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Solubility Properties of Chlorhexidine Salts

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Chlorhexidine (CHX) is effective in treating oral bacterial infections. The solubility was shown to be highly dependent on the salt present in solution. Gluconate enhances the amount of CHX diacetate in solution possibly through mixed micelles formation, because the solubility product is such that the concentration of CHX will exceed the critical micelle concentration. However, only low concentrations of CHX dichloride can be obtained, which is not appreciably solubilized by gluconate ions. The low concentration of CHX that can be achieved in physiological concentrations of chloride in the oral cavity may be problematic for dental, slow release formulations.

Keywords chlorhexidine; chlorhexidine digluconate; chlorhexidine diacetate; chlorhexidine dichloride; micelle; solubility product

INTRODUCTION

Endodontic disease is caused primarily by bacteria that infect the dental pulp tissue within the tooth (Wu, Dummer, & Wesselink, 2006). Root canal therapy is the recommended treatment for such diseases, which consists of accessing the root canal system and removing the diseased pulp tissue from the tooth. With infected root canals, it is very important to prevent re-growth of bacteria in the pulp chamber. Presently, irrigation and intra-canal medication methods reduce the incidence of re-infection, but bacteria remaining in the root canal or dentin present a significant problem for complete eradication.

Chlorhexidine (CHX, Figure 1) is an antimicrobial agent that is used in endodontic therapy (Dolby, Gunnarsson, Kronberg, & Wikner, 1972; Gjermo, 1989; Senior, 1976; Wang & Peng, 2006). It is effective against both gram-positive and gram-negative microbes, and its cationic guanidium groups are believed to bind to negatively charged bacterial cell walls, acting as both a bacteriostatic and a bacteriocidal agent. However, to prevent the re-emergence of bacteria and achieve long-term antimicrobial activity, the presence of CHX may be needed until the root canal system is permanently sealed. This varies between 2 and 4 weeks, depending on when a return visit to the dentist can be scheduled.

Currently, a 2% CHX aqueous solution is used when treating root canals (Zebnder, 2006). Because the free base is essentially insoluble and only exists at very low hydrogen ion concentrations (pH > 12), CHX is used as the salt form, commercially available as CHX diacetate, dihydrochloride, or digluconate. Saturated solutions of CHX diacetate (CHX-Ac_2) and dihydrochloride (CHX-Cl_2) have concentrations of CHX of 2 and 0.2%, respectively (Nerurkar, Zentner, & Rytting, 1995). The digluconate salt is available at a much higher concentration of 20% (wt/vol) and is used clinically as a diluted solution (2%) (Gjermo, 1989). Saliva contains a relatively high concentration of chloride ions, >150 mM, which would be expected to cause the precipitation of CHX-Cl_2 in the oral cavity due to the low solubility product (Leach, 1979). Because the free concentration of CHX has not been determined in clinical trials, the free concentration of CHX in the oral cavity or tooth that provides adequate antimicrobial activity (i.e., the minimum inhibitory concentration or MIC) remains unclear. In addition to the complex ionic equilibria, CHX as a diacetate or digluconate solution has been shown to undergo self-association (Heard & Ashworth, 1968). The formation of aggregates greatly influences the resulting concentration of CHX in solution.

In this study, the solubility of the CHX in mixtures with sodium chloride (NaCl), sodium acetate, and sodium gluconate was determined. The observed solubility was highly dependent on the type and concentration of salt present in solution. In addition, achieving a high concentration of CHX appears to require that the monomer be present at a concentration greater than that required to produce self-association.

THEORY

CHX, 1,1′-hexamethylene-bis-5-(4-chlorophenyl)biguanidide, is a symmetric molecule with two ionizable guanidide moieties. The pK_a values are 2.2 and 10.3, thus making it dicationic over

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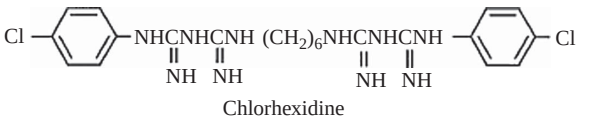

FIGURE 1. Chemical structure of chlorhexidine.

the entire range of physiological pH values (Nerurkar et al., 1995). For the three commonly used salts, digluconate, diacetate, and dihydrochloride, the solubility products may be written as

$$K_{sp}(\mathrm{CHX-X_2}) = K_1K_2 = [\mathrm{CHX^{2+}}][\mathrm{X^-}]^2$$

where X represents Ac, G, or Cl, and the n and $n + 1$ equilibrium constants represent the dissolution of the first and second ion. The reported K_{sp} of the dihydrochloride salt (2.1×10^{-8} M^3) is lower than the diacetate (2×10^{-4} M^3) at 37ºC (Nerurkar et al., 1995).

MATERIALS AND METHODS

Analytical/reagent grade CHX dihydrochloride, CHX digluconate (20%, wt/vol), sodium gluconate, and calcium gluconate were purchased from Sigma Chemical (St. Louis, MO, USA) and used as received. CHX diacetate hydrate was purchased from Acros Organics (Geel, Belgium) and sodium acetate was purchased from Fischer Biosciences (Fair Lawn, NY, USA).

Solubility Measurements

The concentration of CHX was determined as a function of counterion (chloride, acetate, or gluconate) concentration. For the first set of experiments, solid CHX dihydrochloride was placed into 20-mL scintillation vials or 1.5-mL centrifuge tubes with a range of concentrations of sodium gluconate and calcium gluconate. After equilibrating for at least 48 h, the suspensions were centrifuged at $28{,}000 \times g$ for 10 min, and aliquot of the supernatant was removed. Following dilution if necessary, the UV absorbance at 255 nm was measured, and the concentration of CHX in solution was determined by interpolation of the absorption using an appropriate standard curve.

Alternatively, 20% (wt/vol) CHX digluconate solutions with or without addition sodium gluconate were diluted with a NaCl solution to yield a series of samples in which either the total chloride concentration was constant with varying gluconate concentration or the total gluconate concentration was constant with varying chloride concentration. The solubility product of CHX diacetate was estimated by adding sodium acetate to a CHX diacetate solution, measuring the CHX in solution, and fitting the results to the expression, $[\mathrm{CHX}] = K_{sp}/[\mathrm{Ac^-}]^2$.

The standard curves for CHX diacetate and digluconate salt were both linear. However, the gluconate solution yielded a lower absorptivity, 22.8 ± 0.60/cm mg/mL (231 nm) and 27.42 ± 0.48/cm mg/mL (254 nm), in comparison with the acetate solution, which was 44.5 ± 2.3/cm mg/mL (255 nm).

RESULTS AND DISCUSSION

In the first study, excess solid CHX-Cl_2 was placed into solution with increasing concentrations of sodium or calcium gluconate, and the results are given in Figure 2. The concentration of CHX was very low, consistent with the low value of the solubility product of CHX-Cl_2. With increasing concentration of gluconate, the concentration of CHX increased modestly from about 1.4 mM to a little over 1.6 mM. This concentration corresponds to a solubility product of 2.7×10^{-9} M^3, which is lower but consistent with the literature value, which was determined at 25ºC (Nerurkar et al., 1995). The small increase in the concentration with increasing gluconate concentration does not appear to reflect a specific interaction, but rather corresponds to an increase in the solubility product secondary to an increase in the ionic strength as would be expected from the falling activity coefficients.

In the second set of experiments, increasing volumes of a NaCl solution were added to a fixed volume and concentration of CHX-G_2 present as a solution. With the addition of NaCl, a precipitate was immediately observed. Following equilibration, the CHX concentration remaining in solution was determined. In Figure 3, the CHX remaining in solution is given as a function of the concentration of NaCl added to the test tube. The three sets of data correspond to three different initial concentrations of CHX-G_2, which also corresponds to three different gluconate concentrations. As the amount of added NaCl is increased, the concentration of CHX remaining in solution decreased. Moreover, the concentration of CHX remaining in solution was greater for the solutions with the higher initial concentration of CHX-G_2.

In comparing the data, the set with the lowest concentration of CHX-G_2 displayed a dramatic decrease in CHX after 100 to

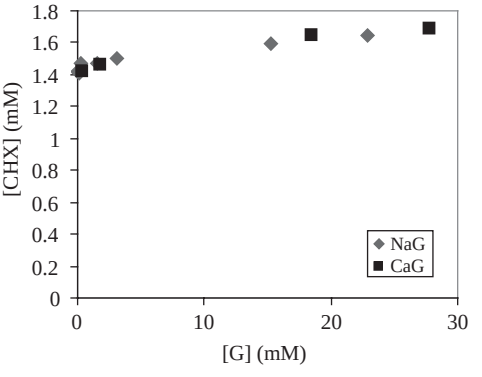

FIGURE 2. Chlorhexidine (CHX) concentration as a function of added (♦) sodium gluconate and (■) calcium digluconate gluconate (based on moles of gluconate) in the presence of excess CHX dihydrochloride.

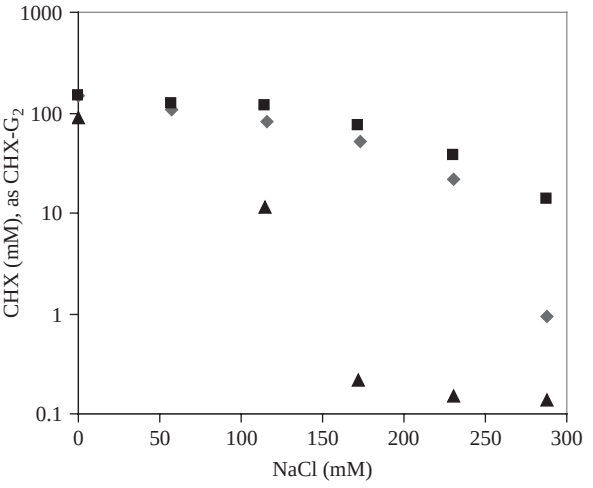

FIGURE 3. CHX-G_2 remaining in solution as a function of added sodium chloride concentration with the initial concentration of gluconate of (▲) 74 mM, (♦) 145 mM and (■) 148 mM.

200 mM of NaCl was added. At the higher initial concentrations of CHX-G_2, the decrease occurred at a higher added NaCl concentration. It appears that with the addition of a low concentration of NaCl, CHX-Cl_2 precipitates but a significant fraction of the CHX remains in solution. Presumably with the precipitation of CHX-Cl_2, the concentration of chloride ions remaining in solution is small, which allows a greater concentration of CHX to remain in solution. As the concentration of added NaCl is increased, more CHX-Cl_2 will precipitate. As the concentration of added NaCl approaches twice the value of the concentration of the initial CHX-G_2 in solution, a dramatic decrease in CHX concentration is observed. At this point, the chloride ion concentration remaining in solution is sufficiently high to control the solubility product. Moreover, this point will clearly be dependent on the initial concentration of CHX-G_2 placed in solution.

The next set of experiments involved preparing CHX-G_2 solutions with increasing concentrations of sodium gluconate, to which a fixed concentration of NaCl was added. In Figure 4A, the concentration of added NaCl was 15.4 mM, and the CHX concentration remaining in solution was near 50 mM and independent of gluconate concentration. As above, it appears that CHX-Cl_2 precipitated, thereby depleting the chloride concentration in solution to permit the CHX concentration to remain at 50 mM.

In Figure 4B, the concentration of added NaCl was much higher at 239 mM. In this case, the concentration of CHX remaining in solution was much lower, 0.1–0.16 mM, but was dependent on gluconate concentration in a manner similar to that seen above. Consistent with the solubility product of CHX-Cl_2 controlling the CHX in solution, by increasing the chloride ion concentration by a factor of 15, the CHX concentration fell by a factor of 15^2. With the higher concentration of added NaCl, which then precipitated with CHX, the ionic strength of the solution is actually lower and thus the effect of gluconate on increasing the solubility product is again evident as shown in Figure 3.

The next experiment involved determining the solubility product of CHX-Ac_2. The results are shown in Figure 5, where the concentration of CHX is plotted as the reciprocal of the square of the acetate concentration in solution. The total concentration of acetate in solution was taken as the sum of the concentration of added sodium acetate and the concentration of CHX-Ac_2. A linear relationship was found, and the slope, 1.66×10^{-4} M^3, is equal to the solubility product. This value agrees very well with the reported solubility product from the measured solubility of CHX-Ac_2, considering that there was no correction for the change in the activity coefficients (Nerurkar et al., 1995).

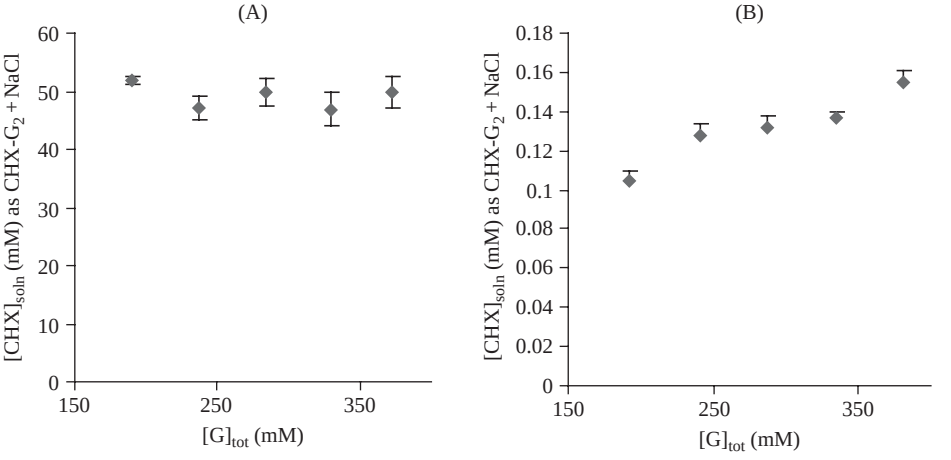

FIGURE 4. CHX concentration in solution as a function of added digluconate concentration in the presence of an initial concentration of sodium chloride of (A) 15.4 mM and (B) 239 mM.

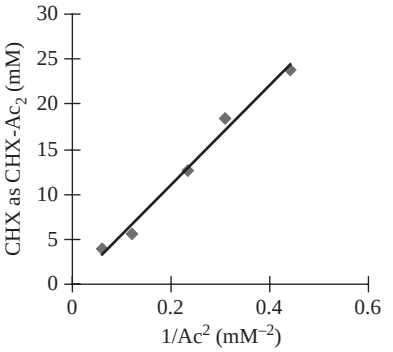

FIGURE 5. Chlorhexidine concentration given as a function of the reciprocal of the square of the acetate concentration at room temperature.

The above experiment provided the concentration of CHX with changing sodium acetate. To examine the effect of changing both the acetate and gluconate concentrations simultaneously, a series of solutions were combined. For these three related series, five different concentrations of sodium acetate ranging from 0 to 200 mM were placed in the solution, whereas the initial concentration of CHX-G_2 was kept constant at 0, 50, or 100 mM. The concentration of CHX remaining in solution is given in the three-dimensional plot as a function of total added gluconate, 0, 100, or 200 mM, and total added acetate (Figure 6). This appears in a three-dimensional bar graph as a series of bars parallel to the acetate concentration axis. Another series was also prepared where the initial concentration of CHX-Ac_2 was kept constant at 100 mM, and the six different concentrations of sodium gluconate were added ranging between 0 and 200 mM. This appears in the bar graph as an orthogonal set to the above three sets. These sets of samples were allowed to equilibrate at room temperature (23°C; Figure 6) whereas another group of sets was equilibrated at 37ºC (Figure 7).

First, it is apparent that the concentration of CHX in solution is greater when the concentration of acetate is low and the concentration of gluconate is high. This perhaps is expected from the dramatic differences in the solubility products of CHX-G_2 and CHX-Ac_2. The dramatic difference in the solubility products is a consequence of the favorable interactions between gluconate and CHX that also gives rise to self-association. Second, in comparing the results from samples with gluconate concentrations of 0, 100, and 200 mM, the observed concentration of CHX increases with increasing gluconate. This indicates that CHX-Ac_2 is solubilized by CHX-G_2 or these two species form mixed aggregates, that is, aggregates composed of both species. It appears that CHX-Ac_2 can exist in solution at a sufficiently high concentration that is above the concentration of aggregation (critical micelle concentration) to form mixed micelles. That was not the case for CHX-Cl_2, where the high concentrations of CHX were not observed.

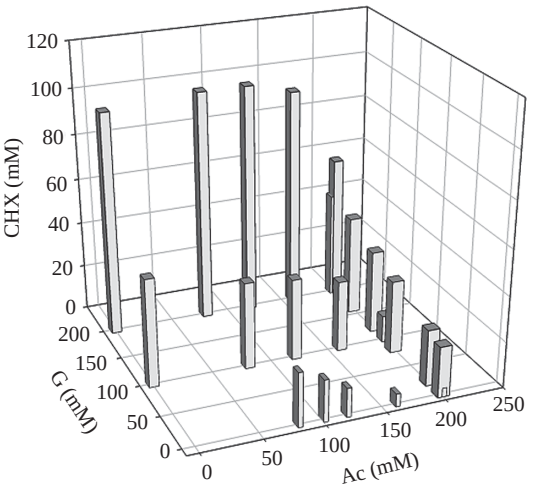

FIGURE 6. Concentrations of CHX as a function of added gluconate and acetate concentrations at room temperature.

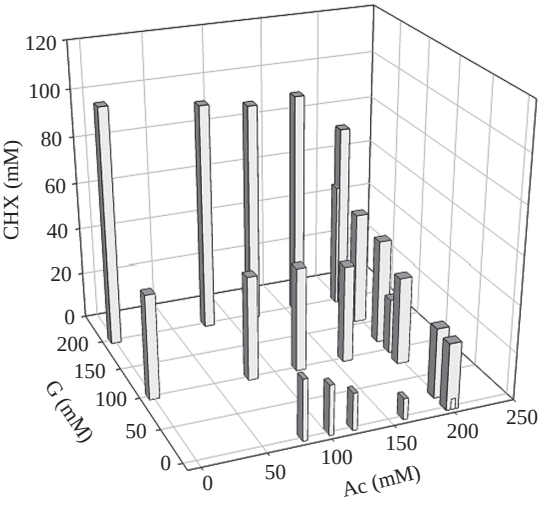

FIGURE 7. Concentrations of CHX as a function of added gluconate and acetate concentrations at 37ºC.

It should be noted that in the data set of constant acetate concentration of 200 mM, there are two overlapping points. For the point at 200 mM acetate and 0 mM gluconate, the sample with lower concentration was prepared with solid CHX-Ac_2 and 200 mM added sodium acetate, whereas the sample with a higher concentration was prepared with just solid CHX-Ac_2. The former sample thus has nearly 200 mM higher concentration of sodium ions that may have caused a reduction in the observed CHX concentration.

Another duplicative point occurs at 200 mM acetate and 200 mM gluconate. For the sample with a higher measured

concentration, CHX was added as a gluconate solution and then was precipitated with sodium acetate. In contrast, the sample with a lower concentration was prepared with solid CHX-Ac_2 and a solution of sodium gluconate was added. Given the discrepancy between the two observed concentrations, it appears that equilibrium was not achieved.

The above study was also carried out at 37ºC (Figure 7). Here similar trends with respect to CHX solubility with varying counterion concentration were observed but generally the CHX concentration was greater at the elevated temperature. In addition, the discrepancy between the concentrations observed with the sample of identical composition but with different starting materials is smaller, suggesting these samples are closer to equilibrium.

These results have a number of important implications for the use of CHX and the development of drug delivery systems for preventing dental infections. High solution concentrations of CHX can be achieved through the use of CHX digluconate. In addition, reasonably high concentrations of CHX can be produced with the diacetate species provided digluconate is included. The high concentration appears to involve solubilization of the diacetate species within the digluconate aggregate to form a mixed micelle, because the acetate concentration will be sufficiently low to allow CHX to exceed the critical micelle concentration (Heard & Ashworth, 1968). However, only low concentrations of CHX can be achieved in the presence of chloride ions, and CHX dichloride was not appreciably solubilized by gluconate ions. It appears that the solubility product of CHX dichloride limits the concentration of CHX to that below the critical micelle concentration and thereby prevents micelle formation.

When CHX solutions are used in the oral cavity, they will come into contact with saliva that contains chloride ions (Dolby et al., 1972; Gjermo, 1989; Wang & Peng, 2006). As such, CHX dichloride will precipitate in accordance to the solubility product. However, relatively high concentrations of CHX may be achieved due to the depletion of chloride ions. This will be favored by the use of a large volume of rinsing solution coupled with a low volume of saliva and thus low number of moles of chloride ions. With respect to the development of drug delivery systems, care must be taken in evaluating the release rate (Carlo et al., 2006; Farkas, Kiss, & Zelko, 2007; Gong et al., 2007; Jones, Lorimer, McCoy, & Gorman, 2008; Kiremitci, Ciftci, Ozalp, & Gumusderelioglu, 2007; Nerurkar et al., 1995; Riggs, Braden, & Patel, 2000). Specifically, a low release rate may fail to achieve a sufficiently high concentration of CHX, because the CHX would come immediately into contact with a physiological concentration of chloride and precipitate to a concentration dictated by the solubility product. This concentration would be below the minimum effective concentration for antimicrobial activity.

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