Mitchener, 1975).

1.3.4 Health Effects after Dermal Exposure

Death: No reliable human or animal studies were located regarding dermal exposure.

Systemic and Immunological Toxicity: Nickel contact allergy is the main problem concerning dermal exposure to nickel. A study conducted by Uter *et al.* (2003) revealed that 15.5% of 74,940 patients tested suffer from contact dermatitis. The study was carried out in contact dermatitis units in Germany and Austria with a patch test using nickel sulfate, 5% in petrolatum. Another study by Menné (1994) shows that sweat can also release nickel chloride from nickel alloys (including jewellery) and is more allergenic than nickel sulfate. Moreover, Menné found that the number of people with nickel sensitisation increases after prolonged dermal contact with nickel.

Neurological Toxicity: No reliable human or animal studies were located regarding dermal exposure.

Reproductive and Developmental Toxicity: No reliable human or animal studies were located regarding dermal exposure.

Carcinogenicity: No reliable human or animal studies were located regarding dermal exposure.

1.4 HUMAN EXPOSURE

For the general population, the major source of nickel exposure is from foods and beverages (ATSDR, 2005). The highest concentrations can be found in chocolate, nuts, oatmeal and soybeans. The overall oral daily intake is around 130 µg nickel, compared to 0.06 µg through breathing the nickel which occurs naturally in air (EA, 2009). Nickel uptake also takes place every time a cigarette is smoked. Each cigarette contains 1 to 3 µg, of which 10 to 20% is released into the smoke that is inhaled (ATSDR, 2005).

1.4.1 Occupational Exposure

The highest concentration of nickel is found in industries which are working with nickel. The level and kind of exposure depends largely on the work areas in these industries. The concentration ranges in different industries are summarised in Table 1.4.

Table 1.4: Airborne nickel concentration in different industries (ATSDR, 2005).

Industry	Concentration range (µg m^{-3})
Laterite mining and smelting	4-420
Stainless steel workers	<1-189
Foundry operators	LOD-900
Electroplating	<2-<16
Nickel-cadmium battery manufacture	20-1,910
Nickel catalyst production from nickel sulfate	1-1,240
Production of nickel salts from nickel or nickel oxide	9-590
Production of wrought nickel and alloy from metal powder	1-60,000

LOD – Limit of Detection

Unfortunately, only one study on the level in a nickel mine was located, from Riddle, Oregon. Furthermore, the mine was decommissioned in 2001 (Kuck, 2002) and the data are from the year 1984. However, it can be seen in Table 1.4 that the concentration in air of other work areas may be, partly, much higher than in a nickel mine. This is due to the fact that nickel in ore only ranges from 1% to 3% and 0.2% to 0.3% in sulfide ores and oxide ores, respectively (Warner, 1984). Hence, the nickel concentration in air may also be relatively low. In general, workers assigned to operations involving grinding, welding and handling powder are exposed to the highest concentration of airborne levels of nickel. In Table 1.5 the distribution of four different nickel forms is illustrated. During crushing and grinding, which can be expected to take place in a mine, most nickel occurs as sulfidic nickel. In the smelting and roasting process, the majority of nickel is present in form of oxidic nickel, whereas in all other processes it is soluble nickel.

Table 1.5: Nickel levels in air and distributions of different forms of nickel as a proportion (by weight) of total nickel in selected departments and time periods at a nickel refinery in Norway (Grimsrud et al., 2002).

Department and period	Total nickel in Air (mg m^{-3})	Proportion of total nickel			
		Soluble nickel	Sulfidic nickel	Metallic nickel	Oxidic nickel
Crushing and grinding					
1990-1994	0.7-1.4	0.12	0.72	0.11	0.04
Smelter					
1910-1929	4.0	0.10	0.05	0.01	0.84
1930-1950	4.0	0.10	0.05	0.08	0.77
1951-1977	2.6-4.4	0.10	0.04	0.18	0.68
Calcining, smelting					
1951-1977	1.5-3.4	0.10	0.05	0.01	0.84
1978-1994	0.5	0.12	0.13	0.01	0.74
Roasting					
1910-1977	1.9-5.3	0.10	0.15	0.03	0.72
1978-1994	0.4	0.15	0.05	0.00	0.80
Copper leaching					
1910-1994	0.1-1.5	0.49	0.01	0.01	0.49
Copper electrolysis					
1910-1994	0.03-0.2	0.80	0.04	0.04	0.13
Copper cementation					
1927-1977	0.6-1.2	0.45	0.05	0.45	0.05
Electrolytic purification					
1927-1977	0.2-0.5	0.80	0.03	0.15	0.02
1978-1994	0.03-0.2	0.98	0.01	0.00	0.01
Nickel electrolysis					
1910-1977	0.1-0.2	0.87	0.05	0.01	0.08
1978-1994	0.03-0.1	0.83	0.04	0.02	0.11

1.5 LEGISLATION CONCERNING NICKEL EXPOSURE

For nickel exposure from air, water and food it is more common to establish guidelines rather than legal limits. In the United Kingdom, the dietary intake should not exceed 4.3 µg kg^{1} b.w. per day and concentration in air should be as low as 0.02 mg m^{-3} (EA, 2009). The tolerable daily intake proposed by the World Health Organisation is 12 µg kg^{-1} b.w. per day (WHO, 2006) and the lifetime risk of developing cancer from nickel in air is 1 in 100,000 at a concentration of 25 ng m^{-3} (WHO, 2000). Furthermore, there is the European Directive 94/27/EC to limit nickel in jewellery and other materials which may be in contact with skin (EU, 1994).

For occupational exposure, legal limits have been set in many countries (Nickel Institute, 2008). A selection of different limits can be seen in Table 1.6, with Finland and South Africa having active nickel mines. As emanates from the data, there are separate limits for different nickel compounds and the limits within the European Union are not uniform.

Table 1.6: Occupational exposure limits established by some major jurisdiction (Adapted from Nickel Institute, 2008).

Country/Body	Status of Standard	Values of Standards[1] (mg Ni m^{-3})				Reference
		Metallic Nickel	Insoluble Nickel Species	Soluble Nickel Species	Nickel Carbonyl	
Austria	Current	0.05[2] [2.0 [3]][2]	0.05[2] [2.0 [3]][2]	0.05[2] [2.0 [3]][2]	0.35 [1.4 [3]]	GKV 2007, 2010
Finland	Current	1.0	0.1	0.1	0.007 [0.021 [3]]	Nickel Institute, 2008
South Africa	Current	0.5	0.5 [0.1 for subsulfide]	0.1	[0.24 [3]]	Nickel Institute, 2008
United Kingdom	Current	0.5	0.5	0.1	0.24[3]	HPA, 2009
United States	Current[4]	1.0[5]	1.0	1.0	0.007	Nickel Institute, 2008

1: 8-hour time-weighted average unless otherwise noted. All values refer to 'total' nickel unless otherwise noted.

2: This threshold limit value applies to nickel metal and alloys, nickel sulfide, sulfidic ores, oxidic nickel, and nickel carbonate in inhalable dust, as well as any nickel compound in the form of inhalable droplets.

3: STEL=15-minutes, short-term standard.

4: In 1989, OSHA reduced the PEL for soluble nickel compounds to 0.1 mg Ni m^{-3}. However, in July 1992, the U. S. Eleventh Circuit Court of Appeals set aside and remanded the entire Air Contaminants Standard on the ground that OSHA's generic approach dealing with over 400 chemicals, including nickel, in a single rulemaking effectively precluded OSHA from making the substance-specific findings of significant risk and the industry-specific findings of technological and economic feasibility that are required by the Occupational Safety and Health Act. Accordingly, the PEL for soluble nickel compounds reverted to a level of 1.0 mg Ni m^{-3}, the same as the PEL for nickel metal and insoluble nickel compounds. The PEL for soluble nickel compounds may, however, be lower than 1.0 mg Ni m^{-3} in individual states that have obtained OSHA's approval.

5: PEL = Permissible exposure limit.

1.6 NICKEL CONCENTRATION IN URINE

As defined in the Encyclopædia Britannica (2010), the main constituents in urine are water, urea, sodium, chloride, sulphate, potassium and phosphate. The exact composition of urine and plasma can be seen in Table 1.7.

Table 1.7: Relative composition of plasma and urine in normal men (Adapted from Encyclopædia Britannica, 2010).

	plasma g L^{-1}	urine g L^{-1}	concentration in urine	molecular formula
Water	900–930	950	—	H_20
Protein	70–85	—	—	
Urea	0.3	20	×60	CH_4N_2O
Uric acid	0.02	0.3	×15	$C_5H_4N_4O_3$
Glucose	1	—	—	$C_6H_{12}O_6$
Creatinine	0.01	1	×100	$C_4H_7N_3O$
Sodium	3.2	6	×2	Na
Potassium	0.2	1.5	×7	K
Calcium	0.1	0.15	×1.5	Ca
Magnesium	0.025	0.1	×4	Mg
Chloride	3.7	6	×2	Cl
Phosphate	0.03	1.2	×40	PO^{3-}_4
Sulfate	0.03	1.8	×60	$[SO_4]^{2-}$
Ammonia	0.001	0.5	×500	NH_3

Urine is the by-product of the kidneys, when they excrete waste from plasma, which is part of the blood. The pH value of urine of healthy people is in the range from 4.8 to 8.4 (Lide, 2008). High values are mainly found among vegetarians.

In section 1.3, it was discussed that absorbed nickel, either through inhalation, ingestion or skin contact, is mainly excreted in urine. To estimate the range of concentration in urine, studies on healthy adults, people with some kind of nickel history and workers in the nickel industry were reviewed. In Table 1.8 it is apparent that the mean concentration of nickel in healthy adults is below 5 µg L^{-1} and hardly any sample exceeded 10 µg L^{-1} (µg g^{-1} creatinine). However, Ohashi *et al.* (2006) have reported concentrations of up to 57 µg L^{-1}.

Table 1.8: Nickel concentrations in urine of healthy, adult persons (Adapted from Templeton et al., 1994).

Country	No.	Mean ± S.D.	Range	Units	Reference
USA	32	2.0 + 1.5	0.5-6.0	µg L^{-1}	Sunderman *et al.*, 1986b
Finland	299	4.1*	10.0°	µg L^{-1}	Kiilunen *et al.*, 1987
France	55	1.59 + 1.67	NS	µg^{-1} creatinine	Elias *et al.*, 1989
USA	44	1.5 ± 0.2	<0.5-4.6	µg^{-1} creatinine	Sunderman *et al.*, 1989b
Italy	878	0.9	0.1-3.9	µg L^{-1}	Minoia *et al.*, 1990
Taiwan	30	3.2 ± 1.7	1.2-7.8	µg L^{-1}	Lin, 1991
China	6	n.s.	0.9-4.7	µg L^{-1}	Yang *et al.*, 1998
Austria	100	0.85	0.01-9.48	µg^{-1} creatinine	Zeiner *et al.*, 2006
Japan	1000	2.1	<LOD-57	µg L^{-1}	Ohashi *et al.*, 2006

* Geometric Mean
° 95 th Percentile
LOD – Limit of Detection

For the purpose of comparison, data from people with some kind of nickel history were also looked at. In Table 1.9, the nickel concentration in urine of people with dermatological diseases (correlated to nickel) and people who live next to a nickel processing industry are listed. The range is from 0.2 up to 121.77 µg g^{-1} creatinine. The results are comparable to the concentration of healthy people, except for one study of people living next to various electroplating factories (Chang *et al.*, 2006). In their study, the 95th percentile value is up to 16.63 µg g^{-1} creatinine and individual results up to 121.77 µg g^{-1} creatinine.

Table 1.9: Nickel concentrations in urine of adult persons with a nickel exposure history.

Country	No.	Mean (S.D.)	Range	95th Percentile	Units	History	Reference
Germany	163	n.s.	0.2-46.1	3.9 (µg L^{-1})	µg g^{-1} creatinine	Dermatological patients	Schwegler *et al.*, 2009
Taiwan	522	6.08–6.74	0.93-121.77	16.63	µg g^{-1} creatinine	Residents of areas with a high density of electroplating factories	Chang *et al.*, 2006
Russia	371	4.9	0.3-61.9	n.s.	µg L^{-1}	Populations living in the proximity of a Russian nickel refinery	Smith-Sivertsen *et al.*, 1998
	418	2.8	0.3-24.2	n.s.	µg L^{-1}		
Denmark	35	1.1 (1.5)	<LOD-5.2	n.s.	µg g^{-1} creatinine	Patients with nickel eczema	Christensen *et al.*, 1999

LOD – Limit of Detection

The nickel concentration of workers in the nickel industry was also evaluated and can be seen in Table 1.10. Two points are obvious. First, the concentrations are partly much higher than in the general population. Second, the mean nickel concentration in urine of occupational exposed workers is mostly between 10 µg L^{-1} and 30 µg L^{-1} (µg g^{-1} creatinine levels are normally lower, anyway). Unfortunately, the results are from different countries, industries and years and, hence, are not fully comparable.

Table 1.10: Nickel concentrations in urine of workers in the nickel industry.

Country	No.	Mean ± SD	Range	Units	Working Area	Reference
USA/Canada	6	11.7 ± 7.7	3.4-25.0	µg L^{-1}	Nickel battery workers	Bernaki *et al.*, 1978
USA/Canada	21	27.5 ± 21.2	3.6-65.0	µg L^{-1}	Nickel platers	Bernaki *et al.*, 1978
USA/Canada	15	222 ± 226	8.6-813.0	µg L^{-1}	Nickel refinery workers	Bernaki *et al.*, 1978
Germany	103	18.5 ± 28,5	0.1-209.4	µg L^{-1}	Stainless steel welders	Angerer and Lehnert, 1990
Italy	6	17.85 ± 11.74	5.8-36.2	µg L^{-1}	Hard metal workers	Scansetti *et al.*, 1998
Italy	6	24.36 ± 17.04	6.4-48.0	µg L^{-1}	Hard metal workers	Scansetti *et al.*, 1998
Russia	14	10.3	28.6*	µg L^{-1}	Smelting	Smith-Sivertsen *et al.*, 1998
Russia	11	5.5	18.2*	µg L^{-1}	Ore roasting	Smith-Sivertsen *et al.*, 1998
Russia	8	6.2	25*	µg L^{-1}	Ore milling, concentration, and flotation	Smith-Sivertsen *et al.*, 1998
Taiwan	23	36.6 ± 16.5	17.0-79.5	µg L^{-1}	Steel production workers	Horng *et al.*, 2003
Taiwan	23	29.8 ± 13.1	4.45-51.0	µg L^{-1}	Quality control workers	Horng *et al.*, 2003
Pakistan	56	9.47 ± 2.95	4.63-18.39	µg L^{-1}	Production workers	Afridi *et al.*, 2006
Russia	96	4.0	0.68-28.0	µg g^{-1} creatinine	Welders	Ellingsen *et al.*, 2006
Norway	9	3,72	0.25-11.1	µg g^{-1} creatinine	Stainless steel grinders	Stridsklev *et al.*, 2007

Percent with ≥10 µgL^{-1}

In conclusion of the last section, the normal level of nickel in urine is up to approximately 10 µg L^{-1}. Among residents in close proximity to nickel processing industry and among workers in those factories, the values are higher. Unfortunately, studies on mine workers were not located.

1.6.1 Correlation of Nickel Exposure and Nickel Concentration in Urine

Correlation of nickel in air and urine: The correlation of nickel in air and nickel concentration in urine depends on the nickel compounds in air and on the time the urine samples are collected. The influences of both factors on the nickel concentration in urine are discussed below.

If the samples should be collected before or after a shift is not clear at the moment. A study from Oliveira *et al.* (2000) recommends collecting post-shift sampling to reach the highest correlation for exposure to nickel salts. However, most of the other studies that investigated the correlation of nickel in air and urine used pre-shift urine samples (Sunderman *et al.*, 1986a; Werner *et al.*, 1999; Yokota *et al.*, 2007). The only point which is evident, is that the concentration of nickel in urine changes over the course of the day. However, some studies are reporting higher pre-shift values (Bernaki *et al.*, 1980; Roels *et al.*, 1993) and others higher post-shift values (Roels *et al.*, 1993; Oliveira *et al.*, 2000; Yokota *et al.*, 2007). These differences are probably dependent on the nickel uptake, which is related to the compound and its characteristics (e.g. size).

As can be seen in Figure 1.3, exposure to insoluble nickel compounds correlates with the concentration in urine. The samples were taken from hydrogen plant workers (H), engineering department workers (E), kiln workers (K), powder plant workers (P) and wet treatment plant workers ($W_{(A)}$). The two groups of workers exposed to high levels of nickel were wearing protective clothes ($P_{(0)}$ and ($W_{(A)}$)).

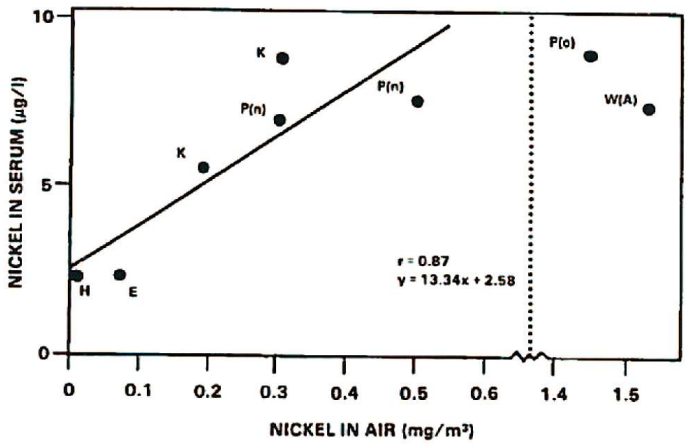


Figure 1.3: Relationship between nickel concentration in pre-shift urine specimens and personal air samples from Welsh nickel refinery workers, exposed to insoluble nickel compounds (Sunderman et al., 1986a).

A significant correlation of soluble nickel compounds and nickel concentration in urine among different industries is illustrated in Figure 1.4.

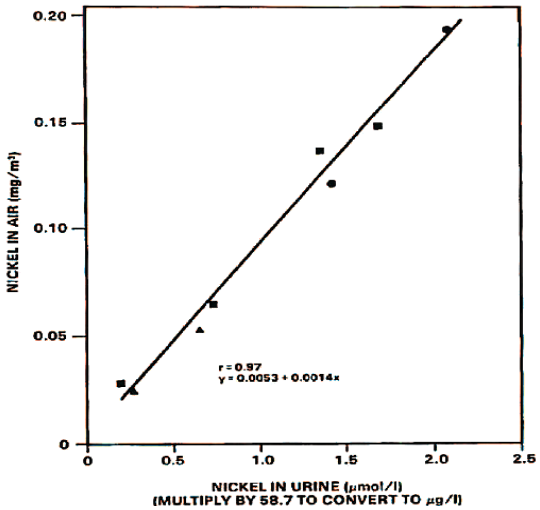


Figure 1.4: Weekly mean concentration in air samples and urine samples of workers exposed to soluble nickel salts. Squares and circles: nickel plant workers; Triangles: electrolytic nickel refinery workers (Sunderman et al., 1986a).

Furthermore, nickel sulfate particularly shows a positive correlation (Oliveira *et al.*, 2000). Concerning nickel hydroxide, more research is necessary. Recent results were inconclusive due to the use of respiratory protection (Yokota *et al.*, 2007).

However, the most interesting is the total nickel concentration in air and its relationship to the nickel concentration in urine. A good correlation of workers from a nickel-cadmium battery factory can be seen in Figure 1.5.

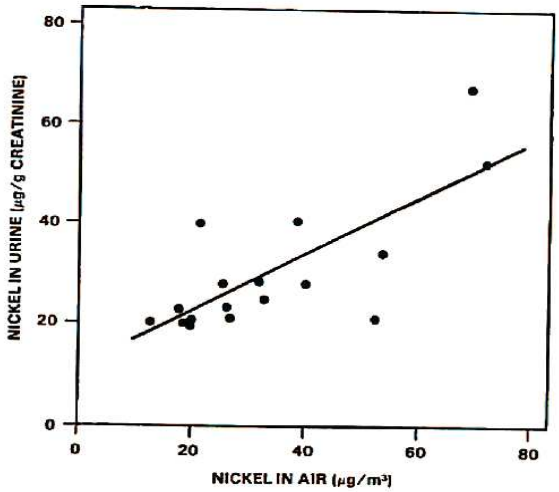


Figure 1.5: Relationship between mean nickel concentrations in personal air samples and urine samples (Sunderman et al., 1986a).

On the contrary to these results, studies from a nickel refinery in Finland (Kiilunen *et al.*, 1997a) and from different industries in Norway (Hogetveit *et al.*, 1978) showed no, or only a poor, correlation.

Although otherwise indicated by Kiilunen and Hogetveit and their teams, other studies recommend the use of urine to assess the exposure to nickel in air (Angerer and Lehnert, 1990; Werner *et al.*, 1999; Oliveira *et al.*, 2000), but also to evaluate a combined exposure through inhalation and ingestion (McNeely *et al.*, 1972; Sunderman *et al.*, 1986a). The combined exposure evaluation is necessary due to the fact that nickel ingestion can falsify the nickel inhalation exposure assessment. There are two routes which have to be taken into account. First, ingestion of contaminated dust is an often overlooked route of exposure (Sunderman *et al.*, 1986a). Second, the environment around nickel mines may also be contaminated with nickel and, hence, water and food (Heikkinen *et al.*, 2002) or lifestyle, dietary habits, and socioeconomic conditions lead to an increased intake (Smith-Sivertsen *et al.*, 1998).

Correlation of nickel in blood and urine: To relate health risk to the nickel concentration in urine, it is also important to evaluate the relationship with the concentrations in blood or plasma. Whereas the correlation of nickel in plasma and urine was not statistically significant in non-exposed people (Torjussen and Andersen, 1979), there is a correlation in exposed workers in miscellaneous industries. Correlation was reported from stainless steel welders (Angerer and Lehnert, 1990), plant workers doing crushing, roasting, smelting and electrolysis (Torjussen and Andersen, 1979) and nickel refinery workers (Bernacki *et al.*, 1978). The results of the latter can be seen in Figure 1.6. As shown in the graph there is a perfect correlation in the whole scope of nickel concentrations in urine up to nearly 400 $\mu g\ L^{-1}$.

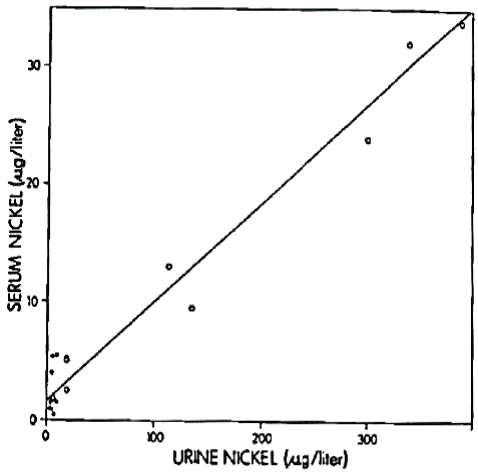


Figure 1.6: Correlation between nickel concentration in the serum and urine of non-exposed industrial workers (solid circles) and nickel refinery workers (open circles). (Adapted from Bernacki et al., 1978).

Conclusion: In conclusion of the previous section, the concentration of nickel in air and urine may not correlate. On the one hand this is because workers may wear protective clothes (see Figure 1.3: $P_{(0)}$ and ($W_{(A)}$)). On the other hand, the results can be influenced by the ingestion of high amounts of nickel in water and food (Kiilunen *et al.*, 1997b) and also from the absorption characteristics of the different nickel compounds (ATSDR, 2005). Generally, a simultaneous measurement of nickel compounds and concentration in air is recommended, because these values have to be known when urine is used for exposure assessment (Sunderman, 1993).

However, the results from the relationship of nickel concentration in blood and urine are statistically significant. This is due to the fact that nickel ingestion and protective clothes no longer play a role. Hence, the determination of nickel concentration in urine may not be completely related to exposure in air, but to nickel concentrations within the human circulatory system. Once again, simultaneous measurement of nickel compounds and concentration in air is recommended, because nickel stored in the respiratory tract is not taken into account.

1.7 ANALYTICAL NICKEL DETECTION TECHNIQUES

1.7.1 Introduction

In the field of trace nickel analysis in body fluids, there are some standard determination methods on the one hand, but on the other there are some efforts to develop a rapid, cheap and portable technique.

The standard analytical techniques for nickel are (Sunderman, 1993; ATSDR, 2005):

- Electrothermal Atomic Absorption Spectrometry (EAAS)
- Inductively Coupled Plasma - Atomic Emission Spectroscopy (ICP-AES)
- Inductively Coupled Plasma - Mass Spectrometry (ICP-MS)
- Isotope Dilution - Mass Spectrometry (ID-MS)

However, the ICP-AES shows the second worst accuracy among all metals in urine (±102%) as reported by NIOSH (2003). Hence, this method will not be discussed in detail.

Although the standard analytical techniques are not cheap and portable, they are discussed in this section because they are more precise or are even classified as reference techniques. Hence, they are used to compare the accuracy of newly developed sensors.

The areas in which rapid, portable and cheap sensors are developed at the moment can be roughly divided into:

- Spectroscopic Detection Techniques
- Bioanalytical Detection Techniques
- Electrochemical Detection Techniques

Most of these rapid, portable and cheap sensor do not focus on nickel detection in urine. However, they are discussed to gain information about the state-of-the-art of science and technology and to find the best available nickel detection technique. This method can then be adapted for nickel detection in urine.

1.7.2 Standard Analytical Nickel Detection Techniques

1.7.2.1 Electrothermal Atomic Absorption Spectrometry

The analyte is atomised in a chamber at a temperature of 2000°C to 3000°C (Higson, 2003). The most popular form uses a graphite surface as the nebuliser. Once a potential is applied, current flows and the graphite tube heats up. The atomisation normally takes place in more than one step and its efficiency is nearly 100%, compared to 0.1% for systems based on flames. During the procedure a beam of light is sent from one end of the tube to the other. The principle experimental set-up can be seen in Figure 1.7. For cooling purposes there is also a connection to an inert gas source. This spectrum of the beam is influenced by the atoms present in the sample. A defined wave length can stimulate electrons to higher energy levels, comparable with the spectroscopic sensors described in the next section (see also Figure 1.9). Hence, the energy is absorbed and the absorption coefficient correlates with the amount of nickel in the sample solution.

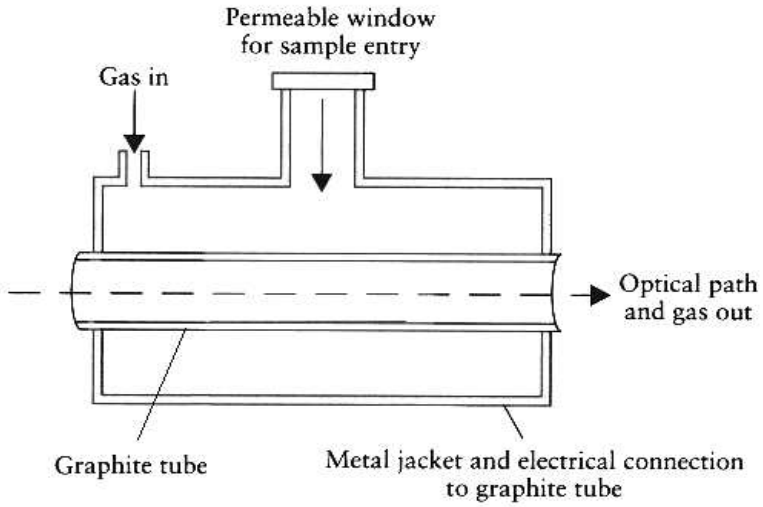


Figure 1.7: Principle experimental set-up for the EAAS (Adapted from Higson, 2003).

The most important wave length affected by nickel is 232.00 nm (Patnaik, 2004). The detailed procedure for nickel in serum and urine is described by Todorovska *et al.* (2002). The reported limit of detection is 0.2 µg L^{-1}.

The stabilised temperature graphite furnace atomic adsorption procedure for nickel in urine was published by Oliveira *et al.* (2000). For this kind of technique, measures are taken to optimise the analytical method and minimise interferences in the graphite tube. The limit of detection is 0.56 µg L^{-1}.

1.7.2.2 Inductively Coupled Plasma - Mass Spectrometry

The principle design of an ICP torch can be seen in Figure 1.8. At the beginning the sample solution is pressed through a nebuliser and mixed with an inert gas (usually argon). The mixture stream is then directed to a tube, which is surrounded by a helical coil. This load coil is connected to a high-power radio-frequency source. The stream of gas is ignited and maintained through the induction from the load coil. Ionisation takes place and the stream reaches temperatures up to 10,000 K. Now the stream is accelerated to a Mass Spectrometer (MS) with the help of an electromagnetic field.

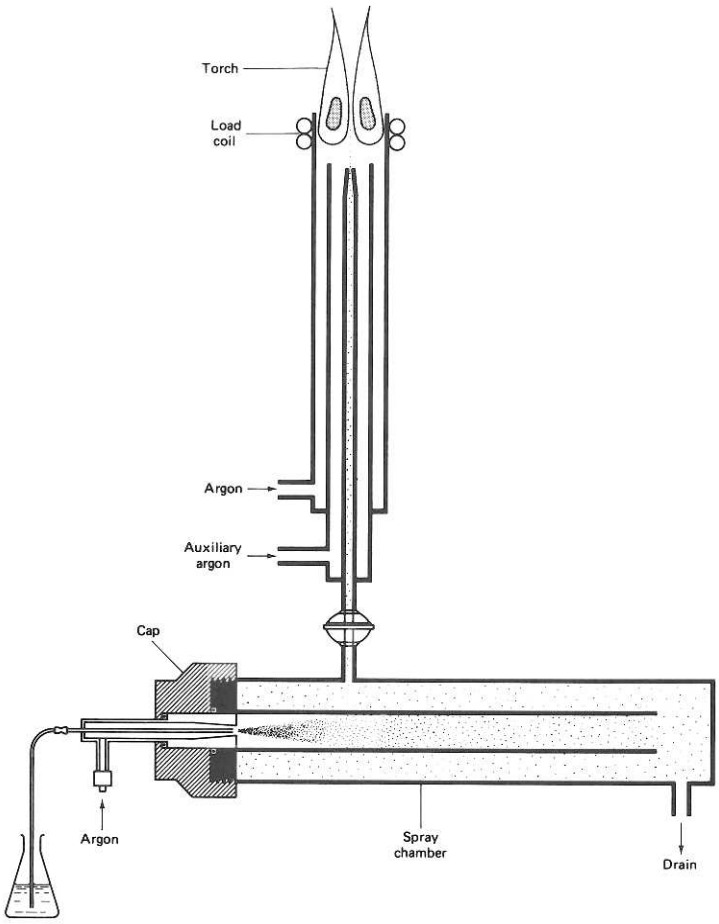


Figure 1.8: Schematic diagram of an inductively coupled plasma torch without MS (Hargis, 1988).

In the MS the different properties and concentrations of the sample solution can be determined. Various techniques are available (Higson, 2003). The principle they all have in common is that they separate the ionised samples according to their mass-to-charge ratio after they have been accelerated in an electromagnetic field. Ions with the greatest mass will travel with the lowest velocity and those with the lowest mass with the highest velocity. As an ion analyser electron multiplier, a Faraday cup or scintillation detectors may be used.

The ICP-MS method for the detection of nickel in urine struggles with strong interference from calcium, sodium and potassium (Vaughan and Templeton., 1990). Hence, this technique has a limit of detection of just 1 µg L^{-1}.

1.7.2.3 Isotope Dilution - Mass Spectrometry

The principle approach of an ID-MS is described by the Royal Society of Chemistry Analytical Methods Committee (2002) as follows (adopted for nickel): a nickel solution with the highest purity possible has to be prepared. These nickel atoms are then labelled, for example radioactively. Afterwards a known weight of this solution is added to a defined amount of the analyte sample, which is sometimes referred to as spiking the sample. After the mixture is equilibrated for some time it is introduced into a MS. The ratio of analyte to its labelled analogue can be measured and the mass of non-labelled nickel (M_I) calculated with the following formula (Higson, 2003):

$$M_I = \frac{R_T}{R_M}(M_M - M_T) \qquad (1)$$

With M_M as the mass of the isolated and purified mixture, M_T as the mass of the trace and R_T and R_M as the count rates for the tracer and mixture, respectively.

ID-MS is a very accurate method with a limit of detection of 1µg L^{-1}, but the approach is difficult, expensive and laborious (Sunderman, 1993). The procedure for nickel in body fluids was published by Aggarwal *et al.* (1989a and 1989b).

1.7.3 Spectroscopic Nickel Detection Techniques

1.7.3.1 Introduction

Spectroscopy is a very popular analytic measurement technique. The methods are based on light and other forms of electromagnetic radiation, including radio frequency, microwave, infrared, visible light, ultraviolet, X-ray and γ-ray. All techniques using any kind of electromagnetic radiation are summarised by the term spectroscopy. The techniques can be distinguished between whether the radiation is simply absorbed or the absorbed radiation is also emitted again, which is called photoluminescence.

The principle components for spectroscopy are a source, a wave length selector, the sample, a detector and a signal processor and readout (Skoog *et al.*, 2000). The source and detector depend on the type of radiation. For visible light this can be some kind of lamp and a photon detector, including phototubes, photomultiplier and photodiodes.

The two principles and their applications in determining nickel as described in the literature are discussed in detail below.

1.7.3.2 Absorption Methods

Principle: The physical principle can be seen in Figure 1.9. The radiation is gleamed through the sample with a defined power (a). On the atomic or molecule level the analyte is stimulated and undergoes a transition from its ground state to a higher state or excited state (b).

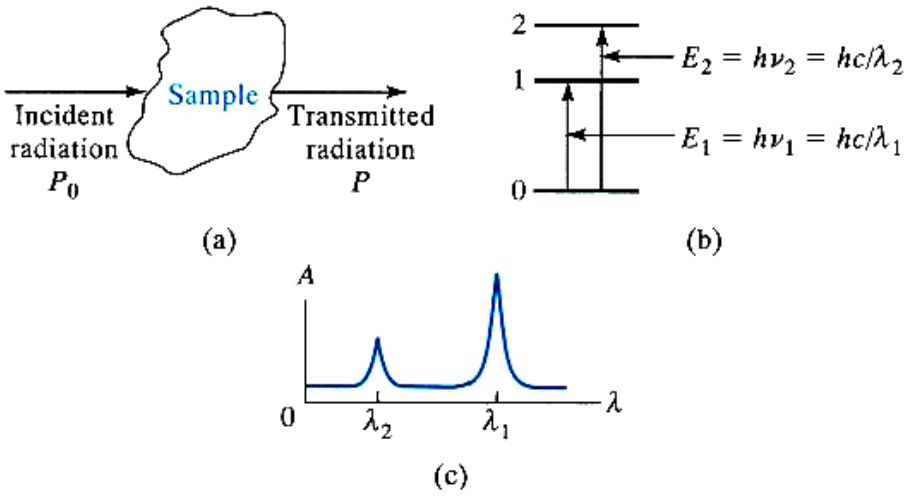


Figure 1.9: The absorption principle for different wave lengths (Skoog et al., 2000).

As illustrated there are different higher states and each one correlates with a defined wave length. The energy needed for the stimulation, or the loss of power from the primary radiation can be calculated as follows:

$$E = h \cdot v = \frac{h \cdot c}{\lambda} \quad (2)$$

With E as energy, h as Planck constant, *v* as frequency, c as speed of light and λ as wave length.

The loss of energy is related to the excited state and leads to an absorption spectrum as shown at (c). The analyte returns radiationless to its ground state, and the energy is otherwise released, such as through heat or vibration.

Available Sensors: Various sensors can be found in the literature which are able to detect nickel in solution using visible light (Madden *et al.*, 1996; Ensafi and Bakhshi, 2003; Amini *et al.*, 2004; Kaur and Kumar, 2007; Li *et al.*, 2010). The principle they all have in common is that they use a chemical with an affinity to form a complex with Ni(II) and, hence, change the maximum absorption peak of the solution. This change is used to determine the nickel concentration. Unfortunately, all of them are strongly influenced by the pH value. This characteristic is highly undesirable for a fluid like urine with a pH range from 4.8 – 8.4 (Lide, 2008). Furthermore, the limit of detection is in no case better than 30 µg L^{-1} (Ensafi and Bakhshi, 2003) and in general a good selectivity was not observed. The only exception is an *1-aminoanthracene-9,10-dione* based chromogenic sensor described by Kaur and Kumar (2007) which is solely selective for nickel and copper. A sensor on the basis *2-(5-bromo-2-pyridylazo)-5-(diethylamino)phenol* in Nafion to bind nickel was developed in 2004 by Resendiz *et al.*. It shows, among all heavy metals, the highest affinity for nickel. However, the design was only enhanced for metals others than nickel.

The same properties were reported from a sensor detecting ultraviolet light by Baxter (2001).

1.7.3.3 Photoluminescence Methods

Principle: A similar principle to the absorption method is photoluminescence. As illustrated in Figure 1.10 (a) radiation is once again gleamed through the sample and once again the analyte becomes stimulated from its ground state to an excited state (b). However, the resulting radiation is more complex compared to the simple absorption technique. The analyte is transformed from the excited state to a lower energy level, or even back to its original state. This transition is no longer radiationless but causes a photon to be emitted. Whether emission takes place or not depends on the chemical used. At (c) the emitted spectrum is shown. The wave length depends on the difference between the energy levels and can be calculated with equation number 2. Photoluminescence can be distinguished more precisely in fluorescence and phosphorescence. Simply described, the difference is that in fluorescence the transition of the analyte back to a lower level takes place immediately, compared to phosphorescence where the emission that takes place is delayed (Skoog *et al.*, 2000).

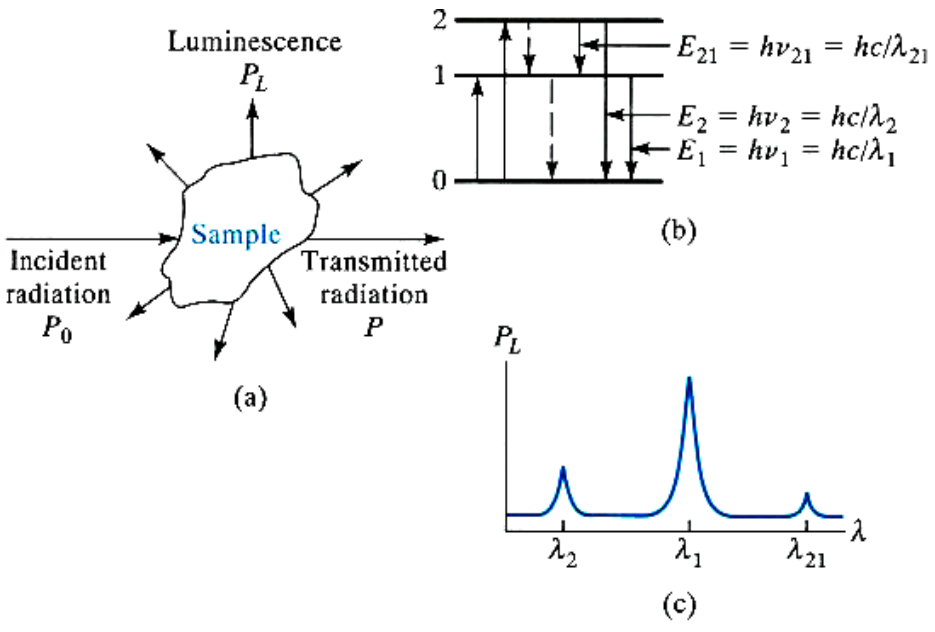


Figure 1.10: The photoluminescence principle for different wave lengths (Skoog et al., 2000).

In principle, chemicals can be used as a photoluminescence sensor for nickel; however, most of these kind of sensors use a biological element. This element changes its mainly fluorescent characteristic correlated to the concentration. This can have an inhibiting as well as enhancing effect. Located sensors using a biological component are discussed in the next section (bioanalytical nickel detection methods).

Available Sensors: Three photoluminescence sensors, on the basis of chemicals, were located in the literature. A first generation polyamidoamine dendrimer with a peripheral *4-dimethylaminoethylamine-1,8-naphthalimide* group sensor from Grabchev *et al.* (2003) has poor selectivity and a limit of detection in the range of mg L^{-1}. A sensor based on *3-hydroxy-3-phenyl-1-o-carboxyphenyltriazene* is able to detect concentrations of nickel down to 2.9 µg L^{-1} (Ressalan and Iyer, 2005). However, the influence is non-linear and the sensor is also selective for zinc and copper. To solve the problem of selectivity, a minimal size sensor array was developed (Palacios *et al.*, 2008). Ten different heavy metals were determined simultaneously by evaluating their effect on various chromophores which were conjugated on *8-hydroxyquinoline*. Since this technique is not yet fully developed the concentration of each heavy metal can not be determined properly at the present time.

1.7.4 Bioanalytical Nickel Detection Techniques

1.7.4.1 Introduction

Biosensors are “analytical devices incorporating a biological material, a biologically derived material or a biomimic as the recognition molecules, which is either intimately associated with or integrated within a physiochemical transducer or transducing microsystems” (Tothill and Turner, 2003). Normally the concentration of an analyte or a group of analytes is transformed into a proportional electronic signal. The principle can be seen in Figure 1.11.

Measurement of the biological reaction is done with a transducer, for which electrochemical, calorimetric, optical or mass determination sensors are used. This principle, in many cases allows, the design of small, fast, sensitive and selective sensors.

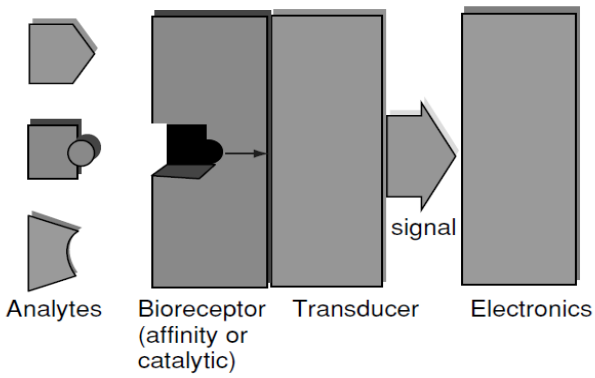


Figure 1.11: Principle arrangement of a biosensor (Tothill and Turner, 2003).

A slightly different concept, compared to a biosensor, is a bioassay, which is defined by the IUPAC (1997) as "a procedure for determining the concentration or biological activity of a substance (e.g. vitamin, hormone, plant growth factor, antibiotic) by measuring its effect on an organism or tissue compared to a standard preparation".

For the detection of nickel, biosensors on the basis of DNA, proteins, organelles and cells, and also methods using cells and organelles in bioassays, have been located in the literature. Most of the techniques are not relevant for the detection of nickel in urine. However, some sensors have good characteristics regarding nickel and will be described in detail.

1.7.4.2 Biosensors

Since DNA has four different binding sites for metal ions, it can be used to detect nickel (Bin and Kraatz, 2009). However, these sensors have poor selectivity and the detection range has in all cases not yet been determined. The same is also true for the organelle sensors from Erat and Siegel (2008), which detect the inhibitory effect on the self-splicing reaction of group II intron S*c*.ai5γ in the yeast mitochondrial. Detailed properties of all DNA and organelle sensors can be seen in Table 1.11.

Table 1.11: List of biosensors for nickel detection and their most important properties.

Biological Element		Principle	Transducer System	Selectivity	Sensitivity [µg L^{-1}]	Reference
DNA		Denaturation	SPR	Poor	n. s.	Wood and Lee, 2005
DNA		Dye which attachs	Spectroscopic	Poor	n. s.	Breimer *et al.*, 2003
DNA		Oxidation	Electrochemical	Poor	n. s.	Oliveira *et al.*, 2008
DNA		Impedance change	Electrochemical	Poor	n. s.	Bin and Kraatz, 2009
DNA		Adsorption patterns	Electrochemical	Poor	n. s.	Chiorcea-Paquim *et al.*, 2009
DNA		Denaturation	AFM	Poor	n. s.	Chiorcea-Paquim *et al.*, 2009
Organelle	Group II intron Sc.ai5γ	Inhibition	n. a.°	Poor	n. s.*	Erat and Siegel, 2008
Protein	Glutathione	Complex Formation	Electrochemical	Poor	147 – 587	Húska *et al.*, 2007
Protein	Mammalian metallothionein	Absorption change	SPR	Poor	0.6 – 5900	Wu and Lin, 2004
Protein	Bovine serum albumin	Absorption change	SPR	Poor	no response	Wu and Lin, 2005
Protein	*E.coli* nickel binding protein	Inhibition	Spectroscopic	Excellent	4.7 – n.s.	Salins *et. al.*, 2002
Enzyme	Carbonic anhydrase II	Inhibition	Spectroscopic	Poor	n. s.	McCall and Fierke, 2000
Enzyme	Jack bean urease	Inhibition	FET	Poor	117 – n.s.	Soldakin *et al.*, 2000
Enzyme	Urease	Inhibition	Spectroscopic	Poor	3000 – n.s.	Preininger and Wolfbeis, 1996
Enzyme	Urease	Inhibition	Spectroscopic	Poor	n. s.	Gabrovska and Godjevargova , 2009
Enzyme	Jack bean urease	Inhibition	n. a.°	Poor	3,000 – 70,000	Zaborska *et al.*, 2001
Enzyme	Alcohol oxidase	Inhibition	Electrochemical	Poor	n. s.	Compagnone *et al.*, 2001
Enzyme	Glycerol-3-P oxidase	Inhibition	Electrochemical	Poor	n. s.	Compagnone *et al.*, 2001
Enzyme	Sarcosine oxidase	Inhibition	Electrochemical	Good	n. s.	Compagnone *et al.*, 2001
Cell	*Ralstonia eutropha*	Genetic modified	Spectroscopic	Excellent	5.87 – 3,520	Tibazarwa *et al.*, 2001
Cell	*Bacillus sphaericus*	Inhibition	Electrochemical	n. s.	2 – 40	Verma and Singh, 2006
Cell	Lung cells (human and rat)	Inhibition	n. a.°	Poor	600 – 117,000	Riley *et al.*, 2005

* main focus was on a different chemical
°no fast transducer element developed yet
n. a. - not applicable
n. s. - not specified
AFM - Atomic Force Microscopy; FET - Field Effect Transistors; SPR - Surface Plasmon Resonance

In principle all the various kinds of proteins can be the basis of a protein based biosensor (Tothill, 2001), but there is an overwhelming amount of published papers examining the inhibitory effect of metals, such as nickel, on enzymes. In Table 1.11, different protein based sensors with their properties are listed. Most of these have very poor characteristics concerning nickel. All enzymatic based biosensors, for example, work solely on the principle of inhibiting the enzymatic catalysis ability. Although the catalysis is highly selective, the inhibition effect is not selective at all (Luque de Castro and Herrera, 2003). Hence, selectivity is in general not good in these types of sensors. However, there are two exceptions. The fluorescence sensor from Salins *et al.* (2002), based on the nickel binding protein, shows a high sensitivity, the detection limit is 4.7 µg L^{-1}, but has only a moderate selectivity. Cobalt concentration only up to 10 folds higher than nickel can be tolerated. This value is higher for other heavy metals. The sensor works with a fluorophore linked to the nickel binding protein of *E.coli* and a fibre optic sensor device. Unfortunately, the response time is very long and in the range of a couple of minutes. The second example is an oxidase enzyme, based on an amperometric sensor (Compagnone *et al.*, 2001). Assessed was the inhibitory effect of different heavy metals on alcohol oxidase, glycerol-3-P oxidase, and sarcosine oxidase. The concentration added was 10 mg L^{-1} for each metal and the incubation time was as long as 30 minutes. The results can be seen in Table 1.12. In particular, sarcosine oxidase is somewhat selective for nickel. However, the concentration range was not evaluated and the response time is very long.

Table 1.12: Relative activity of the oxidase enzymes (Adapted from Compagnone et al., 2001).

Heavy Metal	Alcohol Oxidase	Glycerol-3-P Oxidase	Sarcosine Oxidase
Arsenic	108	92	98
Cadmium	102	93	105
Chromium	103	97	108
Mercury	0[a]	0	81[b]
Lead	98	94	106
Nickel	88[c]	101	22
Copper	22	95	96
Zinc	96	100	112
Tin	101	99	86
Molybdenum	95	97	96
Vanadium	20	93	75[d]
Selenium	108	99	93

[a]At 0.25 ppm = 58%.
[b]At 4 ppm = 100%.
[c]At 5 ppm = 100%.

As it can be seen in Table 1.11, three cell based biosensors were also located. However, the only sensor with good properties concerning nickel detection is the one described by Tibazarwa *et al.* (2001). For the construction, the *ralstonia eutropha* strain AE2515 was used. Emitted bioluminescence correlates highly with nickel and cobalt. The response is linear in the range from 5.87 µg L^{-1} to 3.52 mg L^{-1} (0.1 µM to 60 µM) nickel concentration. Zinc, cadmium, manganese, copper and chromium do not influence the sensor. Sensitivity decreases in the presence of 117.87 mg L^{-1} (2 mM) cobalt or more.

1.7.4.3 Bioassays

No bioassays technique located in the literature is able to correlate the effects of observing, either inhibiting or enhancing cell activity, to a specific chemical or metal, respectively. It does not matter whether single cells are used, *e.g.* Kelly *et al.* (2004) who inserted the Shk1 *lux* gene in different bacteria, or the *anoxybacillus amylolyticus* bacteria (Poli *et al.*, 2009) or the *anabaena doliolum* bacteria (Shukla *et al.*, 2009), or plants, like the marine *dinoflagellate pyrocystis lunula* (Heimann *et al.*, 2002).

1.7.5 Electrochemical Nickel Detection Techniques

1.7.5.1 Introduction

With electroanalytical techniques, it is possible to measure electrical quantities, such as charge, potential, and current which are related to chemical parameters (Wang, 2006). One of the most important chemical reactions is that of oxidation-reduction, the so-called redox reaction (Hargis, 1988). During this reaction, one reactant loses an electron, which means there is an increase in the oxidation number. This free electron passes to a second reactant. The first step is described by oxidation, whereas the gain of an electron is called reduction (Skoog *et al.*, 2000):

$$Oxidised\ \ form\ (O) + ne^- \leftrightarrow\ \ Reduced\ \ form\ (R) \qquad (3)$$

At the working electrode, the analyte in solution becomes oxidised or reduced at the surface of the electrode. The separation of charges at the surface leads to the development of a potential (E). The potential is defined by the Nernst equation:

$$E = E^0 - \frac{RT}{nF} \ln \frac{aR}{aO} \quad (4)$$

The potential is measured compared to the potential E^0 of standard hydrogen reference electrode (SHE), which is defined to have zero volt. R is the gas constant (8.31441 J K^{-1} mol^{-1}), T the temperature [K], n the number of electrons, F the Faraday constant (96,485 C) and aR and aO the activities of all reactants at the electrode surface.

Since the standard hydrogen reference electrode is not a convenient electrode (Hargis, 1988) and hydrogen gas, flow regulators and associated pressure are needed, other electrodes are more practical for routine measurement. These are namely the saturated calomel electrode (SCE) and the silver-silver chloride electrode (SSCE). However, the SCE has two disadvantages. On the one hand it contains mercury, which is toxic and dangerous for the environment (HPA, 2007), and on the other hand it can not be built as small as a SSCE (Hargis, 1988). These are the reasons why the SSCE is one of the most common choices as a reference electrode. The chemical reaction at the SSCE surface is:

$$AgCl(s) + e^- \leftrightarrow Ag^0(s) + Cl^- \quad (5)$$

The reference potential of the SSCE depends on different factors. The crucial ones are the temperature and the chloride concentration of the solution in which the electrode is immersed. Tables with the value of the potential of different test arrangements are published elsewhere (Patnaik, 2004). At a temperature of 20°C and in a 1.0 M solution of potassium chloride, the potential is, for example, 0.22557 V.

The desired properties of the working electrode depend strongly on the electroanalytical technique which is used and, hence, the design of the electrode. In general these properties are, to be environmental friendly and to prevent any interaction of the surface during the chemical reaction (inert). The second point made here is a problem because mercury containing electrodes are quite effective and, hence, very popular. Mercury readily interacts with a huge amount of other heavy metals (formation of amalgam), is liquid at room temperature and resists the evolution of hydrogen (Higson, 2003). However, new environmentally friendly and easily disposable electrodes are available and are discussed in detail in the sections about the different methods.

There are plenty of different test arrangements described in the literature. The two main important techniques are potentiometry and controlled potential ones (Wang, 2006). For the first method the potential between the working and the reference electrode is measured. In the second, the current is measured as the applied potential is varied, which is called voltammetry. If there is a constant potential applied it is called amperometry. Within these methods it is common to use a third electrode, the counter electrode, for the current to flow to or from the working electrode. This is done to keep the potential at the reference electrode stable and make an accurate measurement possible. Furthermore the techniques can be distinguished more precisely in methods such as, ion-selective electrodes, potentiometric titration, linear potential sweep voltammetry, cyclic potential sweep voltammetry, stripping voltammetry, amperometric titration, chronopotentiometry, *etc*. During the literature review on nickel detection methods, it became apparent that the main focus of research is on ion-selective electrodes and stripping voltammetry. Hence, these two techniques are the most promising to make the development of a rapid and cheap measurement method for nickel concentration in urine feasible, and will be discussed in detail in the following two sub sections.

1.7.5.2 Ion-Selective Electrodes

Principle: This kind of technique is at the moment quite popular in environmental monitoring, clinical diagnostics and physiology (Wang, 2006). The measurement principle is simple, but actually measures the activity of the ion analyte and not directly the concentration. However, such electrodes are inexpensive, have a wide linear range, are not influenced by colour or turbidity and are not destructive (Wang, 2006). Moreover, they are also adaptable to a small sample volume (Singh and Harsh-Vardhan, 1995). All these properties make an ion-selective electrode (ISE) suitable for a rapid and cheap detection method for nickel in urine.

The most important part is an ion semi-permeable membrane. This membrane separates the working electrode from the solution. However, the ion analyte can still pass the membrane and, hence, the activity at the surface of the working electrode can be determined by measuring the potential against a reference electrode. The principle test arrangement can be seen in Figure 1.12.

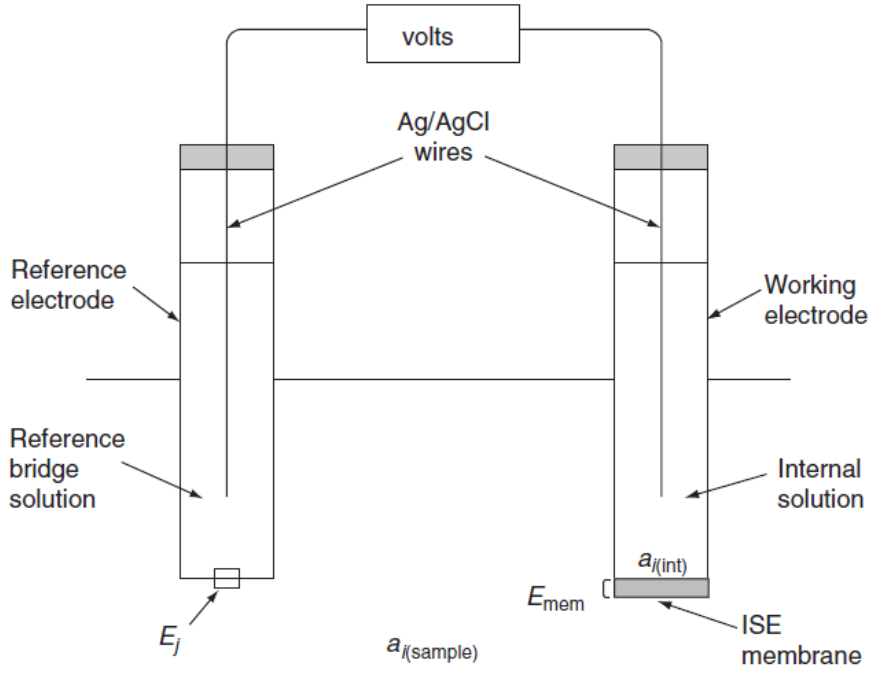


Figure 1.12: Principle arrangement of an electrochemical cell for potentiometric measurements (Wang, 2006).

And a symbol image of an analyser with an ion-selective electrode can be seen in Figure 1.13. However, since no commercially available nickel sensor was found, this sensor for measurement of the pH value and temperature was used.

Figure 1.13: Symbol image of an ion selective electrode analyser (GlobalWater, 2010).

Available Sensors: In the 1990's, macrocyclic compounds were found to bind metal cations selectively and can be used as an ionosphere to transport nickel. Among the first researchers who used macrocyclic compounds embedded in a polyvinyl chloride (PVC) matrix to determine nickel were Singh and Harsh-Vardhan (1995). The sensor was selective to nickel, compared to other heavy metals, but unfortunately not to potassium and ammonium which are found in high concentrations in urine. Hence, it is not applicable for the detection of nickel in urine. However, the research proceeded and a whole range of different carriers for nickel, most importantly on the basis of crown ethers, cryptands and macrocycles, were developed. A list of all published papers found on nickel selective membranes and their properties can be found in the appendix (Appendix A). The three sensors which show the least permeability to other ions occurring in urine (see table 1.7 for the exact composition of urine) and other heavy metals and can operate in the pH range of urine, 4.8 – 8.4 (Lide, 2008), are listed in Table 1.13.

The first sensor uses 3-hydroxy-N-{2-[(3-hydroxy-N-phenylbutyrimidoyl)-amino]-phenyl}-N-phenylbutyramidine, the second one Glyoxal-bis(S-benzyldithiocarbazate) and third one 2,9-(2-methoxyaniline)$_2$-4,11-Me$_2$-[14]-1,4,8,11-tetraene-1,5,8,12-N$_4$ as ionosphere for the PVC based membrane. The first and third sensor from Table 1.13, which were developed by Gupta *et al.* (2008) and Singh *et al.* (2009), respectively, fit reasonably in matters of pH range and specificity, and also use a silver-silver chloride electrode as working and reference electrode. The second sensor from Ma *et al.* (2009) struggles not only with the pH range, but also uses a saturated calomel electrode as working electrode. Moreover, the selectivity of nickel compared to sodium was not evaluated, which is a crucial factor for measurements with urine. All three sensors from Table 1.13 were successfully used for potentiometric titration and also to detect nickel levels in some groceries, such as chocolate, milk, wine and different juices. The main crucial point is the sensitivity of the ion-selective electrodes. The linear range and detection limit in µg L^{-1} are also presented in Table 1.13. As can be seen, the second sensor has problems detecting 10 µg L^{-1} nickel in solution and the performance of the first sensor is not much better. With the third sensor, a detection below 10 µg L^{-1} is possible.

Table 1.13: Properties of different membranes permeable for nickel.

	Sensor 1	Sensor 2	Sensor 3
Ionosphere	3-hydroxy-N-{2-[(3-hydroxy-N-phenylbutyrimidoyl)-amino]-phenyl}-N-phenylbutyramidine	Glyoxal-bis(S-benzyldithiocarbazate)	2,9-(2-methoxyaniline)$_2$-4,11-Me$_2$-[14]-1,4,8,11-tetraene-1,5,8,12-N$_4$ coated in graphite
Composition	Ionosphere: PVC: STPB: CN	Ionosphere: PVC: NPOE	Ionosphere: PVC: STPB: TBP
in the ratio	5:150:5:150	1.7:31:67.3	6:90:5:100
Working concentration range [M] ([µg L^{-1}])	$1.6 \times 10^{-7} – 5.0 \times 10^{-2}$ ($9.4 – 2.93 \times 10^{6}$)	2.8 x 10-7 – 1.0 x 10-1 ($16 – 5.8 \times 10^{6}$)	$7.7 \times 10^{-8} – 1.0 \times 10^{-1}$ ($4.5 – 5.8 \times 10^{6}$)
Limit of Detection [M] ([µg L^{-1}])	1.0×10^{-7} (5.9)	1.2×10^{-7} (7.0)	2.7×10^{-8} (1.6)
Slope [mV/Decade]	30.0	31.9	29.5
Response Time [s]	10	25	8
Independent from pH-Value	2.5 – 9.5	4.0 – 7.5	3.0 – 8.0
Lifetime	4 months	3 months	5 months
Selectivity coefficients compared to nickel*:	mainly all other heavy metals in the range of 10^{-2}, Na^+, K^+ around 10^{-3}	Ag^+ 8.1×10^{-3}, all others below, Na^+ n.s.	Na^+, Cu^{2+}, Co^{2+} and Cd^{2+} around 5.0×10^{-3}, others below
References	Gupta *et al.* (2008)	Ma *et al.* (2009)	Singh *et al.* (2009)

* Selectivity coefficients were measured and calculated by different methods and are not completely comparable

CN	Chloronaphthalene – Solvent
NPOE	2-nitrophenyl octyl ether – Solvent
PVC	Polyvinyl chloride – Matrix Basis
STPB	Sodium tetraphenyl borate – Anion Excluder
TBP	Tributyl phosphate – Solvent

Jain *et al.* (2005) were able to measure nickel concentration in urine, with their PVC based membrane using *N*-(2-hydroxybenzylidene)-*N*-(2-picolyl)ethylenediamine as ionosphere. However, the pH range is very narrow and too low for urine, and the lower working concentration limit is two orders of magnitude higher than those of the three sensors discussed above. For detection in urine, it was necessary to add nickel artificially and pretreat the urine sample using wet digestion. Nevertheless the selectivity is comparable, which proves that a measurement is theoretically possible with an ion-selective electrode.

1.7.5.3 Stripping Voltammetry

Principle: Voltammetry is a very easy and inexpensive electrochemical technique to detect metals (Wang, 2006). Figure 1.14 illustrates the principle composition of a three electrode system used for voltammetric measurements. Most sensors control the potential at the working electrode, which is regulated by comparing the potential between the working and reference electrode (determined with the voltmeter in Figure 1.14) with the desired potential that is applied (E_{app}). Sometimes, though, the current is controlled instead, but this principle is not described any further. During the controlled potential measurement the current which yields is measured (amperemeter in Figure 1.14). If the applied potential is varied the results are displayed in a voltammogram, where the current is related against the applied potential.

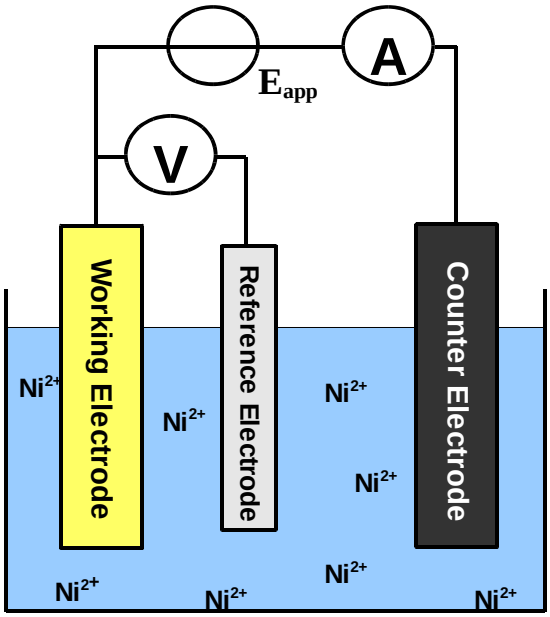


Figure 1.14: Principle arrangement of a three electrode system.

A sensor for voltammetric measurements normally consists of a three electrode system, which is described above. The schematic diagram of three electrode potentiostat, which is the electronic part of the sensor, can be seen in Figure 1.15. During the measurement procedure the potential at the working electrode (compared to the reference electrode) is controlled and the current is measured. The working electrode is in contact with the analyte and facilitate the charge to or from the analyte. This current, caused by the half cell reactivity of the analyte at the working electrode, is measured with a current amplifier, which is usually a current to potential converter. Furthermore, a scan amplifier provides the desired potential at the working. Therefore, the potential at the working electrode is determined compared to another half cell which provides a known and constant potential, the reference electrode. It is crucial that at no point current is passed from or to the reference electrode, because this would influence the chemical equilibrium and, hence, change its half cell potential and falsify the measurment. The determined potential between the working and the reference electrode is then compared to the desired potential applied to the sensor (E_{app}). To regulate the potential at the working electrode the counter electrode passes the current needed to maintain the potential at the desired level, which is applied at the sensor (E_{app}). This is either done by oxidising or reducing the electrolyte at the surface of the counter electrode by varying the potential of the electrode by the scan amplifier.

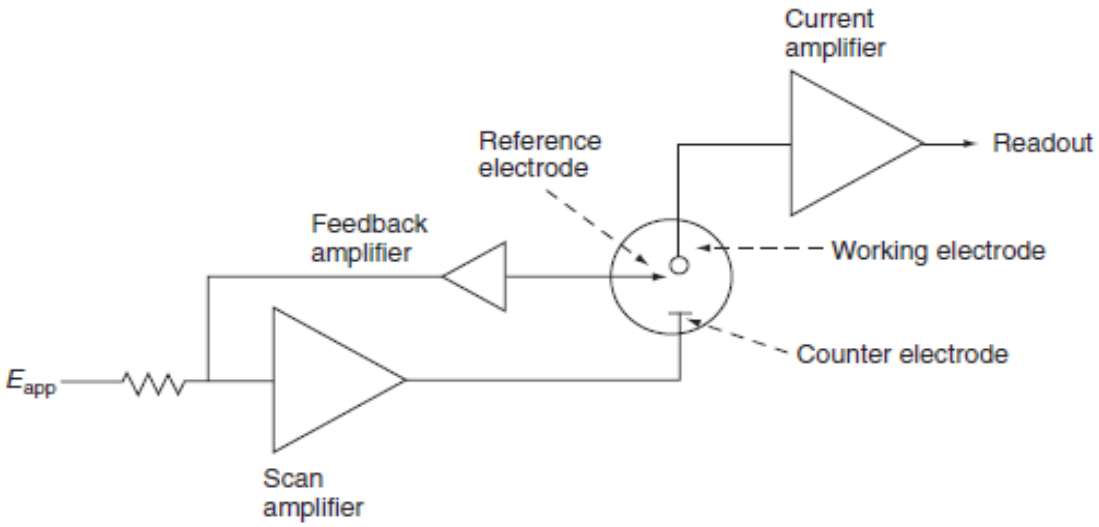


Figure 1.15: Schematic diagram of a three electrode potentiostat (Wang, 2006).

The surface and composition of working electrode strongly influences the performance of sensors which are used for voltammetric measurements (Wang, 1994). The basis of the most popular electrodes used for the determination of metals is mercury, for example the hanging mercury drop electrode (HMDE) (Higson, 2003) or the mercury film electrode (MFE) (Kokkinos *et al.*, 2008). To design an environmental friendly sensor an alternative has to be used, such as the very popular gold electrode. Hence, sensors containing mercury are not considered in this thesis.

All the different kinds of voltammetric methods are described in the literature, such as stripping voltammetry (Higson, 2003; Patnaik, 2004; Wang, 2006). This is a very sensitive and inexpensive method to detect trace metals (Wang, 2006). The high sensitivity is due to the fact that the first step of the measurement is a pre-concentration of the metals at the working electrode using a chelating agent. Unfortunately, the design of a proper stripping voltammetric method for nickel ions is complex for several reasons. One of the most important reason is that the reduction of Ni(II) may be irreversible and, once reduced, nickel shows a strong tendency to form compounds with other metals, which influences the stripping procedure and, hence, falsifies the results (Baldwin *et al.*, 1986). One method which does not struggles with these kind of problems is adsorptive stripping voltammetry (AdSV). This is the reason why for the detection of nickel this technique is by far the most common one described in the literature. Moreover, AdSV is a very powerful method to determine analyte concentrations down to parts per billion (Higson ,2003). The lower detection limit may be as low as 1×10^{-10} M, when no contamination takes place (Wang, 2006).

Before the determination of the nickel concentration using adsorptive stripping voltammetry a chelating agent has to be added to the sample solution. For nickel primarily dimethylglyoxime (DMG) is used (Wang, 2006). This ligand forms a complex with nickel. The following reaction takes place (as suggested by Baxter *et al.*, 1998):

$$Ni^{2+} + 2\,dmgH_2 \leftrightarrow Ni(dmgH)_2 + 2H^+ \qquad (6)$$

The actual measurement procedure is separated into two steps. These two steps are called accumulation and stripping. The principle measurement procedure (a) and the resulting voltammogram (b) can be seen in Figure 1.16.

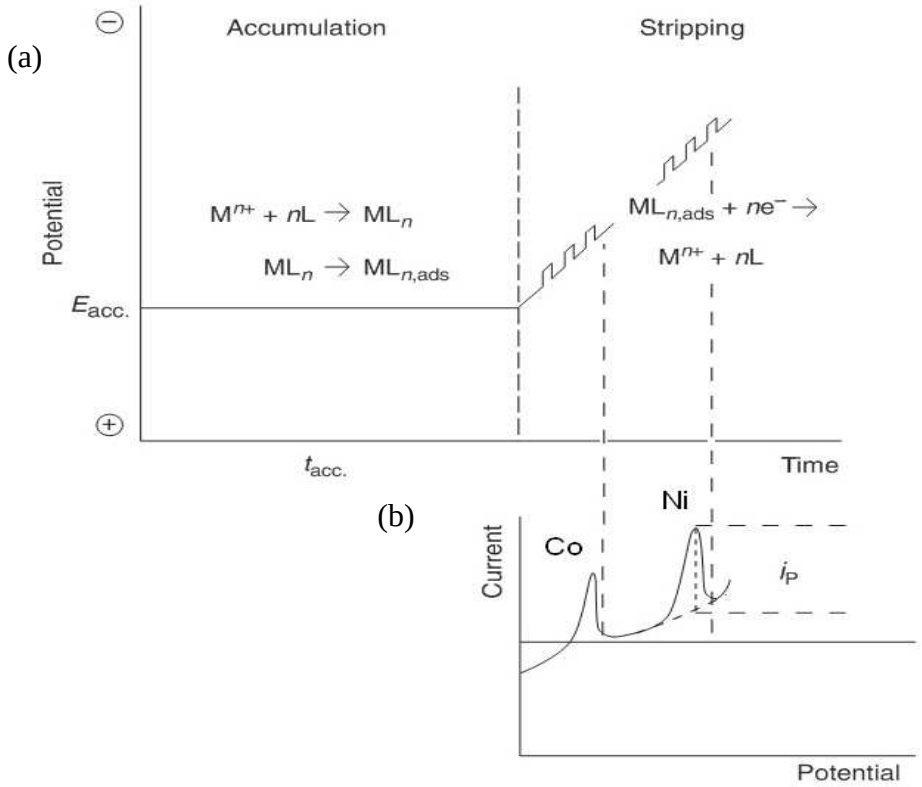


Figure 1.16: Adsorptive stripping voltammetry: (a) Accumulation and stripping steps in adsorptive stripping measurements of a metal ion (M^{n+}) in the presence of an appropriate chelate agent (L), (b) along with the resulting voltammogram (adapted from Wang, 2006).

During the accumulation step a constant negative potential is applied, which causes the analyte (nickel-DMG complex) to be adsorbed to the working electrode. The maximum adsorbed density depends on the surface concentration and size of the adsorbed complex (Wang, 2006). The following reaction takes place (as suggested by Baxter *et al.*, 1998):

$$Ni\,(dmgH)_2 \quad \leftrightarrow \quad Ni\,(dmgH)_{2,adsorbed} \tag{7}$$

When enough of the analyte is adsorbed to the surface of the working electrode a negative going potential scan is initiated, which is called stripping. During this step the complex reduces and dissolutes from the working electrode again at a certain potential.

The reduction of the nickel-DMG complexes generates electrons, which are increasing the current. This can be measured and displayed in a voltammogram. Since the binding energy of each metal is different from the others, each metal dissolves at a different potential. Hence, the potential at which the current is increasing can be related to a specific metal. Moreover, the current peak area can be related to the metal concentration (see Figure 1.16 (b)). During the reduction of the analyte following reaction takes place (as suggested by Baxter *et al.*, 1998):

$$Ni\,(dmgH)_{2,adsorbed} + 2\,e^{-} + 2H^{+} \rightarrow Ni + (dmgH_2)_{desorbed} \quad (8)$$

Available Sensors: The use of chemically modified electrodes has been reported for nickel analysis where a carbon paste is mixed with DMG and used as the working electrode (Baldwin *et al.*, 1986). If the DMG ligand is also added to the solution, a complex with Ni(II) is formed and can be detected. The DMG ligand is still used today to detect nickel with adsorptive stripping voltammetry; however, the materials used for the working electrode are bismuth (Wang and Lu, 2000; Hutton *et al.*, 2005; Kokkinos *et al.*, 2008) and lead (Korolczuk and Tyszczuk, 2006; Tyszczuk and Korolczuk, 2007). The bismuth film electrode (BFE) and lead film electrode (LFE) may increase sensitivity and selectivity. Lead, of course, is not environmentally friendly, especially not compared to bismuth which can be considered to have this characteristic (Wang and Lu, 2000). Recently a new electrode based on antimony was developed (Jovanovski *et al.*, 2009; Kokkinos *et al.*, 2009). The antimony film electrode (SbFE) can also be used to detect nickel. However, the toxicity data of antimony are limited, but it is considered to be less toxic than mercury (Kokkinos *et al.*, 2009).

A list of all located sensors in the literature that use stripping voltammetry for nickel detection can be seen in Table 1.14. In this table the most important properties of the sensors are illustrated as well as their applications in the detection of nickel concentration.

Table 1.14: Properties of different sensors using adsorptive stripping voltammetry for nickel detection.

Reference*	Electrode	Design	Measurement Procedure	LOD (µg L^{-1})	Accu-mulation Time	Properties of Ammonia Buffer Solution	Applications
Wang *et al.* (1996)	DMG-Carbon ink	Screen-printed sensor	DPV	5	30 s	0.1M, pH 9.2	River water sample
Wang and Lu (2000)	Bismuth ex situ	Glassy carbon electrode	AdSV	0.8	180 s	0.01M, pH 9.0	n.a.
Hutton *et al.* (2003)°	Bismuth ex situ	Glassy carbon disk	AdSV, CP	0.26	60 s	0.01M, pH 9.2	n.a.
Morfobos et al. (2004)°	Bismuth ex situ	Rotating disc	SWV	0.1	300 s	0.2 M, pH 9.2	duraluminium, iron ore and water sample
Hutton *et al.* (2005)°	Bismuth ex situ	Microelectrode	SWV	0.09	120 s	0.01M, pH 9.2	n.a.
Hutton *et al.* (2006)°	Bismuth ex situ	Microelectrode	DPV, SWV	0.056	120 s	0.01M, pH 9.2	Saliva, sweat, aqueous humour and cerebrospinal fluid
Legeai *et al.* (2006)	Bismuth ex situ	Copper substrate	SWV	0.58	900s	0.01M, pH 9.0	industrial electrolytic baths, ground water and tap water
Kokkinos *et al.* (2008)	Bismuth	Cell-on-a-chip	SWV	0.1	90 s	0.1M, pH 9.2	River water sample
Jovanovski *et al.* (2009)#	Antimony ex situ	Glassy carbon electrode	SWV	n.s.	30 s	0.01M, pH 9.2	n.a.
Kokkinos *et al.* (2009)	Antimony	Cell-on-a-chip	SWV	0.2	60 s	0.1M, pH 9.2	n.a.

* All studies used DMG as complexing agent

° Simultaneous determination of cobalt

Main focus on lead and cadmium

AdSV Absorptive Stripping Voltammetry

CP Chronopotentiometry

DPV Differential pulse voltammetry

SWV Square wave voltammetry

Adsorptive stripping voltammetry for the detection of nickel ions, with DMG as a ligand, is already an established technique. In the 1990's carbon ink based working electrodes were used. Wang *et al.* (1996) described the use of a disposable nickel screen-printed electrode on the basis of a DMG-containing carbon ink. The sensor is able to detect Ni(II) in a linear range from 5 µg L^{-1} to 200 µg L^{-1} Ni(II) after a 30 second accumulation time period. Furthermore, the sensor was successfully tested to detect nickel in a river water sample. In the same year this kind of arrangement was successfully used to determine nickel, cobalt, mercury and palladium simultaneously. After 120 seconds' pre-concentration, a detection limit of 0.5 µg L^{-1} is feasible. This technique was also used for rice, hair and tea samples.

Wang and Lu (2000) described an AdSV sensor with an adsorption time of 180 seconds and a linear working range from 0.8 µg L^{-1} to 80 µg L^{-1}. Hutton *et al.* (2003) were able to detect cobalt and nickel ions simultaneously. The detection limit for Ni(II) was 0.25 µg L^{-1} after an accumulation time of 60 seconds, in the presence of 2 µg L^{-1} cobalt. However, a chronopotentiometric mode was used, instead of AdSV. Morofobos *et al.* (2004) measured the nickel concentration of duraluminium, iron ore and water with a bismuth rotating disc electrode after an accumulation time of 300 seconds. With a bismuth film microelectrode, nickel determination is possible in the presence of cobalt, cadmium and lead ions (Hutton *et al.*, 2005). The detection limit of nickel is 90 ng L^{-1} when pre-concentrated for 120 seconds. Measurement of nickel in an AdSV mode was possible in a solution containing 20 µg L^{-1} nickel, cobalt, cadmium and lead. This technique was also used to measure successfully nickel concentrations in simulated and real body fluids (Hutton *et al.*, 2006). The properties of the electrode are a low limit of detection of 56 ng L^{-1} after an adsorptive time of 60 seconds. Cobalt was measured simultaneously. The bismuth film electrode was successfully tested in simulated saliva and sweat, and was not compromised in complex biological matrices of cerebrospinal fluid and aqueous humour. After an accumulation time of 900 seconds it is possible to measure the nickel concentration of industrial electrolytic baths, ground water and tap water, with a copper electrode plated with bismuth (Legeai *et al.*, 2006). Kokkinos *et al.* (2008) described a disposable cell-on-chip bismuth film electrode. After 90 seconds of pre-concentration, the linear range is from 0.1 µg L^{-1} to 40 µg L^{-1} in square wave AdSV mode. The interference of other metals was also investigated. Lead, mercury, copper,

iron, aluminium, cadmium, thallium, calcium and manganese do not influence the results (error ± 10%) up to a 100 fold higher concentration than nickel. For cobalt only a 10 fold higher concentration is tolerable. Furthermore, the sensor was successfully tested to detect nickel in a river water sample.

More recently Jovanovski *et al.* (2009) and Kokkinos *et al.* (2009) used an antimony film electrode for nickel analyses. However, Kokkinos *et al.* (2009) researched mainly the characterisation of the electrode (wide pH range is possible) and Jovanovski *et al.* (2009) had their main focus on lead and cadmium. A high sensitivity was observed already after only 30 seconds of accumulation.

1.8 NICKEL DETECTION IN URINE

The state-of-the-art analytical techniques used for nickel detection in biological samples, such as urine, are atomic absorption spectrometry and inductively coupled plasma - atomic emission spectroscopy (ICP-AES) (ATSDR, 2005). Both detection techniques are laboratory based and hence, have a long turn around time. However, in particular voltammetric methods, which are highly sensitive, precise and accurate, are becoming important for nickel determination in biological materials (ATSDR, 2005).

So far, different electrochemical techniques have been used for nickel determination in urine, such as amperometry (Bond *et al.*, 1986), ion-selective electrodes (Jain *et al.*, 2005) and adsorptive stripping voltammetry (Mahajan and Kaur, 1996; Horng *et al.*, 2003). In all these methods either wet digestion or acidification was used as pretreatment of the urine samples. This was done because in urine, surface active substances are present (Bond *et al.*, 1986). It is also known that heavy metals interact with proteins in human body fluids (Wang, 1982), which can lead to unexpected interferences. These interferences can be minimised using wet digestion or acidification (Bond *et al.*, 1986). However, it is proven that many heavy metals, *e.g.* thallium, cadmium, lead and copper, can be detected in urine without any pretreatment using stripping voltammetry (Wang, 1982). For example Lund and Eriksen (1979) used differential pulse anodic stripping voltammetry to detect cadmium and lead in urine without any pretreatment, even the pH was not adjusted. A significant increase of the peak current was reported at temperature levels of 40°C compared to 25°C. It was

achieved to measure concentrations of 0.6 µg L^{-1} cadmium and 17 µg L^{-1} lead in real urine samples. An even higher sensitivity was reported after urine was digested and interference of organic compounds (see Table 1.7 for all major compounds in urine) was minimised. The authors recommended using some kind of sample pretreatment before measurement. However, such a pretreatment of the urine sample introduces a high risk of contamination, and is very time consuming and expensive (Bond and Reust, 1984). These arguments are the reason why a measurement without any pretreatment is desired for a rapid, cheap and portable nickel sensor.

1.8.1 Commercially Available Portable Detection Techniques

During the online research no portable commercially available sensor was located for nickel exposure assessment in urine. The research was focused on websites in English and German. Only laboratory systems, as described in the section above, with a procedure description for nickel concentration measurement in urine are currently available.

However, as discussed in the sections above regarding spectroscopic, bioanalytical and electroanalytical sensors (chapters 1.7.3 to 1.7.5), there are various papers published, which deal with the determination of nickel concentration in different media. Although mainly water, air and soil, some also focus on human body fluids.

Furthermore, there are also sensors commercially available that detect nickel in water and soil, for example the QUANTOFIX® Nickel dip sticks (Machery-Nagel, 2009a) and VISOCOLOR® ECO Nickel test kits (Machery-Nagel, 2009b). These sensors use dimethylglyoxime (DMG) powder, which binds with nickel and changes the colour to red (Machery-Nagel, 2009b). The results can be compared with a colour scale. Although they can be used for a huge pH range (2 to 7), they are influenced by other heavy metals, *i.e.* mainly iron and cobalt (Machery-Nagel, 2010), and the dip stick has a limit of detection of 1 mg L^{-1} and the test kit of 100 µg L^{-1}. However, the sensitivity depicted in these devices is not sufficient for urine analysis.

1.9 AIMS AND OBJECTIVES

It is beyond question that nickel and its compounds are toxic and carcinogenic to humans, especially after exposure to nickel in air. In particular workers in the nickel industry, from mining to refining, may be exposed to high concentrations. Hence, they have to be protected and in fact the legal exposure limits have been already reduced in recent years, at least in industrialised countries. To monitor these limits, urine is in the focus of interest. It is not only easy to collect urine but it is also non-invasive and a targeted exposure measurement for each individual.

However, urine is a complex medium and it is difficult to measure trace metals in it. Hence, powerful methods like EAAS, ICP-MS or ID-MS are currently used to determine the nickel concentration in urine. Since these techniques are expensive, time consuming and laboratory based, there is a need for a cheap, rapid and portable alternative. Various methods were evaluated in the introduction. In summary, spectroscopic methods are not sensitive enough and biosensors are struggling with low selectivity. Fortunately, electrochemical techniques have better properties. These are, on the one hand, ion-selective electrodes and, on the other hand, stripping voltammetric based sensors. In particular, the latter shows great promise for a highly selective and sensitive *in situ* measurement of nickel.

The aim of the work presented in this thesis is concerned with the development of an electrochemical sensor, based on AdSV, for the detection of nickel in urine. The envisaged device is a disposable three electrode screen-printed sensor attached to a portable electrochemical analyser. The device transduces the response of nickel ions into an electrical signal (voltammogram), which can be evaluated.

1.9.1 Specific Aims

- Assess optimal operational parameters for a bare carbon working electrode
- Design a screen-printed sensor with a bismuth film working electrode

- Assess the potential of using a gold film working electrode
- Enhance the detection performance of the best sensor design to detect nickel concentrations in the range from 5 to 150 µg L^{-1}
- Apply the best sensor design in a direct nickel measurement of a spiked real urine sample

CHAPTER 2

EXPERIMENTAL

2.1 DETECTION OF NICKEL (II) USING SCREEN-PRINTED MACRO-ELECTRODES

2.1.1 Reagents and Solutions

All chemicals were of analytical grade and used as received. Ammonia solution (25%) and ammonium chloride (NH_4Cl) were obtained from Merck (Darmstadt, Germany). Bismuth(III) oxide (Bi_2O_3), dimethylglyoxime ($C_4H_8N_2O_2$), ethanol (95%, C_2H_5OH), L-Histidine, Nafion®, nickel atomic absorption standard solution (1000 mg L^{-1} in 2% nitric acid solution), nitric acid (69%, HNO_3), potassium ferrocyanide ($C_6N_6FeK_4$) and HPLC water were received from Sigma-Aldrich (Gillingham, Dorset, United Kingdom). The carbon ink was a E423-SS graphite-based ink from Acheson Colloids (Plymouth, United Kingdom). The determination of the pH value was done with a pH-meter from Hanna Instruments (Portugal).

All laboratory glassware and tubes were of analytic grade. Glassware was washed using nitric acid (1%) and rinsed with HPLC water.

A dimethylglyoxime (DMG) stock solution (0.02 M) was prepared by dissolving an appropriate amount of DMG in ethanol (95%). For the electrolyte, appropriate amounts of the ammonia solution (25%) and ammonium chloride were mixed together to obtain an ammonia buffer solution with a pH value of 9.2 and a concentration of 0.2 M.

The preparation details of all solutions, with the exact production process and used volumes, can be found in Appendix B.

2.1.2 Apparatus

For square-wave adsorptive stripping voltammetric (SWV) and cyclic voltammetry (CV) measurements a laptop-controlled PalmSens (software version 4.1) driven by PSTrace for PalmSens and EmStat version 1.4.1.0 (PalmSens Instruments B. V., The Netherlands) was used. The PalmSens device can be seen in Figure 2.1 and the experimental set-up used is shown in Figure 2.2.

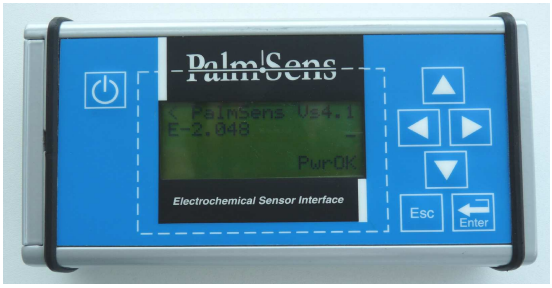


Figure 2.1: Photo of the PalmSens device.

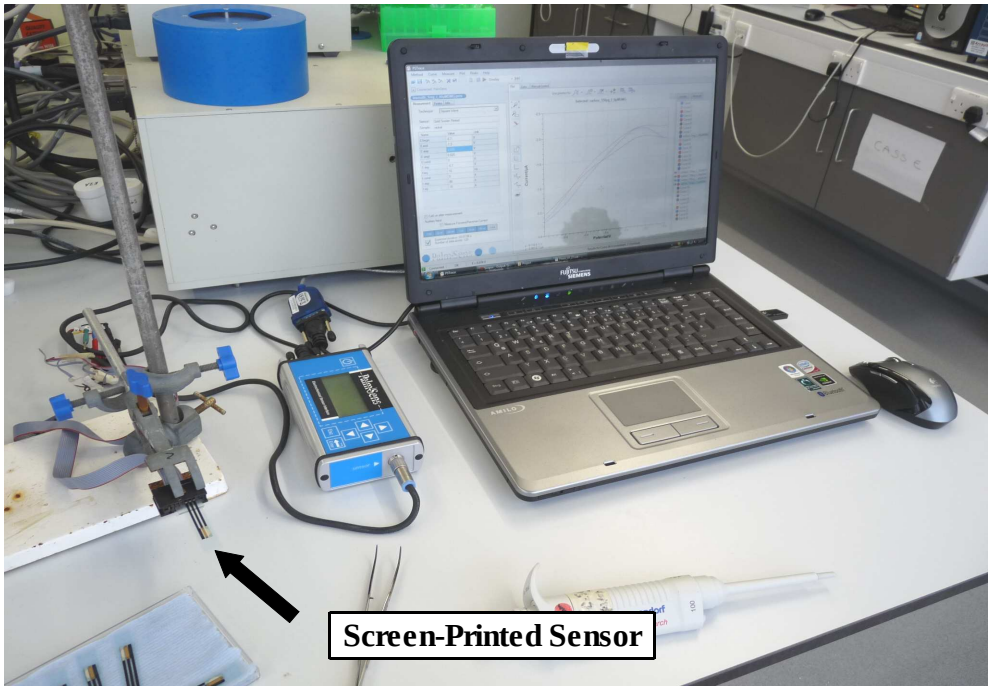


Figure 2.2: Photo of the experimental set-up.

2.1.3 Electrodes and Electrodes Preparation

Two types of screen-printed electrodes were used in this work. The first was printed using the fabrication facilities at DuPont Limited (Bristol, United Kingdom) through a collaboration programme between Cranfield and DuPont. The DuPont sensors were, during fabrication, either dried conventionally in the oven or with infra-red light (IR). The exact composition details of the electrodes are described elsewhere (Abdul Kadir and Tothill, 2010). The second type of sensor was commercially available and purchased from EcoBioServices and Research S.R.L. (Florence, Italy). (see Figure 2.3)

(a)

(b)

Figure 2.3: The gold screen-printed sensors used; a: DuPont; b: Florence.

The design and dimensions of the DuPont sensor are shown in Figure 2.4.

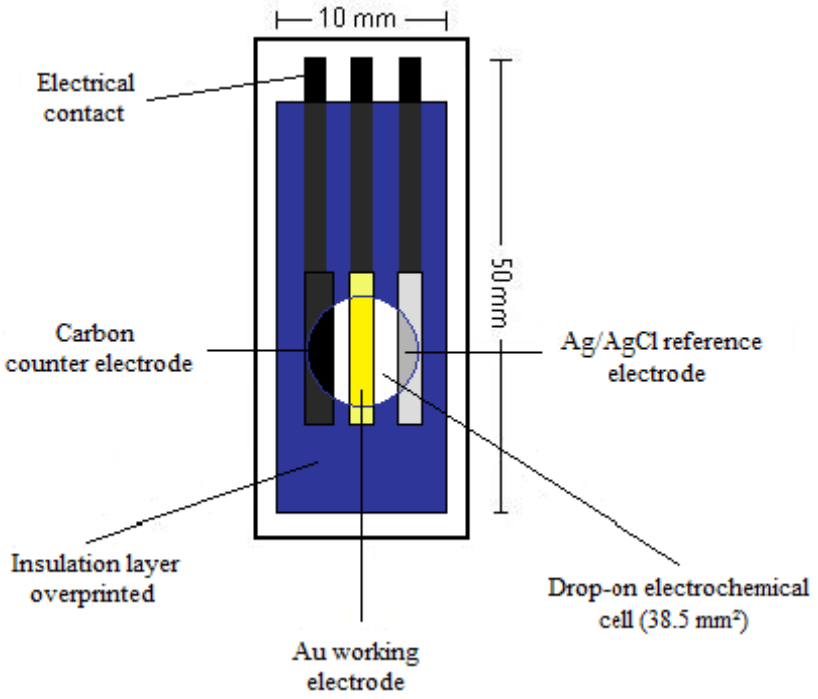


Figure 2.4: Design of the DuPont screen-printed sensor (Adapted from Kadara, 2004).

All procedures described below were the same for the oven dried and IR dried DuPont sensors as well as for the Florence sensors.

Before using the screen-printed sensors, they were cleaned by heating them for 30 minutes at 120°C, then washed with HPLC water and dried with nitrogen gas (N_2).

Gold Film Electrode: The gold film electrodes (GFE) were used without any subsequent treatment after the washing.

Bare Carbon Electrode: To obtain a bare carbon electrode (BCE) the circuit terminals of the CE and WE were changed. Hence, the gold film electrode was used as CE and the carbon electrode as WE.

Bismuth Film Electrode: To obtain a bismuth film screen-printed carbon electrode, the screen-printed sensors were prepared as described above for the unmodified bare electrodes. Afterwards carbon ink with a bismuth(III) oxide content of 2, 5 or 10% was mixed in a mortar and then homogenised (Kadara and Tothill, 2008). This mixture was then brushed on the carbon electrode and dried at 120°C for 60 minutes (Figure 2.5). Following the heating, the sensors were washed with HPLC water and dried with N_2. Once again the circuit terminals of the CE and WE were changed to use the modified carbon electrode as WE. These sensors are referred to as bismuth film electrodes (BFE).

Figure 2.5: Electrochemical cell of a DuPont sensor with a bismuth film brushed on the carbon electrode.

Bare Carbon Electrode covered with Nafion: Nafion modified electrodes (Palchetti *et al.*, 2000) were prepared by drop-coating 1 µl of Nafion (5% w.t. in alcohol and water) with a microliter pipette onto the working or reference electrode, respectively. To cover the whole electrochemical cell on the screen-printed sensor, 5 µl of Nafion were used. The sensors were then left to dry at room temperature for at least ten minutes. The sensors treated with Nafion were solely operated as BCEs.

2.1.4 Measurement Procedures

All measurements were carried out by placing a 100 µl sample drop on the sensor. Particular emphasis was placed on covering the whole electrochemical drop-on cell (see Figure 2.4) with the sample drop. If not specified otherwise, for each measurement a new sensor was used.

Electrochemical characterisation of the electrodes was investigated using cyclic voltammetry and potassium ferrocyanide as the benchmark compound. Therefore, a 2 mM solution of potassium ferrocyanide in an ammonia buffer (0.2 M, pH 9.2) was prepared. The scanning was carried out in the potential range from -0.6 to 1 V and with a varying scan rate from 10 to 40 mV s^{-1}.

The nickel peaks were determined using SWV. The exact operating conditions are listed in Table 2.1. The experiments were carried out using either DuPont BCE, BCE covered with Nafion, BFE, or GFE sensors or Florence BCEs. The nickel solution samples were prepared by diluting various concentrations (0 – 200 µg L^{-1}) of the nickel standard solution in the 0.2 M ammonia buffer solution. If not stated otherwise 30 µM DMG (from the DMG stock solution), as complexing agent, was also added to the sample solution.

Table 2.1: Operating Conditions for SWV (As used by Hutton et al., 2005 and 2006).

Square Wave Voltammetry Operating Conditions	
Conditioning Potential	0V
Duration	0s
Deposition	-0.7V
Duration	90s*°
Equilibration Time	15s#
Cell off after Measurement	Yes
Frequency	10Hz
Initial Potential	-0.7V
End Potential	-1.3V
Step Potential (Scan Rate)	5mV
Amplitude	25mV
Standby Potential	0V

* if not stated otherwise
° taken for nickel determination (without cobalt)
for stirred and unstirred experiments

All experiments were conducted under standard laboratory conditions. Furthermore, for all procedures a CoSHH (Control of Substances Hazardous to Health) and environmental risk assessment were carried out to meet Cranfield Health's Code of Practice specifications and legal requirements.

2.2 DETECTION OF NICKEL (II) USING SCREEN-PRINTED MICROBAND-ELECTRODES

2.2.1 Reagents and Solutions

Reagents and solutions were the same as used in Section 2.1.1. The preparation details of all solutions, with the exact production process and volumes used, can be found in Appendix B.

2.2.2 Apparatus

The experimental set-up was similar to the one described in Section 2.1.2, with the difference that the sensor was put in a glass beaker placed on a magnetic stirrer.

2.2.3 Electrodes and Electrodes Preparation

The screen-printed sensors fabricated by DuPont Limited (Bristol, United Kingdom) were used. Further details about the sensor can be found in Section 2.1.3.

All procedures described below were the same for the oven dried and IR dried sensors.

The front part of the sensor was cut away using a scissors (Dudeney, 2008). The separating line was directly at the upper end of the drop-on-cell (see Figure 2.6). A photo of the front and top view of the microband-electrode can be seen in Figure 2.7.

Gold Film Microband-Electrodes: The gold film microband-electrodes (GFME) were used without any subsequent treatment after the washing.

Bare Carbon Microband-Electrode: To obtain a bare carbon microband-electrode (BCME) the circuit terminals of the CE and WE were changed. Hence, the gold film electrode was used as CE and the carbon electrode as WE.

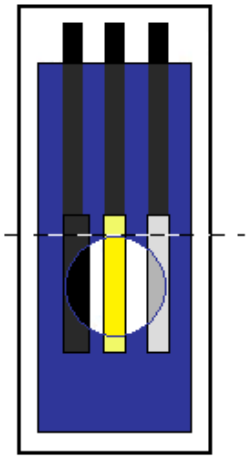
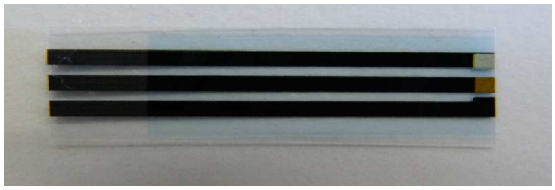


Figure 2.6: Position of the separating line for the production of a microband-electrode.

(a)



(b)

Figure 2.7: Photo of a microband-electrode; a: Top view; b: Frontal view.

2.2.4 Measurement Procedures

The nickel peaks were determined using SWV and electrochemical characterisation of the electrodes was carried out with CV. The exact operating conditions for SWV are listed in Table 2.1. The experiments were carried out using either BCMEs or GFMEs. All measurements were carried out by placing the sensor in a 10 ml glass beaker containing 7 ml of the sample solution. Under stirring conditions the solution was stirred at 350 rpm during the 90 second deposition time. Equilibrium and measurement took place in quiescent solution.

The electrochemical characterisation was carried out in an ammonia buffer solution (0.2 M, pH 9.2) with 2 mM potassium ferrocyanide as benchmark. The scans were carried out in the potential range from -0.6 to 1 V. Unlike for the nickel measurement described below, for each scan rate (10 to 40 mV s^{-1}) a new sensor was used.

For the nickel measurement, the nickel concentration in the 0.2 M ammonia buffer solution was successively increased from 0 to 200 µg L^{-1} and was measured using the same sensor without any treatment from one concentration step to the next. Furthermore, at the beginning of the measurement, before 30 µM DMG (from the DMG stock solution) as complexing agent was added to the sample, a blank voltammogram was recorded by measuring the plain buffer solution.

2.3 DETECTION OF NICKEL IN URINE

2.3.1 Reagents and Solutions

Reagents and solutions were nearly the same as used in Section 2.1.1. However, different ammonia buffer solution concentrations (0.01 M, 0.1 M, 0.2 M, 0.267 M and 0.5 M) were used. The preparation details of all solutions, with the exact production process and used volumes, can be found in Appendix B.

2.3.2 Apparatus

The experimental set-up was the same as described in Sections 2.1.2 and 2.2.2 for the macro-electrode and microband-electrode, respectively.

2.3.3 Sample and Sample Preparation

The urine samples were collected from a 26 year old male volunteer (80kg, healthy, non-occupationally exposed to nickel). Early morning urine was collected in a standard glass laboratory vessel. Urine was kept in the fridge until used and all measurements took place within three days of collection.

Before the actual measurement, the urine sample was diluted with ammonia buffer solution. For the buffer capability measurements, urine was diluted in the ammonia buffer solution in a ratio of 1:3, in buffer concentrations of 0.01, 0.1, 0.2, 0.267 or 0.5 M to adjust the pH value of urine to 9.2. For the nickel measurements, urine was diluted in various amounts (0, 1, 2.5, 5, 7.5, 10, or 25%) in a 0.267 M buffer solution, to evaluate the influence of interference at different urine concentrations. Into the sample, 150 µg L^{-1} of the nickel stock solution and, if not stated otherwise, 30 µM DMG as complexing agent, were added.

2.3.4 Measurement Procedures

The pH values were determined with a pH-meter from Hanna Instruments (Portugal), which was calibrated before each measurement. All electrochemical experiments were either carried out using BCEs covered with Nafion or BCMEs (both IR dried). The nickel peaks were determined using SWV. The exact operating conditions can be found in Table 2.1.

The macro-electrode nickel measurements were carried out by placing a 100 µl sample drop on the sensor. Particular emphasis was placed on covering the whole electrochemical drop-on cell (see Figure 2.4) with the sample drop. For each measurement a new sensor was used.

The microband-electrode nickel measurements were carried out by placing the sensor in a 10 ml glass beaker containing 7 ml of the sample solution. For each measurement a new sensor was used.

CHAPTER 3

RESULTS

3.1 DETECTION OF NICKEL (II) USING SCREEN-PRINTED MACRO-ELECTRODES

3.1.1 Electrochemical Characterisation of the Screen-Printed Macro-Electrodes

To characterise the working electrode (WE), cyclic voltammetry was used. For a 2 mM potassium ferrocyanide solution in ammonia buffer and a scan rate of 30 mV s^{-1}, the cyclic voltammograms at the bare carbon electrode (BCE), bismuth film electrode (BFE) and gold film electrode (GFE) are depicted in Figure 3.1. The peak-to-peak separations obtained for the BCE, BFE and GFE were 0.555 V, 0.410 V and 0.445 V, respectively.

To evaluate the electrochemical behaviour of potassium ferrocyanide at the BCE, BFE and GFE, cyclic voltammograms were also obtained at scan rates of 10, 20, 30 and 40 mV s^{-1} (Figure 3.2).

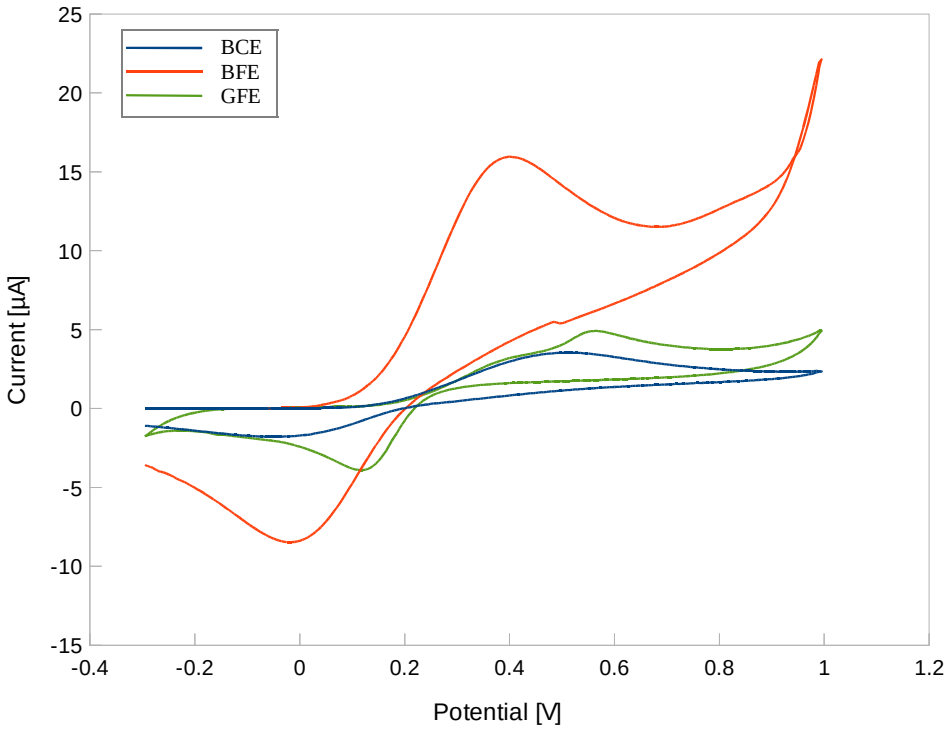


Figure 3.1: Comparison of the cyclic voltammograms of 2 mM potassium ferrocyanide in ammonia buffer solution. BCE, BFE and GFE (Scan rate: 30 mV s^{-1}, all sensors IR dried).

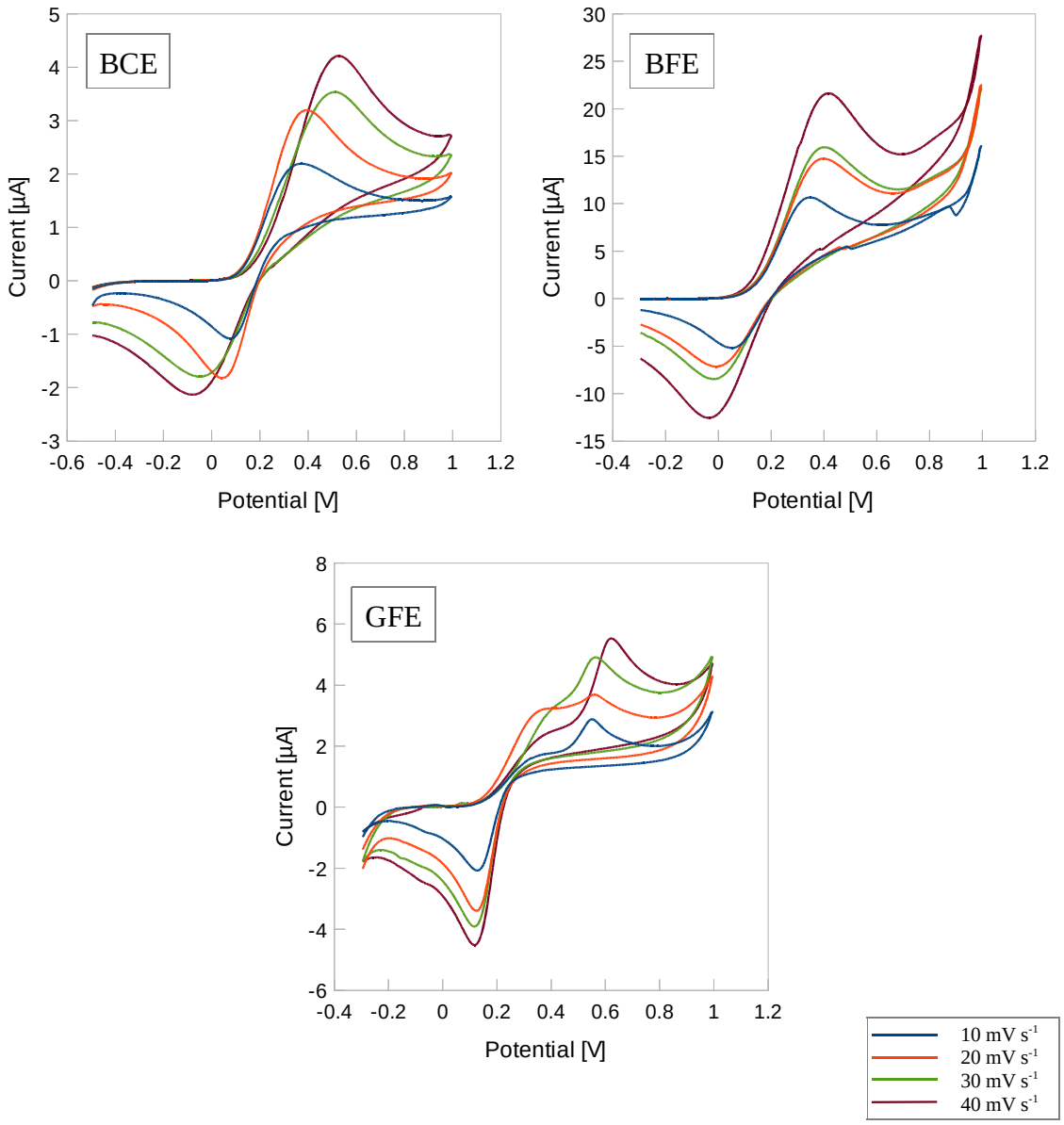


Figure 3.2: Cyclic voltammograms showing the variation in scan rates (10, 20, 30 and 40 mV s^{-1}) for 2 mM potassium ferrocyanide in ammonia buffer solution. Top left: BCE; Top right: BFE; Bottom: GFE. All sensors IR dried.

3.1.2 Background Signal of the Electrolyte

The background signal of the ammonia buffer solution at concentrations of 0.01 M and 0.2 M were evaluated. This was carried out because normally low concentrated buffers are used for adsorptive stripping analysis of nickel, to minimise interference effects. However, to buffer urine to a pH value of 9.2, a higher concentration is required.

In Figure 3.3 the background signal of the ammonia buffer solutions at the BCE, BFE and GFE are depicted. BCE and BFE show a slightly increased background signal at the 0.2 M concentrated buffer, compared to the 0.01 M ammonia buffer. The GFE, on the other hand, shows a strong increase of the background level. The BCE electrode is not only the one with the lowest background signal but also becomes more flat when the buffer concentration is increased.

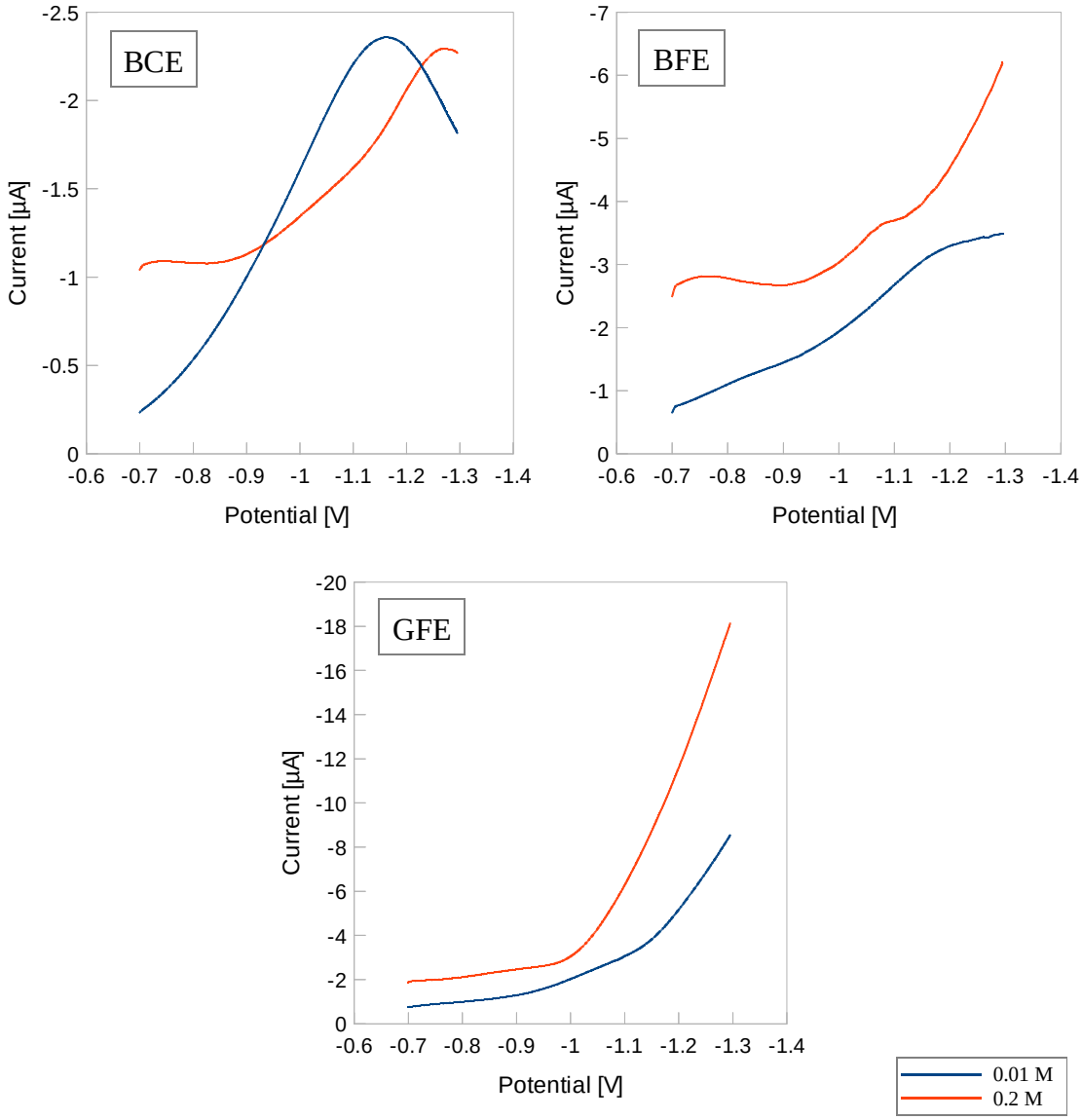


Figure 3.3: Difference of the ammonia buffer solution (pH 9.2, with DMG) background signal of a 0.01 M and 0.2 M concentration. Top left: BCE; Top right: BFE; Bottom: GFE. All sensor IR dried.

A stable background signal is essential for an accurate and repeatable determination of the nickel concentration. To assess the reproducibility of the background signal, two measurements were conducted with the buffer and three measurements with the buffer and 30 µM dimethylglyoxime (DMG) at each electrode (oven and IR dried sensors). The results for the BCE, BFE and GFE are illustrated in Figures 3.4, 3.5 and 3.6, respectively. As can be seen, the signals vary at all electrodes and the signal levels are in general higher at the oven dried sensors. The results at the GFE seem to be the most uniform. However, this is mainly because the signals are much higher than the background signals at the BCE and BFE and, hence, appear better when compared to the other electrodes.

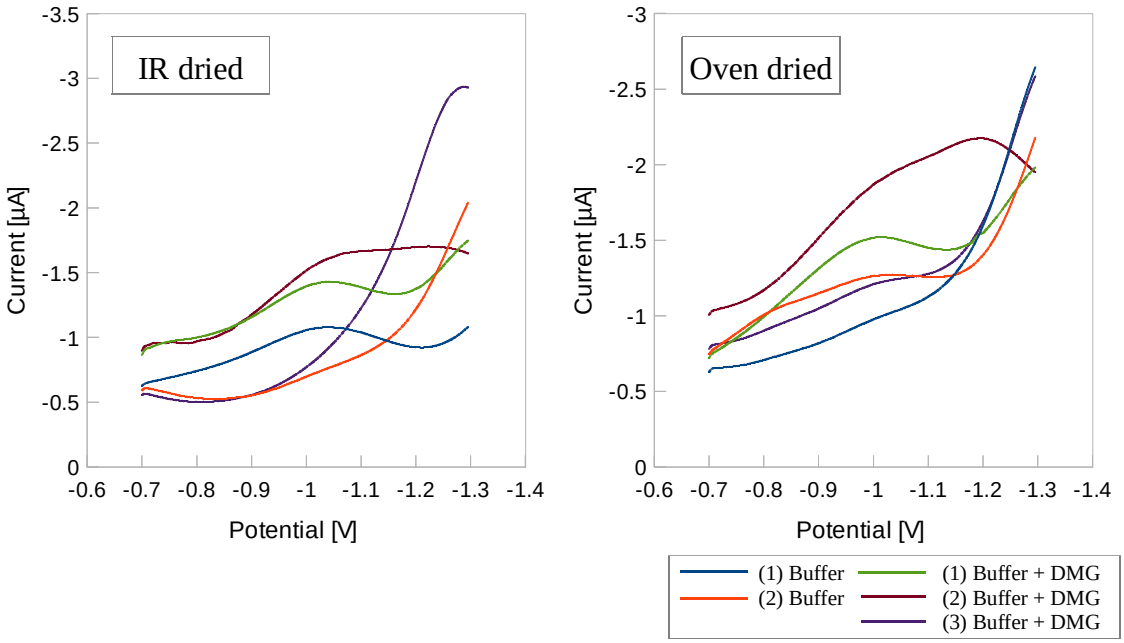


Figure 3.4: Reproducibility of the ammonia buffer solution background signal when measured at a BCE. Two measurements of plain buffer and three measurements of buffer with DMG. Left: IR dried sensor; Right: Oven dried sensor.

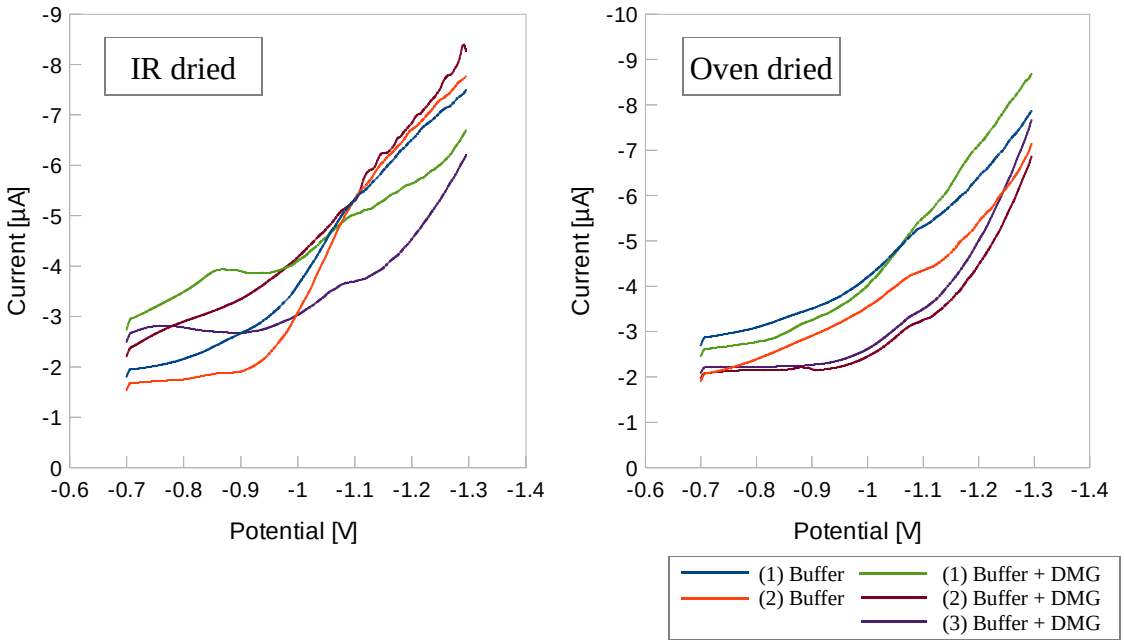


Figure 3.5: Reproducibility of the ammonia buffer solution background signal when measured at a BFE. Two measurements of plain buffer and three measurements of buffer with DMG. Left: IR dried sensor; Right: Oven dried sensor.

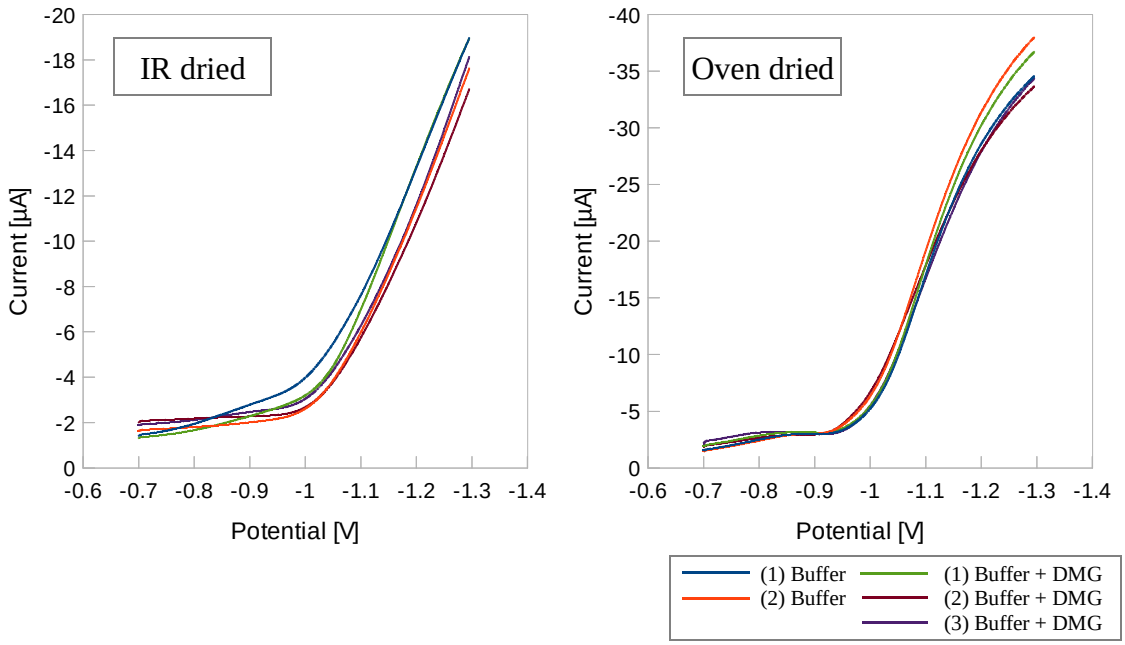


Figure 3.6: Reproducibility of the ammonia buffer solution background signal when measured at a GFE. Two measurements of plain buffer and three measurements of buffer with DMG. Left: IR dried sensor; Right: Oven dried sensor.

To gain more information about the background signal, it was also obtained from a longer potential range at the BCE, BFE and GFE. Figure 3.7 displays the signals from -0.7 V to -2.0 V in ammonia buffer solution with DMG. It can be seen that at all three electrodes there is a huge interference peak. The signals are quite similar; the only difference is that the potential at which they are appearing is shifting towards lower potentials, starting from the highest potential at the BCE (-1.93 V), over the BFE (-1.75 V) to the lowest potential at the GFE (-1.53 V).

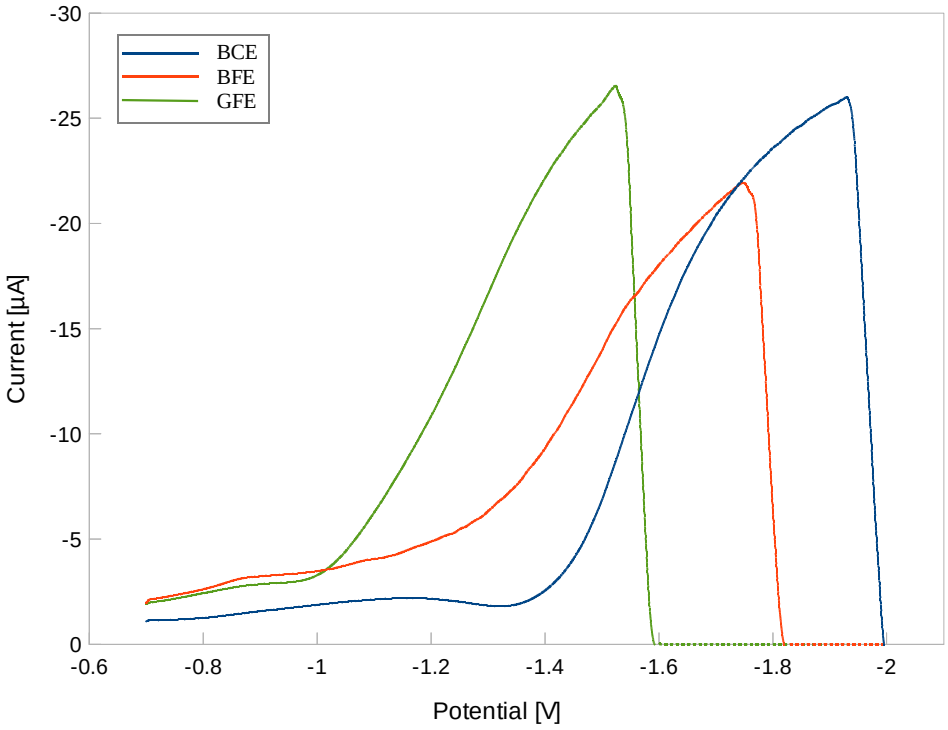


Figure 3.7: Extended background signal of the ammonia buffer solution (with DMG) from -0.7 V to -2.0 V at the BCE, BFE and GFE (all sensors IR dried).

In conclusion, of the last section, the background signal is highly variable. The best behaviour was observed at the BCE and the poorest at the GFE.

3.1.3 Assessment and Optimisation of the Sensors Operational Parameters

A number of variables have an influence on the adsorption capability of the nickel at the electrode. The most important, which could be influenced, were optimised. These are the bismuth concentration in the BFE, the DMG concentration and the deposition time.

Three different bismuth concentrations (2, 5 and 10%) of the BFE were evaluated by measuring 150 µg L^{-1} nickel in ammonia buffer solution containing 30 µM DMG. As can be seen in Figure 3.8, with increasing bismuth concentration the influence of interference phenomena also rises. In fact, only at 2% bismuth concentration is it possible to see a nickel peak around -1.2 V, with a peak area of 62.3 nAV. Hence, for all BFE a bismuth concentration of 2% was used.

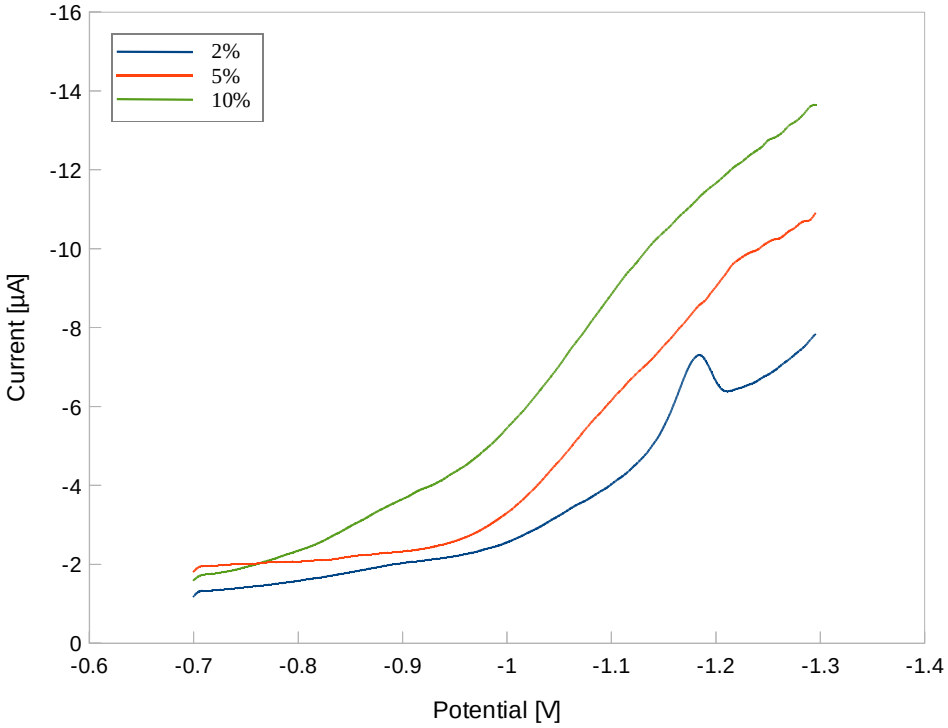


Figure 3.8: Comparison of BFEs with an amount of 2, 5 and 10% bismuth. (Ammonia buffer with DMG and 150 µg L^{-1} nickel, all sensors IR dried).

Sufficient DMG in the sample solution is necessary to allow each nickel ion to complex with two DMG molecules and, hence, be detectable at the electrode. Concentrations of 0, 10, 20, 30 and 40 µM were added and measured at the BCE, BFE and GFE, and the results are depicted in Figures 3.9, 3.10 and 3.11, respectively. Unfortunately, due to strong interference it was not possible to compare the nickel peak areas accurately and, hence, only the graphs are displayed. The highest nickel peak (area: 67.34 nAV) was obtained with an IR dried BCE and 30 µM DMG at -1.15 V. Therefore all other experiments were conducted using 30 µM DMG. The signals of the oven dried BCEs and IR and oven dried BFEs are strongly influenced by interference phenomena. The largest nickel peaks are 39.49 nAV with 30 µM DMG and 27.16 nAV and 28.67 nAV

using 40 µM DMG, respectively. However, the nickel peaks still appear at these electrodes, whereas there are no nickel peaks at all at the GFEs. Due to the totally missing nickel peak at the GFE, no more investigations were conducted at the GFE.

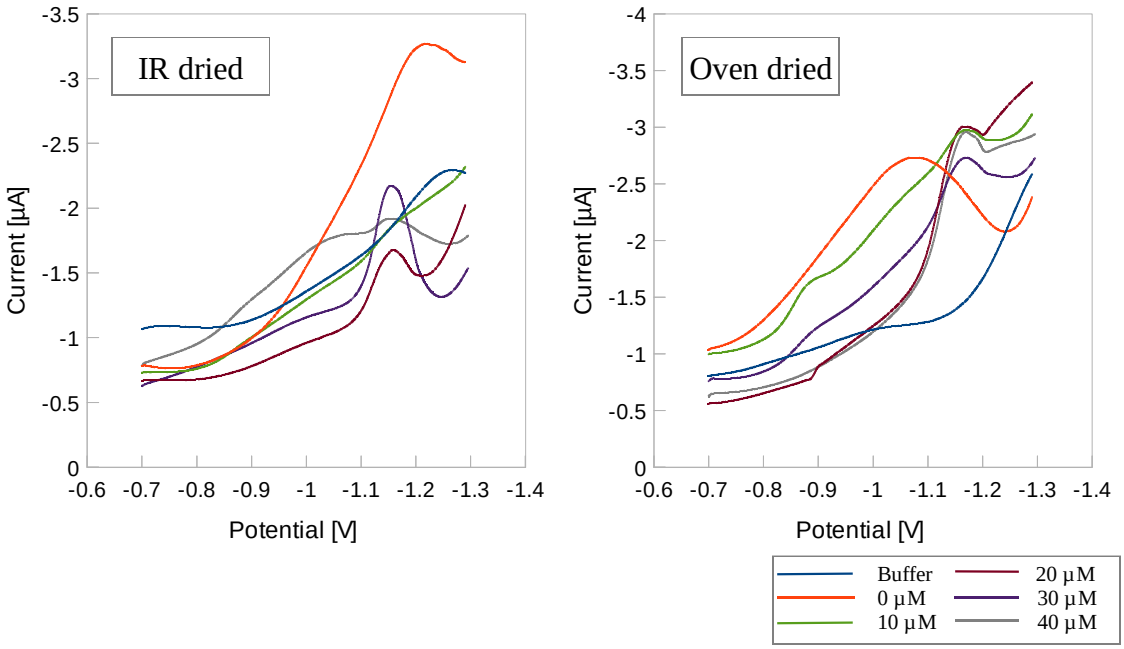


Figure 3.9: Effect of DMG concentration (0, 10, 20, 30 and 40 µM) on the nickel peak area (150 µg L^{-1} nickel) when measured with a BCE (Buffer measurement without any nickel and DMG). Left: IR dried sensors; Right: Oven dried sensors.

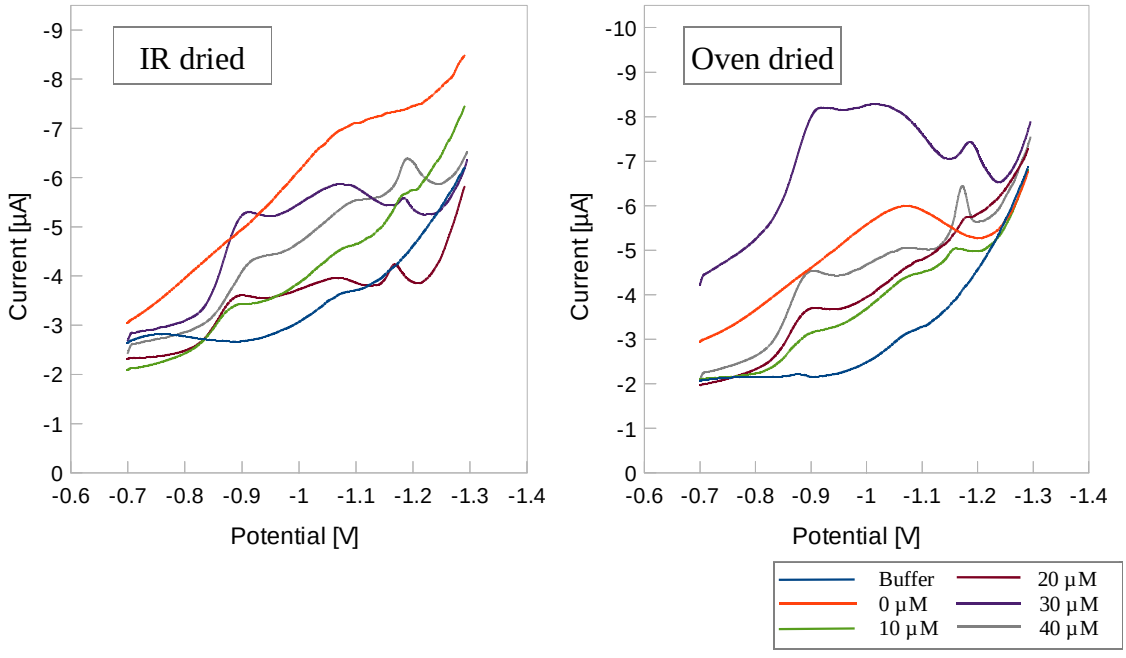


Figure 3.10: Effect of DMG concentration (0, 10, 20, 30 and 40 µM) on the nickel peak area (150 µg L^{-1} nickel) when measured with a BFE (Buffer measurement without any nickel and DMG). Left: IR dried sensors; Right: Oven dried sensors.

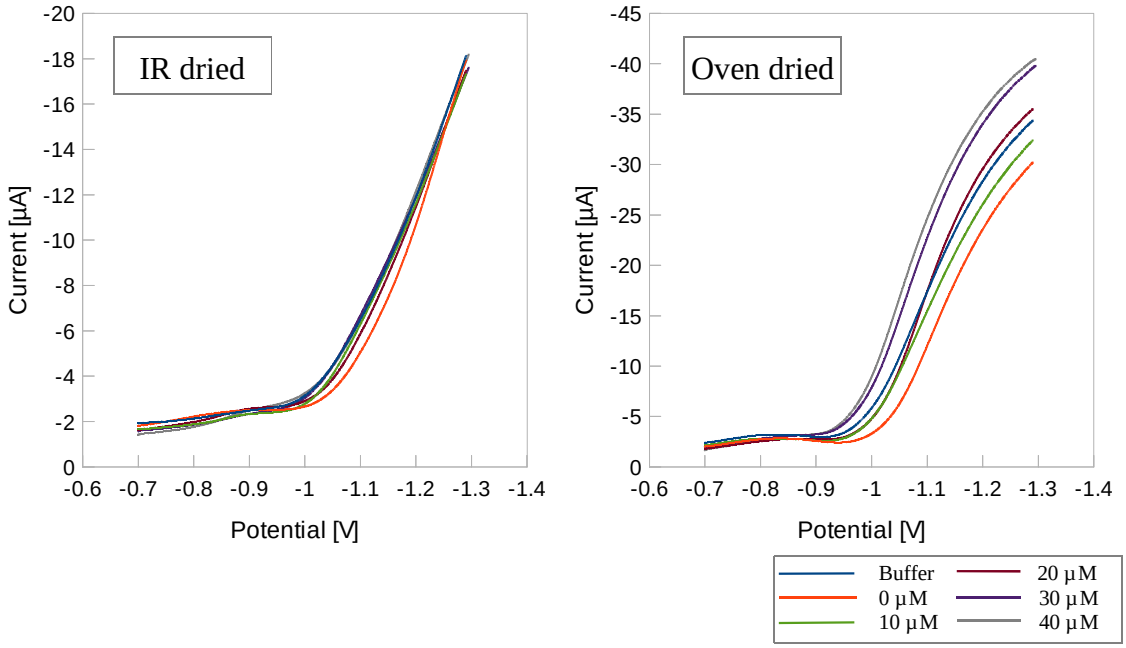


Figure 3.11: Effect of DMG concentration (0, 10, 20, 30 and 40 µM) on the nickel peak area (150 µg L^{-1} nickel) when measured with a GFE (Buffer measurement without any nickel and DMG). Left: IR dried sensors; Right: Oven dried sensors.

An optimal deposition time ensures the pre-concentration of a maximum of nickel-DMG complexes at the electrode in as short a period as possible. To assess the optimal deposition time two different experiments were conducted. Relatively short deposition times of 30, 90, 150, 210 and 270 seconds were used with the BCE sensor. The results, which are shown in Figure 3.12, indicate strong variations in the signal achieved. The greatest nickel peak areas were obtained with a deposition time of 90 (67.34 nAV), 150 (51.92 nAV) and 210 (38.90 nAV) seconds.

On the other hand more information about the background signal was obtained. Therefore, long deposition times up to 22.5 minutes and a larger potential range down to -2.0 V were examined. The results are illustrated in Figure 3.13. It can be seen that with a longer deposition time the peak of the background signal is shifting towards lower potentials and then covering the nickel peak at -1.15 V. Furthermore, a second interference peak is emerging between -0.8 and -1.0 V.

To minimise interference caused by long deposition times, a deposition time of 90 seconds was used for further experiments.

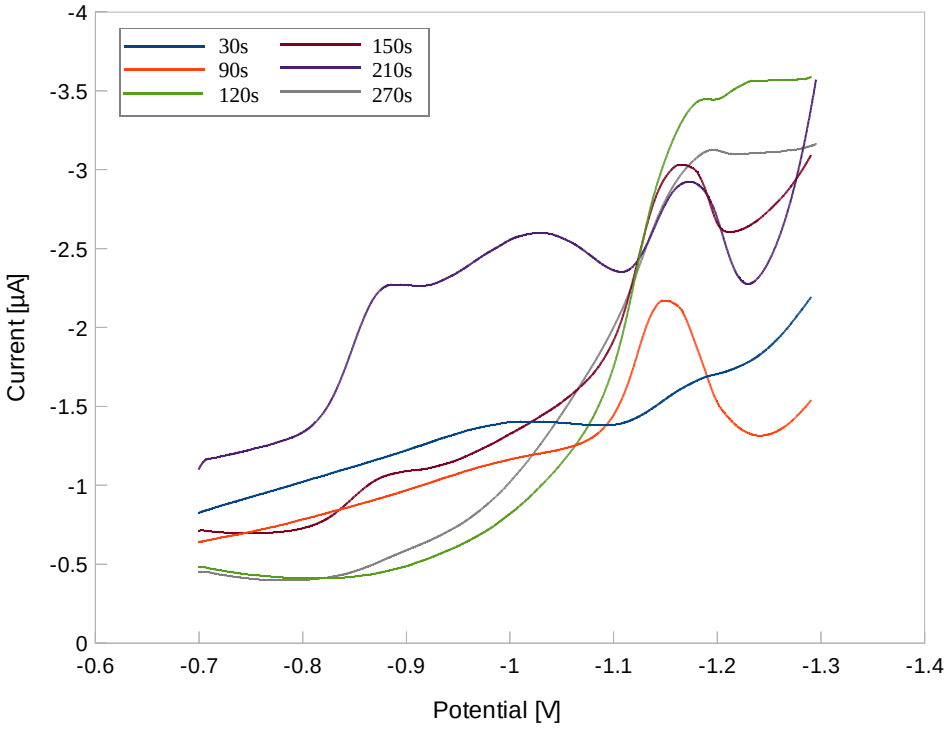


Figure 3.12: Effect of deposition time (30, 90, 120, 150, 210 and 270 seconds) on the nickel peak area (150 µg L^{-1} nickel) when measured with a BCE (all sensors IR dried).

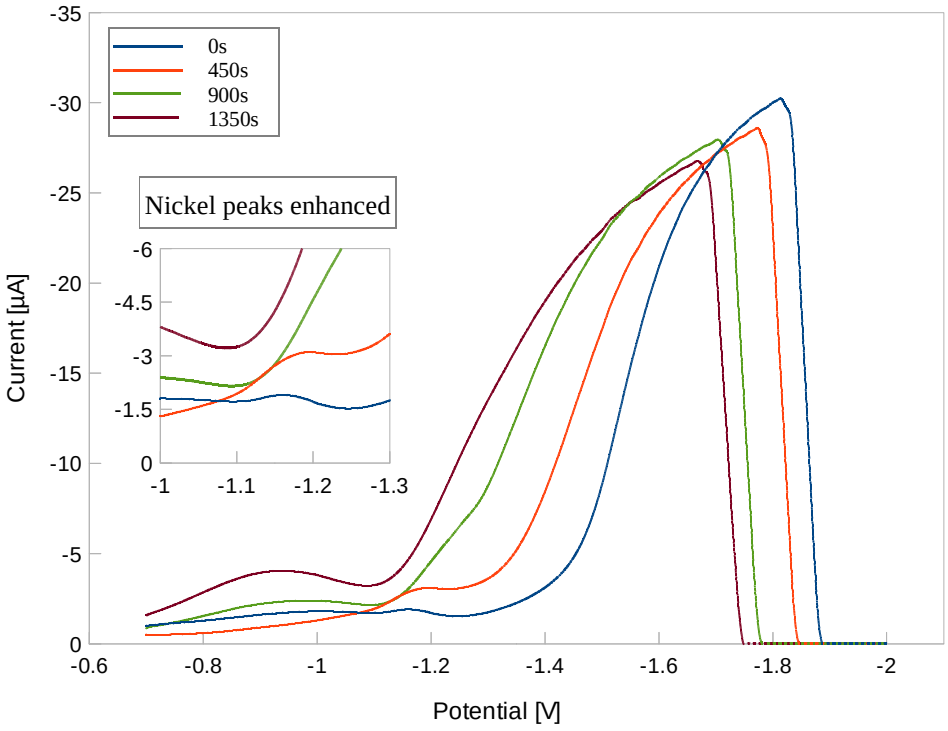


Figure 3.13: Effect on the background signal and nickel peak area (150 µg L^{-1} nickel) when measured with a BCE and very long deposition times of 0, 450, 900 and 1350 seconds. The enhanced graph on the left depicts the effect of time on the nickel peak (at -1.15 V) in more detail. (all sensors IR dried).

3.1.4 Measurement Reproducibility

The reproducibility of the measurements was tested by three experiments. First, an ammonia buffer (with DMG) measurement was conducted and then the sensor was washed with HPLC water and dried with N_2. Afterwards the same sensor was once again used to measure 150 µg L^{-1} nickel added to the same solution. The results of the two different sensors measurements at each electrode (BCE, BFE and GFE) are depicted in Figure 3.14. At all electrodes no nickel peak is appearing after washing and reusing the sensor and, in general, the signal levels are becoming much lower. This indicates that something important, such as silver, is dissolving from the sensor.

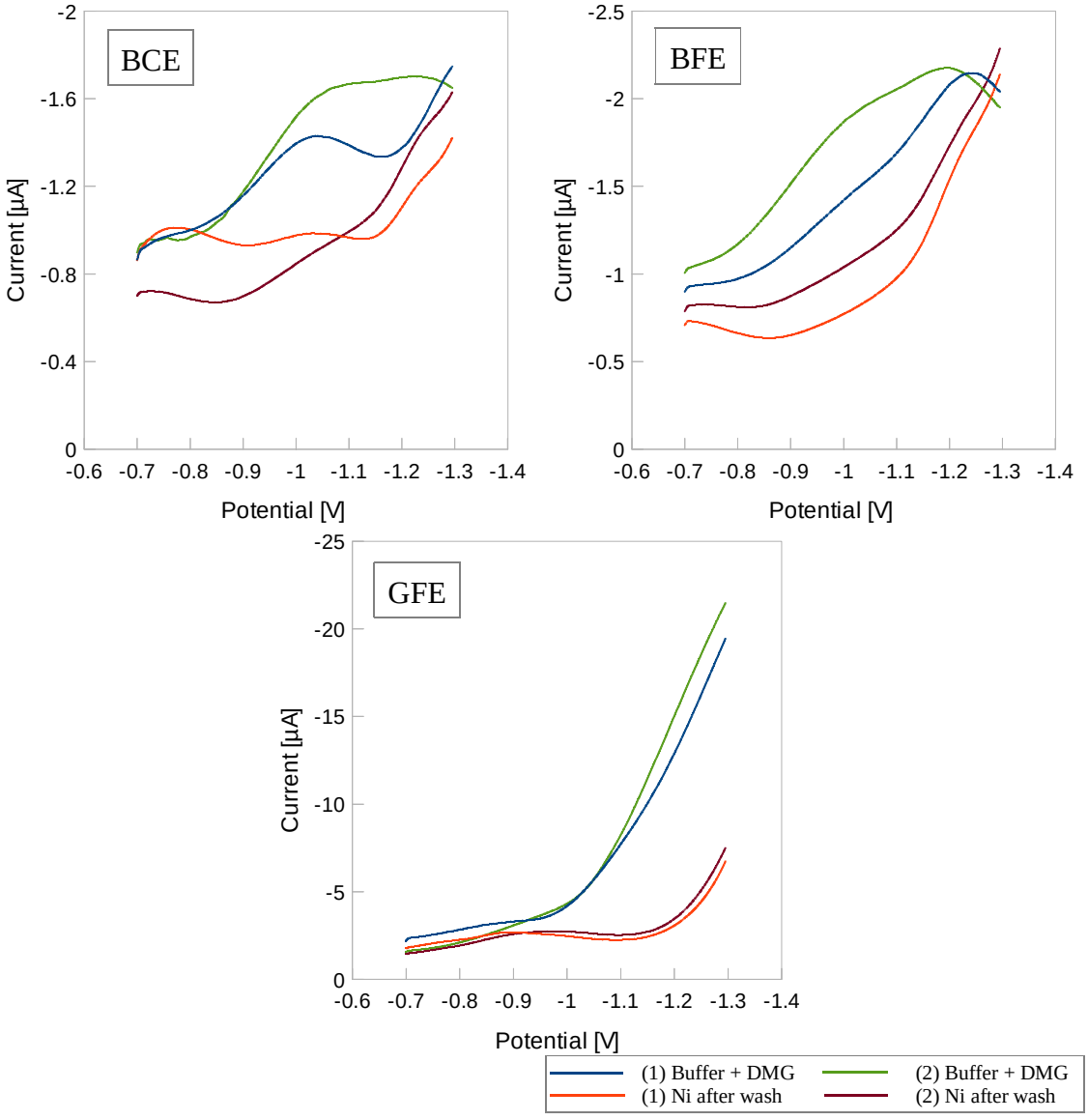


Figure 3.14: Effect on a BCE (top left), BFE (top right) and GFE (bottom) when washed after a buffer measurement and afterwards reused to measure nickel (150 µg L^{-1} nickel, all sensors IR dried). 1: First sensor; 2: Second sensor.

Second, two measurements with buffers (with added DMG) and three measurements with 150 µg L^{-1} nickel conducted at BCEs and BFEs. Once again, for each measurement, a new sensor was used. Whereas, at the BCE a well-defined nickel peak appears, at the BFE the results are more random (Figure 3.15). However, at both electrodes the results cannot be considered as repeatable.

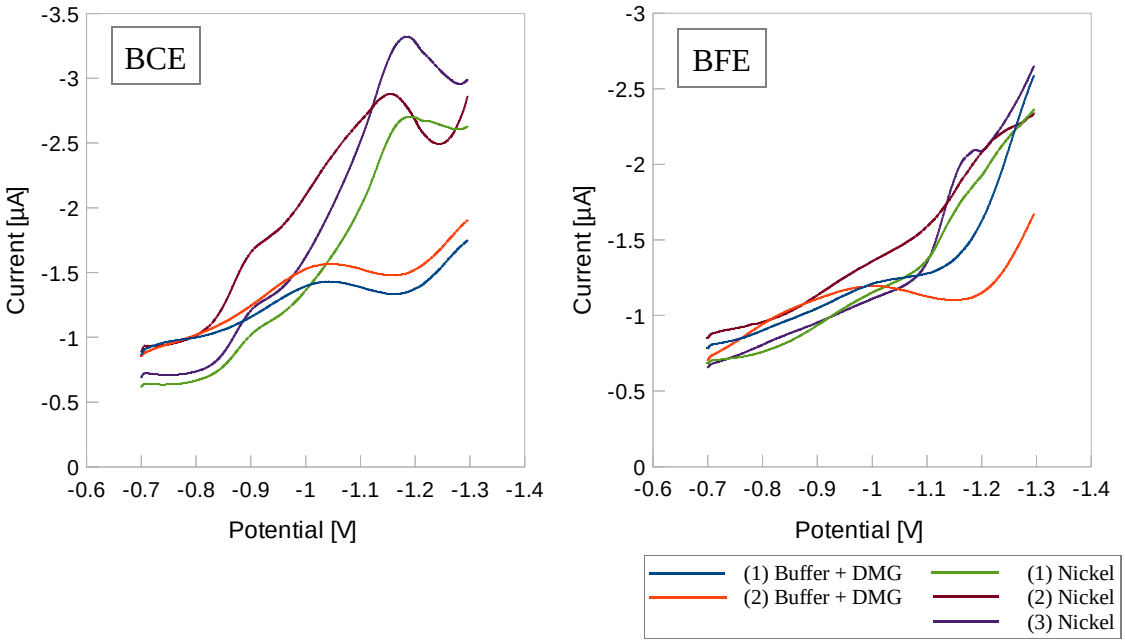


Figure 3.15: Reproducibility of two buffer (with DMG) measurements and three nickel measurements (150 µg L^{-1}) at the BCE (left) and BFE (right) (all sensors IR dried).

The final experiment was to look at the effect of time on the nickel-DMG complex forming. Therefore a single measurement of 1 mg L^{-1} nickel at an IR dried BCE was conducted and then the sample solution was kept in an upright position overnight without moving. On the next day the measurement was repeated with a sample drop from the upper part of the solution with a different sensor. In addition a photo was taken of the solution. Both can be seen in Figure 3.15. Once the nickel forms a complex with the DMG they start to drop to the bottom of the tube and no nickel peak measurement is possible in the mixed solution.

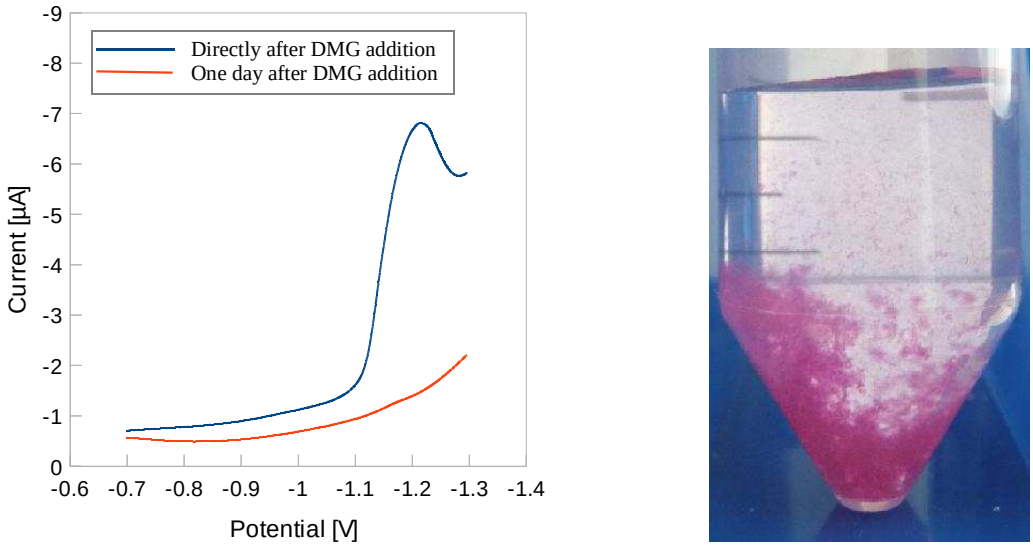


Figure 3.16: Effect of time on the nickel-DMG complex (1 mg L^{-1} Nickel). Left: Influence on the nickel peak measurement. Right: Photo of the nickel-DMG complexes dropped to the bottom of the tube after one day.

3.1.5 Nickel Detection Using the Florence Sensor

The performance of the Florence sensor for nickel detection was also investigated. The sensor used was based on a gold working electrode. However, the carbon counter electrode was used as working electrode. Florence sensors, which are distributed by PalmSens Instruments B. V., were used and stripped in the potential range from -0.7 V to -2.0 V to see the whole background signal. The results are depicted in Figure 3.17, which show measurements of ammonia buffer, 30 µM DMG and a 150 µg L^{-1} nickel. For every measurement a new sensor was used. However, nothing more than a high interference signal was measured. In fact, during the measurement, the dissolving of the silver of the reference electrode was observable.

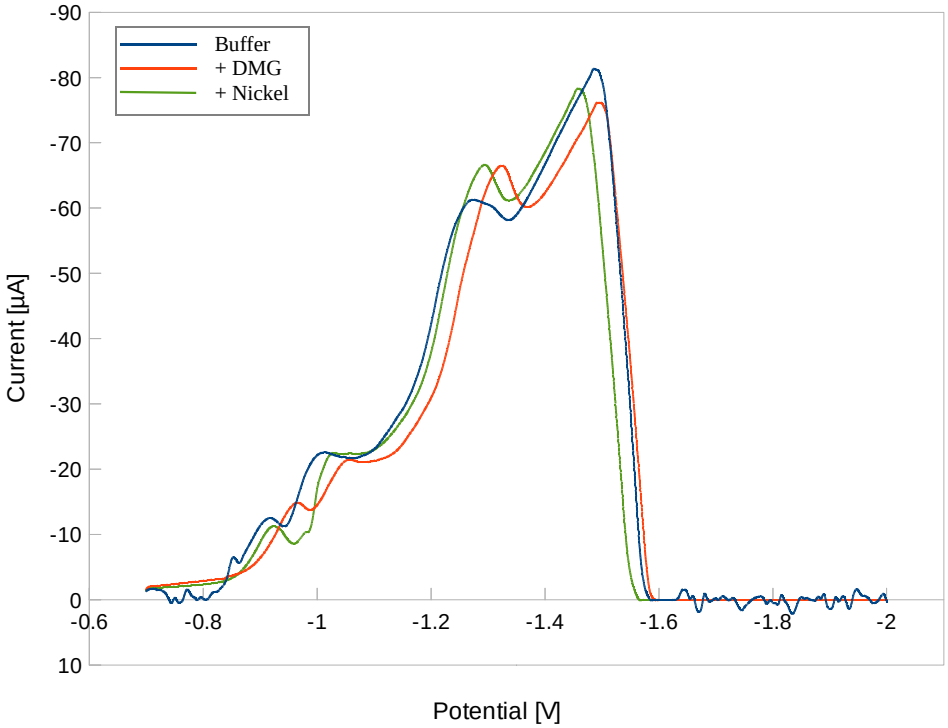


Figure 3.17: Performance of the Florence sensor in a 150 µg L^{-1} nickel and DMG, DMG and plain buffer solution.

3.1.6 Interference Mitigation Using Nafion

Since the BCE sensors are giving the best results for nickel detection these were further examined by using Nafion as an interference barrier (Palchetti *et al.*, 2000; Kadara, 2004). To minimise interference and increase reproducibility the sensors were covered with Nafion and the performance was evaluated. In Figure 3.18 a 150 µg L^{-1} nickel measurement was conducted with Nafion either on the whole drop-on-cell or the reference electrode only or the working electrode only. The best results were observed when Nafion was deposited on the working electrode. Hence, a reproducibility experiment was carried out with Nafion on the working electrode. Three sensors without and three covered with Nafion were used to measure 150 µg L^{-1} nickel. The results are illustrated in Figure 3.19.

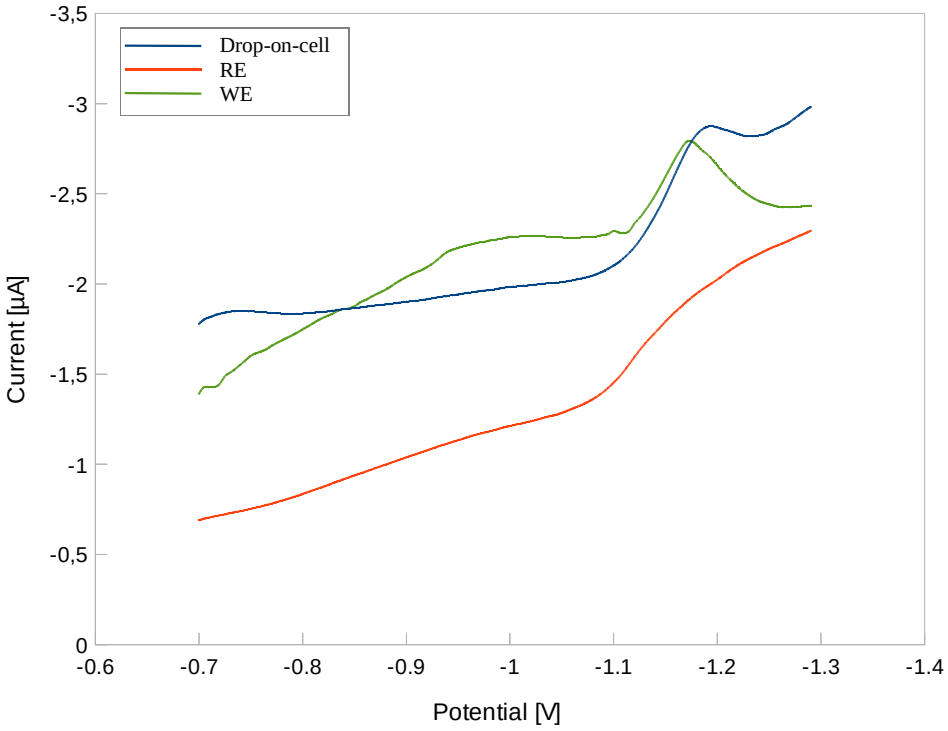


Figure 3.18: Comparison of a 150 µg L^{-1} nickel measurement when Nafion is deposited on the whole drop-on-cell, RE and WE of the BCE (IR dried sensors).

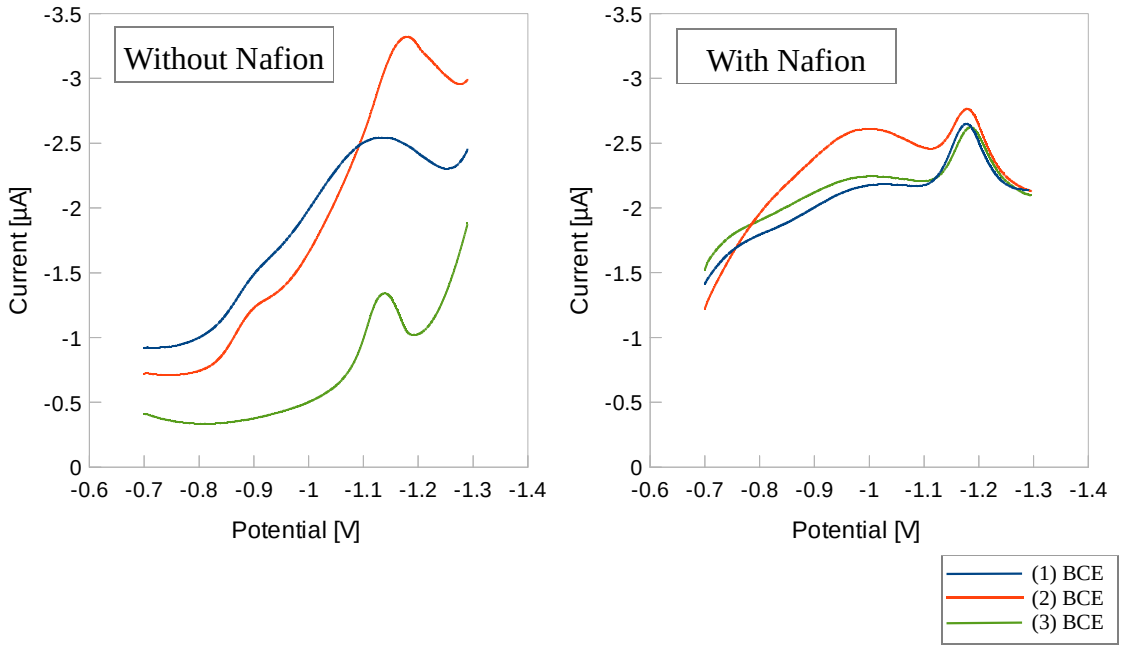


Figure 3.19: Reproducibility of the nickel peak (150 µg L^{-1}) voltammogram when measured three times with a BCE not covered with Nafion (left) and three times with a BCE covered with Nafion (right); (all sensors IR dried).

In Table 3.1 the nickel peak areas and the average nickel peak area are compared. The nickel peaks at the untreated electrodes are higher compared to the electrodes covered with Nafion. However, the standard deviations of the nickel peak areas are ±39.19 nAV at the untreated electrodes and just ±2.22 nAV at the treated electrodes, which shows that the signal is much more repeatable when using Nafion. Hence, in all further experiments the sensors were treated with Nafion.

Table 3.1: Comparison of the nickel peak area of three uncovered and three covered BCE with Nafion (150 µg L^{-1} nickel, IR dried sensors).

Sensor	Peak Area untreated BCE [nAV]	Peak Area BCE with Nafion on WE [nAV]
1	73.3	33.4
2	115.4	29.6
3	37.1	33.5
Average (SD)	53.72 (39.19)	32.17 (2.22)

3.1.7 Effect of L-Histidine on the Nickel Peak Area

Metal-binding proteins and peptides are known to form strong complexes with metals and can improve measurement performance by enhancing the peak area of heavy metals in the voltammogram (Gooding *et al.*, 2001; Md Noh, 2005). One peptide with a high affinity to form a complex with nickel is L-Histidine (Sarkar, 1984).

To investigate the effect of L-Histidine on the nickel peak area, it was added to a sample solution with 150 µg L^{-1} nickel to complex with nickel. However, neither with nor without 30 µM DMG in the sample solution was an increase of the peak area observed at any L-Histidine concentrations (0, 5 and 10 mM). In fact, no nickel peak can be seen after the adding of L-Histidine (Figure 3.20).

This may need to be investigated further since it seems that the nickel complexed with the L-Histidine and did not give a clear peak. Different operational conditions, especially other potentials, may need to be used in further tests.

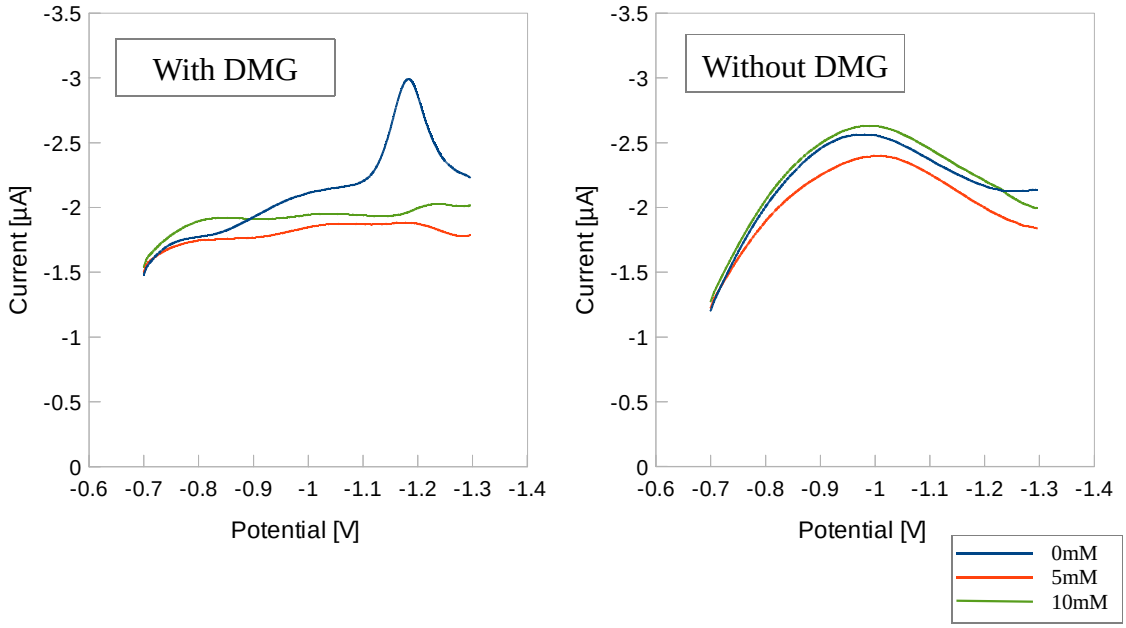


Figure 3.20: Effect on the peak area when L-Histidine is added in the amount of 0, 5 and 10 mM to the solution (IR dried BCEs covered with Nafion, 150 µg L^{-1}). Left: With DMG; Right: Without DMG.

3.1.8 Calibration Plot, Limit of Detection and Precision

The best sensor design, BCE covered with Nafion, was used to generate a calibration plot and determine the limit of detection and precision.

Figure 3.21 displays the voltammograms of the nickel peaks for increasing concentrations of nickel in ammonia buffer solution containing 30 µM DMG. For the measurement, IR dried BCEs covered with Nafion were used. For the calibration plot (Figure 3.22) each concentration was measured three times, each time with a new sensor. A linear plot was obtained in the concentration range from 5 to 200 µg L^{-1} (R^2 = 0.99) and a limit of detection of 3 µg L^{-1}, based on the use of S/N = 3 criterion (of the ammonia buffer sample with DMG), was determined. The variability, especially at high concentration, is quite high. More research is necessary to ensure a good reproducibility. This can done, for example, with a second Nafion layer.

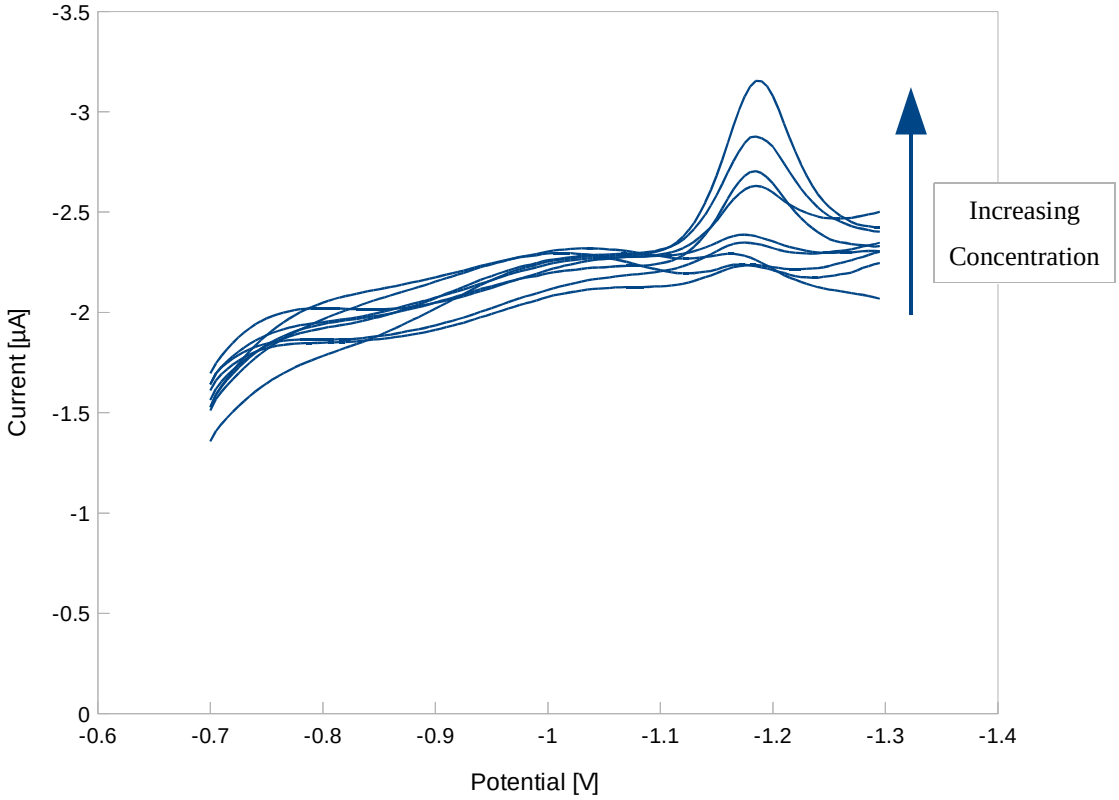


Figure 3.21: Voltammograms of 0, 1, 5, 10, 20, 50, 100, 150, 200 µg L^{-1} nickel at an IR dried BCE covered with Nafion.

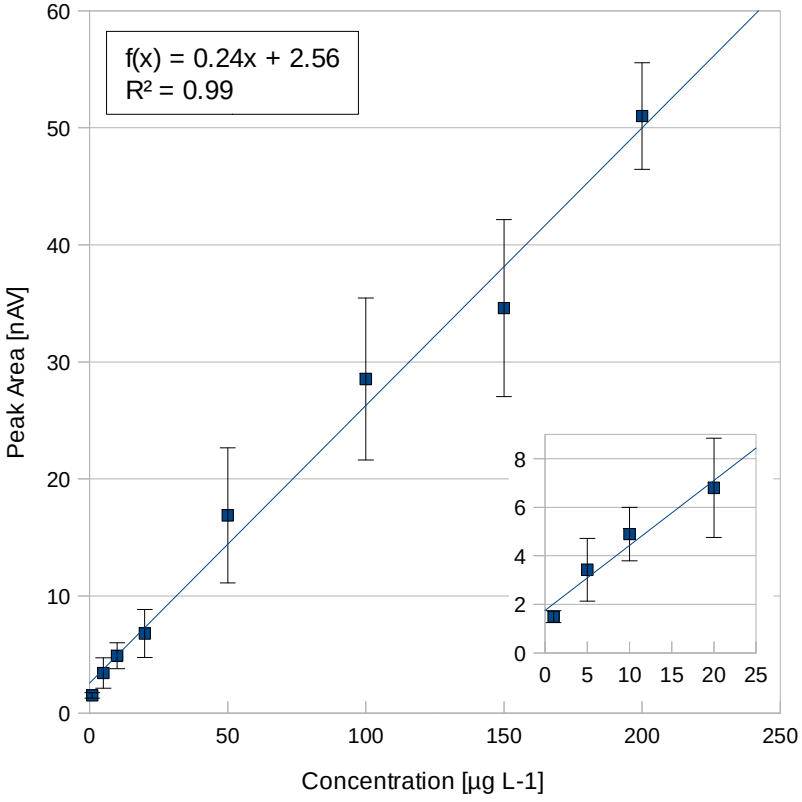


Figure 3.22: Calibration plot for nickel using BCEs covered with Nafion (Error bars = 1 SD, n = 3, IR dried sensors).

3.2 DETECTION OF NICKEL (II) USING SCREEN-PRINTED MICROBAND-ELECTRODES

3.2.1 Electrochemical Characterisation of the Screen-Printed Microband-Electrodes

Cyclic voltammetry was used to characterise the working electrode of the micro-band electrodes. Therefore 2 mM of potassium ferrocyanide in 0.2 M ammonia buffer were prepared. The cyclic voltammograms of the bare carbon microband-electrode (BCME) and the gold film microband-electrode were compared at a scan rate of 30 mV s^{-1} in Figure 3.23. At both electrodes neither an oxidation peak nor a reduction peak is evolving.

To evaluate the electrochemical behaviour of potassium ferrocyanide at the BCME and GFME, cyclic voltammograms were also obtained at scan rates of 10, 20, 30 and 40 mV s^{-1} (Figure 3.24).

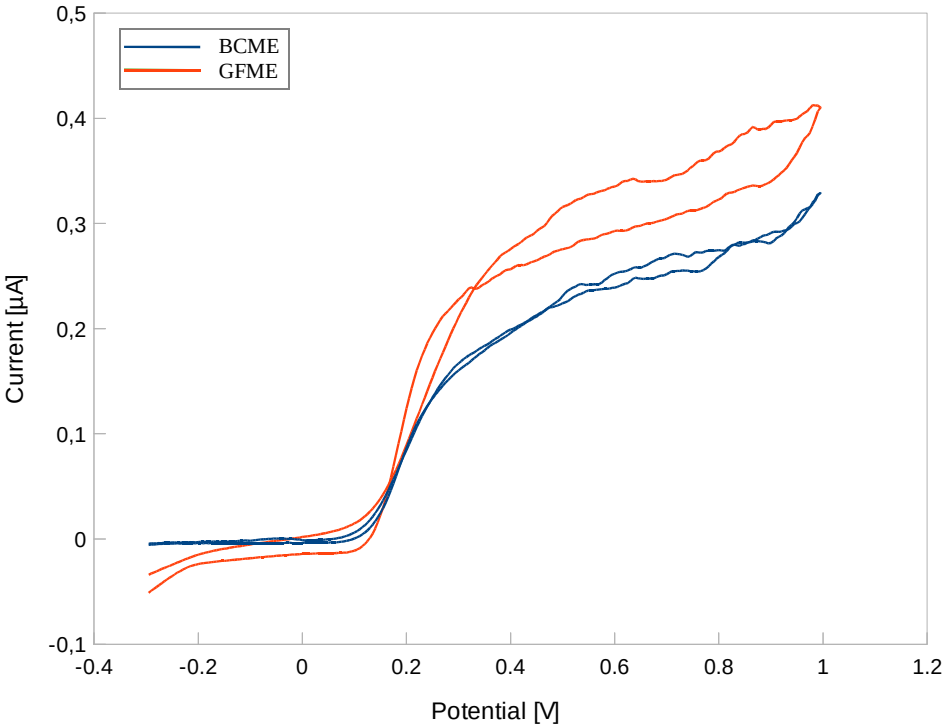


Figure 3.23: Comparison of the cyclic voltammograms of 2 mM potassium ferrocyanide in ammonia buffer solution. BCME and GFME (Scan rate: 30 mV s^{-1}, both sensors IR dried).

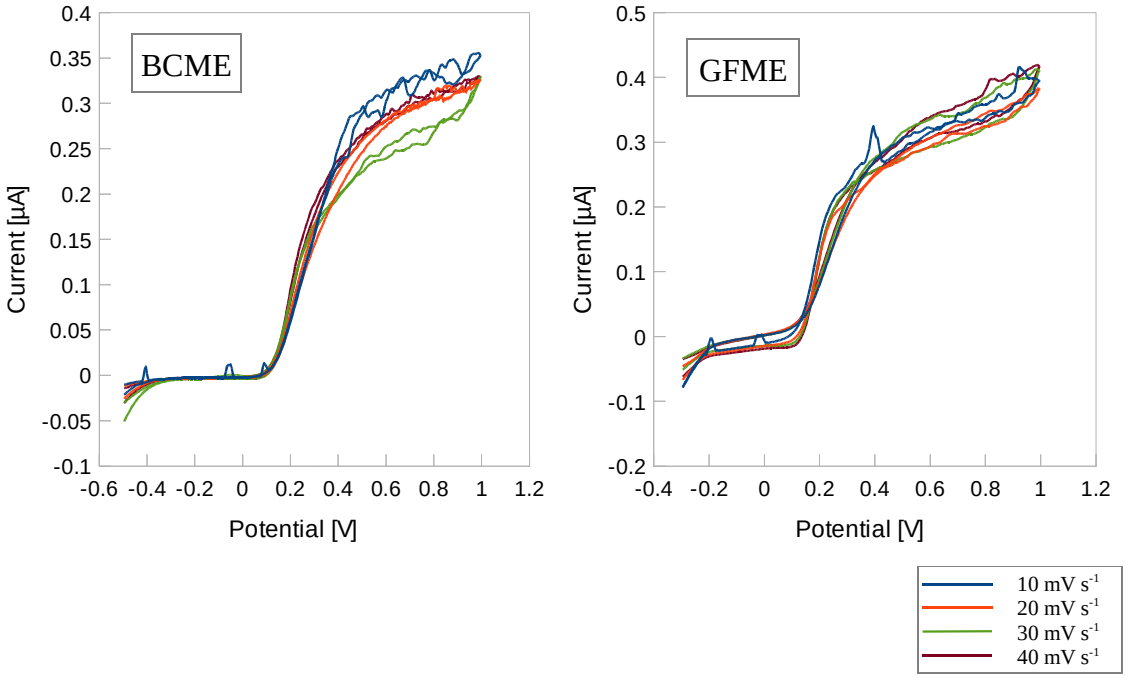


Figure 3.24: Cyclic voltammograms showing the variation in scan rates (10, 20, 30 and 40 mV s^{-1}) for 2 mM potassium ferrocyanide in ammonia buffer solution. Left: BCME; Right: GFME. All sensors IR dried.

3.2.2 Capability of a Gold Film Microband-Electrode

Figure 3.25 shows the results of a 150 µg L^{-1} nickel measurement at oven and IR dried GFME. All measurements were conducted with the same electrode in a stirred sample solution. First the buffer was measured, then DMG and afterwards nickel was added. The background signal at -1.15 V is high and the nickel peak area at 150 µg L^{-1} are as low as 2.39 and 8.38 nAV at the oven and IR dried electrodes, respectively.

By using the microband-electrode, the nickel peak can be seen, when compared to the macro-electrode sensor, due to the reduction of the background signal. However, it is still much lower than the bare carbon microband-electrode which is described below.

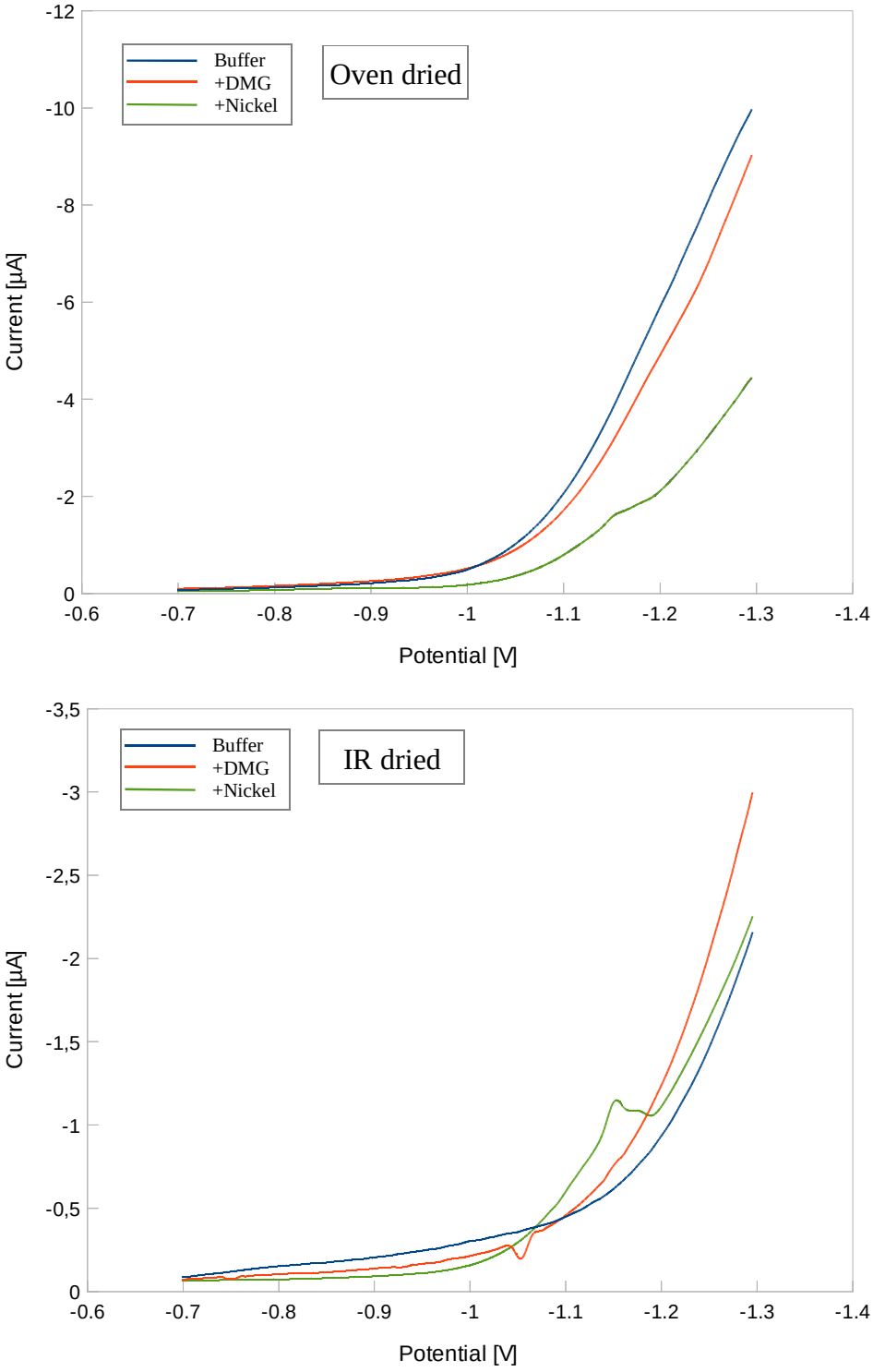


Figure 3.25: Capability of an oven dried (top) and an IR dried (bottom) GFME for nickel measurement (150 µg L^{-1} Nickel).

3.2.3 Calibration Plot, Limit of Detection and Precision of the Bare Carbon Microband-Electrode

Figure 3.26 displays the voltammograms of nickel peaks for increasing concentrations of nickel in ammonia buffer solution containing 30 µM DMG at the oven and IR dried BCME under stirring conditions. The calibration plots for three measurements are depicted in Figure 3.27. A linear plot was obtained in the concentration range of 1 to 200 µg L^{-1} (R^2 = 0.93) and 1 to 200 µg L^{-1} (R^2 = 0.98) at the oven and IR dried electrodes, respectively. The limits of detection were determined with 0.22 µg L^{-1} at the oven dried electrode and 0.36 µg L^{-1} at the IR dried electrode, based on the use of S/N = 3 criterion (of the ammonia buffer sample with DMG).

The voltammograms of nickel peaks were also recorded when the sample was not stirred. The nickel was successively increased in an ammonia buffer solution containing 30 µM DMG at the oven and IR dried bare carbon microband-electrode (BCME). The results are illustrated in Figure 3.28. Since the signal at the oven dried electrode was too small for a proper measurement (first clear peak appears at 100 µg L^{-1} nickel at two different sensors), in Figure 3.29 only the calibration plot of the IR dried sensor can be found. Due to a measurement error for one 1 µg L^{-1} nickel measurement, only two, instead of three values, were used. A linear plot was obtained in the concentration range from 1 to 200 µg L^{-1} (R^2 = 0.99) and the limit of detection was determined at 0.9 µg L^{-1}, based on the use of S/N = 3 criterion (of the ammonia buffer sample with DMG).

For all sensors a strong variability of the results was observed, especially at high concentrations. To increase the reproducibility, further investigations are necessary, most importantly an automatic production of the microband-electrodes to ensure the same surface area of all electrodes.

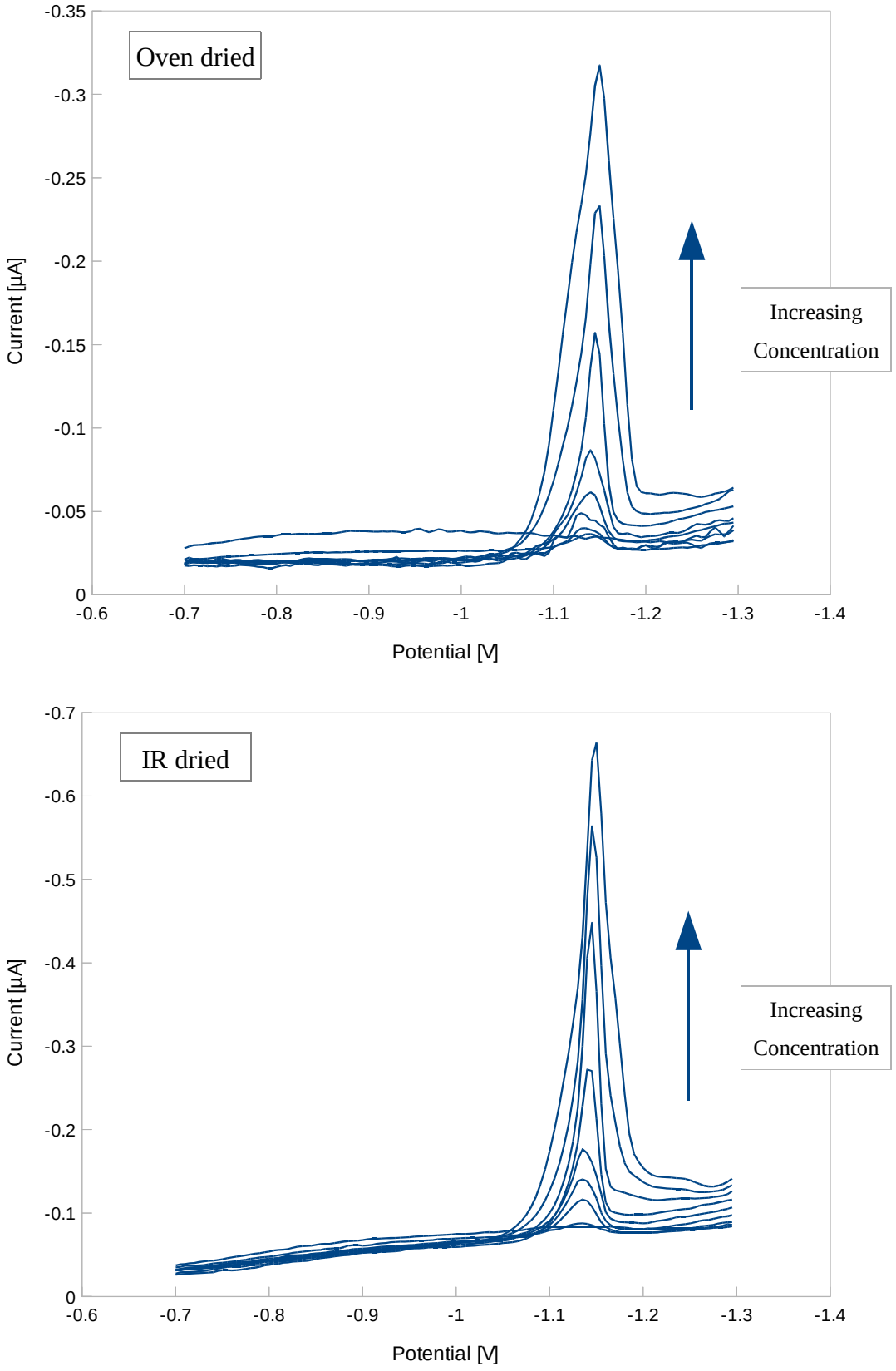


Figure 3.26: Voltammograms of 0, 1, 5, 10, 20, 50, 100, 150, 200 µg L^{-1} nickel at an oven dried (top) and IR dried (bottom) BCME (Stirred).

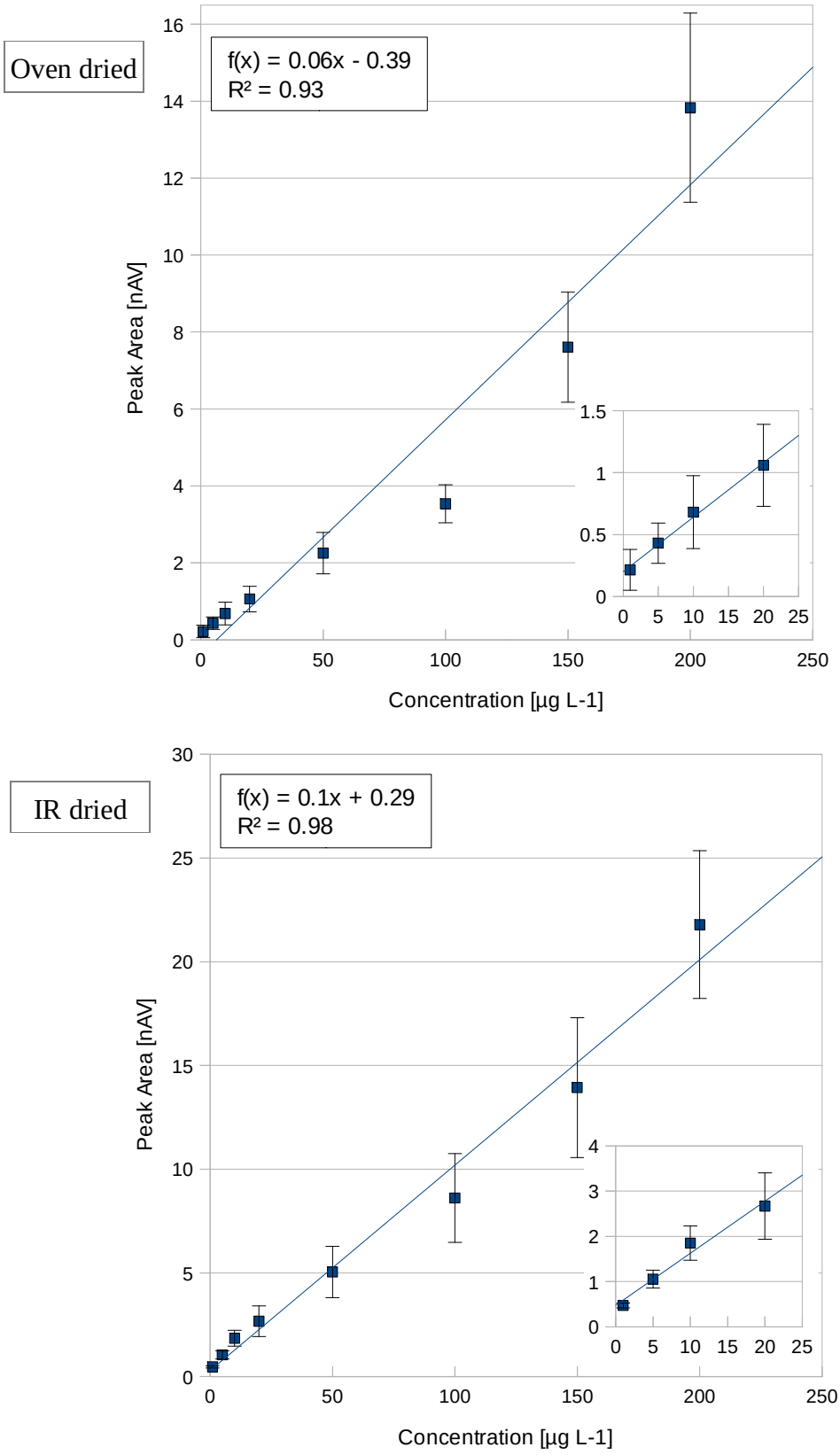


Figure 3.27: Calibration plot for nickel using oven dried (top) and IR dried (bottom) BCMEs (Error bars = 1 SD, n = 3).

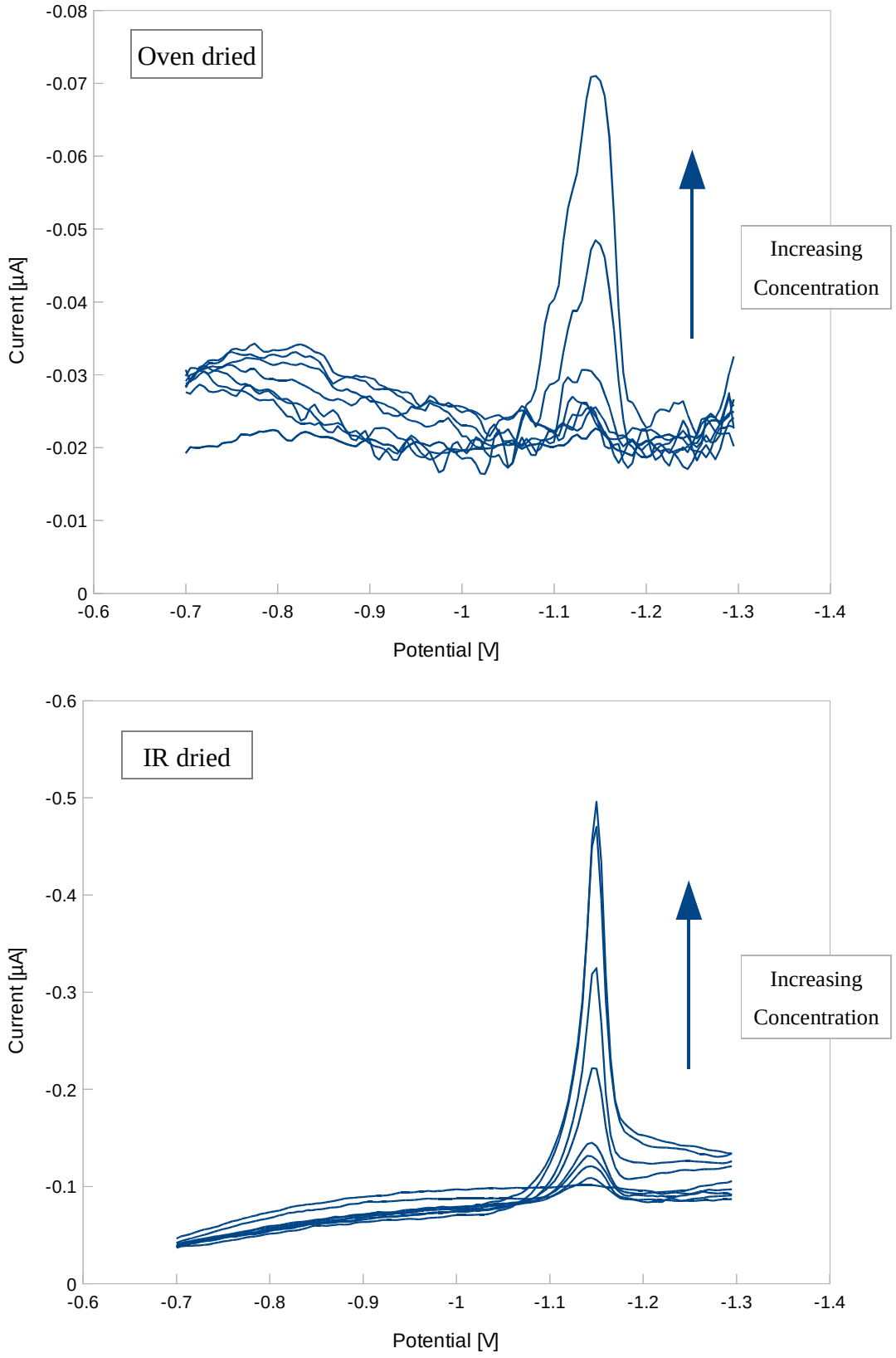


Figure 3.28: Voltammograms of 0, 1, 5, 10, 20, 50, 100, 150, 200 µg L^{-1} nickel at an oven dried (top) and IR dried (bottom) BCME (Unstirred).

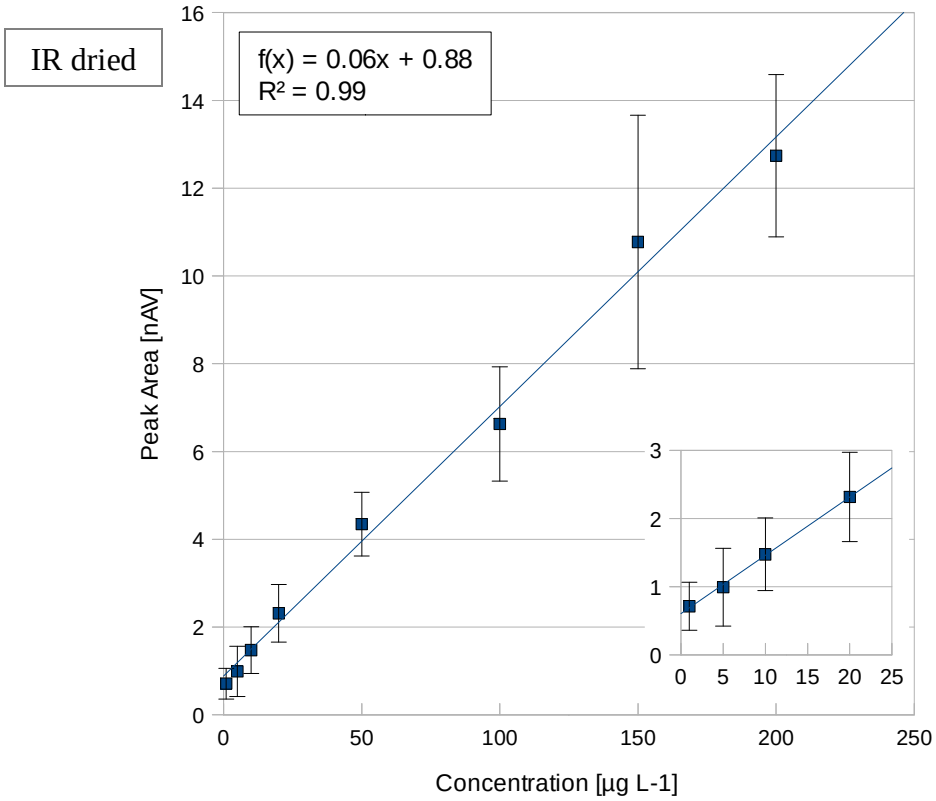


Figure 3.29: Calibration plot for nickel using IR dried BCMEs (Error bars = 1 SD, n = 3, unstirred).

3.3 DETECTION OF NICKEL IN URINE

3.3.1 Assessment of the Optimal Buffer Concentration

The buffer performance of a 0.01, 0.1, 0.2, 0.267 and 0.5 M ammonia buffer solution in urine was examined. The urine sample had a pH value of 5.4 and was diluted 1:3 in the buffer 25% (Hutton *et al.*, 2006), meaning for each part of urine three parts buffer were added. In Table 3.2 the results are presented. The lowest concentrated ammonia buffer solution which was able to buffer the urine sample to a pH value of 9.2 was the 2.67 M one. Once the 2.67 M ammonia buffer is mixed with urine, due to dilution, the ammonia buffer concentration in the mixed sample is adjusted to 0.2 M. This is the reason why, for all experiments assessing the optimal design and operational parameters, a 0.2 M ammonia buffer solution was used.

Table 3.2: Buffer availability of different ammonia buffer solution concentrations when mixed with urine (pH 5.4, 25% urine).

Before mixing with urine		After mixing with urine	
Ammonia buffer solution concentration	pH-Value	Ammonia buffer solution concentration	pH-Value
0.01 M	9.2	0.0075 M	5.8
0.1 M	9.2	0.75 M	7.7
0.2 M	9.2	0.15 M	9.15
0.267 M	9.2	0.2 M	9.2
0.5 M	9.2	0.375 M	9.2

3.3.2 Nickel Measurement in Spiked Urine Samples

Urine (pH 6.0) was diluted (1:3) in a 0.267 M ammonia buffer solution and was spiked with 150 µg L^{-1} nickel and 30 µM DMG. This sample was measured with a BCE covered with Nafion and a BCME (not stirred). In Figure 3.30 the results are displayed and compared with the same sample without nickel and without nickel and DMG. Around -1.15 V, which was determined as specific potential for nickel, no peak is appearing at both electrodes. At the BCE, the voltammograms of the nickel, DMG and plain buffer measurement do not really differ, whereas at the BCME the background signal is decreasing when DMG is added and even more when nickel is also added.

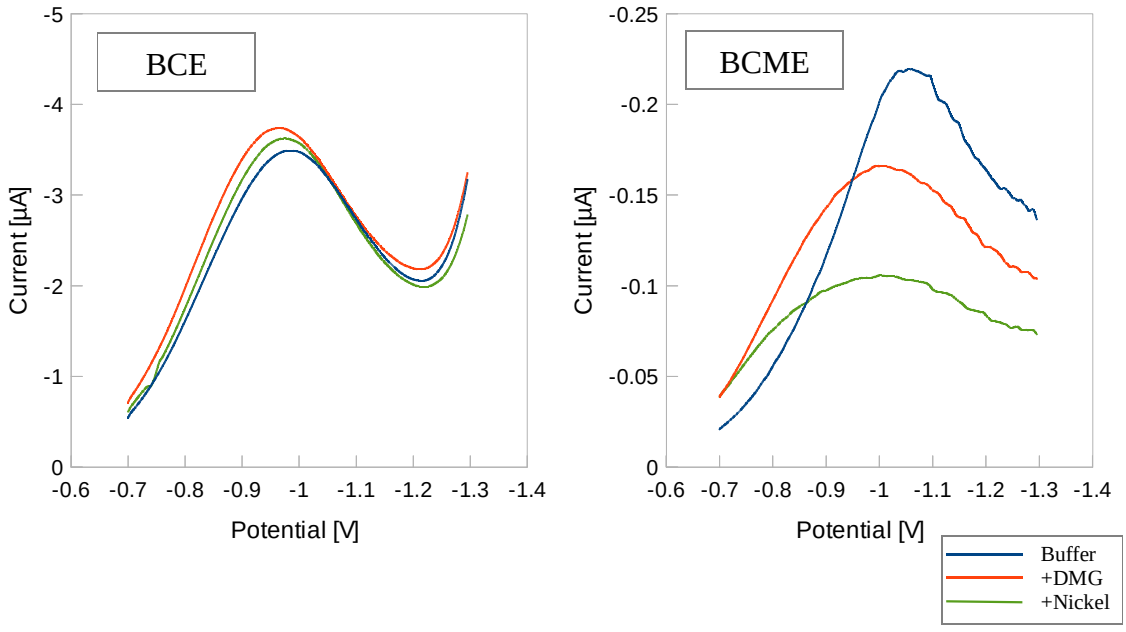


Figure 3.30: Plain buffer, buffer with DMG and 150 µg L^{-1} nickel measurement in a spiked and diluted (1:3) urine sample (pH 6.0) with BCEs covered with Nafion (left) and BCMEs (right, unstirred); (all sensors IR dried).

To minimise the background signal, the urine sample was diluted to 0, 1, 2.5, 5, 7.7, 10 and 25% in a 0.267 M ammonia buffer solution. The sample was spiked with 150 µg L^{-1} nickel and 30 µM DMG. Table 3.3 shows the theoretical nickel concentration in urine, when the dilution due to the buffer is considered. For example a concentration of 1,500 µg L^{-1} of nickel in urine is necessary to achieve 150 µg L^{-1} nickel in the mixed sample, when urine is diluted to 10%.

Table 3.3: Calculated theoretical nickel concentrations in urine, when dilution factor is considered.

Dilution of urine in buffer	Nickel Concentration in Sample [µg L^{-1}]	Corresponding Concentration in Urine [µg L^{-1}]
0%	150	n.a.
1%	150	15,000
2.5%	150	6,000
5%	150	3,000
7.5%	150	2,000
10%	150	1,500
25%	150	600

In Table 3.4 the nickel peak areas of the different dilution ratios are listed and the proportion compared to the nickel peak area in the buffer (0% urine). At the BCE covered with Nafion, a peak (0.93 nAV) is visible of up to 2.5% urine, whereas at the BCME a peak (0.33 nAV) is occurring of up to 5% urine. The voltammograms of all dilution ratios and of the BCE and BCME are depicted in Figure 3.31. Nickel peaks are occurring around -1.2 V at the BCE and around -1.15 V at the BCME. As can be seen, a direct measurement is just possible in very limited circumstances. Hence, further research is necessary to make a rapid nickel measurement in urine feasible.

Table 3.4: Nickel peak areas of diluted samples (0, 1, 2.5, 5, 7.5, 10 and 25% urine in ammonia buffer solution) and a comparison to the nickel peak area of the undiluted sample as a percentage.

	Macro-Electrode		**Microband-Electrode**	
Dilution of urine in buffer	Nickel Peak Area [nAV]	Area compared to 0% dilution [%]	Nickel Peak Area [nAV]	Area compared to 0% dilution [%]
0%	27.42	100	7.56	100
1%	2.92	10.65	1.63	21.56
2.5%	0.93	3.39	0.65	8.6
5%	0	0	0.33	4.37
7.5%	0	0	0	0
10%	0	0	0	0
25%	0	0	0	0

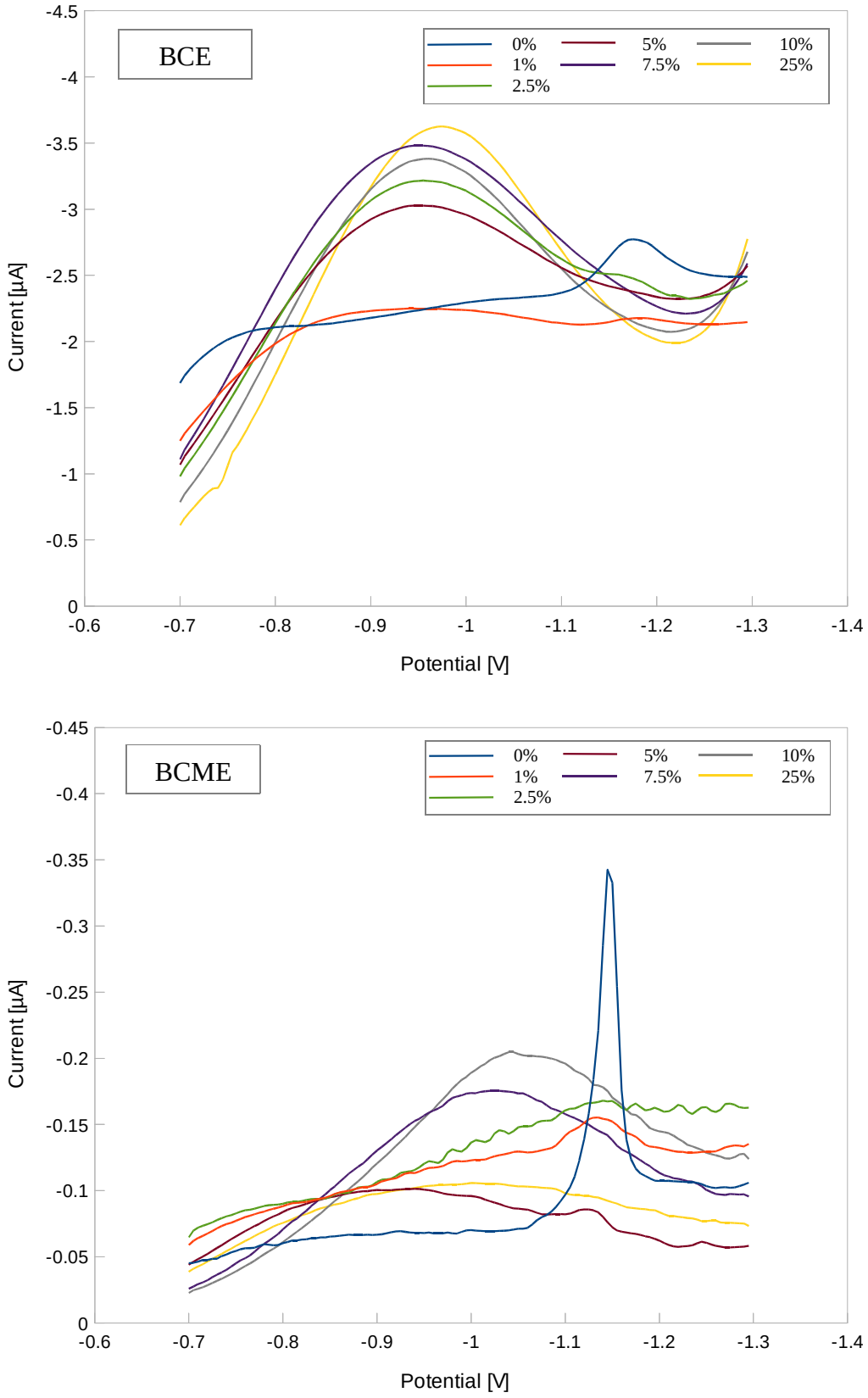


Figure 3.31: Comparison of nickel measurement voltammograms (150 µg L^{-1} with DMG) at different dilutions ratios (0, 1, 2.5, 5, 7.5, 10 and 25%) of urine (pH 6.0) in ammonia buffer solution (0.267 M, pH 9.2) at the BCE (top) and BCME (bottom); (all sensors IR dried).

CHAPTER 4

DISCUSSION

4.1 DETECTION OF NICKEL (II) USING SCREEN-PRINTED MACRO-ELECTRODES

4.1.1 Electrochemical Characterisation of the Screen-Printed Macro-Electrodes

To obtain information about the electrochemical reactions at the electrodes they were characterised using cyclic voltammetry and potassium ferrocyanide in an ammonia buffer solution as the benchmark. Potassium ferrocyanide was used as the benchmark chemical because the oxidation from ferrocyanide to ferricyanide is an almost reversible reaction (Scholz, 2002). These results can be used to compare the performance of the electrodes with other electrodes or in different buffers.

Only one oxidation peak and one reduction peak appeared in the voltammograms, which is a sign that the reaction of potassium ferrocyanide in an ammonia buffer solution at the electrodes is a one electron process. At no electrode, neither at the bare carbon electrode (BCE) nor bismuth film electrode (BFE) nor gold film electrode (GFE) can the redox reaction of potassium ferrocyanide be considered to be reversible. This can be seen in the cyclic voltammograms in two ways. On the one hand this is because the peak-to-peak separation is in all cases much higher than 59 mV, which is normally achieved by a fast reversible one electron process (Wang, 2006). Furthermore, the voltammograms are also more drawn out compared to a reversible process. On the other hand the position of the oxidation peak is slightly shifting to a higher potential with increased scan rates at all three electrodes. This indicates for all electrodes that the redox reaction is quasi-reversible (Wang, 2006).

The oxidation peak currents are indicating that most oxidation processes (loss of an electron) of potassium ferrocyanide are taking place at the BFE followed, after a huge distance, by the GFE and then the BCE. Especially, the high discrepancy between the BFE and the two other electrodes can be explained by the preparation process. The BCE and GFE remained untreated and have approximately the same surface area, except for small differences because the electrodes are to a different degree covered by the isolating layer, which covers more of the carbon electrode compared to the gold electrode (see Figure 2.3: Design of the screen-printed sensor). To obtain a bismuth film, a layer was brushed on the carbon electrode. This process resulted in a huge

increase of surface area of the BFE. When the surface area is increased there is also more space to exchange electrons; hence, a higher oxidation, and also reduction, peak current is entailed.

4.1.2 Background Signal of the Electrolyte

To achieve high sensitivity and also reproducibility, a flat background signal of the supporting electrolyte is desired. Most of the papers dealing with nickel detection using adsorptive stripping voltammetry (AdSV) have a low concentrated ammonia buffer solution of around 0.01 M (Wang and Lu, 2000; Hutton *et al.*, 2003, 2005, 2006; Legeai *at al.*, 2006; Jovanovski *et al.*, 2009) to minimise the influence of the electrolyte. To buffer the pH-value of urine properly, a concentration of 0.267 M was necessary, which is adjusted to 0.2 M by mixing the buffer 3:1 with urine (see also Section 3.3.1 and 4.3.1 for the results and discussion about the optimal buffer concentration for urine). For that reason all experiments were conducted with a 0.2 M buffer solution.

The relatively high ammonia buffer solution concentration is the reason why the difference in the background signal of a 0.01 M and 0.2 M buffer concentration was first investigated. The increase is especially obvious at the GFE. Here the background signal is increasing by a factor of two around -1.0 V, where the nickel peak is supposed to occur (Kokkinos *et al.*, 2008). A slight increase in this potential range can also be found at the BFE. At the BCE there is even a decrease of the background level around the nickel peak potential. However, when the background signal is determined not only until a potential of -1.3 V but down to -2.0 V, is it becoming obvious that at each electrode there is a huge interference peak, which is just appearing at different potentials. Whereas for the BCE this peak does not play any role for the nickel measurement, it is already an issue at the BFE and totally covers the nickel peak potential (around -1.0 V) at the GFE. Moreover, after the interference peak reaches its maximum it is dropping immediately to zero, this point identifies the maximal potential which can be applied. As described by Kokkinos *et al.* (2008) in ammonia buffer solution hydrogen evolution occurs at potentials lower than approximately -1.4 V. As it was assessed, this effect mainly appears at the GFE and makes an accurate measurement nearly impossible.

The background signal measured of the supporting electrolyte is not repeatable at any electrode used. This was evident for both IR and also oven dried sensors. When two sensors tested with buffer and three sensors tested with buffer with DMG and the signals were compared it became obvious that the differences are large and random. Furthermore, there is nearly no difference in the appearance of the signals whether DMG was added or not. The differences amongst the measurements at all six electrodes are between 2 and 3 µA. This large discrepancy is unacceptable for a desired accuracy down to a current of a few nA. The mismatch at the BFEs can be partly explained by the imprecise production process. During this process the carbon-bismuth layer is brushed on the bare carbon electrode, which results in a different electrode surface structure for each single sensor. However, the BCEs and GFEs are not manipulated and should produce a repeatable measurement. These effects were further investigated during the actual nickel measurements. At least, it cannot be excluded that nickel peaks of different measurements are comparable and the electrolyte is just causing an offset, which can be easily deducted from the signal.

4.1.3 Assessment and Optimisation of the Sensors Operational Parameters

All literature located dealing with the AdSV of nickel follow similar measurement procedures (Wang *et al.*, 1996; Wang and Lu, 2000; Hutton *et al.*, 2003, 2005, 2006; Morfobos *et al.*, 2004; Kokkinos *et al.*, 2008). Hence, the operational parameters served as default settings, especially, those of Hutton *et al.* (2005 and 2006), who were able to measure the nickel concentration in various body fluids (aqueous humour, cerebrospinal fluid, saliva and sweat).

Most sensors commonly used for the stripping analysis of nickel, use mercury for the interfacial accumulation of complexes on the electrode (Wang and Lu, 2000). Unfortunately, mercury is not an environmentally friendly material. For many heavy metals applications, such as nickel, bismuth can be used as a mercury substitute without any loss of detection performance (Wang, 2005; Kokkinos and Economou, 2008). As discussed in the introduction chapter, there are various reports on bismuth based sensors for the detection of nickel. Next to bismuth film electrodes (Wang and Lu, 2000; Hutton *et al.*, 2003; Morfobos *et al.*, 2004; Legeai *et al.*, 2006) and bismuth film micro-

electrodes (Hutton *et al.*, 2005, 2006; Kokkinos *et al.*, 2008) there is also a report of a small disposable cell-on-a-chip bismuth film sensor (Kokkinos *et al.*, 2008).

However, to the best knowledge of the author there has never been a bismuth film screen-printed sensor used for the detection of nickel. Hence, for the carbon-bismuth powder paste data from bismuth film screen-printed sensors for the detection of lead, cadmium and zinc (Królicka *et al.*, 2002, Pauliukaitė *et al.*, 2002; Kadara and Tothill, 2008) were used. The performance of the BFE containing bismuth concentrations of 2, 5 and 10% was investigated. With increasing bismuth concentration, the background interference peak is also playing a more important role at around -1.0 V. Whereas with 2% a clear nickel peak is appearing close to -1.2 V, the peak is masked by interference phenomena at 5 and 10%. Hence, for all other experiments, a BFE with a bismuth concentration of 2% was used.

The maximal nickel concentration measured was 200 µg L^{-1}, which is converted 3.4 µM. Since each nickel ion is complexing with two DMG molecules (Ma *et al.*, 1997) a DMG concentration of 6.8 µM should be sufficient. However, there is the risk that some DMG molecules are complexing with other molecules and heavy metals, especially cobalt (Ma *et al.*, 1997). This is why a higher concentration may be necessary. It was already reported by Kadara (2004) and Dudeney (2008), who were working with the same sensor design, that silver ions are dissolving from the surface of the screen-printed sensor itself. Hence, for measurements in the buffer, the influence of silver ions are of particular interest. Therefore, 150 µg L^{-1} nickel, the maximum concentration, which was desired to be detectable, was measured with either 0, 10, 20, 30 or 40 µM DMG at all three electrodes. An optimal DMG concentration of 30 µM was determined at both BCEs, the IR and oven dried one, and 40 µM at both BFEs. Once again the signals at the BFE are quite random, which can be explained either by the imprecise production process or with the higher sensitivity to interference from other ions, which are probably dissolving from the sensor. At both GFEs no nickel peak was observed, and at all DMG concentrations the signals are comparable to the blank sample. Furthermore, at all electrodes (BCE, BFE and GFE) the results of the IR dried sensors are much better than of the oven dried in this case. The nickel peaks, when appearing, are larger and the background signal is lower. After discussing this with the producer of the sensor, DuPont Ltd. (Bristol), the most likely explanation of this

outcome is the composition of the electrodes at the surface. After conventional drying in the oven more silver, gold or carbon can be found at the surface of the electrodes than the polymers, used to retain the silver, gold or carbon. For IR dried it is *vice versa*, compared to the sensors dried in the oven. This results in less interfering compounds dissolving from the sensor surface .

At this point in the project, it was decided not to investigate the nickel measurement performance of the GFE as well as all oven dried sensors any further, due to the strong influence of interferences at these electrodes.

The optimal deposition time was investigated with two different experiments with the BCE: on the one hand with relative short deposition times up to 270 seconds, and on the other hand with very long deposition times of 450, 900 and 1350 seconds and a longer stripping potential range down to -2.0 V. The most interesting result is that with a prolonged deposition time the huge interference peak is moving towards lower potentials; hence, covering the nickel peak area at -1.15 V. Moreover, a second interference peak is emerging from -0.8 to -1.0 V. This peak can also be seen after a deposition time of 150 and 210 seconds. To avoid these interference phenomena and because the nickel peak has the highest area after 90 seconds, this deposition time was used for all other experiments.

The increasing interference peak with increased deposition time can also be explained using the effect of dissolved silver ions from the reference electrode. If the sample drop is longer on the surface of the electrodes, there is also more time to dissolve the silver. Another explanation is described by Kokkinos *et al.* (2008). In ammonia buffer solution -1.4 V is the minimal potential before hydrogen evolution takes place. However, Legeai *et al.* (2006) also used ammonia buffer to detect nickel but with longer deposition times, up to 900 seconds, and did not report any increased interference problems after very long deposition times. The authors were aware of hydrogen evolution at potentials below -1.4 V and took measures to avoid them, such as pulse plating of the bismuth on the electrode to achieve a more homogeneous appearance. Hence, next to silver dissolution, one contribution to the interference peak at very low potentials below -1.4 V could be hydrogen evolution due to an inhomogeneous surface appearance of the electrodes.

Due to the high variability at the BFE it was not possible to conduct a reasonable and significant experiment regarding the assessment of the optimal deposition time.

4.1.4 Measurement Reproducibility

It was observed in the experiments of the previous sections that the signal of the ammonia buffer solution with or without DMG and nickel is very variable. To evaluate the reproducibility three different experiments were conducted.

In the first one, the sensor was washed after a buffer measurement and the same sensor was then used to measure 150 µg L^{-1} nickel. Neither at the BCE nor BFE nor GFE was it possible to obtain any nickel peak after one washing procedure. Furthermore, the current of the signal is decreasing, especially at the GFE. Hence, it is not possible to reuse the sensor. More important, however, is the effect behind this observation. It was already reported that the silver of the reference electrode is dissolving at extreme pH values (pH value around 2) of the very same screen-printed sensor design used for this project (Dudeney, 2008). Assuming that the interference peak is mainly caused by the silver of the reference electrode, it is washed away after the first measurement. This would explain both observations: on the one hand the inability to measure any nickel after the washing procedure, due to the lack of a proper reference potential, and on the other hand the massive decreasing background signal, especially at the GFE, which is in particular influenced by interference.

The second experiment investigated the reproducibility of a 150 µg L^{-1} nickel measurement at the BCE and BFE. Whereas at the BCE a distinct nickel peak is appearing and the signal can clearly be distinguished from the blank, at the BFE these observations were not made. This can once again be explained either by the imprecise production process or by the higher sensitivity to other ions, which are probably dissolving from the sensor.

However, at this point in the project it was decided not to investigate the nickel measurement performance of the BFE any further.

The influence of time on the nickel-DMG complex was investigated in the third experiment. It became apparent that with time the complexes are dropping to the bottom of the solution after one day. Then a nickel measurement from a sample of the upper

part of the solution, even at concentrations of 1 mg L^{-1}, is no longer possible. Hence, it is recommended to shake the solution well, before the sample drop is collected and to measure the sample on the same day the DMG is added to the solution.

4.1.5 Nickel Detection Using the Florence Sensor

Due to the fact that all measurements so far were influenced by strong interference phenomena, probably caused by the sensor itself, it was decided to investigate other commercial sensors. Therefore, Florence sensors directly distributed by PalmSens Instruments B. V. (The Netherlands) were purchased. The sensors were used with the carbon electrode as working electrode and all operational parameters were the same as used for all other experiments; the only difference was that the signal was once again obtained in an enlarged potential range down to -2.0 V. However, instead of obtaining better results, the outcome was much worse compared to the DuPont sensors. A large interference peak was masking a possible nickel peak and the signals did not differ whether plain buffer alone, or with DMG, or with DMG and nickel, was measured. In fact, it was literally possible to see the silver dissolving from the reference electrode and drifting to the working electrode during the measurement. At -1.6 V the dissolving of the silver stopped and at the same time the signal dropped immediately to 0 A, concrete proof that the silver of the reference electrode is a significant part of the interference peak. Furthermore, when all the silver is dissolved the signal is dropping to zero and further measurements with the same sensor are probably no longer possible. Therefore this sensor was not used in further experiments.

4.1.6 Interference Mitigation Using Nafion

The change to Florence sensors resulted in amplifying the problems caused by interference rather than solving them. Hence, different approaches were used to solve the interference problems at the DuPont sensor. The first was covering the electrodes with Nafion to minimise interference. This technique was already successfully used during voltammetric measurements of trace metals at mercury film electrodes (Brett and Fungaro, 2000), gold film electrodes (Dudeney, 2008) and also bare carbon electrodes (Palchetti *et al.*, 2000; Kadara, 2004). An immobilised Nafion membrane prevents negatively charged ions, such as Cl^- and the Ag-Cl complexes, from penetrating the

electrode (Dudeney, 2008). Positively charged ions such as Ni^{2+} can pass through this membrane without any problems. However, reports of the behaviour of the uncharged nickel-DMG complex at the membrane were not found.

The neatest nickel peaks were obtained when the working electrode was covered with Nafion but not the reference electrode or the whole drop-on-cell. Hence, BCEs where the working electrode were covered with Nafion were used to compare the performance with unmodified BCEs. Although interference peaks are still occurring in the voltammograms of BCEs covered with Nafion, the results are much more repeatable compared to unmodified BCEs. The reproducibility of triplicate peak area measurement is 32.17±2.22 nAV (±6.9%) and 53.72±39.19 nAV (±73.0%), respectively. Other effects which were observed are a shift of the potential of the nickel peak from -1.15 V to -1.2 V and an increased offset of the nickel peak. Whereas at an unmodified BCE without interference, the background signal is around -0.5 A, this offset is around -2.0 A at BCE covered with Nafion. The shift of the nickel peak potential was already observed before and is related to the function of the ionophore (Palchetti *et al.*, 2000). There was no explanation found for the increased offset; however, this is without much doubt also somehow related to the Nafion membrane.

4.1.7 Effect of L-Histidine on the Nickel Peak Area

Another approach to improve the measurement performance is to increase the nickel peak area. One way this can be done is by the use of metal-binding proteins (metalloproteins). These proteins and peptides are important for the detoxification process of many organisms and are produced in response to the presence of a specific metal. This specificity and the ability to release the metal ions from other complexes make them ideal to improve the measurement performance of metal ions in biological fluids (Gooding *et al.*, 2001; Md Noh, 2005). The peptides, or proteins form strong complexes with the metals, and this can result in an increased peak area. As described by Sarkar (1984), nickel has a high affinity to form complexes with L-Histidine (see Figure 4.1 for the chemical structure). Hence, the influence of L-Histidine on the peak area was investigated.

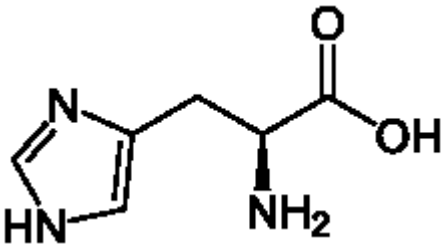


Figure 4.1: Chemical structure of L-Histidine.

For the measurements, 150 µg L^{-1} nickel was added to ammonia buffer solution with and without 30 µM DMG. For the experiment without any DMG no nickel peak was observed at all in L-Histidine concentrations. There are several possible explanations for this observation, for example either the wrong supporting electrolyte or wrong sensor design or wrong operational parameters; however, it is most likely the potential range.

Highly interesting is the fact that in the sample solution containing DMG the nickel peak is also disappearing after adding L-Histidine. This proves that nickel ions are complexing with organic compounds in solution, which influences the complex formation of nickel and DMG, and can have a massively negative effect on the nickel peak area.

4.1.8 Calibration Plot, Limit of Detection and Precision

The sensor design with the best performance so far, BCE covered with Nafion, was used to generate a calibration plot. This was done to determine the linear range, limit of detection and precision of the sensor with a series of three repetitive measurements of each concentration. The limit of detection was calculated at 3 µg L^{-1} (based on the use of S/N = 3 criterion) and a linear plot was obtained between 5 and 200 µg L^{-1} (R^2 = 0.99). This performance characteristics are not sensitive enough, because the urine sample is diluted at least 1:3 and the aim is to measure nickel concentrations in urine of at least 5 µg L^{-1}. Hence, when the dilution factor is considered, a linear range from 1.25 to 50 µg L^{-1} would be necessary. Moreover, the signal is still fluctuating, especially at high nickel concentrations, which is caused by interference phenomena. As a result the

standard deviation is up to 34.69±7.57 nAV (±21.89%), which was obtained at a concentration of 150 µg L^{-1} nickel. One way to reduce this variability may be a second Nafion layer.

Wang (2006) described that at high concentrations the linearity of the calibration plot can be influenced due to buffer effects of the adsorbed complexes on the surface of the electrode. However, this effect was not observed at the BCE in the evaluated concentration range.

4.2 DETECTION OF NICKEL (II) USING SCREEN-PRINTED MICROBAND-ELECTRODES

The sensor with the best performance regarding nickel detection found in the literature was developed by Hutton *et al.* (2005 and 2006). Using a micro-electrode and AdSV it was possible to achieve a limit of detection of 0.056 µg L^{-1} nickel, a good linearity (R^2 > 0.995) and an excellent reproducibility (SD of 3.8% at 0.5 µg L^{-1} nickel). Authier *et al.* (2001) and Chang and Zen (2006) describe a method to make microband-electrodes out of screen-printed sensors. In both papers the three electrodes, reference, working and counter electrode, are stacked and separated by insulating layers, like a sandwich. The sandwich of layers is then cut through, leaving just the ink edge exposed (see Figure 4.2). This approach was successfully used for the detection of arsenic with the DuPont screen-printed sensors by Dudeney (2008). The miniaturisation of the surface area of the electrodes was reported as an effective method to mitigate interference caused by silver dissolving from the reference electrode. The limit of detection was enhanced from 10 µg L^{-1} arsenic using the gold film macro-electrode to estimated 1 µg L^{-1} arsenic with the gold film microband-electrode. Hence, to increase sensitivity and reproducibility, the approach to cut away the drop-on-cell and use instead the cross sectional area of the conductive layers for the actual measurement was also adapted for the nickel measurement.

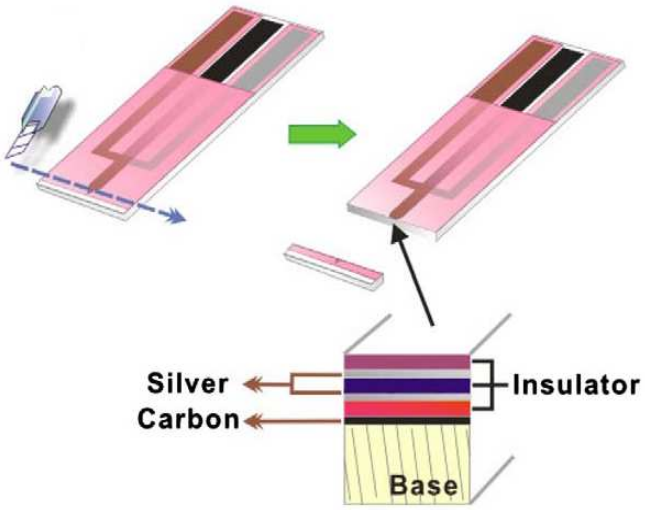


Figure 4.2: Principle production process to obtain a microband-electrode from a screen-printed sensor (Adapted from Chang and Zen, 2006)

4.2.1 Electrochemical Characterisation of the Screen-Printed Microband-Electrodes

An explanation of cyclic voltammetry and the most important information which can be obtained are described in Section 3.1.1 about the electrochemical characterisation of the macro-electrode.

At the bare carbon microband-electrodes (BCMEs) and gold film microband-electrodes (GFMEs) no oxidation or reduction peak was obtained. Furthermore, the voltammograms at all scan rates (10, 20, 30 and 40 mV s^{-1}) are comparable. Even the difference of the signals of the BCME and GFME is marginal, the latter is just slightly more sensitive. Hence, the redox reaction of potassium ferrocyanide in ammonia buffer solution at both microband-electrodes can be considered as irreversible. This can be seen in unchanging signals at different scan rates and in the missing peak of the backward shift (Wang, 2006).

4.2.2 Capability of a Gold Film Microband-Electrode

The capability of the GFME was evaluated by measuring 150 µg L^{-1} nickel in ammonia buffer solution containing DMG. Neither at the oven dried nor at the IR dried GFME was a useful nickel concentration determination possible. However, it was possible to obtain a small nickel peak at -1.15 V of 2.39 nAV and 8.38 nAV, respectively. A positive observation from this experiment is the reduction of the size of the interference peak to a degree, where it is no longer masking the nickel peak, which can be considered as huge progress in mitigating silver interference.

Although it was possible to prove that nickel can be determined at a gold film, the results did not reveal a feasible reason to investigate the nickel measurement performance of the GFME any further.

4.2.3 Calibration Plot, Limit of Detection and Precision of the Bare Carbon Microband-Electrode

At the beginning, the measurements at both BCMEs, oven and IR dried, were carried out under stirring conditions, as described by Hutton *et al.* (2005, 2006). The performance at the oven dried BCME was poor, with a linear plot from 1 to 200 µg L^{-1} (R^2 = 0.93) nickel and a maximum standard deviation of 13.83±2.46 nAV (±17.79%) at 200 µg L^{-1}. The only beneficial attribute is a very low limit of detection of 0.22 µg L^{-1} (based on the use of S/N = 3 criterion). A better performance was obtained at the IR dried BCME. The limit of detection is 0.36 µg L^{-1} (based on the use of S/N = 3 criterion) and also quite low, a theoretical urine dilution down to 7.2% in buffer is possible and, hence, achieve a lower detection limit of 5 µg L^{-1} nickel in urine. A linear plot was obtained for all concentrations evaluated from 1 to 200 µg L^{-1} nickel (R^2 = 0.98). However, high standard deviations were determined, with the worst result of 21.79±3.56 nAV (±16.34%) at a concentration of 200 µg L^{-1}. At the oven and IR dried BCME, the standard deviation is increasing with the nickel concentration. It was also observed that the results during one measurement series were quite linear and this variation is mainly caused by averaging the results of different sensors. This is probably caused by the imprecise preparation process of the microband-electrodes. It was already reported by Chang and Zen (2006) that manual cutting is resulting in a low

reproducibility and that the cut would be probably more repeatable if done by a machine. Dudeney (2008) used several different methods to prepare the microband-electrode, including scissors, knife, CO_2 laser and roller. The best results were reported when using the roller; however, it was speculated that the use of an ultrasonic or drill cutter could lead to better defined edges.

Due to the fact that the measurement procedure should be as simple as possible, the measurements were repeated without stirring. At the oven dried BCME it is was not possible to differentiate the nickel peak from the blank sample up to concentrations of 50 µg L^{-1}, in two different measurement series with two sensors. The first clear nickel peak was obtained at 100 µg L^{-1}. Hence, it was decided that the results were not promising enough to conduct three measurement series and create a calibration plot. The outcome at the IR dried electrode, however, was significantly better. A limit of detection of 0.9 µg L^{-1} nickel (based on the use of S/N = 3 criterion), which means a dilution down to 18% urine in buffer is possible, and a linear plot from 1 to 200 µg L^{-1} (R^2 = 0.99) makes the BCME the most suitable design for nickel detection in urine. The only negative attribute is once again the reproducibility, with a maximum standard deviation at 150 µg L^{-1} of 10.77±2.89 nAV (±26.83%). As discussed in the last paragraph this is probably caused by the imprecise preparation process of the microband-electrodes.

At all BCMEs the influence of interference on the results was minimal but the precision was rather minimised by the imprecise manual cutting using scissors.

The maximum detection limit of AdSV is defined by buffer effects of the adsorbed complexes at the surface of the electrode (Wang, 2006). This effect is reported by Wang *et al.* (1996) (carbon electrode containing DMG: over 200 µg L^{-1}) and Wang and Lu (2000) (bismuth film electrode: over 100 µg L^{-1}), who used a deposition time of 30 seconds for the detection of nickel. However, at all BCME designs and operating conditions a linear plot was obtained until 200 µg L^{-1}.

4.3 DETECTION OF NICKEL IN URINE

4.3.1 Assessment of the Optimal Buffer Concentration

Different electrochemical techniques have already been used to determine nickel concentration in urine. However, the reports are rare and the results are unsatisfactory regarding the aims of this research. Bond *et al.* (1986) and Jain *et al.* (2005) were measuring nickel in urine down to 10 µg L^{-1} and 400 µg L^{-1} with amperometry and an ion-selective electrode, respectively. Unfortunately, both have a limit of detection above the required 5 µg L^{-1}. Mahajan and Kaur (1996) and Horng *et al.* (2003) have successfully determined nickel concentration in urine using differential pulse adsorptive stripping voltammetry. The LOD were 5 µg L^{-1} and 0.69 µg L^{-1}, respectively. However, in all experiments a complex pretreatment of the urine sample was carried out, which was either acidification or wet digestion. Furthermore, except for the ion-selective electrode, where a silver-silver chloride working electrode was used, a working electrode containing mercury was used.

Since there is no information available about rapid electrochemical nickel determination in urine without pretreatment, the papers of Hutton *et al.* (2005 and 2006) were used as guidelines. They were measuring spiked simulated and real human body fluids. This was on the one hand simulated sweat and saliva and on the other hand real aqueous humour and cerebrospinal fluid. The only sample pretreatment used was a dilution of 1:3 in ammonia buffer solution with a concentration of 0.01 M and a pH of 9.2. Square-wave voltammetry was used and nickel concentrations of at least 0.25 µg L^{-1} in sweat and saliva and 4 µg L^{-1} in aqueous humour and cerebrospinal fluid were measured.

Since urine can have a pH value from 4.8 up to 8.4 (Lide, 2008) a higher concentrated ammonia buffer than used by Hutton *et al.* (2005 and 2006) is necessary. A stable pH value is necessary for a repeatable measurement and a value between 8 and 9 is found to be optimal for nickel and DMG to form a complex, which results in high peak currents (Zhang *et al.*, 1996). Unfortunately, a quite high ammonia buffer concentration is necessary to buffer urine to a pH value of 9.2. From three urine samples the one with the lowest pH value (5.4) was used, because it was closest to the worst case scenario of

4.8. The lowest concentration found to buffer the urine sample properly to 9.2 was 0.267 M. In fact, the concentration is slightly higher than necessary in order to consider the worst case scenario of 4.8.

Once the ammonia buffer is mixed with urine, the ammonia concentration is decreasing in relation to the dilution ratio. The mixed sample, which is used for the actual electrochemical measurement with the screen-printed sensor, has an ammonia concentration of 0.2 M, when urine and an ammonia buffer solution with 2.67 M are mixed in the ratio 1:3. This is the reason why a 0.2 M ammonia buffer solution was used for all previous experiments for the assessment and optimisation of the performance of the screen-printed sensors.

4.3.2 Nickel Measurement in Spiked Urine Samples

The two most convenient sensor designs were used to measure the nickel concentration in a spiked urine sample. These were, on the one hand, the BCE covered with Nafion and, on the other hand, the BCME (unstirred). At 150 µg L^{-1} nickel, which is the highest concentration of the desired detection range, and 30 µM DMG it was not possible to detect a nickel peak in the voltammogram neither at the BCE nor at the BCME. However, at around -1.0 V a huge interference peak is emerging. Surface active substances are probably causing this peak, which are occurring in urine and are interacting at the electrode and, hence, are causing interference (Bond *et al.*, 1986).

To minimise interference from chemical and organic compounds present in urine, the dilution factor was increased. Especially, in the voltammograms of the BCE covered with Nafion, it is apparent that the interference peak is already at its maximum at just 2.5% urine. The interference peak at the BCME is, in relation, slightly smaller, but therefore more random. The smallest detectable nickel peak was as low as 2.5% urine at the BCE and 5% at the BCME. These results are not really satisfying, especially when the high concentration of nickel is considered, which was not spiked in the urine sample itself but in the diluted samples. Including the dilution factor, this would mean a nickel concentration in urine of 6,000 µg L^{-1} and 3,000 µg L^{-1}, respectively.

It was already possible to determine thallium, cadmium, lead and copper in urine without any pretreatment by using stripping voltammetry (Wang, 1982). Hence, the interference peak in urine is probably not the only reason for the missing nickel peak in the voltammograms. The most likely explanation for the failure of the nickel measurement in urine is that a chemical or organic compound is influencing the complex formation of nickel and DMG. In fact, it is known that heavy metal ions are binding with proteins in biological samples (Wang, 1982; Lin *et al.*, 1999), which leads to a decreased voltammetric response (Wang, 1982). Unfortunately, no study regarding the appearance, fate or behaviour of nickel in urine was found. However, as already discussed in Section 4.1.7, L-Histidine for example inhibits the formation of the nickel-DMG complex. Hence, there may be a similar compound in urine influencing the reaction that is responsible for this complexation.

For further investigation two possible rapid and easy ways to extract the nickel ions from the urine sample were discovered in the scientific literature. The first is ion-selective membranes, the very same that are used for ion-selective electrodes. These membranes consist of macrocyclic compounds embedded in polyvinyl chloride, which bind metal cations selectively and carry them through the matrix (Singh and Harsh-Vardhan, 1995). The ionosphere 2,9-(2-methoxyaniline)$_2$-4,11-Me$_2$-[14]-1,4,8,11-tetraene-1,5,8,12-N$_4$ for example is at least 1000-fold more selective for nickel ions than for any other heavy metal or other ions appearing in urine, such as calcium, potassium and sodium (Singh *et al.*, 2009). Hence, when placed in front of the drop-on-cell of the screen-printed sensor, these membranes are minimising interference from chemical and organic compounds and, moreover, also other heavy metals. However, nickel has to be present as an ion, and its exact form in urine is not known. This is a possible way, if DMG rather than nickel is influenced in urine.

The second, more promising way, to determine nickel in urine directly, is to use electromagnetic electrodes. Yantasee *et al.* (2008) used superparamagnetic iron oxide (Fe_3O_4) nanoparticles to extract lead from urine. The schematic can be seen in Figure 4.3. On these nanoparticles dimercaptosuccinic acid (DMSA) is immobilised (c), which has a high selectivity of and affinity to different heavy metals, such as lead. This affinity is utilised to release lead from the complexes it forms in urine and bind lead ions. Then the nanoparticles are extracted with a magnetic field directly applied at the electrode (b),

followed directly by stripping voltammetry in an acidic medium (a). As described by Sarkar (1984) nickel has a high affinity to albumin, L-Histidine, and α2-macroglobulin. Hence, they may be immobilised on the superparamagnetic iron oxide nanoparticles to extract nickel ions from urine. The complex formation of nickel and L-Histidine in buffer was already discussed in Section 4.1.7, which makes it an ideal starting point for further research, next to dimercaptosuccinic acid. Moreover, nickel itself is already ferromagnetic, and if the nickel-L-Histidine complex has this characteristic as well, superparamagnetic iron oxide nanoparticles are maybe not necessary. This technique is the appropriate way if nickel rather than DMG is influenced in urine.

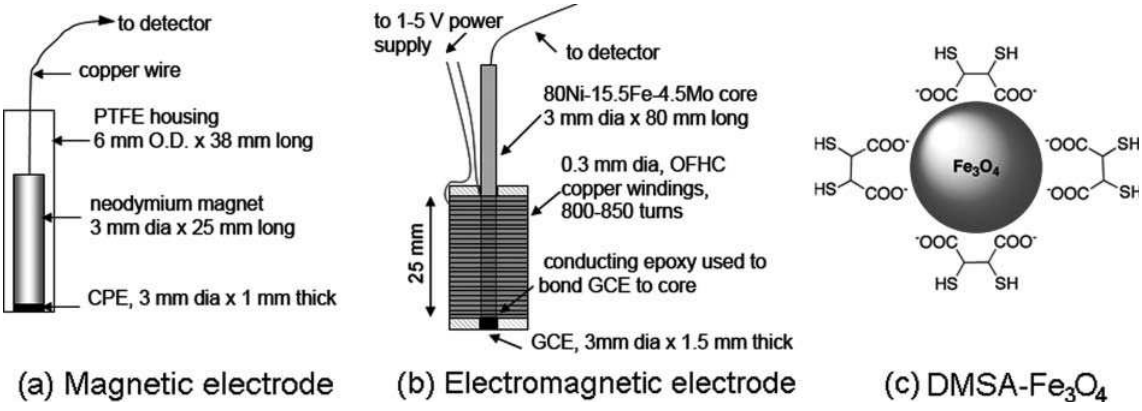


Figure 4.3: Schematics of (a) magnetic electrode and (b) electromagnetic electrode which preconcentrate metal ions using (c) DMSA–Fe_3O_4 nanoparticles (Yantasee et al., 2008).

CHAPTER 5

CONCLUSION AND FUTURE WORK

5.1 CONCLUSION

Nickel is present in various applications and also in food and beverages and is known to be toxic and carcinogenic to humans (ATSDR, 2005). It has already been repeatedly demonstrated that urine can serve as an accurate analyte for the assessment of nickel exposure due to inhalation and ingestion (Sunderman *et al.*, 1986a; McNeely *et al.*, 1972; Angerer and Lehnert, 1990; Werner *et al.*, 1999; Oliveira *et al.*, 2000). At the moment atomic absorption spectrometry and inductively coupled plasma-atomic emission spectroscopy are the most commonly used techniques to determine nickel concentration in urine (ATSDR, 2005). Numerous rapid, cheap and portable nickel detection methods were located in the literature. They can roughly be divided into spectroscopic, bioanalytical and electrochemical detection methods. The most promising approach for the development of a nickel sensor for urine is to use a technique based on voltammetry. In the desired detectable concentration range from 5 to 150 µg L^{-1} nickel, the most convenient method is adsorptive stripping voltammetry, which can provide, especially for the detection of nickel ions, sensitive, selective, precise and accurate results. The ideal chelating complex was found to be dimethylglyoxime (DMG).

During the assessment of the optimal operational parameters in ammonia buffer solution it was observed that the screen-printed sensor, provided by DuPont Ltd. (Bristol, United Kingdom) was not capable of operating under the high pH value of 9.2, which is needed for the complex formation of nickel and DMG. Apparently, component parts of the electrodes were dissolving during the measurement procedure, mainly silver from the reference electrode. These interference phenomena caused a high variability of the voltammograms and made a precise and repeatable measurement impossible.

To suppress the influence of interference on the signal, four different strategies were excogitated and their success regarding interference mitigation evaluated.

First, the DuPont sensors were changed to Florence ones. However, their performance was even worse and the nickel peak was totally masked by a huge interference peak.

Second, the working electrode was covered with Nafion. This membrane prevents negatively charged ions, such as Cl^- and the Ag-Cl complexes, from penetrating the

electrode. This approach led to relatively good results. A linear plot from 5 to 200 µg L^{-1} (R^2 = 0.99) and a limit of detection of 3.0 µg L^{-1} were achieved.

Third, it was tried to increase the peak area by adding L-Histidine to the sample solution. It is known that L-Histidine has a high affinity to bind with nickel. The increased size of the adsorbed complex at the surface of the electrode could have led to a higher current at the stripping potential of nickel. However, no nickel peak was obtained at all and it was not possible to determine the exact reason for this observation. Furthermore, L-Histidine was preventing the complex formation of nickel and DMG.

Fourth, the areas of the electrodes were minimised by producing microband-electrodes. With this approach the best results were achieved. The interference phenomena were nearly eliminated and a linear plot was obtained in the range from 1 to 200 µg L^{-1} (R^2 = 0.99). A limit of detection was determined of 0.9 µg L^{-1}. However, only a poor reproducibility was achieved, very probably caused by the imprecise production process.

All results discussed above were obtained using IR dried bare carbon electrodes. Oven dried electrodes had more problems in coping with the high pH value of the buffer solution and interference played an even greater role. This is probably caused by the different surface structure of the electrodes originating from the drying process. The performance of the bismuth film electrodes was highly negatively influenced by the imprecise production process. Gold film electrodes were determined not to be suitable for a stripping analysis of nickel, due to hydrogen evolution at relative low potentials compared to the carbon and bismuth electrodes.

The sensors showing the best performance (BCE cover with Nafion and BCME) were applied in a spiked urine sample. Unfortunately, it was not possible to directly measure the nickel concentration in urine. The major reason for the impossibility of performing a direct nickel measurement is highly likely caused by organic compounds occurring in urine. They are binding with nickel ions and, hence, influencing the complex formation of nickel and DMG, as it was illustrated with L-Histidine.

5.2 FUTURE WORK

Although it was possible to design successfully a sensor for a concentration range from 5 to 150 µg L^{-1} nickel, some improvements are still necessary. The performance of the bare carbon microband-electrode can be enhanced in various way. At the moment the main problem is reproducibility, which can be achieved with a more precise cut, to obtain well defined edges and comparable surface areas of the electrodes. Furthermore, the working electrode of the microband-electrode can be covered with Nafion to reduce interference further. A possible interference of gold ions can be completely prevented by using a carbon based counter electrode. The working electrode can be manipulated much more accurately during the production of the sensor either with bismuth or antimony to improve sensitivity, or with DMG to avoid its addition to the urine sample and, thus, simplify the measurement procedure. Further research is also necessary in the field of metal-binding proteins and peptides, such as L-Histidine, and their influence on the nickel peak area as well as the optimal measurement operating conditions, which remain unknown.

However, the major obstacles were occurring during the direct nickel measurement in urine. Since the nickel ions are probably binding to organic compounds in urine it is crucial to develop a method to extract the nickel ions from the urine sample. The most promising technique located in the literature is the use of superparamagnetic nanoparticles and extracting the nickel ions with a magnet as described by Yantasee *et al.* (2008) for the direct measurement of lead in urine. Next to dimercaptosuccinic acid it would be worth investigating the efficiency of L-Histidine and α2-macroglobulin to extract nickel from urine, when immobilised on superparamagnetic nanoparticles.

Once the sensor is successfully operating in urine, the influence of other chemicals, in particular heavy metals, on the nickel peak has to be investigated, especially, if a not particularly selective technique is used to extract nickel ions from urine. Furthermore, the sensor has to be tested in different urine samples, with all possible pH ranges and from occupationally exposed people, to ensure that nickel measurements are not only possible in spiked urine samples. The results of real samples have to be compared with standard methods, such as atomic absorption spectrometry or inductively coupled plasma-atomic emission spectroscopy (ICP-AES) to evaluate the accuracy. At the end,

when all problems have been solved, the sensor can be tested in a clinical trial and, if successful in the trial, the sensor may be used commercially for the rapid assessment of human nickel exposure.

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APPENDICES

Appendix A: Table of properties* of different membranes permeable for Ni(II)

Sensor	Matrix	Nickel(II) carrier:	Anion Excluder	Plasticizing solvent mediator	Other	Working concentration range [M]	Slope [mV decade^{-1}]
1	PVC	3,7-bis(2-aminoethyl)-1,3,5,7-tetraazabicyclo[3.3l.l nonane (batn)	STPB	DBP, DOP		$1 \times 10^{-5} – 1 \times 10^{-1}$	25.5 – 28.5
2	PVC	8-methyl-1,3,6,8,10,13,15-heptaazatricyclo[13.1.1.1^{13,15}]octadecane (mho)	STPB	DBP, DOP		$1 \times 10^{-5} – 1 \times 10^{-1}$	25.0 – 29.5
3	PVC	1,3,6,9,11,14-hexaazatricyclo-[12.2.1.1^{6,9}]octadecane (ho)	STPB	DBP, DOP		$1 \times 10^{-5} – 1 \times 10^{-1}$	25.5 – 29.5
4	PVC	1,3,6,10,12,15-hexaazatricyclo[13.3.1.1^{6,10}]eicosane(he)	STPB	DBP, DOP		$1 \times 10^{-5} – 1 \times 10^{-1}$	25.5 – 29.0
5	PVC and PS	5,7,7,12,14,14-hexamethyl-1,4,8,11-tetraazacyclotetradeca-4,11-diene diperchlorate	STPB	DBP, DOP		$5 \times 10^{-6} – 5 \times 10^{-2}$	25.0 – 30.0
6	PVC and PS	3,5,7,7,10,12,14,14-octamethyl-1,4,&11-tetraazacyclotetradeca-4,11-diene diperchlorate	STPB	DBP, DOP		$3 \times 10^{-6} – 5 \times 10^{-2}$	27.0 – 31.0
7	PVC	5,10,15,20-tetraphenylporphyrin	STPB	CN, DBP, DBBP, DOP, TEP		$1.4 \times 10^{-5} – 1.0 \times 10^{-1}$	24.0 – 53.9
8	PVC[#]	5,10,15,20-tetra(4-methylphenyl) porphyrin	STPB	CN, DBP, DBBP, DOP, TEP		$5.6 \times 10^{-6} – 1.0 \times 10^{-1}$	30.1 – 40.0
9	PVC	1,10-dibenzyl-1,10-diaza-18-crown-6	STPB	NPOE	OA, THF	$2.0 \times 10^{-5} – 5.5 \times 10^{-3}$	29.8
10	PVC	Dibenzodiaza-15-crown-4	n. a.	DBP, DMS, DOP, NPOE	THF	$3.0 \times 10^{-7} – 7.8 \times 10^{-2}$	15.8 – 40.2
11	PVC	5,7,12,14-Tetramethyldibenzotetraazaannulene	STPB	DBP, DBBP, DOP, CN, TBP, TEP		$7.9 \times 10^{-6} – 1.0 \times 10^{-1}$	30.0 – 40.0
12	PVC	2-methyl-4-(4-methoxy phenyl)-2,6-diphenyl-2*H*-thiopyran	STPB	BA, DBP, DOP	OA, THF	$2.0 \times 10^{-5} – 5.0 \times 10^{-2}$	6.0 – 29.5
13	PVC	1,5-diphenylthiocarbazone	n. a.	DBP, DBS, NPOE	DZ, THF	$5.0 \times 10^{-6} – 1.0 \times 10^{-2}$	21.0 – 28.6

Table continued:

Sensor	Response time	pH working range	Reported selectivity coefficients of other ions compared to Ni^{2+} °	Lifetime	Analytic application	Reference
1	20s – 2min	2.8 – 5.5	Various (K^+ and NH_4^+ close to 1, Na^+ 10^{-1})	4 months	Effluent from an electroplating works	Singh and Harsh-Vardhan, 1995
2	25s – 1.5min	2.8 – 5.5	Various (K^+ and NH_4^+ close to 1, Na^+ 10^{-1})	4 months	Effluent from an electroplating works	Singh and Harsh-Vardhan, 1995
3	35s – 2min	2.8 – 5.5	Various (K^+ and NH_4^+ close to 1, Na^+ 10^{-1})	4 months	Effluent from an electroplating works	Singh and Harsh-Vardhan, 1995
4	20s – 3min	2.8 – 5.5	Various (K^+ and NH_4^+ close to 1, Na^+ 10^{-1})	4 months	Effluent from an electroplating works	Singh and Harsh-Vardhan, 1995
5	10s – 45s	2.8 – 5.5	Various (Na^+ close to 1, K^+ and NH^{4+} 2 x 10^{-2})	n.s.	Potentiometric titration	Jain *et al.*, 1997
6	10s – 40s	2.8 – 5.5	Various (Na^+ close to 1, K^+ and NH^{4+} 2 x 10^{-2})	n.s.	Potentiometric titration	Jain *et al.*, 1997
7	15s – 90s	2.5 – 7.4	Various (Na^+ up to 6.8 x 10^{-1}, K^+ and NH^{4+} up to 3.5 x 10^{-2})	2 months	Potentiometric titration and chocolate	Gupta *et al.*, 1997
8	25s – 2min	2.5 – 7.4	Various (Na^+ up to 6.8 x 10^{-1}, K^+ and NH^{4+} up to 3.5 x 10^{-2})	2 months	Potentiometric titration and chocolate	Gupta *et al.*, 1997
9	30s	4.0 – 8.0	Various (polyvalent cations 10^{-2}, univalent cations 10^{-1}) Na^+ n.s.	> 6 weeks	Potentiometric titration	Mousavi *et al.*, 2000
10	< 20s	3.0 – 6.0	Various (Ag^+ and Pb^{2+} 5.0 x 10^{-1}, Mg^{2+}, Ca^{2+} and Co^{2+} around of 10^{-2})	> 2 months	Potentiometric titration and chocolate	Shamsipur and Yahya Kazemi, 2000
11	10s – 22s	2.7 – 7.6	Various (Na^+ 5.0 x 10^{-1}, K^+, Ag^{2+} and NH_4^+ up to 3.1 x 10^{-2})	6 months	Potentiometric titration and chocolate	Gupta *et al.*, 2000
12	around 10s	3.0 – 6.0	Various (Co^{2+} 3.85 x 10^{-2}) Na^+, K^+, Ca^+ n.s.	> 8 weeks	Effluent from an electroplating works	Ganjali *et al.*, 2000
13	40s – 45 s	3.0 – 3.0 (?)	Various (Fe^{2+} and Tl^+ 1.2 x 10^{-1}) Na^+, K^+, Ca^+ n.s.	2 months	n. a.	Abbaspour and Izadyar, 2001

Table continued:

Sensor	Matrix	Nickel(II) carrier:	Anion Excluder	Plasticizing solvent mediator	Other	Working concentration range [M]	Slope [mV decade^{-1}]
14	PS	3,4:11,12-dibenzo-2,5,10,13-tetraoxo-1,6,9,14-tetraazacyclohexadecane	n. a.	n. a.		$3.16 \times 10^{-6} – 1.00 \times 10^{-1}$	25.7 – 31.9
15	PVC	Benzylbis(thiosemicarbazone) coated in graphite	STPB, KTCB	BA, DBP	OA, THF	$1.0 \times 10^{-7} – 1.0 \times 10^{-2}$	3.5 – 29.1
16	PVC	Nickel-5,7,12,14-tetramethyldibenzo[b,i]-1,4,8,11-tetraazacyclotetradecane	STPB	CN, DBP, DBBP, DOP, TBP	THF	$7.08 \times 10^{-6} – 1.0 \times 10^{-1}$	29.8 – 37.0
17	PVC[#]	1,3,7,9,13,15,19,21-Octaazapentacyclooctacosane directly coated on a platium wire electrode	STPB	DBP	THF	$1.0 \times 10^{-6} – 1.0 \times 10^{-1}$	30.5
18	PVC	(2-mercapto-4-methylphenyl)-2-benzamido-3-ethoxy-thiopropenoate	STPB	DBP, DMS, DOP, NPOE	THF	$1.0 \times 10^{-7} – 1.0 \times 10^{-2}$	3.1 – 29.5
19	PVC	*N*,*N*-bis-(4-dimethylamino-benzylidene)-benzene-1,2-diamine	STPB	DBP, DMS, DOP, NPOE	THF	$2.0 \times 10^{-7} – 1.0 \times 10^{-2}$	2.0 – 29.0
20	PVC	4,4',4",4"'-21*H*,23*H*-porphine–5,10,15,20 –tetrayl) tetrakis (benzoic acid)	STPB	CN, DBP, DBBP, DOP		$2.0 \times 10^{-6} – 1.0 \times 10^{-1}$	26.0 – 35.3
21	PVC	2,3,7,8,12,13,17,18-octamethyl-21*H*, 23*H*-porphine	STPB	CN, DBP, DBBP, DOP		$1.0 \times 10^{-5} – 1.0 \times 10^{-1}$	29.0 – 38.8
22	PVC	2,5-thiophenylbis(5-*tert*-butyl-1,3-benzexazole)	KTCB	BA, DOP, NPOE	OA, THF	$1.0 \times 10^{-8} – 1.0 \times 10^{-3}$	n. a.
23	PVC	3,4:12,13-dibenzo-1,6,10,15-tetraazacyclooctadecane	STPB	DBP, DBBP, TBP, TEP		$1.26 \times 10^{-5} – 1.0 \times 10^{-1}$	28.5 – 32.0
24	PVC	*N*-(2-hydroxybenzyl)-*N*-(2-hydroxybenzylidene)ethylenediamine Ni(II)	STPB	CN, DEP, DOS, TEP		$6.3 \times 10^{-6} – 5.0 \times 10^{-2}$	28.0 – 31.0
25	PVC	*N*-(2-hydroxybenzylidene)-N-(2-picolyl)ethylenediamine Ni(II)	STPB	CN, DEP, DOS, TEP		$3.2 \times 10^{-6} – 5.0 \times 10^{-2}$	27.0 – 33.0
26	PVC	5,11,17,23,29,35-Hexakis-t-octyl-37,38,39,40,41,42-hexakis(N-phenylthiocarbamoylmethoxy)calix[6]arene	STPB	DBP		$5.0 \times 10^{-6} – 1.0 \times 10^{-1}$	8.97 – 34.87

Table continued:

Sensor	Response time	pH working range	Reported selectivity coefficients of other ions compared to Ni^{2+} °	Lifetime	Analytic application	Reference
14	10s – 30s	2.5 – 7.0	Various (K^+ and NH_4^+ around to 5 x 10^{-1}, Na^+ at least 10^{-1})	2 months	Effluent from an electroplating works	Singh *et al.*, 2001
15	around 15s	4.0 – 7.0	Various (Cd^{2+} and Ag^{2+} 5.0 x 10^{-3}, others below 9.0 x 10^{-4})	> 3 months	Potentiometric titration and effluent from an electroplating works	Ganjali *et al.*, 2002
16	12s – 40s	2.0 – 7.5	Various (Na^+ 6.8 x 10^{-1}, others below 9.0 x 10^{-2})	6 months	Potentiometric titration and effluent from an electroplating works	Gupta *et al.*, 2002
17	5s- 40s	3.0 – 6.0	Various (all below 1.1 x 10^{-2})	> 2 months	Potentiometric titration, chocolate and milk	Mazloum *et al.*, 2002
18	15s	5.0 – 8.5	Various (Co^{2+} 2.5 x 10^{-2}, Ag^+ 1.7 x 10^{-2}, all others below) Na^+ n.s.	> 4 weeks	Potentiometric titration	Mashhadizadeh and Momeni, 2003
19	5s – 10s	4.5 – 9.0	Various (Ag^+ 1 x 10^{-1} and Hg^{2+} 3 x 10^{-2}, all others below) Na^+ n.s.	Around 2 months	Potentiometric titration and different water samples	Mashhadizadeh *et al.*, 2003
20	10s – 60s	2.0 – 7.0	Various (Co^{2+} 2.3 x 10^{-1}, Na^+ 9.0 x 10^{-2} and K^+ 5.3 x 10^{-2})	6 months	Potentiometric titration and effluent from an electroplating works	Singh and Bhatnagar, 2003
21	10s – 60s	2.0 – 7.0	Various (Co^{2+} 4.8 x 10^{-1} and Na^+ 8.2 x 10^{-2})	6 months	Potentiometric titration and effluent from an electroplating works	Singh and Bhatnagar, 2003
22	< 40s	4.0 – 9.0	Various (poor to other heavy metals, eg.: nearly the same than Co^{2+})	> 2 months	Potentiometric titration and effluent from an electroplating works	Shamsipur *et al.*, 2004
23	18s – 30s	2.5 – 7.5	Various (all heavy metals below 7.22 x 10^{-2}) Na,K,Mg,Ca n.s.	About 4 months	Potentiometric titration, chocolate and milk	Singh and Singh, 2005
24	15s – 45s	2.2 – 5.9	Various (all below 5.3 x 10^{-3})	4 months	**Urine**, chocolate and electroplating waste water samples	Jain *et al.*, 2005
25	27s – 30s	2.2 – 5.9	Various (all below 2.9 x 10^{-3})	4 months	**Urine**, chocolate and electroplating waste water samples	Jain *et al.*, 2005
26	< 15s	3.5 – 7.5	Various (Co^{2+} and Hg^{2+} even more selective)	> 1 month	Effluent from an electroplating works	Belhamel *et al.*, 2005

Table continued:

Sensor	Matrix	Nickel(II) carrier:	Anion Excluder	Plasticizing solvent mediator	Other	Working concentration range [M]	Slope [mV decade^{-1}]
27	PVC	*N*1,*N*2-bis((naphthalen-1-yl)-methylene)ethane-1,2-diamine	STPB	DBP, DMS, DOA, DOS, DOP	OA, THF	$1.3 \times 10^{-6} – 1.0 \times 10^{-1}$	12.3 – 29.9
28	PVC	(2*E*, 3*E*)-2*H*-1,4-benzothiazine-2,3(4*H*)-dione dioxime	STPB	DBP, DOS, DOP, TBP	THF	$1.0 \times 10^{-6} – 1.0 \times 10^{0}$	29.3
29	PVC	(1E,4E)-N^1,N^2-bis(thiophen-2-yl)methylene)-ethane-1,2-diamine	STPB	DBP, DBS, DMS, DOA, DOP, DOS	OA, THF	$1.8 \times 10^{-6} – 1.0 \times 10^{-1}$	12.8 – 29.5
30	PVC	Dibenzo[*e*,*k*]-2,3,8,9-tetraphenyl-1,4,7,10-tetraazacyclododeca-1,3,5,7,9,11-hexaene	STPB	CN, DBBP, DOP, TEP		$3.98 \times 10^{-6} – 1.0 \times 10^{-1}$	28.5 – 33.5
31	PVC	meso-tetrakis-{4-[tris-(4-allyl dimethylsilyl-phenyl)-silyl]-phenyl}porphyrin	STPB	CN, DBBP, DBP, DOP, TBP		$2.5 \times 10^{-6} – 1.0 \times 10^{-1}$	29.4 – 33.0
32	PVC	$(sal)_2$trien	STPB	CN, DBBP, DBP, DOP, TBP		$5.0 \times 10^{-6} – 1.0 \times 10^{-1}$	29.1 – 36.4
33	PVC	Dibenzo-18-crown-6	STPB	CN, DBBP, DBP, DOP, TBP, TEP		$1.0 \times 10^{-5} – 1.0 \times 10^{-1}$	29.5 – 34.0
34	PVC	3-hydroxy-N-{2-[(3-hydroxy-N-phenylbutyrimidoyl)-amino]-phenyl}-N-phenylbutyramidine	STPB	CN, DBBP, DBP, DOP, TBP	THF	$1.6 \times 10^{-7} – 5.0 \times 10^{-2}$	26.0 – 33.3
35	PVC	Bis-4-(ethyliminomethyl)naphthalene-1-ol	STPB	CN, DBBP, DBP, DOP, TBP	THF	$5.2 \times 10^{-6} – 5.0 \times 10^{-2}$	28.0 – 32.5
36	PVC	5-methoxy-5,6-diphenyl-4,5 dihydro-3(2H)-pyridazinethione	STPB	DBP, DOP	THF	$1.0 \times 10^{-6} – 1.0 \times 10^{-2}$	10.2 – 31.2
37	PVC	Glyoxal-bis(S-benzyldithiocarbazate)	n. a.	DOP, NPOE, TBP	HTAB	$2.8 \times 10^{-7} – 1.0 \times 10^{-1}$	24.6 – 43.8
38	PVC	2,9-(2-methoxyaniline)2-4,11-Me2-[14]-1,4,8,11-tetraene-1,5,8,12-N4 polymeric membrane electrode	STPB	CN, DBP, DOP, NPOE, TBP		$4.6 \times 10^{-7} – 1.0 \times 10^{-1}$	29.5 – 44.3
39	PVC	2,9-(2-methoxyaniline)$_2$-4,11-Me$_2$-[14]-1,4,8,11-tetraene-1,5,8,12-N_4 coated in graphite	STPB	CN, DBP, DOP, NPOE, TBP		$7.7 \times 10^{-8} – 1.0 \times 10^{-1}$	29.5

Table continued:

Sensor	Response time	pH working range	Reported selectivity coefficients of other ions compared to Ni^{2+} °	Lifetime	Analytic application	Reference
27	15s – 65s	3.6 – 7.4	Various (Na^+ 9.2 x 10^{-2}, others below 9.1 x 10^{-3})	4 months	Vegetable oil, chocolate and electroplating waste water samples	Kumar *et al.*, 2006
28	<10s	2.0 – 6.5	Various (Ag^+, Na^+, K^+ and NH^{4+} around 2 x 10^{-1})	> 4 months	Potentiometric titration and different water samples	Yari *et al.*, 2006
29	11s – 40s	3.2 – 7.9	Various (Pb^{2+}, Ag^{2+},Na^+ and K^+ around 1.5 x 10^{-2}, all others below)	4 months	Potentiometric titration, chocolate and electroplating waste water samples	Kumar *et al.*, 2007
30	8s – 30s	2.5 – 7.7	Various (Co^{2+} 3.8 x 10^{-1}, Na^+, Cu^{2+} and Cd^{2+} around 2.0 x 10^{-2})	4 months	Potentiometric titration, chocolate and milk	Singh and Saxena, 2007
31	8s – 20s	2.0 – 5.5	Various (Zn^{2+} 9.0 x 10-3, all others below)	4 months	Potentiometric titration and chocolate	Gupta *et al.*, 2007a
32	10s – 25s	2.0 – 5.5	Various (Zn^{2+} 1.5 x 10-2, Na^+ 2.0 x 10^{-3}, all others below)	2 months	Potentiometric titration and chocolate	Gupta *et al.*, 2007a
33	25s – 60s	2.6 – 6.8	Various (Na^+ 5.5 x 10^{-1}, Cd^{2+} 3.0 x 10^{-1}, all others in the range of 10^{-2})	4 months	Potentiometric titration and chocolate	Gupta *et al.*, 2007b
34	10s – 16s	2.5 – 9.5	Various (other heavy metals in the range of 10^{-2}, Na^+, K^+ around 10^{-3})	4 months	Potentiometric titration, chocolate and some plants	Gupta *et al.*, 2008
35	11s – 15s	2.5 – 9.5	Various (mainly all other heavy metals in the range of 10^{-2}, Na^+ 1.0 x 10^{-2})	n.s.	Potentiometric titration, chocolate and some plants	Gupta *et al.*, 2008
36	< 15s	4.0 – 6.0	Various (Co^{2+} 6.1 x 10^{-1}, all others in the range 10^{-3} and below)	> 45 days	Potentiometric titration and electroplating waste water samples	Zamani *et al.*, 2008
37	25s – 30s	4.0 – 7.5	Various (Ag^+ 8.1 x 10^{-3}, all others below) Na^+ n.s.	3 months	Potentiometric titration, chocolate and milk	Ma *et al.*, 2009
38	10s	3.0 – 8.0	Various (Na^+, Cu^{2+}, Co^{2+} and Cd^{2+} around 5.0 x 10^{-2}, others below)	4 months	Potentiometric titration, wine and juices	Singh *et al.*, 2009
39	8s – 20s	3.0 – 8.0	Various (Na^+, Cu^{2+}, Co^{2+} and Cd^{2+} around 5.0 x 10^{-3}, others below)	5 months	Potentiometric titration, wine and juices	Singh *et al.*, 2009

Table continued:

* Properties depending on the mixture of the compounds in the membrane; normally properties of the mixture with the best performance are listed

[#] Only one mixture arrangement was used

°Selectivity coefficients were measured and calcul ated by different methods and are not completely comparable

Abbreviations:

BA	Butylacetate
CN	Chloronaphthalene
DBBP	Dibutyl butylphosphonate
DBP	Dibutyl phthalate
DBS	Dibutyl sebacate
DEP	Diethylphthalate
DMS	Dimethyl sebacate
DOA	Dioctyl adipate
DOP	Dioctyl phthalat
DOS	Bis(2-ethylhexyl)sebacate
DZ	Dithizone
HTAB	Hexadecyltrimethylammonium bromide
KTCB	Potassium tetrakis(p-chlorophenyl borate)
NPOE	2-nitrophenyl octyl ether
OA	Oleic acid
PS	Polystyrene
PVC	Polyvinyl chloride
STPB	Sodium tetraphenyl borate
TBP	Tributyl phosphate
TEP	Tris(2-ethylhexyl) phosphate
THF	Tetrahydrofuran

Appendix A: References:

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Appendix B: Solution preparation details

In alphabetic order:

- **Ammonia buffer solution (pH 9.2, various concentrations, ca. 500 ml)**

1) Ammonia solution (250 ml)

	0.01 M	0.1 M	0.2 M	0.267 M	0.5 M
Ammonia (25%, 13.4 M)	0.19 ml	1.87 ml	3.74 ml	4.99 ml	9.35 ml
HPLC water	249.81 ml	248.13 ml	246.26 ml	245.01 ml	240.65 ml

2) Ammonium chloride solution* (250 ml)

	0.01 M	0.1 M	0.2 M	0.267 M	0.5 M
Ammonium chloride (FW: 53.49 g mol^{-1})	0.13 g	1.34 g	2.68 g	3.58 g	6.7 g
HPLC water	249.92 ml	249.12 ml	248.25 ml	247.66 ml	245.62 ml

* based on a density of 1.53 g cm^{-3}

3) Afterwards the ammonia solution and ammonium chloride solution were mixed together until a pH value of 9.2 was obtained (approximately 1:1)

- **DMG stock solution* (0.02 M, 250 ml)**

DMG (FW: 116.12 g mol^{-1})	0.58 g
Ethanol (95%)	249.58 ml

* based on a density of 1.37 g cm^{-3}

Appendix B: Solution preparation details

- **L-Histidine solution* (5 and 10 mM, 50 ml)**

	5 mM	10mM
L-Histidine (FW: 155.15 g mol^{-1})	38.8 mg	77.5 mg
Ammonia buffer solution (0.2 M)	50 ml	50 ml

* density unknown; because of the small L-Histidine quantities neglected

- **Nickel stock solution (1 mg L^{-1}, 40 ml)**

Nickel stock solution (1 g L^{-1})	40 µl
Ammonia buffer solution (0.2 M or 0.267 M)	39.96 ml

- **Nitric acid wash buffer (1%, 500 ml)**

Nitric acid solution (69%)	7.25 ml
HPLC water	492.75 ml

- **Potassium ferrocyanide solution* (2 mM, 50 ml)**

Potassium ferrocyanide (FW: 422.4 g mol^{-1})	42.24 mg
Ammonia buffer solution (0.2 M)	49.977 ml

* based on a density of 1.85 g cm^{-3}