Development of Chlorhexidine Thermosensitive Gels as a Mouth Antiseptic

Sumalee WANNACHAIYASIT¹* and Thawatchai PHAECHAMUD²

¹Department of Biopharmacy, Faculty of Pharmacy, Silpakorn University, Nakhon Pathom, 73000 Thailand
²Department of Pharmaceutical Technology, Faculty of Pharmacy, Silpakorn University, Nakhon Pathom, 73000 Thailand

Abstract

Chlorhexidine is a widely used antiseptic with a broad spectrum of antimicrobial activities. The aim of this research is to develop a chlorhexidine thermosensitive gel as a mouth antiseptic by using lutrol F127 as a gelling agent. Due to its thermosensitive properties, lutrol has a sol-gel transition ability to change from solution stage at low temperature to the viscous gel at high temperature; these characteristics provide extended exposure time to the oral cavity. In order to achieve optimal viscosity and thermal response, the suitable concentrations of lutrol and chlorhexidine were 25% and 0.25% w/w, respectively. The chlorhexidine thermosensitive gel showed antimicrobial activity against S. aureus, E. coli, and C. albicans. In vitro release of chlorhexidine was investigated in phosphate buffer pH 6.2 at 37°C. The release of chlorhexidine was 94% after 180 minutes and followed zero order kinetics.

Key words: Chlorhexidine, Lutrol, Thermosensitive gel

Introduction

Chlorhexidine is a bis-biguanide antiseptic for skin and mucous membranes. It has been used by dental professionals for plaque control and for the treatment of gingival inflammation. Chlorhexidine has a broad spectrum of antimicrobial activity against a wide variety of bacteria (both gram-positive and gram-negative) and fungi. Its mechanism of action is damaging the inner membrane.(4-5, 8)

Lutrol F127 is poloxamer with tri-block structure of (polyethylene oxide)x - (polypropylene oxide)y -(polyethylene oxide)x. Lutrol-based system has thermosensitive property that the system is able to change from solution stage at low temperature to the viscous gel at high temperature.¹⁰ Lutrol is a useful polymeric carrier for many agents and used as a thickening agent. It has been developed as local anesthetic gels for sustained pain relief and as delivery system for ophthalmic drugs.¹¹ It is low toxic, mucomimetic¹², therefore it is a good candidate for mouth antiseptic gel.

In this study, chlorhexidine gel was developed using lutrol F127 as a gelling agent. The concentrations of lutrol were varied to achieve optimal viscosity and thermal response. The antimicrobial activity of gels containing various concentrations of chlorhexidine was studied. The sol-gel transition was investigated at 4°C and 28°C. In vitro release of chlorhexidine was investigated in phosphate buffer pH 6.2 at 37°C. The antimicrobial activity of chlorhexidine gels against Staphylococcus aureus, Escherichia coli, and Candida albicans was performed.

Materials and Experimental Procedures

Materials

Chlorhexidine gluconate was from Poligono Industrial de Celra, Girona, Spain. Lutrol F127 (Poloxamer 407) was purchased from VITA Co., Ltd, Bangkok, Thailand. Acesulfame potassium was obtained from PC Drug, Bangkok, Thailand. Saboraud dextrose agar and Saboraud dextrose broth were purchased from Becton Dickinson and company, MD, USA. Tryptic soy agar and Tryptic soy broth were from Hardy Diagnostics, CA, USA.

Preparation of Chlorhexidine Lutrol-Based Thermosensitive Gels

To investigate the optimal amount of lutrol F127 for developing the thermosensitive gel, various concentrations of lutrol solutions (10, 15, 20, 25, 30% w/w) were prepared by dissolving lutrol in distilled water at 4-5°C to obtain clear
solutions. The stock solution of chlorhexidine gluconate (20% w/w) was subsequently added into the polymer solution to obtain chlorhexidine’s final concentration of 0.25% w/w. The physical properties of the gels (color, homogeneity, taste) were observed. The pH values of the gels were measured. The sol-gel transition was observed at 4°C and 28°C. The viscosity response to temperature was investigated at various temperatures (20-50°C) using a viscometer (Brookfield Engineering Laboratories, USA).

Lutrol (25 % w/w) thermosensitive gels with various concentrations of chlorhexidine (0.063, 0.125, 0.25, 0.5, 1 % w/w) were prepared to examine the effect of chlorhexidine concentrations on the antimicrobial activity against S. aureus, E. coli, and C. albicans.

Acesulfame (potassium salt) was added into chlorhexidine gel as a sweetener to overcome the bitter taste of chlorhexidine. Different concentrations of acesulfame (0.05, 0.1, 0.15, 0.2% w/w) were added.

Antimicrobial Activity Test

The abilities to inhibit the growth of microbes (S. aureus and C. albicans) of lutrol (25 % w/w) gels containing various concentrations of chlorhexidine (0.063, 0.125, 0.25, 0.5, 1 % w/w) were determined by using an agar-cup diffusion method (n=3). Petridishes were filled with melted agar medium. After the agar settled, the microorganisms were streaked on the whole agar surface by using cotton swabs. The cups filled with samples were placed on the agar surface. The plates were incubated at 37°C for bacteria or at 25°C for fungi. The inhibition zone was measured.

The chlorhexidine (0.25%) lutrol (25%) gel with acesulfame was also tested to determine antimicrobial activity against S. aureus, E. coli, and C. albicans.

In Vitro Release Study

The release of free chlorhexidine from lutrol (25 % w/w) gel containing 0.25% chlorhexidine was studied in phosphate buffer pH 6.2, at 37°C by membraneless method.(3) The chlorhexidine lutrol gel was weighed in a small glass cup. The cup was then placed in a glass bottle. The bottle was filled with preheated phosphate buffer pH 6.2. The bottle was shaken by using an incubation shaker (speed of 200 rpm) (SI4, Shel Lab, Cornelius, USA) and the temperature was maintained at 37°C. At various time intervals, the solution was taken and replaced with fresh buffer. The released amount of chlorhexidine was measured at 255 nm using a UV-VIS spectrophotometer (Perkin-Elmer, Germany).

Stability Study

The lutrol (25 % w/w) gel containing 0.25% chlorhexidine and 0.1% acesulfame was evaluated for stability by freeze-thaw process. The formulation was placed in refrigerator at 4°C for 24 hours and then in hot air oven at 40°C, 65% RH for 24 hours. The process was repeated five times. The physicochemical properties were observed and the amount of chlorhexidine released from the gel was measured using a UV-VIS spectrophotometer.

Results and Discussion

Determination of the Physicochemical Properties and Antimicrobial Activity of Chlorhexidine Thermosensitive Gels

Chlorhexidine (0.25% w/w) formulations with various concentrations of lutrol (10, 15, 20, 25, 30% w/w) were prepared to examine the viscosity response to temperature. All formulations had bitter taste of chlorhexidine and pH of approximately 5.5. The formulations with lutrol of 10% and 15% w/w were clear solution at 4°C and 28°C and became gels at 37°C. However, formulation with lutrol of 30% w/w showed precipitation at 28°C. The viscosity-temperature graph for the formulations with 15%, 20% and 25% lutrol are shown in Figure 1. The results showed that the formulation with 15% lutrol had very low viscosity. The formulations with 20% and 25% lutrol increased viscosity when the temperature increased and were able to form gels at 37°C; however, the gel with 25% lutrol had higher viscosity (7413 cps.) than that with 20% lutrol (827 cps.) at 37°C. The results were in accordance with the property of lutrol that its viscosity was mainly influenced by the concentration and temperature.(3) Higher viscosity could prolong exposure time in oral cavity of an antiseptic. Therefore, the optimal amount of lutrol was 25% w/w.
Development of Chlorhexidine Thermosensitive Gels as a Mouth Antiseptic

To investigate the effect of chlorhexidine concentrations on the antimicrobial activity, various concentrations of chlorhexidine (0.063, 0.125, 0.25, 0.5, 1 % w/w) in lutrol (25% w/w) gel were prepared. The results showed that the antimicrobial activity against *S. aureus* and *C. albicans* increased with an increase in chlorhexidine concentration (p<0.05) (Table 1). Therefore, antimicrobial activity of chlorhexidine was concentration-dependent as previously reported.(6) However, the antimicrobial activity of chlorhexidine of 0.25% and 0.5% was similar (p>0.05). Chlorhexidine is frequently used between 0.2% and 0.5%. Due to bitter taste of chlorhexidine, amount of 0.25% was chosen with good antimicrobial activity. Acesulfame was added into chlorhexidine gel as a sweetener as it is safe and acceptable non-nutritive sweetener.(7) Acesulfame of 0.05% could not eliminate bitterness. The formulation with 0.1% and 0.15% acesulfame had similar taste; sweet without bitterness. The formulation with 0.2% was bitter-sweet. Hence, acesulfame of 0.1% w/w was chosen because it was the minimum amount that effectively overcame the bitter taste of chlorhexidine and did not affect the antimicrobial activity of the gel against *S. aureus*, *E. coli* and *C. albicans* (p>0.05) (Table 2). The mechanism of chlorhexidine is damaging the bacterial cytoplasmic inner membrane, precipitating protein and nucleic acid. It is reported that chlorhexidine damages the cell wall of gram-positive bacteria, the outer cell membrane of gram-negative bacteria, and the cytoplasmic membrane of yeasts. Consequently, it is undoubted that the chlorhexidine lutrol gel could inhibit the growth of *S. aureus*, *E. coli* and *C. albicans* that represented for gram-positive, gram-negative bacteria, and yeasts, respectively.

![Figure 1. Viscosity-temperature graph for the formulations with 15%, 20% and 25% lutrol. (n=3).](image)

**Table 1.** The antimicrobial activity of lutrol (25% w/w) gels containing various concentrations of chlorhexidine against *S. aureus* and *C. albicans*. (n=3).

<table>
<thead>
<tr>
<th>concentrations of chlorhexidine in gel (% w/w)</th>
<th>clear zone; cm (mean ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>0.063</td>
<td>16.0 ± 1.0</td>
</tr>
<tr>
<td>0.125</td>
<td>20.0 ± 0.0</td>
</tr>
<tr>
<td>0.250</td>
<td>22.3 ± 1.5</td>
</tr>
<tr>
<td>0.50</td>
<td>24.0 ± 1.0</td>
</tr>
<tr>
<td>1.0</td>
<td>25.0 ± 0.0</td>
</tr>
</tbody>
</table>

**Table 2.** The antimicrobial activity of chlorhexidine (0.25% w/w) lutrol (25% w/w) gel with/ without 0.1% w/w acesulfame potassium against *S. aureus*, *E. coli* and *C. albicans*. (n=3).

<table>
<thead>
<tr>
<th>Formulations</th>
<th>clear zone; cm (mean ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorhexidine lutrol gel with 0.1% w/w</td>
<td></td>
</tr>
<tr>
<td>acesulfame potassium</td>
<td>13.0 ± 1.0</td>
</tr>
<tr>
<td>without 0.1% w/w acesulfame potassium</td>
<td>13.3 ± 1.2</td>
</tr>
<tr>
<td>Lutrol gel base (no acesulfame, no</td>
<td></td>
</tr>
<tr>
<td>chlorhexidine)</td>
<td>0.0 ± 0.0</td>
</tr>
</tbody>
</table>

**In Vitro Release Study**

The release of free chlorhexidine from lutrol (25 % w/w) gel containing 0.25% chlorhexidine was performed in phosphate buffer pH 6.2 at 37°C. Figure 2 shows that 94% of chlorhexidine was released from the formulation after 180 minutes. The release profile was zero order kinetics with r² value of 0.9442. The study indicated that the lutrol thermosensitive gel could prolong the release of chlorhexidine. Extended release of chlorhexidine as well as prolonged retention time of the gel formulation would provide long-term antimicrobial activity of this antiseptic in the mouth.

![Figure 2. Release profile of chlorhexidine from the chlorhexidine (0.25% w/w) lutrol (25% w/w) gel. (n=3).](image)
Stability Study

Chlorhexidine is a cationic antiseptic (9) and lutrol is a nonionic block copolymer (3), therefore these substances are compatible with each other. Chlorhexidine was not stable at high temperature. Chlorhexidine might degrade to para-chloraniline at temperature above 70°C (6). As a result, freeze-thaw was chosen as the stress condition for stability study of chlorhexidine lutrol gel. The lutrol (25 % w/w) gel containing 0.25% chlorhexidine and 0.1% acesulfame was subjected to five freeze-thaw cycles. The gel was kept at 4°C for 24 hours and subsequently at 40°C for 24 hours. After five freeze-thaw cycles, the formulation remained in good appearances and accumulated chlorhexidine released from the gel was measured and was found more than 96 % indicating good stability of the formulation after five freeze-thaw cycles.

Conclusions

The development of chlorhexidine thermosensitive gel was successful. The gel containing 0.25% w/w chlorhexidine, 25% w/w lutrol, 0.1% w/w acesulfame had good physical properties without bitter taste and could form high viscous gel at body temperature. This chlorhexidine gel could be a promising antiseptic for oral cavity with prolonged antimicrobial activity.

Acknowledgements

Authors would like to thank Jirapong Tiamkao, Chanapak Lerdpinyochaitharworn, Nattapong Chinsukserm, Pattanasak Maimongkol, Juree Charoenteeraboon.

References


