TOTAL AND BIOAVAILABLE FLUORIDE CONCENTRATIONS IN COMMERCIALLY AVAILABLE TOOTHPASTES IN SOUTH AFRICA

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ABSTRACT

High caries burden coupled with a lack of water and salt fluoridation make it imperative that toothpastes marketed to the South African consumer demonstrate adequate caries inhibition. It is generally accepted that an effective toothpaste contain between 1000ppm and 1500ppm total fluoride (TF) and that of this at least 1000ppm F should be in free available form. Studies conducted in other countries have demonstrated that toothpastes often do not conform to this regulation, indicating the need for quality assessments to be undertaken. **Objectives:** This study therefore aimed to determine total and free available (potentially bioavailable) fluoride concentrations in 28 adult, fluoridated toothpastes marketed to the South Africa consumer. Labeling practices were also assessed. **Materials & Methods:** Convenience samples were purchased from a major pharmaceutical and food retailer located in each of the five metropolitan areas of South Africa. Information detailed on the packaging was evaluated for compliance with national standards. Total and bioavailable fluoride concentrations were determined potentiometrically, in quadruplicate, following acid hydrolysis of the samples using a Combination Fluoride Ion Selective Electrode, calibrated with standards containing 0.0625ppm F to 6.25ppm F. **Results:** Although TF content on analysis was found to be statistically significantly lower than manufacturer declaration ($1.2x10^{-7;}$ $p \leq 0.05$), 78.6% still contained adequate free, available F levels. Relative mean available fluoride content for toothpastes formulated with a calcium-based abrasive was 85,5% as opposed to 98.7% for those containing silica. Partial alignment with national labeling statutes was observed for all toothpastes. **Conclusion:** Most commercially available toothpastes are adequately formulated to provide anti-cariogenic activity, but consumers should be advised against the use of products containing calcium. Improvements to national standards and stricter regulation of labeling practices are required.

KEYWORDS: Toothpaste**,** Total Fluoride**,** Available Fluoride**,** Soluble Fluoride**,** Labeling Practices

DECLARATION

I declare that *Total And Bioavailable Fluoride Concentrations In Commercially Available Toothpastes In South Africa* is my own work, that it has not been submitted before for any degree or examination in any other university, and that all the sources that I have used or quoted have been indicated and acknowledged as complete references.

Lesley Ross Vorster October 2015

Signed: 

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TABLE OF CONTENTS

		Page
Title Page		i
Abstract		ii
Key Words		ii
Declaration		iii
Acknowledgements		iv
List of Tables		vii
List of figures		viii
CHAPTER 1	**Introduction**	1
CHAPTER 2	**Literature Review**	5
	2.1 Introduction	5
	2.2 Toothpaste Formulation	6
	2.3 Cariostatic Mechanism of Action of Fluoride	7
	2.4 An Effective Fluoride Toothpaste	13
	2.5 Factors Reducing Toothpaste Fluoride Availability	14
	2.6 Findings of Recent Toothpaste Quality Assurance Studies	16
	2.7 Basic Principles of ISE	19
	2.8 Conclusion	22
CHAPTER 3	**Aim & Objectives**	23
	3.1 Aim & Objectives	23
	3.2 Research Hypothesis	23
CHAPTER 4	**Methodology**	24
	4.1 Fluoride Toothpaste Samples	24
	4.2 Section A: Fluoride Measurements	26
	4.2.1 Fluoride Definitions	26
	4.2.2 Determination of [TF] of sample	28
	4.2.3 Determination of [FI] of sample	34
	4.2.4 Determination of [TSF] of sample	37

	4.2.5 Determination of [IF] of sample	39
	4.2.6 Data Analysis	39
	4.2.7 Validity of Results	40
	4.3 Section B: Labeling Practices	44
	4.4 Ethical Considerations	44
	4.5 Declaration of Interest	44
CHAPTER 5	**Results**	45
	5.1 Section A: Fluoride Analyses	45
	5.2 Section B: Labeling Practices	58
CHAPTER 6	**Discussion**	77
	6.1 Section A: Fluoride Analyses	77
	6.2 Section B: Labeling Practices	85
CHAPTER 7	**Conclusions & Future Recommendations**	96
BIBLIOGRAPHY		99
APPENDICES	Appendix I: Fluoridated Toothpaste Samples – Gold Standard List	111
	Appendix II: Raw Data Collection Sheet: Labeling Practices – Gold Standard Sampling List	112

LIST OF TABLES

		Page
Table 1:	Population Count and Density for the 8 Metropolitan Municipalities of South Africa	24
Table 2:	Key to Definitions and Abbreviations for Fluoride Concentrations Analysed	27
Table 3:	Preparation of Fluoride Standards	31
Table 4:	Total and Bioavailable fluoride concentrations for toothpastes purchased in Cape Town and containing hydrated Silica ($SiO2.nH_2O$) as the abrasive	48
Table 5:	Total and Bioavailable fluoride concentrations for toothpastes purchased in Johannesburg and containing hydrated Silica ($SiO2.nH_2O$) as the abrasive	48
Table 6:	Total and Bioavailable fluoride concentrations for toothpastes purchased in Cape Town and containing a calcium-based abrasive	49
Table 7:	Total and Bioavailable fluoride concentrations for toothpastes purchased in Johannesburg and containing a calcium-based abrasive	49
Table 8:	Difference between [TF] and [TSF] for Initial (elevated pH) and Re-Test (pH5-5.5) Analyses	56
Table 9:	Sample Size - Toothpaste Type And Number Per Site	62
Table 10:	Total Sample Compliance With Sans Packaging and Marking Guidelines	63
Table 11:	Compliance With Sans Marking Guidelines Per Product	66
Table 12:	Compliance With ISO 11609:2010 Marking Guidelines	74

LIST OF FIGURES

		Page
Figure 1:	a. Jenway Combination Fluoride Ion Selective Electrode (924-305). b. Immersed in analyte solution and connected to a mV meter (Orion EA-740).	20
Figure 2:	Example Standard Calibration Curve constructed using Microsoft Excel	32
Figure 3:	Schematic Overview - Potentiometric Determination of Fluoride in Aqueous Solutions of Fluoridated dentifrices using Ion Selective Electrode	43
Figure 4:	Cluster bar graph depicting concentration (ppm F) declared by manufacturer and that found on analyses.	50
Figure 5:	Concentration (ppm F) of total and total soluble fluoride found on analyses for toothpastes formulated with hydrated silica as the abrasive.	51
Figure 6:	Concentration (ppm F) of total and total soluble fluoride on analyses for toothpastes Formulated with a calcium-based abrasive.	52
Figure 7:	Adhesive label evident on outer carton	69
Figure 8:	Two different toothpastes having the same distributor and bearing same batch identification	69
Figure 9:	Two different toothpastes having the same distributor, purchased from different retailers in Cape Town, bearing the same batch identification	69
Figure 10:	Samples of Colgate Active Salt	75
Figure 11:	Expiry and Manufacture Dates	75

CHAPTER 1: INTRODUCTION

"Universal access to fluoride for dental health is a part of the basic human right to health."

(WHO, 2006)

Despite extensive knowledge regarding aetiology, prevention and treatment, the caries pandemic persists and continues to impact negatively on population health and well-being through its sequalae of pain, chronic infection, food avoidance, work and school absenteeism, loss of income, physical and learning difficulties (Thema and Singh, 2013:1). According to a 2010 report on the, 'Global Burden of Disease Study', 3.9 billion people suffered from oral conditions. Of the 291 systemic and oral conditions evaluated, untreated caries of the permanent and deciduous dentition ranked as the first and 10th most common condition affecting the global community, having a prevalence of 35% and 9% respectively (Marcenes *et al.*, 2013:593). Epidemiological data presented by the World Health Organization, however, demonstrated a favourable decline in caries incidence and prevalence within industrialized nations over the past 20 years, attributable to public health measures and improved accessibility to preventive oral health systems, including widespread exposure to fluoridated dentifrices. The converse was true of the developing nations (Petersen, 2005:662).

Currently, within the South African context no recent, reliable caries-related epidemiological information is available with respect to adult population subsets as the last national oral health survey undertaken focused predominantly on children (Singh, 2011:259). The most recent of these, 'The Children's Oral Health Survey', conducted between 1999 and 2002 found that 45% to 60% of children needed restorative care, with the mean number of teeth requiring treatment per child ranging between 2 and 3.5. Application of the Unmet Treatment Need Index demonstrated that 80% of childhood caries remained untreated (van Wyk and van Wyk, 2004:373).

The global burden posed by oral conditions may be expressed empirically in terms of disability adjusted life years (DALY), which is the sum of years of life lost (YLL) due to premature mortality and years lived with disability (YLD) (Murray *et al.*, 2002 cited in Marcenes *et al.*, 2013:592). According to the 2010 global burden of untreated caries, severe periodontitis and tooth loss report, untreated caries was found to be the second most leading cause of DALYs in 7 regions, Southern Sub-Saharan Africa recognized as one of these regions (Marcenes *et al.*, 2013:594). This, combined with the fact that South Africa is categorized as a developing, newly industrialized country points to high and increasing caries burden among the population.

The World Health Organization (WHO, 1994:1) and a group of experts that convened in 2006 for the 'Global Consultation on Oral Health through Fluoride' concluded that exposure to appropriate amounts of fluoride is the most effective and pragmatic caries-inhibitory measure (Benzian *et al.*, 2012:213). Multiple clinical trials have repeatedly validated the anti-caries efficacy of fluoridated dentifrices (Stookey, 1990 cited in Zero, 2006:2; WHO, FDI, IADR, Chinese Stomatological Association, 2011:14). As a result the noteworthy decline in caries prevalence within developed nations is primarily attributed to the use of these products (Hargreaves *et al.*, 1983 and Jenkins, 1985 cited in Zero, 2006:2). This evidence supported outcomes of a joint meeting between the WHO, FDI World Dental Federation and International Association for Dental Research (IADR), which stated that Fluoridated dentifrices are the most widely accessible form of fluoride internationally, demonstrate anti-cariogenic properties and are safe despite exposure to fluoride from alternate sources (Benzian *et al.*, 2012:213). Increased accessibility to fluoridated toothpastes, therefore, may be considered a viable means of reducing caries burden in populations.

Within South Africa, fluoridated toothpastes are the only realistic caries control measure since the less expensive methods of water and salt fluoridation are not feasible due to inadequate infrastructure and restricted financial and technological resources (Goldman *et al.*, 2008:2). It is therefore imperative that fluoridated dentifrices marketed to the South African consumer are effective.

A systematic review of the literature has demonstrated a statistically significant positive relationship between caries-reducing benefits and fluoride content in

toothpastes having total fluoride concentrations (sum of soluble/bioavailable and insoluble fluoride) in excess of 1100ppm F (Walsh *et al.*, 2010:2). Typically three fluoride species are encountered in the fluoridated toothpastes, namely, total, soluble and insoluble fluoride. Total fluoride refers to all fluoride that is present in the formulation, while soluble fluoride refers to free ionic fluoride (F^-) and/or ionizable fluoride (PO_3F^{2-}) as present in monofluorophosphate-containing products having the potential to interact with enamel at the tooth surface interface and so disrupt the caries process, while insoluble fluoride is that fraction bound to other components of the formulation, typically calcium (CaF_2), to form an insoluble compound incapable of exerting caries inhibitory activity (Cury, 2015) (Table 2). A truly ideal formulation of a toothpaste may be regarded as one in which total fluoride concentration equates to soluble/bioavailable fluoride content. According to van Loveren *et al.* (2005:225), however, a minimum concentration of 1000ppm free ionic and/or ionizable fluoride (soluble/available) needs to dissociate during brushing and prior to expectoration in order for enamel re-mineralizing properties to be realized. Frequently chemical interaction between toothpaste constituents leads to the formation of insoluble and therefore caries inactive fluoride compounds, indicating that chemical compatibility between toothpaste constituents is of paramount importance to efficacy (Cury *et al.*, 2010:396). Similarly, time and temperature influence the caries inhibitory activity exerted. Fluoridated dentifrices have been found to loose approximately 25% of free available fluoride after one year when stored at 22^oC and 35% after the same time period when stored at 29^oC. For this reason toothpastes, especially commercially available brands are considered to have a 3-year shelf life (Benzian *et al.*, 2012:219).

From this discussion around efficacy it is evident that it is pertinent to evaluate toothpastes not only in terms of fluoride concentration, but also with respect to the provision of consumer information through appropriate labeling practices. This need for quality assurance analyses is further supported by the findings of recent studies conducted within non-established market economy countries that uncovered discrepancies between actual fluoride concentration obtained on laboratory analysis and that declared on the packaging (Kikwilu *et al.*, 2008:294; Van Loveren *et al.*, 2003:229) as well as the existence of counterfeit products (Colgate Palmolive, 2007). Historically analytical methods used in quantification of fluoride content in samples have included spectrophotometry, gas chromatography, ion chromatography, capillary

electrophoresis, atomic absorption and photon activation (Liteplo *et al.*, 2002:18). High costs associated with these analytical techniques, however, limit accessibility in resource challenged contexts, such as, South Africa. Consequently a shift towards an inexpensive, time-efficient potentiometric technique has been observed (Adejumo *et al.*, 2009:47).

Recent studies have shown this method to be suitably sensitive in fluoride ion detection (Benzian *et al.*, 2012: 215; Cury *et al.*, 2010:397; Kikwilu *et al.*, 2008: 295; Van Loveren *et al.*, 2005:226). In consideration of these findings as well as the lack of water and salt fluoridation and high caries burden experienced within South Africa, toothpaste quality control studies, using the cost-effective, reliable ion selective electrode method, can be regarded as a necessity.

The present study therefore aimed to determine total and bioavailable fluoride concentrations in 28 adult, commercially available fluoridated toothpastes in South Africa using the fluoride ion-selective electrode method (ISE). Toothpastes incorporated in the study were conveniently purchased from a major pharmaceutical and food retailer located in the Cape Town and Johannesburg metropolitan areas. Test samples for fluoride analyses were prepared in quadruplicate using direct acid hydrolysis according to a protocol described by Cury *et al.* (2010:397).

Another issue relevant to the quality of fluoridated toothpastes concerns the provision of consumer information through correct labeling. This study therefore also evaluated the quality of consumer information declared on the packaging regarding fluoride and abrasive type, expiry or manufacture dates and lot or batch numbers.

Labeling practices and fluoride content measurements obtained on analysis were then assessed for conformity with regulations stipulated in 1302, edition 1.1, of the South African National Standards documentation (SABS, 2008).

Chemical formulation of the fluoridated dentifrices, mechanism of action of fluoride on the mineralized dental tissues, discussion of the ion selective electrode theory as well as a critical review and interpretation of related studies are discussed extensively in the literature review chapter.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

The fluoridated toothpastes are undisputedly one of the most significant public health measures in the fight against dental caries available today (König, 1993 cited in Hashizume *et al.*, 2003:198). Overwhelming evidence dating from as far back as the 1970's has repeatedly demonstrated the anti-caries efficacy of these products with the prominent decline in caries prevalence within the developed countries ascribed to the widespread use of fluoridated toothpastes (Bratthall *et al.*, 1996: 416; Hargreaves, 1983 cited in Zero, 2006; Jenkins, 1985:1298). Although the developing countries have not enjoyed similar declines in caries prevalence and incidence (Petersen, 2005:662), recent research has commented on dramatic improvements in the distribution and use of fluoridated toothpastes in non-established market economy countries and similar epidemiological trends are therefore anticipated (van Loveren *et al.*, 2005:225). In fact Cury *et al.* (2004:171) found that the decline in caries prevalence in the developing country of Brazil may be attributed to the large-scale introduction of fluoridated toothpastes after 1990. Perhaps the best evidence in support of the anti-caries efficacy of the fluoridated toothpastes emanates from a meta-analysis of 70 clinical trials undertaken by the Cochrane Collaboration for Oral Health that reported a 23% reduction in the DMFT (decayed, missing and filled teeth) 3-year increment of the permanent dentition, independent of background water fluoridation, among children and adolescents on exposure to fluoridated toothpastes on a regular basis (Marinho *et al.*, 2004). The caries inhibitory effect was also found to be both dose-related and time-dependent, improving with increased fluoride concentrations, brushing frequency and supervised brushing, especially evident among those presenting with a high caries index.

2.2 Toothpaste Formulation

Fluoridated toothpastes are typically formulated with water, an active fluoride compound, abrasives, binding agents, humectants, surfactants, colourants, preservatives, flavourants and sweeteners (Zero, 2006). Recently, in addition to fluoride other active agents indicated for the management of hypersensitivity, plaque, gingivitis, calculus and/or oral malodour are being incorporated into the toothpaste formulation (ADA, 2005:7). Despite the fact that these newer toothpastes advertise multiple health benefit claims in labeling of the product, according to Zero (2006) the only claim associated with a long-term health benefit is the caries inhibitory activity provided by fluoride.

In South Africa Fluoride is added to adult toothpastes in a maximum 0.15% concentration (University of Pretoria and South African Legal Information Institute, 2014:11) as sodium fluoride (NaF), sodium monofluorophosphate (Na_2PO_3F), stannous Fluoride (SnF_2), amine fluoride (NH_4F) or a combination of these compounds. Irrespective of chemical form used, various fluoride species are present in toothpastes. These include total fluoride (TF), insoluble/inactive fluoride (IF) and total soluble fluoride (TSF) in ionic free form (F^-) for the NaF, SnF_2 and NH_4F-containing products or ionizable form (PO_3F^{2-}) for those formulated with Na_2PO_3F (Cury *et al.*, 2010:397). The caries inhibitory properties of the fluoridated toothpastes are reliant on the total soluble, also termed potentially available fluoride content (i.e. fraction of total fluoride that is chemically soluble in the formulation). During use however, it is quite probable that only a fraction of the potentially available fluoride, referred to as the potentially bioavailable fluoride, reaches the enamel/oral fluid interface to allow for disruption of the caries process and is absorbed through the gastro-intestinal tract (Cury, 2015). An ideal formulation is accepted as one in which fluoride bioavailability is equivalent to fluoride availability/TSF content which, in turn, is equivalent to the TF concentration (i.e. all F incorporated into the formulation is available in active, soluble form).

2.3 Cariostatic Mechanism of Action of Fluoride

Historically, the anticariogenic activity of fluoride was attributed to the incorporation of fluoride into the crystalline lattice of the dental hard tissues during odontogenesis, producing a tissue with decreased solubility in an acidic environment (Rošin-Grget *et al.*, 2001:703; ten Cate, 1999:325). Contrary to this, current evidence indicates that the primary caries preventive mechanisms of action of fluoride are post eruptive through topical effects and include the inhibition of demineralization, promotion of remineralization and disruption of metabolic enzyme activity of plaque bacteria (Featherstone, 1999:32; Goldstep, 2012; Lussi *et al.*, 2012:1030).

In order to fully appreciate the mechanism of action of fluoride on the mineralized dental tissues an understanding of the nature of tooth mineral and processes taking place during acidogenic challenge are required.

2.3.1 Nature of tooth mineral

The dental hard tissues are primarily inorganic, with enamel, dentine and cementum consisting of 97%, 70% and 65% hydroxyapatite (HAP) by weight respectively (Jansen van Rensburg, 1995:289). The physico-chemical properties of an apatite are such that iso-ionic substitutions can occur within the crystalline lattice without loss of essential identity (Ten Cate and Featherstone, 1991:284; Jansen van Rensburg, 1995:487). The dental hard tissues, however, do not consist of pure HAP $\{Ca_{10}(PO_4)_6(OH)_2\}$ but rather a highly substituted carbonated/impure hydroxyapatite $\{Ca_{10-x}(Na)_x(PO4)_{6-y}(CO3)_Z(OH)_{2-u}(F)_u\}$ (Featherstone, 1999:33). This carbonated hydroxyapatite is Ca^{2+} deficient due to substitution by other ions such as, Na^+, Mg^+, Zn^+, consists of 3% to 6% CO_3^{2-} by weight and has a greater susceptibility to acid dissolution than pure HAP (LeGeros *et al.*, 1969:5). CO_3^{2-} typically occupies phosphate (PO_4^{3-}) positions within the lattice and in so doing creates disturbances in the regular array of ions in the crystal lattice (Featherstone, 1999:33). These substitutions are found to take place during odontogenesis.

On the other hand F^- confers a certain caries resistance on the dental hard tissue by reducing the relative amounts of carbonated apatite (Rošin-Grget and Linčir, 2001:704). The partial substitution of fluoride ions (F^-) for hydroxyl (OH^-) groups in hydroxyapatite results in molecular contracture and a more regular and stable apatitic structure that effectively reduces the surface area exposed to the action of acids and so increases resistance to acid dissolution (Rošin-Grget and Linčir, 2001:704). If all OH^- groups present in pure hydroxyapatite are replaced by F^-, the resultant mineral is Fluorapatite (FAP) {$Ca_{10}(PO_4)6F_2$} (Featherstone, 1999:33). Pure FAP (i.e. complete substitution) never occurs. Approximately only 10% of OH^- groups are replaced by F^- in surface enamel producing a hydroxyapatite-fluorapatite (FHAP) blend (Rošin-Grget and Linčir, 2001:704). This percentage drops even further at a depth of 50μm (Lussi *et al.*, 2012:1030). The FHAP present, however, displays "fluorapatite-like" behavior conferring an increased resistance to acid dissolution on the dental hard tissues.

2.3.2 Acidogenic Challenge

Dental caries may be defined as the most common human affliction characterized by demineralization, cavitation and destruction of the dental hard tissues in response to microbial activity (Heasman and McCracken, 2007:42). In fact it is the result of an imbalance between two independent processes, namely, sub-surface demineralization of enamel following bacterial acid production and remineralization, the natural repair of the acid induced enamel lesions by saliva and plaque fluid occurring at pHs approaching neutral, with demineralization being favoured (ten Cate and Featherstone 1991:284). These two processes can be described in terms of the solubility isotherms of HAP. At a neutral intra-oral pH the plaque fluid (i.e. the fluid in the immediate vicinity of the carbonated HAP of enamel or exposed dentine and cementum) is found to be supersaturated with respect to the carbonated HAP (i.e. the concentrations of Ca^{2+}, PO_4^{3-}, OH^- and F^- are greater than that encountered in, for example, surface enamel), creating a driving force for mineral deposition into or remineralization of tooth structure (Featherstone, 1999:34). On cariogenic attack, however, the organic acids produced by acidogenic bacteria on exposure to a carbohydrate-rich substrate dissociate increasing the H+ ion concentration within the plaque fluid, which, in turn, reduces the OH^- ion content thereof.

In addition the H^+ ions are found to protonate phosphates within the plaque fluid to form either HPO_4^{2-} or more commonly H_2PO4^-. As the PO_4^{3-} content of the plaque fluid declines at low pHs, PO_4^{3-} ions and later OH^- groups dissolve out of tooth structure in an attempt to establish an equilibrium in ion concentrations at the interface between plaque fluid and the dental hard tissue. This process eventually results in a leaching of Ca^{2+} from the dental hard tissue as well and a process of demineralization occurs (Lussi *et al.*, 2012:1031). Simply stated, as the pH of the plaque fluid declines below the critical pH, for HAP identified as 5,5, the point at which Ca^{2+}, $PO4^{3-}$, OH^- and F^- ion concentrations between the plaque fluid and surface enamel are in equilibrium, the plaque fluid becomes under-saturated with respect to HAP and so shifts the driving force within enamel to mineral dissolution/demineralization (Goldstep, 2012). It follows therefore that as the critical pH of FHAP is defined as 4.7, presence of FHAP in surface enamel will retard the dissolution process occurring at the enamel/plaque fluid interface (Lussi *et al.*, 2012:1032).

2.3.3 Inhibition of Demineralization

As was stated in the introductory comments, however, FHAP incorporated into the apatitic structure of the dental hard tissues confers only a minor caries preventive effect. The major cariostatic mechanism of F is attributed to F in solution surrounding the tooth (Featherstone, 199:31; Lussi *et al.*, 2012:1031; Rošin-Grget and Linčir, 2001:703; ten Cate and Featherstone, 1991:283) Brushing with a fluoridated toothpaste introduces ionic and/or ionizable fluoride (PO_3F^{2-}), depending on formulation used, into saliva and the plaque fluid. Ionizable fluoride, if present, then undergoes a process of acid hydrolysis within the oral cavity making ionic F^- available. If during cariogenic challenge, ionic F^- is present in the plaque fluid, this F^- penetrates together with the bacterial acids into the sub-surface of the tooth, adsorbs strongly to the carbonated HAP and protects it against further acid dissolution (Featherstone, 1999:34). This adsorbtion not only provides a direct barrier to dissolution, but also establishes a dynamic equilibrium with the F^- content of the plaque fluid. Eventually the fluid in the immediate vicinity of the crystals may reach supersaturation with respect to FHAP and consequently mineral reprecipitation into tooth structure will be observed (Lussi *et al.*, 2012:1032).

2.3.4 Calcium Fluoride

In the presence of calcium (Ca^{2+}) and phosphate (PO_4^{3-}) ions as is typically encountered in saliva and plaque fluid, ionic fluoride interacts with enamel to produce either "firmly bound" (fluorapatite) or "loosely bound" (calcium fluoride) fluoride (Rošin-Grget and Linčir, 2001:705). Formation of fluorapatite or calcium fluoride (CaF_2) is dependent on the solubility isotherms, namely, environmental pH and concentration of each ionic species (Ten Cate and Featherstone, 1991:284).

Chronic exposure to low fluoride concentrations, between 0.01 and 10ppm F, form systemic or latent topical sources leads to the formation and growth of FAP crystals ("firmly-bound" F) according to the following chemical equations (Rošin-Grget and Linčir, 2001:705):

Iso-ionic exchange of F^- for OH^- in hydroxyapatite:

$$Ca_{10}(PO_4)_6(OH)_2 + 2F^- \rightarrow Ca_{10}(PO_4)_6F_2 + 2OH-$$

Crystal growth from supersaturated solutions:

$$10Ca^{2+} + 6PO_4^{3-} + 2F- \rightarrow Ca_{10}(PO_4)_6F_2$$

At a low intra-oral pH and at increasing fluoride concentrations, ranging between 100 and 10000ppm F, occurring, for example, on exposure to a fluoridated toothpaste an additional chemical reaction leading to the formation of CaF_2 ("loosely-bound" F) becomes evident (Rošin-Grget and Linčir, 2001:705):

Hydroxyapatite dissolution with CaF_2 formation:

$$Ca_{10}(PO_4)_6(OH)_2 + 20F^- \rightarrow 10CaF_2 + 6PO_4^{3-} + 2OH^-$$

CaF_2 is regarded as the essential component in caries inhibition (Lussi *et al.*, 2012:1032). It has been found that the greater the F concentration of the surrounding solution, the lower the pH and the longer the contact time, the greater amounts of CaF_2 are formed (Saxegaard and Rölla, 1988:534). During cariogenic challenge (i.e. low pH) enamel solubility increases. Enamel dissolution, in turn, releases Ca^{2+} from the crystal lattice making it available for substantial CaF_2 production in the presence of F^- ions.

The CaF_2 precipitates on sound and demineralized enamel (incipient lesions), within plaque and stagnation areas such as aproximal surfaces. Within plaque, incipient lesions and stagnation sites Ca^{2+} concentrations are high in response to high concentrations of bacterial acids and the resultant enamel dissolution.

As saliva is under-saturated with respect to CaF_2, however, the precipitated CaF_2 dissociates/dissolves gradually over time leading to a caries-prophylactically relevant increase in F concentration of plaque fluid and saliva (Lussi *et al.*, 2012:1032). CaF_2, therefore, only acts as a temporary F reservoir and it is for this reason that regular twice daily brushing is recommended in order to constantly replenish this reservoir. CaF_2 does however resist dissolution to a certain extent at a neutral pH (Rölla and Ekstrand, 1996 cited in Lussi *et al.*, 2012:1032). This resistance has been ascribed to the interaction of CaF_2 with phosphate or proteins contained within the acquired pellicle (Rošin-Grget and Linčir, 2001:708). This phosphate-induced inhibition of CaF_2 dissolution is pH dependent and decreases below pH 5 which is of significance in the caries process (Lagerlöf *et al.*, 1988:447). During acidogenic challenge, therefore, as pH declines below a value of 5, CaF_2 dissociates rapidly making large numbers of F^- ions available. These F^- ions have the potential to inhibit demineralization (through adsorbtion to the crystalline lattice) and promote remineralization (through supersaturation of the plaque fluid). In fact CaF_2 is considered the primary source of F^- during acidic challenge (Lussi *et al.*, 2012:1032).

2.3.5 Promoting Remineralization

Following acidogenic challenge, as pH returns to neutral in response to salivary buffering, enamel sub-surface dissolution decreases. With increasing pH the oral fluid becomes supersaturated with Ca^{2+} and PO_4^{3-} providing a driving force for reprecipitation of mineral into tooth structure (Featherstone, 1999:34). As the critical pH of FHAP is 4.7, lower than that for HAP at 5.5, supersaturation of the oral fluid with respect to FHAP will occur first as long as F^- is present in the oral fluid. Consequently during remineralization a redistribution of mineral phases takes place with FHAP content increasing preferentially over carbonated HAP (Lussi *et al.*, 2012:1032).

According to ten Cate and Featherstone (1991:287) partially demineralized hydroxyapatite crystals act as a nidus for remineralization, while F- present in the oral fluid accelerates this remineralization process, by adsorbing to the demineralized crystal surface and attracting Ca^{2+}. This, in turn, functions to bring Ca^{2+} and PO_4^{3-} together with F^- preferentially incorporated into the chemical reaction that then takes place, while CO_3^{2-} is excluded. Consequently a blended FHAP enamel surface having a lower solubility in contrast to initial carbonated hydroxyapatite is produced (Featherstone, 1999:35). In other words, any future acidogenic challenge will need to be of a long duration and extremely strong before remineralized enamel dissolves, demonstrating how F^- not only **promotes remineralization, but also inhibits demineralization.**

2.3.6 Antimicrobial activity

Ionic F^- does not have the ability to cross the bacterial cell wall. Under acidic conditions however it combines with H^+ to form HF, which readily diffuses across the bacterial cell wall. Once within the bacterium, HF dissociates (Goldstep, 2012). The H^+ ions lead to an over-acidification of the cytoplasm, which inhibits glucose transport within the bacterium, while the F^- ions disrupt enolase and proton-releasing adenosine triphosphatase enzymatic activity (Sutton *et al.*, 1987:2602).

Another antimicrobial mechanism of action proposed was the prevention of bacterial colonization of the tooth surface following a fluoride pre-treatment, for example, brushing with a fluoridated toothpaste (Lussi *et al.*, 2012:1033). Many studies have since disputed this theory, but there is some evidence that the cations of F^- compounds, in particular, stannous and amine fluoride do interfere with bacterial colonization (van der Mei *et al.*, 2008:19).

Ten Cate and van Loveren (1999) cited in Lussi *et al.* (2012:1034) found that chronic exposure of bacterial plaque to F^- lead to a decrease in acidogenicity. These antimicrobial effects, however, are only evident at extremely high F concentrations, far in excess of those encountered in the oral cavity. Consequently the cariostatic effects of inhibition of demineralization and promotion of remineralization are of greater significance from a clinical perspective (ten Cate, 1999:327).

2.4 An Effective Fluoridated Toothpaste

It is universally accepted among the dental and scientific disciplines that exposure to an optimum level of fluoride confers caries protective benefits upon the dentition (Benzian *et al.*, 2012: 213; Cury *et al.*, 2010:396; van Loveren *et al.*, 2005: 224; Marinho *et al.*, cited in Zero, 2006). As for most substances, fluoride ingestion in excess of optimum, however, may be detrimental to systemic health. Numerous studies have implicated fluoride in the aetiology of diverse pathologies, including, dental and skeletal fluorosis (Tokalioglu *et al.*, 2004:204), osteoarthritis, impaired cognitive development in children due to neurotoxicity (Varner *et al.*, 1998:296), impaired immune functioning, birth defects, perinatal deaths and acute, fatal toxicity characterized by disruption of enzyme functioning (Adejumo *et al.*, 2009:47). Under normal conditions of health, chronic toxicity is rare as fluoride excretion predominantly via urine and to a lesser extent sweat, saliva, milk and digestive juices is effective with excretory rates increasing in relation to increased ingestion (Jansen van Rensburg, 1995: 482). As a result toothpaste fluoride concentrations are regulated by numerous authorities and it is internationally accepted that an effective, safe, fluoridated toothpaste (i.e. one demonstrating caries inhibitory activity while minimizing potentially negative effects) contain between 1100ppm and 1500ppm total fluoride (Benzian *et al.*, 2012:220; Kikwilu *et al.*,2008:298) or more specifically a minimum of 1000ppm free, available fluoride (Cury *et al.*, 2010:396; van Loveren *et al.*, 2003:229; Walsh *et al.*, 2010:3). Furthermore, the internationally recognized and accepted ISO11609:2010 framework goes on to define that an adequate fluoridated toothpaste is not only one in which total fluoride content does not exceed 1500ppm, but is also appropriately labeled with the word dentifrice, name and contact of the distributor or manufacturer, a tracking code, both a production and expiration date, list of ingredients and a cautionary note pertaining to use in paediatric consumers (European Commision for Standardization, 2010:4).

Conversely, a systematic review of the literature undertaken by Walsh *et al.* (2010:3) reveals that toothpastes containing fluoride at concentrations up to 500ppm provide no statistically significant caries inhibitory effect in contrast to placebo. Based on these findings, "Delivering Better Oral Health: An Evidence Based Toolkit" developed by the department of health in the United Kingdom recommends that in

order for anticariogenic activity to be realized, paediatric consumers less than 3 years of age should be exposed to only a smear of toothpaste containing no less than 1000ppm and for children between 3 and 6 years a pea-size amount containing between 1350ppm and 1500ppm fluoride. In children it is vital that the stipulated amount be used to limit fluorosis risks (Public Health England, 2014:17).

2.5 Factors Reducing Toothpaste Fluoride Availability

A number of factors, however, are known to decrease the fluoride availability and therefore the cariostatic mechanism of action of fluoridated toothpastes.

2.5.1 Chemical Incompatibility

Inactivation of F in toothpastes occurs readily due to its chemical reactivity (Forward, 1980:257). Abrasive systems are incorporated into toothpastes to regulate, halt or prevent, dental staining. The most commonly used abrasives include calcium carbonate, dicalcium phosphate, silica and sodium bicarbonate (Zero, 2006). Extensive evidence, dating as far back as the first clinical trials to evaluate the anti-caries efficacy of fluoridated toothpastes (Bibby, 1945 cited in Zero, 2006) demonstrated that abrasives influenced fluoride availability and rate of fluoride release in response to chemical incompatibility between these components (Zero, 2006). In other words free fluoride ions, the most electronegative and reactive of the elements, have the tendency to interact with other constituents, typically calcium ions to form an insoluble compound, such as, calcium fluoride (CaF_2). This precipitated (CaF_2) fluoride will not be solubilized in the mouth during brushing and prior to expectoration and is as a result unavailable for caries inhibition (Forward, 1980:258).

Typically when sodium monofluorophosphate is used as the active ingredient in the toothpaste formulation it is combined with a Ca-based abrasive as it is assumed that as F^- ions are covalently bonded to phosphate within monofluorophosphate (PO_3F^{2-}), it is unlikely that ionic F will be made available for complexation with Ca^{2+} of the abrasive (de Oliveira Conde *et al.*, 2003:248; Pessan *et al.*, 2011 cited in Benzian, 2012:298). Recent analytical studies have, however, repeatedly demonstrated that this bond is not stable and that over time PO_3F^{2-} undergoes hydrolysis to release F^-

that then interacts with Ca^{2+}, effectively reducing the fluoride availability of the toothpaste (Benzian *et al.*, 2012:216; Cury *et al.*,2010:399; Kikwilu *et al.*, 2008:298). Consequently, manufacturers have been seen to formulate the Na_2PO_3F/CaCO3-containing toothpastes with the maximum permissible total fluoride concentration of 1500ppm F to account for this anticipated loss in fluoride availability (de Oliviera *et al.*, 2003:252).

2.5.2 Ageing

Consistently within the literature reference is made to the fact that as a toothpaste ages fluoride availability and therefore potential bioavailability (cariostatic activity) declines, particularly evident among the Na_2PO_3F/CaCO3-containing toothpastes presumably in response to PO_3F^{2-} hydrolysis over time and F- inactivation on complexation with Ca2+ (Hashizume *et al.*,2003:198; van Loveren, 2003:229; Thakkar *et al.*, 2015:3) . Furthermore this decrease is accelerated on storage at excessive ambient temperatures and may occur to such an extent that fluoride availability declines to sub-therapeutic levels, below 1000ppm (Benzian *et al.*, 2012:219; de Freitas, 1984: 30; de Oliviera *et al.*, 2003:252).

2.5.3 Counterfeit Products

Unfortunately there have been increasing incidents of counterfeit/fake products leaching into the market (Colgate Palmolive, 2007). These products are frequently indistinguishable from the genuine toothpaste (Benzian *et al.*, 2012:217) and are often unscrupulously formulated with toxic compounds, most notably diethylene glycol, and only negligible or trace amounts of fluoride (Enoch, 2007; Hood, 2008). Diethylene glycol ingestion has been implicated in renal and neurological toxicities and in the absence of appropriate and timeous supportive care may be fatal (Schep *et al.*, 2009:525). Existence of such products is therefore profoundly significant to consumers and regulating authorities.

Due to the multiple factors impacting on toothpaste fluoride availability as well as the sequelae associated with excessive fluoride ingestion it is pertinent in terms of the public health implications that toothpaste quality assurance studies be undertaken and is a topic that has garnered increasing interest with numerous studies now reported in the literature. The predominant analytical technique used for quantification of fluoride content within these studies involved direct potentiometric measurement using ion selective electrode. Variations in sample preparation methodology are noted between studies. Similarly, as the caries epidemic has reached crisis status within developing countries (Marcenes *et al.*, 2013:594; Petersen, 2005:662), a large number of these studies have focused on analysis of toothpastes marketed within these nations.

2.6 Findings of Recent Toothpaste Quality Assurance Studies

Results from a study that analyzed toothpastes derived from both a developed as well as developing countries showed disparities in toothpaste quality (anti-caries efficacy) between socio-economic strata. In this study all (excluding one) toothpastes sampled in the Netherlands (the developed nation) were found to contain a mean, relative free available fluoride concentration of 94% in contrast to those obtained from the developing countries, of Brunei, Cambodia, Laos and Suriname which on analysis were found to only contain a mean, relative free available fluoride content of 53.2% for the Na2PO3F/Ca-containing toothpastes and 90.4% for those formulated with NaF/Silica (Benzian *et al.*, 2012:216). Of the 19 adult toothpastes sampled in the Netherlands, 13 contained an available fluoride content in excess of 1000ppm F, the minimum level presumed necessary for anti-caries benefits to be realized. It must, however, be noted that although the study classed the toothpastes as adult products, of 19 some were indicated for use by children five years and older and were therefore manufactured with a slightly lower, mean total fluoride content of 1102.78ppm F. Consequently, in most instances the formulation proved ideal with free available fluoride content equivalent to total fluoride concentration. Conversely, it was found that only 9.5% of the toothpastes evaluated from the developing nations actually contained free available fluoride in excess of 1000ppm.

Similarly, a study undertaken by van Loveren *et al.* (2003:228) to analyse the total and free available fluoride content of toothpastes sampled in several non-established market economy countries found that 50% of the total sample contained less than 780ppm free available fluoride, considerably less than the critical 1000ppm F level. Unfortunately within this study no mention was made with respect to abrasive agent. Likewise, substandard free available fluoride contents were noted for Tanzanian and Nigerian toothpastes. The Tanzanian products were found to only contain between 66ppm and 384ppm free available fluoride (Kikwilu *et al.*, 2008:296), while the Nigerian study which restricted analyses to total fluoride content alone, found that only 3 of the 11 adult toothpastes sampled actually conformed with the total permissible fluoride levels, ranging between 825ppm F to 1250ppm F, as prescribed by the Standards Organization of Nigeria, containing 925.78ppm F, 903.76ppm F and 903.33ppm F.

The remaining 9 toothpastes all presented with total fluoride concentrations ranging between 348.79ppm F and 792.7ppm F (Adejumo *et al.*, 2009:48). As the available fluoride content of a toothpaste is typically slightly less than total fluoride concentration, it may be assumed that for all toothpastes sampled in Nigeria the free, available fluoride concentrations were insufficient to disrupt the caries process. A recent, 2015, study evaluating 5 commercially available adult toothpastes in India found that the total fluoride content for all 5 toothpastes conformed to the maximum permissible level of 1000ppm. On average the mean, relative free available fluoride content was calculated at 86.7% for the 3 $Na_2PO_3F/CaCO_3$-containing toothpastes sampled and 96.5% for the 2 remaining NaF/SiO_2-based products. Therefore, as all toothpastes sampled conformed to the maximum permissible total fluoride concentration of 1000ppm, the available fluoride content in all instances (marginally so in some) was below the critical 1000ppm level (Thakkar *et al.*, 2015:3). Although this discussion has focused primarily on adult dentifrices, a study reporting on Brazilian paediatric toothpastes favourably concluded that 78% of toothpastes analysed contained free available fluoride above the critical 1000ppm F level (Cury *et al.*, 2010:399). In general, however, it may be accepted that toothpastes originating from or marketed within developing countries, where caries burden is greatest, are of inferior quality, especially in relation to $Na_2PO_3F/CaCO_3$-containing toothpastes.

Commercial availability of these sub-optimal products in the developing countries represents a lack of appropriate regulation by authorities and may be regarded as a contributory factor driving oral health disparities between nations.

Regrettably toothpastes formulated with Na_2PO_3F and $CaCO_3$ as abrasive are the preferred vehicle for topical fluoride delivery within these nations (Jones *et al.*, 2005:673) and as is evidenced in the literature demonstrates marked declines in fluoride availability with ageing. Stability studies undertaken in Japan and Brazil both concluded that although total fluoride content remains stable over time, total soluble (available) fluoride content is unstable, will decrease over time with a greater fraction precipitating as insoluble fluoride. This change tends to be restricted to the Na_2PO_3F/Ca-based products. The Brazilian study demonstrated a 23% increase in the insoluble fluoride fraction of the $Na_2PO_3F/CaCO_3$ toothpastes, evaluated at baseline and after 12 months storage at ambient temperatures (de Oliveira Conde, 2003:252). These results were corroborated by the Japanese study which found an average 11.6% increase in insoluble fluoride content relative to total fluoride content for $Na_2PO_3F/CaCO_3$-containing toothpastes when stored under comparable conditions for a year (Hashizume *et al.*, 2003: 196).

Another factor relevant to the quality/efficacy of fluoridated toothpastes is the provision of appropriate consumer information through labeling. As a result a number of studies mentioned reflected on this aspect as well. Benzian *et al.* (2012:220) found that, in general, toothpastes sampled from the countries of Cambodia, Laos, Brunei and Suriname failed to meet minimum marking requirements as stipulated in the ISO11609:2010 framework. Toothpastes analyzed in the recent Indian study were, however found to comply with national regulations (Thakkar *et al.*, 2015:2). Unfortunately this study does not detail these regulations and as marking practices vary widely between countries, reporting on alignment with ISO 11609:2010 guidelines would have been more pertinent.

As the studies discussed have all applied the ion selective electrode method in analysis of fluoride availability, a brief discussion of the theory governing this technique will now follow.

2.7 Basic Principles of Ion Selective Electrode (ISE)

The ion selective electrode is reputed to be a sensitive, accurate and inexpensive analytical tool that allows for the determination of the ionic concentration of a specific ion in solution in 3 simple steps that require:

1. Construction of a calibration curve according to a series of standard solutions of known concentration;
2. Immersion of the electrode in the analyte solution; and
3. Reading of the answer directly from a millivolt (mV) meter.

In general ion selective electrodes range between 5mm to 15mm in diameter and 5cm to 10cm in length, are constructed with an outer plastic tube and an ion-selective membrane affixed to one end. The membrane is arranged in such a manner that only the external surface can come into contact with the analyte solution. The other terminal is fitted with a gold plated pin for connection to the mV meter. The internal connections are completed by a solid-state system, liquid or gel electrolyte (Rundle, 2015). In the present study a Jenway combination fluoride ISE (FISE) was used and is described by the manufacturer as consisting of solid-state crystalline membrane with an integral non-refillable driTEK gel/reference (Jenway, no date). Solid-state crystalline membranes typically consist of a single lanthanum Fluoride (LaF_3) crystal that has been doped with Europium Fluoride (EuF_2). Doping refers to the process in which a small number of La^{3+} ions are replaced by Eu^{2+} ions in order to decrease the LaF_3 crystal resistance.

The only ionic interferent of the FISE is OH^- ions which are seen to interact with the LaF_3 crystal to form $La(OH)_3$ and in the process release extra F^- ions into the analyte solution, exaggerating the effective concentration and providing a false reading (Rundle, 2015). This interference however, is readily eliminated through addition of a pH buffer (TISAB II) that regulates pH in the range of 5 to 5.5 and so ensures a low OH^- ion concentration in the analyte solution (Method 9214, 1996:2).

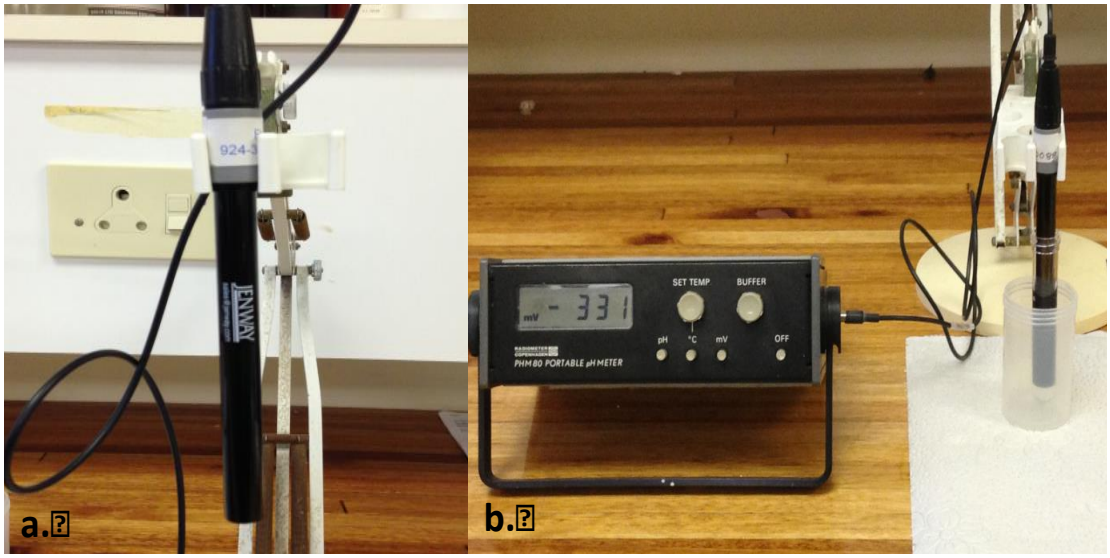

Figure 1: a. Jenway Combination Fluoride Ion Selective Electrode (924-305) b. Immersed in analyte solution and connected to a mV meter (Orion EA-740)

The basic principles governing the use and function of ISEs are as follows (Rundle, 2015):

1. The sensitive membrane selectively allows for the passage of a specific ion, in this instance the F^- ion, to the exclusion of all others.
2. On immersion into the analyte (F-containing) solution, external F- ions will diffuse across the membrane until equilibrium is established between the external and internal concentrations.
3. As a result a charge accumulates on the internal surface of the membrane. This charge, due to equilibrium conditions, is proportional to the number of F^- ions in the external solution.
4. Current flow is negligible under equilibrium conditions and as a result the potential difference developed across the membrane can only be measured relative to a separate and stable reference system which is also in contact with the analyte solution, but unaffected by it. A mV meter is then used to record this potential difference.
5. The potential difference that develops across the membrane is found to be directly proportional to the logarithm of the ionic concentration in the analyte solution.
6. In order to determine the potential difference that has developed across the membrane it is necessary for the ISE to be immersed in the analyte solution together with a separate reference electrode and that the two electrodes are

connected by a mV meter. At equilibrium electrons (e^-) removed from the analyte solution by the ISE membrane are balanced by an equal and opposite charge at the reference interface resulting in a deviation from the initial stable reference voltage, which, in turn, is registered on the voltmeter.

7. The relationship between the ionic concentration and electrode potential is given by the Nernst equation:

$$\mathbf{E = E^0 + (2.303RT/\ nF)\ x\ Log(A)}$$

Where:

E = the total potential (in mV) developed between the sensing and reference electrodes.

E^0 = is a constant which is characteristic of the particular ISE/reference pair.

2.303 = the conversion factor from natural to base10 logarithm.

R = the Gas Constant (8.314 joules/degree/mole).

T = the Absolute Temperature.

n = the charge on the ion (with sign).

F = the Faraday Constant (96,500 coulombs).

Log(A) = the logarithm of the activity of the measured ion.

2.303RT/nF = Slope of the line (from the straight line plot of E versus log(A) which is the basis of the ISE calibration curve). In general, a F ISE is considered to be operating correctly when the slope of the calibration curve lies in the range of 54mV to 60mV per decade change in concentration at a temperature of 25^0C (Method 9214, 1996:4).

8. The electrode response at extremely low fluoride concentrations is poor and it is for this reason that the linearity of the calibration curve is lost below 0.05 ppm (0.05µg F/ml) (Cury, 2015).
9. In practice the slope of the calibration curve varies in response to temperature changes, contamination of the membrane or when the analyte solution contains the ion of interest at the detection limit of the electrode.

2.8 Conclusion

In accordance with current literature this study therefore aimed to assess the quality of the commercially available fluoridated toothpastes in South Africa using the ISE method. Description of the sample preparation methodology used and calibration of the fluoride electrode is detailed in chapter 4, 'Methodology'.

Another factor pertinent to the quality of toothpaste is the provision of adequate consumer information through labeling of the product. Packing and marking practices were therefore also evaluated for compliance with national standards.

CHAPTER 3: AIMS, OBJECTIVES & RESEARCH HYPOTHESIS

3.1 Aim and Objectives

The present study aims to determine fluoride bioavailability in commercially available toothpastes in South Africa and to evaluate labeling practices and provision of consumer information.

In order to achieve these aims a number of objectives are identified as follows:

1. To determine potentially available (total soluble) fluoride concentration in commercially available toothpastes in South Africa using the fluoride ion sensitive electrode (ISE) method.
2. To determine total fluoride concentration in commercially available toothpastes in South Africa using the fluoride ISE method.
3. To evaluate information provided on toothpaste sample packaging (either outer carton or tube itself).
4. To determine whether sample-labeling practices align with South African National Standards (SANS) guidelines.

3.2 Research Hypothesis

Commercially available toothpastes in South Africa will comply with SANS (South African National Standards) labeling requirements and there will be no significant difference between fluoride content declared and that found to be present on analysis.

CHAPTER 4: METHODOLOGY

4.1 Fluoride Toothpaste Samples

Fluoridated dentifrices were conveniently purchased from a major pharmaceutical (Dis-Chem) and major food (Pick 'n Pay) retailer located in each of the five metropolitan municipalities of South Africa, namely, City of Johannesburg, City of Cape Town, eThekwini, Nelson Mandela Bay and Mangaung. As of the 1st of September 2010 the municipal demarcation board defined eight metropolitan municipalities, three located within Gauteng, two in the Eastern Cape and one each for the provinces of Western Cape, KwaZulu Natal and Free State. Sampling was restricted to the administrative seat of one metropolitan municipality per province. As the fundamental aim of this study was to evaluate fluoridated dentifrices marketed to South African consumers, sampling within Gauteng and Eastern Cape was limited to the seat, identified according to results of the 2011 census, as having highest population number and density (Table 1).

Table 1: Population Count and Density for the 8 Metropolitan Municipalities of South Africa (Statistics South Africa, 2012:2)

Province	Municipality	Seat	Population	Population Density (per km^2)
Gauteng	City of Johannesburg	Johannesburg	4 434 827	2695,9
	Ekuruhleni	Germiston	3 178 470	1652,0
	City of Tshwane	Pretoria	2 921 488	460,4
Western Cape	City of Cape Town	Cape Town	3 740 026	1520,3
Eastern Cape	Nelson Mandela Bay	Port Elizabeth	1 152 115	588,1
	Buffalo City	East London	755 200	297,8
Free State	Mangaung	Bloemfontein	747 431	118,9
KwaZulu Natal	eThekwini	Durban	3 442 361	1501,9

Initially, paediatric and adult fluoridated toothpastes, excluding herbal brands and those claiming to have tooth-whitening properties, were conveniently purchased off the shelf of a Dis-Chem outlet located in metro Cape Town. The toothpastes sampled were used to construct a list that provided the 'gold standard', to direct sampling from the other retailers and provinces included in the study (Appendix IA).

Dis-Chem was selected as the retailer on which to base the 'gold-standard' sample list as it is a specialist pharmaceutical chain stocking the widest diversity of toothpaste brands and type per brand. Similarly, Pick 'n Pay was selected for incorporation into the study as according to investment analyst Funeka Benja (2012:8), of the four major South African food retailers, Pick 'n Pay enjoys greatest market share. As many toothpaste types as possible, according to the 'gold standard' list were purchased conveniently off the shelves of all retailers and cities (metropolitan municipality seats) involved in the study. All toothpaste samples collected were included in evaluation of labeling practices.

On the other hand as the scientific and dental communities define an effective fluoridated toothpaste as one containing a minimum of 1000ppm free available fluoride, fluoride analyses were restricted to the adult toothpastes reflected on the gold standard sampling list (Appendix IA). A total of 28 toothpastes were therefore tested, 14 conveniently purchased from a Dis-Chem outlet, a pharmaceutical retailer, located in Cape Town and the remaining 14 from Pick 'n Pay hypermarket, a food retailer, located in Johannesburg. These geographical sites were selected on the basis of greatest population number (Table 1).

Toothpastes to be analysed for fluoride content were codified from 1 to 28 and on days that fluoride analyses were undertaken, codes were randomly drawn from a hat.

4.2 SECTION A: FLUORIDE MEASUREMENTS

All tests for fluoride content were conducted within 6 to 7 months of the dates of purchase of the toothpaste samples.

4.2.1 Fluoride Definitions

For the purposes of this study, three (3) forms/species of fluoride, namely, total, total soluble and insoluble, present in dentifrices were analysed. Prior to defining each of these forms it is important to distinguish between potentially available and potentially bioavailable fluoride. Potentially available fluoride refers to the fraction of total fluoride that is chemically soluble in the toothpaste formulation whereas potentially bioavailable fluoride is the fraction of potentially available fluoride that may be released into the oral cavity during brushing and prior to expectoration. Bioavailable fluoride is that component of the toothpaste that actually exerts caries inhibitory activity and may be absorbed through the gastrointestinal tract, increasing fluorosis risk in paediatric consumers. As this study was restricted to an *in-vitro*/laboratory analysis of toothpaste fluoride content using direct acid hydrolysis and ion selective electrode method, **results obtained for total soluble fluoride determination were regarded as an indicator of fluoride bioavailability** although total soluble fluoride in the strict sense refers to potentially available fluoride. In an ideal situation the assumption can be taken that fluoride bioavailability should equate to potentially available fluoride concentration, which, in turn, should be equivalent to total fluoride content. The term, total soluble fluoride (TSF) is further defined as either the fluoride ion concentration [F^-] (where square brackets indicate concentration), chemically soluble fluoride in sodium fluoride (NaF), amine fluoride (NH_4F) or stannous fluoride (SnF_2) containing dentifrices or the sum of [F^-] and monofluorophosphate ion concentration [FPO_3^{2-}] in sodium monofluorophosphate-containing (Na_2PO_3F) samples. In terms of TSF, this distinction is made as *in vivo* FPO_3^{2-} also possesses the ability to interact with enamel, following hydrolysis by saliva and dental biofilm, and so influence de-/re-mineralization processes (van Loveren *et al.*, 2005: 228). [FPO_3^{2-}] ion concentration therefore needs to be measured when assessing fluoride bioavailability *in vitro.*

As the fluoride ISE is predominantly sensitive to free F^- ions (ignoring for now the anion interferents and OH^- ions) it is essential, prior to analysis and during sample preparation, to hydrolyse bonds between the PO_4^+ and F^- ions in FPO_3^{2-} allowing for the release of ionic fluoride and enabling detection by the ISE. For this reason $Na_2PO_3F^-$ containing samples were subjected to a slightly different analytical procedure, discussed under the subheading, 'Fluoride Content Analyses'. Total fluoride concentration [TF], on the other hand, is regarded as the sum of [TSF] and insoluble fluoride [IF] concentrations, where IF refers to fluoride bound to the abrasive agent and/or other components of the toothpaste formulation (i.e. chemically inactive fluoride that does not contribute towards the anticariogenic action of the toothpaste).

Table 2: Key to Definitions and Abbreviations for Fluoride Concentrations Analysed

	Fluoride Type	Abbrv.	Chemical Forms of F in Dentifrices	Definition
1.	Total Fluoride Concentration	[TF]	$NaF/NH_4F/SnF_2$ Na_2PO_3F	TF = FI + IF TF = TSF + IF
2.	Total Soluble (Potentially Available) Fluoride Concentration	[TSF]	$NaF/NH_4F/SnF_2$ Na_2PO_3F	TSF = F^- TSF = $F^- + PO_3F^{2-}$
3.	Insoluble Fluoride	[IF]	$NaF/NH_4F/SnF_2$ Na_2PO_3F	IF = TF – F^- IF = TF – (F^- + PO_3F^{2-})

*Bioavailability is defined as the degree to which a drug is made available at the site of physiological activity (Merriam-Webster, 2015).

Fluoride concentrations (total, total soluble, insoluble) were determined potentiometrically using a Jenway Fluoride Combination Ion Selective Electrode (924-305) coupled to a PHM80 portable pH/mV meter (Orion EA-740). Samples were prepared according to a protocol described by Cury *et al.* (2010:397).

4.2.2 Determination of Total Fluoride [TF] Content of Sample

For all fluoride analyses undertaken only plastic lab-ware, reagent grade chemicals and double distilled water were used. Use of plastic is essential as fluoride ions interact with and adsorb to glass and so effectively reduce recorded potentials when, as in the present study, fluoride concentrations are determined potentiometrically (Specht, 1956:1015).

4.2.2.1 Sample Preparation and Dilution

Prior to opening, the toothpaste tube was repeatedly squeezed from top to bottom to achieve a homogenous distribution of contents. The first few grams extruded from the tube was discarded as contents are often not adequately homogenized in this region. Test samples were then prepared in quadruplicate as follows:

- Approximately 110mg (0,11g) to 130mg (0.13g) of toothpaste was homogenized in 10ml (10g) of double distilled water by shaking vigorously by hand for 30seconds. The assay tubes were then examined to ensure that no toothpaste remnants adhered to the sides. Variations in weight between trials were controlled for through calculation of a dilution factor specific to each toothpaste weighing.
- 0.25ml of this toothpaste-water slurry was then taken for estimation of TF content

To each (i.e. 4) 0.25ml slurry, 0.25ml of 2M hydrochloric (HCL) acid was added. The resultant toothpaste-HCl acid suspension was then subjected to 45^{o}C for an hour, allowing for hydrolysis and release of complexed F. Thereafter 0.5ml of a 1M sodium hydroxide (NaOH) solution was added to neutralize the HCl. Following neutralization, 1ml TISAB II buffer [0.3M potassium hydrogen phthalate (C8K5KO4), 0.21M KOH, 1M KNO3] was added, adhering to the 50:50 required sample:TISAB ratio as stipulated by the manufacturer.

The ISE actually measures the activity of the analyte as opposed to the concentration. The activity of an ion in solution is a measure of the number of ions taking part in any given reaction, in this case the number of F ions interacting with the ISE membrane and therefore the magnitude of the voltage developed by the electrode (Rundle, 2015). This number is always less than the effective number of ions present in solution (i.e. concentration) because the mobility of the ions is impeded by inter-ionic interactions with other ions in the solution. The higher the concentration of other ions, whether the same or different from the analyte species (i.e. the ionic strength of the solution) then the stronger is the retarding effect and the greater is the difference between activity and concentration. This difference between activity and concentration is overcome through addition of total ionic strength adjustment buffer (TISAB) to both test samples and standards (discussed later) in order to equalize ionic strength and hence activity coefficients, allowing for concentrations to be read directly from calibration curves (Rundle, 2015).

Decomplexing (chelating) agents and pH buffers are also included in TISAB II and function to minimize the effect of interfering ions and variable pH (Campbell, 1987:698). Polyvalent cations, Al^{3+}, Fe^{3+}, Si^{4+} and Mg^{2+} interfere by forming complexes with F that cannot be measured by ISE. The chelating agent incorporated into TISAB buffer eliminates this interference by the polyvalent cations (EPA, 1996:1). Similarly, adjustment of pH to a value between 5 and 5.5 is required as the fluoride ISE is sensitive to the presence of OH^- ions at high pH, meaning that the potential reading obtained would be greater than that obtained for F alone (Campbell, 1987:698). On the other hand at an acidic pH, H^+ ions complex with F forming HF or HF_2^- and so preclude detection by ISE. Consequently adjustment to a slightly acidic/slightly basic range is absolutely essential prior to fluoride content determination (PASCO Scientific, 1997:7).

Once prepared, sample pH and temperature were recorded. If pH did not fall in the range of 5 to 5.5, the sample was discarded and re-prepared. Likewise to ensure the accuracy of results obtained, the temperature of all samples were monitored and millivolt potentials only recorded when the samples had equilibrated to room temperature and fell within 1^0C of the temperature recorded for the standards on which the calibration curve was constructed *(discussed later).*

This requirement was strictly adhered to as a difference of 1^0C is known to result in a 2% measurement error (i.e. higher temperatures reduce ionic activity of the analyte at the ISE membrane and therefore the lower the concentration recorded) (EPA, 1996:2; PASCO Scientific, 1997:5). Sample millivoltage (mV) potential was then determined using a combination fluoride ion selective electrode coupled to an ion analyzer, previously calibrated with fluoride standards containing 0.0625ppm F to 6.25ppm F, prepared with the same reagents, namely, 2M HCl, 1M NaOH and TISAB II, in the same ratios as the samples. This calibration range was selected as it covered the anticipated range of the samples.

4.2.2.2 Preparation of 2M HCl

The 2M HCl acid solution used was prepared from a 37% ACS (American Chemical Society) reagent grade HCl stock solution. This stock solution has a 12M (molar) concentration (i.e. 12 moles of HCl per 1L of H_2O, where one mole refers to the weight, in grams, of $6{,}022 \times 10^{23}$ (Avogadro's constant) molecules of HCl. HCl has a molecular weight of 36,46g.mol^{-1}. Therefore in order to prepare a 2M HCl solution from 37% HCl, dividing 1000ml by 12M gives 83,33ml.mol^{-1}, meaning that 83,33ml of this stock solution made up to 1L by the addition of water would provide a 1M solution. Thus in preparation of a 2M solution, 16,7ml 0f 37% HCl was added to 67ml double distilled water, stirred and then made up to 100ml with double distilled water.

4.2.2.3 Preparation of 1M NaOH

NaOH beads of size 20 to 40 mesh and reagent grade 97% was used to prepare the 1M NaOH solution. As NaOH has a molecular weight of 40g.mol^{-1}, 10g NaOH was dissolved in 250ml double distilled water. This is an exothermic reaction and once the solution had cooled to room temperature it was then passed through filter paper to obtain a clear filtrate which was then stored in an air tight plastic container.

4.2.2.4 Preparation of Fluoride Standards and Calibration of the Fluoride Electrode

A calibration range between 0.5ppm and 100ppm fluoride in double distilled water was prepared. A 1000ppm NaF solution was used as standard, from which eight serial dilutions, having a final volume of 10ml each, were prepared as follows:

Table 3: Preparation of Fluoride Standards	
100ppm	1ml of 1000ppm standard + 9ml distilled H2O
50ppm	0.5ml of 1000ppm standard + 9.5ml distilled H2O
25ppm	0.25ml of 1000ppm standard + 9.75ml distilled H2O
10ppm	0.1ml of 1000ppm standard + 9.9ml distilled H2O
5ppm	1ml of 50ppm standard + 9ml distilled H2O
2.5ppm	1ml of 25ppm standard + 9ml distilled H2O
1ppm	1ml of 10ppm standard + 9ml distilled H2O
0.5ppm	0.1ml of 50ppm standard + 9.9ml distilled H2O

Prior to determination of millivolt potential for each standard dilution, the dilutions were treated according to the same procedure as described for the test samples, namely, 0.25ml 2M HCl was added to 0.25ml of the standard and left at 45^0C for an hour. Thereafter 0.5ml 1M NaOH and 1ml TISAB II were added to yield a final volume of 2ml. Each standard was therefore diluted by an additional factor of 8, yielding an effective calibration range from 0.0625ppm F (0.0625µg/ml) to 12.5ppm F (12.5µg/ml). Millivolt potential of the standard dilutions was determined using the fluoride combination ion selective electrode (ISE), starting from lowest and progressing to highest concentration. Prior to use the protective cap was removed from the electrode and the electrode pre-soaked by immersion in a 10ppm fluoride solution for 5 minutes. Following immersion, the electrode was rinsed with double distilled water and blotted dry with facial tissues. This rinsing procedure was then repeated to prevent any carry over or cross contamination of the standards.

Similarly in recording the mV potential of the standard dilutions, cross contamination was prevented through rinsing of the electrode tip with double distilled water and blotting dry with a facial tissue between measurements. On drying, the electrode tip was blotted in such a manner so as to avoid electrostatic charge. A linear regression based on the NaF standard dilutions, between fluoride concentration in ppm, on the $\log_{10}$ scale, and mV potential, on the linear axis was constructed using Microsoft Excel software (Figure 2: Example of standard calibration curve).

After initial calibration of the electrode, an initial calibration verification (ICV) was undertaken through analysis of a 1.25ppm F check standard, prepared from a second/independent 10ppm F stock solution. As per requirements stipulated in method 9214 (1996:4) it was ensured that the ICV recovery lay between 90% and 110% relative to the 1.25ppm F calibration standard.

Electrode recalibration was performed on each day that fluoride analyses were undertaken.

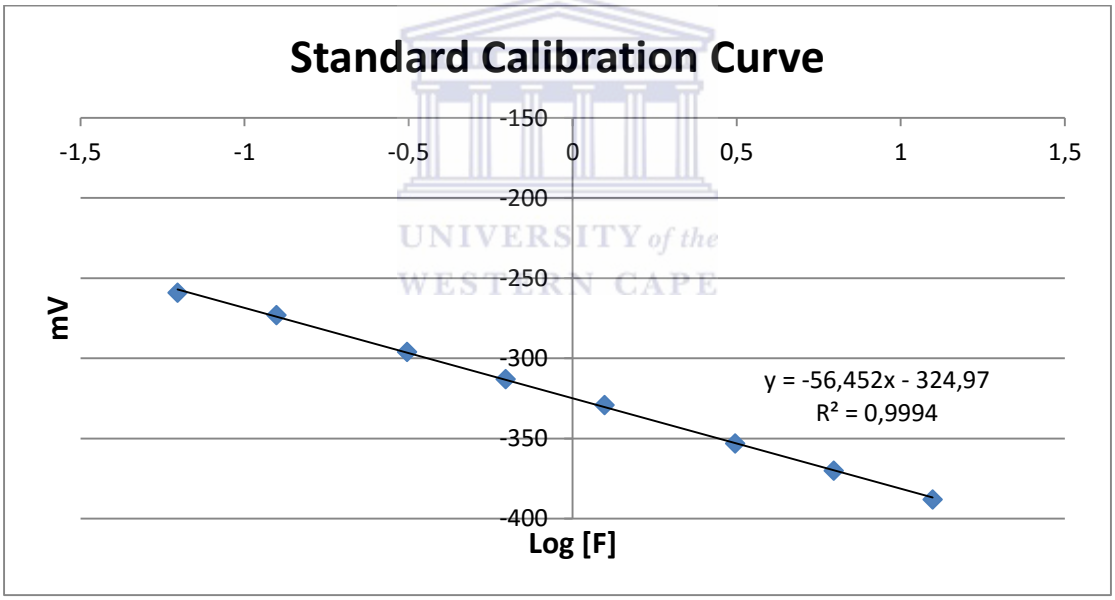

Figure 2: Example Standard Calibration Curve constructed using Microsoft Excel.
The slope is given by -56.452 and provides a good indicator that the electrode is working correctly. In general, the slope of the calibration curve should lie in the range of a 54mV to 60mV change for every decade increase in [F]. The y-intercept is at -324.97. R^2 refers to the coefficient of determination, the square root of which is the coefficient of correlation (r) and is equivalent to 0.9997, very close to +1 demonstrating the strength of the linearity between the two variables. The fluoride ion electrode always demonstrates a straight-line relationship between concentration and mV potential. Only below concentrations of 0.05ppm F, close to the detection limit of the electrode, is linearity lost.

4.2.2.5 Total Fluoride [TF] Measurement of Samples

Following construction of the calibration curves, total fluoride concentration of the prepared, diluted test samples were determined by placing the electrode into the samples and recording the stable mV value obtained. On average it took 2.5 minutes for a stable reading to be attained. On attainment of the apparent stable reading, the mV value was not recorded until the result had remained stable/unchanged for at least 20seconds. These values were then entered onto the calibration curve and the corresponding ppm result read off the x-axis. As the initial toothpaste samples were, for example, initially diluted by a factor of 91.9x (i.e. 0,11g of toothpaste homogenized in 10g distilled water) and then further diluted by an additional factor of 8 on test preparation (i.e. 0.25ml of sample in 0.25ml 2M HCL, 0.5ml 1M NaOH and 1ml TISABII) the measured concentration was smaller by the total factor of dilution (i.e. 91.9 x 8 = 735.2x) than the concentration in the tube. The real concentration was therefore back calculated by multiplying the ppm result obtained by the dilution factor, for example, 1.4ppm F x 735.2 = 1029.28ppm F.

As for the measurement of the standard dilutions, the electrode was rinsed with double distilled water between measurements and blotted dry with facial tissues. After every fourth measurement a continuing calibration verification (CCV) was undertaken using the 1.25ppm F standard from the middle of the calibration range. If any drift in mV potential away from the initial recorded value was observed, the electrode was recalibrated and the last 4 measurements repeated. Consistency on CCV confirmed the accuracy of electrode function and validity of the results being documented.

Prior to testing pH and temperature were evaluated to confirm that pH fell between 5 and 5.5 and that temperature was constant across all samples and standards.

4.2.3 Determination of Ionic Fluoride [FI] Content of Sample

(* [FI] was assessed for all toothpaste samples irrespective of the chemical form of F declared. [FI] for the NaF, NH_4F and SnF_2 containing toothpastes was taken to represent the [TSF]/Available fluoride content)

4.2.3.1 Sample Preparation and Dilution

The samples were prepared in the same manner as to that described for total fluoride analysis (i.e. dissolution of approximately 0.11g toothpaste in 10g double distilled water). The homogenous toothpaste-water slurry obtained was then sedimented by centrifugation at 2800rpm for 45 minutes, to remove the insoluble fluoride (i.e. fluoride bound to abrasive agent or other constituents of the toothpaste formulation) fraction. Insoluble fluoride, also referred to as inactive fluoride does not contribute to caries inhibitory activity (i.e. it is not bioavailable). Therefore, following sedimentation only soluble/available fluoride remained in suspension. Quadruplicate samples were prepared by taking 0.25ml of the suspension obtained following centrifugation and adding 1ml TISAB II, 0.25ml 2M HCl and 0.5ml NaOH specifically in that sequence. Temperature and pH of all test samples were then analysed using a pH electrode to ensure that temperature and pH were consistent across the samples and standards. The millivolt potential for each sample was then determined using a fluoride ISE previously calibrated with fluoride standards ranging in concentration from 0.0625ppm F to 12.5ppm F.

4.2.3.2 Preparation of Fluoride Standards and Calibration of the Fluoride Electrode

The fluoride standards were prepared by serial dilution of 1000ppm NaF solution according to the technique as described for total fluoride content analysis. Once again the standard dilutions were treated in the same manner and with the same reagents as the test samples. In this instance (i.e. determination of [FI]), however, as for preparation of samples, the standard dilutions were not incubated at 45^0C for an hour. Neutralization proceeded immediately following the addition of 0.25ml 2M HCl through the addition of 0.5ml 1M NaOH.

In order to construct the calibration curve (linear regression between [F] concentration on the logarithmic scale against millivolt potential on the linear axis), millivolt potential for each standard dilution, progressing from least to most concentrated, was determined using the combination fluoride ISE attached to the mV meter. Temperature and pH of the standards were checked for equivalency prior to testing. In conducting these analyses, cross contamination between standards was prevented by rinsing the electrode tip with double distilled water and blotting dry with facial tissues between measurements.

From this discussion it is evident that a separate calibration curve was constructed for ionic fluoride content determination to ensure that both the standard dilutions and ionic fluoride test samples were treated in an identical manner, protecting the accuracy of the concentration results achieved.

4.2.3.3 Ionic Fluoride [FI] Measurement of Samples

Following construction of the calibration curve and ICV, [FI] of the prepared toothpaste suspensions (i.e. test samples) were determined by placing the electrode into the samples and recording the stable mV value obtained. These values were then entered onto the FI calibration curve and the corresponding ppm result read off the x-axis. Once again as described for determination of the total fluoride content of the tests, the true fluoride ion concentration was obtained by multiplying the ppm F result obtained on analysis by the dilution factor for that test.

As for total fluoride content analyses the electrode tip was rinsed with double distilled water and blotted dry with facial tissues between measurements and a CCV was undertaken after every fourth reading.

Motivation as to why [FI] concentration was used as measurement of [TSF] concentration for the NaF, NH_4F and SnF_2 containing toothpastes is that firstly and theoretically, [TSF] is known to equate to [FI]. Secondly as these analyses were undertaken during winter it was found that for any given testing session the ambient temperatures and therefore test and standard temperatures changed by as much as 2^0C. With this change in temperature mV potential was seen to drift.

As soon as potential drifted by 1mV the electrode was recalibrated and a new linear regression/calibration curve constructed. All tests analysed from that point on were then calculated off the 'new' linear regression. This was done chiefly to minimize error introduced by the limitations of the mV meter used. As the mV meter did not have a 0.1mV resolution a 1mV drift in potential equated to an approximate 50ppm F change in concentration, thus introducing significant error. If one considers that each reading took approximately 2.5 minutes to stabilize and that 16 tests, 8 standards, 1 ICV and 4 CCVs needed to be read, time required to complete just the [TSF] analyses, at a minimum, amounted to 73 minutes, the limit at about which fluctuations in mV potential were observed, meaning that [TF] would have been read off a different calibration curve. It was therefore decided that the [FI] for the NaF, NH_4F and SnF2-containing toothpastes would be recorded as the [TSF] and read immediately off an independent calibration curve constructed from standards prepared in the same manner as the tests.

Thirdly [FI] tests could commence immediately (i.e. there was no need to incubate standards and samples at 45^0C for and hour) and served to effectively stream-line and improve the convenience of the entire toothpaste testing procedure.

Fourthly, it is assumed that [FI] in solution without incubating under strongly acidic conditions for an hour would be more closely related to dissolution of the toothpaste in the oral cavity during brushing and therefore provide a better indicator of the fluoride availability/potential bioavailability of the toothpaste.

4.2.4 Determination of Total Soluble/Available Fluoride [TSF] Content of Sample

(*This technique was applied to the Na_2PO_3F-containing toothpaste samples. Results obtained indicated the sum of [F^-] and [PO_3F^{2-}])

4.2.4.1 Sample Preparation and Dilution

Once again, the samples were prepared in the same manner as that described for TF and FI analysis (i.e. dissolution of 0.11g toothpaste in 10g double distilled water). As for [FI] determination, the homogenous toothpaste-water slurry obtained was sedimented by centrifugation at 2800rpm for 45 minutes, allowing for precipitation of the insoluble fluoride fraction. Following sedimentation only soluble/available fluoride remained in suspension. It must however be borne in mind that for Na_2PO_3F-containing samples, FPO_3^{2-} ions are also soluble, present in suspension, and bioavailable (i.e. having the ability to interact directly with enamel influencing de-/reminerlization processes). Consequently suspensions generated from Na_2PO_3F-containing toothpastes were left for an hour at 45^0C following 0.25ml 2M HCl addition to allow for complete acid hydrolysis of the bonds between F^- and PO_4^{3-} ions in FPO_3^{2-}, releasing free F ions into solution to permit detection by the fluoride ion-selective electrode. Subsequent to incubation the HCl was neutralized through addition of 0.5ml 1M NaOH with sample ionic strength and pH standardization achieved through addition of 1ml TISAB II. As for TF and IF analyses, samples were prepared in quadruplicate.

Once the samples had been left to equilibrate to room temperature, temperature and pH were assessed to ensure that these variables were comparable between samples and standards. The millivolt potential for each sample was then determined using a fluoride ISE previously calibrated with fluoride standards ranging in concentration from 0.0625ppm F to 12.5ppm F.

4.2.4.2 Preparation of Fluoride Standards and Calibration of the Fluoride Electrode

The same calibration curve used for [TF] determination was applied to [TSF] analyses provided that assessments were being undertaken on the same day. The F electrode was re-calibrated on each day that [TF] and [TSF] analyses were performed. For full details on preparation of fluoride standards and calibration of the fluoride electrode, please refer to the discussion under the same sub-heading within the section titled, 'Determination of Total Fluoride (TF) Content of Sample'.

4.2.4.3 Total Soluble/Bioavailable Fluoride [TSF] Measurement of Samples

Following construction of the calibration curve and ICV, [TSF] of the prepared toothpaste suspensions (i.e. test samples) were determined by placing the electrode into the samples and recording the stable mV value obtained. These values were then entered onto the TSF calibration curve and the corresponding ppm result read off the logarithmic scale. As the initial toothpaste samples were diluted, the measured concentration was smaller by the factor of dilution than the concentration in the tube. The real concentration was therefore calculated by multiplying the ppm result obtained on analysis by the dilution factor. The results (i.e. [TSF]) obtained in this manner for the Na_2PO_3F-containing samples reflect the sum of [FI] and [FPO_3^{2-}]. Therefore in order to determine [FPO_3^{2-}] in isolation, the value obtained for [FI] of the sample was subtracted from that obtained for [TSF] (i.e. [FPO_3^{2-}] = [TSF] – [FI]) (Table 2).

As for TF and FI content analyses the electrode tip was rinsed with double distilled water and blotted dry with facial tissues between measurements. After every fourth measurement a continuing calibration verification was undertaken using the 1.25ppm F standard.

4.2.5 Determination of Insoluble Fluoride [IF] Content of Sample

[IF] of the samples were determined by subtracting [TSF] form [TF] values obtained on analyses. The [FI] was representative of [TSF] for the NaF, NH_4F and SnF_2 containing samples, while the [TSF] for the Na_2PO_3F-containing was representative of the sum of [FI] and [PO_3F^{2-}] values obtained (Table 2).

4.2.6 Data Analysis

As discussed, two linear regressions, one based on [TF] and the other [FI], were constructed by plotting electrode response on the linear (y-axis) against analyte concentration on the logarithmic (x-axis) using Microsoft Excel Software. These calibration curves were then used to quantify analyte concentration of an unknown sample directly from the electrode response of the sample.

The slope, intercept and correlation coefficient for the standards were calculated by performing a linear regression analysis of the data in Excel. A slope between -54mV to -60mV per decade of F ion concentration confirmed that the electrode was functioning correctly.

The electrode response values (millivolt potentials) for the samples were recorded and converted to concentration values using the following formula:

Conc. (ppm) = 10^(electrode response(mV) – intercept/slope), where

Intercept: y=mx + b; and

Slope = $\Delta y/\Delta x$

Each sample was analyzed four times for reliability. The mean and standard deviation for the TF, FI and TSF were calculated using Microsoft Excel

4.2.7 Validity of the results

In summary a number of internal controls were used to validate the accuracy of the fluoride analyses results obtained and a coefficient of variation, based on standard deviation, equal to or less than 5% was considered acceptable.

The internal controls employed included:

1. Initial calibration verification (ICV): After initial calibration of the electrode, a calibration verification was undertaken through analysis of a 1.25ppm F check standard, prepared from a second/independent 10ppm F stock solution. As per requirements stipulated by the EPA in method 9214 (1996:4) it was ensured that the ICV recovery lay between 90% and 110% relative to the 10ppm F calibration standard.

2. Continuous Calibration Verification (CCV): After every 4 measurements (i.e. one weighing measured in quadruplicate) a CCV was undertaken using the 1.25ppm F calibration standard. As soon as a 1mV drift in potential was observed, the electrode was re-calibrated and the last 4 analyses undertaken repeated. As prescribed by the methodology used the electrode was always calibrated twice, once for determination of ionic fluoride content and the other for determination of the total and total soluble fluoride content. If, however, sample analyses for total and total soluble fluoride exceeded a duration of 2 hours it was found that re-calibration became necessary and for this reason the electrode was calibrated up to a maximum of 3 times on any day that fluoride assessments were undertaken.

3. Slope of Calibration Curve: Prior to sample analysis the gradient of the calibration curve was checked for compliance with manufacturer's specifications and lay in the range of 54mV to 60mV change per decade increase in fluoride concentration. The correlation coefficient (r) for each calibration curve was calculated to check for linearity between the 2 variables (i.e. log of fluoride concentration and mV potential).

In general the electrode demonstrates a linear response at fluoride concentrations greater than 0.05ppm (Thermo Scientific, 2011:15). The *r*-values obtained for the calibration curves averaged 0.9998 demonstrating an almost perfect straight-line relationship, as was expected. Deviation away from a value of 1 was due to decreased non-linear electrode response observed on measuring the 0.0625ppm F standard (i.e. close to detection limit of 0.05ppm F, below which the relationship between the variables becomes non-linear).

4. In addition to the internal controls, other parameters that were also strictly controlled included:

    a. Temperature: As temperature changes influence electrode potentials, all samples and standards were allowed to equilibrate to room temperature ($\pm 1^0$C). A 2% measurement error has been reported in the literature for a 1^0C change in temperature (Boyle, 1997:5).

    b. pH and Ionic Strength: The pH and Ionic strength between standards and samples were controlled for by the addition of TISABII. TISABII functioned to buffer pH to between 5 and 5.5 and ensured that a similar high activity coefficient was achieved between different samples and standards to allow for comparisons to be made.

    c. Rinsing and Drying: The electrode was double rinsed and blotted (to eliminate electrostatic charge) dry with a facial tissue between measurements. This prevented any cross-contamination between samples or standards.

    d. Air bubbles: On Immersion the membrane interface was checked for the presence of air bubbles and removed and re-immersed until no trapped air was evident.

e. Endpoint time: All millivolt readings were recorded after approximately 2.5 minutes, once the reading had stabilized and had remained stable for at least 20 seconds.

f. Slurrying Time: All toothpastes were slurried with double distilled water by shaking vigorously by hand for exactly 30 seconds immediately prior to extraction of 0.25ml aliquots for total fluoride determination. After slurrying the assay tubes were visually checked to ensure that no toothpaste adhered to the side of the assay tube or had precipitated out.

g. Centrifuge Speed and Time: All toothpaste suspensions were sedimented by centrifugation for 45 minutes at 2800 r/min prior to removal of 0.25ml aliquots of the supernatant for ionic and total soluble fluoride determinations.

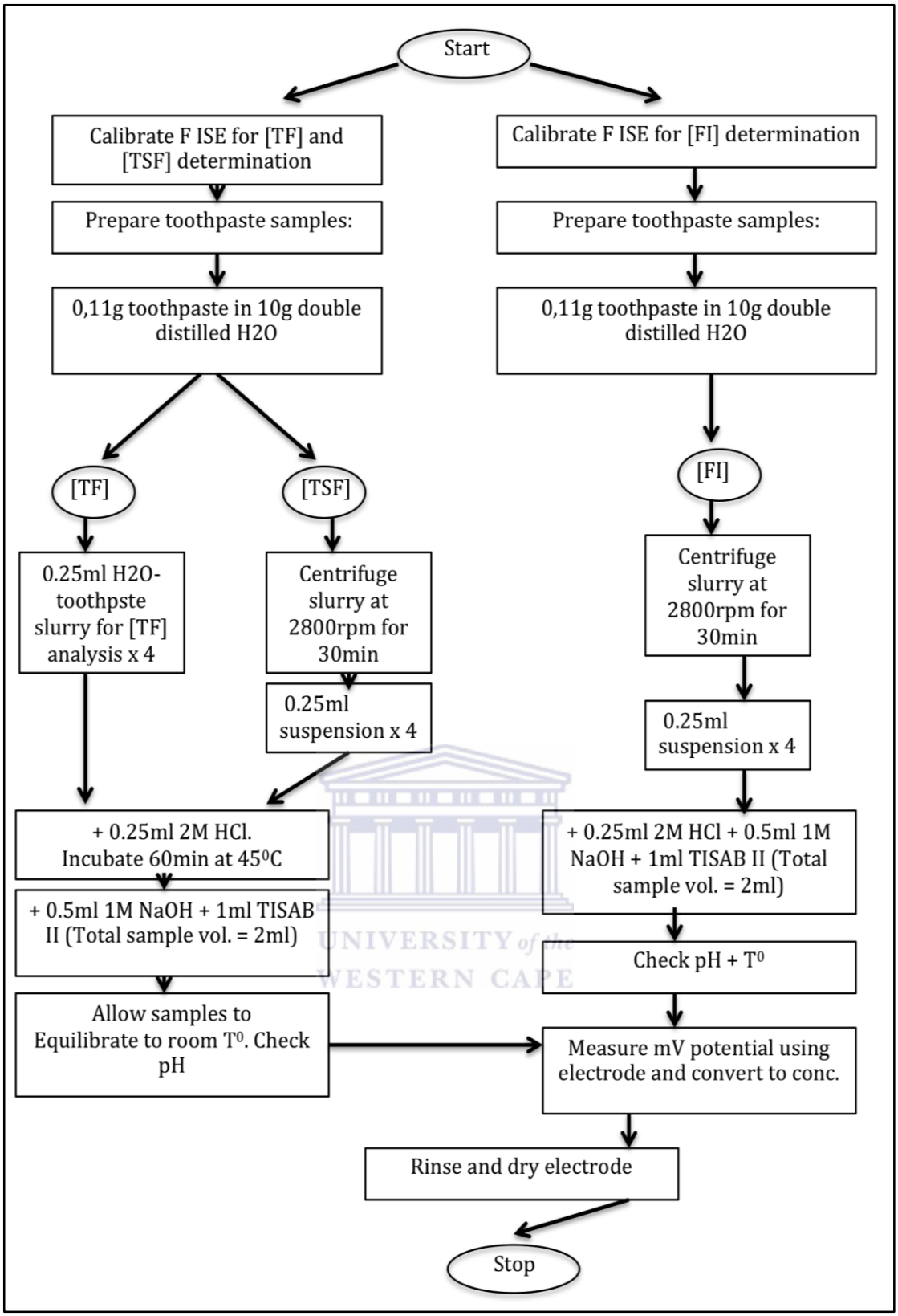

Figure 3: Schematic Overview - Potentiometric Determination of Fluoride in Aqueous Solutions of Fluoridated dentifrices using Ion Selective Electrode

4.3 SECTION B: LABELING PRACTICES

The packaging of all toothpaste samples, the outer carton and inner tube was assessed for conformity with the South African National Standards (SABS, 2008). In terms of these standards the information evaluated included: country of production, content description, the presence of a key identifier, product descriptor and batch identification. In addition labels were scrutinized for the descriptive name (chemical form) of fluoride and abrasive component, fluoride concentration expressed in parts per million (ppm) and expiry date. If fluoride concentration was expressed as a percent of volume (%w/v) or weight (%w/w), these values were converted to ppm. If only the production date was specified, expiry was recorded as three years later.

4.4 Ethical considerations

Written approval for the study, including both the fluoride analyses and evaluation of toothpaste labeling practices, was obtained from the Senate Research Ethics Committee of the University of the Western Cape (registration number: 15/1/7).

4.5 Declaration of interest

The author has no conflict of interest to declare.

CHAPTER 5: RESULTS

5.1 SECTION A: FLUORIDE ANALYSES RESULTS

5.1.1 Introduction

Currently as the scientific and dental communities define an effective fluoridated toothpaste as one containing a minimum of 1000ppm free available fluoride, fluoride analyses were restricted to the adult toothpastes reflected on the gold standard sampling list (Appendix I). A total of 28 toothpastes were tested, 14 conveniently purchased from a Dis-Chem outlet, a pharmaceutical retailer, located in Cape Town and the remaining 14 from Pick 'n Pay hypermarket, a food retailer, located in Johannesburg. These geographical sites were selected on the basis of greatest population number (Table 1).

The 28 toothpastes included in the study were codified from 1 to 28. Codes are not indicative of the sequence in which the toothpastes were analysed.

5.1.2 Sample Composition

A majority, 57.14% contained NaF, 28.57% Na_2PO_3F, 3.57% NH_4F, 7.14% a combination of NaF and Na_2PO_3F, and 3.57% a combination of SnF_2 and NaF. Most, 93.8% of the NaF-containing toothpastes, 25% of the Na_2PO_3F-containing samples, the only sample containing a combination of SnF_2 and NaF and 50% of the samples containing a combination of NaF and Na_2PO_3F evaluated were formulated with hydrated silica as the abrasive agent (Tables 4 and 5). All remaining samples, excluding 1 formulated with sodium bicarbonate ($NaHCO_3$) and silica (SiO_2) as abrasives, incorporated a calcium-based abrasive either as calcium carbonate ($CaCO_3$) or dicalcium phosphate($CaHPO_4$) (Tables 6 and 7). As the toothpaste formulated with NaHCO3 and SiO_2 abrasives also contained calcium sulphate ($CaSO_4$), which according to the manufacturer is indicated for enamel remineralization and for which the phrase, "liquid calcium technology™" was coined, this toothpaste was placed into the category of toothpastes having a calcium-based abrasive.

Presence of $CaSO_4$, from results obtained, appeared to exert the same influence as a calcium-based abrasive, reducing total soluble (potentially bioavailable) fluoride content and therefore increasing insoluble fluoride fraction in the formulation. This toothpaste coded 27 (Enamel Care) presented with a mean insoluble fluoride concentration of 386.1ppm (sd ±7.7) in contrast to the other calcium-containing toothpastes (n=6) having a mean 131.9ppm (sd ±117.9) insoluble fluoride content (Tables 6 and 7, 'mean [IF] in analysis'), all compared to the SiO_2-containing samples, excluding toothpastes coded 10 and 21, that presented with a mean insoluble fluoride fraction of 16.1ppm (sd ±24.9) (Tables 4 and 5, 'mean [IF] in analysis). Reasons for exclusion of toothpastes 10 and 21 are discussed hereunder.

5.1.3 Stability

All toothpastes excluding 2, 1 from each of the Cape Town and Johannesburg sampling sites, formulated with NaF in combination with hydrated silica as the abrasive, were analysed prior to expiry. Increasingly evidence within the literature indicates that soluble/available (potentially bioavailable) fluoride is unstable, with concentration declining over time and in relation to the ambient conditions under which that toothpaste is stored (de Oliviera Conde *et al.*, 2003:249; Hashizume *et al.*, 2003: 197). In general, however, this decline is not noted for products formulated with a Si-based abrasive and is typically limited to the Ca-containing toothpastes. Despite having surpassed expiry, it is for this reason that it was decided to still analyse toothpastes coded 10 and 21 (Pepsodent Complete 8 and Dis-Chem Dentalmate), to establish whether findings of the present study correspond with those reported in the literature. The mean, available (soluble) fluoride content relative to total fluoride concentration obtained on analyses of these samples were 93.8% and 96.2% respectively, while mean, available fluoride content, for the remaining, non-expired NaF/Silica-based toothpastes analysed (n=13) was calculated at 98.7% (sd ±2.1) (Tables 4 and 5, column, '[TSF] as % [F]'), suggesting that stability changes may not necessarily be limited to Ca-containing products.

In addition, toothpaste coded 10 expired in March of this year (2015) while toothpaste coded 21 expired in June (a 3-month difference). The relative mean, soluble (potentially bioavailable) fluoride concentration is markedly lower for that sample

having expired in March (93.9%) as opposed to the sample bearing the June expiry date (96.2%). Even though relative mean, available fluoride concentrations for these 2 toothpastes were lower than those obtained for the non-expired NaF/SiO_2 products, these toothpastes still presented with sufficient concentrations, 1300.4ppm (sd ±37.7) and 1132ppm (sd ±28.88) of available (potentially bioavailable) fluoride to be effective in preventing dental caries (Figure 5, toothpastes coded 10 and 21).

As these 2 toothpastes were expired, results obtained on analyses thereof were excluded from calculations for mean total soluble (potentially bioavailable) fluoride concentrations for the entire sample (n=26). Interestingly, even though evaluation of affordability was not an objective of the present study, these were 2 of the cheapest toothpastes sampled, costing R9.95 and R5.95 respectively.

5.1.4 Total and Total Soluble (potentially Bioavailable) Fluoride Concentrations

Tables 4, 5, 6 and 7, list the total and total soluble (available) fluoride concentrations determined potentiometrically following direct acid hydrolysis of the samples. This data is displayed graphically in Figures 5 and 6. In general, the total soluble/available fluoride concentrations were slightly less than the total fluoride concentrations obtained on analysis.

A minority, 23.1% (n=26) presented with sub-therapeutic fluoride levels (total soluble/potentially available F <1000ppm), while 1 toothpaste (3.8%), coded 5, contained a mean total fluoride concentration of 1514,9ppm (sd ±48.2) in excess of the declared 1490ppm F and in excess of the South African and internationally accepted norm in terms of maximum permissible fluoride levels that a toothpaste may contain. The mean available (potentially bioavailable) fluoride content, however, conformed to manufacture's specifications and was recorded as 1473ppm (sd ±37.6).

Table 4: Total and Bioavailable fluoride concentrations for toothpastes puchased in Cape Town and containing hydrated Silica (SiO2.nH2O) as the abrasive																		
TP Code	No. of Det.	Toothpaste Type	Expiry	Chemical form of F	[TF] declared (ppm)	Mean [TF] in analysis (ppm)	SD	Rel. SD	[TSF] in analysis (ppm)					Rel. SD	[TSF] as % [TF]	Mean [IF] in analysis (ppm)	% Diff. [TF] declared vs. analysis	% Diff [TF] vs. [TSF] in analysis
									[FI] in analysis	SD	[PO3F2-] in analysis	Total	SD					
1	12	Aquafresh All-in-one	09/2016	NaF	-	1371,2	± 70,2	5,1	1358,7	± 70,5	0	1358,7	± 70,5	5,2	99,1	12,5		0,9
2	12	Aquafresh Extreme Clean	07/2016	NaF	1450	1223,5	± 64,4	5,3	1175,2	± 59,6	0	1175,2	± 59,6	5,1	96,1	48,3	15,6	3,9
3	8	Colgate Total 12	04/2017	NaF	1450	1349,66	± 61	4,5	1349	± 35,8	0	1349,02	± 35,8	2,7	100,0	0,6	6,9	0,0
4	16	Elgydium Sensitive	09/2015	NH_4F	1250	1125,3	± 26,6	2,4	1100,4	± 27,2	0	1100,4	± 27,2	2,5	97,8	24,9	10,0	2,2
5	8	GUM Caries Protect	07/2017	Na_2PO_3F + NaF	1490	1514,9	± 48,2	3,2	1328,7	± 5,8	144,5	1473,2	± 37,6	2,6	97,2	41,7	-1,7	2,8
6	16	Mentadent P Protection	04/2017	NaF	1450	1239,54	± 40,3	3,3	1184	± 38,1	0	1184,02	± 38,1	3,2	95,5	55,5	14,5	4,5
7	8	Oral B Pro-Expert	04/2016	SnF2 + NaF	1450	1203,3	± 20,7	1,7	1220,4	± 21,9	0	1220,4	± 21,9	1,8	101,4	-17,1	17,0	-1,4
8	12	Sensodyne Cool Gel	07/2016	NaF	1400	1349,8	± 69,6	5,2	1368,7	± 47,3	0	1368,7	± 47,3	3,5	101,4	-18,9	3,6	-1,4
9	8	Sensodyne Repair & Protect	05/2016	Na_2PO_3F	1450	1342,9	± 34,6	2,6	47,8	± 3,3	1277,3	1325,1	± 34,4	2,6	98,7	17,8	7,4	1,3
10	12	Pepsodent Complete 8	03/2015	NaF	1450	1385,74	± 59,2	4,3	1300,4	± 42	0	1300,4	± 42	3,2	93,8	85,3	4,4	6,2
Table 5: Total and Bioavailable fluoride concentrations for toothpastes puchased in Johannesburg and containing hydrated Silica (SiO2.nH2O) as the abrasive																		
11	16	Aquafresh Ultimate	08/2016	NaF	1450	1259,2	± 19,7	1,6	1218,4	± 21	0	1218,35	± 21	1,7	96,76	40,83	13,2	3,2
12	16	Colgate MaxFresh	04/2017	NaF	1000	897,3	± 36	4,0	866,88	± 25,6	0	866,88	± 25,6	3,0	96,61	30,44	10,3	3,4
13	16	Colgate ProGum Health	05/2016	NaF	1450	1276,5	± 38,2	3,0	1291,5	± 35,1	0	1291,52	± 35,1	2,7	101,2	-14,98	12,0	-1,2
14	16	Colgate Sensitive MultiProte	09/2016	Na_2PO_3F	1000	908,0	± 24	2,6	73,31	± 3	844,27	917,58	± 35,6	3,9	101,1	-9,56	9,2	-1,1
15	8	Close Up Deep Action	06/2017	NaF	1450	1318,3	± 32,6	2,5	1266,1	± 21,8	0	1266,1	± 21,8	1,7	96,04	52,2	9,1	4,0
16	16	Mentadent P Gel Protection	07/2017	NaF	1450	1238,7	± 42,3	3,4	1223,2	± 33,7	0	1223,2	± 33,7	2,8	98,75	15,49	14,6	1,3
17	8	Mentadent P Micro Granules	08/2017	NaF	1450	1151,8	± 53,5	4,6	1140,1	± 54,8	0	1140,1	± 54,8	4,8	98,98	11,7	20,6	1,0
18	8	Mentadent P Sensitive	07/2016	Na_2PO_3F	1000	960,0	± 26,1	2,7	109,2	± 3,4	870,4	979,6	± 14,7	1,5	102	-19,6	4,0	-2,0
19	16	Sensodyne Multi Care	07/2016	NaF	1400	1340,6	± 31,1	2,3	1333,1	± 25,8	0	1333,05	± 25,8	1,9	99,44	7,51	4,2	0,6
20	16	Sensodyne Rapid Action	04/2016	NaF	1040	981,5	± 25,2	2,6	954,71	± 21,5	0	954,71	± 21,5	2,3	97,27	26,8	5,6	2,7
21	12	Dis-Chem Dentalmate	06/2015	NaF	-	1176,9	± 42,7	3,6	1132	± 30,2	0	1131,97	± 30,2	2,7	96,18	44,94		3,8

KEY: TP = Toothpaste; TF = Total Fluoride; TSF = Total Soluble Fluoride; FI= Fluoride Ion; IF = Insoluble Fluoride; NaF=Sodium Fluoride; HN_4F = Amine Fluoride; Na_2PO_3F = Sodium Monofluorophosphate; SnF_2 = Stannous Fluoride; $PO3F^{2-}$ = Monofluorophosphate Ion. Square brackets [ ] denote concentration.

Table 6: Total and Bioavailable fluoride concentrations for toothpastes puchased in Cape Town and containing a calcium-based abrasive																		
TP Code	No. of Det.	Toothpaste Type	Expiry	Chemical form of F	[TF] declared (ppm)	Mean [TF] in analysis (ppm)	SD	Rel. SD	[TSF] in analysis (ppm)					Rel. SD	[TSF] as % [TF]	Mean [IF] in analysis (ppm)	% Diff. [TF] declared vs. analysis	% Diff [TF] vs. [TSF] in analysis
									[FI] in analysis	SD	[PO3F2-] in analysis	Total	SD					
22	8	Colgate Active Salt	10/2015	Na_2PO_3F	1000	960,1	± 21	2,2	157,4	± 6,7	536,1	693,5	± 14,2	2,0	72,2	266,6	4,0	27,8
23	16	Colgate Gel	09/2016	Na_2PO_3F + NaF	1450	1305,9	± 59,1	4,5	418,3	± 19,5	890,2	1308,5	± 40	3,1	100,2	-2,6	9,9	-0,2
24	12	Colgate Max. Cavity Protecti	07/2016	Na_2PO_3F	1450	1244,4	± 32,9	2,6	102,1	± 5,7	1045	1147,1	± 38,1	3,3	92,2	97,3	14,2	7,8
25	8	Colgate Pro-Relief Sensitive	04/2017	Na_2PO_3F	1450	1435,5	± 26,1	1,8	225,3	± 5,8	956,3	1181,6	± 15,9	1,3	82,3	253,9	1	17,7
Table 7: Total and Bioavailable fluoride concentrations for toothpastes puchased in Johannesburg and containing a calcium-based abrasive																		
26	16	Colgate Triple Action	10/2016	Na_2PO_3F	1450	1387,5	± 52	3,7	116,7	± 10	1101	1217,7	± 17,9	1,5	87,8	169,8	4,3	12,2
27	8	Enamel Care	08/2016	NaF	1100	1077	± 36,5	3,4	690,9	± 14,2	0	690,9	± 14,2	2,1	64,2	386,1	2,1	35,8
28	16	Pepsodent	03/2016	Na_2PO_3F	1450	1356,06	± 38,3	2,8	357,56	± 11,6	992,06	1349,62	± 50,8	3,8	99,5	6,44	6,5	0,5

KEY: TP = Toothpaste; TF = Total Fluoride; TSF = Total Soluble Fluoride; FI= Fluoride Ion; IF = Insoluble Fluoride; NaF=Sodium Fluoride; HN_4F = Amine Fluoride; Na_2PO_3F = Sodium Monofluorophosphate; SnF_2 = Stannous Fluoride; $PO3F^{2-}$ = Monofluorophosphate Ion. Square brackets [ ] denote concentration.

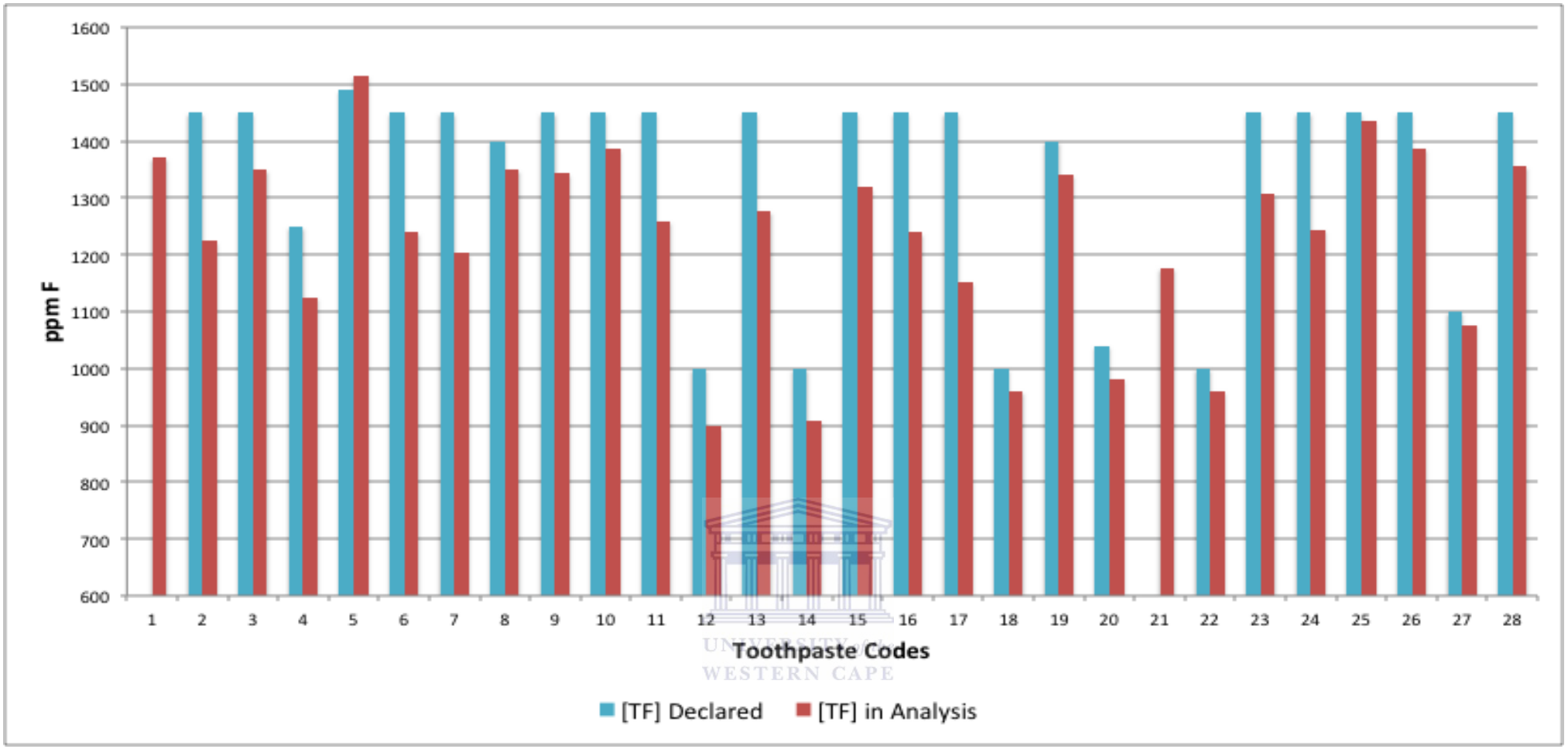

Figure 4: Cluster bar graph depicting concentration (ppm F) declared by manufacturer and that found on analyses. A total fluoride content was not declared for toothpastes coded 1 and 21.

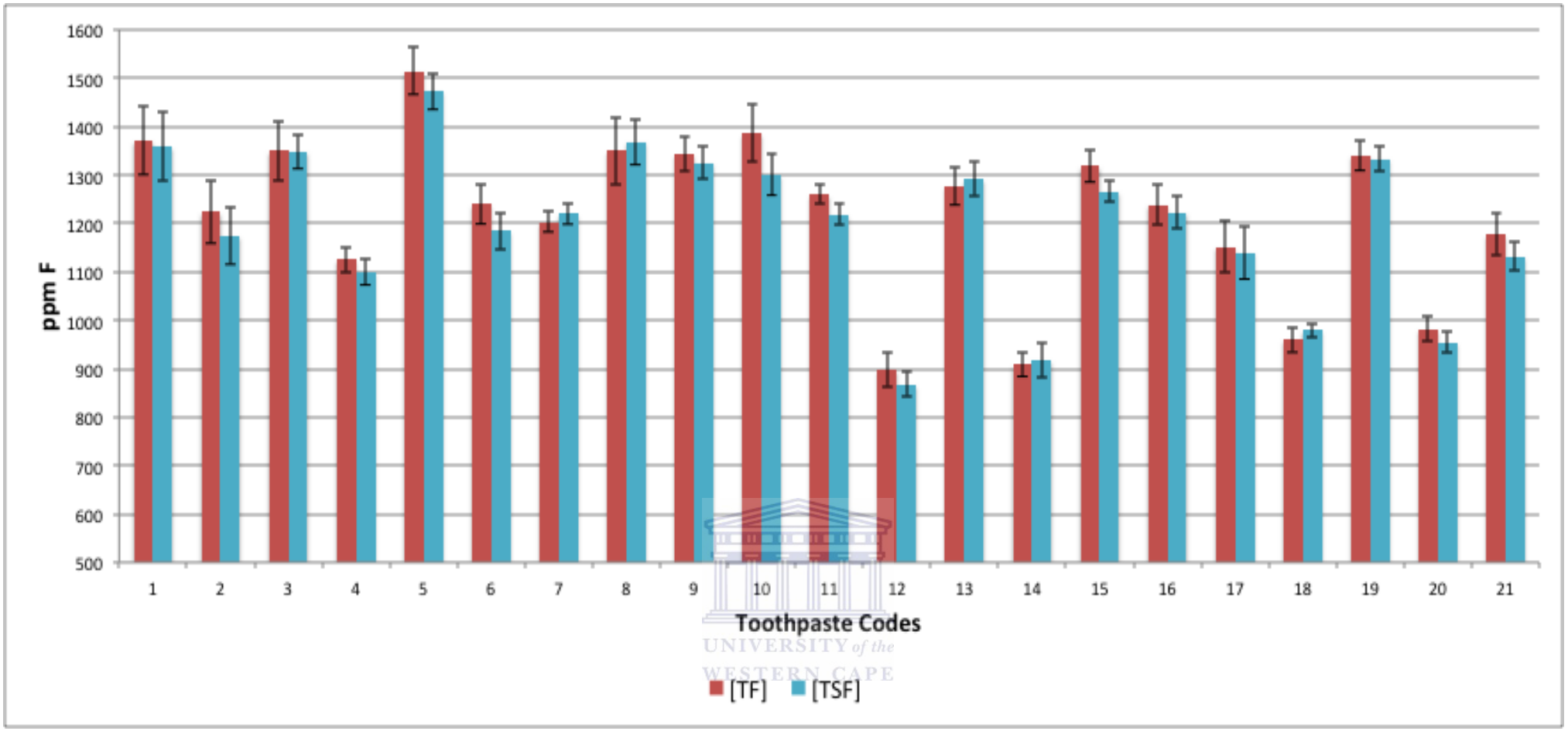

Figure 5: Concentration (ppm F) of total and total soluble fluoride found on analyses for toothpastes formulated with hydrated silica as the abrasive. Toothpaste coded 5 presented with total fluoride concentration in excess of maximum permissible levels of 1500ppm. Toothpastes 12, 14, 18 and 20 presented with sub-therapeutic fluoride concentrations ([TSF]<1000ppm). Error bars indicate 1 SD of uncertainty.

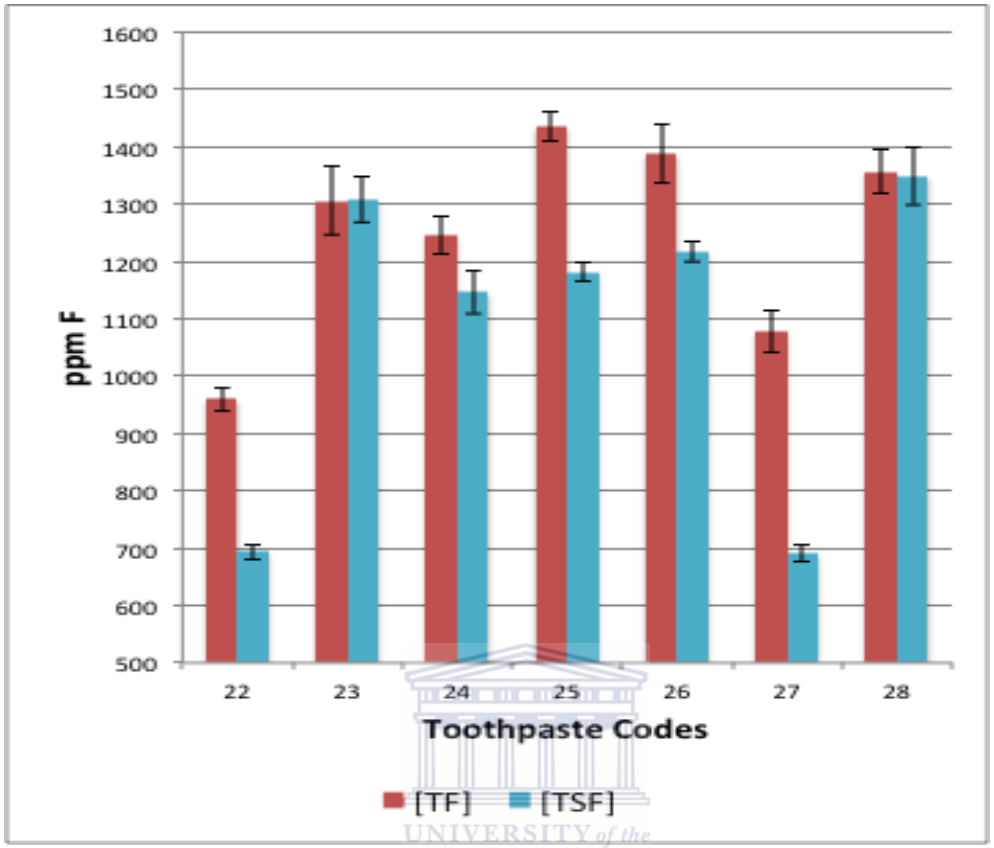

Figure 6: Concentration (ppm F) of total and total soluble fluoride on analyses for toothpastes Formulated with a calcium-based abrasive. Toothpastes 22 and 27 presented with sub-therapeutic fluoride concentrations ([TSF]<1000ppm). Error bars indicate 1 SD of uncertainty.

Further examination of the label indicates that this toothpaste should have been formulated with a 0.41% Na_2PO_3F and 0.21% NaF mass fraction, corresponding to a 539.5ppm Na_2PO_3F and 954.5ppm NaF content. On analysis, however, mean PO_3F^{2-} and F^- concentrations were recorded as 144.5ppm and 1328.7ppm respectively, not in keeping with stipulated ratios.

Two toothpastes coded 1 and 21**,** namely Aquafresh all-in-one and Dis-Chem Dentalmate, did not declare fluoride content (Figure 4; note absence of TF declared bar for toothpaste codes 1 and 21). On analysis mean total fluoride concentrations were found to be 1371.2ppm (sd ±70.2) and 1176.9ppm (sd ±42.7) respectively. Mean total fluoride concentrations ranging from 1151,8ppm (sd ±53.5) to 1387.5ppm (sd ±52) and 897.3ppm (sd ±36) to 960.1ppm (sd ±21) were obtained for 17 toothpastes declaring a 1450ppm F and 4 toothpastes declaring a 1000ppm F content respectively. Similarly, 1 toothpaste declaring a 1250ppm F, a 1100ppm F and 1040ppm F content were found on analysis to contain mean total fluoride concentrations of 1125.3ppm (sd ±26.6), 1077ppm (sd ±36.5) and 981.5ppm (sd ±25.2) respectively. The mean total soluble (available) content relative to mean total fluoride concentration (Tables 4,5,6 and 7; Figure 5 and 6) obtained for all toothpastes evaluated (n=26) was 95.1% (sd ±9.1), while relative mean soluble fluoride content for toothpastes formulated with a calcium-based abrasive was 85,5% (sd ±13.6; n=7) (Tables 6 and 7; Figure 6) opposed to 98.7% (sd ±2.1; n=19) for those containing a silica-based abrasive (Tables 4 and 5; Figure 5).

Similarly, relative mean available fluoride concentrations for toothpastes containing a SiO_2-based abrasive purchased in Cape Town and Johannesburg were found to be comparable and were calculated at 98.6% (sd ±2.1; n=9) and 98.8% (sd ±2.1; n=10) respectively (Tables 4 and 5). On the other hand a greater disparity in terms of relative mean available fluoride content was observed between these 2 groups for toothpastes containing a calcium compound (Tables 6 and 7, '[TSF] as % [TF]). Relative, mean available fluoride content obtained for the calcium-containing toothpastes purchased in Cape Town was 86,7% (sd ±12.1), while that for the Johannesburg samples was 83.8% (sd ±18).

These findings therefore strongly agree with evidence located in the literature which states that soluble/available fluoride content is unstable and declines on ageing of the product, especially evident among Ca-containing toothpastes. Conversely, the soluble/available fluoride content of Si-based toothpastes, irrespective of whether it is in F^- or PO_3F^{2-} remains relatively stable over time (de Oliveira Conde *et al.*, 2003:253; Hashizume *et al.*, 2003:198). These results, once again are representative of the chemical incompatibility between $Ca2^+$ and F^- in the formulation, further motivated by the fact that toothpaste coded 27, formulated with $NaF/CaSO_4$ presented with the lowest relative mean soluble (potentially bioavailable) fluoride concentration of 64.2% (Table 7; Figure 6 toothpaste code 27). Typically a calcium-containing compound is not combined with NaF as NaF dissociates readily releasing F^- having the potential to complex with Ca^{2+} (Cury, 2010:399; Thakkar *et al.*, 2015:3).

Chemical incompatibility between Ca^{2+} and F^- or monofluorophosphate ions (PO_3F^{2-}) is well documented in the literature. Historically, even as late as 2005, it was maintained by certain authorities that this incompatibility did not occur when Na_2PO_3F was combined with a calcium-containing abrasive in the toothpaste formulation. Consequently most toothpastes containing Na_2PO_3F are typically combined with $CaCO_3$ as the abrasive (Thakkar *et al.*, 2015:3). It is however interesting to note that in response to the findings of current research which raise doubts as to chemical compatibility between PO_3F^{2-} and Ca^{2+} there has been a shift away from this thinking as is demonstrated by the chemical composition of toothpastes analysed within the present study. If the chemical composition of the study toothpastes are examined (Tables 4 and 5, 'chemical form of fluoride'), it is found that 4 toothpastes, coded 5, 9, 14 and 18, (n=28) containing Na_2PO_3F are in fact formulated with a SiO_2 abrasive, with all 4 samples presenting with therapeutic soluble (>1000ppm F) and relative soluble (available), fluoride concentrations of 97.2%; 98.7%; 101.1% and 102%. These results indicate that all of the fluoride incorporated into the toothpaste formulation is in free available form (i.e. an ideal formulation). Conversely, toothpaste coded 27, formulated with $NaF/CaSO_4$ presented with a sub-therapeutic soluble (<1000ppm F) and relative mean soluble fluoride content of only 64.2% (Table 7).

Application of a paired t-test to recorded data found that mean total fluoride content on analysis was statistically significantly lower than manufacturer declaration ($p=1.2x10^{-7}$) (Figure 4). Similarly, mean total soluble (available) fluoride concentrations were found to be statistically significantly lower than mean total fluoride concentrations obtained on analysis ($p=0.0008$) at the 0.05 significance level (Figures 5 and 6). The Null hypothesis was therefore rejected in both instances. Mean percentage difference between total fluoride content on analysis and that declared was 8.6% (sd ±5.5), while percent difference between total soluble (potentially available) and total fluoride on analysis was 4.9% (sd ±9.1). Only 5 (17.9%) and 6 (21.4%) of the 28 toothpastes analysed presented with mean total and mean available fluoride concentrations below the critical 1000ppm F concentration required for anti-caries activity respectively (Figures 5 and 6).

5.1.5 Accuracy and Reproducibility

Results reflected in Tables 4, 5, 6 and 7 (column, 'No of Det.') represent the mean of either 16, 12 or 8 determinations with the standard deviations reflected in the columns alongside. The 16 determinations for each of the total, ionic and total soluble (in the case of Na_2PO_3F –containing samples) fluoride ion species, were obtained from quadruplicate analyses of a toothpaste (i.e. 4 separate weighings of a toothpaste were suspended in double distilled water from which 4 aliquots were then taken for total, ionic and/or soluble fluoride assessments). All toothpastes (n=28) were initially subjected to quadruplicate analyses.

Results for certain toothpastes displayed in Tables 4, 5, 6 and 7, however, are based on 8 determinations (i.e. duplicate weighings analysed in quadruplicate) alone. These toothpastes were re-tested due to slightly elevated pH values, ranging between pH5.6-5.8, obtained for the total fluoride trials. The same pH discrepancies were not observed for the soluble/available fluoride trials prepared for that same toothpaste and consequently the decision was taken to still analyse these samples. Interestingly, the results obtained on analysis of these samples corresponded closely with the re-tests and in fact total and total soluble fluoride concentrations recorded slightly lower than for the re-tests, an unexpected result as it would be assumed that excess OH^- ions would have been detected by the fluoride electrode producing elevated, false readings

(Table 8). In keeping with stipulated protocol parameters, however, only the re-test results are incorporated in calculations of mean total and total soluble fluoride concentrations for the entire sample. These results therefore suggest that interference by OH^- was negligible in the 5.6 to 5.8 pH range. Due to time and resource limitations, toothpaste re-tests were restricted to 8 determinations.

Table 8: Difference between [TF] and [TSF] for Initial (elevated pH) and Re-Test (pH5-5.5) Analyses

TP Code	[TF]int. (ppm)	[TF]re-test (ppm)	[TF]int.-[TF]re-test (ppm)	[TSF]int. (ppm)	[TSF]re-test (ppm)	[TSF]int.-[TSF]re-test (ppm)
3	1340,6	1349,66	-9,06	1353,7	1349	4,7
5	1470	1514,9	-44,9	1388,7	1473,2	-84,5
7	1224,9	1203,3	21,6	1173,3	1220,4	-47,1
9	1279,2	1342,9	-63,7	1318	1325,1	-7,1
15	1108,9	1318,3	-209,4	1074,7	1266,1	-191,4
22	959,5	960,1	-0,6	698	693,5	4,5
25	1366,6	1435,5	-68,9	1089,2	1181,6	-92,4
27	902,3	1077	-174,7	579,3	690,9	-111,6

For those toothpastes codified 1, 2, 8, 10, 21 and 24, results displayed in tables 4, 5 and 6 represent the mean of 12 determinations. For each of these toothpastes, millivolt potential/fluoride concentration for 1 of the 4 weighings/suspensions initially prepared, recorded markedly lower than for the remaining 3 suspensions, lying at approximately 1.5 to 1.6 standard deviations from mean total fluoride content, generating a coefficient variation greater than 5% and therefore falling outside of the current study's acceptance criteria. For each of these toothpastes, exclusion of these results (i.e. 4 determinations from a single weighing), produced a coefficient of variation less than or equal to 5% (Tables 4, 5 and 6; column 'Relative SD'), corresponding with defined acceptance parameters. Consequently these results were omitted from general reporting of data. If, however all 16 initial determinations are factored into the analyses of these samples it was found that coefficient variations in excess of 5% were predominantly associated with analysis of mean total fluoride content and not total soluble or ionic fluoride concentrations and this then raises questions regarding the repeatability of the assay in terms of this variable ([TF]).

The error bars displayed on Figures 5 and 6, represent 1 standard deviation of uncertainty from mean total and total soluble fluoride concentrations for each of the 28 toothpastes sampled. In general, the shorter the error bar, the greater was the concentration of readings on which the mean was calculated and consequently the more certain/accurate the mean value was expected to be. Length of the error bar also provides a measure of the degree of methodological precision and reproducibility between assays within a toothpaste sample. From Figures 5 and 6, error bars are visibly narrower for soluble/available fluoride determinations as opposed to total fluoride determinations, providing evidence that the fluoride electrode displays a greater degree of sensitivity/accuracy in soluble fluoride determinations (i.e. those from which the insoluble fluoride fraction has been removed) and in fact if the standard deviations for all determinations (total, total soluble and ionic fluoride) are analysed (Tables 4, 5, 6 and 7; 'SD columns') it is found that the fluoride electrode displays greatest accuracy in ionic fluoride detection for Na_2PO_3F-containing samples. Findings of the current study therefore suggest that for direct analysis with fluoride ion selective electrode, that the electrode is best suited to ionic fluoride detection and that for total fluoride analyses, especially if high degree of accuracy is required, determination should be confined to direct potentiometric analysis following sample preparation according to a microdiffusion technique, such as, acid-hexamethyldisiloxane (HMDS) diffusion or depending on accessibility gas chromatographic method so as to control for any interference (fluoride complexation with cations present in the formulation).

A 1mV difference equated to an approximate 50ppm F concentration difference. A greater degree of accuracy of results would have been obtained if the voltmeter used had had a 0.1mV resolution.

5.1.6 Conclusion

In conclusion and in answering the first two major objectives of this study, namely, determination of total and total soluble (potentially bioavailable) fluoride content of toothpastes marketed to the South African consumer it was found that 78.6% of toothpastes sampled presented with adequate total and potentially bioavailable fluoride concentrations and therefore the ability to disrupt the caries process.

5.2 SECTION B: LABELING PRACTICES RESULTS

5.2.1 Introduction

In addition to fluoride content of toothpastes, another factor relevant to the quality of the product is the packaging and marking practices that are employed. Packaging is fundamental in protecting the product against contamination from microorganisms, moisture and dehydration while appropriate labeling practices that do not confuse or mislead by implication, provides a platform for effective communication with and education of the consumer.

Consequently two major objectives of the present study were to analyse labeling practices in terms of the information provided and conformity with prescribed marking regulations as stipulated within the South African National Standards (SANS 1302:2008).

According to the Standards Act, 2008 (Act no. 8 of 2008), the mandate of the South African Bureau of Standards (SABS) includes the development, maintenance and promotion of South African national standards as well as provision of compliance assessment services to industry in order to advance South Africa's international economic standing and competitiveness while protecting the health and safety of civil society, the environment and ensuring responsible utilization of resources (SABS, 2012:4). Standards generated by the SABS serve as a framework within which all stakeholders should operate to ensure production of goods or provision of services of consistent quality. Affairs of the SABS are governed by the Board of the SABS, whose members are appointed by the minister of trade and industry (SABS, 2012:3).

5.2.2 South African National standard – Toothpaste

SANS 1302:2008 (edition 1.1) documentation pertaining to toothpastes was ratified by the National Committee SABS SC271B, Cosmetics – Toothpastes, was published in June 2008 and supersedes SANS 1302:1980 (edition 1). Packing and marking regulations as stipulated within this documentation are summarized hereunder (SABS 1302, 2008).

Toothpaste is to be packaged in leak-proof, collapsible tubes bearing the total nominal volume or in the case of composite packs (i.e. two or more toothpastes of equivalent or differing net volume(s) and in which the tubes are separated from one another) the total nominal volume of the pack. The volumes to be used are specified as any volume up to 15ml and 35ml, 50ml, 75ml, 100ml, 125ml, 150ml, 175ml and 200ml thereafter.

Individual and composite packs are to be packaged in such a manner that affords protection against contamination and damage during transportation, handling and storage. Bulk packages must comprise individual tubes of equivalent net volume and batch identification, where batch refers to a quantity of toothpaste that is manufactured from the same ingredients and filled into tubes during one continuous cycle of operations or composite packs of tubes of equivalent nominal volumes and batch numbers.

In terms of tube labeling, in addition to nominal volume, the name of the toothpaste, the name and contact information of the manufacturer or responsible distributor, a description of content, batch number and if the product is fluoridated the phrase, “Fluoride Toothpaste” or “Fluoride Dental Crème” is to be displayed. This phrase is to be marked in a durable and intelligible manner with a font size of 3mm and must be readily visible against the background. Similarly all marking requirements, excluding batch identification, as stipulated for the tube are to be applied in marking of the outer carton, packaging of composite packs and bulk packages.

It is of interest to note that the SANS 1302:2008 marking guidelines differ in certain respects from those stipulated in the internationally recognized and accepted ISO 11609:2010 framework. Consideration of ISO 11609:2010 in the context of this study is pertinent due to the global marketing of products not manufactured in South Africa, yet available to local consumers. Consequently labeling practices were also evaluated in terms of the ISO11609:2010 regulations.

All SANS 1302:2008 labeling requirements are represented in the ISO 11609:2010 guidelines set forth for marking of the primary package/tube. The latter however does deviate away from SANS 1302:2008 in that it requires an expiry date to be reflected if product shelf-life is less than 30 months, that a cautionary note pertaining to use in paediatric consumers below six years of age be displayed in instances where total fluoride concentration exceeds 1000ppm and inclusion of a readily identifiable production date in the batch identification/tracking code (European Organization for Standardization, 2010:4). Additionally, although specifications in terms of fluoride content are comparable between the two documents and require that total fluoride concentration not exceed a mass fraction of 0,15% (or 1500ppm) (European Organization for Standardization, 2010:2; University of Pretoria and South African Legal Information Institute, 2014:11), ISO 11609:2010 requires that the chemical for of fluoride and total fluoride concentration in micrograms per gram and/or percent by mass be declared in labeling of the product, while SANS 1302:2008 merely requires that the words, “Fluoride Toothpaste” or “Fluoride Dental Créme” be reflected (European Organization for Standardization, 2010:4; SANS 1302, 2008:6). In both documents, no mention is made with respect to the minimum soluble fluoride content that needs to be maintained in the formulation.

5.2.3 Sample Size

As discussed within chapter 4, "Research Design and Methodology", sub-heading, "fluoride toothpaste samples", initially, paediatric and adult fluoridated toothpastes, excluding herbal brands and those claiming to have tooth-whitening properties, were conveniently purchased off the shelf of a Dis-Chem outlet located in metro Cape Town. The toothpastes sampled in this manner were used to construct a 'gold standard' list that functioned to direct sampling from the other retailers and 5 provinces included in the study.

A total of 300 toothpastes (n=300) were purchased across ten sampling sites, namely, two retailers, Dischem and Pick 'n Pay, per region/province. Refer to Appendix II and Table 9 for a complete listing of toothpaste numbers, brands and types sampled and assessed per site.

Each toothpaste/sample (n=300) was evaluated in terms of packing and marking regulations (variables) stipulated in the SANS 1302:2008 (edition 1.1) documentation. As a number of these variables, toothpaste brand and name, nominal volume, manufacturer or distributor contact details, content description and the presence of the phrase 'fluoride toothpaste' were and are identical for the same toothpaste types irrespective of sampling site, wherever possible, to improve ease of interpretation, the data obtained was reported in terms of the 'gold standard' list alone, having a total sample size (n) of 38. Results of the SANS compliance analysis for total sample and per product are summarized in Tables 10 and 11 respectively.

Table 9: Sample Size - Toothpaste Type And Number Per Site											
Toothpaste Samples	**Sampling Sites**										
	BD	**BP**	**CD**	**CP**	**DD**	**DP**	**JD**	**JP**	**PD**	**PP**	**TOTAL**
Paediatric Toothpastes											
Aquafresh Milk Teeth 0-3yrs	1	1	1	1	1	1	1	1	1	1	10
Aquafresh Little Teeth 4-6yrs	1	1	1	1	1	1	1	1	1	1	10
Aquafresh Big Teeth >6yrs	1	1	1	1	1	1	1	1	1	1	10
Colgate 0-2yrs	1	1	1	1	1	1	1	1	1	1	10
Colgate 2-5yrs	1		1			1	1	1	1	1	7
Colgate >5yrs	1	1	1	1	1		1	1	1	1	9
Dis-Chem Dentalmate Kids			1				1				2
Elgydium Kids 2-6yrs	1		1		1		1		1		5
Mentadent P Kids	1	1	1	1	1		1	1	1	1	9
Nature Fresh Junior Toothpaste			1				1		1		3
Adult Toothpastes											
Aquafresh all-in-one Protection	1	1	1	1	1	1	1	1	1	1	10
Aquafresh Extreme Clean	1		1	1	1	1		1	1	1	8
Aquafresh Ultimate	1		1	1	1		1	1	1	1	8
Close Up Deep Action	1		1		1	1	1		1		6
Colgate Active Salt		1	1	1			1		1		5
Colgate Gel		1	1		1		1	1	1	1	7
Colgate MaxFresh	1	1	1	1	1	1	1	1	1	1	10
Colgate Maximum Cavity Protection	1	1	1	1	1	1	1	1	1	1	10
Colgate Sensitive Multiprotection		1	1	1	1	1	1	1	1	1	9
Colgate Sensitive Pro-Relief		1	1	1	1	1	1	1	1	1	9
Colgate Total 12		1	1			1	1		1	1	6
Colgate Pro-Gum Health	1	1	1	1	1	1	1	1	1	1	10
Colgate Triple Action	1	1	1	1	1	1	1	1	1	1	10
Dis-Chem Dentalmate	1		1		1		1		1		5
Elgydium Sensitive	1		1		1		1		1		5
Enamel Care			1		1		1		1		4
GUM Caries Protect			1				1		1		3
Mentadent P Gel Protection	1	1	1		1	1	1	1	1	1	9
Mentadent P Micro Granules	1	1	1	1	1	1	1	1	1	1	10
Mentadent P Protection	1	1	1	1	1	1	1	1	1	1	10
Mentadent P Sensitive	1	1	1	1	1	1	1	1	1	1	10
Oral B Pro-Expert		1	1	1	1		1	1	1	1	8
Pepsodent	1		1	1		1	1		1		6
Pepsodent Complete 8	1		1	1	1		1		1	1	7
Sensodyne Cool Gel	1	1	1	1	1	1	1	1	1	1	10
Sensodyne Multi Care	1	1	1	1	1	1	1	1	1	1	10
Sensodyne Rapid Action	1	1	1	1	1	1	1	1	1	1	10
Sensodyne Repair & Protect	1	1	1	1	1	1	1	1	1	1	10
Sample number per site	28	25	38	26	31	24	37	26	37	28	**300**

***KEY:** 1st Letter refers to site, namely, B (Bloemfontein), C (Cape Town), D (Durban), J (Johannesburg) and P (Port Elizabeth). 2nd Letter refers to retailer, namely, D (Dischem) and P (Pick 'n Pay), for example, CD implying Cape Town Dischem

Table 10: Total Sample Compliance With Sans Packaging and Marking Guidelines					
Sample size (n) = 300					
Variables				**Number Compliant**	**Percentage Compliance**
1	**Collapsible, "leak-proof" tubes:**			300	100
2	**Presence of outer carton**			299	99
3	**Batch/Lot Identification:**	Box		271	90,3
		Tube		**254**	**84,7**
	"Gold Standard" Sampling List: Sample size (n) = 38				
4	**Toothpaste Brand & Name:**			38	100
5	**Nominal Volume (ml):**			35	92,1
6	**Address Manufacturer/Distributor:**			38	100
7	**Content Description:**			34	89,4
8	**Product Descriptor - Phrase "Fluoride Toothpaste":**	Box	Present	22	57,9
			Font size 3mm	8	21,1
			Distinct	16	42,1
			All Applied	8	21,1
		Tube	**Present**	**24**	**63,2**
			Font size 3mm	**0**	**0**
			Distinct	**24**	**63,2**
			All Applied	0	0
		Both		0	0

***As variables 4 to 8 are identical for the same toothpaste brands and types across the sampling range statistics pertaining only to the gold standard sampling list are reflected. As the outer packaging or carton is typically discarded after purchase, results relative to tube labeling are highlighted, emphasizing the significance of the primary package in communicating with, education of and therefore protection of the consumer.**

5.2.4 Compliance with SANS

In evaluating compliance with prescribed packaging and marking specifications stipulated in the South African national standard, specifications within this framework were subdivided into 8 variables. Variables 1 and 2 pertained to packaging, while the remaining variables, 3 to 8, related to labeling/marking (Table 10). As labeling regulations pertain to both the tube and outer carton, labeling compliance analyses were undertaken for both. Conformity with each of the packing and marking variables is discussed in the following text.

No toothpastes (n=300) sampled applied all, eight, regulations/variables framed under the current SANS guidelines. A majority, 71,05% (n=38) of fluoridated toothpastes reflected on the gold standard list adhered to 5 of the 6 (83.3%) marking requirements (Table 11). The least compliant toothpaste, Dis-Chem Dentalmate Kids applied only two dictates, namely product name and distributor address, neither of which have any bearing on consumer education around product safety or potential health benefits.

5.2.4.1 Variables 1 and 2: Packaging (Table 10)

All toothpastes (n=300) complied with packaging specifications in terms of being contained within "leak-proof", collapsible tubes. Likewise, all samples, excluding 'Nature Fresh Junior Toothpaste' (3; n=300) were packaged in individual outer cartons. The packaging of 'Nature Fresh Junior Toothpaste' was restricted to a tube alone and it may therefore be assumed that this compromises the degree to which the toothpaste is protected against contamination and degradation under normal conditions of transportation, handling and storage. In interpretation of the results, absence of an outer carton, in terms of this product, was taken into account in calculation of box marking statistics.

5.2.4.2 Variable 3: Batch Identification (Table 10 and 11)

According to SANS 1302:2008 (edition 1.1), batch may be defined as a quantity of toothpaste manufactured from the same materials during a single, continuous cycle of operations, while lot refers to 100 toothpastes or more, produced by the same manufacturer and bearing an identical nominal volume and batch identification that may be submitted at a specific point in time for quality assurance purposes (SABS, 2008:3). According to this documentation and as reflected in the introductory discussion it is only necessary for the batch identification to be displayed on the primary packaging, taken to mean the container, in this instance the tube, that is in direct contact with the product. Paradoxically fewer toothpastes sampled, 84,67% (n=300) presented with a tube batch identification as opposed to the not required representation on the box, present on 90,33% of boxes (Table 10 variable 3).

Non-compliance was primarily attributed to the illegibility of this information with numbers being smudged on the outer box, tube or imprint superimposed onto corrugated, terminal tube seal. Certainly, embossing of this information onto the tube complies with durability requirements, but depending on technique employed disregards visibility regulations. Questions need to be investigated as to the influence on batch identification visibility of the sequencing of tube manufacture and marking procedures (i.e. imprinting batch number concurrently, pre- or post- sealing of tube terminal) and appropriate recommendations made.

Notably, although unnecessary in terms of specified standards, batch identification was omitted from the marking of the outer package for all Oral-B Pro-Expert and Mentadent P Kids toothpastes (10; n=300), Dentalmate Adult (5; n=300), Dentalmate Kids (2; n=300) and 1 of 5 Colgate Active Salt toothpastes sampled. Similarly batch identification for 1 of 9 Colgate >5yrs and 1 of 6 Close Up Deep action toothpastes sampled were omitted in calculation of box marking statistics due to illegibility/smudging of the information.

On the other hand batch number illegibility was the key reason for breach of this dictate in terms of tube marking with all Colgate Total 12 (6 of 6), 9 of 10 Colgate MaxFresh and Colgate Pro-Gum Health, 2 of 10 Colgate Maximum Cavity Protection, 4 of 9 Colgate Sensitive Multiprotection, 1 of 10 Mentadent P Micro Granules, 1 of 9 Mentadent P Gel Protection, 1 of 8 Aquafresh Ultimate, 6 of 10 Colgate 0-2 years and 3 of 7 Colgate 2-5 years toothpastes sampled, presenting with unreadable numbers (Table 9). The fact that not all tubes of the same brand and type sampled were involved indicates a need for improvements in quality assurance procedures. These statistics also demonstrate that illegibility of batch identification information occurs frequently and that although these products contravene policy and ideally should not reach the shelf, withdrawal would have significant financial implications for manufacturers. Major concern therefore centers around inability to track and trace these items through the supply chain and impact that this may have on consumer trust.

Fluoridated Toothpaste Samples	Table 11: Compliance With Sans Marking Guidelines Per Product Sample Size (n) = 38 Batch Identification *Tube Only*	Product Name	Nominal Volume	Manufacturer Address	Content Description	Product Descriptor	Number of Regulations Applied	% Compliance per Product
Aquafresh Milk Teeth 0-3yrs	1	1	1	1	1	0	5	83,3
Aquafresh Little Teeth 4-6yrs	1	1	1	1	1	0	5	83,3
Aquafresh Big Teeth >6yrs	1	1	1	1	1	0	5	83,3
Colgate 0-2yrs	0	1	1	1	1	0	4	66,7
Colgate 2-5yrs	1	1	1	1	1	0	5	83,3
Colgate >5yrs	1	1	1	1	1	0	5	83,3
Dis-Chem Dentalmate Kids	0	1	0	1	0	0	2	33,3
Elgydium Kids 2-6yrs	1	1	1	1	1	0	5	83,3
Mentadent P Kids	1	1	1	1	1	0	5	83,3
Nature Fresh Junior Toothpaste	1	1	1	1	0	0	4	66,7
							0	0,0
Aquafresh all-in-one Protection	1	1	1	1	0	0	4	66,7
Aquafresh Extreme Clean	1	1	1	1	1	0	5	83,3
Aquafresh Ultimate	1	1	1	1	1	0	5	83,3
Close Up Deep Action	1	1	0	1	1	0	4	66,7
Colgate Active Salt	1	1	1	1	1	0	5	83,3
Colgate Gel	1	1	1	1	1	0	5	83,3
Colgate MaxFresh	0	1	1	1	1	0	4	66,7
Colgate Maximum Cavity Protection	0	1	1	1	1	0	4	66,7
Colgate Sensitive Multiprotection	1	1	1	1	1	0	5	83,3

Table 11: Compliance with SANS Marking Guidelines Cont'd								
Fluoridated Toothpaste Samples	**Batch Identification** ***Tube Only***	**Product Name**	**Nominal Volume**	**Manufacturer Address**	**Content Description**	**Product Descriptor**	**Number of Regulations Applied**	**% Compliance per Product**
Colgate Sensitive Pro-Relief	1	1	1	1	1	0	5	83,3
Colgate Total 12	0	1	1	1	1	0	4	66,7
Colgate Pro-Gum Health	0	1	1	1	1	0	4	66,7
Colgate Triple Action	1	1	1	1	1	0	5	83,3
Dis-Chem Dentalmate	1	1	1	1	0	0	4	66,7
Elgydium Sensitive	1	1	1	1	1	0	5	83,3
Enamel Care	1	1	0	1	1	0	4	66,7
GUM Caries Protect	1	1	1	1	1	0	5	83,3
Mentadent P Gel Protection	1	1	1	1	1	0	5	83,3
Mentadent P Micro Granules	1	1	1	1	1	0	5	83,3
Mentadent P Protection	1	1	1	1	1	0	5	83,3
Mentadent P Sensitive	1	1	1	1	1	0	5	83,3
Oral B Pro-Expert	1	1	1	1	1	0	5	83,3
Pepsodent	1	1	1	1	1	0	5	83,3
Pepsodent Complete 8	1	1	1	1	1	0	5	83,3
Sensodyne Cool Gel	1	1	1	1	1	0	5	83,3
Sensodyne Multi Care	1	1	1	1	1	0	5	83,3
Sensodyne Rapid Action	1	1	1	1	1	0	5	83,3
Sensodyne Repair & Protect	1	1	1	1	1	0	5	83,3
Total Compliant per Regulation	32	38	35	38	34	0		
Proportion Compliant per Regulation	84,2	100	92,1	100	89,5	0		

Another irregularity encountered in evaluation of batch identification that represents an absence of effective quality control and that would have significant implications with respect to consumer trust due to lack of product traceability through the supply chain is the supply of different products (Sensodyne Cool Gel and Sensodyne Multi Care) bearing the same batch identification. Three products, one sample of Cool Gel obtained from Bloemfontein Dis-Chem and two samples of Multi Care purchased from Dis-Chem outlets located in Bloemfonteim and Johannesburg presented with 4321 as the batch identification on the outer carton and 064321 on the tube with all samples due to expire 07/2016 (Figure 8). Sensodyne Cool Gel and Multi Care are two different products with Cool Gel presenting as a blue gel and Multi Care a white paste. Additionally if the ingredients list is analysed, the lists for both products correspond except for that of Cinnamal, or Hexyl Cinnamaldehyde a synthetic aroma commonly used as an additive in cosmetics (Cosmetics Information Organization, 2015), with CI 42090 and CI 77891 being incorporated into the formulation of Multi Care and CI 42090 alone being incorporated into that of Cool Gel. If according to SANS 1302:2008 (edition 1.1), a batch is defined as a quantity of toothpaste manufactured from the same materials during a single, continuous cycle of operations then these two products are definitely not from the same batch. In addition fluoride content labeling irregularities were noted for the Cool Gel (Bloemfontein Dis-Chem) product. An adhesive label reflecting the toothpaste composition had been stuck over that of the outer carton (Figure 7). This label indicated a NaF concentration of 1450ppm while marking on the underlying box only declared a 1400ppm NaF content, the latter being reflected on tube marking as well. If what was reflected on the adhesive label is correct, this provides further evidence that Cool Gel and Multi Care are indeed two different products otherwise a similar adhesive label should have been applied to the packaging of the Multi Care samples bearing the same batch identification, unless the discrepancies in batch identification precluded appropriate tracking of product through the supply chain? All Sensodyne Cool Gel products manufactured after this date have retained the 1400ppm NaF content marking in labeling of both the outer carton and tube. The question then arises as to whether amendments to fluoride concentration in this instance were consequent to internal quality control processes that identified an elevated fluoride content for this batch? Should the elevated concentration then not have been corrected on both the outer carton and tube and for all products bearing the same batch identification (Figure 7)?

The fact that amendments were only made to the Cool Gel product seem to suggest that the Multi Care products were the toothpastes bearing the incorrect batch identification.

Figure 7: Adhesive label evident on outer carton. No label evident on the tube, the marking on which declares a 1400ppm F content as opposed to 1450ppm F declaration on the adhesive label.

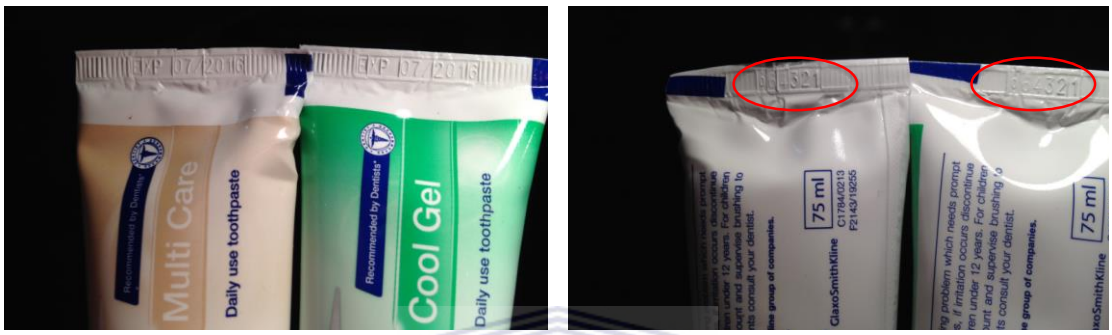

Figure 8: Two different toothpastes having the same distributor and bearing the same batch identification (064321) on the tube.

Figure 9: Two different toothpastes having the same distributor, purchased from different retailers in Cape Town, bearing the same batch identification (104343).

A similar discrepancy with respect to batch identification was noted for a sample of Cool Gel and Multi Care purchased from Cape Town Dis-Chem and Pick 'n Pay respectively. Both these samples were marked with 104343 as the batch identification on the tube and an expiry date of 08/2016 (Figure 9).

5.2.4.3 Variables 4 and 6: Toothpaste Brand and Name; Manufacturer or Distributor Address (Table 10)

All toothpastes sampled (n=300) presented with a brand name or key identifier by which the consumer recognizes the product and a manufacturer or distributor address

5.2.4.4 Variable 5: Nominal Volume (Table 11; Appendix II)

A minority, 7,89% of toothpastes, namely, Dischem Dentalmate Kids, Close Up Deep Action and Enamel Care, sampled (n=38), did not display nominal volume. Quantity was still however declared in terms of weight in grams, with weights of 70g, 125g and 115g reflected respectively. Close-Up Deep Action is manufactured in Vietnam, Enamel Care in the United Kingdom, while place of manufacture for Dischem Dentalmate Kids is not indicated, although it is suspected that the latter may be manufactured in Bulgaria for distribution by Dis-Chem South Africa as the latest Dentalmate sensitive and whitening toothpastes do indicate Bulgaria as place of origin. Place of manufacture is considered significant as these products do comply with ISO 11609:2010 regulations that allow for quantity to be expressed as a net volume in milliliters or weight in grams (European Committee for Standardization, 2010:4). ISO norms although internationally agreed and recognized have not been adopted within the South African context.

5.2.4.5 Variable 7: Content Description (Table 10)

The ‘content description’ regulation implies through use of the term ‘description’ that a listing of all ingredients in conjunction with an explanation of the concentration and function or purpose of each ingredient be provided. Possibly due to space limitations imposed by the size of the outer carton and tube and requirements that all information be presented in a visible and legible manner, majority of toothpastes sampled confined content ‘description’ to a listing of constituents and frequently an indication of the active ingredient, in most cases (excluding the desensitizing products) the chemical form of fluoride incorporated in conjunction with fluoride concentration.

The presence of these elements (i.e. constituent listing that included the abrasive agent, chemical form of fluoride and declaration of total fluoride concentration) was taken to indicate compliance with this edict. A majority, 89.47% of the sample (n=38) provided a product description or rather a listing of ingredients (Table 10) in marking of the outer carton. Nonobservance was noted for Dis-Chem Dentalmate paediatric and adult toothpastes, Nature Fresh Junior toothpaste and Aquafresh all-in-one protection (Table 11). Dis-Chem Dentalmate Kids and Nature Fresh Junior toothpaste declared no information with respect to formulation and as a result no indication of the abrasive agent and fluoride content or chemical form, suffice to say that fluoride was present, while Dis-Chem Dentalmate adult toothpaste and Aquafresh all-in-one protection did not stipulate fluoride concentration.

As abrasives influence fluoride bioavailability and rate of fluoride release, reflection of the abrasive agent in the list of ingredients is of paramount importance. Fortuitously 94.7% of toothpastes sampled (n=38) displayed the abrasive in listing of the constituents, although it is not designated as the abrasive meaning that the lay person may have difficulty in recognizing it as such (Appendix II).

5.2.4.6 Variable 8: Product Descriptor (Table 10 and 11)

No toothpastes sampled were found to conform to the product descriptor regulation (i.e. presence of the phrase 'fluoride toothpaste' with font size of 3mm and distinct from the background) in marking of the tube. Eight (n=38), however, did meet specifications in marking of the box/outer carton, these included Mentadent P Kids, Colgate Active Salt, Colgate Gel, Colgate MaxFresh, Colgate Maximum Cavity Protection, Colgate Sensitive Pro-Relief, Colgate Total 12 and Colgate Triple Action, demonstrating greatest/overwhelming compliance by the Colgate brand as well as locally manufactured products. Of the products listed, Mentadent P Kids, Colgate Gel, Colgate Maximum Cavity Protection and Colgate Triple Action are manufactured in South Africa. Presumably observance of this regulation among local products is due to greater familiarity with the national standards. These results were obtained in terms of a strict application of the ruling (i.e. precise conformity with all aspects of this variable).

A less rigorous application of this standard increases compliance percentage from 0% to 7,89% and 21,1% to 28,95% (Table 10) in terms of tube and box labeling respectively as other locally manufactured products, specifically Mentadent P Protection, Mentadent P Gel Protection and Mentadent P Micro Granules display the descriptor, “Fluoride Toothpaste” in a durable and visible manner with the exception that a 4mm font size is used in marking of both the tube and box (Appendix II). Toothpastes sampled that demonstrated complete compliance with specifications apart from a 2mm or smaller font size were omitted from this lenient interpretation as it can be argued that visibility, due to diminished font size, was significantly reduced.

Likewise, if those that conformed with size and visibility parameters, but displayed alternative phrases such as “with fluoride”, “fluoride” and the concentration or “active element: fluoride” as opposed to required terminology were also incorporated in terms of a less rigorous application of the ruling then further improvements in box marking compliance statistics from 28,95% to 44,74% was recorded. These samples included the internationally manufactured Colgate paediatric products as well as the paediatric and adult Dentalmate products and were considered for inclusion as the watchword “fluoride” was used. The paediatric and adult Elgydium products displaying the phrase “toothpaste gel with fluorinol” were however excluded as it may be asserted that the layperson would not understand what is meant by the term “fluorinol” (Appendix II). Once again place of manufacture was regarded as significant because as per the ISO 11609:2010 directive only the word “dentifrice” or equivalent need be marked (European Committee for Standardization, 2010:4).

5.2.5 Strength of SANS 1302:2008 (ed. 1.1)

In contrast to current labeling practices employed in marking the primary packaging of most samples and in relation to ISO 11609:2010 guidelines (European Committee for Standardization, 2010:4), a number of frank omissions are evident from SANS 1302:2008 (edition 1.1) regulations. These omissions pertain to key information, namely, expiry date, chemical form of fluoride, total fluoride concentration, cautionary note regarding use in paediatric consumers, storage instructions and guidelines for use, the provision of which can be regarded as mandatory in terms of product safety and health benefits and therefore fulfillment of full consumer protection. Take note that the two later variables, storage instructions and guidelines for use are also absent from the ISO11609:2010 framework. Consequently, each sample was also evaluated in terms of these omitted variables. Refer to Table 12 for a summary of results obtained for these analyses.

As is evident from Table 12, application of the 'additional' ISO labeling regulations (i.e. those not mentioned within SANS) in marking of the primary package is widely divergent with results ranging form 65,8% (n=38) for cautionary note regarding use in paediatric consumers to 99% (n=300) for 'expiry date'. The 1% failing to present an expiry date may be accounted for by the fact that ISO11609:2010 guidelines only require that an expiry date be reflected if product stability is less than 30 months. This, however, was not a finding of the present study as the three toothpastes failing to present with this information do in fact, as indicated by the manufacturer, have a 24 month stability.

ISO labeling regulations pertain to the tube alone. It is therefore noteworthy that only 77.3% of toothpastes sampled displayed an expiry date in marking of the outer carton. Products lacking a stability declaration on the box included Colgate MaxFresh, Colgate Pro-Gum Health, both the paediatric and adult Dis-Chem Dentalmate toothpastes, Mentadent P Kids, Nature Fresh Junior toothpaste in which packaging, as discussed previously, is limited to a tube only and a few samples (not all) of Colgate Active Salt (Appendix II).

Table 12: Compliance With ISO 11609:2010 Marking Guidelines				
	Sample size (n) = 300			
Variables			**Number Compliant**	**Percentage Compliance**
1	Stability/Expiry Date	Box	232	77,3
		Tube	**297**	**99**
	"Gold Standard" Sampling List: Sample size (n) = 38			
2	Chemical Form of Fluoride		36	94,7
3	Total Fluoride Concentration		34	89,5
4	Safety Notice re. use in Paediatric Consumers	Box	35	92,1
		Tube	**25**	**65,8**
		Both	25	65,8
Additional information provided by manufacturers not covered in SANS 1302:2008 and ISO11609:2010 Frameworks.				
5	Directions for use	Box	26	68,4
		Tube	**18**	**47,4**
		Both	13	34,2
6	Storage Instructions	Box	12	31.6
		Tube	**10**	**26.3**
		Both	10	26.3

***As variables 2 to 6 are identical for the same toothpaste brands and types across the sampling range statistics pertaining only to the gold standard sampling list are reflected. As the outer packaging or carton is typically discarded after purchase, results relative to tube labeling are highlighted, emphasizing the significance of the primary package in communicating with, education of and therefore protection of the consumer.**

Four of a total of five samples of Colgate Active Salt, bearing differing lot/batch identification numbers, purchased form Dis-Chem Port Elizabeth (manufactured 8/13), Dis-Chem Johannesburg (manufactured 07/14), Pick ‘n Pay Cape Town (manufactured 10/14) and Bloemfontein Pick ‘n Pay (manufactured 10/14) stipulated a manufacture date alone in marking of the outer carton (Figure 10, 4 toothpaste tubes not circled present in same order as discussed in text). There were two exceptions - one sample purchased from Dis-Chem Cape Town that displayed a manufacture (11/13) and expiry date (10/2015) on both the outer carton (Figure 10, circled product) and tube and a second sample obtained from Dis-Chem Port Elizabeth that presented with an expiry date (07/15) on the tube (Figure 11), but not the outer carton which declared a manufacture date (08/13) alone. The 3 remaining samples, namely, one from Dis-Chem Johannesburg, Pick ‘n Pay Bloemfontein and Cape Town only declared a manufacture date on both the outer carton and tube.

A two-year product stability was declared for those presenting with an expiry date. A common manufacturer, namely, Colgate-Palmolive India, produces all samples of Colgate Active Salt distributed within South Africa. Why then the discrepancy? Was it that regulations were amended after 10/13 or were these specific lots requested for internal or external quality assurance purposes either by authorities located within Colgate Palmolive or national standards regulators? In terms of this product it is vitally important that information with respect to product stability be stipulated due to the 24 month shelf-life and because as per manufacturer declaration this toothpaste contains a maximum total Na_2PO_3F concentration of 1000ppm F (minimum content required for anti-caries activity) and is formulated in combination with a $CaCO_3$ abrasive. In general, Na_2PO_3F/CaCO3-containing products, due to chemical incompatibility, degrade to a greater degree than NaF/Silica based products implying that if an insoluble fluoride fraction is present (the greater the fraction closer to expiration), fluoride bioavailability will most assuredly present at sub-therapeutic levels (Cury *et al.*, 2010:399). An assumption supported by the findings of the fluoride analyses. On analysis this toothpaste was found to contain a mean total soluble F content of 693.5ppm F and thus a mean insoluble fraction of 266.6ppm F.

Figure 10: Samples of Colgate Active Salt all displaying a manufacture date, excluding one (circled) displaying both a manufacture and expiry date.

Figure 11: Expiry dates only evident present on only two toothpaste tubes (circled). The remaining 3 tubes bear manufacture date alone.

Interestingly the ISO guidelines do require that a tracking code, taken to mean batch identification, be displayed on the tube and that this tracking code must include an intelligible production date. If the gold standard list, raw data collection sheets are examined (Appendix II) very few toothpastes are found to comply with this standard.

5.2.6 Conclusion

In conclusion and in answering objectives 3 and 4 of the present study, partial compliance with SANS 1302:2008 (ed. 1.1) marking requirements was observed for all toothpastes sampled. Due to the global marketing of toothpastes, however, a large number of manufacturers were also found to partially conform to ISO11609:2010 marking criteria.

CHAPTER 6: DISCUSSION

6.1 SECTION A: FLUORIDE ANALYSES

6.1.1 Introduction

Numerous clinical trials have consistently demonstrated the caries reducing benefits of fluoridated toothpastes (Bratthall *et al.*, 1996: 416; Hargreaves, 1983 cited in Zero, 2006; Jenkins, 1985:1298). Due to increasing accessibility within non-established market economies (van Loveren *et al.*, 2003:225), fluoridated toothpastes have gained global recognition as the principle means for delivering topical fluoride and obtaining caries preventive benefits (Zero, 2006). Although clinical trials are regarded as the gold standard for evaluating the anti-caries efficacy of a fluoridated toothpaste, the time and expense associated with such an undertaking has led to the development of numerous laboratory-based procedures for analysis of the total and total soluble (available) fluoride content of toothpastes, the latter providing an indirect measure of potential fluoride bioavailability and therefore anti-caries efficacy (Grobler *et al.*, 1983:475). The potentiometric determination of fluoride using ion-selective electrode is one such analytical technique that has been proven to be accurate, inexpensive and convenient and was therefore employed in the present study to make observances with respect to the efficacy of the fluoridated toothpastes sampled.

6.1.2 Study Limitations

Due to a number of factors results for the present study should be interpreted with caution. These are as follows:

Convenience Sampling: As a convenience, non-systematic sampling technique was used to obtain toothpastes incorporated in this study and due to the small sample size, results obtained for the fluoride analyses are representative of the actual toothpastes tested and cannot be used to draw inferences regarding fluoride content of toothpaste brands or types.

Sample Preparation: Currently multiple sample preparation methodologies for direct analysis of fluoride content using ion selective electrode are reported in the literature. Consequently, the technique used in the present study may differ from that used by other researchers meaning that the data obtained cannot be compared to that published by other studies. To allow for comparisons between multi-center studies as well as diverse formulations there is thus a significant need for development of a standardized, reliable and convenient analytical technique.

Voltmeter Accuracy: The voltmeter used did not read to decimal places meaning that the last significant figure was in the unit's position (ones' place). This then limited the accuracy or "trueness" of the value measured to its' actual value and therefore the precision of the experiment (ability to obtain the same value on repetition). On average a 1mV change in reading equated to a 50ppm F concentration difference and so introduced measurement error.

Sample Size: To validate the accuracy of the results obtained there is a need to repeat the analyses using a larger sample of toothpastes.

International Labeling Variations: In response to local standards and regulating authorities, large variations in marking practices are observed between different countries. The packing and labeling practices documented therefore apply to the South African context alone. It cannot be assumed that the same marking practices will be evident for the same toothpaste brands and/or types marketed in other countries.

6.1.3 Accuracy of Fluoride Analyses

Despite limitations introduced by the equipment used, a number of internal controls, as discussed in chapter 4, 'Methodology' were used to validate the analyses and a coefficient of variation, based on standard deviation, equal to or less than 5% was considered acceptable. As per Zady (2009) a coefficient variation of 5% or less serves as an indicator of good method performance.

6.1.4 Fluoride Content and Anti-Caries Efficacy

It is universally accepted among the dental and scientific disciplines that exposure to an optimum level of fluoride confers caries protective benefits upon the dentition (Benzian *et al.*, 2012: 213; Cury *et al.*, 2010:396; van Loveren *et al.*, 2005: 224; Marinho *et al.*, cited in Zero, 2006). As for most substances, fluoride ingestion in excess of optimum, however, may be detrimental to systemic health. Numerous studies have implicated fluoride in the aetiology of diverse pathologies, including, dental and skeletal fluorosis (Tokalioglu *et al.*, 2004:204), osteoarthritis, impaired cognitive development in children due to neurotoxicity (Varner *et al.*, 1998:296), impaired immune functioning, birth defects, perinatal deaths and acute, fatal toxicity characterized by disruption of enzyme functioning (Adejumo *et al.*, 2009:47). Under normal conditions of health, chronic toxicity is rare as fluoride excretion predominantly via urine and to a lesser extent sweat, saliva, milk and digestive juices is effective with excretory rates increasing in relation to increased ingestion (Jansen van Rensburg, 1995: 482). As a result toothpaste fluoride concentrations are regulated by numerous authorities and it is internationally accepted that an effective, safe, fluoridated toothpaste (i.e. one demonstrating caries inhibitory activity while minimizing potentially negative effects) contain between 1100ppm and 1500ppm total fluoride (Benzian *et al.*, 2012:220; Kikwilu *et al.*,2008:298) or more specifically a minimum of 1000ppm available (potentially bioavailable) fluoride (Cury *et al.*, 2010:396; van Loveren *et al.*, 2003:229; Walsh *et al.*, 2010:3).

Conversely, a systematic review of the literature undertaken by Walsh *et al.* (2010:3) reported that toothpastes containing fluoride at concentrations up to 500ppm provide no statistically significant caries inhibitory effect in contrast to placebo. Based on these findings, "Delivering Better Oral Health: An Evidence Based Toolkit" developed by the department of health in the UK recommends that for anti-cariogenic activity to be realized, paediatric consumers less than 3 years of age should be exposed to only a smear of toothpaste containing no less than 1000ppm and for children between 3 and 6 years a pea-size amount containing between 1350ppm and 1500ppm fluoride. In children it is vital that the stipulated amount be used to limit fluorosis risks (Public Health England, 2014:17). It is for this reason that the decision was taken to restrict fluoride analyses to the adult dentifrices in the present study.

Deficiencies in terms of total fluoride concentration relative to manufacturer declaration were found for all but one of the toothpastes analysed (n=28) and may therefore, be regarded as safe when used appropriately by the consumer. Despite this finding, only 21.4% actually presented with sub-therapeutic total soluble (potentially bioavailable) fluoride concentrations (<1000ppm F) (Figure 5 and 6). These sub-standard toothpastes were all formulated with, according to manufacturer declaration, a total of 1100ppm F or 1000ppm F. Although general inferences with respect to fluoride content of toothpaste brands and/or types cannot be made from the fluoride analyses results of the present study, the results do seem to suggest that most adult fluoridated toothpastes marketed to the South African consumer are indeed effective and that the ongoing caries crisis is most likely associated with social determinants.

Within the South African context the Medicines and Related Substances Act 101 of 1965 (R510) merely prescribes that the total fluoride concentration of a toothpaste shall not exceed a mass fraction of 0.15% (1500ppm). No acknowledgement is made with respect to the differing fluoride species (University of Pretoria and South African Legal Information Institute, 2014:11). This norm aligns with the guideline stipulated in the internationally recognized and accepted ISO11609:2010 framework (Estonian Centre for Standardization, 2010:2). Contrary to this, regulations stipulated in the Federal Drug Administration's anti-caries monograph on fluoride dentifrice products (Federal Register 21 CFR 355.10) prescribes that sodium monofluorophosphate and sodium fluoride-containing dentifrices be formulated with a total fluoride content between 850ppm and 1150ppm and fluoride availability of 800ppm (Zero, 2006). The reasoning underpinning divergence from this internationally recognized standard remains unclear. It is, however, worth noting, conceivably due to the detrimental side effects associated with excessive fluoride ingestion as well as the litigious atmosphere within which American medical professionals operate, that toothpastes are classified as an over-the-counter drug as opposed to a cosmetic as is the case in South Africa. It is also noteworthy that the Americans acknowledge the presence and occurrence of different fluoride species, namely, total and soluble/available of which the available value is of greatest importance as it represents the fraction of total concentration that disrupts the caries process at the enamel/oral fluid interface, even though this concentration is regulated at 800ppm, below the critical level of 1000ppm F considered necessary for caries inhibition.

6.1.5 Factors Influencing Fluoride Bioavailability

6.1.5.1 Chemical Incompatibility

Abrasive systems are incorporated into toothpastes to regulate, halt or prevent, dental staining. The most commonly used abrasives include calcium carbonate, dicalcium phosphate, silica and sodium bicarbonate (Zero, 2006). Extensive evidence, dating as far back as the first clinical trials undertaken to evaluate the anti-caries efficacy of fluoridated dentifrices (Bibby, 1945 cited in Zero, 2006) demonstrated that abrasives influenced fluoride bioavailability and rate of fluoride release in response to chemical incompatibility between these components (Zero, 2006). In other words free fluoride ions, the most electronegative and reactive of the elements, have the tendency to interact with other constituents of the formulation, typically calcium ions when present to form an insoluble compound, such as, calcium fluoride (CaF_2) that will not dissociate in the oral cavity to provide anti-caries activity (i.e. disruption of the caries process is reliant on the presence of free, ionic fluoride).

Recent analytical studies have also repeatedly demonstrated that fluoride bioavailability is markedly reduced in toothpastes formulated with a calcium-containing abrasive over those incorporating a silica-based abrasive (Kikwilu *et al.*, 2008:298). A study undertaken by Benzian *et al.* (2012:216) to analyze the total and bioavailable fluoride content of toothpastes from Brunei, Cambodia, Laos, Suriname and the Netherlands found that mean fluoride bioavailability in the calcium-containing toothpastes was 53,2%, statistically significantly lower than the mean of 90,4% obtained for the silica-containing products. Similarly research conducted by Cury *et al.* (2010:399) found that for approximately half (50%) of the sample evaluated, fluoride bioavailability was lower than total concentration measured. This finding was primarily restricted to the sodium monofluorophosphate (Na_2PO_3F) combined with calcium carbonate ($CaCO_3$) as the abrasive containing products and was ascribed to chemical incompatibility between these components. An insoluble fluoride fraction, in the form of CaF_2, was therefore reported for the $PO_3F^{2-}/CaCO_3$-containing toothpastes, while a similar influence was not observed for the NaF-containing products formulated with silica as the abrasive (Cury *et al.*, 2010:399).

Findings of the present study therefore correspond with current literature, with relative mean soluble (potentially bioavailable) fluoride content for toothpastes formulated with a calcium-based abrasive calculated at 85,5% (sd ±13.6; n=7) (Tables 6 and 7; Figure 6) as opposed to 98.7% (sd ±2.1; n=19) for those containing a silica-based abrasive (Tables 4 and 5; Figure 5). Fortunately a majority, 75% (n=28), of toothpastes analysed contained hydrated silica as the abrasive. For the remaining 25% formulated with a calcium-based abrasive, 42.3% were locally manufactured and 71% contained a combination of $Na_2PO_3F/CaCO_3$. Interestingly, all toothpastes containing a calcium-based abrasive, excluding one, were manufactured in developing countries. An overwhelming body of evidence pointing to low-quality (inefficacy) of the $Na_2PO_3F/CaCO_3$ toothpastes now exists. As a large number of toothpastes are still formulated in this manner there is a significant need to educate consumers around the influence of abrasives in toothpastes and to avoid Ca-containing products.

Questions then arise as to why Na_2PO_3F/ $CaCO_3$-containing products are still being manufactured? Historically a meta-analysis of 19 clinical trials investigating the use of PO_3F^{2-}containing toothpastes reported by Forward (1980 cited in Grobler *et al.*, 1983:475) found a statistically significant reduction in the caries increment, while more recently a statement published within the Bulletin of the WHO in an article titled, "The effective use of fluorides in public health" recommended that due to low-cost and ease of accessibility of precipitated $CaCO_3$ over the other recognized abrasives that this be the abrasive of choice (Jones *et al.*, 2005:673). Admittedly the numerous recent studies referred to were conducted subsequent to publication of the 2005 edition of the WHO bulletin, but it is also pertinent to note that the Indonesian clinical trial on which this WHO article was based, demonstrated incompatibility between $CaCO_3$ and fluoride within toothpastes, but went on to state that incorporation of a stabilizer into Na_2PO_3F-containing toothpastes prevented formation of insoluble CaF_2 (Adyatmaka *et al.*, 1998:8). Likewise as F^- ions are covalently bonded to phosphate within monofluorophosphate (PO_3F^{2-}) it seemed correct to assume that this relationship was stable, precluded dissociation and therefore availability of free, ionic fluoride with the potential to interact with Ca.

Consequently the misconception that Na_2PO_3F is chemically compatible with Ca-containing abrasives persists (Pessan *et al.*, 2011 cited in Benzian, 2012:298). A need to dispel this misbelief, particularly among toothpaste manufacturers, exists and as such it appears advisable that the WHO issue an updated review, based on current research. Despite the need for revisions with respect to the use of $CaCO_3$ and in fact any calcium compound in toothpastes it must be noted that increasing numbers of toothpastes are now being formulated with a Na_2PO_3F/SiO_2 combination as is evidenced by the present study in which 19% of the silica-containing products presented with this formulation.

6.1.5.2 Stability/Ageing

Additionally it has been shown, in particular among Na_2PO_3F/CaCO3-containing toothpastes, that fluoride availability (potential bioavailability) decreases over time, the rate of decrease, in turn, influenced by storage conditions and that this fraction may actually decline to sub-therapeutic levels, below 1000ppm (de Oliviera *et al.*, 2003:252). Once again similar observances were made with respect to the present study.

Two of the NaF/SiO_2 toothpastes sampled were close to expiry at the time of purchase and had just expired on analysis and were determined to contain the first and third largest mean insoluble fluoride fractions of 85.3ppm F and 44.9ppm F for the silica-based products (Table 4 and 5). Although it would be ideal to compare these fluoride analyses results with non-expired samples, the results may suggest that stability changes are not only limited to calcium-based toothpastes and that on ageing and surpassing of expiry date in Si-based products that fluoride availability will eventually decline. It is therefore recommended that further toothpaste stability studies be undertaken. Fortunately, however, total soluble (potentially bioavailable) fluoride content was in excess of the 1000ppm F limit required for anti-caries efficacy.

Similarly, the most aged calcium-based product contained the second highest insoluble fluoride fraction of 266.6ppm F. Notably, this product, although close to expiry had not expired by the time of fluoride analysis and the insoluble fluoride fraction was already dramatically larger in the order of 181.3ppm F and 221.7ppm F

than that observed for the expired NaF/SiO_2 toothpastes. Additionally in contrast to the expired NaF/SiO_2, the available (potentially bioavailable) fluoride concentration measured was insufficient to provide caries inhibitory activity.

Regrettably South African regulations do not stipulate the minimum available fluoride content that needs to be maintained in the toothpaste formulation. Similar regulatory gaps are also reported for Brazilian and European standards and legislation (Cury *et al.*, 2010:399).

6.1.6 Conclusion

In conclusion and in answering the first 2 major objectives of the current study, namely, determination of total and total soluble (potentially bioavailable) fluoride content of toothpastes marketed to the South African consumer it was found that 78.6% of toothpastes sampled presented with adequate total and potentially bioavailable fluoride concentrations and therefore the ability to disrupt the caries process. On the other hand a relatively large fraction of the sample, 21.4% (n=28) contained substandard fluoride concentrations, which has implications with respect to anti-caries efficacy, and depending on popularity may predispose consumers to a public health burden especially as fluoride intake is not balanced through background water fluoridation. It is therefore recommended that consumer surveys be undertaken in order to examine fluoridated toothpaste use among South Africans and to obtain an indication as to affordability and market share of each toothpaste type.

6.2 SECTION B: LABELING PRACTICES

6.2.1 Introduction

Partial compliance with packing and marking regulations as stipulated in the SANS 1302:2008 (edition 1.1) was observed for all toothpastes sampled (Table 11). All toothpastes (n=300) complied with packing specifications in terms of being contained within "leak-proof", collapsible tubes. Likewise, all samples, excluding 'Nature Fresh Junior Toothpaste' (3; n=300) were packaged in individual outer cartons. A majority, 71.05% (n=38) of fluoridated toothpastes reflected on the gold standard list adhered to a maximum of 5 of the 6 (83.3%) marking requirements (Table 11). The least compliant toothpaste, Dis-Chem Dentalmate Kids applied only two dictates, namely product name and distributor address, neither of which has any bearing on consumer education around product safety or potential health benefits.

Despite the fact that batch identification permits traceability through the supply chain the only two other marking variables included within the SANS parameters that may be regarded as critical in terms of consumer protection are those pertaining to product descriptor and content description and will be reflected on in greater detail (Table 10).

6.2.2 Content Description (including fluoride declaration) and Product Descriptor

In the interests of protecting the consumer against harm from the product and ensuring that intended health gains are attained, appropriate labeling that includes a readily visible product descriptor ('fluoride toothpaste') and quantity declaration of not only total but also bioavailable fluoride content is imperative. Within the present study no toothpastes were found to conform to the product descriptor regulation (Table 10), while 93.3% of the toothpastes evaluated in terms of labeling (n=300) declared a total fluoride concentration. No declarations were made with respect to total soluble (potentially bioavailable) fluoride content.

Two probable explanations as to why bioavailable content is not declared in marking of the packaging could firstly stem from the fact that regulating authorities fail to recognize or adequately define differing fluoride species. This is supported by the fact that the SANS 1302:2008 (edition 1.1) only provides guidance with respect to analysis of total fluoride content while the Medicines and Related Substances Act 101 of 1965 (R510) merely prescribes the maximum total fluoride (0.15%) that a toothpaste may contain (University of Pretoria and South African Legal Information Institute, 2014:11). Secondly, fluoride bioavailability decreases over time in response to chemical incompatibility between toothpaste constituents (Cury *et al.*, 2010:399) and the ambient conditions under which the toothpaste is stored and handled (Benzian *et al.*, 2012:219), meaning that any declaration in terms of fluoride bioavailability is of questionable integrity. As anti-caries efficacy is reliant on total soluble (potentially bioavailable) fluoride content it is recommended that as per the FDA's anti-caries monograph that manufacturers formulate toothpastes in such a manner that will ensure maintenance of a minimum total soluble (available) fluoride content for the duration of a product's shelf-life when stored and handled appropriately, that this minimum total soluble fluoride concentration be declared on the label in conjunction with storage instructions. It may however be anticipated that if this recommendation is to be met, additional analyses would need to be undertaken by manufactures, new formulations may need to be developed and labels would need to be updated, all of which have significant financial implications.

Listing of the bioavailable content although essential in terms of consumer protection, is complex, starting with the need for regulating authorities to clearly define and acknowledge the presence of differing fluoride species and for manufacturers to have a complete understanding of fluoridated toothpaste chemistry (i.e. the interaction of fluoride with other constituents of the toothpaste formulation and the impact of environmental conditions on these processes) to allow for amendments to existing or generation of new standards/norms and policies.

A majority, 89.47% of the sample (n=38) provided a product description or rather a listing of ingredients (Table 10) in marking of the outer carton.

Understandably, full product description (i.e. listing of constituents in conjunction with its' function) is best practice in empowering consumers to make informed choices, but impractical in terms of packaging. It is therefore advisable that in order to circumvent misinterpretation that wording of the standard be amended to a 'complete listing of ingredients' that must include a statement with regards to the chemical form of fluoride, fluoride content declaration and designation of abrasive agent(s) or to consider regulations in terms of product information sheets/inserts. Notably both the Elgydium Kids and Elgydium Sensitive toothpastes manufactured in France and formulated with amine fluoride (flurinol) as the active ingredient are supplied to the consumers together with a product information sheet. Provision of information sheets would however markedly increase costs and benefits in terms of consumer protection would have to be offset against affordability. Additionally, ISO 11609:2010 standards only require that "a complete listing of ingredients according to the International Nomenclature of Cosmetic Ingredients (INCI)" be reflected (European Committee for Standardization, 2010:4). As toothpastes are classified as cosmetic products within the South African context use of this phrasing may result from the fact that the SABS regulates packaging and marking subject to the 'Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No. 54 of 1972; Regulation R1555). In the preface of this document, included within the list of definitions, describe is said to include 'to advertise or label'.

Extensive evidence, dating as far back as the first clinical trials undertaken to evaluate the anti-caries efficacy of fluoridated dentifrices (Bibby, 1945 cited in Zero, 2006) demonstrated that abrasives influenced fluoride bioavailability and rate of fluoride release in response to chemical incompatibility between these components (Zero, 2006). Recent analytical studies have also repeatedly demonstrated that fluoride bioavailability declines markedly over time especially in toothpastes formulated with a calcium-containing abrasive as opposed to a silica-based abrasive or when subjected to excessive temperatures (Kikwilu *et al.*, 2008:298). In the interests of consumer protection and realization of intended health gains it is therefore imperative that consumers be educated with respect to abrasive agents within the toothpaste formulation, be alerted to the fact that anti-caries efficacy declines over time and markedly so with improper handling and storage, highlighting a need for identifying the abrasive agent(s) and legibly displaying the expiry date and storage instructions

on the label. Fortunately, results from the present study showed that 94.74% (n=38) and 77.3% (n=300) of toothpastes sampled displayed the abrasive in listing of the constituents (although it is not indicated as the abrasive meaning that the lay person would have difficulty in recognizing it as such) and an expiry date respectively, while negatively, only 26.32% (n=38) displayed storage instructions in marking of either the tube or outer carton (Appendix II).

6.2.3 Expiry

As fluoride bioavailability (total soluble fluoride content) is reliant on age of the product and as bioavailability is a measure of efficacy it is fundamentally important that the stability of the toothpaste be specified through provision of an expiry date and it is for this reason that this variable, although not mentioned within SANS parameters, will receive special mention here.

Declaration of an expiry date as opposed to date of manufacture is preferable as the layperson is not necessarily aware that toothpastes have a shelf life and that the normal duration for which a product may be considered viable and safe for use is 2 years. Additionally, conflicting information presents within the literature with respect to product stability. Some researchers refer to a 3-year shelf life (Benzian *et al.*, 2012:214), while the ADA (American Dental association) stipulates a 2-year product stability (ADA, 2015).

6.2.4 Impact of Low-Cost Products on Labeling Quality

Notably, although affordability was not investigated in the present study, Dis-Chem Dentalmate paediatric and adult toothpastes were the cheapest of the toothpastes sampled, costing R5.95. The Dis-Chem Dentalmate adult toothpaste, however, applied 4 of the 6 SANS marking variables and included a nominal volume as well as batch identification in addition to the two variables indicated for the Dis-Chem Dentalmate Kids toothpaste in marking of the outer carton. Once again, information inconsequential to product efficacy and safety other than the fact that batch identification permits traceability through the supply chain. Although provision of an affordable product is to be applauded, it may be conjectured that this represents a

business strategy aimed at expansion into and taking greater share of the informal market through targeting of lower LSM (living standards measures) consumers. Likewise it would be logical to assume that the sub-optimal labeling practices observed for these 'no-name' brand toothpastes reduce production costs, increasing affordability. According to Armer (2013,27) noncompliance with the 'new' food labeling laws introduced after the consumer protection bill came into effect in 2011 results in part due to expense associated with updating and editing of labels.

South Africa has a progressive constitution that speaks of inclusiveness, equality and equity (Constitutional Assembly, 1996:3). Provision of an unsafe product due to lack of essential information may be regarded as unconstitutional, violates the consumer protection bill which advocates complete transparency in marking of a product (Government Gazette, 2009:2), both of which would serve to increase the health burden among those of low socio-economic status, re-enforcing health inequities between population groups.

Furthermore all samples of Dis-Chem Dentalmate Adult toothpaste conveniently purchased in the period between December 2014 and January 2015 from Dis-Chem retailers located in the 5 major metropolitan areas of South Africa were close to expiry, declaring a 06/2015 expiration date. Low cost may therefore represent a strategy to increase sales prior to expiration or that cheaper ingredients have been used (Benzian *et al.*, 2012:219). As it is well documented in the literature that fluoride bioavailability and therefore anti-caries efficacy declines over time, sale of this product would then mean that a less effective product is reaching the unsuspecting consumer.

6.2.5 Comparison with Current Literature

Findings from analysis of labeling practices, in the present study therefore concurred with those of Kikwilu *et al.* (2008:298) that reported inadequate/incomplete labeling for all toothpastes evaluated in the study. Divergence away from the findings of Kikwilu *et al.* (2008:298) is however noted in terms of declaration of the abrasive agent. Majority of the toothpastes analysed in the present study did include the abrasive in listing of the ingredients although it was not designated as such, while the Kikwilu *et al.* (2008:298) study reported that the abrasive was not always indicated and if a calcium compound was used as the abrasive the compound was not specified.

As the presence of calcium-containing abrasives impacts on product efficacy, failure to disclose and define the abrasive agent incorporated into the formulation represents a lack of transparency and withholding of essential information from the consumer and so defeats the provisions of the 'Consumer Protection Bill'. Results were also contradictory to those of a study undertaken by Benzian *et al.* (2012:220) which found, in general, that marking pertaining to expiry date, chemical form of fluoride and fluoride concentration for toothpastes purchased in the developing countries of Brunei, Cambodia, Laos and Suriname were absent (i.e. failure to adhere to minimum ISO 11609 labeling regulations). Fortuitously a majority of toothpastes marketed to the South African consumer and assessed within the present study adhered to these minimum requirements which due to the inclusion of parameters pertaining to declaration of an expiry date, chemical form of fluoride, total fluoride concentration and cautionary note regarding use in paediatric consumers can certainly be considered a stronger standard that that of the SABS.

6.2.6 South African Statutory Requirements

Some of these omissions, however, may be accounted for by a clause inserted into the SANS 1302:2008 (edition 1.1) documentation, immediately below the heading, "4 Packing and Marking" which stipulates that all packing and marking practices must comply with current national legislation, namely the 'Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No. 54 of 1972; Regulation R1555), the primary statute regulating the sale, manufacture, import and export of these substances. In recent years, especially since the ratification of the consumer protection bill (Act No. 68 of 2008) in April 2010 and operationalization in March 2011, consumer rights and protection have garnered increasing attention. In association with this upsurge, amendments to this initial act, 'Foodstuffs, Cosmetics and Disinfectants Amendment Act, 2007 (Act No. 39 of 2007) and food labeling regulations (R146 of 2010) have been promulgated and focuses, in particular, on revisions to labeling regulations as adequate marking is a means to empowering the consumer to make informed choices and respecting his/her right to autonomy.

The Cosmetics, Toiletries and Fragrance Association of South Africa (CTFA) states that in keeping with 'new' cosmetics labeling legislation certain compulsory information, namely, brand name, product descriptor, directions for use, cautionary statements, ingredient listing and quantity declaration be displayed in marking of a product (CTFA, 2014). Once again no mention is made with respect to product stability/expiry, chemical form of fluoride and concentration all of which are paramount to consumer safety. As food, cosmetics and disinfectants are regulated under the same act it is noteworthy that the 'new' Food labeling regulations, R146 (2010), do require that an expiry/use-by date be conspicuously displayed (Department of Health, 2010:17). Regulations pertaining to permissible fluoride content are located in the Medicines and Related Substances Act 101 of 1965 (R510) and states that fluoride concentration shall not exceed a mass fraction of 0.15% (University of Pretoria and South African Legal Information Institute, 2014:11). This, however controls manufacture and formulation with no mention made as to representation on the label. No reference is made within this act with respect to the different fluoride ion species or chemical form of fluoride.

According to the CTFA (2014), South Africa ascribes to the European regulations and ingredient annexures to facilitate import and export and provides an additional explanation as to why toothpaste labeling practices do not conform completely with SANS parameters and local legislation, but also partially adhere to ISO11609:2010 specifications.

Appropriate packaging and marking is therefore a very complex process when one considers the multitude of acts and amendments governing the sale, manufacture and advertisement of toothpastes and difficulties associated with comprehending the intricacies of legal discourse therein. In summary, the SABS regulates packaging and marking as well as quantity declarations subject to the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No. 54 of 1972; Regulation R1555) and the Trade Metrology Act (Act No. 77 of 1973) respectively, while fluoride content is managed in accordance with the Medicines and Related Substances Act (Act 101 of 1965; Regulation R510).

Additionally the National Consumer Commission oversees improvements to standards of consumer information and prohibition of unfair marketing practices, such as, ambiguous and misleading labeling and advertising (National Consumer Commission, 2014). A feasible recommendation therefore, is that all regulations pertaining to manufacture, marking and packaging be clearly and comprehensibly documented in bullet format within SANS guidelines in a manner that provides no leeway for interpretation and as a result protects both the consumer against harm from the product as well as the manufacturer/distributor from legal liability.

Despite these statutory requirements, the South African cosmetics industry is self-regulated meaning that manufacturers and/or distributors are responsible for 'in-house' quality assurance (CTFA, 2014). Periodic inspection by external authorities such as SANS must occur and sale of these products despite non-compliance demonstrates an inability of authorities to impose restrictions. Reasons cited for non-compliance with food labeling laws (R146) included a lack of awareness around labeling regulations or misinterpretation of certain aspects of it, purposeful disregard of legislative details in an attempt to remain competitive, high costs associated with compliance due to required laboratory testing and/or assistance provided by

consultants and lawyers in legal interpretations, large expense incurred in updating and changing labels and poor enforcement of regulations (Armer, 2013:27).

As foodstuffs and cosmetics are regulated subject to Act 54 of 1972, all reasons discussed in terms of food labeling noncompliance are applicable to this research context as well. Lack of awareness and misinterpretation of legislation may stem from the fact that as a majority of toothpastes are imported, manufacturers lack familiarity with local laws (although it is anticipated that the major brands would have 'in-house' legal departments) and due to the multitude of acts governing marking procedures. Editing labels in an effort to achieve compliance is not only expensive but runs the risk of altering the key identifier by which the consumer recognizes the product, reducing sales thereby reducing competitiveness meaning that norms are ignored.

Product labeling is an effective marketing tool and label laws were introduced frequently in the past, in particular, in relation to the food industry, manufacturers were exploiting this, misleading consumers by ambiguous and dishonest statements to provide a product with a competitive advantage and increased market share. Labeling laws therefore function to prohibit unfair marketing strategies, provide manufacturers with an equal platform from which to promote a product and protect the consumer (de Villiers, 2009:13).

In recent years the South African oral health industry has undergone a shift in consumer demand from concerns around plaque removal and caries prevention towards protection, restoration and promotion of the health of the dentition and aesthetics, a trend reflected in an increasing demand for more sophisticated and targeted products (Euromonitor, 2015). As per a representative of the Dis-Chem pharmaceutical retailer, the company's five (5) top toothpaste sellers include Aquafresh Extreme Clean Whitening, Colgate Optic White, Oral B Pro-Expert, Sensodyne Repair and Protect and Colgate Sugar Free Acid (Dis-Chem, 2015). Although the 'whitening toothpastes' were excluded from this study, use of the terminology "tooth whitening" is a prime example of a 'half-truth' that labeling laws attempt to circumvent.

Over-the-counter tooth whitening dentifrices are typically manufactured with increased abrasivity and function to remove extrinsic staining and so 'whiten' the tooth'. Long-term or excessive use would be detrimental to oral health due to enamel and dentinal abrasion leading to increased sensitivity (Demarco *et al.*, 2009:65). Perhaps more appropriately, these products should be advertised as stain removers or inhibitors and indicated for short-term use. Unfortunately the terminology stain-remover would not generate the same selling potential as tooth whitener. Zero (2006) reported similar trends with respect to the American oral health industry and went on to state that as marketing yields higher returns in contrast to investing into research and development of new classes of products, toothpaste manufacturers have a greater incentive to pursue non-therapeutic cosmetic claims. As the South African cosmetics industry is self-regulating it seems logical to anticipate that competitors would report each other and that this self-policing approach would function to enforce label laws.

Contrary to this and in response to a mature toothpaste retail market and increase in unit costs due to devaluation of the South African Rand, key players seem to have adopted similar marketing strategies in an attempt to gain a competitive advantage and so claim market share from one another with concerns around sales superseding consumer protection (Euromonitor, 2015). It is for this reason that all toothpastes claiming to provide a certain benefit, such as, tooth whitening, desensitization, improvements in gingival health, increased interdental cleaning and so on, that these claims be substantiated by way of the ingredients list. It is therefore recommended that the South African Dental Association (SADA) as noted for the American Dental Association (ADA) develop acceptance program guidelines for fluoridated toothpastes and that under the label sub-heading active ingredient that not only the chemical form of fluoride be reflected but that the other ingredient(s) purported to provide the advertised health or cosmetic gain be identified.

6.2.7 Conclusion

In conclusion and in answering objectives 3 and 4 of the present study, partial compliance with SANS 1302:2008 (ed. 1.1) marking requirements was observed for all toothpastes sampled. SANS 1302:2008 may, however, be regarded as a weak standard when compared to ISO 11609:2010, as marking requirements with respect to information considered mandatory in terms of consumer protection are omitted. Due to the global marketing of toothpastes, however, a large number of manufacturers were found to also partially conform to ISO11609:2010 labeling guidelines which, in addition, regulates the provision of essential consumer information omitted from SANS 1302:2008. Some manufacturers even went further to provide additional information, such as, directions for use and storage instructions (Table 12), not regulated by either the SANS or ISO marking criteria. As a result the quality of consumer information provided on most of the toothpastes evaluated was considered satisfactory.

Emanating from this discussion and in the interest of consumer protection, it is viable to recommend that at a minimum, SANS documentation be revised to align with ISO guidelines or alternatively complete adoption and implementation of the ISO framework within the South African context. Despite the fact that the ISO framework includes labeling regulations (omitted from current SANS) pertaining to information considered mandatory in terms of consumer protection and attainment of intended health gains, this standard may also be regarded as suboptimal due to omission of norms pertaining to designation of abrasive agent, recognition and quantity declaration of differing fluoride species, directions for use and appropriate storage instructions. Optimally, therefore, it is advisable that in addition to adoption of the ISO framework within the South African context, that national standards and policies be amended to embrace these variables as well.

In a consumer-oriented environment in which labeling regulations are set to become more stringent and in which consumers are ‘wising’ up to their rights, threat of liability and legal action should provide sufficient motivation for manufacturers to become compliant.

CHAPTER 7: CONCLUSIONS & FUTURE RECOMMENDATIONS

Despite the universal adoption and infusion of fluoridated toothpastes into all, urban and rural, communities, alarming oral health statistics are repeatedly and frequently published - 3.9 billion suffering from oral disease of which dental caries of the permanent dentition is the most prevalent and the most common, chronic disease of childhood (Marcenes *et al.*, 2013:593). Fluoridated toothpastes, however, do not provide a "cure-all" and merely represent one, although profoundly significant, tool available in the anti-caries arsenal and this certainly makes sense – a multifactorial strategy will be required to control a disease that is multifactorial by nature (Jenkins, 1985:1297).

The results of the present study certainly re-iterate this sentiment. Although the fluoride analyses results in the strict sense only apply to the toothpastes evaluated, if inferences could be drawn, these would indicate that based on *in-vitro* analysis the majority of commercially available adult fluoridated toothpastes are effective anticariogenic agents and when the study is considered in its' entirety, not only from a fluoride content perspective, but also in relation to the quality of consumer information provided in labeling of the product, and yet the South African oral health statistics are comparatively more dismal than for the developed world (Allukian, 2008:S82).

In order for population-based health gains in response to fluoridated toothpastes to be realized, it requires that the population as a whole have access to effective products and that these be used on a regular, daily basis. This then raises questions with respect to the true accessibility of toothpastes within South Africa, namely, is it affordable to the average consumer and is oral health literacy levels sufficient to encourage appropriate, recommended use?

The fluoride analyses results concurred with the findings of recent research. Once again it was proven that toothpastes with a calcium-based abrasive demonstrate a lower and often sub-therapeutic free available and therefore potentially bioavailable fluoride concentration and points to the fact that such formulations should be

disbanded (Benzian *et al.*, 2012:216; Cury *et al.*, 2010:399; de Oliviera *et al.*, 2003:252; Kikwilu *et al.*, 2008:298). This, however, will require the WHO to take cognizance of the increasing body of evidence, revise and amend their 2005 recommendations which stated that $CaCO_3$ be used as the abrasive of choice due to low cost and ready accessibility especially within resource challenged environments (Jones *et al.*, 2005:673). An inferior product within these contexts represents a contributory factor driving oral health disparities between nations, more especially the developed and developing worlds. In fact most of the products manufactured locally and assessed within this study were formulated with a $Na_2PO_3F/CaCO_3$ combination. In the interim, however, there is a need for consumers to be made aware of the implications of using a calcium-containing toothpaste. Consumers therefore not only need to be educated with respect to their own individual brushing practices and behavior, but also around the product in terms of stability and that the total fluoride content reflected in marking of the product is not necessarily a true indication of the actual fluoride dose that they are receiving. As echoed in the literature the presence of free available fluoride in toothpastes is completely ignored within South Africa and constitutes a withholding of essential consumer information. Undoubtedly product quality is reliant on drafting regulations pertaining to the minimum available fluoride content that needs to be maintained in the formulation.

In the absence of water fluoridation and in light of the caries crisis it is an ethical imperative for manufacturers to guarantee the quality of their toothpaste, for regulating authorities to develop appropriate standards and policies that will enable population-wide access to effective toothpastes and for regulators to have the ability to impose restrictions should the need arise. Appropriate marking of a product is central to its' quality as it provides a platform for effective communication with and education of the consumer, even more so in the South African context where large numbers of the population due to socioeconomic and political factors are underserved, have limited access to oral health services and therefore rely heavily on the information provided with the product. Even though all toothpastes evaluated in this study only demonstrated partial compliance with national labeling standards it was concluded that the quality of consumer information provided was satisfactory. This is due to the fact that a majority, in addition, somewhat adhered to the internationally recognized and accepted ISO 11609:2010 framework which can be regarded as a

stronger standard than the South African one as it includes parameters, completely omitted from the South African National Standards, pertaining to provision of information that may be regarded as mandatory in terms of consumer protection, namely, declaration of an expiry date, chemical form of fluoride, total fluoride concentration and cautionary note regarding use in paediatric consumers (European Committee for Standardization, 2010; SABS, 2008). There is thus a significant need for an expert committee to reconvene and revise the current national standards.

Bearing in mind the principle action areas of the Ottawa Charter for health promotion (WHO, 2015), from this discussion it becomes clearly evident how the institution of new public policies, such as development of policies to manage affordability and large-scale delivery of fluoridated toothpastes in conjunction with appropriate educational campaigns and revisions to the current guidelines regulating the sale and manufacture of toothpastes will aid in establishment of a supportive environment not only for oral but also systemic health.

From the findings of the present study, the following recommendations are suggested:

- Revision of and amendments to current national standards;
- Introduction of policy stipulating the minimum soluble fluoride content that needs to be maintained in the toothpaste formulation for the duration of shelf life, with this value reflected in marking of the product;
- Introduction of policy to restrict or prohibit incorporation of calcium-containing abrasives in product manufacture;
- Introduction of policies to ensure more stringent regulation of fluoridated toothpaste quality;
- Nationwide surveys to identify factors influencing accessibility to fluoridated toothpastes; and
- Advocacy for introduction of policies to facilitate population-based delivery of effective fluoridated toothpastes in conjunction with educational campaigns.

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APPENDIX I: ***Fluoridated Toothpaste Samples – Gold Standard List***

FLUORIDATED TOOTHPASTE SAMPLES			
		Active Ingredient*	Ppm
Children's Toothpaste	Aquafresh Milk Teeth 0-3yrs	NaF	500
	Aquafresh Little Teeth 4-6yrs	NaF	1000
	Aquafresh Big Teeth >6yrs	NaF	1400
	Colgate 0-2yrs	NaF	500
	Colgate 2-5yrs	NaF	500
	Colgate >5yrs	NaF	1450
	Elgydium Kids 2-6yrs	NH_4F	250
	Mentadent P Kids	NaF	1000
	Nature Fresh Junior Toothpaste	Homeopathic Fluoride??	
Adult Toothpaste	Aquafresh all-in-one protection	NaF	??
	Aquafresh Extreme Clean	NaF	1450
	Aquafresh Ultimate	NaF	1450
	Close Up Deep Action	NaF	1450
	Colgate Active Salt	Na_2PO_3F	1000
	Colgate Gel	Na_2PO_3F+ NaF	1000+450
	Colgate MaxFresh	NaF	1000
	Colgate Maximum Cavity Protection	Na_2PO_3F	1450
	Colgate Sensitive Multiprotection	Na_2PO_3F	1000
	Colgate Sensitive Pro-Relief	Na_2PO_3F	1450
	Colgate Total 12	NaF	1450
	Colgate Pro-Gum Health	NaF	1450
	Colgate Triple Action	Na_2PO_3F	1450
	Dis-Chem Dentalmate	??	??
	Elgydium Sensitive	NH_4F	1250
	Enamel Care	NaF	1100
	Gum Caries Protect	Na_2PO_3F+ NaF	1490
	Mentadent P Gel Protection	NaF	1450
	Mentadent P Micro Granules	NaF	1450
	Mentadent P Protection	NaF	1450
	Mentadent P Sensitive	Na_2PO_3F	1000
	Oral B Pro-Expert	NaF +SnF_2	350+1100
	Pepsodent	Na_2PO_3F	1450
	Pepsodent Complete 8	NaF	1450
	Sensodyne Cool Gel	NaF	1400
	Sensodyne Multi Care	NaF	1400
	Sensodyne Rapid Action	??	1040
	Sensodyne Repair & Protect	Na_2PO_3F	1450

*NH_4F = Flurinol/Amine Fluoride; SnF_2 = Stannous Fluoride; NaF = Sodium Fluoride; Na_2PO_3F = Sodium Monofluorophosphate

APPENDIX II: *Raw Data Collection Sheet: Labelling Practices – Gold Standard Sampling List*

Fluoridated Toothpaste Samples	Country of Production/Distributor's Address	Abrasive Agent	Phrase: "Fluoride Toothpaste"/"Fluoride Dental Créme"							
			Box				Tube			
			Present (Y/N)	*3mm Font Size (Y/N)*	*Distinct from Background (Y/N)*	*Compliant*	*Present (Y/N)*	*3mm Font Size*	*Distinct*	*Compliant*
Aquafresh Milk Teeth 0-3yrs	South Africa	Hydrated Silica ($SiO_2.nH_2O$)	1	1mm	0	0	1	0.5mm	1	0
Aquafresh Little Teeth 4-6yrs	South Africa	Hydrated Silica ($SiO_2.nH_2O$)	1	1mm	0	0	1	0.5mm	1	0
Aquafresh Big Teeth >6yrs	South Africa	Hydrated Silica (SiO2.nH2O)	1	1mm	0	0	1	0.5mm	1	0
Colgate 0-2yrs	China	Hydrated Silica (SiO2.nH2O)	F and Conc.	3mm	1	1	1	1.0mm	1	0
Colgate 2-5yrs	China	Hydrated Silica (SiO2.nH2O)	F and Conc.	3mm	1	1	1	1.0mm	1	0
Colgate >5yrs	Poland	Hydrated Silica (SiO2.nH2O)	F and Conc.	3mm	1	1	1	1.0mm	1	0
Dis-Chem Dentalmate Kids	?	?	ctive element:	3mm	1	1	0	0	0	0
Elgydium Kids 2-6yrs	France	Silica (SiO_2)	aste Gel With F	3mm	1	1	1	2.0mm	1	0
Mentadent P Kids	South Africa	Hydrated Silica (SiO2.nH2O)	1	3mm	1	1	1	2.0mm	1	0
Nature Fresh Junior Toothpaste	South Africa	?	0	N/A	N/A	0	0	0	0	0
Aquafresh all-in-one Protection	South Africa	Hydrated Silica ($SiO_2.nH_2O$)	1	1mm	1	0	1	2.0mm	1	0
Aquafresh Extreme Clean	South Africa	Hydrated Silica ($SiO_2.nH_2O$)	1	1mm	1	0	1	2.0mm	1	0
Aquafresh Ultimate	South Africa	Hydrated Silica (SiO2.nH2O)	1	1mm	0	0	0	0	0	0
Close Up Deep Action	Vietnam	Hydrated Silica ($SiO_2.nH_2O$)	0	N/A	N/A	0	0	0	0	0
Colgate Active Salt	India	Calcium Carbonate ($CaCO_3$) + Hy	1	3mm	1	1	1	2.0mm	1	0
Colgate Gel	South Africa	Dicalcium Phosphate ($CaHPO_4.2H_2O$) + Hydrated Silica ($SiO_2.nH_2O$)	1	3mm	1	1	0	0	0	0
Colgate MaxFresh	Thailand	Hydrated Silica ($SiO_2.nH_2O$)	1	3mm	1	1	0	0	0	0
Colgate Maximum Cavity Protection	South Africa	Calcium Carbonate ($CaCO_3$)	1	3mm	1	1	0	0	0	0
Colgate Sensitive Multiprotection	Thailand	Hydrated Silica ($SiO_2.nH_2O$)	0	N/A	N/A	0	0	0	0	0
Colgate Sensitive Pro-Relief	Brazil	Calcium Carbonate ($CaCO_3$)	1	3mm	1	1	1	2.0mm	1	0
Colgate Total 12	China	Hydrated Silica ($SiO_2.nH_2O$)	1	3mm	1	1	0	0	0	0
Colgate Pro-Gum Health	China	Hydrated Silica ($SiO_2.nH_2O$)	1	2mm	1	0	0	0	0	0
Colgate Triple Action	South Africa	Calcium Carbonate ($CaCO_3$) + Hydrated Silica ($SiO_2.nH_2O$)	1	3mm	1	1	1	2.0mm	1	0
Dis-Chem Dentalmate	South Africa	Hydrated Silica ($SiO_2.nH_2O$)	ctive element:	5mm	1	1	1	5.0mm	1	0
Elgydium Sensitive	France	Hydrated Silica ($SiO_2.nH_2O$)	aste Gel With F	3mm	1	1	1	2.0mm	1	0
Enamel Care	United Kingdom	Calcium Sulphate($CaSO_4$) + Sodium Bicarbonate ($NaHCO_3$) + Silica (SiO_2)	0	N/A	N/A	0	0	0	0	0
GUM Caries Protect	Spain	Hydrated Silica ($SiO_2.nH_2O$)	0	N/A	N/A	0	0	0	0	0
Mentadent P Gel Protection	South Africa	Hydrated Silica ($SiO_2.nH_2O$)	1	4mm	1	1	1	4.0mm	1	0
Mentadent P Micro Granules	South Africa	Hydrated Silica ($SiO_2.nH_2O$)	1	4mm	1	1	1	4.0mm	1	0
Mentadent P Protection	South Africa	Hydrated Silica ($SiO_2.nH_2O$)	1	4mm	1	1	1	4.0mm	1	0
Mentadent P Sensitive	South Africa	Hydrated Silica ($SiO_2.nH_2O$)	1	4mm	1	1	1	4.0mm	1	0
Oral B Pro-Expert	Germany	Hydrated Silica ($SiO_2.nH_2O$)	1	2mm	1	0	1	2.0mm	1	0
Pepsodent	Vietnam	Calcium Carbonate ($CaCO_3$) + Hydrated Silica ($SiO_2.nH_2O$) + Calcium Glycerophosphate(CaGP)	0	N/A	N/A	0	0	0	0	0
Pepsodent Complete 8	Vietnam	Hydrated Silica ($SiO_2.nH_2O$)	0	N/A	N/A	0	0	0	0	0
Sensodyne Cool Gel	South Africa	Hydrated Silica ($SiO_2.nH_2O$)	1	2mm	N	0	1	2.0mm	1	0
Sensodyne Multi Care	South Africa	Hydrated Silica ($SiO_2.nH_2O$)	1	2mm	N	0	1	2.0mm	1	0
Sensodyne Rapid Action	South Africa	Hydrated Silica ($SiO_2.nH_2O$)	with Fluoride	2mm	1	0	1	2.0mm	1	0
Sensodyne Repair & Protect	South Africa	Silica (SiO_2)	with Fluoride	2mm	1	0	1	2.0mm	1	0
						19	24		24	0

Fluoridated Toothpaste Samples	Fluoride Type	[F] ppm	Instructions for use			Warning Paediatric Consumers Box			Storage Instructions		
			Box	*Tube*	*Both*	*Box*	*Tube*	*Both*	*Box*	*Tube*	*Both*
Aquafresh Milk Teeth 0-3yrs	NaF	500	1	0	0	1	0	0	0	0	0
Aquafresh Little Teeth 4-6yrs	NaF	1000	1	0	0	1	0	0	0	0	0
Aquafresh Big Teeth >6yrs	NaF	1400	1	0	0	1	0	0	0	0	0
Colgate 0-2yrs	NaF	500	1	0	0	1	1	1	0	0	0
Colgate 2-5yrs	NaF	500	1	0	0	1	1	1	0	0	0
Colgate >5yrs	NaF	1450	1	0	0	1	1	1	0	0	0
Dis-Chem Dentalmate Kids	?	?	0	0	0	0	0	0	0	0	0
Elgydium Kids 2-6yrs	NH_4F	250	1	1	1	1	0	0	0	0	0
Mentadent P Kids	NaF	1000	0	1	0	1	1	1	0	0	0
Nature Fresh Junior Toothpaste	Homeopathic F ?	?	0	1	0	0	0	0	0	0	0
Aquafresh all-in-one Protection	NaF	?	1	1	1	1	1	1	0	0	0
Aquafresh Extreme Clean	NaF	1450	1	1	1	1	1	1	0	0	0
Aquafresh Ultimate	NaF	1450	1	1	1	1	1	1	0	0	0
Close Up Deep Action	NaF	1450	0	0	0	1	1	1	1 (cool dry place)	1	1
Colgate Active Salt	Na_2PO_3F	1000	1	0	0	1	1	1	0	0	0
Colgate Gel	Na_2PO_3F + NaF	1000 + 450	1	0	0	1	0	0	0	0	0
Colgate MaxFresh	NaF	1000	1	0	0	1	0	1	0	0	0
Colgate Maximum Cavity Protection	Na_2PO_3F	1450	1	0	0	1	0	0	1 (cool dry place away from direct heat or sunlight)	0	0
Colgate Sensitive Multiprotection	Na_2PO_3F	1000	1	0	0	1	1	1	0	0	0
Colgate Sensitive Pro-Relief	Na_2PO_3F	1450	1	1	1	1	1	1	1 (keep cap closed when not in use)	1	1
Colgate Total 12	NaF	1450	1	0	0	1	1	1	1 (cool dry place away from direct heat or sunlight)	1	1
Colgate Pro-Gum Health	NaF	1450	1	1	1	1	1	1	1 (cool dry place away from direct heat or sunlight)	1	1
Colgate Triple Action	Na_2PO_3F	1450	1	0	0	1	0	0	1 (cool dry place away from direct heat or sunlight)	0	0
Dis-Chem Dentalmate	NaF	?	0	0	0	0	0	0	0	0	0
Elgydium Sensitive	NH_4F	1250	1	1	1	1	1	1	0	0	0
Enamel Care	NaF	1100	1	1	1	1	1	1	1(works best when stored at normal room temperature >20 and moisture contamination is prevented by replacing cap)	1	1
GUM Caries Protect	Na_2PO_3F + NaF	1490	0	0	0	1	0	0	0	0	0
Mentadent P Gel Protection	NaF	1450	0	1	0	1	1	1	0	0	0
Mentadent P Micro Granules	NaF	1450	0	1	0	1	1	1	0	0	0
Mentadent P Protection	NaF	1450	0	1	0	1	1	1	0	0	0
Mentadent P Sensitive	Na_2PO_3F	1000	1	1	1	1	0	0	1 (store below 25)	1	1
Oral B Pro-Expert	SnF_2 + NaF	1100 + 350	0	0	0	1	1	1	0	0	0
Pepsodent	Na_2PO_3F	1450	0	0	0	1	1	1	1 (cool dry place away from direct heat or sunlight)	1	1
Pepsodent Complete 8	NaF	1450	0	0	0	1	1	1	1 (cool dry place away from direct heat or sunlight)	1	1
Sensodyne Cool Gel	NaF	1400	1	1	1	1	1	1	0	0	0
Sensodyne Multi Care	NaF	1400	1	1	1	1	1	1	0	0	0
Sensodyne Rapid Action	NaF	1040	1	1	1	1	1	1	1 (store below 30)	1	1
Sensodyne Repair & Protect	Na_2PO_3F	1450	1	1	1	1	1	1	1 (store below 25)	1	1
			26	18	13	35	25	26	12	10	10

Fluoridated Toothpaste Samples	Expiry Date				Purchase Date	Lot/Batch Identification			Volume (ml)
		Box	*Tube*	*Both*		*Box*	*Tube*	*Both*	
Aquafresh Milk Teeth 0-3yrs	07.2016	1	1	1	30.11.2014	4314	114314	1	50
Aquafresh Little Teeth 4-6yrs	06.2016	1	1	1	30.11.2014	4285	114285	1	50
Aquafresh Big Teeth >6yrs	06.2016	1	1	1	30.11.2014	4291	124291	1	50
Colgate 0-2yrs	01.2017	1	1	1	30.11.2014	4010CN123F	illigible	0	50
Colgate 2-5yrs	07.2016	1	1	1	30.11.2014	3195CN121H	3195CN121H	1	50
Colgate >5yrs	07.2017	1	1	0	30.11.2014	CP(L)4223PL1133	CP(L)4223PL1132	1	50
Dis-Chem Dentalmate Kids	01.2017	0	1	1	16.01.2015	?	?	0	70g
Elgydium Kids 2-6yrs	09.2015	1	1	0	30.11.2014	VOO250	VOO250	1	50
Mentadent P Kids	05.2017	0	1	0	30.11.2014		5038	1	50
Nature Fresh Junior Toothpaste	09.2016	0	1	0	30.11.2014		01360/00000001	0	75
Aquafresh all-in-one Protection	09.2016	1	1	1	30.11.2014	4365	104365	1	100
Aquafresh Extreme Clean	07.2016	1	1	1	30.11.2014	4311	64311	1	75
Aquafresh Ultimate	08.2016	1	1	1	30.11.2014	4352	54352	1	75
Close Up Deep Action	12.2016	1	1	1	30.11.2014	MN051213	MN051213	1	125g
Colgate Active Salt	10.2015	1	1	1	30.11.2014	113B04F21	113B04F21	1	75
Colgate Gel	09.2016	1	1	1	30.11.2014	14248ZA1021	14248ZA1012	1	100
Colgate MaxFresh	07.2017	0	1	0	30.11.2014	4192TH111M	illigible	0	75
Colgate Maximum Cavity Protection	07.2016	1	1	1	30.11.2014	14203ZA1034	illigible	0	100
Colgate Sensitive Multiprotection	07.2016	1	1	1	30.11.2014	3184TH111H	3184TH111H2	1	75
Colgate Sensitive Pro-Relief	04.2017	1	1	1	16.01.2015	4112BR121C	4112BR12CB	1	75
Colgate Total 12	04.2017	1	1	1	30.11.2014	4116CN123F	illigible	0	75
Colgate Pro-Gum Health	05.2016	0	1	0	30.11.2014	4123CN121N	illigible	0	75
Colgate Triple Action	08.2016	1	1	1	30.11.2014	14237ZA1022	14237ZA1022	1	100
Dis-Chem Dentalmate	06.2015	0	1	0	30.11.2014		1062012	0	75
Elgydium Sensitive	09.2015	1	1	1	30.11.2014	V03579	V03579	1	75
Enamel Care	10.2015	1	1	1	30.11.2014	FE2326	FE2326	1	115g
GUM Caries Protect	07.2017	1	1	1	30.11.2014	109	109	1	75
Mentadent P Gel Protection	04.2017	1	1	1	30.11.2014	40227	O227	1	100
Mentadent P Micro Granules	08.2017	1	1	1	30.11.2014	40634	O634	1	100
Mentadent P Protection	05.2017	1	1	1	30.11.2014	45001	5001	1	100
Mentadent P Sensitive	07.2016	1	1	1	30.11.2014	40601	O601	1	100
Oral B Pro-Expert	04.2016	1	1	1	30.11.2014		4149B4	0	75
Pepsodent	03.2016	1	1	1	30.11.2014	MN010314	MN010314	1	150
Pepsodent Complete 8	03.2015	1	1	1	30.11.2014	MM270312	MM270312	1	100
Sensodyne Cool Gel	07.2016	1	1	1	30.11.2014	4322	o64322	1	75
Sensodyne Multi Care	08.2016	1	1	1	30.11.2014	4343	104343	1	75
Sensodyne Rapid Action	04.2016	1	1	1	30.11.2014	14127B	14127B 1	1	75
Sensodyne Repair & Protect	05.2016	1	1	1	30.11.2014	254A	254A	1	75
		32	38	31				29	