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Spectrophotometric Evaluation of Potassium Nitrate Penetration Into the Pulp Cavity

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Clinical Relevance

The tooth is permeable to potassium nitrate–containing formulations with an application time of 30 minutes.

SUMMARY

Objectives: The aim of this study was to evaluate the penetration level of potassium nitrate–containing desensitizers or whitening materials into the pulp cavity with regard to the concentration and viscosity of the formulation.

Methods and Materials: Fifty extracted human molar teeth were prepared and randomized into five groups of 10 specimens each. The control received a 30-minute treatment without any treatment material; the other four groups corresponded to treatment with DayWhite, a 14% hydrogen peroxide whitening material containing potassium nitrate; PreviDent 5000 Sensitive, a desensitizing toothpaste; Relief ACP, a desensitizing gel; or UltraEZ, a desensitizing gel. Potassium nitrate penetration levels were measured spectrophotometrically based on the Griess assay method. Treatment materials were measured for viscosity as a function of shear rate through the use of a cone-and-plate rheometer.

Results: Nitrate penetration levels were significantly different among the five groups (*p*$<$0.0001, Kruskal-Wallis test). After adjustment for multiple comparisons using an overall 0.05 level of type I error, the distribution of nitrate penetration values was found to differ significantly among all groups with the exception of DayWhite (median: 10.72 μM) and Ultra-

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DOI: 10.2341/14-214-L

EZ (median: 9.22 μM), which differed significantly from other groups but not from each other. The highest levels of nitrate penetration value were observed for PreviDent (median: 27.61 μM) followed by Relief ACP (median: 19.64 μM). The lowest penetration level was observed for the control group (median: 3.41 μM). Stable end-point viscosities of 11.43 ± 0.67 Pa/s, 1.33 ± 0.06 Pa/s, 0.85 ± 0.09 Pa/s, and 0.40 ± 0.01 Pa/s were observed for UltraEZ, ReliefACP, DayWhite, and PreviDent, respectively.

Conclusion: Potassium nitrate included in different formulations can penetrate the enamel and dentin within 30 minutes. The level of potassium nitrate penetration is influenced by concentration and may also be partly affected by the viscosity of the material as well as other constituents of proprietary preparations.

INTRODUCTION

Tooth whitening represents the most common elective dental procedure as a growing number of people envision a Hollywood smile highly driven by media emphasis on beauty and health.[1] It has proven to be safe and effective when properly diagnosed, treatment planned, and supervised by the dentist. The wide range of techniques available—in-office whitening, dentist-supervised at-home whitening, over-the-counter whitening, and do-it-yourself whitening—reflects its popularity.[2] The efficacy and side effects are related to the techniques, with tooth sensitivity being the most frequently reported side effect associated with all of the approaches.[3]

Whitening-induced tooth sensitivity commonly presents itself as generalized sensitivity to cold stimuli but often also occurs as a spontaneous sharp, shooting pain limited to one or few teeth.[4] The incidence of tooth sensitivity ranges from 0% to 75%, and the degree can vary from mild to intolerable, leading some patients to discontinue treatment.[5] A clinical trial by Jorgensen and Carroll[6] concluded that about half of the patients performing whitening will experience mild sensitivity, 10% will have moderate sensitivity, and 4% will have severe sensitivity. Despite the fact that sensitivity is only transient and will resolve almost immediately on completion of the whitening procedure, it adversely affects patient compliance and satisfaction with the whitening experience. Therefore, efforts to elucidate the etiology and develop strategies for the prevention and treatment of whitening-induced tooth sensitivity continue to be a central issue to be addressed in tooth-whitening procedures.

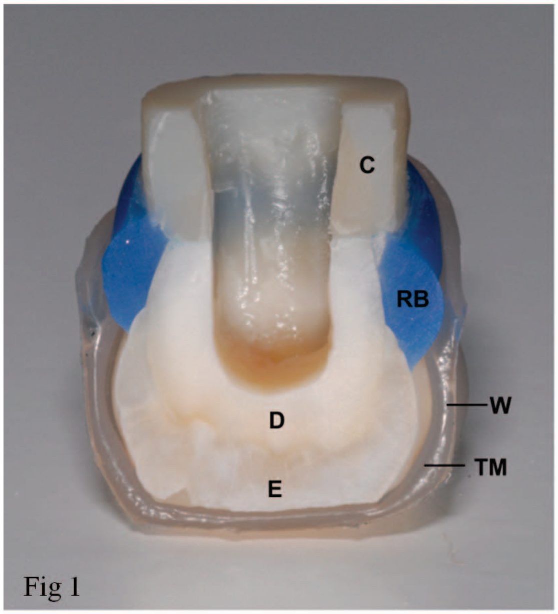
The management of tooth sensitivity related to whitening has been addressed using a variety of approaches based on hydrodynamic theory. According to Brännström's theory, dentinal fluid expands, contracts, or flows within dentinal tubules under the influence of thermal, evaporative, or osmotic changes and stimulates pressure-sensitive nerve receptors (A-δ fibers) that transmit the stimulus and produce the perception of pain.[7] Agents such as fluoride and amorphous calcium salts are generally used to occlude the permeability of dentinal tubules, preventing the flow of dentinal fluid. Another common desensitizing approach is to decrease the activity of dentinal sensory nerve fibers and prevent pain signal transmission to the central nervous system.

The mode of desensitizing action of potassium nitrate is that it affects the extracellular potassium ion concentration, which is the principal determinant of the nerve resting electrical potential. When the potassium ion concentration is increased above the normal physiologic level, the cell depolarizes, creating a period of inactivation. However, because of the properties of membrane gates, this period does not last long, and the action potentials begin to occur again.[8,9]

It has been demonstrated that hydrogen peroxide in the whitening gel readily penetrates into the pulp cavity within 5 to 15 minutes when applied to the external surface of the tooth.[10,11] The penetration is facilitated by the use of higher peroxide concentration, heat, light, and younger age of the tooth.[11-14] It may be assumed that potassium nitrate will penetrate into the tooth structure similar to hydrogen peroxide to exert the depolarizing effect on the dental nerve fibers.

Currently, potassium nitrate is available in varying concentrations in desensitizing gels or toothpastes, usually in conjunction with sodium fluoride or amorphous calcium phosphate. Several manufacturers have also incorporated potassium nitrate in their whitening formulations. These materials need to flow easily on insertion but should have a viscosity at low stresses to stay in place on teeth that is related to the rheological properties of the material.[15] However, little information is available regarding the penetration potential of potassium nitrate into the pulp with regard to the concentration and physical characteristics of the gels and pastes used.

The purpose of this study was to evaluate the potential penetration of potassium nitrate–contain-



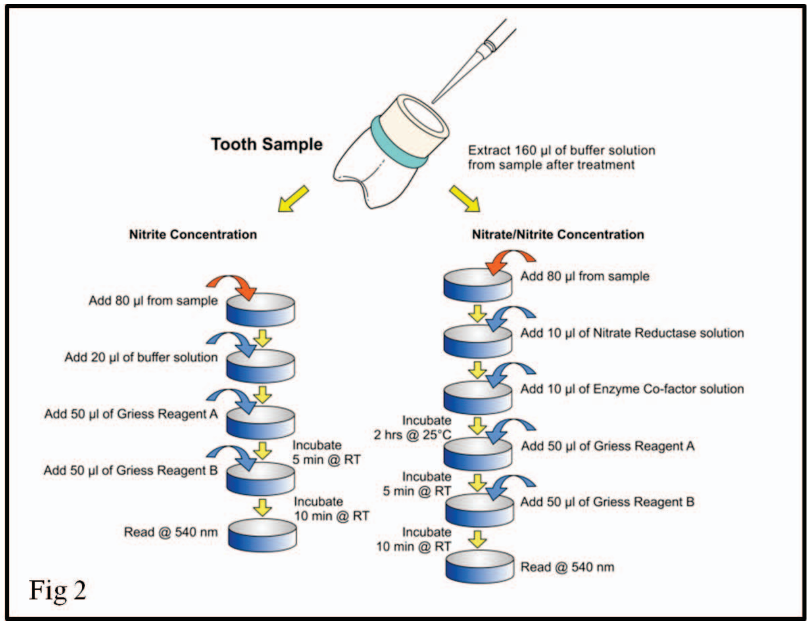


Figure 1. *Photograph showing cross section of tooth specimen preparation and material application.*
Figure 2. *Diagram of colorimetric assay.*

ing desensitizers or whitening materials into the pulp cavity. The hypotheses to be evaluated were the following:

1. There will be significant potassium nitrate penetration into the pulp cavity when compared to the control.
2. Potassium nitrate penetration into the pulp cavity will be concentration dependent.
3. Potassium nitrate penetration into the pulp cavity will be dependent on the viscosity of the treatment material.

METHODS AND MATERIALS

Sample Selection and Preparation

Fifty extracted human molar teeth were collected prior to the study and stored in 0.2% thymol (Sigma-Aldrich, St Louis, MO, USA) in distilled water at 4°C. Calculus was removed with a sickle scaler and any surface debris cleaned with plain pumice (Preppies, Whip Mix Corporation, Louisville, KY, USA) and purple prophy cups (Young Dental, Earth City, MO, USA). All teeth were observed for the absence of developmental anomalies, caries, existing restorations, deep crack lines, or severe attrition. The roots were trimmed 3 mm apical to the cemento-enamel junction, the pulp was removed, and the pulp chamber was enlarged with a pointed tapered diamond bur (NeoDiamond, Microcopy, Kennesaw, GA, USA) to maintain a standardized outer wall tooth thickness of 2 mm. Tooth thickness was measured from the outer surface to the outer boundary of the pulp cavity at the cross-sectioned root 3 mm below the cemento-enamel junction using an electronic digital caliper (Harbor Freight Tools, Pittsburgh, PA, USA). Molar teeth were selected for the study to enable the retention of 200 µL of phosphate-buffered saline in the pulp cavity that was required for the spectrophotometric assay of nitrate and nitrite. Additionally, the root surface was built up with flowable resin (Estelite Flow, Tokuyama Corp, Tokyo, Japan) to aid the retention of phosphate buffer and prevent any spilling out of the pulp chamber. The occlusal pit and fissures were sealed with flowable resin to prevent any leakage of the buffer out of the cavity. A resin barrier (OpalDam, Ultradent Products, Inc, South Jordan, UT, USA) was placed 2 mm apical to the cemento-enamel junction and light cured for 20 seconds (Elipar S10 LED curing light, 3M ESPE, St Paul, MN, USA) to limit the treatment material to the coronal surface of the tooth (Figure 1).

Application Protocol by Group

Specimens were randomized into five groups of 10 specimens each and are summarized in Table 1. Each treatment material (0.3 mg) was applied on the external coronal surface and covered with a low-density polyethylene wrap (Saran Premium Wrap, S.C. Johnson & Son, Inc, Racine, WI, USA) to

Table 1: *Desensitizers and Whitening Agents Used by Group*

Group	Brand Name	KNO_3 (%)	Viscosity (Pa/s)[a]
Control	NA	NA	NA
DayW	DayWhite	2.9	0.85 ± 0.09
PrevD	PreviDent 5000 Sensitive	5	0.40 ± 0.01
Relief	Relief ACP	5	1.33 ± 0.06
Ultra	UltraEZ	3	11.43 ± 0.67

Abbreviations: KNO_3, potassium nitrate; NA, not applicable.
[a] Stable viscosity (10-point average ending at 400 L/s).

simulate the placement of a custom-fabricated tray (Figure 1). The control received a 30-minute treatment with the wrap but without any treatment material applied. Group DayW was treated with DayWhite, a 14% hydrogen peroxide whitening material containing potassium nitrate (Philips Oral Health Care, Stamford, CT, USA); group PrevD with PreviDent 5000 Sensitive, a desensitizing toothpaste (Colgate-Palmolive, New York, NY, USA); group Relief with Relief ACP, a desensitizing gel (Philips Oral Health Care); and group Ultra with UltraEZ, a desensitizing gel (Ultradent Products).

The pulp cavities were rinsed twice with 100 μL of distilled water and dried with coarse paper points prior to the placement of 200 μL of buffer solution. The buffer solution acted as a stabilizing agent of potassium nitrate that might have diffused into the pulp cavity. All teeth were kept in a closed humid chamber (General Glassblowing Co, Richmond, CA, USA) during the treatment time (30 minutes) at 37°C with 100% relative humidity.

Spectrophotometric Assay of Nitrate/Nitrite

A nitrate/nitrite assay kit (Sigma-Aldrich) composed of nitrate and nitrite standard solutions, buffer solution, nitrate reductase, enzyme cofactors, and Griess dyes was used for the colorimetric determination of nitrate in the samples. The standard solutions were mixed with the buffer solution to yield final concentrations of 0, 25, 50, and 100 μM of nitrite and nitrate/nitrite to establish the calibration curves.

Figure 2 illustrates the colorimetric nitrate/nitrite assay procedure. Retrieving 80 μL of buffer solution from the pulp cavity and transferring it to a 96-well cell culture plate determined the nitrite concentration in the sample. Subsequently, 20 μL of buffer solution and 50 μL of Griess reagent A and B were added and mixed. The mixture was incubated for 10 minutes at room temperature and the absorbance read at 540 nm with a microplate reader (Power Wave X-I, BioTek, Winooski, VT, USA). The nitrite concentration in the sample solution was determined from the nitrite calibration curve.

The nitrite and nitrate concentration in the samples was determined by retrieving 80 μL of buffer solution from the pulp cavity and transferring it to a 96-well cell culture plate. Subsequently, 10 μL of nitrate reductase solution and 10 μL of enzyme cofactor solution were added, mixed, and incubated at 25°C for 2 hours. After incubation, 50 μL of Griess reagent A and B were added and mixed. The mixture was incubated for an additional 10 minutes at room temperature and the absorbance read at 540 nm with a microplate reader. The nitrate and nitrite concentration in the sample solution was determined from the nitrate/nitrite calibration curve. The final nitrate concentration in the sample was obtained by the following equation:

$$[\text{Nitrate}] = [\text{Nitrate/Nitrite}] - [\text{Nitrite}]$$

Measurement of Viscosity

Samples were measured for viscosity as a function of shear rate through the use of a cone-and-plate rheometer (Haake Rheostress 1, Thermo Scientific, Nashville, TN, USA) with temperature controls. For viscosity testing, 0.8 mL of sample was probed using a 35-mm-diameter/4-degree-angle stainless-steel cone plate at a gap height of 0.140 mm. Controlled shear rate ramp-ups were performed, a method that continuously raises the frequency of plate rotation while recording the sample viscosity in predefined time increments. These flow ramp-ups were performed over 3 minutes over a range of shear rates from 0.1 to 400 L/s, allowing for the viscosity curves to stabilize at higher shear rates. Sample temperature was controlled at 37°C throughout experimentation. Viscosity curves were generated for each of the four samples, and stable viscosity values were obtained.

Data Analysis

The nonparametric Kruskal-Wallis test was used to assess group differences because of nonnormality and variance heterogeneity associated with the distribution of potassium nitrate penetration values among the five treatment groups. All possible pairwise comparisons of treatment groups were made using the modification of the Tukey method as modified by Conover[16] to adjust for multiple comparisons in conjunction with an overall 0.05 level

Table 2: *Nitrate Penetration Descriptors by Group (μM).*					
Group	**Control**	**DayW**	**PrevD**	**Relief**	**Ultra**
n	10	10	10	10	10
Mean	4.41	11.14	30.15	19.64	9.71
Standard deviation	2.93	2.49	8.20	3.49	3.88
Median	3.41	10.72	27.61	19.64	9.22
Minimum	0.39	6.82	18.07	11.45	3.64
Maximum	8.51	15.97	43.76	24.72	17.94

of type I error. Descriptive statistics and box plots were generated for each treatment group.

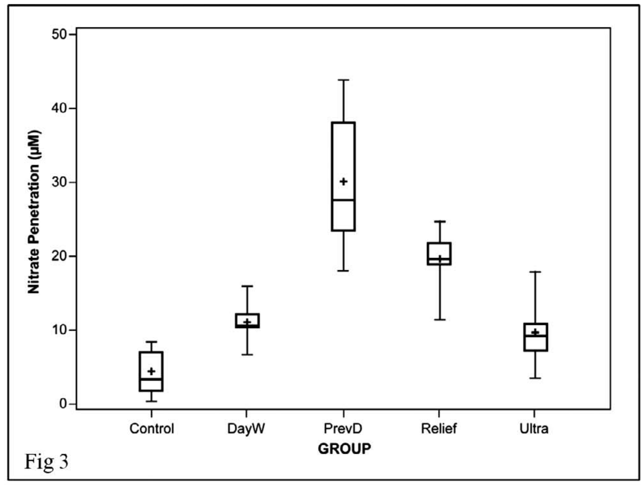
RESULTS

Potassium Nitrate Penetration

Descriptors of nitrate concentration are given for each of the five treatment groups in Table 2, and nitrate distributions are characterized graphically by box plots in Figure 3. The data provided strong evidence of differences in the distribution of potassium nitrate penetration among the five treatment groups ($p<0.0001$, Kruskal-Wallis test). After adjustment for multiple comparisons using an overall 0.05 level of type I error, the distributions of nitrate penetration values were found to differ significantly among all groups with the exception of DayWhite and UltraEZ, which could not be said to differ from each other, although each differed significantly from the other three groups. The highest levels of nitrate penetration value were observed for PreviDent (median: 27.61 μM); this distribution significantly differed from that of Relief (median: 19.64 μM). Both differed significantly from both DayWhite (median: 10.72 μM) and UltraEZ (median: 9.22 μM), although results for DayWhite and UltraEZ could not be said to differ from each other. The lowest penetration level was observed for the control group (median: 3.41 μM), which was significantly lower than for all the other treatment groups.

Viscosity

Figure 4 shows the average sample viscosity curves (n=3) for UltraEZ, ReliefACP, DayWhite, and PreviDent plotted against the shear rates of the ramp-up experiments. Stable end-point viscosities of 11.43 ± 0.67, 1.33 ± 0.06, 0.85 ± 0.09, and 0.40 ± 0.01 Pa/s were observed for UltraEZ, ReliefACP, DayWhite, and PreviDent, respectively (Table 1). Shear thinning behavior was observed for all samples. This decreasing order of sample viscosities can be observed over all shear rates investigated, with the exception of the similarities between DayWhite and



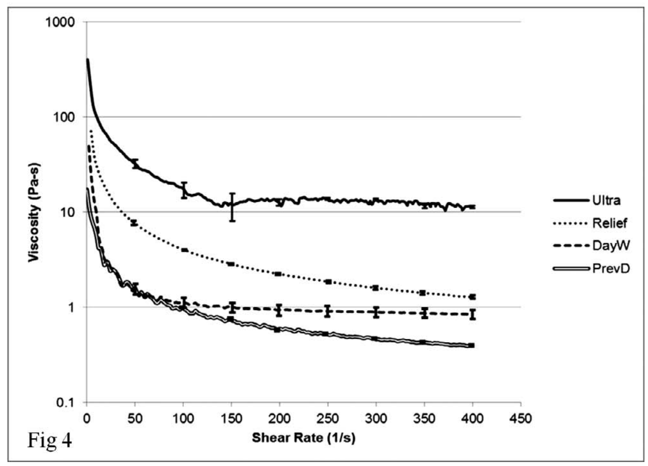


Figure 3. *Box plots of nitrate penetration by group.*
Figure 4. *Controlled shear rate viscosity ramp-up curves by treatment group.*

PreviDent viscosities from 0.1 to 100 L/s. Intra-sample standard deviations were relatively small for viscosity curve data over all shear rates investigated.

DISCUSSION

It is evident that whitening materials have to penetrate throughout the tooth to exert the whitening effect on chromogens within the enamel and dentin. Although numerous *in vitro* studies have shown that hydrogen peroxide readily penetrates into the pulp cavity, considering the potential toxic effects that hydrogen peroxide might have on the pulp, it is still debatable whether a high penetration amount is actually desired or needed for superior whitening efficacy.[17] The penetration of potassium nitrate into the tooth structure is expected to be similar to hydrogen peroxide to reach the pulp and exert its calming action on the nerve receptors located mainly in the inner dentin. However, there has been no study in the literature reporting on the assay of potassium nitrate–containing whitening agents or desensitizers. This is somewhat surprising considering the variety of currently marketed delivery methods of potassium nitrate–containing dentrifices, desensitizers for professional use, and whitening gels.

This study explored the penetration potential of potassium nitrate with different concentrations and formulations. The findings supported the hypothesis that there would be significant penetration as compared to the control group. There was also higher penetration with increasing potassium nitrate concentration so that the second hypothesis was also supported. It is important to mention that even the control group exhibited trace amounts of nitrate (median: 3.41 μM). This may be explained by the fact that potassium nitrate is commonly used in food preparation, specifically cold meats and other delicatessen products.[18] There is also some nitrate (maximum contaminant level goals [MCLG]: 10 ppm) and nitrite (MCLG: 1 ppm) in our drinking water so that accumulation of trace amounts of potassium nitrate may be expected in human extracted teeth.[19] The results related to concentration confirm that the flux of molecules follow Fick's second law, in which diffusion is proportional to surface area, the diffusion coefficient, and concentration and inversely related to diffusion distance. It is also in agreement with studies that showed that penetration of hydrogen peroxide was concentration dependent.[10,12] The highest potassium nitrate concentration of 5% used in this study is also the maximum concentration allowed by the U.S. Food and Drug Administration to be used in desensitizing toothpastes because this material and concentration have the best scientific evidence for treating tooth sensitivity.[4]

The current protocol for the treatment and prevention of whitening-induced sensitivity can be either passive or active. Passive treatment involves using a lower concentration of whitening agent, decreasing the treatment time, or increasing the treatment interval, whereas active treatment involves the application of 3% to 5% potassium nitrate in the tray for 10 to 30 minutes before or after bleaching.[20-22] Brushing with a potassium nitrate–containing toothpaste for 2 weeks prior to the whitening and continuing this brushing throughout the treatment has been shown to have an additional benefit in the reduction of sensitivity.[23] Tray delivery of desensitizing toothpaste such as PreviDent 5000 Sensitive can also be effective; however, caution has been advised because toothpastes contain sodium lauryl sulfate (SLS), the primary ingredient in cleaning and hygiene products that creates foaming. SLS has been associated with increased aphthous ulcers and contact gingival irritation in some patients and also has been shown to remove the smear layer, inviting more sensitivity.[4] Manufacturers have also incorporated potassium nitrate into their whitening formula, and studies have shown that this formula indeed showed significantly less sensitivity with regard to incidence and severity.[24-27] Our study confirmed that all approaches are valid in terms of potassium nitrate penetration with an application time of 30 minutes. However, like any other medication to relieve pain, it is important to determine the onset, duration, and proper dosage of potassium nitrate for maximum efficacy, and these considerations should be further investigated in future studies.

There are also other factors to consider when evaluating potassium nitrate penetration levels, one of which is the viscosity of the material. Wille and others[15] suggested that whitening agents should fulfill specific rheological properties to withstand shear stress so that they do not flow out of the tray and are not easily washed away or swallowed. Therefore, with the use of a square root transformation of potassium nitrate penetration values to achieve conformance to model assumptions, we also explored possible relationships between potassium nitrate penetration and the potassium nitrate concentration and viscosity of the four noncontrol proprietary treatments to address our third hypothesis of whether potassium nitrate penetration will be viscosity dependent. No clear trends to support our third hypothesis could be identified based on these explorations using linear modeling, as there was evidence of significant interaction between potassium nitrate concentration and viscosity.

Although DayWhite and UltraEZ contain comparable concentrations of potassium nitrate (2.9% vs 3%, respectively), DayWhite has a lower viscosity than UltraEZ (0.85 vs 11.43 Pa/s). On the basis of the viscosity difference, it would have been expected that diffusion through DayWhite would have been more rapid than diffusion through UltraEZ; however, the concentrations of nitrate found in the buffer in the pulp cavities were nearly identical. The fact that each proprietary product is distinct in a variety of ways suggests that other factors may be exerting a modulating influence. One difference between these two preparations is that DayWhite contains hydrogen peroxide, while UltraEZ does not. Hydrogen peroxide is sufficiently volatile as to produce local evaporative cooling. This potential thermal effect could slow diffusion in DayWhite and provide a plausible explanation to our findings. It is also noteworthy to point out that within the desensitizers (PreviDent, Relief ACP, and UltraEZ), there was a strong tendency of viscosity dependency: the lower the viscosity, the higher the penetration levels. This is in accordance with a study that evaluated the effect of viscosity on the penetration depth of resin-based materials into the tooth structure. The study showed deeper penetration into the tooth structure with the material of lower viscosity.[28]

This *in vitro* model is representative of the *in vivo* process, although it is not known how closely it would compare to the *in vivo* absorption of potassium nitrate in teeth with vital pulps exhibiting positive pulpal pressure during the application process. It is also noteworthy to point out that teeth were mechanically altered to encompass 200 μL of phosphate-buffered saline in the pulp cavity that may have affected the permeability of potassium nitrate. Based on these findings, further studies should be employed to evaluate the significance of potassium nitrate penetration levels at the nerve endings and the clinical significance of these penetration levels. The best potassium nitrate delivery method and application time should be assessed to ultimately suggest a whitening protocol with minimal tooth sensitivity and maximum whitening efficacy.

CONCLUSIONS

Within the limitations of this *in vitro* study, the following can be concluded:

1. Potassium nitrate included in desensitizers, dentrifices, and whitening materials, when applied to the external surface of the tooth, can penetrate the enamel and dentin within 30 minutes.
2. The level of potassium nitrate penetration is influenced by concentration and may also be partly affected by the viscosity of the material as well as other constituents of proprietary preparations.

Acknowledgment

The authors thank Patricia Conrad at Technology and Media Services for designing the illustrations.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the University of Iowa. The approval code for this study is 201404750. This study was conducted at the University of Iowa.

Conflict of Interest

The authors of have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

(Accepted 7 January 2015)

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