A New Method to Assess Available Chlorine in Small Volumes of Liquid

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Abstract

Introduction: There is no robust and simple way to quantify available chlorine from small volumes such as human root canals. Therefore, a new method was developed and assessed. **Methods:** Standardized size-40 paper points were soaked in a 15% (w/v) potassium iodide solution for 1 minute. Subsequently, the paper points were placed in an incubator and dried at 110°C for 4 hours. The paper points (n = 5 per measurement) were then dipped in different concentrations of NaOCl and photographed under standardized conditions in RAW format. The pictures were imported to image processing software and adjusted to the standardized background. The red, green, and blue levels of the paper points were assessed at a predefined area. Inverse regression was used to determine NaOCl concentration from red, green, and blue values, with both explanatory and outcome variables log-transformed to base 10. **Results:** The red value measurements were chosen for further analysis based on a comparison of the coefficient of determination (R^2) and a residual analysis. The method was applied to concentrations of NaOCl between 0.0001% and 1% (R^2 = 0.92). In this range, NaOCl concentrations could be assessed with an error not larger than 3-fold the determined concentration. **Conclusions:** The present method proved to be robust to determine the order of magnitude of available chlorine that is present in a small volume. This should be useful for endodontic research. *(J Endod 2014;40:534–537)*

Key Words

Hypochlorite, method development, NaOCl, paper point

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Sodium hypochlorite (NaOCl) is the main agent used in root canal treatments to fight infection in the pulp space. This is because of its unique capacity to dissolve necrotic tissue remnants and biofilms (1). Activation schemes to render NaOCl solutions more efficient in debriding root canals are of great clinical interest. These procedures and techniques have been discussed in detail (2). However, one clinically important issue has gained relatively little attention in this context: for how long does a hypochlorite solution stay reactive when applied in the root canal space?

The active component of NaOCl solutions used in dentistry is the free available chlorine, which is in the form of the OCl^- ion (1). It would be interesting to know how much available chlorine is used during a given time under certain conditions. In other words, how much active chlorine remains after a specific procedure, and how frequently does the irrigant need to be replaced? This question has been addressed in bovine teeth after activating NaOCl solutions with pulsed laser light or ultrasound (3). However, bovine teeth have a much higher root canal volume than human counterparts. More importantly, their canal wall area per volume, which can react with the OCl^-, is considerably smaller than in human teeth. Human root canals have a volume in the range of 1–3 mm^3 (4), which equals 1–3 μL irrigant that can be placed therein. Measuring free available chlorine in such small volumes is full of methodological pitfalls. Redox titration is not possible because it relies on drops of the titrant, which are added to the analyte (4). One drop of an aqueous solution is at least 20 μL. Alternatively, standard assays to quantify active chlorine in aqueous solutions, for instance the N, N-diethyl-p-phenylenediamine assay (5), could be used. These sensitive assays were invented to determine chlorine in swimming pools and drinking water, ie, for chlorine concentrations in the order of magnitude of 0.0001%. However, the commonly used concentrations of NaOCl solutions used for root canal treatments range between 1% and 6% (1). The irrigant taken from the root canal, which cannot be pipetted or weighed precisely, has got to be diluted for determination by using those assays, thus multiplying the error of measurement.

After many unsuccessful attempts of tackling the problem of chlorine determination in human root canals, a simple assay was invented, which is based on the reaction used for iodine titration. This article presents the development of a robust method to quantify available chlorine from small volumes by using paper points impregnated with potassium iodide.

Materials and Methods

General Principle of the Assay

The assay presented here is based on the reaction:

$$OCl^- + 2I^- + 2H^- \rightarrow Cl^- + I_2 + H_2O$$

The reaction between sodium hypochlorite (NaOCl or NaClO) and potassium iodide (KI), which are both colorless, results in the generation of elemental iodine (I_2), which is brown. The idea was that the brown coloration should increase with the concentration of NaOCl. Paper points of an ISO size 40 (ORBIS Dental, Münster, Germany) were placed in an incubator (HORO Dr Hofmann GmbH, Ostfildern, Germany) at 110°C for 1 hour to remove any residual moisture. Subsequently, the paper points were soaked in 15% KI solution for 1 minute. A pilot study showed that this concentration of KI gave stable results in the measurement range of the assay. The paper points were then placed into the incubator and dried at 110°C for 4 hours. After 4 hours, the fluid that had been soaked up by the paper points had completely evaporated, which was verified by using a precision balance (Mettler Toledo, Greifensee, Switzerland). The KI-impregnated paper points were then dipped in different concentrations of NaOCl for 10 seconds and photographed under standardized conditions.

Camera Setup

A Nikon (Tokyo, Japan) D200 camera with Nikon AF-S Micro Nikkor 105-mm 1:2.8-gauge lens and a Sigma EM-140 DG Ring-flash was mounted on a custom-made stand in a darkened room. The images were taken at fixed camera settings (white balance, focus, flash, and ISO) and with a standardized grey background (Digi Grey, www.digigrey.com). The pictures were saved in RAW format.

Color Reading

The pictures were transferred to Photoshop Element (Adobe Systems, San Jose, CA). Images were corrected to the standardized grey background in Camera RAW 6.5 (Adobe Systems). The tip of the paper point was identified. By using the DigitalColor Metric program (Apple Computer, Cupertino, CA) at the settings ×8 magnification and Adobe RGB, the mean red, green, and blue (RGB) levels were measured in an area 287–299 pixels from the tip of the paper point. The RGB value readings by the DigitalColor Metric program and Photoshop Element were compared in a pilot study. No difference was found between the programs. The reason for using DigitalColor Metric was based on convenience (easier to find the middle of the paper point) and measurement error reduction (mean RGB values from larger area).

NaOCl Dilutions and Measurement

A technical-grade 5.5% NaOCl solution (Kantonsapotheke, Zürich, Switzerland) was diluted in a 10-fold dilution series down to 0.000055% NaOCl with distilled water. Five KI-impregnated paper points were immersed for 10 seconds in each of the 6 NaOCl concentrations, and the images of the respective paper points were taken within 2 minutes. A 2-fold dilution series was also performed from 2% NaOCl down to 0.000245% NaOCl (14 NaOCl concentrations) and evaluated as previously described. The initial concentrations of both solutions were verified by titration (6). The data of both dilution series were combined in the results.

Data Presentation and Statistical Analysis

The mean RGB values and standard deviation were plotted in a graph, and the $\log_{10}$ values were calculated and plotted. For the NaOCl concentration the main predictive variable was the RGB levels. Because the RGB color value is subject to measurement error, it cannot be examined as an explanatory variable. Instead, it was used in an inverse regression as dependent variable, and the NaOCl concentration was used as explanatory variable. Formula (A) shows the regression model:

$$Y_i = \beta_0 + \beta_i \cdot x_i + \in_i \quad \text{(A)}$$

Here Y_i refers to the RGB level between 0 and 255 (red, green, or blue) for a given NaOCl concentration level x_i, and $\in_i$ is the measurement error, assumed to have constant variance.

Both the explanatory and the dependent variables were log-transformed to the base 10. Inverse regression is conducted with an estimate of $x^{(0)}$ for an observed value of $Y^{(0)}$. A prediction interval for $x^{(0)}$ could be obtained by the set of all x values, for which the corresponding prediction interval for Y contained the observed value $Y^{(0)}$ (7). The best response variable Y_i (red, green, or blue) was selected on the basis of the coefficient of determination R^2. Visualizing the residuals $\in_i$ helped to check the assumptions in Formula (A). The x-axis represented the fitted values of the regression model, and y-axis values are residuals. In a valid model, the residuals have a constant variance and a mean of 0, with respect to the fitted values.

All analyses were performed in the R programming language (R Development Core Team; R Foundation for Statistical Computing, Vienna, Austria).

Results

A previous experiment evaluated the impact of KI concentration in the solution the paper points were impregnated with. On the basis of these observations, a 15% KI solution was used for all additional experiments. This concentration gave a clear dose response, which could be appreciated visually in a concentration range from 1% down to 0.001% NaOCl (Fig. 1).

Inverse regression was performed on separate data sets of RGB value measurements. Each data set contained 100 observations, 5 for each concentration. The concentrations were taken from 2-fold and 10-fold experiments, and the range of concentrations was from 0.000055%–5.5%. However, to fit the regression model (A), outliers corresponding to concentrations < 0.0001% or concentrations > 1% were removed. This step helped to fulfill the assumptions of linearity. The inverse regression models for RGB measurements (Fig. 2, upper panels) yielded R^2 values of 0.92 (red), 0.91 (green), and 0.90 (blue). Visual residual analysis (Fig. 2) showed clusters of observations

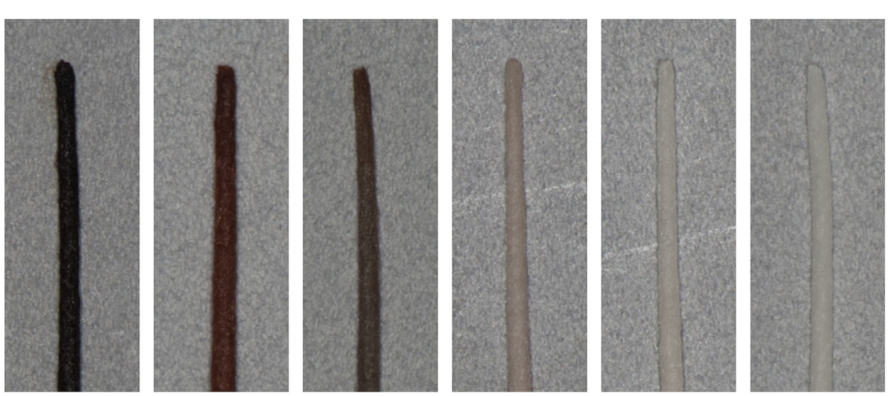

Figure 1. Photographs of paper points impregnated with 15% KI solution. Points were dipped for 10 seconds in NaOCl solution of different concentrations or distilled water. Note the correlation between the brown shade and NaOCl concentration (w/v).