The influence of pH on the *in vitro* permeation of platinum through human skin

Y van Nieuwenhuizen

22764844

BSc, BSc (Hons)

Mini-dissertation submitted in partial fulfilment of the requirements for the degree *Magister Scientiae* in Occupational Hygiene at the Potchefstroom Campus of the North-West University

Supervisor:	Prof A Franken
Co-supervisor:	Prof JL du Plessis
Assistant-supervisor:	Prof J du Plessis

December 2016

Acknowledgements

The author would like to thank everyone who contributed to this study, especially the following persons:

- Prof A. Franken, for being a remarkable supervisor and the guidance she provided throughout this study as well as for sharing her knowledge.
- Prof J.L. du Plessis, for his professional opinion, direction, advice and calmness during this study.
- Prof J. du Plessis, for her professional feedback during the study.
- Miss S.J. Jansen van Rensburg, for the additional help and guidance as well as the patience she had during this study.
- My parents, for their unconditional love, support and motivation throughout the duration of my studies.
- Mr W.E. Jordaan, for his support and encouragement.
- Miss M. Keyter and Miss L. Myburgh, for their much appreciated help and assistance in the laboratory.
- All the doctors, nurses and administrative staff at the hospitals and the patients for their willingness to contribute towards this study and for donating skin.
- The National Research Foundation for funding this research project.

Preface

In this mini-dissertation the article format is used. The reference style in the mini-dissertation follows the guidelines of *Toxicology in Vitro,* the journal chosen for potential publication and used throughout for uniformity. Regarding the references, this journal has no strict requirements for the reference format and can be in any style but should be consistent. The reference list must however be in alphabetical order by name of first author and thereafter chronological, if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication. Details on the requirements of the specific referencing aspects can be found in Chapter 3.

The outline of this mini-dissertation is as follows:

Chapter 1 is an introductory chapter and provides the necessary background with regard to the study. It includes the problem statement, aims and hypothesis of the study.

Chapter 2 presents a basic summary of the relevant literature regarding platinum group metals and specifically platinum, and the possible health effects thereof. It also contains the information regarding the skin structure, its barrier function and skin surface pH. Also critically discussed is the influence of pH on the ionisation of metals and permeation through the skin.

Chapter 3 is the article to be submitted for publication. It includes background information, the materials and methods used, results obtained, a discussion thereof as well as a conclusion.

Chapter 4 is the concluding chapter containing a further discussion, the overall conclusions, recommendations for occupational settings and future studies, and the limitations of this study.

Chapter 5 is the appendix and includes the report from the language editor and the ethical approval certificate.

The National Research Foundation (NRF) Thuthuka Funding Grant (UID: 94113), awarded in 2015, funded this research study.

Disclaimer: Any opinion, finding and conclusion or recommendation expressed in this material is that of the author(s) and the NRF does not accept any liability in this regard.

Author contributions

This study was planned and executed by a team of researchers. The contribution of each researcher is described as follows:

Ms Y van Nieuwenhuizen (Author): Responsible for planning, design and writing of the mini-dissertation under the supervision of Prof A Franken and Prof JL du Plessis, as well as researching and reviewing of the relevant literature, collection of data and interpretation of the results.

Prof A Franken (Supervisor): Involved in all aspects of this study, specifically supervising the design and planning of the experimental method, critically reviewing the mini-dissertation and guiding the interpretation of results and the writing of the mini-dissertation.

Prof JL du Plessis (Co-supervisor): Contributed towards the design and planning of the sampling method; responsible for critically reviewing the mini-dissertation and guiding the interpretation of results and the writing of the mini-dissertation.

Prof J du Plessis (Assistant-supervisor): Responsible for critically reviewing and supervising the writing of the mini-dissertation.

The following is a statement from the researchers involved, confirming each individual`s role in this study:

I declare that I have approved the above-mentioned study and that my role in the completion thereof as indicated above is representative of my actual contribution. I hereby give my consent that it may be published as part of Y van Nieuwenhuizen's MSc Occupational Hygiene mini-dissertation.

_____

Ms Y van Nieuwenhuizen (MSc Student)

_____

Prof A Franken (Supervisor)

_____

Prof JL du Plessis (Co-supervisor)

_____

Prof J du Plessis (Assistant-supervisor)

Table of Contents

List of Tables

Chapter 3

List of Figures

Chapter 2 **page**

Chapter 3

Abstract

Background: At platinum mines and refineries, and other industries such as the catalytic industry, workers are at risk of being potentially exposed via dermal contact to soluble platinum salts, which are known sensitisers and allergy-eliciting compounds. The availability of information regarding the permeability of soluble platinum salts through intact human skin and its health effects specifically on the skin is limited. The permeation of platinum was confirmed only at a pH of 6.5 but the influence of a lower pH on platinum permeation has not yet been investigated. The *in vitro* permeation of metals was investigated in previous studies and showed that a lower pH could lead to an increase in permeation. This could be due to the metals oxidising at a lower pH leading to the formation of permeable ions. Therefore, an acidic environment could potentially increase the permeation of a metal. *Aim:* The aim of this study was to determine and compare the permeation of a soluble platinum salt, potassium tetrachloroplatinate (K_2PtCl_4), at a pH of 4.5 and 6.5. *Method:* Full thickness abdominal skin from two female Caucasian donors, aged 37 and 47, were obtained as biological waste after surgery. The Franz diffusion cell method was used in which the synthetic sweat in the donor compartment of the experimental cells contained 0.3 mg/ml of K_2PtCl_4. The physiological receptor solution was removed at intervals of 1, 2, 6, 12, 18 and 24 hours for analysis. After 24 hours the receptor and donor solution were removed for analysis and the skin chemically digested before analysis. The mass of platinum in the receptor solution was determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The platinum mass in the donor and digested skin solutions were determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). *Results:* Platinum permeated through intact human skin, at both pH levels of 4.5 and 6.5. The mass of platinum that permeated through the skin was 58.09% higher at a pH of 4.5 (34.18 ± 7.79 ng/cm^2) than at a pH of 6.5 (21.62 ± 4.4 ng/cm^2) after 24 hours. The retention of platinum in the skin was statistically significantly higher at a pH of 4.5 (2118.9 ± 62.6 ng/cm^2) than at a pH of 6.5 (1771.3 ± 131.9 ng/cm^2) ($p = 0.02$). The mass of platinum that was retained in the skin was statistically significantly higher than the mass that diffused through the skin ($p \leq 0.001$) at both pH levels. The lag time at a pH of 4.5 (2.47 ± 0.34 h) was 37.25% shorter than at a pH of 6.5 (3.39 ± 0.28 h) and leaned towards being statistically significant ($p = 0.054$). *Conclusion:* A decrease in pH resulted in increased mass of platinum retained inside the skin, which prolongs the exposure time and results in more platinum potentially permeating through the skin. Therefore, an acidic environment, such as a precious metal refinery, poses a greater risk for the permeation of platinum through the skin and the significantly higher risk for retention of platinum inside the skin.

Key words: Skin surface pH, skin permeation, metal, platinum group metals.

Opsomming

Agtergrond: By platinummyne en -raffinaderye soos die katalisatorindustrie, loop werkers die risiko om moontlik blootgestel te word aan oplosbare platinumsoute via die vel, wat bekende sensitiseerders en allergie ontlokkende verbindings is. Die beskikbaarheid van inligting in verband met die vel se deurlaatbaarheid vir oplosbare platinumsoute asook die gesondheidseffekte daarvan is beperk. Dit is reeds bevind dat platinum die vel kan deurdring by 'n pH van 6.5, alhoewel die invloed van 'n laer pH nog nie ondersoek is nie. Die *in vitro*-deurlaatbaarheid van metale was ondersoek in vorige *in vitro*-studies en het getoon dat 'n laer pH moontlik kan lei tot verhoogde deurlaatbaarheid. Dit kan wees as gevolg van metale wat oksideer by 'n laer pH, wat lei tot die vorming van ione wat deurlaatbaar is deur die vel. Dus kan 'n suur-omgewing moontlik die deurlaatbaarheid van die vel vir 'n metaal verhoog. *Doelstellings*: Die doel van die studie was om die deursypeling van 'n oplosbare platinumsout, kaliumtetrachloroplatinaat (K_2PtCl_4), te bepaal en the vergelyk by pH's van 4.5 en 6.5. *Metode:* Voldikte abdominale vel van twee vroulike Kaukasiër-skenkers met ouderdomme 37 en 47 is verkry as biologiese afval na chirurgie. Die Franz-diffusieselmetode is gebruik waar die sintetiese sweet van die eksperimentele selle 0.3 mg/ml K_2PtCl_4 bevat het. Die reseptorvloeistof is by intervalle van 1, 2, 6, 12, 18 en 24 ure verwyder vir analise. Na 24 uur is die reseptor- en skenkeroplossings om die beurt verwyder en die vel is chemies verteer vir analise. Die massa van die platinum in die reseptoroplossings is geanaliseer met Induktief-Gekoppelde Plasma-Massaspektrometrie. Die platinummassa in die skenker en verteerde vel-oplossings is geanaliseer deur middel van Induktief-Gekoppelde Plasma-Optiese Emissiespektrometrie. *Resultate:* Platinum het die vel deurdring by beide pH waardes van 4.5 en 6.5. Die massa platinum wat deurgedring het by pH 4.5 (34.18 ± 7.79 ng/cm^2) was 58.09% hoër as by pH 6.5 (21.62 ± 4.4 ng/cm^2) na 24 uur. Die platinum retensie in die vel was statisties betekenisvol hoër by 'n pH van 4.5 (2118.9 ± 62.6 ng/cm^2) as by 'n pH van 6.5 (1771.3 ± 131.9 ng/cm^2) ($p = 0.02$). Die massa platinum wat in die vel geakkumuleer het was statisties betekenisvol hoër as die massa wat deurbeweeg het ($p \leq 0.001$) vir beide pH's. Die tydsverloop (*lag time*) by 'n pH van 4.5 (2.47 ± 0.34 h) was 37.25% vinniger as die na-yling (*lag time*) vir pH 6.5 (3.39 ± 0.28 h) en het geneig na statistiese betekenisvolheid ($p = 0.054$). *Gevolgtrekking:* Die afname in pH het 'n toename in platinumretensie in die vel veroorsaak, wat die blootstellingstyd verleng en moontlik 'n toename in platinumdiffusie tot gevolg gehad het. Dus, sal 'n suuromgewing soos by 'n raffinadery 'n groter risiko inhou vir platinum om deur die vel te beweeg en die aansienlike risiko inhou vir platinumretensie in die vel.

Sleutelwoorde: vel-oppervlak-pH, vel-deurlaatbaarheid, metaal, platinumgroepmetale.

Chapter 1 – Introduction

1.1 General Introduction

The platinum group metals (PGMs) are six closely related metals with very similar chemical properties and are widely used in emission control catalysts as the active ingredient that convert hazardous emissions to less hazardous substances (Ash *et al.,* 2014). Platinum, the most well-known and widely used PGM, is a rare and durable metal with exceptional catalytic properties (Cawthorn, 1999; Gómez *et al*., 2000; Merget and Rosner, 2001; Ash *et al.,* 2014). Due to platinum's major commercial significance, it is also used in numerous other industries such as jewellery and glass production, catalyst manufacturing as well as in the petroleum and medical industries (Cristaudo *et al.,* 2005; Wiseman and Zereini, 2009; Zereini *et al*., 2012). The remaining PGMs include rhodium, iridium, osmium, ruthenium and palladium (Cawthorn, 1999).

The PGM mining sector of South Africa is considered to be one of the largest components of the South African mining industry and is a substantial contributor to the economy (Chamber of Mines, 2010, 2014). The Chamber of Mines (2010) reported that 54.80% of the three most prominent PGMs (platinum, rhodium and palladium) were supplied by South Africa in 2009, along with South Africa being the worldwide leading producer of platinum (76.50%) and rhodium (86.10%). There was however a significant decrease in platinum production from 2011 to 2012 due to a devastating strike that shook the industry. Nevertheless, in 2013 there was a 6.6% increase in the platinum production from 2012 in South Africa and accounted for 71.80% of the worldwide platinum production that year (Chamber of Mines, 2014). The Chamber of Mines (2014) stated that the largest number of workers in the mining industry were employed at PGM mines with 191 261 workers.

The occupational exposure of workers to the various forms of platinum and other PGMs in the aforementioned industries, as well as the mining and refining processes, takes place on a regular basis (Cleare *et al.,* 1976; Rao and Reddi, 2000; Chang *et al*., 2012). The exposure to PGMs can occur through various exposure routes, namely inhalation, ingestion or through the skin. Therefore, the gradual increase in the production and use of platinum and other PGMs could lead to the increased exposure of miners and refinery workers via any route, which could pose adverse implications to their health. Their exposure via any route can be re-occurring, resulting in the accumulation of PGMs in the body, which may contribute to adverse health effects (Boscolo *et al*., 2004; Sartorelli *et al*., 2012).

Dermal exposure has only recently become a research topic; therefore, reliable experimental data on the dermal permeation of PGMs, specifically platinum, is lacking (Sartorelli *et al*., 2012). Dermal exposure and/or the permeability through the skin of certain metals, such as nickel,

cobalt, chromium, silver and gold have been investigated (Larese Filon *et al*., 2004, 2007, 2009, 2011, 2012; Du Plessis *et al.,* 2013). Research performed on platinum primarily focused on the occupational exposure to airborne particulate matter (Gómez *et al*., 2000, 2001; Wiseman and Zereini, 2009; Zereini *et al*., 2012). Platinum salts, specifically halogenated complexes, have been established to be potent sensitisers and are allergenic to humans (Hunter *et al.,* 1945; Boscolo *et al*., 2004). Niezborala and Garnier (1996) and Merget and Rosner (2001) reported that halogenated platinum salts, more specifically hexa- and tetrachloroplatinates, induce toxicity or hypersensitivity reactions i.e. respiratory symptoms such as asthma as well as dermal symptoms such as contact urticaria and to a lesser degree, contact dermatitis. Maynard *et al.* (1997) reported sensitisation in workers exposed to airborne soluble platinum below the respirable occupational exposure limit and suggested that the dermal route contributed to total exposure, possibly playing a role in sensitisation. Although concluding and evidential information on the dermal health effects of platinum is scarce, available data implies that the platinum derived from many industrial processes is merely in oxidic or metallic forms, which is unlikely to cause sensitisation (Boscolo *et al*., 2004). However, Franken *et al*. (2014) provided evidence after completing an *in vitro* study that there is the risk of platinum permeating through intact human skin when workers are exposed to soluble platinum.

As the skin provides a possible route of exposure, it also has a defensive function responsible for preventing or minimising the permeation of substances from the external environment (Darlenski and Fluhr, 2012). This defensive function of the skin is due to the skin acting as an effective but not absolute barrier (Byford, 2009). This barrier function is achieved by two means, firstly by the prevention of water- and nutrient loss from the inside and secondly, through protecting the body against hazardous substances and xenobiotics on the outside (Machado *et al*., 2010; Rice and Mauro, 2013). Many factors may influence this barrier function and have an impact on the permeation rate of substances (Hostýnek *et al.*, 2006). In this particular study pH is the specific factor investigated, which could potentially influence the permeation of metal ions. The skin surface pH normally ranges between 4 and 6.5, but may be lower in some cases (Yosipovitch *et al*., 1998). The skin surface pH is essential in regulating the function of enzymes responsible for renewing the skin barrier and maintenance of keratinisation (Schmid-Wendtner and Korting, 2006; Stefaniak *et al*., 2013).

The acidic and basic properties of substances can be influenced by pH with regard to their solubility and partitioning in the various skin layers and it can either promote or inhibit its permeation (Wagner *et al*., 2003). Previous studies have indicated that pH influenced the permeation of zinc, chromium and rhodium (Ågren, 1990; Larese Filon *et al*., 2008; Jansen van Rensburg *et al*., 2016). Hatanaka *et al.* (1995) suggested that the permeability of the skin would differ for each ionic species. Therefore, the oxidation state of metal ions can possibly be

influenced by pH and thereby alter the permeation thereof through the skin (Hatanaka *et al.,* 1995; Wagner *et al*., 2003).

The investigation of metals and their permeation characteristics is done with *in vitro* methods, since the defensive function is still present in excised skin and *in vitro* methods have been used extensively to date, specifically for metals (Franz, 1975; Tanojo *et al*., 2001; Larese Filon *et al*., 2007; Rubio *et al*., 2011). For *in vitro* studies to be a more accurate representation of normal intact skin the synthetic sweat needs to be adjusted to a pH that is typical to that of the skin surface (Franz, 1975; Larese Filon *et al*., 2007). The pH of human skin is slightly acidic with normal levels ranging from 4 to 6.5 (Yosipovitch *et al*., 1998). Therefore, researchers performing skin permeation studies previously used a pH of 6.5, in order to represent the normal pH of intact skin (Yosipovitch *et al*., 1998; Larese Filon *et al*., 2004, 2008, 2009; Franken *et al*., 2014). However, the workplace conditions could be more acidic, so it was proposed that some metals should be investigated at a lower pH as it could potentially favour its permeation (Larese Filon *et al*., 2004, 2007). Larese Filon *et al*. (2008) found that the permeation of chromium was influenced by a change in pH. Chromium, one of the metals that was investigated, could not permeate the skin at a pH of 6.5. At a pH of 4.5 it was, however, able to oxidise and therefore permeate through the skin (Larese Filon *et al*., 2008). Therefore, it is important to determine the permeation behaviour of metals at different but relevant pH levels.

Franken *et al*. (2014) has already established that platinum in the salt form can permeate through the skin at a pH of 6.5. Jansen van Rensburg *et al*. (2016) found that the permeation of rhodium through the skin at a pH of 4.5 increased compared to a pH of 6.5. This study is designed to determine whether a lower pH would influence the permeation of a platinum salt, more specifically K_2PtCl_4, through intact Caucasian skin. This salt is used due to it being soluble and dissolving to form the sensitiser, tetrachloroplatinate [$PtCl_4^{2-}$].

1.2 Research Aims and Objectives

1.2.1 General aims:

The general aim of this study is to investigate the influence of pH on the *in vitro* skin permeation of a soluble platinum salt through intact Caucasian skin.

1.2.2 Specific objectives:

- To investigate the *in vitro* permeation of platinum (K_2PtCl_4) through Caucasian skin at a pH of 4.5 and 6.5 over a 24 hour period by using the Franz diffusion cell method.

- To establish if there is any statistical significant difference between the permeation of platinum through the skin at a pH of 4.5 and a pH of 6.5.

1.3 Hypothesis

Previous *in vitro* permeation studies were conducted at a pH of 6.5. However, it was proposed by Larese Filon *et al*. (2008) that lower pH levels such as 4.5, which is still within the range of normal skin pH, could enhance skin permeation. A lower pH of 4.5 resulted in higher permeation values for chromium and rhodium, possibly due to increased oxidation (Larese Filon *et al*., 2008; Jansen van Rensburg *et al*., 2016). It is therefore hypothesised that the *in vitro* permeation of platinum through Caucasian skin at a pH of 4.5 is significantly higher than the permeation at a pH of 6.5.

1.4 References

Ågren, M.S., 1990. Percutaneous absorption of zinc from zinc oxide applied topically to intact skin in man. Dermatologica. 180, 36-39.

Ash, P.W., Boyd, D.A., Hyde, T.I., *et al*. 2014. Local structure and speciation of platinum in fresh and road-aged North American sourced vehicle emissions catalysts: an x-ray absorption spectroscopic study. Environ. Sci. Technol. 48, 3658-3665.

Boscolo, P., Giampaolo, L.D., Reale, M., *et al*. 2004. Different effects of platinum, palladium and rhodium salts on lymphocyte proliferation and cytokine release. Ann. Clin. Lab. Sci. 34, 299-306.

Byford, T., 2009. Environmental Health Criteria 235: Dermal Absorption. Int. J. Environ. Stud. 66, 662-788.

Cawthorn, R.G., 1999. The platinum and palladium resources of the Bushveld complex. S. Afr. J. Sci. 95, 481-489.

Chamber of Mines, South Africa., 2010. Facts and figures 2009/2010. Available at: URL:http://chamberofmines.org.za/media-room/facts-and-figures (accessed 20 May 2015)

Chamber of Mines, South Africa., 2014. Facts and figures 2013/2014. Available at: https://commondatastorage.googleapis.com/comsa/f_f_2014_final.pdf (accessed 20 May 2015)

Chang, Y.C., Chen, C.P., Chen, C.C., 2012. Predicting the skin permeability of chemical substances using a quantitative structure-activity relationship. Procedia. Eng. 45, 875-879.

Christaudo, A., Sera, F., Severino, V., *et al*. 2005. Occupational hypersensitivity to metal salts, including platinum, in the secondary industry. Allergy. 60,159-164.

Cleare, M.J., Hughes, E.G., Jacoby, B., *et al*. 1976. Immediate (type I) allergenic responses to platinum compounds. Clin. Allergy. 6, 183-195.

Darlenski, R., Fluhr, J.W., 2012. Influence of skin type, race, sex and anatomic location on epidermal barrier function. Clin. Dermatol. 30, 269-273.

Du Plessis, J.L., Eloff, F.C., Engelbrecht, S., *et al*. 2013. Dermal exposure and changes in skin barrier function of base metal refinery workers co-exposed to cobalt and nickel. Occup. Health. Southern. Africa. 19, 6-12.

Franken, A., Eloff, F.C., Du Plessis, J., *et al.* 2014. *In vitro* permeation of platinum and rhodium through Caucasian skin. Toxicol. *In Vitro.* 28, 1396-1401.

Franz, T.J., 1975. Percutaneous absorption on the relevance of *in vitro* data. J. Invest. Dermatol. 64, 190-195.

Gómez, M.B., Gómez, M.M., Palacios, M.A., 2000. Control of interferences in the determination of Pt, Pd and Rh in airborne particulate matter by inductively coupled plasma mass spectrometry. Anal. Chim. Acta. 404, 285-294.

Gómez, B., Gómez, M., Sanchez, J.L., *et al*. 2001. Platinum and rhodium distribution in airborne particulate matter and road dust. Sci. Total. Environ. 269, 131-144.

Hatanaka, T., Morigaki, S., Aiba, T., *et al*. 1995. Effect of pH on skin permeability of a zwitterionic drug, cephalexin. Int. J. Pharmaceut. 125, 195-203.

Hostýnek, J.J., Dreher, F., Maibach, H.I., 2006. Human stratum corneum penetration by copper: *In vivo* study after occlusive and semi-occlusive application of the metal as powder. Food. Chem. Toxicol. 44, 1539-1543.

Hunter, D., Milton, R., Perry, K.M.A., 1945. Asthma caused by the complex salts of platinum. Brit. J. Ind. Med. 2, 92-98.

Jansen van Rensburg, S.J., Franken, A., Du Plessis, J., *et al*. 2016. The influence of pH on the *in vitro* permeation of rhodium through human skin. Toxicol. Ind. Health. 1-8. DOI 10.1177/0748233716675218 (Available online)

Larese Filon, F., Maina, G., Adami, G., *et al*. 2004. *In vitro* percutaneous absorption of cobalt. Int. Arch. Environ. Health. 7, 85-89.

Larese Filon, F., Gianpiero, A., Venier, M., *et al*. 2007. *In vitro* percutaneous absorption of metal compounds. Toxicol. Lett. 170, 49-56.

Larese Filon, F., D'Agostin, F., Crosera, M., *et al*. 2008. *In vitro* percutaneous absorption of chromium powder and the effect of skin cleanser. Toxicol. *In Vitro.* 22, 1562-1567.

Larese Filon, F., D'Agostin, F., Crosera, M., *et al*. 2009. *In vitro* absorption of metal powders through intact and damaged human skin. Toxicol. *In Vitro.* 23, 574-579.

Larese Filon, F., Crosera, M., Adami, G., *et al*. 2011. Human skin penetration of gold nanoparticles through intact and damaged skin. Nanotoxicology. 5, 493-501.

Larese Filon, F., Crosera, M., Timeus, E., *et al*. 2012. Human skin penetration of cobalt nanoparticles through intact and damaged skin. Toxicol. *In Vitro.* 27, 121-127.

Machado, M., Hadgraft, J., Lane, M.E., 2010. Assessment of the variation of skin barrier function with anatomical site, age, gender and ethnicity. Int. J. Cosmetic. Sci. 32, 397-409.

Maynard, A.D., Northage, C., Hemingway, M., *et al*. 1997. Measurement of short-term exposure to airborne soluble platinum in the platinum industry. Ann. Occup. Hyg. 41, 77-94.

Merget, R., Rosner, G., 2001. Evaluation of the health risk of platinum group metals emitted from automotive catalytic converters. Sci. Total. Environ. 270, 165-173.

Niezborala, M., Garnier, R., 1996. Allergy to complex platinum salts: A historical prospective cohort study. Occup. Environ. Med. 53, 252-257.

Rao, C.R.M., Reddi, G.S., 2000. Platinum group metals (PGM); occurrence, use and recent trends in their determination. Trends. Anal. Chem. 19, 565-586.

Rice, R.H., Mauro, T.M., 2013. Toxic responses of the skin, in: Klaassen, C.D. (Eds.), Casarett & Doull`s toxicology: the basic science of poisons. 8th ed. pp. 839-859. ISBN 978-0-07-176923-5

Rubio, L., Alonso, C., López, O., *et al*. 2011. Barrier function of intact and impaired skin: percutaneous penetration of caffeine and salicylic acid. Int. J. Dermatol. 50, 881-889.

Sartorelli, P., Montomoli, L., Sisinni, A.G., 2012. Percutaneous penetration of metals and their effects on skin. Prevent. Res. 2, 158-164.

Schmid-Wendtner, M.H., Korting, H.C., 2006. The pH of the skin surface and its impact on the barrier function. Skin. Pharmacol. Appl. 19, 296-302.

Stefaniak, A.B., Du Plessis, J., John, S.M., *et al.* 2013. International guidelines for the in vivo assessment of skin properties in non-clinical settings: part 1. pH. Skin. Res. Technol. 19, 59-68.

Tanojo, H., Hostýnek, J.J., Mountford, H.S., *et al*. 2001. *In vitro* permeation of nickel salts through human stratum corneum. Acta. Derm. Venereol. 21, 19-23.

Wagner, H., Kostka, K., Lehr, C., *et al*. 2003. pH profiles in human skin: influence of two *in vitro* test systems for drug delivery testing. Eur. J. Pharm. Biopharm. 55, 57-65.

Wiseman, C.L.S., Zereini, F., 2009. Airborne particulate matter, platinum group elements and human health: a review of recent evidence. Sci. Total. Environ. 407, 2493-2500.

Yosipovitch, G., Xiong, G.L., Erhard, H., *et al.* 1998. Time-dependent variation of the skin barrier function in humans: transepidermal water loss, stratum corneum hydration, skin surface pH and skin temperature. J. Invest. Dermatol. 110, 20-23.

Zereini, F., Alsenz, H., Wiseman, C.L.S., *et al*. 2012. Platinum group elements (Pt, Pd, and Rh) in airborne particulate matter in rural vs. urban areas of Germany: concentrations and spatial patterns of distribution. Sci. Total. Environ. 416, 261-268.

Chapter 2 – Literature Study

This chapter will review the literature available regarding the permeability of metals through intact human skin as well as the influence of skin surface pH on its permeability. The information available on the platinum group metals (PGMs) will be discussed with the focus on platinum, including its physical and chemical properties, occupational exposure and its potential adverse health effects. The physiological organisation of the skin, including the barrier function, will be discussed to achieve a better understanding of how dermal permeation may occur. Several studies on the *in vitro* permeation will be reviewed to investigate the ionisation of metals as well as the possible influence of pH on metal ionisation, and thus the effect of pH on the permeation of these metals. Also to be discussed is the method used to evaluate the permeation of platinum through intact skin, namely the *in vitro* Franz diffusion cell method.

2.1 Platinum group metals

PGMs are six closely related elements due to similar physical and chemical properties and include platinum, rhodium, palladium, ruthenium, iridium and osmium (Cawthorn, 1999; Cristaudo *et al*., 2005). These are noble metals – chemically reactive towards a limited number of materials (Ravindra *et al.,* 2004). PGMs are of the more scarce elements due to their low abundance and the complex extraction and refining processes they require (Bernardis *et al*., 2005). They occur in close association in the earth crust and are often found together in nature as natural alloys in concentrations ranging from 0.4 to 5 µg/kg, with platinum considered the main element (Ravindra *et al.,* 2004; Bernardis *et al*., 2005; Cristaudo *et al*., 2005; Yajun and Xiaozheng, 2012; Zereini *et al*., 2012). In South Africa, PGMs are primarily obtained from the ores being mined, with copper, nickel and cobalt as by-products or from secondary sources which include industrial scrap and pre-used catalysts. In other countries such as Canada, PGMs are treated as the by-products of copper and nickel base-metal mining (Smith *et al.,* 1974; Bernardis *et al*., 2005; Mpinga *et al*., 2015).

All the PGM elements have numerous stable oxidation states that are readily available (Bernardis *et al*., 2005). Ruthenium, rhodium, and palladium occur naturally in higher oxidation states than platinum, osmium and iridium. The simplicity with which PGMs can convert between oxidation states is what gives rise to the rich catalytic chemical properties of these elements (Burch *et al.,* 2002; Bernardis *et al*., 2005). Therefore, PGMs are widely used in the automotive industry, mainly as the active catalyst material in vehicle exhaust catalysts. These PGMs, with platinum used predominantly, provide improved means in controlling the hazardous emissions by removing nitrous oxide, unburned hydrocarbon and carbon monoxide (Burch *et al.,* 2002; Ash *et al.,* 2014). Another characteristic contributing to their significant industrial use is their high melting points, rendering the PGMs chemically inert and therefore resistant to corrosion at

high temperatures. Iridium and ruthenium are used in moderate amounts while osmium is seldomly used (Mpinga *et al*., 2015). Platinum and other PGMs are also utilised in the jewellery and medical industries, as well as the petrochemical industry (Wiseman and Zereini, 2009; Zereini *et al*., 2012; Resano *et al.,* 2015).

The worldwide PGM production is currently dominated by South Africa, rendering South Africa the leading producer of PGMs (Mpinga *et al*., 2015). As seen in Figure 1, the platinum production is the main contributor to the overall PGM production, regardless of the PGM production tendency (Chamber of Mines, 2014). The annual usage of PGMs worldwide in electronic appliances is approximately 11 800 kg of platinum, 22 100 kg of palladium, 200 kg of rhodium, 4500 kg of ruthenium, 680 kg of iridium and less than 100 kg of osmium (Smith *et al.,* 1974; Twigg, 2003). The Chamber of Mines (2010) reported that 54.80% of the three most prominent PGMs, one being platinum, were supplied by South Africa in 2009, along with South Africa being the leading producer of platinum internationally. In 2013, South Africa accounted for 79.50% of the total mineral supply, which included gold, iron ore, coal and PGMs (Chamber of Mines, 2014).

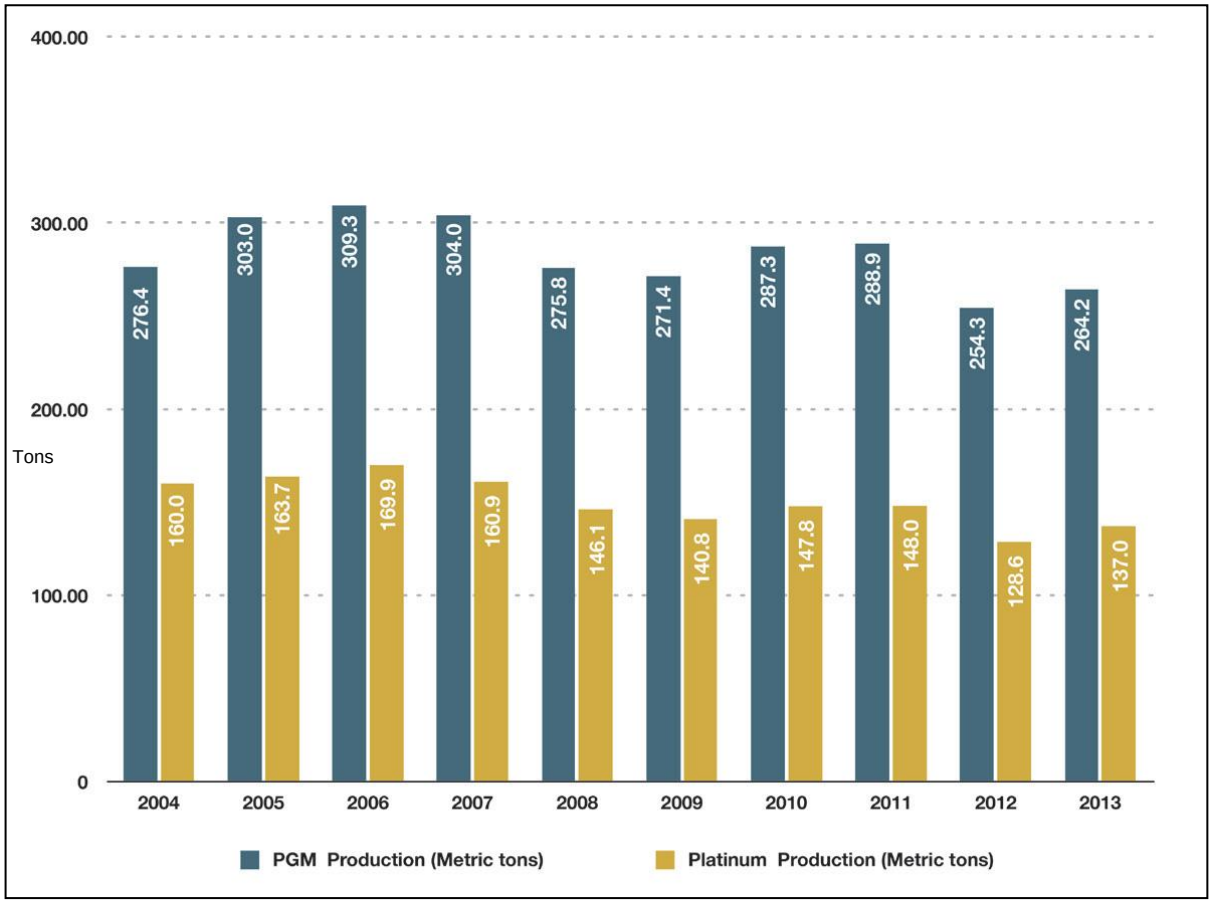


Figure 1: South African PGM and platinum production (Chamber of Mines, 2014)

2.2 Physical and chemical properties of platinum

Platinum's specific chemical and physical properties have attributed to some of its highly sophisticated applications in the technological industries (Cristaudo *et al*., 2005). Platinum is a noble transitional metal, belonging to group VIII of the periodic table, with an atomic number and weight of 78 and 195.09, respectively. It is also a dense, ductile and malleable metal with a grey-white appearance and has six naturally occurring isotopes. The highest oxidation state is Pt^{6+}, but Pt^{2+} and Pt^{4+} are the most stable (Mastromatteo, 1983; WHO, 2000). It was used in the early development of the electric telegraph, incandescent lamps and thermionic valves, among many other applications (Murdoch *et al*., 1986; Bernardis *et al*., 2005; Cristaudo *et al*., 2005). The face centred cubic structure of platinum, similar to that of gold, provides platinum with properties that are analogous to that of gold; therefore the metal is soft, significantly resistant to high temperature oxidation and also corrosion-resistant at high temperatures (Zhang *et al*., 1997; Bernardis *et al*., 2005).

2.3 Occupational exposure to platinum

Due to the various applications of platinum there are several occupations where exposure to platinum or its salt compounds occur on a regular basis. The occupational exposure to these different platinum forms primarily results from the mining, refining and processing thereof (Kielhorn *et al.,* 2002; Cristaudo *et al*., 2005). During the mining of platinum, workers are usually exposed to the free metal or to platinum in extremely insoluble forms, whereas exposure to soluble forms occurs during the refining of platinum (Baker *et al.*, 1990). Exposure may also occur in chemical and electronic industries where emission control catalysts, jewellery and glass are produced, and in laboratories where research is conducted (Cristaudo *et al*., 2005; Wiseman and Zereini, 2009; Goossens *et al*., 2011; Zereini *et al*., 2012). The most common exposure to soluble platinum salts, however, is currently within the platinum refineries and where catalysts are manufactured and recycled (Cleare *et al*., 1976; Boscolo *et al*., 2004; Cristaudo *et al*., 2005).

In the various processes and applications, a worker can be exposed to platinum through various exposure routes. In the field of occupational hygiene, the majority of occupational exposure to PGMs, specifically platinum, was to airborne particulate matter. This is mainly due to inhalation being regarded as the main route of occupational exposure (Gómez *et al*., 2000, 2001; Semple, 2004; Wiseman and Zereini, 2009; Kissel, 2010; Sartorelli *et al*., 2012; Zereini *et al*., 2012). Several studies reported that workers had developed respiratory symptoms following occupational exposure to airborne platinum salts in PGM refineries, where concentrations ranged between 1.7 and 6 $\mu g/m^3$ (Venables *et al*., 1989; Schierl *et al*., 1998). Platinum concentrations in the environment possibly rank as one of the highest due to the platinum

emissions of automobiles worldwide releasing an estimated 0.5 – 1.4 ton annually. The potential total intake of platinum particulate matter with a diameter of ten micrometres or less through inhalation, is thought to be approximately 0.062 ng/m^3 (Schierl 2000; Mauro *et al*., 2015).

Little information is available about the exposure to PGMs and platinum, in particular by means of skin contact and ingestion, as these exposure routes are frequently overlooked (Gómez *et al*., 2000, 2001; Semple, 2004; Wiseman and Zereini, 2009; Zereini *et al*., 2012). Maynard *et al.* (1997) reported respiratory sensitisation of workers even though the airborne concentrations of platinum were below the time-weighted average occupational exposure limit (TWA-OEL), which was the average exposure to a contaminant to which the majority of workers might be exposed without having any adverse effects over an 8 hour period. In a few cases, sensitisation also occurred even though no airborne soluble platinum were detected suggesting that the exposure to airborne platinum was either extremely high for a short period of time or the dermal route may contribute to the total exposure and possibly to respiratory sensitisation (Maynard *et al.,* 1997). A study done by Bello *et al.* (2007) found the development of asthma in workers exposed to isocyanates regardless of the improved control measures resulting in minimal respiratory exposure. Therefore, the authors reviewed the potential role of dermal exposure and observed that the skin might be an influential site of exposure in the development of respiratory sensitisation. Although limited data is available, there is a concern that skin exposure may contribute to respiratory sensitisation with regard to substances able to permeate the skin (Redlich and Karol, 2002; Semple, 2004).

If dermal exposure to substances occurred, it could consequently increase the systemic load or cause local toxicity as well as allergic reactions (Semple, 2004). Dermal exposure may contribute a larger fraction to the overall exposure than initially assumed (Kissel, 2010; Sartorelli *et al*., 2012). Du Plessis *et al.* (2013) assessed workers' dermal exposure to cobalt and nickel in a base metal refinery and found changes in certain skin parameters such as skin hydration, trans-epidermal water loss and skin surface pH, which may be contributing factors to an impaired barrier function causing the permeation of these metals to increase. This, in turn, may increase the dermal exposure due to the less resistance to permeation.

The exposure route least studied is oral exposure, possibly due to the minor contribution in relation to respiratory and dermal exposure. The ingestion of metals, including platinum, is mainly unintentional and occurs either by accident through the diet due to contaminated hands or when consuming soiled water (Kavcar *et al.,* 2009; Muhammad *et al.,* 2011). The exposure via ingestion cannot be excluded or ignored when investigating the overall exposure of a worker. However, the ingestion of contaminants are difficult to assess, but possible to control.

2.4 Health effects

The metallic form of platinum has been considered to be inert regarding biological reactions, but certain soluble platinum salts have been reported to be allergens and sensitisers (Ravindra *et al.,* 2004). The solubility of a compound determines its acute toxicity (Merget and Rosner, 2001). However, when platinum binds with certain ligands it can form complexes which could indirectly induce toxicity. Platinum-ligand complexes that comprise high affinity bonds and remain neutral are completely inactive. Therefore, the allergenicity of the platinum-ligand complex is directly related to the charge and reactivity of the complex and thus the ionisation of the complex (Cleare *et al*., 1976; Merget and Rosner, 2001).

Studies established that a very small group of ionic complexes, usually compounds containing reactive halogen ligands such as a bromide or chloride, are allergy-eliciting compounds (Cleare *et al*., 1976; Merget and Rosner, 2001). Hexa- and tetrachloroplatinates are soluble halogenated platinum salts and pose a major risk to the health of workers as the studies report these complex salts as potent allergens leading to hypersensitivity (Hunter *et al.,* 1945; Bolm-Audorff *et al*., 1992; Bullock, 2010). These platinum salts induce toxicity and hypersensitivity reactions which include respiratory symptoms such as chest tightening and difficult breathing, asthma, rhinoconjuctivitis, coughing, breathlessness and the development of platinosis. Furthermore, dermal symptoms include contact urticaria, dermatitis, eczema, and the inflammation of mucous membranes (Kiilunen and Aitio, 2007). Hunter *et al.* (1945) found respiratory symptoms in 57.14% of the workers working in a platinum refinery, whereas the prevalence of dermal symptoms was only 14.29%. Other health effects can include nausea, abnormal hair loss and an increase in spontaneous abortion (Ravindra *et al*., 2004). The type and severity of symptoms will differ depending on the dose, which is determined by the duration and magnitude of exposure (Rice and Mauro, 2013). Information on the carcinogenicity and mutagenicity in humans has not been reported when exposed to the platinum metal or insoluble or soluble platinum salts (Health Council of the Netherlands, 2008).

2.4.1 Sensitisation

Previously, it was suggested that soluble platinum salts caused sensitisation by stimulating the release of histamine and similar substances and therefore, acted through these substances and not as platinum-protein complexes forming antigens (Parrot *et al*., 1969; Campbell *et al*., 1975). More recent studies have proved otherwise, where metal ions act as haptens (incomplete antigens) with the potential to be immunogenic (Budinger and Hertl, 2000). According to Budinger and Hertl (2000), when the skin comes into contact with the metal ions they bind to carriers in the cell matrix, usually proteins, and induce an immune response. The authors explained a strong proliferative reaction occurring in individuals who are allergic to certain

metals, which indicates the involvement of T-cells in the development of hypersensitivity to metals when the target organ is the skin. This is known as a type I immunoglobulin IgE-mediated response. The T-helper lymphocytes secrete various chemical messengers and induce type I immunity, which is depicted by extreme phagocytic activity (Spellberg and Edwards, 2001). This is the mechanism by which respiratory sensitisation could potentially lead to several other diseases (Boscolo *et al*., 2004; Cristaudo *et al*., 2005).

Several lifestyle factors and hygiene habits of exposed workers may potentially indirectly increase the risk of becoming sensitised. The risk of developing respiratory metal sensitisation increases four to five times in smokers than non-smokers, which may be because of the elevated IgE serum concentrations in smokers (Holt, 1987; Calverley *et al.,* 1995). Another possible explanation could be the presence of free radicals in the smoke and because of the high reactivity of a free radical; it can interact with intercellular lipids and membranes of keratinocytes, causing oxidative stress (Elias, 1983; Dowling *et al.,* 1987; Silwerstein, 1992; Muizzuddin *et al.,* 1997; Knuutinen *et al.,* 2002). The nicotine in cigarettes is a vasoconstrictor which reduces the blood flow to the skin, resulting in tissue ischemia and diminished healing of damaged tissue (Silwerstein, 1992). Chronic alcohol consumption also contributes to delayed healing as alcohol has been associated with various skin diseases including psoriasis and palmer erythema (Brand *et al.,* 2007). Therefore, smoking and alcohol consumption may potentially indirectly cause increased permeation of metals as they damage the skin, thereby impairing the barrier function (Silwerstein, 1992; Brand *et al.,* 2007). Poor hygiene and uncleanliness of workers could prolong their exposure to metals as these metals remain on the skin surface (Bruce *et al*., 1986; Venables *et al*., 1989).

2.5 Skin

The largest organ of the human body, the skin, weighs approximately 5 kg and has an average surface area of 1.5 – 2 m^2 (Godin and Touitou, 2007; Darlenski and Fluhr, 2012). It is a dynamic, heterogeneous organ, separating the internal environment from the external environment and is therefore the only organ constantly exposed to the environment. Significant functions of the skin include controlling the body's temperature through sweat production and participation in the regulation of metabolism and hormones (Byford, 2009; Ngo *et al*., 2009). It is responsible for defence and self-restoration (Benson, 2005; Byford, 2009). These functions are achieved by the ability of the skin to act as an effective, although incomplete, barrier (Byford, 2009).

The major barrier against the permeation of substances is considered to be the stratum corneum, which is mechanically strong and is efficient in resisting a chemical assault (Grasso and Lansdown, 1972). The stratum corneum, the outermost layer, is one of four layers in the

epidermis along with the stratum granulosum, the stratum spinosum and the stratum germinativum. The epidermis also contains appendages, such as sweat glands, hair follicles and sebaceous glands (Byford, 2009). The epidermis, which has no blood supply, is one of three uniquely identifiable skin layers as seen in Figure 2. Directly underneath the epidermis is the dermis, which contains the capillaries and nerve endings as well as the epidermal appendages that may promote the penetration of substances by providing the route of least resistance. The innermost layer of the skin is known as the subcutaneous layer consisting of fat and collagen fibres (Byford, 2009; Ngo *et al*., 2009; Jepps *et al.*, 2013; Rice and Mauro, 2013).

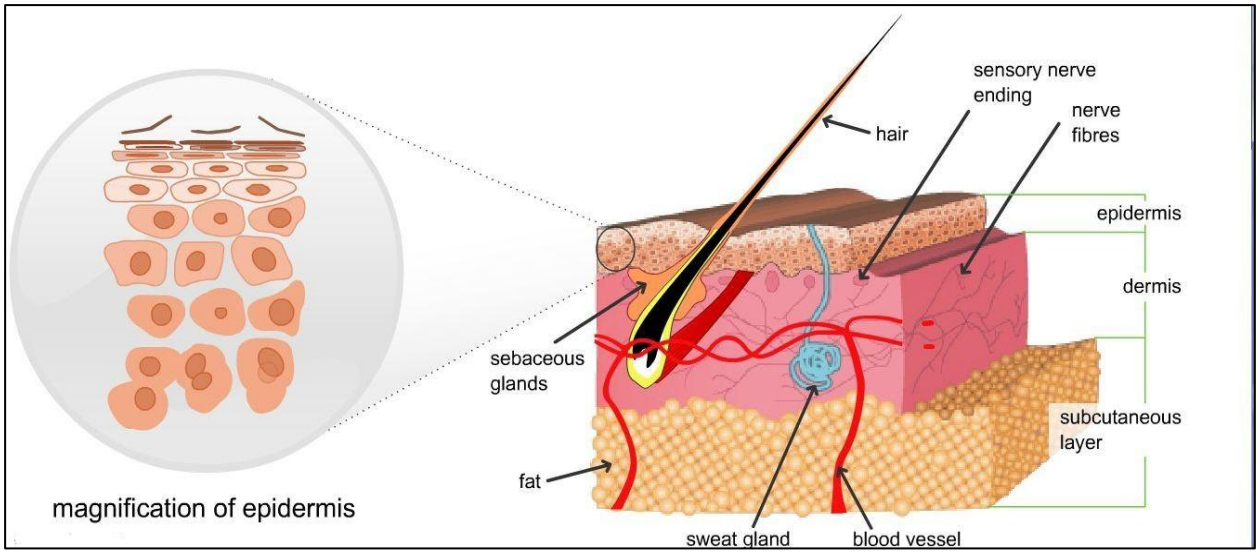


Figure 2: Skin organisation (Raj, 2012)

2.5.1 Skin barrier function

The barrier function is accomplished mainly by the skin's anatomical organisation (see Figure 2), and especially by the specific stratified structure of the stratum corneum (Byford, 2009; Rubio *et al*., 2011). Its structure consists of multiple corneocyte layers surrounded by the extracellular lipid matrix (Benson, 2005; Godin and Touitou, 2007; Lee *et al*., 2010). The multiple layers of corneocytes are enclosed by a cornified envelope of cytoskeletal elements and desmosomes which provides the strength of the skin, while the movement of water and ions is restricted by the highly organised and dense lipid matrix of the extracellular space, which consists of different lipids (Feingold, 2007; Jepps *et al.*, 2013).

The skin functions as a barrier in one of two ways. The first is by preventing the loss of body fluids, also referred to as the inside-out barrier. The second, which relates to this study, is to prevent permeation of hazardous chemicals and xenobiotics, also known as the outside-in barrier (Zhai and Maibach, 2002; Byford, 2009; Rubio *et al*., 2011). When the skin barrier is

disrupted, a quick repair response is initiated to restore a damaged barrier. Within a short period of time, the lamellar bodies from the outer stratum granulosum cells will secrete their contents, causing the formation of new lamellar bodies and the simultaneous synthesis of cholesterol and fatty acids. This aforementioned repair response is produced by a change in the extracellular calcium ion concentration which surrounds the stratum granulosum cells. When the barrier is disrupted, the movement of water increases, transporting the calcium ions towards the skin surface causing the calcium concentration in the extracellular space to decrease, leading to the release of lamellar bodies. The contents of these lamellar bodies are phospholipids, cholesterol glucosylceramides and sphingomyelin as well as a variety of enzymes (Feingold, 2007; Jepps *et al.*, 2013). These lipids in the lamellar bodies are the precursors for the extracellular lipids in the stratum corneum necessary to repair and replace damaged skin (Feingold, 2007).

2.6 Skin surface pH

The skin surface pH is considered to be an important parameter when assessing epidermal functions as well as the barrier function regarding the integrity and health of the skin (Ehlers, 2001; Darlenski and Fluhr, 2012). The skin surface pH has an effect on the dissolution and partition characteristics of substances found on the skin (Stefaniak *et al*., 2013). The pH is the negative logarithm of the free hydrogen ion concentration in an aqueous solution. The value is between one and fourteen, with a value of seven considered as neutral, above seven as alkaline and below seven as acidic (Schmid-Wendtner and Korting, 2006).

Usually the skin surface pH is thought to be acidic, ranging between 4 and 6.5 or even lower in some situations (Yosipovitch *et al*., 1998; Larese Filon *et al*., 2006; Byford, 2009). The presence of water-soluble elements in the stratum corneum, the secretion of sebum, diffusion of carbon dioxide and sweat are just a few factors that can contribute to a more acidic skin surface (Ehlers, 2001; Parra and Paye, 2003; Schmid-Wendtner and Korting, 2006). The physiological pH in the deeper layers of the stratum corneum can increase to 7.4, indicating a pH gradient across the different layers of the skin (Wong, 2014). The pH levels in the extracellular spaces should be maintained in an acidic range as this regulates the enzyme activities which are responsible for the renewal of the skin barrier and could therefore benefit the maintenance of keratinisation (Schmid-Wendtner and Korting, 2006; Stefaniak *et al*., 2013). Therefore, an optimal pH is necessary to stimulate the enzymes indirectly responsible for barrier formation by means of the lamellar bodies (Feingold, 2007). According to Schmid-Wendtner and Korting (2006), the pH of the body's internal environment seems to be more neutral, ranging between 7.35 and 7.46. The acidic nature of the skin surface contributes to a variety of skin functions, which include the regulation and maintenance of skin barrier homeostasis as well as antimicrobial functions and balance. The maintenance of the skin surface pH has an important

role in the integrity of the stratum corneum as well as intercellular cohesion (Kim *et al*., 2006; Schmid-Wendtner and Korting, 2006; Feingold, 2007; Gunathilake *et al.,* 2009; Stefaniak *et al*., 2013).

The skin surface pH of workers, in many occupational environments and settings, may be lower than generally thought due to the acidic environment (Larese Filon *et al*., 2006, 2007, 2008). Workers exposed to nickel and cobalt at a base metal refinery showed a decrease in both skin surface pH and skin hydration and an increase in trans-epidermal water loss. The measured skin surface pH of base metal refinery workers ranged between 5.0 and 6.0 (Du Plessis *et al.,* 2010). This could be indicative of a disrupted barrier or the presence of more acidic substances in the working environment and the active metabolic state of the skin (Feingold, 2007; Larese Filon *et al*., 2007, 2008; Du Plessis *et al.,* 2010). Sartorelli *et al*. (2012) found an increase in permeation of chromium, when applied as potassium dichromate, with increasing skin surface pH. This was most likely due to an impaired barrier function (Sartorelli *et al*., 2012). An increase in skin pH could irritate the physiological 'acid mantle' necessary for protection, interfering with the microbial composition and enzyme activity in the upper epidermis (Gfatter *et al.,* 1997; Feingold, 2007) Hence, the stratum corneum's barrier function, which is effective in minimising or preventing the permeation of substances under normal conditions, could be influenced by a change in the skin surface pH. This could either delay or increase the permeability of substances depending on whether the pH tends to be acidic or alkaline (Schmid-Wendtner and Korting, 2006; Byford, 2009).

2.6.1 Factors influencing skin surface pH

2.6.1.1 Endogenous

Numerous physiological factors can influence the skin surface pH. Age has been known to influence the skin surface pH as the skin adapts and mature postnatal (Schmid-Wendtner and Korting, 2006). Between the ages of 18 and 60, the pH remains relatively constant, whereas after the age of 60, the skin surface pH decreases. Therefore, age does not play any significant role in this particular study, as most workers are within the age range of 18-60. Another factor is the different anatomical sites, which makes it difficult to compare results and data as the skin surface composition is not necessarily constant, leading to great variability. It was established that areas with increased moisture such as the finger webs and submammary folds, have slightly higher pH levels (Schmid-Wendtner and Korting, 2006). Certain skin diseases can also influence the skin surface pH. Atopic dermatitis causes an elevated skin surface pH due to certain mutations causing the formation of dysfunctional filaggrin, a protein necessary to maintain the skin barrier integrity (Stefaniak *et al*., 2013). Skin surface pH can also differ because of racial and genetic background, such as African skin which has a lower skin surface

pH than Caucasian skin (Warrier *et al*., 1996; Berardesca *et al*., 1998; Muizzuddin *et al*., 2010). The effect of the skin`s sebum on pH is moderate and also depends on the amount of sebum (Parra and Paye, 2003). It has been reported that chronological rhythms, such as the circadian rhythms, potentially have an effect on the skin surface pH, with maximum levels in the afternoon and minimum levels during the night (Stefaniak *et al*., 2013).

With regard to metabolic activity, one of the by-products formed is lactic acid and the presence of lactic acid in eccrine sweat causes the skin surface to be more acidic. In contrast, a by-product formed during microbial metabolism is ammonia, which may cause the sweat to be more alkaline. When eccrine sweat evaporates, the ammonia evaporates rapidly causing the skin surface sweat to return to a more acidic state, leading to a decrease in the skin surface pH, indirectly decreasing the protection ability of the skin barrier (Parra and Paye, 2003).

2.6.1.2 Exogenous

With regard to external factors, the most important factors are skin cleansing, hygiene practices, topical applications and occlusion (Gfatter *et al.,* 1997; Schmid-Wendtner and Korting, 2006; Stefaniak *et al*., 2013). Even the use of detergents, especially formulated to have the same pH as the skin surface, tap water and certain alkaline soaps can cause a short-term increase in skin surface pH, whereas acidic soaps can remarkably decrease the skin surface pH (Korting *et al.,* 1992; Gfatter *et al.,* 1997; Schmid-Wendtner and Korting, 2006; Stefaniak *et al*., 2013). Working in an acidic or alkaline environment could also contribute to an altered skin surface pH. The environment ultimately decreases the skin surface pH below normal, potentially leading to the increased formation of permeable contaminants (Tanojo *et al*., 2001; Larese Filon *et al*., 2007). An increase in pH was also observed when occlusion of skin was allowed for several days, which returned to the baseline pH within the first day after removing the occlusive dressing. Therefore, occlusion caused by the use of gloves could hyper-hydrate the skin, resulting in a reduced barrier function (Hartmann, 1983; Schmid-Wendtner and Korting, 2006).

2.7 Permeation through human skin

The movement of substances into the circulatory system through undamaged skin is described as percutaneous absorption and is considered to be a complex process. This particular process can be subdivided into three discrete processes. The first process involves the penetration of substances through the stratum corneum, which is essentially achieved through passive diffusion and is regarded as the major rate-limiting process (Byford, 2009; Jepps *et al*., 2013). The second is permeation, where the substance is transported from the one layer to the next and the third is resorption (absorption) which describes the uptake and transport of the substance in the circulatory system (Byford, 2009).

A substance's affinity for the corneocytes of the stratum corneum and its ability to permeate the cell membrane as well as the substance's affinity for the lipid environment will mainly determine the rate of permeation (Jepps *et al*., 2013). When penetrating the stratum corneum, it can happen via three routes, namely the transcellular route, the intercellular route and the follicular route.

The transcellular route is a series of passive diffusion and partitioning processes through the corneocytes and multiple lipophilic and hydrophilic layers before complete absorption into the skin is achieved (Benson, 2005; Jepps *et al*., 2013). When a substance diffuses between the corneocytes following the intercellular lipid matrix, the substance is permeating via the intercellular route. The follicular pathway, also known as the shunt or appendageal route, is via the appendages, suggesting the substance is entirely bypassing the corneocytes and is transported through sweat and sebaceous glands as well as hair follicles (Byford, 2009). Even though permeation via the appendages supplies an easier route, the contribution thereof to the total permeation through the skin is only approximately 0.1%. This is due to the total amount of appendages in relation to the body's total skin surface are 0.1% and depends on the anatomical site as well as the hair follicle's density and size (Otberg *et al*., 2004). However, for *in vitro* studies the follicular pathway will contribute 0.1% or less to the total permeation; therefore, the majority of the permeation is presumed via the other two pathways (Benson, 2005; Jepps *et al*., 2013). Due to lipophilic sebum, lipid-soluble substances may diffuse into the glands and hair follicles without difficulty, while this route may be challenging for water-soluble substances (Jepps *et al*., 2013).

An amphipathic molecule, which is a molecule that is lipid-soluble on the one end and water-soluble on the other, could permeate the skin more easily because of this unique property (Grasso and Lansdown, 1972; Jepps *et al*., 2013). This follicular route usually supplies a way of absorption through and storage in the skin for larger molecules, such as proteins. These larger molecules can only be removed from the appendages with sebum production and hair growth (Ngo *et al*., 2009; Schneider *et al*., 2009). However, any substance inside the appendages is considered to be outside the body but the clearance of substances from the appendages is slow. Thus, an alteration in the structure of the skin barrier could potentially arise from substances accumulating within these appendages (Schneider *et al*., 2009).

A substance could reach the lower epidermis, also known as viable epidermis, and the underlying layers if it can permeate the stratum corneum, which is the main barrier of the skin. The viable epidermis, which is mainly avascular, can in the presence of certain proteins provide some form of barrier. This barrier is not as efficient as the stratum corneum but it provides some sort of delay to the xenobiotics' diffusion process. The viable epidermis has an increased

water fraction, which is different from the stratum corneum, causing the viable epidermis to be a more effective barrier against the permeation of lipid-soluble substances (Ngo *et al*., 2009; Jepps *et al.*, 2013). When a substance does permeate the viable epidermis, it will be transported to the dermis and subcutaneous layers, which may then lead to the circulatory entry of the substance. The permeation of substances through the dermis is considerably different from the permeation through the epidermis. The dermis is highly vascularised which may contribute to the transport and distribution of substances in the skin. In contrast, the dermis may improve the barrier function due to the binding and sequestration of some substances, limiting the extent to which a substance could permeate through the skin (Jepps *et al.*, 2013). If a substance does however permeate all the skin layers mentioned, it may either accumulate in the subcutaneous layer or it may be distributed through the circulatory system (Ngo *et al*., 2009; Jepps *et al.*, 2013).

2.7.1 Factors influencing skin permeation

There are a variety of factors influencing the permeation of substances through the skin, which can be categorised as individual, environmental or substance related factors (Hoang, 1992; Hostýnek, 2003). Individual factors, also known as endogenous factors, include age, anatomical site and race, including the thickness of the stratum corneum. Environmental factors, also referred to as exogenous factors, include frequency and dose of exposure to substance, temperature, the application of topical agents and the skin pH. Substance related factors include particle size, solubility and polarity (Hostýnek, 2003; Machado *et al*., 2010; Mauro *et al*., 2015). For this particular study, the focus will be on the exogenous factor, namely skin pH and its influence on the permeability of platinum through intact human skin, and therefore will be discussed comprehensively in the following section.

2.7.1.1 Influence of pH on skin permeation

Thune *et al*. (1988) and Zatz (1991) stated that the permeability of the barrier does not change when the pH range is between 3.5 and 8.5. However, this statement of non-change in permeability is in contradiction with the available literature. The information regarding the mechanisms responsible for the possible change in permeability at different pH levels is limited (Sznitowska *et al*., 2001). More recent studies have established the permeation of different substances and metals, and some studies also reported the difference in permeation at different pH levels, mainly due to some substances oxidising at lower or higher pH levels (Larese Filon *et al*., 2004, 2007, 2008; Franken *et al*., 2014; Jansen van Rensburg *et al*., 2016). Hostýnek *et al*. (2006) stated that the permeation of metals might be influenced by the formation of possible soluble compounds and explained that the oxidation of metals could occur because of the sweat possibly acting as an electrolyte. The permeability of the metals through the skin is thus, in

some cases, dependent on the oxidisibility of the metal, which is influenced by the pH of the skin surface. Therefore, the pH of the skin may lead to the formation of metal ions and other compounds that can permeate the skin more easily (Tanojo *et al*., 2001; Hostýnek *et al*., 2006; Larese Filon *et al*., 2007).

The *in vitro* permeability of nickel, cobalt and chromium through the skin was investigated using synthetic sweat with a pH of 6.5. While nickel and cobalt permeated through the skin, the permeation of chromium was limited. This was due to the inability of chromium to be oxidised at such a high pH (Larese Filon *et al*., 2007). A following study investigated the solubility and permeation of chromium in synthetic sweat at a more acidic pH. Results indicated that the dissolution of chromium increased with a decreasing pH and that the permeation occurred due to chromium oxidation. In a subsequent study, Larese Filon *et al*. (2009) stated that certain chemical elements, such as chromium, are more readily ionised in an acidic environment. A one unit decrease in pH could lead to a 10 to 100 fold increase in skin permeation (Larese Filon *et al*., 2009). Several metals such as cobalt, nickel and chromium investigated at a pH of 6.5, were also investigated at a pH of 4.5 and the permeation was found to be higher at a pH of 4.5 (Larese Filon *et al*., 2009). A metal salt rhodium chloride, investigated by Jansen van Rensburg *et al*. (2016), demonstrated an increase in permeation at a pH of 4.5. Ågren (1990) noticed that with a decreasing pH, the zinc flux across healthy human skin increased. It is thus of significant importance to determine the most appropriate pH for the synthetic sweat when performing *in vitro* research with substances and metals.

Presently a pH of 4.5 may be more applicable due to the more acidic work environment in the industry and due to the possible influence pH could have on ionisation (Hostýnek *et al*., 2006; Larese Filon *et al*., 2007, 2008). Hostýnek *et al.* (2006) showed that the pH dependent permeation of certain substances may reveal that the selective permeation property of the skin is dependent on the non-polarity of the ionic species. The ionisation state of a compound, which is determined by the pH of the solution, primarily influences the permeation (Grasso and Lansdown, 1972). The ionisation of metals could also be influenced by the size of the metal particles. Metals as nanoparticles have become a concern as it could influence the ionisation and therefore the rate of permeation. In a recent study done by Mauro *et al*. (2015), the permeation of platinum and rhodium nanoparticles was investigated. These nanoparticles can interact with the skin surface and possibly permeate either as nanoparticles or as ions in sweat. Due to nanoparticles having a high surface to volume ratio, more ions can be released, leading to increased ionisation. The released metal ions could interact with the naturally occurring physiological ions found on the skin surface, such as chloride ions, fatty acids or amino acids. This could produce the formation of soluble and therefore more diffusible compounds (Grasso and Lansdown, 1972; Hostýnek *et al*., 2006; Mauro *et al*., 2015). Mauro *et al*. (2015) found that

the platinum administered as nanoparticles did not permeate intact skin but was rather deposited in the skin.

According to Guy and Hadgraft (1989) molecules in ionised form permeate the skin poorly and Smith (1990) also stated that ionised compounds permeate less rapidly than non-ionised compounds. An ionised molecule gains a charge thereby increasing its polarity and therefore decreasing its ability to permeate the skin. This is further supported by Vecchia and Bunge (2003) who found that the permeability coefficients for the non-ionised molecule were 1-2 times higher than the permeability coefficients of the molecule in ionised form of the same molecule. The relationship between the non-ionised and ionised form of the same molecule depends on the lipid-solubility of the non-ionised form. In different terms, if the non-ionised form of the molecule is more water-soluble, then the permeation rates of the ionised and non-ionised form will be more similar and less similar if the non-ionised form is more lipid-soluble (Hadgraft and Valenta, 2000; Magnusson *et al*., 2004; Kielhorn *et al.,* 2006). In contrast Tanojo *et al*. (2001) found that ionised molecules had a higher permeability due to having a smaller diameter. Among other metals, silver, gold, nickel and cobalt were investigated and the authors found that the ions had a higher permeation than the non-ionised molecule due to the small size of the ions (Larese Filon *et al*., 2004, 2007, 2009, 2011, 2012).

2.8 *In vitro* skin permeation

As mentioned in Section 2.7, when the skin surface comes into contact with a substance, the substance can permeate the skin by means of three routes: transcellular, intercellular and through the appendages (Benson, 2005). These three routes however cannot be separated from one-another because of the lack of a suitable experimental model. Therefore, to investigate the permeation of substances through the skin, several *in vivo* as well as *in vitro* models were developed (Venter *et al*., 2001). Most *in vivo* studies are performed on animals such as mice and rats but it is however, very challenging to correlate the results with human responses (Larese Filon *et al*., 2007). The most widely used and accepted manner to investigate the permeation of substances, including metals, is via *in vitro* methods, specifically the Franz diffusion cell method (Franz, 1975). In studies where the permeation is investigated through *in vitro* method, the appendages play a limited role in the permeation of the substance as the constant hydration of the skin surface causes the skin to swell and in turn, seals off the appendages, therefore permeation is mainly via the transcellular and intercellular routes (Benson, 2005).

These dermal absorption tests are necessary, particularly for toxic and biological active substances as well as substances that can accumulate in the body. It also provides a mechanism for gaining a better understanding of factors influencing the permeation thereof

(Grasso and Lansdown, 1972; Larese Filon *et al*., 2007). Larese Filon *et al*. (2008) used this method to investigate the permeation of nickel, cobalt and chromium, whereas Franken *et al*. (2014) investigated platinum and rhodium.

A Franz diffusion cell has two separate compartments (Figure 3), one being the donor or top compartment and the other the receptor or bottom compartment. The circular skin membrane is wedged, stratum corneum side up, between these two compartments and clamped.

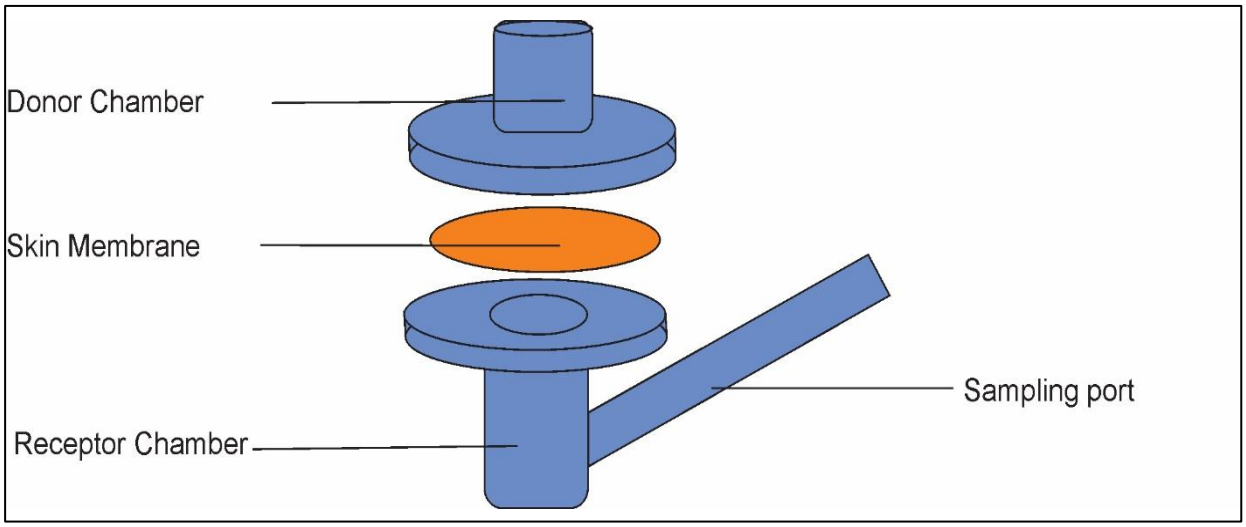


Figure 3: Franz Diffusion Cell

The donor compartment is filled with donor solution, which is the synthetic sweat solution containing the metal that is being investigated and the receptor compartment is filled with a physiological solution. The synthetic sweat is normally adjusted to a pH that is similar to that of the normal skin surface pH in order to simulate normal intact skin conditions as closely as possible (Larese Filon *et al*., 2007). The receptor compartment is filled with a physiological solution, representing the internal environment of the body. Furthermore, for *in vitro* studies to be a realistic representation of the exposure of workers in the working environment, the same conditions must be attained. *In vitro* studies are, as mentioned, normally undemanding to carry out and the experimental conditions can be accurately maintained (Larese Filon *et al*., 1999). This method is applied in a variety of research fields such as toxicokinetics in occupational toxicology and pharmaceutics, which can be regarded as an advantage (Larese Filon *et al*., 2007).

According to Bronaugh and Maibach (1983), there are several more advantages when using *in vitro* methods, which include sampling directly from under the skin a larger number of samples and investigating the skin permeability for highly toxic substances. *In vitro* techniques can be rapid, easy to perform and relatively inexpensive in comparison with other techniques

(Bronaugh and Maibach, 1983; Larese Filon *et al*., 2009). There are however supplementary disadvantages of using this method. Venter *et al.* (2001) proposed the possibility of differences that might develop between excised skin and *in vivo* skin, due to the skin being deprived from all blood supply. Physiological changes could also occur during the removal of the skin and the metabolism of substances in the skin is not taken into account (Venter *et al.,* 2001). However, this method has been used and improved by many researchers, as the benefits of this method outweigh the disadvantages. Not only does it enable the researcher to simulate the workplace conditions but is a good alternative to *in vivo* methods and therefore can be utilised in skin permeability studies (Bronaugh and Maibach, 1983; Larese Filon *et al*., 2006, 2007, 2008; Franken *et al*., 2014; Mauro *et al*., 2015; Jansen van Rensburg *et al*., 2016).

2.9 Summary

Different permeation tendencies were noticed at different pH levels for certain metals. The permeation of platinum at a pH of 6.5 was investigated and it was reported that platinum permeation occurred. However, the influence of pH on platinum permeation is still unclear. Authors reported that the permeation of rhodium through the skin increased at a lower pH. Chromium did not permeate through the skin at a pH of 6.5, but only when subjected to a pH of 4.5, when oxidation occurred and permeation followed. It is possible that the pH could influence the ionisation of metals and potentially lead to the increased permeation of the metal through the skin. Therefore, an acidic working environment could potentially enhance the permeation of platinum through the skin. Thus, this study will simultaneously investigate the *in vitro* permeability of a soluble platinum salt through intact human skin at both a pH of 4.5 and 6.5.

2.10 References

Ågren, M.S., 1990. Percutaneous absorption of zinc from zinc oxide applied topically to intact skin in man. Dermatologica. 180, 36-39.

Ash, P.W., Boyd, D.A., Hyde, T.I., *et al*. 2014. Local structure and speciation of platinum in fresh and road-aged North American sourced vehicle emissions catalysts: an x-ray absorption spectroscopic study. Environ. Sci. Technol. 48, 3658-3665.

Baker, D.B., Gann, P.H., Brooks, S.M., *et al.* 1990. Cross-sectional study of platinum salts sensitization among precious metals refinery workers. Am. J. Ind. Med. 18, 653-664.

Bello, D., Herrick, C.A., Smith, T.J., *et al.* 2007. Skin exposure to isocyanates: reasons for concern. Environ. Health. Persp. 115, 328-335.

Benson, H.A.E., 2005. Transdermal drug delivery: penetration enhancement techniques. Curr. Drug. Deliv. 2, 23-33.

Berardesca, E., Pirot, F., Singh, M., *et al.* 1998. Differences in stratum corneum pH gradient when comparing white and black African-American skin. Br. J. Dermatol. 139, 855-857.

Bernardis, F.L., Grant, R.A., Sherrington, D.C., 2005. A review of methods of separation of the platinum-group metals through their chloro-complexes. React. Funct. Polym. 65, 205-217.

Bolm-Audorff, U., Bienfait, H.G., Burkhard, J., *et al*. 1992. Prevalence of respiratory allergy in a platinum refinery. Int. Arch. Occ. Env. Hea. 64, 257-260.

Boscolo, P., Giampaolo, L.D., Reale, M., *et al*. 2004. Different effects of platinum, palladium and rhodium salts on lymphocyte proliferation and cytokine release. Ann. Clin. Lab. Sci. 34, 299-306.

Brand, R.M., Jendrzejewski, J.L., Charron, A.R., 2007. Potential mechanisms by which a single drink of alcohol can increase transdermal absorption of topically applied substances. Toxicology. 235, 141-149.

Bronaugh, R.L., Maibach, H.I., 1983. Percutaneous absorption of hazardous substances from soil or water, in: Wilhelm, P.K., Zhai, H., Maibach, H.I. (Eds.), Dermatotoxicology. CRC Press. pp. 343-360. ISBN 978-1-84-184857-0

Bruce, M., Scott, N., Lader, M., *et al*. 1986. The phychopharmacological and electrophysiological effects of single doses of caffeine in healthy human subjects. Brit. J. Clin. Pharmaco. 22, 81-87.

Budinger, L., Hertl, M., 2000. Immunologic mechanisms in hypersensitivity reactions to metal ions: an overview. Allergy. 55, 108-115.

Bullock, J., 2010. Chloroplatinate Toxicity: Use and Misunderstanding of Merget. International Precious Metals Institute. USA: Tucson, Arizona. Available at: http://ipmi.org/pdf/Chloroplatinate%20Toxicity%20Merget.pdf (accessed 5 May 2015)

Burch, R., Breen, J.P., Meunier, F.C., 2002. A review on selective reduction of NO_x with hydrocarbons under lean-burn conditions with non-zeolitic oxide and platinum group catalysts. Appl. Cata. B-environ. 39, 283-303.

Byford, T., 2009. Environmental Health Criteria 235: Dermal Absorption. Int. J. Environ. Stud. 66, 662-788.

Calverley, A.E., Rees, D., Dowdeswell, R.J., *et al*., 1995. Platinum salt sensitivity in refinery workers: incidence and effects of smoking and exposure. Occup. Environ. Med. 52, 661-666.

Campbell, K.I., George, E.L., Larry, L., *et al*. 1975. Dermal irritancy of metal compounds. Arch. Environ. Health. 30, 168-170.

Cawthorn, R.G., 1999. The platinum and palladium resources of the Bushveld complex. S. Afr. J. Sci. 95, 481-489.

Chamber of Mines, South Africa., 2010. Facts and figures 2009/2010. Available at: URL:http://chamberofmines.org.za/media-room/facts-and-figures (accessed 5 Aug 2015)

Chamber of Mines, South Africa., 2014. Facts and figures 2013/2014. Available at: https://commondatastorage.googleapis.com/comsa/f_f_2014_final.pdf (accessed 5 Aug 2015)

Christaudo, A., Sera, F., Severino, V., *et al*. 2005. Occupational hypersensitivity to metal salts, including platinum, in the secondary industry. Allergy. 60, 159-164.

Cleare, M.J., Hughes, E.G., Jacoby, B., *et al*. 1976. Immediate (type I) allergenic responses to platinum compounds. Clin. Allergy. 6,183-195.

Darlenski, R., Fluhr, J.W., 2012. Influence of skin type, race, sex and anatomic location on epidermal barrier function. Clin. Dermatol. 30, 269-273.

Dowling, D.T., Stewart, M.E., Wertz, P.W., *et al*. 1987. Skin lipids, an update. J. Invest. Dermatol. 88, 2-6.

Du Plessis, J.L., Eloff, F.C., Badenhorst, C.J., *et al*. 2010. Assessment of dermal exposure and skin condition of workers exposed to nickel at a South African base metal refinery. Ann. Occup. Hyg. 54, 23-30.

Du Plessis, J.L., Eloff, F.C., Engelbrecht, S., *et al*. 2013. Dermal exposure and changes in skin barrier function of base metal refinery workers co-exposed to cobalt and nickel. Occup. Health. Southern. Africa. 1, 6-12.

Ehlers, C., Ivens, U.I., Moller, M.L., *et al*. 2001. Females have lower skin surface pH than men: A study on the influence of gender, forearm site variation, right / left difference and time of the day on the skin surface pH. Skin. Res. Technol. 7, 90-94.

Elias, P.M., 1983. Epidermal lipids, barrier function and desquamation. J. Invest. Dermatol. 80, 44-49.

Feingold, K.R., 2007. The role of epidermal lipids in cutaneous permeability barrier homeostasis. J. Lipid. Res. 48, 2531-2546.

Franken, A., Eloff, F.C., Du Plessis, J., *et al.* 2014. *In vitro* permeation of platinum and rhodium through Caucasian skin. Toxicol. *In Vitro.* 28, 1396-1401.

Franz, T.J., 1975. Percutaneous absorption on the relevance of *in vitro* data. J. Invest. Dermatol. 64, 190-195.

Gfatter, R., Hackl, P., Braun, F., 1997. Effects of soap and detergents on skin surface pH, stratum corneum hydration and fat content in infants. Dermatology. 195, 258-262.

Godin, B., Touitou, E., 2007. Transdermal skin delivery: predictions for humans from in vivo, ex vivo and animal models. Adv. Drug. Deliver. Rev. 59, 1152-1161.

Gómez, M.B., Gómez, M.M., Palacios, M.A., 2000. Control of interferences in the determination of Pt, Pd and Rh in airborne particulate matter by inductively coupled plasma mass spectrometry. Anal. Chim. Acta. 404, 285-294.

Gómez, B., Gómez, M., Sanchez, J.L., *et al*. 2001. Platinum and rhodium distribution in airborne particulate matter and road dust. Sci. Total. Environ. 269, 131-144.

Goossens, A., Cattaert, N., Nemery, B., *et al*. 2011. Occupational allergic contact dermatitis caused by rhodium solutions. Contact. Dermatitis. 64, 158-184.

Grasso, P., Lansdown, A.B.G., 1972. Methods of measuring, and factors affecting, percutaneous absorption. J. Soc. Cosmet. Chem. 23, 481-521.

Gunathilake, R., Schurer, N.Y., Shoo, B.A., *et al*. 2009. pH-Regulated mechanisms account for pigment-type differences in epidermal barrier function. J. Invest. Dermatol. 129, 1719-1729.

Guy, R.H., Hadgraft, J., 1989. Structure-activity correlations in percutaneous absorption, in: Bronaugh, R.L., Maibach, H.I. (Eds.), Percutaneous absorption: Methods – Methodology – Drug Delivery. 2nd ed. New York: Marcel Dekker, Inc. pp. 95-109. ISBN 0-8247-8036-1

Hadgraft, J., Valenta, C., 2000. pH, pK_a and dermal delivery. Int. J. Pharm. 200, 243-247.

Hartmann, A.A., 1983. Effect of occlusion on resident flora, skin-moisture and skin-pH. Arch. Dermatol. Res. 275, 251-254.

Health Council of the Netherlands., 2008. Platinum and platinum compounds. Health based recommended occupational exposure limit. The Hague: HCN; publication no. 2008/12OSH. ISBN 978 90 5549 718 8

Hoang, K.T., 1992. Dermal exposure assessment: principles and applications.US Environmental Protection Agency, Exposure Assessment Group. Washington, DC: Office of Health and Environmental Assessment. EPA/600/8-91/011B Available at: http://www.epa.gov/oppt/exposure/presentations/efast/usepa_1992d_dermalea.pdf (accessed 5 May 2015)

Holt, P.G., 1987. Immune and inflammatory function in cigarette smokers. Thorax. 42, 241-249.

Hostýnek, J.J., 2003. Factors determining percutaneous metal absorption. Food. Chem. Toxicol. 41, 327- 345.

Hostýnek, J.J., Dreher, F., Pelosi, A., *et al*. 2003. Human stratum corneum penetration by nickel. Acta. Derm. Venereol. Suppl. 212, 5-10.

Hostýnek, J.J., Dreher, F., Maibach, H.I., 2006. Human stratum corneum penetration by copper: *In vivo* study after occlusive and semi-occlusive application of the metal as powder. Food. Chem. Toxicol. 44, 1539-1543.

Hunter, D., Milton, R., Perry, K.M.A., 1945. Asthma caused by the complex salts of platinum. Brit. J. Ind. Med. 2, 92-98.

Jansen van Rensburg, S.J., Franken, A., Du Plessis, J., *et al*. 2016. The influence of pH on the *in vitro* permeation of rhodium through human skin. Toxicol. Ind. Health. 1-8. DOI 10.1177/0748233716675218 (Available online)

Jepps, O.G., Dancik, Y., Anissimov, Y.G., *et al*. 2013. Modeling the human skin barrier – towards a better understanding of dermal absorption. Adv. Drug. Deliver. Rev. 65, 152-168.

Kavcar, P., Sofuoglu, A., Sofuoglu, S.C., 2009. A health risk assessment for exposure to trace metals via drinking water ingestion pathway. Int. J. Hyg. Envir. Heal. 212, 216-227.

Kielhorn, J., Melber, C., Keller, D., *et al.* 2002. Palladium – a review of exposure and effects to human health. Int. J. Hyg. Envir. Heal. 205, 417-432.

Kielhorn, J., Melching-Kolluβ, S., Mangelsdorf, I., 2006. IPCS Environmental health criteria for dermal absorption. World health organisation. pp. 10-58. ISBN 978-92-4-157235-4

Kiilunen, M., Aitio, A., 2007. Platinum, in: Nordberg, G.F., Fowler, B.A., Nordberg, M., *et al.,* (Eds.), Handbook on the Toxicology of Metals. Elsevier, London. pp. 777-778. ISBN 978-0-12-369413-3

Kissel, J., 2010. The mismeasure of dermal absorption. J. Expo. Anal. Env. Epid. 21, 302-309.

Knuutinen, A., Kokkonen, N., Risteli, J., *et al*. 2002. Smoking affects collagen synthesis and extracellular matrix turnover in human skin. Brit. J. Dermatol. 146, 588-594.

Korting, H.C., Hubner, K., Greiner, K., *et al.* 1990. Differences in the skin surface pH and bacterial microflora due to the long-term application of synthetic detergent preparations of pH 5.5 and pH 7.0. Acta. Derm. Venereol. 70, 429-457.

Larese Filon, F., Fiorito, A., Adami, G., *et al*. 1999. Skin absorption of glycol ethers. Int. Arch. Occ. Env. Hea. 72, 480-484.

Larese Filon, F., Maina, G., Adami, G., *et al*. 2004. *In vitro* percutaneous absorption of cobalt. Int. Arch.Occ. Env. Hea. 7, 85-89.

Larese Filon, F., Boeniger, M., Giovanni, M., *et al*. 2006. Skin absorption of inorganic lead (PbO) and the effect of skin cleansers. J. Occup. Environ. Med. 48, 692-699.

Larese Filon, F., Gianpiero, A., Venier, M., *et al*. 2007. *In vitro* percutaneous absorption of metal compounds. Toxicol. Lett. 170, 49-56.

Larese Filon, F., D'Agostin, F., Crosera, M., *et al*. 2008. *In vitro* percutaneous absorption of chromium powder and the effect of skin cleanser. Toxicol. *In Vitro.* 22, 1562-1567.

Larese Filon, F., D'Agostin, F., Crosera, M., *et al*. 2009. *In vitro* absorption of metal powders through intact and damaged human skin. Toxicol. *In Vitro.* 23, 574-579.

Larese Filon, F., Crosera, M., Adami, G., *et al*. 2011. Human skin penetration of gold nanoparticles through intact and damaged skin. Nanotoxicology. 5, 493-501.

Larese Filon, F., Crosera, M., Timeus, E., *et al*. 2012. Human skin penetration of cobalt nanoparticles through intact and damaged skin. Toxicol. *In Vitro.* 27, 121-127.

Lee, P.H., Conradi, R., Shanmugasundaram, V., 2010. Development of an in silico model for human skin permeation based on a Franz cell skin permeability assay. Bioorg. Med. Chem. Lett. 20, 69-73.

Machado, M., Hadgraft, J., Lane, M.E., 2010. Assessment of the variation of skin barrier function with anatomical site, age, gender and ethnicity. Int. J. Cosmetic. Sci. 32, 397-409.

Magnusson, B.M., Anissimov, Y.G., Cross, S.E., *et al.* 2004. Molecular size as the main determinant of solute maximum flux across the skin. J. Invest. Dermatol. 122, 993-999.

Mastromatteo, E., 1983. Platinum, alloys and compounds. Encyclop. Occup. Health. Safety. 2, 1723-1724.

Mauro, M., Crosera, M., Bianco, C., *et al*. 2015. Permeation of platinum and rhodium nanoparticles through intact and damaged human skin. J. Nanopart. Res. 17, 253.

Maynard, A.D., Northage, C., Hemingway, M., *et al*. 1997. Measurement of short-term exposure to airborne soluble platinum in the platinum industry. Ann. Occup. Hyg. 41, 77-94.

Merget, R., Rosner, G., 2001. Evaluation of the health risk of platinum group metals emitted from automotive catalytic converters. Sci. Total. Environ. 270, 165-173.

Mpinga, C.N., Eksteen, J.J., Aldrich, C., *et al*. 2015. Direct leach approaches to Platinum Group Metal (PGM) ores and concentrates: A review. Miner. Eng. 78, 93-113.

Muhammad, S., Tahir-Shah, M., Khan, S., 2011. Health risk assessment of heavy metals and their source apportionment in drinking water of Kohistan region, north Pakistan. Microchem. J. 98, 334-343.

Muizzuddin, N., Hellemans, L., Van Overloop, L., *et al*. 2010. Structural and functional differences in barrier properties of African American, Caucasian and East Asian skin. J. Dermatol. Sci. 59, 123-128

Muizzuddin, N., Marenus, K., Vallon, P., *et al*. 1997. Effect of cigarette smoke on skin. J. Soc. Cosmet. Chem. 48, 235-242.

Murdoch, R.D., Pepys, J., Hughes, E.G., 1986. IgE antibody responses to platinum group metals: a large scale refinery survey. Brit. J. Ind. Med. 43, 37-43.

Ngo, M.A., O'Malley, M., Maibach, H.I., 2009. Percutaneous absorption and exposure assessment of pesticides. J. Appl. Toxicol. 30, 91-114.

Otberg, N., Richter, H., Schaefer, H., *et al*. 2004. Variations of hair follicle size and distribution in different body sites. J. Invest. Dermatol. 122, 14-19.

Parra, J.L., Paye, M., 2003. EEMCO Guidance for the in vivo assessment of skin surface pH. Skin. Pharmacol. Appl. 16, 188-202.

Parrot, J.L., Hebert, R., Saindelle, A., *et al*. 1969. Platinum and platinosis. Arch. Environ. Health. 19, 685-691.

Pirot, F., Falson, F., 2004. Skin barrier function, in: Agache, P., Humbert, P. (Eds.), Measuring the skin. pp. 513-524. ISBN 3 540 01771 2

Raj, A., 2012. Know your skin better. Available at: https://arunimaraj.wordpress.com/2012/10/25/know-your-skin-better/ (accessed 11 Nov 2015)

Ravindra, K., Bencs, L., Van Grieken, R., 2004. Platinum group elements in the environment and their health risk. Sci. Total. Environ. 318, 1-43.

Redlich, C.A., Karol, M.H., 2002. Diisocyanate asthma: clinical aspects and immunopathogenesis. Int. Immunopharm. 2, 213-224.

Resano, M., Del Rosario Florez, M., Queralt, I., *et al*. 2015. Determination of palladium, platinum and rhodium in used automobile catalysts and active pharmaceutical ingredients using high-resolution continuum source graphite furnace atomic absorption spectrometry and direct solid sample analysis. Spectrochim. Acta. B. 105, 38-46.

Rice, R.H., Mauro, T.M., 2013. Toxic responses of the skin, in: Klaassen, C.D. (Eds.), Casarett & Doull`s toxicology: the basic science of poisons. 8th ed. pp. 839-859. ISBN 978-0-07-176923-5

Rubio, L., Alonso, C., López, O., *et al*. 2011. Barrier function of intact and impaired skin: percutaneous penetration of caffeine and salicylic acid. Int. J. Dermatol. 50, 881-889.

Sartorelli, P., Montomoli, L., Sisinni, A.G., 2012. Percutaneous penetration of metals and their effects on skin. Prev. Res. 2, 158-164.

Schierl, R., Fries, H.G., Van de Weyer, C., *et al*. 1998. Urinary excretion of platinum from platinum industry workers. Occup. Environ. Med. 55, 138-140.

Schierl, R., 2000. Environmental monitoring of platinum in air and urine. Microchem. J. 67, 245-248.

Schmid-Wendtner, M.H., Korting, H.C., 2006. The pH of the skin surface and its impact on the barrier function. Skin. Pharmacol. Appl. 19, 296-302.

Schneider, M., Stracke, F., Hansen, S., *et al*. 2009. Nanoparticles and their interaction with the dermal barrier. Dermatol. Endocrin. 4, 197-206.

Semple, S., 2004. Dermal exposure to chemicals in the workplace: Just how important is skin absorption? Occup. Environ. Med. 61, 376-382.

Silwerstein, P., 1992. Smoking and wound healing. Am. J. Med. 93, 22-24.

Smith, I.C., Carson, B.L., Ferguson, T.L., 1974. Osmium: An appraisal of environmental exposure. Environ. Health. Persp. 8, 201-213.

Smith, K.L., 1990. Penetrant characteristics influencing skin absorption, in: Kemppainen, B.W., Reifenrath, W.G. (Eds.), Methods for skin absorption. Boca Raton: CRC Press. pp. 23-34. ISBN 0-8493-4651-7

Spellberg, B., Edwards, J.E., 2001. Type 1/Type 2 immunity in infectious diseases. Clin. Infect. Dis. 32, 76-102.

Stefaniak, A.B., Du Plessis, J., John, S.M., *et al.* 2013. International guidelines for the in vivo assessment of skin properties in non-clinical settings: part 1. pH. Skin. Res. Technol. 19, 59-68.

Sznitowska, M., Janicki, S., Baczek, A., 2001. Studies on the effect of pH on the lipoidal route of penetration across stratum corneum. J. Control. Release. 76, 327-335.

Tanojo, H., Hostýnek, J.J., Mountford, H.S., *et al*. 2001. *In vitro* permeation of nickel salts through human stratum corneum. Acta. Derm. Venereol. 21, 19-23.

Thune, P., Nilsen, T., Hanstad, I.K., *et al*. 1988. The water barrier function of the skin in relation to the water content of stratum corneum, pH and skin lipids. Acta. Derm. Venereol. 68, 277-283.

Twigg, M.V., 2003. Automotive Exhaust Emissions Control. Platinum. Metals. Rev. 47, 157-162.

Venables, K.M., Dally, M.B., Nunn, A.J., *et al*. 1989. Smoking and occupational allergy in workers in a platinum refinery. Brit. Med. J. 299, 939-942.

Vecchia, B.E., Bunge, A.L., 2003. Skin absorption databases and predictive equations, in: Guy, R., Hadgraft, J. (Eds.), Transdermal drug delivery. CRC Press: NY. 2nd ed. pp. 57-141. ISBN 978-0-203-90968-3

Venter, J.P., Müller, D.G., Du Plessis, J., *et al*. 2001. A comparative study of an in situ adapted diffusion cell and an *in vitro* Franz diffusion cell method for transdermal absorption of doxylamine. Eur. J. Pharm. Sci. 13, 169-177.

Warrier, A.G., Kligmn, A.M., Harper, R.A., *et al*. 1996. A comparison of black and white skin using non-invasive methods. J. Soc. Cosmet. Chem. 47, 229-240.

Wiseman, C.L.S., Zereini, F., 2009. Airborne particulate matter, platinum group elements and human health: a review of recent evidence. Sci. Total. Environ. 407, 2493-2500.

Wong, T.W., 2014. Electrical, magnetic, photomechanical and cavitational waves to overcome skin barrier for transdermal drug delivery. J. Control. Release. 193, 257-269.

World Health Organization (WHO)., 2000. Inorganic pollutants: Platinum, in: Air quality guidelines for Europe. Copenhagen: WHO. pp. 166-169.

Yajun, W., Xiaozheng, L.E., 2012. Health risk of platinum group elements from automobile catalysts. Procedia. Eng. 45, 1004-1009.

Yosipovitch, G., Xiong, G.L., Erhard, H., *et al.* 1998. Time-dependent variation of the skin barrier function in humans: transepidermal water loss, stratum corneum hydration, skin surface pH and skin temperature. J. Invest. Dermatol. 110, 20-23.

Zatz, J., 1991. Assessment of vehicle factors influencing percutaneous absorption, in: Bronaugh, R.L., Maibach, H.I. (Eds.), *In vitro* percutaneous absorption: Principles, fundamentals and applications. CRC Press: FL. ISBN 978-0-8493-4748-1

Zereini, F., Alsenz, H., Wiseman, C.L.S., *et al*. 2012. Platinum group elements (Pt, Pd, and Rh) in airborne particulate matter in rural vs. urban areas of Germany: concentrations and spatial patterns of distribution. Sci. Total. Environ. 416, 261-268.

Zhai, H., Maibach, H.I., 2002. Occlusion vs. skin barrier function. Skin. Res. Technol. 8, 1-6.

Zhang, M., Zhou, B., Chuang, K.T., 1997. Catalytic deep oxidation of volatile organic compounds over fluorinated carbon supported platinum catalysts at low temperatures. Appl. Cata. B-environ. 13, 123-130.

Chapter 3 – Article

3.1 Instructions to Authors

Toxicology In Vitro publishes papers reporting and interpreting original toxicological research involving the application or development of *in vitro* techniques.

Language: Manuscripts must be in English and the authors should write in a way which is simple and clear. British style and spelling were used in this article and words or phrases which might be unclear in other parts of the world was avoided or clearly explained.

Title, abstract and keywords: The title must be concise and informative. Abbreviations and formulae should be avoided where possible. The abstract should be a self-contained summary of the objectives, results and significance of the study, not exceeding 200 words. After the abstract, a maximum of 6 keywords must be provided. These keywords will be used for indexing purposes.

Structure of paper: There are no strict formatting requirements but all manuscripts must contain the essential elements needed to convey the article, for example Abstract, Keywords, Introduction, Materials and Methods, Results, Conclusions, Figures and Tables with Captions. All pages must be numbered, including the Title Page, which should carry the title of the paper, the surnames and initials of the authors, and the names and address of the institutions where the work was done. The introduction should be a concise and clear statement on the background, purposes and significance of the work. A detailed description will be given of the experimental design and of any new or improved methods and well-established methods and techniques may be identified by reference only.

Units and symbols: The metric system is the standard for all measurements. Test chemicals must be clearly identified, wherever possible, with the aid of CAS Registry.

Figures: This can be photographs, diagrams and charts. Each illustration must have a caption and should comprise of a brief title as well as a description of the illustration. Keep text in the illustrations to a minimum but explain all symbols and abbreviations used.

References: The reference style used by the journal will be AUTHOR INFORMATION PACK 21 Jul 2015 www.elsevier.com/locate/toxinvit 11 applied to the accepted article by Elsevier at the proof stage. Where applicable, author(s) name(s), journal title/book title, chapter title/article title, year of publication, volume number/book chapter and the pagination must be present. Use of DOI is highly encouraged.

All citations in the text should refer to:

Single author: the author's name (without initials, unless there is ambiguity) and the year of publication; two authors: both authors' names and the year of publication; three or more authors: first author's name followed by *'et al*.' and the year of publication. Citations may be made directly (or parenthetically). Groups of references should be listed first alphabetically, then chronologically. Examples: 'as demonstrated (Allan, 2000a, 2000b, 1999; Allan and Jones, 1999). Kramer *et al*. (2010) have recently shown ....'

References should be arranged first alphabetically and then further sorted chronologically if necessary, however the referencing is not stringent but should just be consistent. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.

Examples:

Reference to a journal publication: Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. J. Sci. Commun. 163, 51–59.

Reference to a book: Strunk Jr., W., White, E.B., 2000. The Elements of Style, fourth ed. Longman, New York.

Reference to a chapter in an edited book: Mettam, G.R., Adams, L.B., 2009. How to prepare an electronic version of your article, in: Jones, B.S., Smith, R.Z. (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281–304.

The influence of pH on the *in vitro* permeation of platinum through human skin

YOLANDI VAN NIEUWENHUIZEN[1], ANJA FRANKEN[1], JEANETTA DU PLESSIS[2], JOHANNES L DU PLESSIS[1]

[1]Occupational Hygiene and Health Research Initiative (OHHRI), North-West University, Potchefstroom Campus, South Africa.
[2]Centre of Excellence for Pharmaceutical Sciences, North-West University, Potchefstroom Campus, South Africa.

Prof A Franken

Occupational Hygiene and Health Research Initiative (OHHRI)

North-West University, Potchefstroom Campus

Private Bag X6001

Potchefstroom

2520

South Africa

Tel: 018 299 2437

Fax: 018 299 1053

Email: anja.franken@nwu.ac.za

3.2 Abstract

Platinum, one of the platinum group metals (PGMs), is mined and refined in South Africa, whereafter it is widely utilised in various industries. In these industries workers can potentially be exposed to either metallic platinum or to platinum salts. Exposure to platinum can occur by means of inhalation, ingestion or dermal contact. It has been established that soluble platinum salts can permeate through intact human skin; however, the effect of pH on the permeation of platinum is unknown. Some metals have demonstrated differences in permeability at differing pH levels. Jansen van Rensburg *et al*. (2016) found the permeation of rhodium to be higher at a pH of 4.5 than at a pH of 6.5. Chromium could permeate through the skin at a pH of 4.5, which did not occur at a pH of 6.5. The *in vitro* permeation of platinum through intact skin at a pH of 6.5 has been established and the possibility of platinum accumulating and being retained inside the skin confirmed. However, the permeation of platinum at different pH levels has not yet been investigated. The aim was to determine the *in vitro* skin permeation of a platinum salt, potassium tetrachloroplatinate (K_2PtCl_4) dissolved in synthetic sweat, at a pH 4.5 and 6.5 using the Franz cell diffusion method. A concentration of 0.3 mg/ml platinum was applied and analyses were performed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Platinum permeated through intact human skin, at both pH levels of 4.5 and 6.5. The mass of platinum that permeated through the skin after 24 hours was 58.09% higher at a pH of 4.5 (34.18 ± 7.79 ng/cm^2) than at a pH of 6.5 (21.62 ± 4.4 ng/cm^2). The retention of platinum in the skin was 19.62% higher at a pH of 4.5 (2118.9 ± 62.6 ng/cm^2) than at a pH of 6.5 (1771.3 ± 131.9 ng/cm^2), which was statistically significant ($p = 0.02$). Regardless of the pH, a statistically significant higher percentage of platinum was retained in the skin relative to the percentage that diffused through the skin ($p \leq 0.001$), which indicates that platinum accumulated in the skin irrespective of the environment's acidity. However, the 58.09% increase in platinum permeation that occurred at a pH of 4.5 implies that an acidic environment or lower skin surface pH may enhance platinum permeation and therefore potentially increase the body burden and result in adverse health effects. It is thus suggested that future *in vitro* permeation studies include a pH of 4.5 to simulate the actual conditions in acidic working environments.

Key words: Skin surface pH, skin permeation, metal, platinum group metals.

3.3 Introduction

In South Africa, the platinum group metals (PGMs) mining sector is deemed as one of the largest and contributes largely to the economy of South Africa. Platinum, the most widely used and well-known PGM, has exceptional catalytic properties (Cawthorn, 1999; Gómez *et al*., 2000; Merget and Rosner, 2001; Ash *et al.,* 2014). Platinum is also applied in the chemical, petroleum,

electronic and medical industries as well as glass and jewellery production (Wiseman and Zereini, 2009; Zereini *et al*., 2012). Thus, the increased demand for platinum has led to the increased occupational exposure of workers in platinum refineries and other related industries (Wiseman and Zereini, 2009). Occupational exposure can occur through various routes, namely inhalation, ingestion or potentially through the skin. Due to the main historic focus on respiratory exposure, dermal contact as an exposure route is often overlooked (Boscolo *et al*., 2004; Semple, 2004; Sartorelli *et al*., 2012).

Limited information is available regarding dermal exposure to soluble platinum and the extent to which workers are exposed to platinum salts by means of dermal contact (Tanojo e*t al*., 2001; Bocca and Forte, 2009; Wiseman and Zereini, 2009). The majority of research conducted focused on the respiratory exposure to platinum as airborne particulate matter (Gómez *et al*., 2000, 2001; Sartorelli *et al*., 2012). In a study conducted by Maynard *et al.* (1997) several cases were reported in which workers developed respiratory sensitisation even though the airborne platinum concentration was well below the legislative respiratory occupational exposure limit or no airborne platinum was detected. The authors suggested an alternative exposure route such as the dermal route, potentially contributing to the sensitisation. With an animal study, Dearman *et al*. (1998) found respiratory sensitisation in mice after the topical application of ammonium hexachloroplatinate, which is a soluble platinum salt. Kimber and Dearman (2002) used diisocyanates and reactive dyes to investigate the relevance of the exposure route and concluded that in some cases respiratory sensitisation could result from dermal exposure.

The acute toxicity of a platinum salt depends on its solubility as well as the ability to bind to certain ligands to form complexes (Cleare *et al*., 1976; Merget and Rosner, 2001). Hexa- and tetrachloroplatinates are soluble halogenated platinum salts known to be sensitisers and allergens eliciting hypersensitive reactions which include both respiratory and dermal symptoms (Hunter *et al.,* 1945; Bolm-Audorff *et al*., 1992; Ravindra *et al*., 2004). Potassium tetrachloroplatinate (KPt_2Cl_4), the salt used in this study, is considered to be a soluble platinum compound forming the sensitiser tetrachloroplatinate ($[PtCl_4]^{2-}$) when it reacts with eccrine sweat. (Hunter *et al.,* 1945; Bolm-Audorff *et al*., 1992; Hostýnek *et al*., 2006; Bullock, 2010).

The ability of metals to undergo oxidation and form ions is one of the factors influencing a metal's permeability through human skin (Hostýnek *et al*., 2006). According to Hostýnek *et al*. (2006) electrochemical oxidation occurs due to the sweat acting as an electrolyte which leads to the formation of metal ions. However, Smith (1990) suggested that non-ionised molecules permeate the skin more rapidly due to the lack of charge. In contrast, according to Tanojo *et al*. (2001), the free nickel ions that form in synthetic sweat can permeate the skin more rapidly due to being smaller in size than the non-ionised molecule. However, these ions can also bind to

other ions with opposite charges or to fatty acids and peptides in the stratum corneum thereby decreasing the permeation. This could lead to the metal having an increased retention in the stratum corneum, which contradicts literature regarding the effect of ionisation on permeability. Several metals, including nickel, cobalt, silver and gold, have been found to permeate the skin due to the formation of ions. These ions have a higher permeability than the non-ionised molecule due to its smaller size (Tanojo *et al*., 2001; Larese Filon *et al*., 2004, 2007, 2009, 2011, 2012). Nanoparticles, which are exceptionally small, release more ions due to their high surface to volume ratio (Mauro *et al*., 2015). Mauro *et al*. (2015) investigated the permeation of platinum nanoparticles and found a greater mass of platinum had deposited inside the skin than the amount that permeated.

In an acidic environment, the ionisation of some metals may occur more rapidly and thus a decrease in pH would increase the rate of ions being released and potentially result in a higher permeability (Tanojo *et al*., 2001; Larese Filon *et al*., 2007). This was confirmed for chromium which did not permeate the skin at a pH of 6.5, due to oxidation not taking place (Larese Filon *et al*., 2007). In a following study, the same authors established that chromium permeated the skin at a lower pH, due to oxidation causing the formation of permeable ions (Larese Filon *et al*., 2008). Ågren (1990) also reported an increase in the zinc flux (rate of permeation) when the pH was decreased. Rhodium, which is also a PGM, applied as rhodium chloride permeated the skin more rapidly at a lower pH of 4.5 (Jansen van Rensburg *et al*., 2016).

The permeation characteristics of platinum have not yet been investigated when subjected to different pH levels. Other metals have shown differences in permeation at different pH levels. Thus, the pH could potentially influence the permeability of a metal and therefore the motivation for this study. The permeability of platinum at a pH of 6.5 has been established (Franken *et al.,* 2014); however, according to our knowledge not at a pH of 4.5. Therefore, the aim of this study was to investigate the *in vitro* permeation of a soluble platinum salt, KPt_2Cl_4, through intact Caucasian skin using the Franz diffusion cell method at different pHs, specifically 6.5 and 4.5. This study was unique since permeability was investigated at two different pH levels simultaneously utilizing skin from two different donors.

3.4 Methods and Materials

3.4.1 Chemicals

All the chemicals used were of analytical grade. Ammonia (NH_3) (32%), hydrochloric acid (HCl) (37%) and potassium di-hydrogen phosphate (KH_2PO_4) were purchased from Merck, South Africa. Sodium chloride (NaCl), urea, lactic acid (88 - 92%) and sodium phosphate dibasic (Na_2HPO_4) were purchased from Sigma Aldrich, South Africa. Nitric acid (HNO_3) (65%) and HCl

(33%) were purchased from De Bruyn Spectroscopic Solutions, South Africa. Hydrogen peroxide (H_2O_2) (50%) and acetone were purchased from Associated Chemical Enterprises, South Africa. Potassium tetrachloroplatinate (K_2PtCl_4) was obtained from Johnson Matthey and sponsored by Anglo American Technical Solutions, South Africa. All solutions were prepared with ultrapure water from a Millipore purification system (milliQ).

The synthetic sweat utilised as donor solution consisted of 0.5% NaCl, 0.1% urea and 0.1% lactic acid in 1000 ml milliQ water with the pH adjusted to 4.0 with NH_3. K_2PtCl_4 powder (0.03233 g) was added to 50 ml synthetic sweat, with a final platinum concentration of 0.3 mg/ml. The solution was stirred on a stirring plate for 30 minutes at room temperature to ensure even distribution and dissolution of K_2PtCl_4. The 50 ml solution was split into two equal volumes (25 ml), each separately being corrected for pH with NH_3 to a pH of 4.5 and 6.5, respectively.

The physiological receptor solution (representative of interstitial fluid) consisted of 2.38 g Na_2HPO_4, 0.19 g KH_2PO_4 and 9 g NaCl dissolved in 1000 ml milliQ water. The pH was adjusted with HCl to 7.35.

3.4.2 Preparation of skin membranes

This study was approved by the Health Research Ethics Committee (HREC) of the North-West University, approval number NWU-00202-15-A1. Abdominal skin of Caucasian females was obtained as surgical waste from various hospitals only after written informed consent was obtained from each patient. For this experiment, the abdominal skin from two different donors, aged 37 and 47 years, was used.

3.4.3 Preparation of the *in vitro* diffusion system

The *in vitro* experimental design used to determine the permeation of a platinum salt was adapted from the Franz method (Franz, 1975), which was also used by various researchers (Larese Filon *et al*., 2006, 2007, 2008, 2009; Franken *et al.*, 2014, 2015; Jansen van Rensburg *et al*., 2016). Abdominal skin was collected and stored in a freezer at -20 °C for a period not exceeding six months. After the skin was allowed to thaw, it was prepared by firstly removing the subcutaneous layer of fat with a scalpel without damaging the epidermis (full thickness skin remaining intact). The skin was cut with an iron punch into smaller circular pieces, each with a diameter of 24 mm. The thickness of the skin, which was measured with a Vernier caliper, did not exceed 1 mm. Care was taken to ensure that no damaged skin with stretch marks was included in the permeation area of the skin, as it could influence the permeation rate. Each circular piece of skin was mounted separately on the Franz cell's receptor compartment, with the stratum corneum facing towards the donor compartment and held together with a metal

clamp. To provide a seal between the skin and the two compartments, vacuum grease (Dow Corning, USA) was applied to the Franz diffusion cell. The mean exposed surface area of the skin in the Franz cell when clamped was 1.066 cm^2.

Before commencing with the experiment, the skin integrity was tested. The donor and receptor compartments of the Franz diffusion cell was filled with 0.9% NaCl and placed in a water bath for 30 minutes at 37 °C. Skin integrity was tested using a conductometer (Precision LCR-Meter, LCR-800, GW-Instek) to test the electrical conductivity of the skin. The conductometer was set to measure the resistance in parallel at a frequency of 1 kHz. If the resistance for any cell ranged between 10 – 80 kΩ (kilo Ohm) it was considered acceptable for use and the 28 cells with a value within 8 – 10 kΩ of each other were chosen (Lawrence, 1997; Davies *et al*., 2004). Resistance values below 10 kΩ or above 80 kΩ were considered as an indication of damaged skin, or the diffusion system set up incorrectly and those cells were discarded. After the experiment, the skin integrity was tested again in the same manner.

The receptor compartment was filled with 2 ml of the physiological solution and also contained a magnet to continually stir the solution. The receptor compartment was immersed in water to maintain the temperature at 37 °C in order to simulate normal core body temperature. To prevent the evaporation of the solution, parafilm was used to cover the sampling port. The donor compartments of the experimental cells were filled with 1 ml donor solution (synthetic sweat containing K_2PtCl_4). The donor compartments of the blank cells were filled with 1 ml synthetic sweat, containing no K_2PtCl_4. The donor compartment of all cells was covered with both parafilm and a cap.

3.4.4 Removal of solutions

At selected intervals (1, 2, 6, 12, 18 and 24 hours), the receptor solution was removed for analysis. At each removal the receptor compartment was rinsed with 2 ml of the receptor solution, which was also removed for analysis. Therefore, 4 ml receptor solution was removed into a vial for analysis at each interval. After removal, each receptor compartment was refilled with 2 ml of receptor solution (except after 24 h). At the 24 hour interval, the donor solution was removed. The donor compartment was washed with 1 ml of synthetic sweat, four consecutive times, which was also removed and added to the donor solution. This was done to ensure that no K_2PtCl_4 remained on the skin surface and any K_2PtCl_4 detected in the skin analyses was representative of the platinum retained inside the skin. In total, 5 ml of donor solution and 4 ml of receptor solution were removed for analysis. The skin was removed from the *in vitro* diffusion system after skin integrity was tested and placed into a vial for digestion to determine whether any of the K_2PtCl_4 had accumulated in the skin.

3.4.5 Chemical digestion of skin

Before analysis, each circular piece of skin was chemically digested. This was done by placing each piece of skin in a glass beaker and rinsing it with acetone. The acetone was allowed to evaporate by heating the beaker on a hotplate. HNO_3 was added, which was allowed to evaporate first, followed by the addition of H_2O_2. The HNO_3 and H_2O_2 were used to dissolve the tissue and destroy the organic material in the sample. *Aqua Regia* (1 ml HNO_3 and 3 ml HCl) was added and allowed to evaporate whereafter HCl was added and allowed to evaporate to ensure that any platinum that might have been oxidised was returned to a stable state. The solution was transferred to a 15 ml centrifuge tube and made up to 10 ml with 0.07 M HCl. Six quality control samples were also included to verify reagent and methodology integrity.

3.4.6 Analyses

Three different types of samples with different expected platinum concentrations were sent for analysis. Each sample type required a different calibration matrix for analysis. Physiological solution was used for the receptor solutions' calibration standard, synthetic sweat for the donor solutions' calibration standard and 0.07 M HCl for the skin samples' calibration standard. This was done to maintain consistency between the calibration standard and the samples. New standards were prepared for each analysis procedure.

The platinum concentrations of the donor and skin solutions were determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Spectro Analytical ARCOS, SPECTRO Analytical Instruments GmbH, Germany). The concentrations of platinum in the receptor solutions were determined by using high resolution Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Thermo Scientific Element XR, Thermo Fischer Scientific, Waltham, MA, USA). An internal standard was added to compensate for volume inaccuracies. The receptor solutions were spiked with 10 µg/L rhenium whereas the donor and digested skin solutions were spiked with 4 mg/L yttrium. The calibration curves for the ICP-MS were determined with solutions containing platinum and rhenium with concentrations of 0, 5, 10, 20, 50 and 100 ng/L. The ICP-OES was calibrated with platinum and yttrium solutions of 0, 1, 2, 3, 4, 5, 10 and 20 mg/L.

3.4.7 Data and statistical analyses

The cumulative mass of platinum that permeated each piece of skin was plotted against time and used to calculate the flux (rate of platinum permeability across skin). The data was subjected to curve-fitting (Easyplot, Spiral Software), as developed by Diez-Sales *et al*. (1991). The following equation (Eq. 1) was used to fit the data:

$$Q\ (t) = AKhCv[D\frac{t}{h^2} - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\exp\left(\frac{-D^2\pi^2 t}{h^2}\right)] \qquad \textit{(Eq. 1)}$$

Where

Q(t) – amount of substance permeating the skin within a time period

t – time

K – partition coefficient of a substance (between the vehicle and the skin)

h – diffused path length

C_v – actual concentration of the substance (in the donor compartment)

D – diffusion coefficient of a substance

However, as the time, *t,* approaches infinity, the exponential term becomes negligible and the equation can be simplified to the following (Eq. 2):

$$Q\ (t) = AKhC_v[D\frac{t}{h^2} - \frac{1}{6}] \qquad \textit{(Eq. 2)}$$

Due to the values of *K* and *D* being unknown, *K x h* and D/h_2 were replaced by *α* and *β*, respectively, which was determined by fitting Eq. 2 to the permeation plots. These plots were obtained by curve fitting the graphs using EasyPlot (Spiral Software). Where this curve intercepted with the x-axis, the lag time in hours (h) was determined, which is the minimum time needed to reach a steady flux.

The permeability coefficient *(k_p)* was determined using Eq. 3 and Eq. 4 to calculate the flux *(J).*

$$k_p = \frac{KD}{h}\ (=\ \alpha\beta) \qquad \textit{(Eq. 3)}$$

$$J = k_p C_v \qquad \textit{(Eq. 4)}$$

The flux, *J*, allows for the calculation of K_p, which represents the permeability coefficient. The flux signifies the amount of metal that permeated per area over a unit time (ng/cm^2/h). The cumulative concentration that permeated at selected time intervals is presented as ng/cm^2. In addition to this, the mass retained in the skin after 24 hours was calculated and reported.

Data analyses were performed using Statistica 13.0 (Statsoft Inc.) and Microsoft Excel for Windows. The mean and standard deviation for each pH were determined. After the data had been Box-Cox transformed since the original data were skewed, an independent t-test was used to determine any statistically significant difference in the cumulative mass of platinum, the platinum mass retained, the flux and the lag time between pH 4.5 and pH 6.5 (Box and Cox,

1964). The data is reported as mean ± standard error of means (SEM) and $p \leq 0.05$ was considered statistically significant.

3.5 Results

The permeation of platinum, applied as K_2PtCl_4, through intact human skin was investigated to determine whether there was any significant difference between the permeation at a pH of 4.5 and 6.5. Platinum permeated through intact human skin at both pH levels. As illustrated in Figure 1, an increase in permeation was found over 24 hours. As the exposure time prolonged, the mass of platinum that permeated increased, regardless of the pH. At all the time intervals (1, 2, 6, 12, 18 and 24 hours) the cumulative mass of platinum that permeated through intact human skin was higher at a pH of 4.5 than at a pH of 6.5 with statistically no significant differences between the permeation at different pH levels.

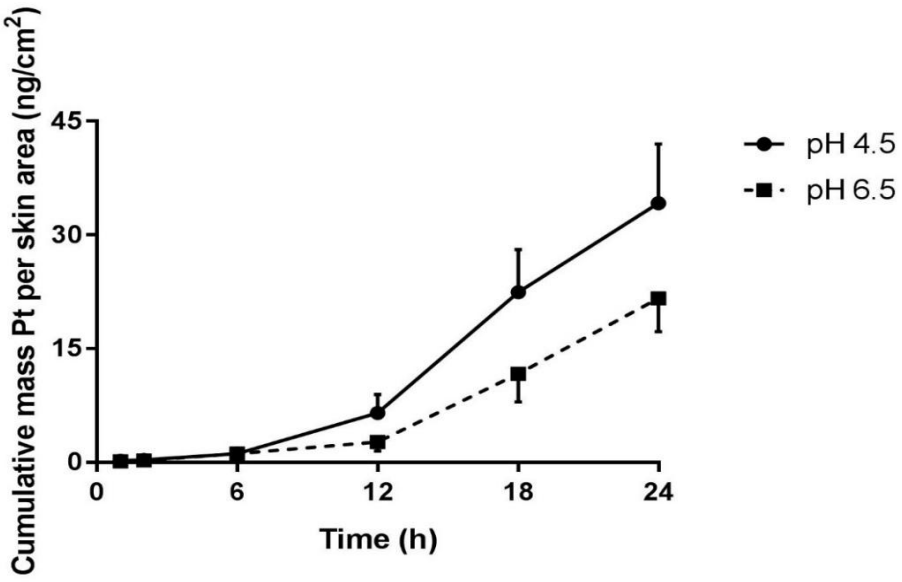


Figure 1: Cumulative mass of platinum that permeated per area of skin at a pH of 4.5 (n = 9) and a pH of 6.5 (n = 9).

Table 1 contains a summary of the mass of platinum that permeated and diffused through the skin, the mass that was retained inside the skin and the flux values as well as the lag time for each pH. The cumulative mass of platinum that permeated through the skin after 24 hours at a pH of 4.5 was 58.09% higher than the mass of platinum that permeated at a pH of 6.5. From 12 hours to 24 hours, the permeation of platinum increased 5.26 times at a pH of 4.5 and 8.04 times at a pH of 6.5, indicating the possible effect of prolonged exposure. At a pH of 4.5, the flux of platinum was 33.75% higher than at a pH of 6.5 with no statistical significance.

The mass of platinum that was retained in the skin at a pH of 4.5 was significantly greater than the mass retained at a pH of 6.5 ($p = 0.02$). Therefore, the percentage of platinum retained in the skin was also statistically significant ($p = 0.02$) greater at pH 4.5 than at pH 6. The percentage of platinum that was retained in the skin was highly significantly greater than the percentage that diffused through the skin ($p \leq 0.001$) at a pH of 4.5 and the same was observed for pH 6.5.

The lag time was less than four hours for both pH levels, with the difference between pH 4.5 and pH 6.5, leaning towards statistical significance ($p = 0.054$) as seen in Table 1. At a pH of 6.5 the lag time was 37.25% higher than at a pH of 4.5.

Table 1: Summary of platinum that permeated through the skin and was retained inside the skin at pH 4.5 and pH 6.5.

	pH 4.5	pH 6.5	p
	Mean ± SEM	**Mean ± SEM**	
Number of cells (n)	9	9	
Cumulative mass permeated 1h (ng/cm^2)	0.19 ± 0.03	0.14 ± 0.01	0.2
Cumulative mass permeated 2h (ng/cm^2)	0.33 ± 0.05	0.24 ± 0.02	0.13
Cumulative mass permeated 6h (ng/cm^2)	1.14 ± 0.41	1.13 ± 0.49	0.35
Cumulative mass permeated 12h (ng/cm^2)	6.50 ± 2.44	2.69 ± 1.21	0.18
Cumulative mass permeated 18h (ng/cm^2)	22.46 ± 5.59	11.67 ± 3.72	0.33
Cumulative mass permeated 24h (ng/cm^2)	34.18 ± 7.79	21.62 ± 4.4	0.50
Mass in skin (ng/cm^2)	**2118.9 ± 62.6**	**1771.3 ± 131.9**	**0.02**
Percentage in skin (%)	**3.4 ± 0.1**	**2.8 ± 0.2**	**0.02**
Mass diffused (ng/cm^2)	6.48 ± 1.81	5.05 ± 1.02	0.50
Percentage diffused (%) ($\times 10^{-3}$)	2.45 ± 0.64	1.75 ± 0.36	0.50
Flux ($ng/cm^2/h$)	1.07 ± 0.3	0.80 ± 0.2	0.44
Lag time (h)	2.47 ± 0.34	3.39 ± 0.28	0.054

3.6 Discussion

The *in vitro* permeation of platinum through intact human skin at pH 4.5 and 6.5 was investigated. It was found that platinum permeated through the skin at both pH levels. Dermal exposure to platinum could lead to permeation at a pH 6.5, as a previous *in vitro* permeation study had established (Franken *et al*. 2014). This study is unique since the permeability of a platinum salt was confirmed simultaneously at pH 4.5 and pH 6.5.

Certain metals previously investigated, demonstrated differences in permeation at different pH levels (Larese Filon *et al*., 2004, 2007; Jansen van Rensburg *et al*., 2016). Therefore, when the environment is more acidic or the chemicals used in conjunction with the platinum production or during refining is acidic, the permeation of these metals could potentially be influenced. Since the influence of pH on platinum permeability was not yet known, it provided the motivation for this study. The skin surface pH, in general, is considered to be of a more acidic nature. It normally ranges from pH 4 to 5.5, but due to physical activity and sweat evaporation, the pH can be even lower (Larese Filon *et al*., 2008, 2009, 2011; Byford, 2009). The skin surface pH also differs with race, as African skin was found to have a lower skin surface pH when compared to other skin types, ranging from 4.3 to 5 (Warrier *et al*., 1996; Berardesca *et al*., 1998; Muizzuddin *et al*., 2010). An acidic environment or lower skin surface pH could result in a potential concentration gradient of the contaminant between the stratum corneum and deeper skin layers (Jepps *et al*., 2013). Larese Filon *et al*. (2009) stated that an one unit decrease in pH could result in a 10 – 100 times increase in permeation of certain substances. Thus, the reason for this study was to determine the influence pH could have on the permeation of platinum and it demonstrated that the cumulative mass of platinum that permeated through Caucasian skin after 24 hours at a pH 4.5 was 58.09% higher than at a pH of 6.5. Although it was not statistically significant, an increase in permeation was observed with a two-unit decrease in pH.

With some metals previously investigated, it was established that the permeation was enhanced by the ability to ionise, which increased the solubility (Hostýnek *et al*., 2006; Larese Filon *et al*., 2007). Free nickel ions permeated the skin more rapidly than non-ionised nickel molecules, due to having a smaller diameter (Tanojo *et al*., 2001). This was also found for cobalt and chromium (Larese Filon *et al*., 2004, 2007). In an acidic environment, and thus at a lower pH, some metals undergo oxidation and would therefore potentially increase the permeation through the skin (Larese Filon *et al*., 2007). A previous *in vitro* study, which investigated a rhodium salt, demonstrated an increase in rhodium permeation and the flux, which is the rate of permeation, to be greater at a lower pH (Jansen van Rensburg *et al*., 2016). A 58.09% higher permeation in platinum and 1.34 times greater flux were found in this study at a pH of 4.5 and is in agreement with the results shown by Jansen van Rensburg *et al*. (2016). The percentage mass of platinum

that diffused through the skin was calculated by the cumulative mass of platinum that permeated through the skin after 24 hours, divided by the original platinum mass at the start of the experiment. The percentage of platinum that diffused at a pH of 4.5 was 40% higher than the percentage that diffused at a pH of 6.5. These results therefore indicate the probability for increased platinum permeation through intact skin in an acidic environment.

The continuous exposure to platinum salts can increase the risk of platinum permeating through intact human skin, as this study has indicated that prolonged exposure resulted in an increased cumulative platinum permeation at both pH 4.5 and 6.5. In occupational settings, an average of eight hours is considered a normal working shift and 12 hours an extended working shift. The permeation at pH 4.5 increased 5.70 times from six to 12 hours and for pH 6.5 the increase was only 2.38 times. This is important to note, especially for workers who have extended shifts or workers who do not wash or clean properly after a normal eight hours shift, as it may result in increased permeation due to the prolonged exposure time. The mass of platinum that permeated through the skin from 12 to 24 hours at both pH levels increased more than five times. The notable increase in platinum permeation from six to 24 hours indicates the probable accumulation and permeation of platinum as a result of prolonged exposure, thereby indicating the potential risk for workers with extended shifts or workers who do not wash properly after leaving the workplace.

The mass of platinum that was retained in the skin after 24 hours at a pH of 4.5 was significantly greater statistically than the mass that was retained at a pH of 6.5 ($p = 0.02$). Furthermore, the percentage of platinum that was retained in the skin after 24 hours was significantly greater than the percentage of platinum that diffused through the skin ($p \leq 0.001$) (99.69% at pH of 4.5 and 99.71% at a pH of 6.5). Hostýnek *et al*. (2003) described that some metals form complexes with the skin causing the formation of insoluble salts, such as aluminium(III), which would lead to the increased accumulation of the metal in the skin. Mauro *et al*. (2015) showed that a greater mass of platinum nanoparticles was retained inside the skin than the mass that permeated through the skin. However, nanoparticles are exceptionally small in diameter and release a large amount of ions (Mauro *et al*., 2015). It was stated by Tanojo *et al*. (2001) that the accumulation of free nickel ions in the skin could be attributed to the binding of the ions to peptides or fatty acids with oppositely charged ions in the stratum corneum, which causes the increased retention of the metal. Potassium tetrachloroplatinate (K_2PtCl_4) is the soluble salt used in this study and one of the ionised compounds forming when K_2PtCl_4 dissociates as chloroplatinate $[PtCl_4]^{2-}$. This ionised compound potentially interacted with other oppositely charged ions in the stratum corneum, resulting in the substantial accumulation of platinum in the stratum corneum, which could prolong the exposure time to platinum. Consequently, potentially greater levels of platinum could diffuse through the skin causing a concentration gradient across the different

skin layers. This can result in platinum being distributed to the vascular network as well as the lymphatic system, from where distribution throughout the body may occur and exert adverse health effects or it may be cleared from the body (Ngo *et al*., 2009; Jepps *et al*., 2013).

Health effects, such as sensitisation, have been reported for airborne platinum that was inhaled. However, respiratory sensitisation was reported when no airborne platinum was detected, suggesting another route of exposure, such as dermal contact, possibly contributing to the sensitisation (Maynard *et al*., 1997). The data from an animal study proved the possibility of developing respiratory sensitisation after dermal exposure to platinum salts (Dearman *et al.*, 1998). Kimber and Dearman (2002) investigated two aspects regarding respiratory sensitisation, one being the role of IgE antibodies and the other was the relevance of the exposure route. Mice that were exposed to ammonium hexachloroplatinate via dermal contact showed elevated levels of eosinophils, which is characteristic of hypersensitivity reactions in the respiratory tract (Dearman *et al*., 1998). The authors concluded that in some cases, respiratory sensitisation could result from dermal exposure (Kimber and Dearman, 2002). Based on the research done by Maynard *et al.* (1997), and Kimber and Dearman (2002), it can be suggested that dermal exposure to respiratory sensitising platinum salts may possibly result in or contribute to respiratory sensitisation.

The relatively short lag time, which is less than four hours for both pH levels, indicates the time needed for platinum to achieve a steady state of permeation through the skin and for the permeation to be optimal. This is in accordance with a previous study where a lag time of less than four hours was observed for platinum (Franken *et al*., 2014). However, the lag time should be approached cautiously due to the unreliability thereof, as the thickness of the membrane and the diffusion coefficient are used to calculate the lag time. The thickness of the membrane may differ greatly between skin samples, making it difficult to reproduce consistent lag time values (Kierstan *et al*., 2001). The lag time for pH 4.5 was only 37.25% shorter than for pH 6.5. This signifies that even though the permeation was higher at a pH of 4.5, steady state can be reached within a few hours even at a higher pH. This steady-state is reached due to the saturation of the skin with the contaminant, in this case platinum (McDougal, 2002). Thus, the lag time indicates the time period needed for a sufficient mass of platinum to accumulate in the skin resulting in optimal permeation through the skin. The lag time is not an indication of the time before permeation occurs as permeation through the skin has already occurred after one hour of exposure.

3.7 Conclusion

The influence of pH on the *in vitro* permeation of platinum through intact human skin at a pH of 4.5 and a pH of 6.5 was established. Although there was an increase in platinum permeation at

both pH levels with the prolonged exposure time, pH 4.5 resulted in 58.09% greater permeation. The retention of platinum inside the skin was significantly greater at a pH of 4.5 ($p = 0.02$). The decrease in the synthetic sweat pH led to an increased permeation as well as an increase in skin retention in platinum accumulating in the skin. This substantial retention may prolong the exposure to platinum and thereby possibly increase the permeation leading to an increased body burden. Thus, an acidic environment could lead to a lower skin surface pH and according to the results could increase the mass of platinum permeating through the skin as well as increase the mass of platinum retained inside the skin. It is therefore important to promote good personal hygiene in the workplace and implement measures to protect the skin barrier, including the skin surface pH and to prevent skin contact with acidic chemicals and sensitising metals.

3.8 Acknowledgements

This study is funded by the NRF Thuthuka Funding Grant (UID: 94113).

Disclaimer: Any opinion, finding and conclusion or recommendation expressed in this material is that of the author(s) and the NRF does not accept any liability in this regard.

3.9 References

Ågren, M.S., 1990. Percutaneous absorption of zinc from zinc oxide applied topically to intact skin in man. Dermatologica. 180, 36-39.

Ash, P.W., Boyd, D.A., Hyde, T.I., *et al*. 2014. Local structure and speciation of platinum in fresh and road-aged North American sourced vehicle emissions catalysts: an x-ray absorption spectroscopic study. Environ. Sci. Technol. 48, 3658-3665.

Berardesca, E., Pirot, F., Singh, M., *et al.* 1998. Differences in stratum corneum pH gradient when comparing white and black African-American skin. Br. J. Dermatol. 139, 855-857.

Bocca, B., Forte, G., 2009. The epidemiology of contact allergy to metals in the general population: prevalence and new evidences. Open. Chem. Biomed. Meth. J. 2, 26-34.

Bolm-Audorff, U., Bienfait, H.G., Burkhard, J., *et al*. 1992. Prevalence of respiratory allergy in a platinum refinery. Int. Arch. Occup. Environ. Health. 64, 257-260.

Boscolo, P., Giampaolo, L.D., Reale, M., *et al*. 2004. Different effects of platinum, palladium and rhodium salts on lymphocyte proliferation and cytokine release. Ann. Clin. Lab. Sci. 34, 299-306.

Box, G.E.P., Cox, D.R., 1964. An analysis of transformations. J. Roy. Stat. Soc. 26, 211-252.

Bullock, J., 2010. Chloroplatinate Toxicity: Use and Misunderstanding of Merget. International Precious Metals Institute. USA: Tucson, Arizona. Available at: http://ipmi.org/pdf/Chloroplatinate%20Toxicity%20Merget.pdf (accessed 5 May 2015)

Byford, T., 2009. Environmental Health Criteria 235: Dermal Absorption. Int. J. Environ. Stud. 66, 662-788.

Cawthorn, R.G., 1999. The platinum and palladium resources of the Bushveld complex. S. Afr. J. Sci. 95, 481-489.

Cleare, M.J., Hughes, E.G., Jacoby, B., *et al*. 1976. Immediate (type I) allergenic responses to platinum compounds. Clin. Allergy. 6,183-195.

Davies, D.J., Ward, R.J., Heylings, J.R., 2004. Multi-species assessment of electrical resistance as a skin integrity marker for *in vitro* percutaneous absorption studies. Toxicol. *In Vitro*. 18, 351-358.

Dearman, R.J., Basketter, D.A., Kimber, I., 1998. Selective induction of type 2 cytokines following topical exposure of mice to platinum salts. Food. Chem. Toxicol. 36, 199-207.

Diez-Sales, O., Copovi, A., Casabo, C.G., *et al.* 1999. A modelistic approach showing the importance of the stagnant aqueous layers in *in vitro* diffusion studies, and *in vitro – in vivo* correlations. Int. J. Pharm. 77, 1-11.

Franken, A., Eloff, F.C., Du Plessis, J., *et al.* 2014. *In vitro* permeation of platinum and rhodium through Caucasian skin. Toxicol. *In. Vitro.* 28, 1396-1401.

Franken, A., Eloff, F.C., Du Plessis, J., *et al.* 2015. *In vitro* permeation of platinum through African and Caucasian skin. Toxicol. Lett. 232, 566-572.

Franz, T.J., 1975. Percutaneous absorption on the relevance of *in vitro* data. J. Invest. Dermatol. 64, 190-195.

Gómez, M.B., Gómez, M.M., Palacios, M.A., 2000. Control of interferences in the determination of Pt, Pd and Rh in airborne particulate matter by inductively coupled plasma mass spectrometry. Anal. Chim. Acta. 404, 285-294.

Gómez, B., Gómez, M., Sanchez, J.L., *et al*. 2001. Platinum and rhodium distribution in airborne particulate matter and road dust. Sci. Total. Environ. 269, 131-144.

Hostýnek, J.J., Dreher, F., Pelosi, A., *et al*. 2003. Human stratum corneum penetration by nickel. Acta. Derm. Venereol. Suppl. 212, 5-10.

Hostýnek, J.J., Dreher, F., Maibach, H.I., 2006. Human stratum corneum penetration by copper: *In vivo* study after occlusive and semi-occlusive application of the metal as powder. Food. Chem. Toxicol. 44, 1539-1543.

Hunter, D., Milton, R., Perry, K.M.A., 1945. Asthma caused by the complex salts of platinum. Br. J. Ind. Med. 2, 92-98.

Jansen van Rensburg, S.J., Franken, A., Du Plessis, J., *et al*. 2016. The influence of pH on the *in vitro* permeation of rhodium through human skin. Toxicol. Ind. Health. 1-8. DOI 10.1177/0748233716675218 (Available online)

Jepps, O.G., Dancik, Y., Anissimov, Y.G., *et al*. 2013. Modeling the human skin barrier – towards a better understanding of dermal absorption. Adv. Drug. Deliver. Rev. 65, 152-168.

Kierstan, K.T.E., Beezer, A.E., Mitchell, J.C., *et al*. 2001. UV-spectrophotometry study of membrane transport processes with a novel diffusion cell. Int. J. Pharm. 229, 87–94.

Kimber, I., Dearman, R.J., 2002. Chemical respiratory allergy: the role of IgE antibody and the relevance of route of exposure. Toxicol. 181, 311-315.

Larese Filon, F., Maina, G., Adami, G., *et al*. 2004. *In vitro* percutaneous absorption of cobalt. Int. Arch. Environ. Health. 7, 85-89.

Larese Filon, F., Boeniger, M., Giovanni, M., *et al*. 2006. Skin absorption of inorganic lead (PbO) and the effect of skin cleansers. J. Occup. Environ. Med. 48, 692-699.

Larese Filon, F., Gianpiero, A., Venier, M., *et al*. 2007. *In vitro* percutaneous absorption of metal compounds. Toxicol. Lett. 170, 49-56.

Larese Filon, F., D'Agostin, F., Crosera, M., *et al*. 2008. *In vitro* percutaneous absorption of chromium powder and the effect of skin cleanser. Toxicol. *In Vitro.* 22, 1562-1567.

Larese Filon, F., D'Agostin, F., Crosera, M., *et al*. 2009. *In vitro* absorption of metal powders through intact and damaged human skin. Toxicol. *In Vitro.* 23, 574-579.

Larese Filon, F., Crosera, M., Adami, G., *et al*. 2011. Human skin penetration of gold nanoparticles through intact and damaged skin. Nanotoxicology. 5, 493-501.

Larese Filon, F., Crosera, M., Timeus, E., *et al*. 2012. Human skin penetration of cobalt nanoparticles through intact and damaged skin. Toxicol. *In Vitro.* 27, 121-127.

Lawrence, J.N., 1997. Electrical resistance and tritiated water permeability as indicators of barrier integrity of *in vitro* human skin. Toxicol. *In Vitro*. 11, 241-249.

Mauro, M., Crosera, M., Bianco, C., *et al*. 2015. Permeation of platinum and rhodium nanoparticles through intact and damaged human skin. J. Nanopart. Res. 17, 253.

Maynard, A.D., Northage, C., Hemingway, M., *et al*. 1997. Measurement of short-term exposure to airborne soluble platinum in the platinum industry. Ann. Occup. Hyg. 41, 77-94.

McDougal, J.N., 2002. Methods for assessing risks of dermal exposure in the workplace. Crit. Rev. Toxicol. 34(2), 291-327.

Merget, R., Rosner, G., 2001. Evaluation of the health risk of platinum group metals emitted from automotive catalytic converters. Sci. Total. Environ. 270, 165-173.

Muizzuddin, N., Hellemans, L., Van Overloop, L., *et al*. 2010. Structural and functional differences in barrier properties of African American, Caucasian and East Asian skin. J. Dermatol. Sci. 59, 123-128.

Ngo, M.A., O'Malley, M., Maibach, H.I., 2009. Percutaneous absorption and exposure assessment of pesticides. J. Appl. Toxicol. 30, 91-114.

Ravindra, K., Bencs, L., Van Grieken, R., 2004. Platinum group elements in the environment and their health risk. Sci. Total. Environ. 318, 1-43.

Sartorelli, P., Montomoli, L., Sisinni, A.G., 2012. Percutaneous penetration of metals and their effects on skin. Prevent. Res. 2, 158-164.

Semple, S., 2004. Dermal exposure to chemicals in the workplace: Just how important is skin absorption? Occup. Environ. Med. 61, 376-382.

Smith, K.L., 1990. Penetrant characteristics influencing skin absorption, in: Kemppainen, B.W., Reifenrath, W.G. (Eds.), Methods for skin absorption. Boca Raton: CRC Press. pp. 23-34. ISBN 0-8493-4651-7

Tanojo, H., Hostýnek, J.J., Mountford, H.S., *et al*. 2001. *In vitro* permeation of nickel salts through human stratum corneum. Acta. Derm. Venereol. 21, 19-23.

Warrier, A.G., Kligmn, A.M., Harper, R.A., *et al*. 1996. A comparison of black and white skin using non-invasive methods. J. Soc. Cosmet. Chem. 47, 229-240.

Wiseman, C.L.S., Zereini, F., 2009. Airborne particulate matter, platinum group elements and human health: a review of recent evidence. Sci. Total. Environ. 407, 2493-2500.

Zereini, F., Alsenz, H., Wiseman, C.L.S., *et al*. 2012. Platinum group elements (Pt, Pd, and Rh) in airborne particulate matter in rural vs. urban areas of Germany: concentrations and spatial patterns of distribution. Sci. Total. Environ. 416, 261-268.

Chapter 4 – Concluding Chapter

4.1 Further discussion

Platinum is the most well-known and widely used metal of the platinum group metals (PGMs) due to its catalytic properties (Cawthorn, 1999; Gómez *et al*., 2000). The mines and refineries as well as various other industries, such as the catalysis, petroleum and medical industries, utilise platinum salts. Workers in these industries could possibly be exposed to platinum by means of skin contact, either directly or indirectly (Schneider *et al*., 1999; Boscolo *et al*., 2004; Sartorelli *et al*., 2012).

Previous *in vitro* studies have investigated the permeation characteristics of various metals such as nickel, cobalt and chromium. These studies have shown that when the metals undergo oxidation under acidic conditions, permeation through intact human skin occurred (Tanojo *et al*., 2001; Larese Filon *et al*., 2004, 2007, 2009, 2011, 2012). With chromium, the oxidation process took place in an acidic environment, as the lower pH caused an increased rate at which metal ions were released (Larese Filon *et al*., 2007).

Before commencing with this study, it had already been established that a platinum salt permeated through intact human skin (Franken *et al*. 2014), whereas the influence of pH on this *in vitro* permeation was unknown. The aim of this study was to determine the influence of pH on the *in vitro* permeation of platinum, applied as K_2PtCl_4. This was investigated in order to characterise the permeation of platinum salts when subjected to different pH levels, as well as the effect thereof due to some working environments being more acidic. Previous *in vitro* studies have indicated that a lower pH level could possibly promote permeation and the retention of these metals inside the skin (Larese Filon *et al*., 2007; Jansen van Rensburg *et al*., 2016). Thus, workers in an acidic environment, or with a lower skin surface pH, may be more at risk when dermal exposure to platinum salts occurs, which may enhance the permeation and accumulation of platinum in the skin. Thus, the risk to the health of workers in the applicable occupational settings should be a concern for the platinum industry.

Soluble platinum salts could cause respiratory sensitisation when inhaled and are also allergens with known skin symptoms (Maynard *et al.,* 1997; Ravindra *et al.,* 2004). Some workers who reported respiratory sensitisation worked where no airborne platinum was detected, suggesting the skin to be contributing to exposure (Maynard *et al.,* 1997). The accumulation of platinum in the skin may possibly contribute to the sensitisation due to the skin forming a platinum reservoir. This reservoir formation increases the duration of exposure time, which allows for more platinum to permeate through the skin, possibly into the vascular system, which may subsequently be distributed throughout the body by means of the circulatory system.

4.2 Conclusion

At both the pH levels of 4.5 and 6.5) the cumulative mass of platinum that permeated through the skin increased with prolonged exposure up to 24 hours. This study has proven that pH does influence the *in vitro* permeation of a platinum salt. The cumulative mass of platinum that permeated through the skin after 24 hours at pH 6.5 was less than the mass that permeated at pH 4.5, although the difference was not statistically significant. The rate of permeation (flux) at a pH of 4.5 was 1.34 times greater than the rate at a pH of 6.5, indicating that platinum permeates quicker at a lower pH. Previous *in vitro* studies used a synthetic sweat with a pH of 6.5, however the working environment could be more acidic. Therefore, a pH of 4.5 could be appropriate in simulating more acidic conditions found in workplaces.

Workers with a prolonged exposure to soluble platinum salts are at risk of platinum permeating through the skin, as the permeation of platinum started before one hour and increased over 24 hours. The risk is even greater for workers working in an acidic environment where the pH is 4.5 or lower, as the lower pH would result in increased platinum permeation through the skin. The cumulative mass that permeated after 24 hours was 58.09% higher at a pH of 4.5 than at a pH of 6.5. The more acidic environment or the lower skin surface pH may be a potential mechanism for platinum to oxidise more rapidly, increasing the rate at which permeable ions are formed (Tanojo *et al*., 2001; Larese Filon *et al*., 2007). The small diameter of ions could potentially increase the permeation thereof. However, investigation of this aspect was not the aim of the study.

Literature indicates that Africans have a skin surface pH ranging from 4.3 to 5, which is lower than other skin types, including Caucasian skin (Berardesca *et al*., 1998; Muizzuddin *et al*., 2010). The results from this study indicated the potential for increased platinum permeation through the skin and retention inside the skin when the skin surface pH decreases. The inherent lower skin surface pH of Africans, together with the acidic environment increases the risk for skin permeation to occur in this work group, since the acidic environment could result in an even lower skin surface pH (Berardesca *et al*., 1998). An *in vitro* investigation comparing African and Caucasian skin permeability established significantly higher platinum permeation through African skin ($p = 0.044$) and retention inside the skin ($p = 0.002$) when compared to Caucasian skin (Franken *et al.,* 2015). Therefore, skin contact with platinum salts is a significant risk for African workers in platinum refineries and workplaces with similar conditions and therefore special precautions needs to be implemented to prevent this exposure.

This study did not only confirm the permeability of platinum through the skin, but has also shown the significant retention of platinum in the skin, regardless of the pH. The retention of platinum at pH 4.5 was significantly higher than the retention at pH 6.5 ($p = 0.02$). The amount of

platinum that diffused through the skin after 24 hours, was substantially less than the amount of platinum that accumulated in the skin. Due to platinum retention, the exposure time potentially increases allowing for more platinum to diffuse through the skin and possibly be distributed throughout the body. The retention of platinum together with a decreased skin surface pH may disrupt the barrier function of the skin, resulting in increased permeation (Kezic and Nielson, 2009; Du Plessis *et al*., 2013). This may be a mechanism of soluble platinum to elicit adverse health effects, such as sensitisation and dermal symptoms which, among others, include contact urticaria and dermatitis.

A lag time of less than four hours was found for both pH 4.5 and pH 6.5, with pH 4.5 having the shorter lag time. However, it should not be assumed, mistakenly, that any exposure time shorter than the lag time of less than four hours is safe, as permeation already occurred after one hour. The lag time only indicates the time that is needed for permeation to reach a steady state. Therefore, the lag time is the time delay before the permeation through the skin is optimal, which is obtained when the skin becomes saturated with the contaminant, resulting in continuous permeation at a constant rate. Therefore, the lag time is the time needed for a sufficient mass of platinum to accumulate in the skin, resulting in continuous platinum permeation through the skin.

This study aimed at investigating the influence of pH on the *in vitro* permeation of a soluble platinum salt through intact skin. The Franz diffusion cell method was successfully used to accomplish objective 1, which was to investigate the *in vitro* permeability of platinum (K_2PtCl_4) through Caucasian skin at both pH levels of 4.5 and 6.5 simultaneously. By simultaneously investigating the permeability at two different pH levels, the study was suited to accomplish objective 2, which was to determine the potential difference in permeability at the different pH levels.

The hypothesis of the study was: ‘It is therefore hypothesised that the *in vitro* permeation of platinum through Caucasian skin at a pH of 4.5 is significantly higher than the permeation at a pH of 6.5. The results from this study indicated that the difference in platinum permeation at the different pH levels did not statistically differ significantly. Therefore, the hypothesis is rejected. However, the study has indicated a significant difference between the mass of platinum retained in the skin at pHs 4.5 and 6.5, with pH 4.5 resulting in significantly higher retention ($p = 0.02$). This retention of platinum inside the skin could prolong the exposure to platinum resulting in increased platinum permeation through the skin, potentially contributing to the adverse health effects of platinum.

4.3 Limitations

This study was subjected to a number of limitations. The mass of platinum initially applied to the skin surface is possibly much higher than the expected mass present in the workplace. Due to the limitation of the analysis method sensitivity, this mass was used in order for the platinum found in the results to be above the detection limit of the analytical method. During this study, the pieces of skin remained static during the entire 24 hours, whereas in an occupational setting, the skin would be subjected to certain mechanical forces, such as bending and stretching, resulting in constant movement. Care was taken not to include damaged skin with stretch marks in the study; however, in the workplace the workers may have damaged skin due to abrasions or cuts, potentially leading to higher permeation values. Under normal working conditions, it is highly unlikely for the skin to be exposed to sweat for 24 hours as the normal working shift is only 8 to 12 hours on average. Thus, an unintended partiality may be created for platinum permeation when considering the skin's hydration state when subjected to synthetic sweat for 24 hours versus the actual exposure conditions in the working environment. However, the *in vitro* methodology is internationally recognised and currently the best method available for simulating actual working conditions; therefore, it is a reliable method to investigate skin permeability of metals.

4.4 Recommendations for occupational settings

For implementing preventative strategies, engineering controls should be applied to prevent or control specifically dermal exposure to platinum. Dermal contact can occur through the contaminant settling out from air, through direct contact with the contaminant or through secondary transfer (contamination). Therefore, all these pathways must be considered when control measures are implemented. Entirely enclosed systems are ideal for hazardous operations, especially enclosing the source to prevent the platinum salt from becoming airborne. However, it is not always possible or practically feasible considering the type of hazardous operations conducted or type of source. An enclosed system aims at separating the worker from the hazard or may even reduce the number of workers exposed. If the control of a hazard is considered by means of extraction ventilation, then the particle size and transport velocity of the particles to be extracted should be taken into account. Regardless of the effectiveness an enclosed system may offer, some particles could possibly still escape, resulting in skin contamination. Thus special precautionary measures must be implemented to prevent or minimise dermal contact through either airborne contaminants settling out or direct contact. It should include keeping surfaces clean to avoid secondary skin contamination and providing adequate washing facilities, to which workers have access throughout the entire shift (Schneider *et al*., 1999).

When engineering controls are not possible or sufficient in reducing the exposure to platinum, administrative controls should be considered. This can include the training of workers on how to avoid skin contact and on the handling of contaminated PPE without contaminating themselves. The regular rotation of workers can be implemented so that no worker is exposed to platinum during evry shift, and also the installation of access control to limit the number of workers in that specific area. Further administrative controls can include written procedures for appropriate work practices, a scheduled maintenance and cleaning plan for the working area as well as the equipment being used. This can be helpful in preventing unnecessary exposure. Another important administrative control is good housekeeping, keeping the working area and equipment clean on a daily basis. Where practicable, replace manual labour (sweeping) with mechanised equipment (mechanised sweeping), to decrease worker involvement. The provision of adequate washing facilities with an alkaline-based soap will enable workers to promptly wash any exposed skin whilst regulating the skin surface pH, especially before lunch to minimise the possible ingestion of platinum, and just after the shift to prevent prolonged exposure.

The last control measure is providing appropriate PPE, which is capable of adequately controlling exposure to platinum, specifically dermal exposure. When selecting the appropriate PPE, the size, comfort thereof, ease of putting on or removing it and also mobility restriction must be considered. The PPE chosen to protect workers against platinum should include impermeable overalls and outer gloves, heavy duty nitrile gloves (3M Tekk Protection nitrile gloves, model no. 90011T), full or half masks (3M 6800 or similar with 3M 6057 cartridges) and these should also be worn continuously during the working shift. When selecting impermeable protective gloves, it should provide effective protection against platinum salts and should consider penetration, degradation and breakthrough. The disposable gloves should be worn underneath the impermeable protective gloves to further improve protection and prevent contamination during the removal of the outer gloves. Training regarding the PPE should be provided on the correct wearing of PPE as well as handling procedures when PPE is contaminated. Written procedures should be available regarding the safe cleaning and disposal of contaminated PPE. Supervision must be provided to ensure workers make use of the provided PPE and wear the PPE correctly. The behaviour of a worker can influence the potential exposure; therefore, it is necessary to promote principles of good personal hygiene (Schneider *et al*., 1999). Workers who understand how exposure and contamination can occur, would be more knowledgeable on how to implement the preventative measures and avoid skin contact.

4.5 Recommendations for future studies

[1]More studies should be conducted regarding the influence of pH on the *in vitro* permeation of metals, more specifically the PGMs (platinum, palladium, rhodium, iridium, ruthenium and osmium) as the available data is limited. The studies that have been completed to determine the ionisation of metals and its permeability at different pHs was not done concurrently and thus experimental differences are included (Larese Filon *et al*., 2007, 2008). The lesser-known PGMs (iridium, osmium, palladium and ruthenium) are not as widely used but it is still of great importance to determine what the hazards are to provide effective protection.

[2]Dissolution studies can be conducted to determine the release of platinum ions at different pH levels to either confirm or refute the probability of platinum to ionise in sweat at the skin interface. This would broaden the current knowledge available on the mechanism of platinum permeation through intact skin. It is essential for more experimental data on platinum products to gain information and a better understanding regarding the permeability of platinum as well as the optimal conditions for maximum permeation. This research can be done on various platinum salts. Other factors that can influence the permeability of the metals, such as the polarity and reactivity, should also be investigated.

[3]Future studies should not only aim at collecting new information and producing more experimental data, but also at minimising any uncertainties pertaining the conditions in which *in vitro* studies should be done, for example the pH that should be used regarding the synthetic sweat as well as the composition of the synthetic sweat. The skin surface pH ranges between 4.5 and 6, where in a working environment the pH value can be below 4.5, depending on the type of work, therefore a pH of 4.5 or below should be included in future *in vitro* studies (Hanson *et al*., 2002; Larese Filon *et al*., 2007, 2008).

[4]Future studies should consider the possibility of mixed exposure. With the mining of platinum, certain base metals such as copper, cobalt and nickel are produced as by-products (Bernardis *et al*., 2005; Mpinga *et al*., 2015). It is more common in the workplace for workers to have mixed exposure than to be exposed to a single substance, or in this case a metal. The possibility of synergistic or additive effects may influence the permeation. Future studies can include the combined exposure of a PGM and a base metal, such as platinum and nickel. These studies can be extended to two PGMs as well as two base metals and can also be done for both intact and damaged human skin.

[5]It is also recommended for future studies to aim at standardising solutions or use solutions that are standardised when considering the composition and pH of the different solutions as well as the storage thereof (e.g. temperature). This should be done to enable the comparison of data

and to make the data as realistic as possible. For example, the receptor solution must be similar to bodily fluids regarding its salt concentration, pH and temperature (Schmid-Wendtner and Korting, 2006; Stefaniak *et al*., 2013).

[6]Future studies should make use of human skin when investigating the *in vitro* permeation of metals to simulate real life conditions. In using full thickness skin, damage to the barrier function is limited, which is caused when the epidermis is removed from the dermis by separation and cutting methods. The thickness of the skin should however, not exceed 1 mm (Sartorelli *et al*., 2000). The use of abdominal human skin is also recommended due to the surface area being larger than the surface area of skin obtained from any other area, such as the breasts and due to anatomical differences, comparison between skin from different anatomical sites is impractical (Schmid-Wendtner and Korting, 2006). The same studies should be repeated on different racial groups due to potential inter-racial differences. Gaining knowledge of the permeation properties of contaminants through different skin types, will enable improved protection specific for each skin type.

4.6 References

Berardesca, E., Pirot, F., Singh, M., *et al.* 1998. Differences in stratum corneum pH gradient when comparing white and black African-American skin. Br. J. Dermatol. 139, 855-857.

Bernardis, F.L., Grant, R.A., Sherrington, D.C., 2005. A review of methods of separation of the platinum-group metals through their chloro-complexes. React. Funct. Polym. 65, 205-217.

Boscolo, P., Giampaolo, L.D., Reale, M., *et al*. 2004. Different effects of platinum, palladium and rhodium salts on lymphocyte proliferation and cytokine release. Ann. Clin. Lab. Sci. 34, 299-306.

Cawthorn, R.G., 1999. The platinum and palladium resources of the Bushveld complex. S. Afr. J. Sci. 95, 481-489.

Du Plessis, J.L., Eloff, F.C., Engelbrecht, S., *et al*. 2013. Dermal exposure and changes in skin barrier function of base metal refinery workers co-exposed to cobalt and nickel. Occup. Health. Southern. Africa. 1, 6-12.

Franken, A., Eloff, F.C., Du Plessis, J., *et al.* 2014. *In vitro* permeation of platinum and rhodium through Caucasian skin. Toxicol. Lett. 232, 1396-1401.

Franken, A., Eloff, F.C., Du Plessis, J., *et al.* 2015. *In vitro* permeation of platinum through African and Caucasian skin. Toxicol. *In. Vitro.* 28, 566-572.

Franken, A., Eloff, F.C., Du Plessis, J., *et al.* 2015. *In vitro* permeation of platinum through African and Caucasian skin. Toxicol. Lett. 232, 566-572.

Gómez, M.B., Gómez, M.M., Palacios, M.A., 2000. Control of interferences in the determination of Pt, Pd and Rh in airborne particulate matter by inductively coupled plasma mass spectrometry. Anal. Chim. Acta. 404, 285-294.

Hanson, K.M., Behne, M.J., Barry, N.P., *et al*. 2002. Two-photon fluorescence lifetime imaging of the stratum corneum pH gradient. Biophys. J. 83, 1682-1690.

Jansen van Rensburg, S.J., Franken, A., Du Plessis, J., *et al*. 2016. The influence of pH on the *in vitro* permeation of rhodium through human skin. Toxicol. Ind. Health. 1-8. DOI 10.1177/0748233716675218 (Available online)

Kezic, S., Nielson, J.B., 2009. Absorption of chemicals through compromised skin. Int. Arch. Occup. Environ. Health. 82, 677-688.

Larese Filon, F., Maina, G., Adami, G., *et al*. 2004. *In vitro* percutaneous absorption of cobalt. Int. Arch. Environ. Health. 7, 85-89.

Larese Filon, F., Gianpiero, A., Venier, M., *et al*. 2007. *In vitro* percutaneous absorption of metal compounds. Toxicol. Lett. 170, 49-56.

Larese Filon, F., D'Agostin, F., Crosera, M., *et al*. 2008. *In vitro* percutaneous absorption of chromium powder and the effect of skin cleanser. Toxicol. *In Vitro.* 22, 1562-1567.

Larese Filon, F., D'Agostin, F., Crosera, M., *et al*. 2009. Human skin penetration of silver nanoparticles through intact and damaged skin. Toxicol. 225, 33-37.

Larese Filon, F., Crosera, M., Adami, G., *et al*. 2011. Human skin penetration of gold nanoparticles through intact and damaged skin. Nanotoxicology. 5, 493-501.

Larese Filon, F., Crosera, M., Timeus, E., *et al*. 2012. Human skin penetration of cobalt nanoparticles through intact and damaged skin. Toxicol. *In Vitro.* 27, 121-127.

Maynard, A.D., Northage, C., Hemingway, M., *et al*. 1997. Measurement of short-term exposure to airborne soluble platinum in the platinum industry. Ann. Occup. Hyg. 41, 77-94.

Mpinga, C.N., Eksteen, J.J., Aldrich, C., *et al*. 2015. Direct leach approaches to Platinum Group Metal (PGM) ores and concentrates: A review. Miner. Eng. 78, 93-113.

Muizzuddin, N., Hellemans, L., Van Overloop, L., *et al*. 2010. Structural and functional differences in barrier properties of African American, Caucasian and East Asian skin. J. Dermatol. Sci. 59, 123-128.

Ravindra, K., Bencs, L., Van Grieken, R., 2004. Platinum group elements in the environment and their health risk. Sci. Total. Environ. 318, 1-43.

Sartorelli, P., Anderson, H.R., Angerer, J., *et al*. 2000. Percutaneous penetration studies for risk assessment. Environ. Toxicol. Pharmacol. 8, 133-152.

Sartorelli, P., Montomoli, L., Sisinni, A.G., 2012. Percutaneous penetration of metals and their effects on skin. Prev. Res. 2, 158-164.

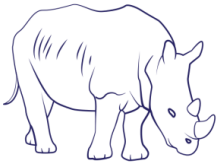
Schneider, T., Vermeulen, R., Brouwer, D. H., *et al*. 1999. Conceptual model for assessment of dermal exposure. Occup. Environ. Med. 56, 765-773.

Schmid-Wendtner, M.H., Korting, H.C., 2006. The pH of the skin surface and its impact on the barrier function. Skin. Pharmacol. Appl. 19, 296-302.

Stefaniak, A.B., Du Plessis, J., John, S.M., *et al.* 2013. International guidelines for the in vivo assessment of skin properties in non-clinical settings: part 1. pH. Skin. Res. Technol. 19, 59-68.

Tanojo, H., Hostýnek, J.J., Mountford, H.S., *et al*. 2001. *In vitro* permeation of nickel salts through human stratum corneum. Acta. Derm. Venereol. 21, 19-23.

Chapter 5 – Annexure



TECHLANG (Pty) Ltd

Text Editing / Translation

2012/032470/07

Certificate of Editing

This is to certify that the Mini-Dissertation:

The influence of pH on the in vitro permeation of platinum through human skin

by

Y van Nieuwenhuizen

has been edited by me for English language usage.

Prof SW Vorster
BA (Hons), MMet, MSc (Eng), PhD
Accredited Member No. 1000923
South African Translators' Institute

Cell 072 719 7025
schalk.vorster@nwu.ac.za

Date: 02 December 2016

NORTH-WEST UNIVERSITY
YUNIBESITI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT

Private Bag X6001, Potchefstroom
South Africa 2520

Tel: (018) 299-4900
Faks: (018) 299-4910
Web: http://www.nwu.ac.za

Institutional Research Ethics Regulatory Committee
Tel +27 18 299 4849
Email Ethics@nwu.ac.za

ETHICS APPROVAL CERTIFICATE OF PROJECT

Based on approval by **Health Research Ethics Committee (HREC)**, the North-West University Institutional Research Ethics Regulatory Committee (NWU-IRERC) hereby approves your project as indicated below. This implies that the NWU-IRERC grants its permission that, provided the special conditions specified below are met and pending any other authorisation that may be necessary, the project may be initiated, using the ethics number below.

Project title: ***In vitro*** **human skin permeation of platinum group metals.**

Project Leader: Dr A Franken

Ethics number:

N	W	U	-	0	0	2	0	2	-	1	5	-	A	1
Institution				Project Number						Year			Status	

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Approval date: 2016-02-01 **Expiry date**: 2017-01-31 **Risk** **Minimal**

Special conditions of the approval (if any): None

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following:

- *The project leader (principle investigator) must report in the prescribed format to the NWU-IRERC:*
  - *annually (or as otherwise requested) on the progress of the project,*
  - *without any delay in case of any adverse event (or any matter that interrupts sound ethical principles) during the course of the project.*
- *The approval applies strictly to the protocol as stipulated in the application form. Would any changes to the protocol be deemed necessary during the course of the project, the project leader must apply for approval of these changes at the NWU-RERC. Would there be deviated from the project protocol without the necessary approval of such changes, the ethics approval is immediately and automatically forfeited.*
- *The date of approval indicates the first date that the project may be started. Would the project have to continue after the expiry date, a new application must be made to the NWU-IRERC and new approval received before or on the expiry date.*
- *In the interest of ethical responsibility the NWU-IRERC retains the right to:*
  - *request access to any information or data at any time during the course or after completion of the project;*
  - *withdraw or postpone approval if:*
    - *any unethical principles or practices of the project are revealed or suspected,*
    - *it becomes apparent that any relevant information was withheld from the NWU-IRERC or that information has been false or misrepresented,*
    - *the required annual report and reporting of adverse events was not done timely and accurately,*
    - *new institutional rules, national legislation or international conventions deem it necessary.*

The IRERC would like to remain at your service as scientist and researcher, and wishes you well with your project. Please do not hesitate to contact the IRERC for any further enquiries or requests for assistance.

Yours sincerely

Prof LA Du Plessis

Digitally signed by Prof LA Du Plessis
DN: cn=Prof LA Du Plessis, o=North-West University, ou=Campus Rector, email=Linda.DuPlessis@nwu.ac.za, c=ZA
Date: 2016.02.02 08:16:25 +02'00'

Prof Linda du Plessis
Chair NWU Institutional Research Ethics Regulatory Committee (IRERC)

NORTH-WEST UNIVERSITY
YUNIBESITI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT

Private Bag X6001, Potchefstroom,
South Africa, 2520

Tel: (018) 299-4900
Faks: (018) 299-4910
Web: http://www.nwu.ac.za

Institutional Research Ethics Regulatory Committee

Tel: +27 18 299 4849

Email : Ethics@nwu.ac.za

ETHICS APPROVAL CERTIFICATE OF PROJECT

Based on approval on 04/06/2016 by **Health Research Ethics Committee (HREC)** after being reviewed at a meeting held on **10/03/2016**, the North-West University Institutional Research Ethics Regulatory Committee (NWU-IRERC) hereby **approves** your project as indicated below. This implies that the NWU-IRERC grants its permission that, provided the special conditions specified below are met and pending any other authorisation that may be necessary, the project may be initiated, using the ethics number below.

Project title: *In vitro* human skin permeation of platinum group metals

Sub-study title: The influence of pH on the in vitro permeation of platinum through human skin

Project Leader/Supervisor: Prof A Franken
Student: Y van Nieuwenhuizen

Ethics number:

N	W	U	-	0	0	2	0	2	-	1	5	-	A	1
Institution				Project Number						Year			Status	

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Application Type: Sub-study

Commencement date: 04/06/2016 **Risk:** **Minimal**

Special conditions of the approval (if applicable):

- Translation of the informed consent document to the languages applicable to the study participants should be submitted to the HREC (if applicable).
- Any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the HREC. Ethics approval is required BEFORE approval can be obtained from these authorities.

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following:

- *The project leader (principle investigator) must report in the prescribed format to the NWU-IRERC via HREC:*
  - *annually (or as otherwise requested) on the progress of the project, and upon completion of the project*
  - *without any delay in case of any adverse event (or any matter that interrupts sound ethical principles) during the course of the project.*
  - *Annually a number of projects may be randomly selected for an external audit.*
- *The approval applies strictly to the protocol as stipulated in the application form. Would any changes to the protocol be deemed necessary during the course of the project, the project leader must apply for approval of these changes at the HREC. Would there be deviated from the project protocol without the necessary approval of such changes, the ethics approval is immediately and automatically forfeited.*
- *The date of approval indicates the first date that the project may be started. Would the project have to continue after the expiry date, a new application must be made to the NWU-IRERC via HREC and new approval received before or on the expiry date.*
- *In the interest of ethical responsibility the NWU-IRERC and HREC retains the right to:*
  - *request access to any information or data at any time during the course or after completion of the project;*
  - *to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process.*
  - *withdraw or postpone approval if:*
    - *any unethical principles or practices of the project are revealed or suspected,*
    - *it becomes apparent that any relevant information was withheld from the HREC or that information has been false or misrepresented,*
    - *the required annual report and reporting of adverse events was not done timely and accurately,*
    - *new institutional rules, national legislation or international conventions deem it necessary.*
- *HREC can be contacted for any report templates Ethics-Monitoring@nwu.ac.za or further assistance via Ethics-HRECApply@nwu.ac.za; 018 299 1206.*

The IRERC would like to remain at your service as scientist and researcher, and wishes you well with your project. Please do not hesitate to contact the IRERC or HREC for any further enquiries or requests for assistance.

Yours sincerely

Linda du Plessis

Digitally signed by Linda du Plessis
DN: cn=Linda du Plessis, o=NWU, ou=Vaal Triangle Campus, email=linda.duplessis@nwu.ac.za, c=ZA
Date: 2016.06.12 16:20:09 +02'00'

Prof Linda du Plessis

Chair NWU Institutional Research Ethics Regulatory Committee (IRERC)