

Stability of Fortified Cefazolin Ophthalmic Solutions Prepared in Artificial Tears Containing Surfactant-Based Versus Oxidant-Based Preservatives

Theerasak Rojanarata,¹ Junlathip Tankul,¹ Chayanee Woranaipinich,¹ Paweena Potawanich,¹ Samarwadee Plianwong,¹ Sirinart Sakulma,² and Choedchai Saehuan³

Abstract

Objective: The aim of this study was to investigate the stability of fortified cefazolin sodium ophthalmic solutions (50 mg mL⁻¹) extemporaneously prepared in commercial artificial tears containing 2 different types of preservatives, namely the surfactants and oxidants.

Methods: Fortified cefazolin sodium solutions were prepared by reconstituting cefazolin for injection with sterile water and further diluted with Tears Naturale[®] II or Natear[®], 2 commercial artificial tears containing poly-quaternium-1 and sodium perborate, respectively, as preservatives. The solutions were then kept at room temperature (28°C) or in the refrigerator (4°C). During the 28-day period, the formulations were periodically examined for the physical appearance, pH, and the remaining drug concentrations. The antibacterial potency was evaluated as the minimal inhibitory concentration against *Staphylococcus aureus* strain ATCC 29923 by broth dilution technique. The activity of the preservatives was demonstrated by antimicrobial effectiveness tests. On day 28, the microbial contamination in the preparations was tested.

Results: The stability profiles of cefazolin solutions prepared in Tears Naturale[®] II, Natear[®], and water were not different, but they were significantly influenced by the storage temperature. The refrigerated formulations showed no loss of drug and antibacterial potency as well as alteration of physical appearance and pH throughout the 28 days. In contrast, those kept at room temperature showed gradual change in color and odor. The degradation of drug exceeded 10% from day 3 and the decrease of antibacterial potency could be observed at week 3. All cefazolin solutions prepared in artificial tears retained the antimicrobial activity of preservatives and were free from bacterial and fungal contamination throughout the 28-day period of study.

Conclusions: Cefazolin sodium ophthalmic solutions can be extemporaneously prepared in Tears Naturale[®] II or Natear[®] without the influence from different types of preservatives used in these vehicles. The formulation is physically and chemically stable and retains the antibacterial potency for at least 28 days in the refrigerator.

Introduction

CEFAZOLIN AS SODIUM SALT is a broad spectrum cephalosporin antibiotic that is effective against *Staphylococcus aureus*, a common ocular pathogen causing a variety of infections, such as conjunctivitis, blepharitis, and keratitis.¹ The drug is often prescribed for topical application when *S. aureus* is identified as an etiologic agent.^{2,3} However, in many countries, commercial cefazolin ophthalmic solutions are unavailable or have inadequate drug concentration for the treatment. Thus, hospital pharmacists are typically

asked to extemporaneously prepare fortified cefazolin formulations (30–100 mg mL⁻¹), usually by mixing parenteral or lyophilized cefazolin sodium with compatible vehicles such as sterile water or commercially available artificial tears. In comparison with sterile water, the use of artificial tears as vehicles offers several advantages. It is capable of prolonging the contact time between antibiotics and the corneal tissues because of viscosity. In addition, it extends the shelf life of the multidose formulations by preventing microbial contamination and biodegradation by the action of preservatives.

¹Faculty of Pharmacy, Silpakorn University, Nakhon Pathom, Thailand.

²Pharmacy Department, Banpong Hospital, Ratchaburi, Thailand.

³Faculty of Allied Health Sciences, Naresuan University, Phitsanulok, Thailand.

In pharmaceutical compounding, the compatibility of drugs with other excipients and the stability of the formulation must be considered. In artificial tears, commonly used preservatives such as surfactants and oxidants exert their antimicrobial activity by 2 main mechanisms.⁴ Benzalkonium chloride and polyquaternium (Polyquad[®]) are examples of cationic surfactants that disrupt bacterial cell membrane, resulting in the leakage of cytoplasmic contents.⁵ In another way, oxidative preservatives penetrate cell membrane and oxidize cellular components such as membrane, lipids, proteins, and DNA, thus interfering with cell functions.⁶ Sodium perborate is an example of oxidant used in some brands of eye drops as a disappearing preservative. It is converted to hydrogen peroxide, an oxidative antimicrobial agent, when combined with water. From literature search, there have been numerous reports regarding preservative-induced ocular damage or irritation side effects. However, the information about the effect of these preservatives on the stability of the drug, especially in the cases of extemporaneous preparation, is limited. Whether cationic surfactants would be reactive against anionic drugs such as cefazolin or whether the drugs would undergo oxidative degradation when they coexist with oxidant preservatives leading to shortened shelf life of the prepared formulations is still the question. Therefore, the purpose of this investigation was to determine whether the preparation and prolonged storage of cefazolin sodium in artificial tears containing surfactant-based or oxidant-based preservatives was feasible. In this study, physical and chemical stability as well as antibacterial potency of extemporaneous formulations of cefazolin sodium (50 mg mL⁻¹) prepared in Tears Naturale[®] II and Natear[®], which contained polyquaternium-1 and sodium perborate, respectively, as preservatives, were evaluated for 28 days at room temperature (28°C) and in the refrigerator (4°C).

Methods

Preparation of fortified cefazolin sodium ophthalmic solutions

Fifty milligrams per milliliter of cefazolin ophthalmic solutions were prepared using aseptic techniques by reconstituting 1 g cefazolin for injection (as sodium salt) with 4.5 mL sterile water. Then 1 mL of the solution was taken and further diluted with 3 mL artificial tear vehicles in light-resistant bottles. The two types of artificial tears used in this study were Tears Naturale[®] II (Alcon) and Natear[®] (Silom Medical). The active ingredient in both products was 0.3% hydroxypropyl methylcellulose, but Tears Naturale[®] II used 0.001% Polyquad[®] II (polyquaternium-1), whereas Natear[®] used 0.028% sodium perborate as preservatives. The formulations were clear and colorless after preparation. As stated in the product information from the manufacturers, both artificial tears had an iso-osmolarity. Thus, 50 mg mL⁻¹ cefazolin solutions prepared in these vehicles had a tonicity of about 420 mOsm L⁻¹, which did not exceed the level that can be tolerated by human eyes (600 mOsm L⁻¹).⁷

Chemical stability analysis

The concentration of cefazolin in the formulations was chemically analyzed by high-performance liquid chromatographic (HPLC) system equipped with an Agilent 1100 series and diode array detector (Agilent Technology). The column

was an Agilent Eclipse XCB-C₁₈ (5 μm; 4.6×250 mm). The mobile phase consisted of 10 volumes of acetonitrile and 90 volumes of a solution containing 2.77 g L⁻¹ of disodium hydrogen phosphate and 1.86 g L⁻¹ of citric acid. The flow rate was 1.0 mL min⁻¹ and the injection volume was 20 μL. Detection was performed at 270 nm. Standard solutions were freshly prepared from reference standard cefazolin sodium (purity of 98.4%; Sigma) at 0, 0.05, 0.10, 0.15, 0.20, and 0.25 mg mL⁻¹. Calibration curve of concentrations versus peak area showed a good linear relationship with a correlation coefficient (*r*²) of >0.999. Samples taken were diluted (1:5) with sterile water prior to HPLC injection.

To rule out the possible interference from degradation products of cefazolin, the HPLC method was validated using forced degradation experiments by treating cefazolin sodium solutions under conditions of acid (0.5 M HCl, heated at 45°C), alkaline (0.1 M NaOH, at 25°C), and neutral (water, heated at 80°C) hydrolysis and oxidation (3% H₂O₂). The method proved to be stability indicating, as no degradation peaks coeluted at the same retention time as the intact drug, even in the chromatograms of the degraded samples. Analyte peak purity was also verified by using diode-array detector and ChemStations software.

Determination of antibacterial potency

A methicillin-sensitive, β-lactamase-negative *S. aureus* strain ATCC 29923 was chosen for this study. The microorganism was susceptible to cefazolin sodium at a minimal inhibitory concentration (MIC) of 0.25–1 μg mL⁻¹, as tested by broth dilution, which was described in the National Committee for Clinical Laboratory Standards (2003).⁸ Prior to the experiments, cultures were incubated in trypticase soy broth (TSB) at 37°C until the growth was equivalent to a 0.5 McFarland turbidity standard. The MIC assay was conducted in sterile 24-well microplates using a final volume of 1 mL. Cefazolin sodium solutions were diluted 3,125-fold to a concentration of about 16 μg mL⁻¹ by sterile water before serial 2-fold dilutions were made with TSB down to the desired minimum concentration. For each well, 0.5 mL of bacterial suspension and 0.5 mL of antibacterial formulation were incubated together at 37°C in an aerobic environment for 16 h. The MIC was determined as the lowest antibiotic concentration that inhibited the growth of the organism as measured by a lack of gross turbidity. The antibacterial potency was expressed as titer in "dilution fold." This unit is the degree of dilution for the 3,125-fold prediluted formulation to reach the MIC value. It is proportional to the concentration of the active drug.

Studies of microbial contamination

The test for microbial contamination was done after the formulations were kept until day 28. One milliliter of each formulation was aseptically taken and added into 10 mL of TSB for microbial enrichment. After an overnight incubation, 0.5 mL of inoculated TSB was subcultured onto blood agar and anaerobic blood agar and then incubated at 37°C to detect aerobic and anaerobic bacterial contamination, respectively. Another 0.5 mL aliquot was inoculated onto Sabouraud dextrose agar and then kept at 30°C to check fungal contamination. Anaerobic bacterial contamination was also tested by inoculating 10 mL of thioglycolate broth

with 1 mL of formulation and observing the turbidity. All broths and plates were carefully examined for microbial growth for a period of 7 days.

Stability study

The fortified cefazolin sodium ophthalmic solutions prepared in each vehicle were divided into 2 groups. Five bottles were stored in the dark at 28°C of room condition, whereas the other 5 bottles were kept at 4°C in the refrigerator. During the storage period of 28 days, the physical appearance of solutions, for example, the clarity, color, and odor as well as the pH, was periodically examined. The remaining concentrations of cefazolin sodium were assayed by performing a chemical analysis using HPLC as described and calculated as the percentage remaining drug content based on the following equation:

$$\text{Remaining concentrations} = \frac{\text{Drug concentration at the day of investigation}}{\text{Drug concentration at day 0}} \times 100$$

To investigate the influence of artificial tear vehicles on the stability of cefazolin, the drug solutions were also prepared using sterile water as a vehicle and subjected to the studies in the same way.

Antimicrobial effectiveness test

To demonstrate that the extemporaneously prepared solutions retained the efficacy of antimicrobial preservation during storage and potential multiuse, an antimicrobial effectiveness test was performed following The United States Pharmacopeia 30.⁹ Briefly, the formulations were challenged with the following microorganisms: *Candida albicans* ATCC 10231, *Aspergillus niger* ATCC 16404, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Staphylococcus aureus* ATCC 6538, and stored at 4°C, which was a practical storage temperature for typical extemporaneous ophthalmic preparations. At the specified intervals, the samples were withdrawn from the containers and appropriately diluted to eliminate the residual antimicrobial activity of the products, and the microorganisms were counted. The antimicrobial effectiveness were acceptable if the following criteria were met: for bacteria, not less than 1.0 log reduction from the initial calculated count at 7 days, not less than 3.0 log reduction from the initial count at 14 days, and no increase from the 14 days' count at 28 days; for yeast and molds, no increase from the initial calculated count at 7, 14, and 28 days.

Result

Chemical stability, appearance, and pH

Cefazolin was considered chemically stable if its concentration exceeded 90% of the initial concentration. On analysis using the stability-indicating HPLC, it was found that cefazolin sodium was stable at 4°C in both Tears Naturale® II and Natear® during the 28-day period (Fig. 1). In addition, there were no changes in clarity, color, and pH (Fig. 2) compared with those on the day of preparation. However, under the 28°C storage condition, there was a significant decrease in the drug content with time and the formulations showed losses of drug at more than 10% from day 3 onward

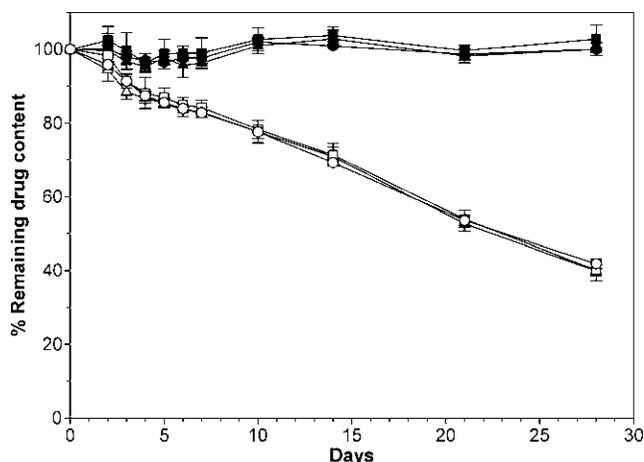


FIG. 1. The percentage of remaining drug content (mean ± standard deviation, n = 5) during 28 days in the formulations prepared in Natear® (▲,△), Tears Naturale® II (■,□), and water (●,○). The formulations stored at 4°C are represented by closed symbols and those kept at 28°C are represented by open symbols.

in all vehicles. The degradation of drug was proven by the decrease of peak area of cefazolin and the occurrence of other peaks belonging to degradation products on the high-performance liquid chromatograms (Fig. 3). The deterioration of drug led to more yellowish color and unpleasant odor albeit the pH of the solutions did not significantly change (Fig. 2). In all solutions that were stored at the same temperature, there was no significant difference in the stability profiles when whatever vehicle (Tears Naturale® II, Natear®, or sterile water) was used.

Antibacterial potency and efficacy of antimicrobial preservation and microbial contamination

Results were found to be similar to those obtained for the chemical stability. As shown in Fig. 4, all formulations on

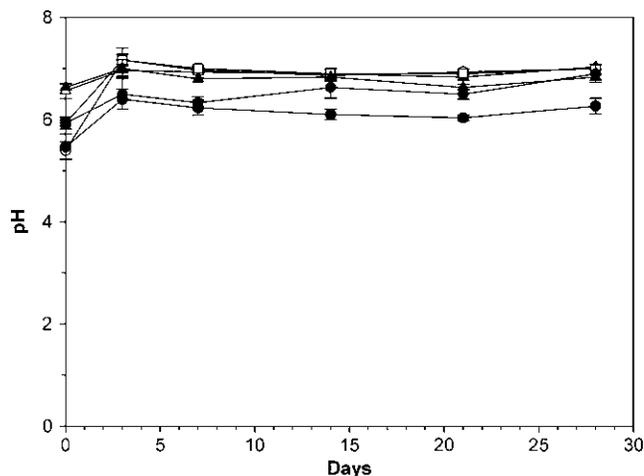


FIG. 2. The pH (mean ± standard deviation, n = 5) of the formulations prepared in Natear® (▲,△), Tears Naturale® II (■, □), and water (●, ○). The formulations stored at 4°C are represented by closed symbols and those kept at 28°C are represented by open symbols.

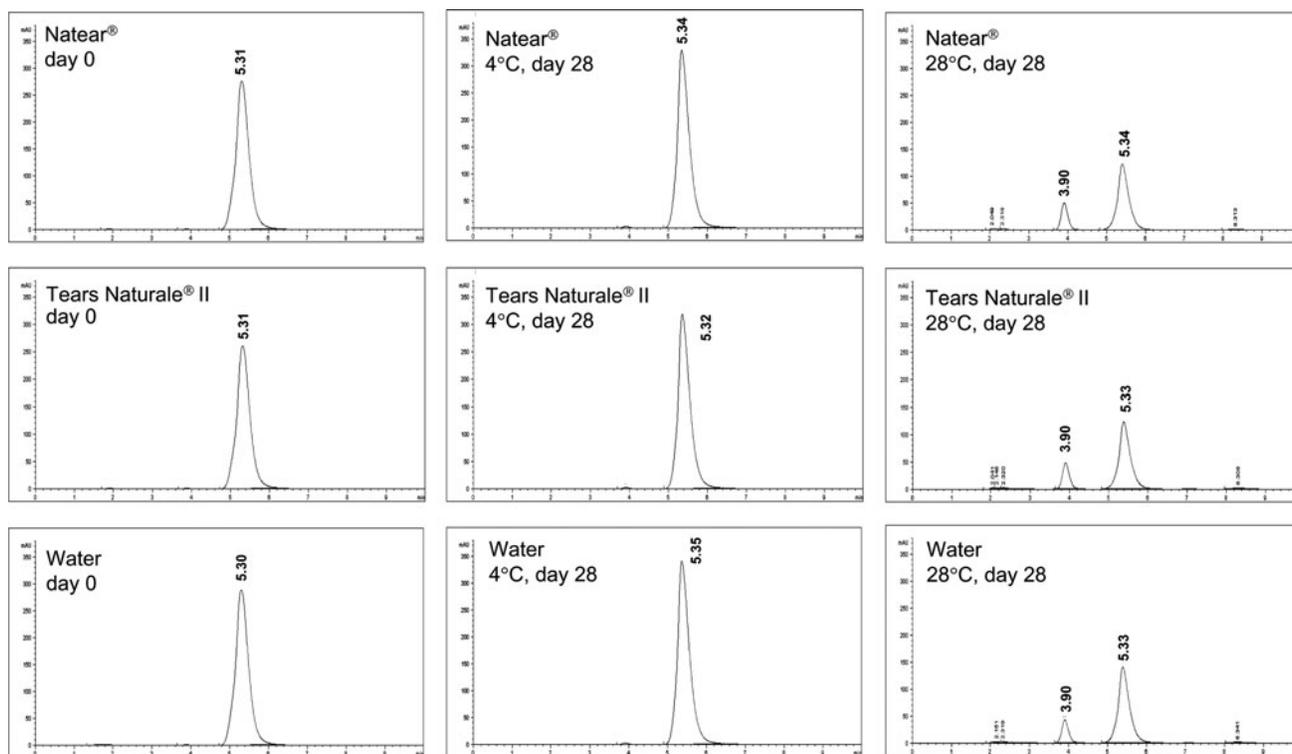


FIG. 3. High-performance liquid chromatograms of cefazolin sodium solution prepared in Natear[®], Tears Naturale[®] II, and water on day 0 and after storage at 4°C and 28°C for 28 days.

day 0 showed the MIC against *S. aureus* ATCC 29923 at the concentration obtained after 16-fold dilutions were made from 3,125-fold prediluted formulations. Throughout the study period, all solutions kept at 4°C retained the antibacterial activity as shown by the same dilution fold to reach the MIC values. In contrast, all samples that were stored at 28°C showed noticeable losses of antibacterial potency after 21 days. These formulations reached the MIC values after they were 8-fold diluted.

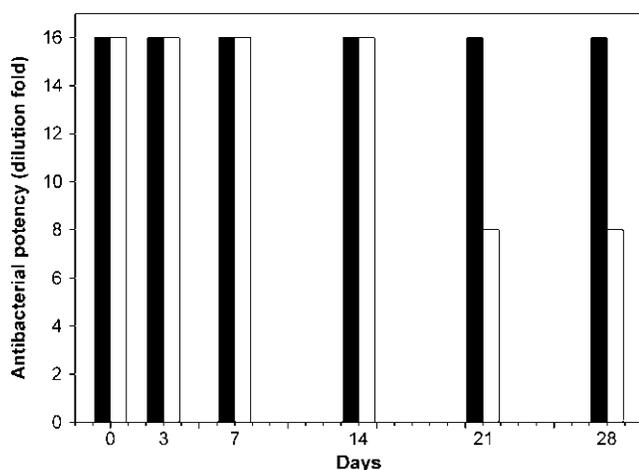


FIG. 4. The antibacterial potency of the formulations stored at 4°C (■) and 28°C (□), expressed in dilution fold for the 3,125-fold prediluted formulation to reach the minimal inhibitory concentration (MIC) value against *Staphylococcus aureus* ATCC 29923.

The antimicrobial effectiveness test demonstrated that the cefazolin solutions prepared in both commercial artificial tears passed the antimicrobial effectiveness test. In addition, no bacterial and fungal contamination was found in any solutions during the study period.

Discussion

Although preservatives prolong the shelf life of the multidose ophthalmic formulations by preventing microbial contamination and biodegradation and by maintaining drug potency, they may adversely react with the drug and alter the potency or accelerate the degradation. Particularly when the medications are to be prepared extemporaneously, this information is necessary for the selection of compatible vehicles. In the case of cefazolin, degradation is primarily caused by the hydrolysis of the β -lactam ring and the substitution groups; nevertheless, the oxidation of thioether moiety has been reported as a possible way of drug decomposition.¹⁰ This circumstance was observed in our forced degradation experiments where cefazolin solution was treated with 3% hydrogen peroxide. Within 1.5 h, it was found that 50% of the initial drug was rapidly degraded and produced breakdown products that were different from those obtained from the hydrolytic degradation, as evidenced by different peaks on the chromatograms (data not shown).

As sodium perborate exerts its preservative action by releasing hydrogen peroxide to oxidize the contaminated microbes, it is a question whether this reactive oxygen species can also oxidize and degrade cefazolin as it acts in the forced

degradation experiments or not. In addition, the incompatibility between drug and other excipients possessing opposite charges has been known to happen in many cases, leading to reduced drug potency and stability. However, the proof whether the cationic preservative polyquaternium-1 do not react with anionic cefazolin is lacking. Although there are some previous publications informing about the cefazolin stability in several vehicles containing various types of preservatives, for example, benzalkonium chloride, thiomersal, phenylmercuric borate, or chlorhexidine,¹¹⁻¹⁵ no stability data of the cefazolin prepared in the vehicles containing the newer types of preservatives (sodium perborate and polyquaternium-1) have been found. Because of the increasing use of the artificial tear products containing these 2 preservatives, as they cause lower irritation effect on the eye surface, many hospitals have extemporaneously prepared this formulation in these vehicles. Thus, the study of product stability needs to be conducted to provide essential information for the pharmacists.

In the stability assessment of antimicrobial formulations, not only chemical stability as represented by the remaining drug amount quantified by valid stability-indicating assays, but also the actual antimicrobial activity determined by microbiological methods should be evaluated. From the literature review, some works studied the cefazolin stability by assaying the antimicrobial activity, but they did not follow the chemical degradation or evaluate the remaining drug content by the chemical analysis.^{11,12} In these cases, it could not be ensured that the antimicrobial activity found in the assay was from the parent drug or the drug together with its degraded products because degraded products may retain the antimicrobial activity. Although having the activity, those breakdown products may cause toxicity problems when the formulations are used by patients. Vice versa, some works examined the drug stability by performing only chemical analysis and ignoring the antimicrobial activity tests.^{13,14} Further, the study of cefazolin stability conducted by Arici et al. used the measurement of absorbance spectra to quantify the drug concentration.¹⁵ Our preliminary experiment revealed that such spectrophotometric method did not sufficiently indicate the stability, because it could not differentiate the intact cefazolin from its coexisting degraded products. The cefazolin concentrations determined by absorbance measurement were inaccurately higher than the real amount, compared with those obtained from the stability-indicating HPLC method. Consequently, the stability data reported in this work might not be quite accurate. Therefore, to obtain comprehensive and reliable information about drug potency, efficiency, and safety, we conducted a chemical analysis by using a validated stability-indicating HPLC assay in parallel with microbiological studies. In addition, the physical characteristics, the change of pH, the microbial contamination, and the effectiveness of preservatives were monitored throughout the study.

It was found that the cefazolin solutions prepared in this study degraded via hydrolytic reaction under the neutral condition at 28°C, as their chromatograms (Fig. 3) were similar to that obtained from the aqueous solution of cefazolin sodium forced to degrade at 80°C (data not shown). Interestingly, although the preservative sodium perborate in Natear[®] could release the active oxidizing agent hydrogen peroxide, it did not affect the drug stability. The drug prepared in Natear[®] and stored at 28°C was degraded via

hydrolysis, but not oxidation, as the high-performance liquid chromatograms of these formulations were distinct from that of the cefazolin solution treated with 3% hydrogen peroxide (data not shown). The comparison of stability profiles of the drug solutions prepared in 2 types of artificial tear versus in water revealed no differences, thus indicating that the ingredients such as hydroxypropyl methylcellulose as well as surfactant or oxidant preservatives at the concentration present in these commercial products did not affect the stability of cefazolin. Therefore, it is feasible for the pharmacists to prepare cefazolin ophthalmic solutions by choosing commercially available artificial tears containing either surfactant-based or oxidant-based preservatives as a vehicle.

The more crucial concern is the storage temperature because it significantly affects the stability. The refrigerated formulations were stable for at least 28 days, whereas those kept at room temperature changed physically and the drug content loss was more than 90% from day 3. Although the antibacterial potency began to decrease afterward at week 3 probably because of the antimicrobial activity of some degraded products, it is not advisable to use these preparations after day 3 unless kept under refrigeration because they may lead to toxicity problems associated with the breakdown products. As shown in the antimicrobial effectiveness test, the required preservative activity in the commercial artificial tear was not diminished by the incorporation of cefazolin. Moreover, the ability to control the growth of *E. coli* ATCC 8739 and *S. aureus* ATCC 6538 was augmented by an intrinsic antibacterial activity of the drug. Throughout the study period, no microbial contamination was detected, ensuring the potential for multiuse. During the investigation, the pH of all formulations was in the range of 5–8, which is usually tolerable by the eyes.

Author Disclosure Statement

The authors have no proprietary interest in the products and no competing financial interests.

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Address correspondence to:

Dr. Theerasak Rojanarata
Department of Pharmaceutical Chemistry
Faculty of Pharmacy
Silpakorn University
Nakhon Pathom 73000
Thailand

E-mail: teerasak@su.ac.th; rtheerasak@yahoo.com