Influence of Mixed Additives on the Physicochemical Properties of a 5.25% Sodium Hypochlorite Solution: An Unsupervised Multivariate Statistical Approach

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Abstract

Introduction: This article reports for the first time the effects of multiple additives (polyethylene glycol 400, Triton X-100, benzalkonium chloride, and ethyl formate) on the surface tension, pH, and viscosity of 5.25% sodium hypochlorite (NaOCl) irrigant solution. Advanced statistical approaches based on unsupervised multivariate analysis (cluster analysis and principal component analysis) were used to quantify the variability of the physicochemical properties of the modified NaOCl solution for the first time in dentistry. **Methods:** Solutions of 5.25% NaOCl were modified with multiple additives in various concentrations, physicochemical parameters were measured at 22°C and 37°C, and the results were statistically analyzed to group the solutions and reveal the effects of additives. **Results:** Cluster analysis and principal component analysis revealed that pH and surface tension were the significant parameters ($P < .05$) for grouping the modified solutions. Four principal components, accounting for 90.6% of the total variance, were associated with flow characteristics (37.3%) determined by polyethylene glycol; the wetting property (22.5% and 10.5%), which was dependent on cationic and nonionic surfactant; and the antimicrobial effect (20.3%) influenced by ethyl formate. Varimax rotation of the principal components showed that the cationic surfactant (benzalkonium chloride) had significantly decreased surface tension compared with the nonionic surfactant (Triton-X). Although ethyl formate was introduced as an odor modifier, it had a significant effect on pH decrease and the occurrence of effervescence with O_2 and hypochlorous acid release. **Conclusions:** The statistical results revealed that the 5.25% NaOCl irrigant solution should be modified with a mixture of 0.1% benzalkonium chloride, 1% ethyl formate, and 7% polyethylene glycol for obtaining a low pH and low surface tension. *(J Endod 2017;■:1–6)*

Key Words

Cluster analysis, pH, principal component analysis, sodium hypochlorite, surface tension, viscosity

Significance

Ethyl formate, benzalkonium chloride, polyethylene glycol, and Triton-X mixture added to 5.25% NaOCl irrigant solution could improve the antimicrobial efficiency, wettability, and flow characteristics by effervescence, decreasing the pH and surface tension, and increasing the viscosity.

Sodium hypochlorite (NaOCl) proved to be the most effective endodontic irrigant because of its excellent antimicrobial efficiency, biofilm disruption, organic tissue dissolution, and debris removal properties (1–5). The biofilm disruption and smear layer removal were found to be dependent on the irrigant flow (6) and the chemical action of hypochlorite ion (ClO^-) (7), whereas the penetration depth was associated with surface tension (8). The antimicrobial efficacy is critically dependent on the concentration and oxidizing action of nondissociated hypochlorous acid (HOCl) species (7). The dissolving capacity of organic matter seems to be determined by the chemical reactions of the highly unstable HOCl with fatty acids and the protein amino group (9). The major drawbacks of NaOCl as an irrigant solution were related to limited penetration into the root canal dentin and canal irregularities as a result of high surface tension (8, 10) as well as a lack of residual antimicrobial activity that facilitates the recolonization of persistent microorganisms (11).

The adjustment of the physicochemical properties (ie, surface tension, viscosity, and pH) of NaOCl with various surfactants/modifiers led to favorable results including an increase in the ability of the irrigant to penetrate passively inside the main root canal (12), a higher penetration depth into the dentinal microtubules (13), an improved tissue dissolution capacity (14), and a stronger antibacterial power (15, 16). There are several commercially available irrigant solutions such as Hypoclean A (Ogna Laboratori Farmaceutici, Muggio, Italy) and Hypoclean B (Ogna Laboratori Farmaceutici) (5.25% NaOCl modified with quaternary ammonium compounds [cetrimide] and polypropylene glycol [17]), and Chlor–Xtra (Vista Dental Products, Racine, WI) (<6% NaOCl modified with Triton X-100 and alkylating agents [18]). The effects of cationic surfactants, such as benzalkonium chloride in the concentra-

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tion range of 0%–12.5%, and nonionic surfactants, such as Triton-X 0%–25% and polyethylene glycol sorbitan monooleate (TWEEN 80; Sigma-Aldrich, St Louis, MO) 0%–25%, on the surface tension and viscosity of 2.4% NaOCl have been previously reported but using a single additive (19, 20).

To the best of our knowledge, the effects of combining multiple additives such as benzalkonium chloride, Triton X-100, polyethylene glycol, and ethyl formate on the physicochemical properties of NaOCl solution have not yet been determined. Therefore, the purpose of this study was to find the influence of combinations of these additives in various concentrations on the pH, surface tension, and viscosity of 5.25% NaOCl. Moreover, powerful unsupervised multivariate data analysis tools, such as cluster analysis (CA) and principal component analysis (PCA), were applied for the first time in the endodontic literature to find the significant correlations among physicochemical parameters, concentration, and type of modifying additive. Thus, the influence of multiple additives on the variability of physicochemical properties of NaOCl solution could be quantified. The novelty of the statistical approach used in this study is supported by a statement in a recent review (21) that common statistics (correlation and regression) were used the most in dental research, whereas the potential of multivariate statistical methods has not been sufficiently explored and, hence, much of the useful information hidden in the data may be lost.

Materials and Methods

Reagents and Solutions

Analytic pure 60% NaOCl solution (Reogecon, Shannon, Ireland), polyethylene glycol 400 (VWR International LLC, Radnor, PA), Triton X-100, benzalkonium chloride ≥95%, ethyl formate ≥97% (Sigma-Aldrich), and Milli-Q water 18.1 MΩ cm (Merk, Darmstadt, Germany) were used for the preparation of the test solutions. Twenty-five solutions of 5.25% NaOCl were modified with additives in the concentration range as indicated in Table 1. Solutions were homogenized, and the physicochemical parameters were measured immediately (n = 3 parallel samples).

TABLE 1. Concentration (%) of Triton-X, Polyethylene Glycol, Benzalkonium Chloride, and Ethyl Formate in the Modified 5.25% Sodium Hypochlorite Solutions

Solution	Triton-X	Polyethylene glycol	Benzalkonium chloride	Ethyl formate
S_1	0.1	1	0.005	0
S_2	2	1	0.005	0
S_3	0.1	7	0.005	0
S_4	2	7	0.005	0
S_5	0.1	1	0.1	0
S_6	2	1	0.1	0
S_7	0.1	7	0.1	0
S_8	2	7	0.1	0
S_9	0.1	1	0.005	1
S_{10}	2	1	0.005	1
S_{11}	0.1	7	0.005	1
S_{12}	2	7	0.005	1
S_{13}	0.1	1	0.1	1
S_{14}	2	1	0.1	1
S_{15}	0.1	7	0.1	1
S_{16}	2	7	0.1	1
S_{17}	0.1	4	0.052	0.5
S_{18}	2	4	0.052	0.5
S_{19}	1.05	1	0.052	0.5
S_{20}	1.05	7	0.052	0.5
S_{21}	1.05	4	0.005	0.5
S_{22}	1.05	4	0.1	0.5
S_{23}	1.05	4	0.052	0
S_{24}	1.05	4	0.052	1
S_{25}	1.05	4	0.052	0.5

Measurement of Physicochemical Parameters

The measurement of pH was performed at 22°C according to the European Pharmacopoeia requirements using an EasyPlus precision titrator (Mettler-Toledo International Inc, Columbus, OH) (22). A low range tuning fork vibration viscometer (SV-100; A&D Company, Tokyo, Japan) was used for the dynamic viscosity measurements at 22°C according to the European Pharmacopoeia protocol (23). The dynamic surface tension was determined by the pendant drop method (24) using the DSA100 (Kruss GmbH, Hamburg, Germany) in thermostatic conditions at 22°C and 37°C, respectively. Large needle diameters (1.833, 1.830, and 1.829 mm) were used to obtain the pear-shaped drop, and the details are provided in (Supplemental Figure S1 is available online at www.jendodon.com). A high-precision rheometer (DMA 4500; Anton Paar, Gratz, Austria) was used for the density measurement at 22°C and 37°C. Memory effects were avoided by cleaning the equipment with toluene, acetone, and alcohol after each sample.

Statistical Analysis

A 1-sided t test at a 95% confidence level was used to compare the mean values of parameters for modified 5.25% NaOCl solution with mixtures of additives with those obtained for the nonmodified 5.25% NaOCl. Ward's linkage method and Euclidian distance as a measure of similarity were chosen to group nonmodified and modified NaOCl solutions and their physicochemical parameters, respectively, in CA (25). The PCA was based on the eigenvalues of the correlation matrix on standardized data, and varimax rotation was used to maximize the variation expressed by the principal components. Only the principal components with eigenvalues >1 were retained in order to extract the hidden information on the influence of each additive (26). The influence of each additive was classified as "strong," "moderate," and "weak," corresponding to the absolute loading value of >0.75, 0.50 to 0.75, and 0.30 to 0.50 (27). Multivariate statistical analyses were performed using the Statistica 8.0 software package (StatSoft Inc, Palo Alto, CA).

Results

The Physicochemical Parameters for the 5.25% NaOCl Solutions

The values of the physicochemical parameters are listed in Table 2. The precision of measurements expressed as the relative standard deviation (n = 3 parallel measurements) was in the range of 1%–3%.

A significant decrease in surface tension (mN/m) was observed for all the modified NaOCl solutions down to 29.36 and 28.06 compared with 49.85 and 49.48, the values obtained for the nonmodified 5.25% NaOCl, at both working temperatures. The surface tension values (mN/m) for all the modified 5.25% NaOCl solutions were lower than those of Chlor-Xtra (33.14 ± 0.53) and comparable with those of Hypoclean A (30.00 ± 0.78) and Hypoclean B (29.3 ± 0.56), as reported by Palazzi et al (18). Likewise, a pH decrease to 8.33 for the solutions containing ethyl formate was noted compared with 10.08 for the nonmodified 5.25% NaOCl solution.

The linear regression correlation matrix indicated only a few statistically significant correlations (P < .05) between the physiochemical parameters and additives. A positive correlation was found between viscosity, density, and polyethylene glycol, whereas a negative correlation was found between pH and ethyl formate as indicated in Supplemental Table S1 is available online at www.jendodon.com. However, quantifying the influence of additives on the variability of the physicochemical parameters of the NaOCl solution was difficult without a deeper approach, such as the unsupervised statistical methods that were performed in this study.