ORIGINAL ARTICLE DENTISTRY

Journal of Contemporary Medicine and Dentistry
www.jcmad.com

ISSN [P-2347-4513]
ISSN [O-2349-0799]
Year: 2020
Volume: 8
Issue: 1
48-53

Ultraviolet–Visible Spectrophotometric Analysis of Tooth Whitening Efficiency of Ultraphosphate

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Abstract

Aim: *To compare the tooth whitening efficiency of Sodium Ultraphosphate solution with 30% Hydrogen per oxide and McInnes solution using Ultraviolet–visible spectrophotometry.* ***Methods:*** *60 human extracted central incisors were selected and decoronated. The samples were subjected to an artificial staining procedure for 3 days using freshly prepared coffee extract. The samples were then washed under running water for 10 seconds and randomly divided into 3 groups with 20 samples each. The whitening procedure was carried out for 10 minutes - Group 1 was treated with 10ml of 30 % Hydrogen per oxide solution and Group 2 with 30 ml of McInnes solution and Group 3 with 10ml of 20% 10ml of Sodium Ultraphosphate solution. After 10 minutes the test solutions mixed with the removed caffeine stain from tooth samples were analyzed spectrophotometrically and the difference in the absorbance values of the solutions before and after stain removal is directly proportional to the amount of caffeine present. The results were statistically analyzed by One-way analysis of Variance followed by Tukey's post hoc test.* ***Results:*** *The percentage of caffeine removed by Group 3 - Ultraphosphate solution was significantly higher (55%) then that of Group 1 - Hydrogen per oxide (48%) and Group 2- McInnes solution (2.98%).* ***Conclusion:*** *Our results clearly indicate that Sodium Ultraphosphate could be used as a potential stain remover in severely discolored tooth when compared to H_2O_2 and McInnes since it has got only a strong chelating action on tooth stains, without much adverse effect on dental hard and soft tissues. Development of a novel teeth stain removal system incorporating Sodium Ultraphosphate is expected in future.*

Keywords*: Tooth stains, Bleaching agents, Sodium ultraphospahte, Chelation, UV spectrophotometer*

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Date of Acceptance: 07/04/2020

Introduction

One of the fastest growing areas of aesthetic dentistry today is the management of the discolored and hypoplastic dentition. The demand for an improved appearance and a brighter smile has made tooth whitening a very popular dental procedure. Tooth whitening offers a conservative treatment option and minimally invasive intervention for discolored teeth in comparison to resin bonded composites, porcelain veneers or crowns.[1] Tooth bleaching procedures are based on a chemical oxidation-reduction reaction between the coloring substance (reducing agent) and the bleaching molecule (oxidizing agent).The oxidation reaction yields molecular oxygen, which is capable of penetrating the mineralized dental tissues without altering them and of degrading the pigments responsible for their coloration.[2] The oxygen molecules enter the organic enamel-dentin matrix and dissociate the pigments by modifying the long dark-colored molecular chains and splitting them into smaller and lighter-colored molecules being the stain destroyer. [3] Tooth bleaching was first described in 1877.

Dr. Walter Kane in 1916 used Hydrochloric acid to successfully remove fluorosis stains. In 1937, Ames reported an alternative agent for removing fluorosis using 30% Hydrogen per oxide. [4] McInnes in 1966, reported a technique that combined 5 parts 30% Hydrogen peroxide, 5 parts 36% Hydrochloric acid and 1 part Ethyl Ether for bleaching.[1-3] Despite the increase in the number of tooth whitening agents, Post-Operative sensitivity remains an unchanged adverse effect.[4] Ultraphosphate is an inorganic phosphate (Poly P) due to its chelating properties it aids in stain removal and prevents stain deposition on the tooth surfaces.[5] It has a branched mesh- like structure including a branched PO4 group in the molecule. Ultraphosphate with an average chain length of which is 10 to 30 phosphate residues and in particular, when the average chain length is 15 or more, high whitening effect can be observed by improved chelation. The concentration of phosphate polymers is preferably 3.5 to 20% by weight.[6] The polyphosphate may be added into the composition as a salt like sodium poly phosphate and potassium polyphosphate. This oral composition has high biocompatibility and high usability since it works well under neutral condition of pH 6.5 to 8 (or pH 6.9 to 7.5).[7] Ultraviolet–visible spectrophotometry (UV–Vis or UV/Vis) is routinely used in analytical chemistry for the quantitative determination of different analytes, such as transition metal ions, highly conjugated organic compounds, and biological macromolecules.[8] According to the Beer–Lambert law the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length. [9] Thus, for a fixed path length, UV/Vis spectroscopy can be used to determine the concentration of the absorber in a solution.

Hence the aim of this in- vitro study is to evaluate the Stain removal efficiency of this novel agent Ultraphosphate and is compared with two conventional bleaching agents – 30% Hydrogen peroxide and Mc Innes solution using Spectrophotometer.

Materials and Methods

The materials and the reagents used in the study are mentioned in Table 1.

Table-1: Materials and Reagents used

Materials	Working concentrations	Characteristics
Hydrogen peroxide	30%	Reagent grade
McInnes Solution	10 ml of 30% H_2O_2 10 ml of 36% HCl 2 ml anaesthetic ether	Reagent grade
Sodium Ultraphosphate	20%	Food-additive grade
Nescafe Classico Dark Roast Instant Coffee	5.5 grams	Commercial product

Sample preparation

Sixty human maxillary central incisors extracted for periodontal reasons were collected and stored in 0.9% Sodium chloride. The teeth were decoronated at the CEJ under copious irrigation using a diamond disc and washed under running water. The canal orifices were sealed using tooth- colored acrylic resin. The enamel was roughly exposed by using water resistant paper and surface irregularities were created using round end tapered diamond bur. Final wash with distilled water was done for 10 minutes. (Fig: 1-a, b)

Preparation of coffee extract

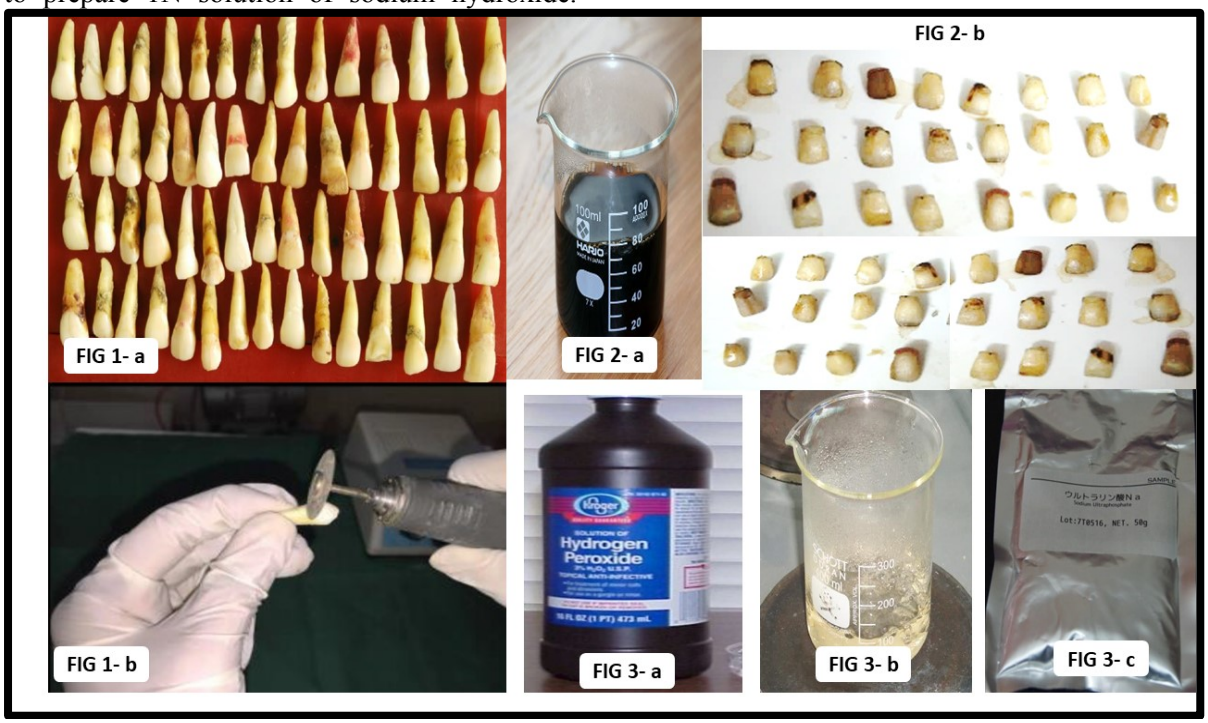
In order to artificially stain the samples, coffee extract solution was prepared by dissolving 5.5grams of instant coffee powder in 80 ml of boiling water at 100˚C (Fig:2 a). The maximum absorbent value (λ max) of this prepared coffee solution was measured using Spectrophotometer. The tooth samples were immersed in the prepared coffee extract for 72 hours. After 3 days the samples were washed with distilled water for 10 seconds and the dried. After drying they were randomly divided into 3 groups of 20 samples each based on the whitening solutions used. (Fig: 2 b)

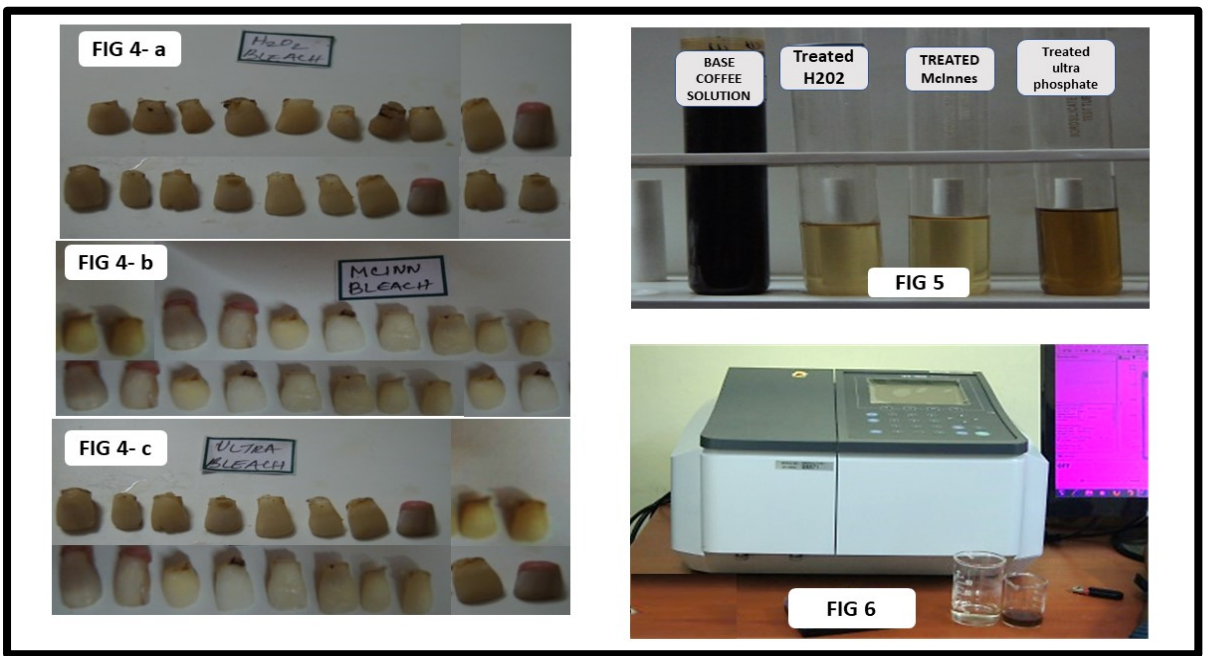
a) Preparation of test solutions

Group 1: 20ml of 30% Hydrogen per oxide solution at pH 5.5 (Fig 3a).

Group 2: McInnes solution was prepared by mixing 10ml 36% Hydrochloric acid. 10ml of 30% Hydrogen peroxide and 2ml of Anesthetic Ether (5:5:1) to a pH 5. (Fig 3b).

Group 3: Type 60 Poly (P) (sodium salts; medium chain Poly (P) of 60 phosphate residues) was obtained from Tokyo, Japan. 2 grams of 40 equivalent sodium hydroxide powder was dissolved in 50ml of distilled water to prepare 1N solution of sodium hydroxide. 10grams of Sodium metaphosphate powder was dissolved in 50ml of distilled water to get 20% solution. 15ml of 1N Sodium hydroxide was triturated with 5ml of 20% Ultraphosphate solution to a neutral pH of 7. (Fig 3c)





Methodology

The three test solutions were subjected to spectrophotometry and the maximum absorbance values (AU-absorbance units) were measured. The tooth samples were immersed into the test solutions for 30 minutes. (Fig 4-a,b,c) The samples were taken out from the solutions and using Spectrophotometer, the maximum absorbance values of the resultant test solution were

evaluated and statistically analyzed (Fig 5). The absorbance values of our test solutions before and after whitening procedure indicates the percentage of caffeine present and the results were tabulated and graphically plotted. (Fig 6)

Statistical Analysis

The SPSS version 20 software (IBM Corp., Armonk NY, USA) was used to perform statistical analyses and non-parametric Friedman test was used to compare the mean absorbance values between the three groups at significance value set at 1%. (Table 2)

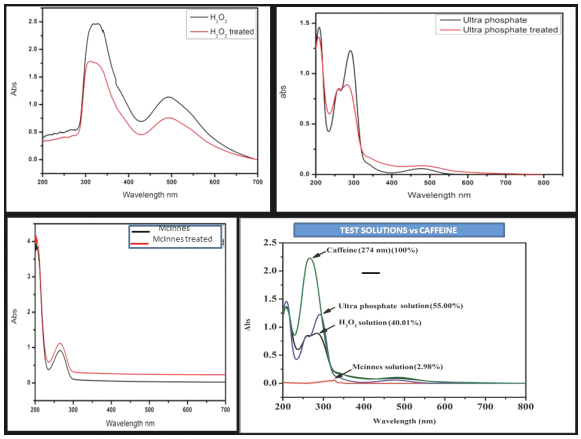
Table- 2: Percentage of stain removal & absorbance values of the test solutions after bleaching

Solutions	Percentage of Caffeine	Absorbance Value
Caffeine	100%	2.2319
Ultraphosphate	55%	1.2277
H_2O_2 Solution	40.01%	0.8930
McInnes	2.98%	0.6671

Results

There was a statistically significant difference in the percentage of stain removal among the three groups (p=<0.001). Post Hoc Analysis with Wilcoxon signed rank test was conducted and the Median (IQR) for the three groups were UPO_4→1.15(1.02-1.25), H_2O_2→0.66 (0.62 - 0.71), McInnes→0.91(0.87-0.98) There was a statistically significant difference between the groups when compared UPO4 & H2O2—Z=-3.883, p=<0.001 and UPO4 & McInnes—Z=-3.920, p=<0.001. The UV – Spectrophotometric absorbance peaks graph clearly shows the amount of caffeine in the group 3 is higher than the other two groups and indicates that Sodium Ultraphosphate removes the caffeine stain effectively without degrading them, thereby showing higher absorbance peaks nearer to caffeine. (Graph: 1)

Graph-1: UV–Vis Spectrophotometry Absorbance Peaks



Discussion

Tooth plays a major role in the beauty and personality of the individual. One of the main reasons for seeking cosmetic dental treatment is the discolored tooth. Bleaching is one of the economical and least invasive aesthetic treatments for lightening of the stains.[10] Various agents have been used traditionally as vital bleaching agents most of them primarily act by oxidation of the organic stains. However, each of them possesses their own advantages and disadvantages. [11]

Hydrogen per oxide was first advocated for the removal of fluorosis stains in the year 1884 by Dr. Harlan. Hydrogen per oxide acts by redox reaction. It is a potent biological oxidant of organic and inorganic compounds through the formation of free radicals, reactive oxygen molecules, and hydrogen peroxide anions. Because of its low molecular weight, hydrogen peroxide can penetrate into and through the enamel to reach the enamel-dentin junction and dentin, capable of releasing oxygen that breaks the double bonds of organic and inorganic compounds of dentin structure.[12] Hydrogen peroxide can destroy stain material itself by producing hydroxyl radical and other oxygen radicals, that's why it is referred as a Bleaching agent. The main disadvantage of hydrogen per oxide is post-operative sensitivity because peroxide dissolves calcium phosphate including Hydroxyapatite - it causes dissolution of the enamel along with stain removal. [13]

In 1966 McInnes reported a technique that combined five parts 30% hydrogen peroxide, five parts 36% hydrochloric acid and one part ethyl ether and applied the solution with a cotton wrapped toothpick to the areas of the

teeth affected by fluorosis stain. After 10 to 15 minutes the teeth were washed with water and neutralized with a sodium bicarbonate paste. However, the abrading nature of the McInnes solution may result in reduced micro-hardness of the enamel structure. In the present study, the stain removal potential of Sodium ultraphosphate was compared with Hydrogen per oxide and McInnes solution because they are the most commonly used agents for bleaching in a clinical setup.[14]

Ultraviolet–visible spectroscopy uses light in the visible and adjacent ranges. The absorption or reflectance in the visible range directly affects the perceived colour of the chemicals involved. In this region of the electromagnetic spectrum, atoms and molecules undergo electronic transitions.[15] Based on the Beer–Lambert law for a fixed path length, UV/Vis spectroscopy was used to determine the concentration of the absorber (caffeine) in the test solutions. The wavelengths of absorption peaks can be correlated with the types of bonds in a given molecule and are valuable in determining the functional groups within a molecule.[16]

The quest for a novel whitening agent with efficient stain removal potency and least effect on enamel and gums formed the basic objective for the formulation of inorganic phosphates.[17] Inorganic polyphosphate (Poly (P)) exists as polymers of orthophosphate units and is widespread in nature, mammalian nuclei, mitochondria, lysosomes and plasma membranes.[18] It is generated by polymerizing two or more PO4 tetrahydras to share an oxygen atom included in other PO4 tetrahydras. The safety aspects have also been shown in the rat, guinea-pig and dog and confirmed in humans. [19] The oral composition realizes dramatically high stain removal effect compared to the case of the peroxides or other phosphate polymers.[20-21] The present study clearly showed that tooth stains can be removed effectively even in neutral pH condition using the salt of phosphate polymers at a predetermined concentration.

In this study ultraphosphate possessed higher tendency for reduction of stain when compared to hydrogen per oxide and McInnes solution. Ultraphosphate could bind to the teeth surface by its high negative charge and it could repel the stains from the tooth because the binding energy between ultraphosphate and teeth is higher than that of the binding between the stain and teeth. This chelating nature of the ultraphosphate could prevent abrasion of the tooth surface and hence the post-operative sensitivity. Since ultraphosphate could not dissociate into free radicals, it is not a bleaching agent. It is a powerful stain remover, but it cannot destroy stain material itself. Still further in vivo studies are needed to substantiate its potential in the clinical scenario.

Conclusion

Within the limitations of this in-vitro study, it could be concluded that the Stain removal efficiency of Sodium Ultraphosphate is comparable to the commonly used bleaching agents, Hydrogen-per-oxide and McInnes solution and it removes the stains without eliciting a bleaching action on the tooth. A paradigm shift to develop a novel aesthetic tooth whitening system with Sodium Ultraphosphate is expected in the near future.

Acknowledgment

This study was conducted at The Department of Medicinal Chemistry, Madurai Kamaraj University, Madurai, Tamil Nadu, India.

Conflict of Interest: *None declared*
Source of Support: *Nil*
Ethical Permission: *Obtained*

References

1. Joiner A. The bleaching of teeth: a review of the literature. J Dent 2006;34:412–19.
2. Dahl JE, Pallesen U. Tooth bleaching-a critical review of the biological aspects. Crit Rev Oral Biol Med 2003;14:292-304.
3. Rotstein I, Li Y. Tooth discoloration and bleaching. In: Ingle's Endodontics 6. Eds.: J. I. Ingle; L. K. Bakland and J. C. Baumgartner, pp. 1383-1399, BC Decker Inc. Hamilton, 2008.
4. American Dental Association Council on Scientific Affairs. Statement on the effectiveness of tooth whitening products. February 2008. Retrieved August 14, 2009,

from http://www.ada.org/1902.aspx [accessed on 13/12/2019].
5. Shiba T, Kawazoe Y, Kato S. Oral composition for removing tooth stain, WO 2012/039452 A1, Sep. 22, 2011.
6. Aoki T, Nakano T, Iwashita T, Sugimoto Y, Ibraham HR, Toba Y, *et al.* Preparation and characterization of micellar calcium phosphate-casein phosphopeptide complex. J Nutr Sci Vitaminol (Tokyo) 1998;44:447-56.
7. Lanigan RS. Final report on the safety assessment of sodium metaphosphate, sodium trimetaphosphate, and sodium hexametaphosphate. Int J Toxicol 2001; 20: 75-89.
8. Brian M. Tissue, Ultraviolet and Visible Absorption Spectroscopy, Characterization of Materials, (1-13), (2012). Wiley Online Library [accessed on 22/12/2019]
9. Chu SJ. Use of a reflectance spectrophotometer in evaluating shade change resulting from tooth-whitening products. J Esthet Restor Dent. 2003;15 Supp l1:S42-48.
10. Mohammed Q. Alqahtani. Tooth-bleaching procedures and their controversial effects: A literature review. Saudi Dent J. 2014 Apr; 26(2): 33–46.
11. Sulieman MA. An overview of tooth-bleaching techniques: chemistry, safety and efficacy. Periodontol 2000. 2008; 48:148-69.
12. Junior MT, Rodrigues CA, Bernardes VL, Araujo TSB, Nicoli GA, et al. Dental Bleaching and New Possibilities - Literature Review. Health Sci J 2018;12(6):600.
13. Mounika A, Mandava J, Roopesh B, Karri G. Clinical evaluation of color change and tooth sensitivity with in-office and home bleaching treatments. Indian J Dent Res 2018;29:423-71.
14. Torres CR, Wiegand A, Sener B, Attin T. Influence of chemical activation of a 35% hydrogen peroxide bleaching gel on its penetration and efficacy – *In vitro* study. J Dent 2010;38:838-46.
15. H E Darshan and N D Shashikiran J. The effect of McInnes solution on enamel and the effect of Tooth mousse on bleached enamel: An *in vitro* study. Conserv Dent. 2008 Apr-Jun; 11(2): 86–91.
16. Vieira Glauco Fioranelli, Arakaki Yuri, Caneppele Taciana Marco Ferraz. Spectrophotometric assessment of the effects of 10% carbamide peroxide on enamel translucency. Braz. oral res. 2008; 22(1):90-95.
17. Yue Hugh Guan- The measurement of tooth whiteness by image analysis and spectrophotometry: A comparison Article *in* Journal of Oral Rehabilitation 32(1):7-15 February 2005.
18. Masahiro Koyasu, Toshikazu SHIBA, Yumi Kawazoe, Atsufumi Manabe Takashi. Ultraphosphate, a potent stain control agent that is effective for both stain removal and prevention of stain deposition. Dental materials journal 2014; 33(2): 252-60.
19. K. Brow Richard Structure Review: the structure of simple phosphate glasses. Journal of Non-Crystalline Solids 2000; 263-264.
20. Shiba T, Tsutsumi K, Ishige K et al. Inorganic polyphosphate and polyphosphate kinase: their novel biological functions and applications. Biochemistry (Mosc) 2000; 65: 315–323.
21. Shiba Toshikazu, Kawazoe Yumi, Kato Shinichi. Oral Composition for Removing Tooth Stain. United States Patent Application 20130189200 2013; [accessed online from http://www.freepatentsonline.com/y2013/0189200.html on 22/01/2020]