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# Shelf-Life of a 2.5% Sodium Hypochlorite Solution as Determined by Arrhenius Equation

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Accelerated stability tests are indicated to assess, within a short time, the degree of chemical degradation that may affect an active substance, either alone or in a formula, under normal storage conditions. This method is based on increased stress conditions to accelerate the rate of chemical degradation. Based on the equation of the straight line obtained as a function of the reaction order (at 50 and 70 °C) and using Arrhenius equation, the speed of the reaction was calculated for the temperature of 20 °C (normal storage conditions). This model of accelerated stability test makes it possible to predict the chemical stability of any active substance at any given moment, as long as the method to quantify the chemical substance is available. As an example of the applicability of Arrhenius equation in accelerated stability tests, a 2.5% sodium hypochlorite solution was analyzed due to its chemical instability. Iodometric titration was used to quantify free residual chlorine in the solutions. Based on data obtained keeping this solution at 50 and 70 °C, using Arrhenius equation and considering 2.0% of free residual chlorine as the minimum acceptable threshold, the shelf-life was equal to 166 days at 20 °C. This model, however, makes it possible to calculate shelf-life at any other given temperature.

Key Words: accelerated stability test, sodium hypochlorite solution, shelf-life, kinetics, chemical stability, irrigation solution.

## INTRODUCTION

Sodium hypochlorite solutions are used all over the world due to their antimicrobial characteristics. They are also used in tissue dissolution (1) in spite of their limited use in endotoxin inactivation (2), and are indicated as disinfectant or antiseptic agents in several health care areas. Two substances may be formed when sodium hypochlorite is dissolved in water, depending on the pH of the solution: hypochlorous acid (HOCl) and hypochlorite ion ($OCl^-$). Hypochlorous acid is responsible for most of the effective antimicrobial action, although hypochlorite ion may also contribute to it. Antiseptic/disinfectant action is due to chlorination of microbial plasma proteins/enzymatic systems.

Vianna et al. (3) and Estrela et al. (4,5) showed that sodium hypochlorite is more bactericidal than chlorhexidine gel. In terms of tissue dissolution, efficiency is proportional to hypochlorite concentration in the solution (6,7).

Sodium hypochlorite solutions have low cost, and are very efficient as tissue solvents (8) and in the reduction of endodontic microbiota (9) even at low concentrations. However, they are chemically unstable (10,11). Stability may be defined as the extent to which a solution is able to maintain, within specified ranges, the same properties and characteristics it possessed at the moment it was prepared (identity, concentration, quality, purity and physicochemical properties) throughout its shelf-life. Chemical instability may be evidenced, in some situations, by means of changes in physical and/or sensory characteristics of the preparation. However, chemical degradation is frequently imperceptible in terms of these aspects, and may only be determined by quantitative analysis of the active principle and/or other components of the solution (12).

Stability tests are used to evaluate the behavior of drugs as a function of time and to use data obtained in this evaluation to determine shelf-life, mainly in relation to the concentration of the active substance in the raw

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material or final preparation.

Some authors have studied the stability of solutions containing sodium hypochlorite. Pécora et al. (13) analyzed free residual chloride by means of titration in 5% sodium hypochlorite solutions. Johnson and Remeikis (11) observed that chemical stability of sodium hypochlorite is adversely affected by exposure to high temperature, light, air, and the presence of organic and inorganic contaminants. Clarkson at al. (14) analyzed the decrease in free residual chloride in different sodium hypochlorite solutions stored in conditions similar to actual clinical use.

Although no solution is stable for an indefinite period of time, the speed of chemical reactions is variable. Stability tests are able to determine if physicochemical characteristics of different formulas are preserved. Accelerated stability tests may be used to monitor degradation reactions and determine shelf-life under normal storage conditions. Different from long-term stability tests, which are carried out under normal storage and usage conditions, accelerated stability tests use stress storage conditions, such as high temperature, to increase chemical degradation and accelerate physical changes observed in substances and/or characteristics of the solution. This established procedure yields, in a short period of time, results that make it possible to calculate shelf-life of the solution. Using data collected in the stability tests, the concentration of the active substance may be determined at any moment in time by means of mathematical equations (12,15).

The order of the reaction and the rate constant may be determined based on the decrease in concentration of the active substance over time. Together with Arrhenius equation, these values may be used to calculate shelf-life for solutions submitted both to normal storage conditions and to other conditions of interest (12,15).

The purpose of the present study was to develop an accelerated stability test for a 2.5% sodium hypochlorite solution. This solution was chosen due to its chemical instability, since it may be a model for accelerated stability tests and/or shelf-life determination for sodium hypochlorite as raw material and as a component of other formulas, and for other active compounds alone or as parts of different preparations.

## MATERIAL AND METHODS

A hypochlorite solution with 25 mg/mL of free residual chlorine was used. Free residual chlorine was determined by titration, and solution was placed in 1,000 mL amber glass flasks. Two incubators were stabilized at 50 and 70 °C ± 2 °C before the beginning of the study. Samples were placed in duplicate in the 2 incubators, and flasks were only opened at the moment of analysis.

Iodometric titration of free residual chlorine was performed for 7 days at 70 °C and 23 days at 50 °C, using the method described in the British Pharmacopoeia (16). Titration solution was 0.1 N sodium thiosulfate (hyposulfite) previously standardized with potassium dichromate.

Time limits (shelf-life) for the use of solutions are determined by quality control specifications, such as the concentration of free residual chlorine. In this trial, the minimum threshold was considered to be equal to 20 mg/mL of free residual chlorine.

One of the parameters used to validate analytical methods is precision measurement, expressed as a variation coefficient, which indicates the degree of reproducibility, that is, the level of agreement between results obtained in several repetitions of the method. In the present study, the variation coefficient was determined by 20 analyses of one sample (17).

Data obtained based on the quantitative analyzes were plotted in graphs of concentration versus time for each of the temperatures analyzed. Correlation coefficients and the reaction order were both determined.

Based on the straight lines obtained, Arrhenius equation (equation 1) was used to determine the degradation rate of the samples at each temperature. This procedure determined the degree of chemical degradation that would occur under normal storage conditions, or at any other given temperature.

$$K = A \,.\, e^{-\Delta Ha / RT} \quad \text{(Equation 1)}$$

Where:
K = specific reaction rate
A = frequency
Δ Ha = activation energy
R = ideal gas constant
T = absolute temperature in which the phenomenon occurs (in Kelvin)

This model of accelerated stability study demonstrates the influence of temperature in the speed of a chemical reaction, according to classic laws of chemical kinetics (12,15).

## RESULTS

Precision of an analytical method is usually expressed as standard deviation or relative standard deviation (coefficient of variation). In the present study, standard deviation was equal to 0.06485, variation coefficient, 0.26% and standard error, 0.015. These values are much lower than those officially established (17).

Graphs were drawn based on the degradation curve of free residual chlorine (y axis) *vs*. time (x axis) at each temperature; the straight line yielded determined the reaction order.

The reaction order is related to the equation of the straight line in its reduced form, that is, as represented by the expression y = ax + b (a = angular coefficient, the slope of the straight line in relation to x axis. In the study, it represents K, the reaction rate; and b = linear coefficient, the intercept of the straight line and y axis).

Degradation reactions may be of different orders, that is, the reaction order is determined by best correlation coefficient obtained between a given function *vs*. time. In this study, concentration *vs*. time yielded the best correlation for 70 and 50 $^oC$ (respectively, 0.9931 and 0.9953); therefore, a zero order reaction. In a zero order reaction, decomposition rate is independent of reagent concentration, that is, the rate is constant (12,15).

Figures 1 and 2 show the degradation curves of free residual chlorine as a function of time. The straight line equation was determined by the mean straight line in each situation using least squares fitting. Based on the equation of the straight line, it is possible to calculate the concentration of the active principle at any given time.

Using the speed constant obtained with samples submitted to the 2 different conditions together with Arrhenius equation (equation 1), it is possible to calculate the speed constant at any temperature.

$\Delta$ Ha values were determined (equation 2) by means of experimental K values ($K_1$ and $K_2$), for temperatures $T_1$ and $T_2$.

$$\text{Log } K_1 / K_2 = \Delta \text{ Ha} / 2.303 \,.\, R \,.\, T_1 \,.\, T_2 / T_1 - T_2$$ (Equation 2)

if $\Delta$ Ha values are known, log A (equation 3) may be determined. Any experimentally determined T and K pairs may be used.

$$\text{Log } K = \log A - \Delta \text{ Ha} / 2.303 \,.\, R \,.\, 1 / T$$ (Equation 3)

In the present study, $K_{70}$ and $K_{50}$ were used to determine the activation energy ($\Delta$ Ha) of the reaction, which was equal to 14.82 kcal $mol^{-1}$ $degree^{-1}$. Log A was obtained using log $K_{70}$, equal to 9.5309058. Based on these results, the speed constant at $20^oC$ was equal to $K_{20} = 0.030165$ $day^{-1}$.

Shelf-life in terms of a decrease in free residual chlorine from 25 to 20 mg/mL was determined using equation 4, based on the zero order reaction kinetics.

$$t = Co - C / K$$ (equation 4)

Where:
t = time (days)
Co = initial concentration (25 mg/mL)
C = final concentration (20 mg/mL)

Equation 4 estimated that it would take 166 days at $20^oC$ for the 25 mg/mL sodium hypochlorite solution

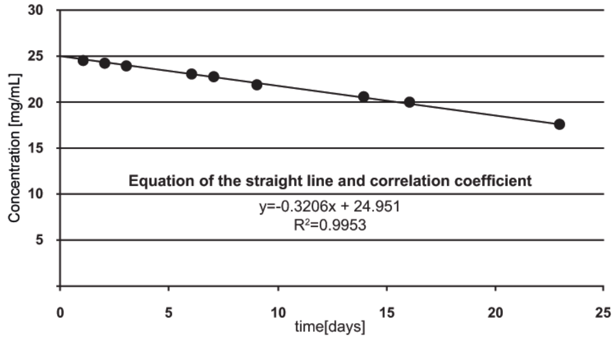

Figure 1. Concentration of free residual chlorine (temperature=50°C).

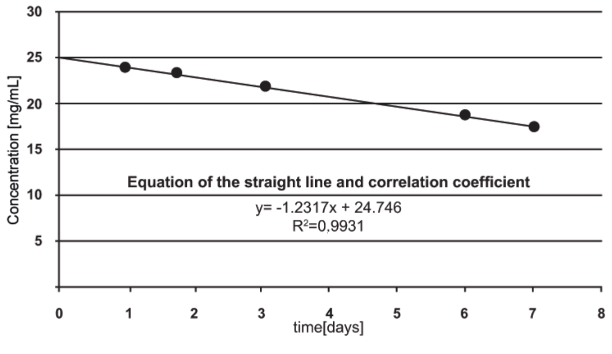

Figure 2. Concentration of free residual chlorine (temperature = 70 ºC).

to reach 20 mg/mL of free residual chlorine. Both studied conditions showed zero order kinetics.

## DISCUSSION

Studies on the activity of sodium hypochlorite solutions as tissue solvents and bactericidal compounds are very popular nowadays (6,7). However, chlorine is highly unstable in these solutions (10,13) mainly due to factors such as presence of organic material (1,6), pH (9), temperature (18), and presence of oxygen or light (19).

The shelf-life of a given pharmaceutical presentation is variable and depends on the degradation of its main component. This fact underscores the importance of using sodium hypochlorite solutions showing free residual chloride content within specified ranges, as determined by pharmacopoeia monographs, both for sodium hypochlorite as an active principle and as a component of more complex solutions.

Pécora et al. (13) evaluated the degradation of sodium hypochlorite solutions using titration to assess that chlorine content of the samples was similar to minimum thresholds (titration is a simple and reproducible method for the analysis of chlorine content in sodium hypochlorite solutions). However, shelf-life would be determined much more quickly if the method proposed here was used, provided that chlorine degradation showed a straight line with a viable correlation coefficient.

Nicoletti and Magalhães (19) evaluated the chemical stability of three different commercial bleach solutions and analyzed the influence of oxygen in open flasks, confirming that oxygen has an important role in the degradation of this solution.

Two kinds of factors may be responsible for changes in pharmaceutical products: external (extrinsic) factors, such as time, temperature, light, oxygen, carbon dioxide, primary packaging, humidity and microbial agents; and internal (intrinsic) factors, that is, those related to the nature of the drug, such as oxidation, hydrolysis, racemization, decarboxylation, incompatibilities, polymerization and disammination (12).

Knowledge of the kinetics of the degradation reaction of a chemical compound either isolated or in a formula, is essential for stability studies. Kinetics of the reaction is determined by the concentration of the active substance in the formula under given conditions. Accelerated stability tests use increased reaction kinetics produced by higher temperatures, which in turn increase the speed of the reactions affecting substances in the formula. This study model may be used when shelf-life is unknown, no matter the active principle it contains, provided there is a methodology to quantify its concentration. Samples subjected to high temperatures show increased rate of degradation of the active substance in the formula, making it possible to measure this degradation in a short time. The importance of this study may be underscored by the fact that a preparation may have different shelf-lives depending on the environmental conditions it is submitted to, as occurs in the diversity of temperatures found in the different Brazilian regions.

## RESUMO

Testes acelerados de estabilidade são indicados para avaliar, em um curto período de tempo, o grau de degradação química que poderá afetar uma substância química, isoladamente ou quando inserida em uma fórmula, sob condições normais de armazenamento. Este método está fundamentado na intensificação das condições de estresse para acelerar a velocidade de degradação química. Baseando-se na equação da reta obtida e na ordem de reação determinada (a 50 e 70 $^o$C) e usando a equação de Arrhenius, a velocidade de reação foi calculada para a condição de temperatura de 20$^o$C (condições normais de armazenamento). Este modelo de teste acelerado de estabilidade torna possível a predição da estabilidade química de qualquer substância, em qualquer tempo, desde que o método de quantificação da substância química esteja disponível. Como exemplo da aplicabilidade da equação de Arrhenius em teste acelerado de estabilidade, uma solução de hipoclorito de sódio a 2,5% foi analisada por ser quimicamente instável. A quantificação do cloro residual livre foi determinada através de titulação iodométrica. A partir dos dados obtidos decorrentes das amostras submetidas às temperaturas de 50 e 70 $^o$C e com o emprego da equação de Arrhenius, o tempo de prateleira obtido foi de 166 dias em temperatura de 20 $^o$C, considerando como limite inferior a concentração de 20 mg/mL de cloro residual livre. Este modelo, entretanto, possibilita o cálculo de tempo de prateleira em qualquer outra temperatura de interesse.

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