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Cornea & External Disease

Local Ugandan Production of Stable 0.2% Chlorhexidine Eye Drops

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Purpose: The purpose of this study was to develop a protocol to prepare buffered chlorhexidine (CHX) eye drops (0.2% w/v) in the United Kingdom that can be reproduced at a production facility in Uganda. Buffered CHX eye drops can prevent CHX degradation and improve ocular tolerability during the treatment of fungal keratitis.

Methods: Buffered CHX eye drops in amber glass containers were prepared using sodium acetate buffer at pH 5.90 to 6.75. Two commercial CHX solutions and CHX in water were used as controls. Eye drops were stored at 40°C (70% humidity, 21 months) in the United Kingdom and at ambient temperature in Uganda (30 months). High-performance liquid chromatography was used to determine CHX stability over time, and pH was monitored. Sterility was achieved using an autoclave (121°C, 15 minutes) and water bath (100°C, 30 minutes).

Results: The pH of acetate-buffered CHX eye drops did not change over 21 months at 40°C or at ambient temperature (30 months), whereas the pH of the unbuffered aqueous CHX displayed significant fluctuations, with an increase in acidity. The CHX concentration remained the same in both buffered and unbuffered eye-drop solutions. Eye drops sterilization was successful using an autoclave and a water bath.

Conclusions: Stable, sterile, buffered CHX eye drops (pH 6.75) were successfully prepared first in the United Kingdom and then reproducibly in Uganda. This eye drops can be prepared in a hospital or pharmacy setting with limited resources, thus providing a cost-effective treatment for fungal keratitis.

Translational Relevance: A protocol has been developed to prepare buffered CHX eye drops in low- and middle-income countries to treat fungal keratitis.

Introduction

It has recently been estimated that more than 1 million people develop fungal infection of the cornea (fungal keratitis) worldwide each year.[1] Topical natamycin eye drops (5% w/v) are the first-line treatment for fungal keratitis, and they were recently added to the World Health Organization (WHO) List of Essential Medicines for this indication.[2] However, natamycin eye drops are frequently unavailable or unaffordable in many low- and middle-income countries (LMICs); therefore, many people who develop fungal keratitis go untreated, leading to the loss of the affected eye. There is a need for anti-fungal eye drops that can be locally and reliably manufactured to treat patients in LMICs.

Chlorhexidine (CHX), a widely used broad-spectrum biocide with antifungal and antibacterial action, is also included in the WHO List of Essential Medicines. CHX (4%) is used for skin antisepsis and

topical application to the umbilicus of newborns.[2] CHX solutions (0.1%–0.2% w/v) are widely used around the world as a long-term mouth wash to prevent and treat oral candidiasis (a fungal infection) and for general oral hygiene.[3–5]

CHX has been used in ophthalmology for more than 30 years as an eye-drop preservative (0.01%–0.2%), to sterilize contact lenses, as a preoperative topical antiseptic, and for treating *Acanthamoeba* spp. infections and fungal keratitis (corneal infections).[6–11] Aqueous CHX (0.1% w/v) was found to be an effective, less painful alternative to povidone–iodine to provide prophylaxis antisepsis prior to intravitreal injections.[12] The antifungal properties of CHX can also be beneficial.[13–15] In a study evaluating potential affordable antifungal treatments for keratitis, CHX (0.2%) gave the best results in vitro compared with propamidine (Brolene), povidone–iodine. and polyhexamethylene biguanide.[16]

Subsequently, two pilot randomized controlled trials of CHX for the treatment of fungal keratitis were conducted. Some evidence indicated that CHX eye drops prepared in sterile, unbuffered water (0.2% w/v) were not inferior to natamycin in terms of both showing a favorable response by 5 days and cure by 21 days.[7,8] Overall, a Cochrane systematic review of treatments for fungal keratitis found a statistically non-significant trend favoring CHX over natamycin in treating fungal keratitis by 21 days (relative risk = 0.70; 95% confidence interval, 0.45–1.09) when the data from these two trials were combined.[17]

In our recent non-inferiority trial in Nepal, natamycin was found to be superior; however, CHX still performed very well in the large majority of cases, achieving microbiological control (culture negative) in 85% of cases, compared with 92% for natamycin (P = 0.17) after 7 days of treatment. CHX eye drops can be used as second-line therapy for the treatment of fungal keratitis where natamycin eye drops are not available.[18,19] The CHX eye drops (0.2% in acetate buffer, pH 5.8) used in this trial were a special formulation and were imported from the United Kingdom to the trial site.

Our pilot study in Uganda showed that CHX eye drops (0.2% in acetate buffer, pH 6.7) made locally can be a useful sequential adjunctive therapy for the treatment of fungal keratitis cases that are not responding to natamycin eye drops (5%).[20] The local production of CHX eye drops would be a cost-effective and sustainable solution to ensure the availability of CHX eye drops in LMICs where natamycin eye drops are scarcely available due to the difficulty in manufacturing and costly active pharmaceutical ingredients.

CHX is susceptible to light-initiated degradation and is not stable in solutions with pH values above 7.[21] Clinically used CHX eye drops are prepared using sterile water without controlling pH and have low tonicity. The use of a buffer in eye drops is important for controlling pH and the tonicity of the solution. Eye-drop solutions are well tolerated when the tonicity and pH match those of the lacrimal fluid.[21,22] There are limited options for buffers that can be used for the preparation of CHX eye drops, due to poor CHX compatibility with buffer salts such as phosphates, sulfates, carbonates, and chlorides.[21] The only buffer can be used is sodium acetate, which is known to maintain CHX stability.[23] Sodium acetate buffer is a common excipient used in pharmaceutical preparations and has previously been used to stabilize eye-drop solutions at a lower concentration of 0.02% CHX for the treatment of *Acanthamoeba* keratitis.[23] Sodium acetate has a pKa of 4.6. The buffering range of 20-mM sodium acetate is pH 3.6 to 5.6 which can be increased by using a high salt concentration (141 mM).[23] A desirable pH range for CHX eye drops is pH 6 to 7, with better tolerability being exhibited closer to pH 7 (note that pH values greater than 7 may result in CHX degradation).[24] The aim of this present study was to develop a method and protocol for preparation of sodium acetate–buffered CHX eye drops that can be transferred to a Ugandan hospital eye-drop production unit and reproduced at low cost.

Materials and Methods

Materials

Good Manufacturing Practice (GMP)-grade 20% CHX (#750594) was purchased from Fagron (Rotterdam, The Netherlands). Sodium acetate (#S2889) and acetic acid (#A6283) were purchased from (St. Louis, MO). Honeywell Fluka sodium hydroxide (#S/4920/53) was purchased from Thermo Fisher Scientific (Waltham, MA) and was made up to a 10-M solution by dilution in deionized water. Tryptone soya agar with 5% sheep blood (#EOLAPP1651-P090) and LB miller agar (#102502ZA) were purchased from VWR International (Radnor, PA). We purchased two types of CHX eye drops in current clinical use that are supplied as specials by two undisclosed companies: C_{Aq} (CHX 0.2% in sterile water) and C_{AcB} (CHX 0.2% in acetate buffer). Details of the pilot study measuring the stability of an aqueous commercial CHX eye drops can be found in the Supplementary Materials.

Preparation of Buffered CHX Eye Drops at University College London

All glassware was sterilized by autoclave at 121°C for 15 minutes before being used to prepare the CHX (0.2% w/v) eye drops. Sodium acetate buffer was prepared in triplicate, by addition of sodium acetate (141.4 mM) and acetic acid (7.8 mM) in 1.1 L of type 2 water, purified by reverse osmosis. The pH was adjusted by the addition of sodium hydroxide (10 M) to reach pH 5.90, 6.25, 6.50, and 6.75 (labeled AcB1–4, respectively), and the pH and osmolarity were measured before and after sterilization by autoclaving at 121°C for 15 minutes.

In a grade II laminar flow sterile hood, CHX 0.2% was prepared by dilution of 20% CHX digluconate (10 mL) in acetate buffer (final volume of 1 L). The CHX solutions were prepared in triplicate and filtered through a grade-3 sinter into a conical flask via vacuum filtration and bottled by transferring the solutions into 10-mL amber glass vials. A further three solutions were made that were filtered through a 0.22-µm filter placed on the grade-3 sinter and bottled in 10-mL amber glass vials. The solutions were sterilized by autoclave at 121°C for 15 minutes before conducting the stability studies.

Stability Studies

Sample solutions were incubated at 40°C at 70% humidity alongside clinical control samples prepared as specials by two undisclosed companies. An additional sample of the clinical control samples were stored at 4°C. Aliquots (100 µL) were collected at 0, 1, 2, 3, 4, 8, and 85 weeks, and the pH and concentration of these samples were determined. The concentration of CHX at each time point was measured using high-performance liquid chromatography (HPLC).

Analysis of CHX Eye-Drop Solutions

Osmolality measurements were conducted with a Roebling type 13 osmometer using distilled water and 300-mOsm/kg standard solution as calibrants. The pH of the remaining sample was determined using a Hanna HI1093B pH probe (Hanna Instruments, Smithfield, RI). Aliquots (20 µL) from each sample were diluted in 180 µL deionized water for HPLC analysis.

The high-performance liquid chromatography (HPLC) system was an Agilent 2400 equipped with ultraviolet detector and fitted with a Supelco Discovery HS C18 column (150 mm × 4.6 mm; 5 µm) (#568522-U; Agilent Technologies, Santa Clara, CA) and guard column. A method was developed in-house using a gradient aqueous mobile phase containing 0.1% trifluoracetic acid and increasing acetonitrile from 20% to 70% over 17 minutes, an approach that provided good separation between CHX and the expected degradation product 4-chloroaniline peaks. Total run time was 20 minutes, and the retention time of CHX was 8.9 minutes.

Sterility Testing

Eye-drop solutions (10 mL) were sterilized by passing the solution through (1) fresh sinter, (2) fresh sinter and a 0.22-µm membrane, or (3) a disposable 0.22-µm syringe filter. Eye-drop solutions were then incubated for 85 weeks and were then analyzed. To isolate any microbial growth from the CHX solution, each 10-mL solution was passed through a disposable 0.22-µm filter using a syringe and washed with 10 mL of water to remove chlorhexidine from the filter bed. A 0.5-mL backwash through the filter was then conducted to resuspend any microbes into a solution. The syringe was changed at each step. The backwashed solution (200 µL) was transferred and spread onto both Lysogeny broth (LB) agar and 5% blood agar plates. Sterile water was run as a negative control, and a water solution spiked with *Escherichia coli* was used as positive control. All samples were run in triplicate. After solution drying in a sterile environment for 10 minutes, the plates were incubated at 37°C for 48 hours.

Eye Drops Production at Ruharo Mission Hospital, Uganda

Distilled water was produced onsite by a fixed distillation unit. For eye drops production, distilled water was collected within 2 hours of production into a clean plastic container. All containers used for the preparation of the eye-drop solutions were rinsed three times with distilled water before use by filling with distilled water and discarding.

Acetate buffer was prepared by the addition of sodium acetate (141.4 mM) and acetic acid (7.8 mM) in a mixing bucket. The pH was measured using a portable pH meter (Hanna Halo 12302) and adjusted by dropwise addition of sodium hydroxide (10 M) to reach pH 6.75. CHX 0.2% (100-mL batches) was prepared by dilution of chlorhexidine digluconate (1 mL) in a volumetric flask to a volume of 100 mL. The solution was shaken to mix and filtered through a grade-3 sinter aided by a hand vacuum pump. The solution was dispensed using a Pressmatic bottle top dispenser in 10-mL portions into amber glass bottles. Lids were screwed on firmly by hand, and the bottles sterilized by a water steam bath at 100°C and

atmospheric pressure for 30 minutes. Three batches were made in this way, and the first, middle, and last bottles of each batch were sent for sterility testing.

CHX concentration was determined using a portable Jenway 7205 UV-VIS Spectrophotometer (Cole-Palmer, Vernon Hills, IL) at a wavelength of 280 nm. A standard curve was conducted using CHX solutions prepared by serial dilution in triplicate in concentrations from 0.01 to 0.000115% w/v. Study samples (20 µL) were diluted by 100-fold by the addition of 1.980 mL of water.

Sterility testing was conducted by Hybrid Bioanalytics in Uganda. The total plate count and coliform count were determined. The eye-drop solutions (5 mL) were filtered through a 0.22-µm filter using a filtration funnel. The filter bed was flushed with autoclaved distilled water (100 mL), and the filters were plated onto the Tryptone Glucose Extract nutrient pads and agar for the total plate and coliform counts, respectively. Distilled water (100 mL) was filtered in the same manner and run as a control. The incubation at 35°C for coliforms and total plate count were conducted for 24 and 48 hours, respectively.

Results

Stability of Unbuffered Commercial Aqueous CHX Eye Drops

In our pilot study, commercial unbuffered aqueous CHX eye drops (C_{Aq}) were analyzed for changes in formulation upon storage in refrigerated conditions (4°C) or in a tropical environment using an incubator set at 40°C and 70% relative humidity (Fig. 1). For solutions subjected to both conditions, a decrease in pH was observed over the 12-month study period. Under refrigeration, a decrease of 0.29 pH units was measured, and in tropical conditions the pH decreased by 0.50 units. No significant change in CHX concentration was observed for the solutions in either storage condition.

Stability of Compounded Buffered CHX Eye Drops

Compounded buffered CHX eye drops (AcB) were prepared using sodium acetate buffer made at the same concentration of buffer salts (sodium acetate 141 mM) at different pH values adjusted by the addition of sodium hydroxide (Table). CHX digluconate 20% stock solution was diluted to 0.2% in the sodium acetate buffer solution at each pH value (5.98–6.82) and displayed tonicity within an acceptable range (296–319 mOms/kg) (Table). Compounded unbuffered CHX eye drops (Aq) prepared by dilution in distilled water displayed low tonicity compared to that recommended for topical ophthalmic solutions. The osmolality of the compounded buffered CHX eye drops (AcB) was much greater than that of the compounded unbuffered aqueous eye drops (Aq), which might improve ocular tolerability.

The pH and CHX concentration of the sterilized buffered CHX eye-drop solutions (AcB) were monitored during incubation at 40°C (70% relative humidity) for 21 months. The unbuffered eye drops (Aq and C_{Aq}) (Table) displayed variations in pH over time (Fig. 2). This variability in the pH observed in the case of compounded unbuffered aqueous CHX eye drops (Aq) and commercial unbuffered aqueous CHX

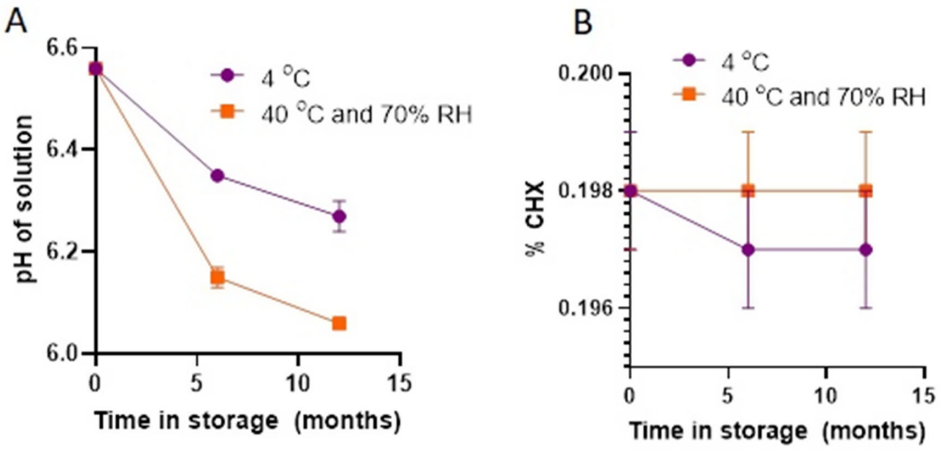

Figure 1. Changes in commercial unbuffered aqueous CHX eye drops (C_{Aq}) over 12 months at different temperatures. (A) Change in pH, and (B) change in CHX concentration.

Table. Osmolality and pH of CHX Eye Drops (0.2%) Prepared in Sodium Acetate Buffer at Varying pH (AcB1–4) or Water (Aq)

Formulation	Initial pH (After AC)	Osmolality (mOsm/kg)
Aq	6.40	5.3 ± 0.6
AcB1	5.98	319.0 ± 67.6
AcB2	6.35	296.3 ± 5.0
AcB3	6.72	303.3 ± 12.5
AcB4	6.82	296.0 ± 3.0
C_{Aq}	5.84	5.0 ± 0
C_{AcB}	5.89	282.0 ± 5.0

AC, autoclave sterilization at 121°C; Aq, compounded unbuffered aqueous CHX eye drops; AcB, compounded buffered CHX eye drops; C_{Aq}, commercial unbuffered aqueous CHX eye drops; C_{AcB}, commercial buffered CHX eye drops. Osmolality data are expressed as mean ± SD ($n = 3$).

eye drops (C_{Aq}) might be due to the degree of water purity or pH or method of purification. Our buffered CHX eye drops (AcB) exhibited a more stable pH over 21 months than the unbuffered preparations, a finding that was similar to that for the clinically used commercial buffered CHX eye drops (C_{AcB}). After terminal sterilization by autoclaving, a minor increase in the pH of the compounded buffered CHX eye drops (AcB) was observed.

HPLC analysis of all analyzed eye drops (AcB1–4, Aq, C_{Aq}, and C_{AcB}) revealed that there was only one peak in the chromatogram corresponding to the intact CHX, which demonstrates that the acetate-buffered CHX eye drops was stable at 40°C and 75% relative humidity over 21 months. No significant change in CHX concentration was observed in either buffered or non-buffered solutions (Fig. 3). For

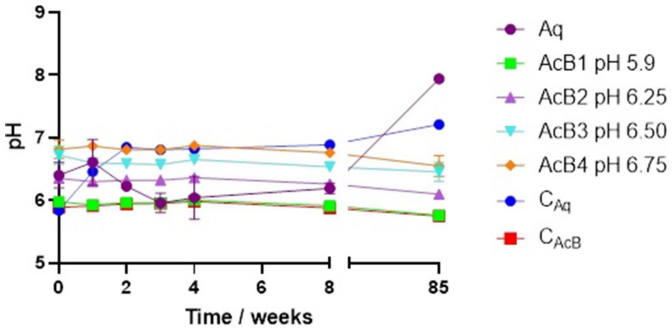

Figure 2. The change in pH of CHX eye drops during storage at 40°C and 70% relative humidity for 85 weeks. Aq is compounded unbuffered aqueous CHX eye drops, AcB is compounded buffered CHX eye drops, C_{Aq} is commercial unbuffered aqueous CHX eye drops, and C_{AcB} is commercial buffered CHX eye drops. Data are expressed as average ± SD ($n = 3$).

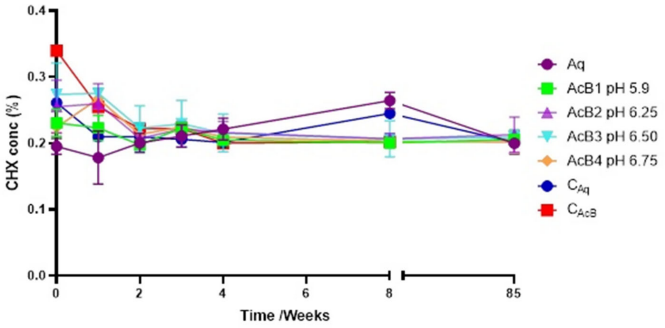

Figure 3. The change of CHX concentration during storage at 40°C and 70% relative humidity for 85 weeks. Aq is compounded unbuffered aqueous CHX eye drops, AcB is compounded buffered CHX eye drops, C_{Aq} is commercial unbuffered aqueous CHX eye drops, and C_{AcB} is commercial buffered CHX eye drops. Data are expressed as average ± SD ($n = 3$).

stability, it is recommended that CHX solutions be maintained at pH 5 to 6 when exposed to temperatures greater than 25°C.[21] The present study indicates that sodium acetate–buffered CHX eye drops (AcB) at a pH range of 5.9 to 6.78 are stable during storage in what are considered to be tropical conditions for up to 21 months.

Sterility of Buffered CHX Eye Drops

Particles and microbial growth in eye-drop solutions are a significant hazard, and solutions are often filtered to ensure that these are kept to a minimum. Considering that filtration devices vary worldwide and assuming that terminal sterilization will be applied using either an autoclave or steam water bath, different filtration procedures were investigated. CHX solutions were prepared and filtered by one of three different filtration techniques: (1) filtration through a glass sinter (porosity grade 3), (2) filtration through a glass sinter with a 0.22-μm membrane placed above, or (3) filteration through a 0.22-μm disposable syringe filter. The sterility of these solutions was assessed following terminal sterilization by autoclave. None of the solutions tested had microbial growth, and the filtration technique had no effect on the overall sterility of the sample. The procedure used in Uganda follows a WHO protocol for the small-scale production of eye drops in local pharmacy settings, which used a grade-3 porosity sinter.[25] Although the 0.22-μm filter membrane is ideal for removing smaller particles, supply can be challenging, and the use of the glass sinter was already in place. We concluded that filtration through the glass sinter with porosity grade 3 was sufficient for removal of large particles and a sterile product could be achieved through the terminal sterilization step.

Figure 4. Images from the Eye Drop Production Department at Ruharo Mission Hospital. *Clockwise from top left*: the external building; the clean production room; the analysis room; and the raw material preparation room.

Protocol Transfer

The local production of CHX eye drops could offers cost-effective benefits for hospitals and communities. We sought to investigate whether the protocol to produce acetate-buffered CHX eye drops (0.2%) could be transferred to Ruharo Mission Hospital in Mbarara, Uganda, and whether the eye drops were of a comparable standard. With the goal of producing batches of eye drops on the multi-liter scale for local patients and for distribution to smaller pharmacies and hospitals further afield, the Eye Drop Production Department at Ruharo Mission Hospital has built a dedicated local eye drop production department (LEPD). Completed in June 2019, the LEPD consists of a dedicated storage area, preparation room, quality control and analysis room, equipment room, and production room (Fig. 4). Currently, many different eye drops are made to treat a variety of eye conditions within the hospital and prescribed to patients. The production room is entered via a small intermediate room that acts as an airlock to prevent dust and disturbance during production.

A key difference from the facilities in the United Kingdom is the lack of a sterile, air-flow controlled environment and temperature maintenance in the Ugandan setting. To compensate for this, the entire Ugandan facility is cleaned thoroughly with detergent, water, and ethanol at the beginning of each week. Following WHO guidelines,[25] the production room surfaces and floors are further cleaned within 2 hours before each production by wiping with distilled water. An additional antimicrobial cleanse is achieved by spraying production surfaces with 70% ethanol. A laminar flow sterile cabinet is planned for installation. The temperature in the eye drops production unit in the Ugandan setting is not controlled, and it fluctuates between 18°C and 30°C. An air-flow–controlled environment with a heating, ventilation, and air conditioning (HVAC) system designed for a class C clean room which contains a 0.4-µm filter that can remove dust and particulates from the air has been recently installed in the eye-drop production facility in Uganda. This HVAC system can provide clean air and temperature control in the eye-drop production facility. Water used for preparation of the buffered CHX eye drops in Uganda is purified using distillation, whereas in the United Kingdom reverse osmosis is used for water purification. Both distillation and reverse osmosis comply with European Pharmacopeia guidelines for purified water use in ophthalmic preparations.[26]

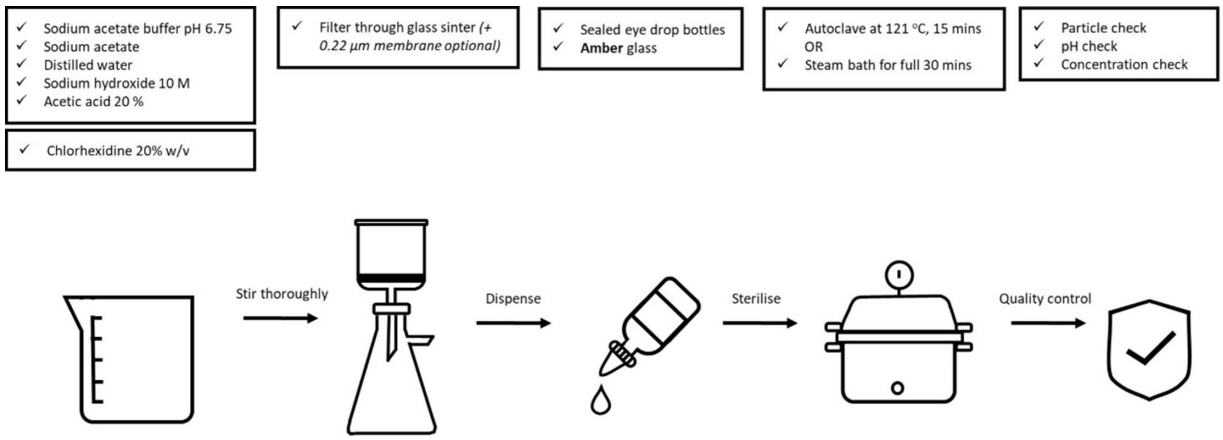

Figure 5. Flow chart of the simplified production of 0.2% CHX eye drops using the Ugandan protocol.

At the Ugandan facility, three batches of CHX 0.2% eye drops (100 mL) were produced using sodium acetate buffer with pH adjusted to 6.7 in triplicate (Fig. 5). Filtration was conducted before bottling through a grade-3 glass sinter filter. Terminal sterilization was achieved by a water steam bath filled with distilled water and boiled for 30 minutes at atmospheric pressure.

Sterility testing of the final samples was conducted using techniques similar to those used in the United Kingdom and following WHO procedures currently used in Uganda.[25] Our results provided evidence that the production of buffered CHX eye drops (0.2%) in a simple clean room followed by steam bath sterilization results in eye drops with sterility standards similar to those for eye drops that were prepared in a type 2 safety cabinet and sterilized by autoclave; no growth was observed. Not only does steam bath sterilization reduce the costs associated with production but the protocol employed in Uganda is also easier for production staff to implement. The use of standard operating procedures (SOPs) has been shown to significantly reduce levels of contamination in rural production sites.[25,27] With this is mind, SOPs were developed and disseminated for every step of production, including pre-production cleaning, production, all equipment used, analysis, sterilization, and line clearance procedures.

The concentrations of the three batches of buffered CHX eye drops prepared in Uganda were determined at the time of production using an ultraviolet–visible light spectrophotometer. The CHX concentration in these eye drops was $0.22\% \pm 0.02\%$. These batches were stored at ambient room temperature (18°C–30°C) for 30 months in Uganda before being shipped to London for analysis by HPLC. These CHX eye drops remained stable (drug strength of $0.19\% \pm 0.01\%$ and pH of 6.98 $\pm$ 0.02; $n = 3$) after storage at ambient room temperature (18°C–30°C) for 30 months in Uganda. There was no presence of degradation products in the CHX eye drops after storage for 30 months at ambient temperature (see Supplementary Materials).

Discussion

Microbial keratitis is a challenging infection to treat and can rapidly result in blindness from destruction and scarring of the cornea or perforation or loss of the eye. Microbial keratitis is caused by various pathogens (bacteria, fungi, viruses, and protozoa) and is a major ophthalmic public health problem in LMICs. In tropical regions, filamentous fungi cause about half of the microbial keratitis that is known to occur.[1] Natamycin eye drops (5%) are the first-line therapy for fungal keratitis; however, they either are scarcely available or are expensive in developing countries. Furthermore, even with intensive topical natamycin treatment, infection might progress to perforation and eye loss. Second-line drugs are often required in severe and non-responding cases. Therefore, there is a need for affordable and effective antifungal eye drops.

CHX is an antiseptic agent with both antibacterial and antifungal properties. It is a widely used broad-spectrum biocide that kills microorganisms through cell membrane disruption.[28] CHX has been used in ophthalmology for more than 30 years as an eye-drop preservative, to sterilize contact lenses, and to treat *Acanthamoeba* and fungal keratitis. CHX is safe and well tolerated at a 0.2% concentration, and CHX is used for treating fungal keratitis in the United Kingdom, Europe, and Sub-Saharan Africa.

Clinically used CHX eye drops (0.2%) are prepared by aqueous dilution from commercially available 20% (w/v) aqueous CHX stock solution. The CHX eye drops are sterilized by filtration through a 0.22-μm membrane filter, essentially in aseptic conditions. There is no need to add a preservative because CHX acts as a preservative. The pH plays an important role in the tolerance of CHX eye drops and should be adjusted to the tear film (pH 6–7). The antimicrobial activity is optimal at slightly acidic pH, and the maximum stability of CHX in aqueous solution is at pH 5.6.

Buffered solutions are commonly used to formulate eye-drop solutions to enhance the stability of the drug and to maintain ocular tolerability. CHX is not compatible with many buffers such as phosphate, citrate, bicarbonate, and borate. Acetate buffer is known to be compatible with CHX[23]; therefore, it was selected to formulate the buffered CHX eye drops for transfer to the facility in Uganda. Sodium acetate buffer is a U.S. Food and Drug Administration (FDA)-approved excipient for use in ophthalmic formulations. Studies by Lin et al.[23] showed that sodium acetate buffer can be used to give the desired tolerable osmolality at a concentration within the concentration limits for non-active ingredients in eye-drop preparations described by the FDA.[29]

The pH of eye drops affects their ocular tolerability. Neutral pH or slightly alkali solutions are better tolerated than acidic solutions. CHX is unstable in basic solutions[21]; therefore, a pH below but close to pH 7 was sought. Acetate-buffered CHX eye drops were prepared at pH 5.98 to 6.82, with an optimum pH of 6.5. The acetate-buffered CHX eye drops displayed stable pH compared to clinically used commercial aqueous CHX eye drops. Furthermore, the osmolality of the acetate-buffered CHX eye drops was within the tolerable range, unlike the clinically used CHX eye drops. Patients tolerate a solution osmolality range of 200 to 600 mOsm/kg.[30]

CHX eye drops prepared locally in hospitals in resource-limited regions in tropical climates are stored at room temperature and not refrigerated. There is a need to ensure the stability of eye-drop formulations in tropical conditions to maintain dose reproducibility and safety. The acetate-buffered CHX eye drops were stable over 21 months at 40°C. The use of sodium acetate buffer in formulating CHX eye drops offers several advantages: (1) ability to maintain a stable CHX solution for at least 21 months, and (2) constant tolerable pH and osmolality upon storage in tropical conditions that mimic clinical settings in developing countries.

A non-inferiority clinical trial of CHX eye drops was conducted in Nepal. CHX eye drops were found to be effective but inferior to natamycin eye drops in treating fungal keratitis.[18] In our recent pilot study in Uganda, patients with recalcitrant fungal keratitis that were not responding to natamycin 5% were treated with CHX eye drops (0.2%, in acetate buffer pH 6.7) with good outcomes.[20] It was possible to formulate the CHX eye drops in the local eye-drop pharmacy production unit following the protocol described in this paper and make it available to these patients. In these patients, CHX eye drops were well tolerated, and most of the patients healed with clear peripheral cornea; no patients needed to discontinue CHX eye drops because of stinging, allergy, or toxicity.[20] In countries where natamycin eye drops have only limited availability or fungal keratitis patients do not respond to natamycin eye drops, CHX eye drops can be used as second-line therapy for the treatment of fungal keratitis.

Local production allows local healthcare providers to be able to provide more affordable and easily accessible fungal keratitis medications for their patients. There is a need to optimize and validate the local Ugandan preparation of CHX eye drops to ensure that the preparation process is reproducible, robust, scalable, and traceable within Uganda. The course of treatment is 6 to 8 weeks and requires three bottles (10 mL each) of the CHX eye drops. The local preparation of the CHX eye drops must be in a form that is ready to use by the health practitioner and patient. Ready-to-use eye drops will reduce dilution errors and contamination. The use of undiluted CHX can result in severe burns and result in impaired vision.[31,32] Several reports have shown that CHX aqueous solution or gel (7.1%) intended for use as an antiseptic for umbilical cord care was wrongly used in the eyes of babies, resulting in corneal chemical injury.[33] The protocol for the preparation of buffered CHX eye drops was successfully validated and transferred to the pharmacy manufacturing site in Uganda (Eye Drop Production Department at Ruharo Mission Hospital) to facilitate local access to the CHX eye drops and to ensure sustainability.

Our recent work showed that the cost of the buffered CHX eye drops prepared in the Ugandan pharmacy setting is affordable to patients.[20] Currently, the starting materials such as CHX and buffer components can be purchased at a reasonably affordable price. However, due to worldwide inflation, the prices might increase, which would affect the sustainability of CHX eye-drop production. For sustainable production of CHX eye drops in the pharmacy setting there is a need to ensure the existence of affordable suppliers for raw materials, amber

glass eye-drop containers, and personal protective equipment.

In countries where fungal keratitis is prevalent and treatment costs are a significant limiting factor contributing to medical interventions not being sought, CHX eye drops is an inexpensive and easily prepared option. Here, we have demonstrated a facile method to produce buffered CHX eye drops that are within the isotonic range of lacrimal fluid, in a pH range compatible with CHX stability and with optimal pH to facilitate tolerability for patients. Furthermore, the stability testing indicates that the eye drops can be stored in tropical regions without refrigeration (at ambient room temperature) for up to 30 months. This work is being published with the aim toward dissemination and implementation of local production of CHX eye drops in other similar facilities worldwide.

Conclusions

Four different CHX (0.2% w/v) eye drops were successfully prepared using acetate buffer at a pH range of 5.9 to 6.75. Higher pH values closer to lacrimal fluid pH are known to exhibit better ocular tolerability; thus, the preparations of CHX 0.2% eye drops buffered at pH 6.75 were considered the most suitable. The buffered CHX eye drops were stable under tropical conditions and did not require a cold chain; it displayed consistent pH values and concentrations. The preparation and sterility of the CHX eye drops were reproducible in both the United Kingdom and Uganda. These encouraging results indicate that sterile buffered CHX eye drops can be prepared in a hospital or pharmacy setting with limited resources. As a result, cost-effective and locally produced treatments for fungal keratitis can be prepared. The main challenge that might affect the local production of CHX eye drops is the possible future rise in prices of raw materials (CHX and buffer compositions), amber glass eye-drop containers, and personal protective equipment which can be overcame by finding affordable suppliers.

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