Effect of Temperature and Length of Storage to Chloramphenicol Eye Drop’s Concentration

A Yugatama¹, R Nurmalinda¹, S Rohmani¹, D E Ermawati¹, and F Prihapsara¹
¹Pharmacy Department, Faculty of Mathematics and Natural Sciences, Sebelas Maret University, Indonesia

Email: adiyugatama.apt@gmail.com; adi.yugatama@staff.uns.ac.id

Abstract. Chloramphenicol is a broad spectrum antibiotic that acts by inhibiting protein synthesis. One of chloramphenicol dosage form which widely found in the marketplace is eyedrops. Drug storage will affect drug stability. This research aimed to know the effect of temperature and length of storage to chloramphenicol eye drop’s concentration. Analysis of chloramphenicol concentration in eyedrops was done by UV spectrophotometry. This analysis was done to eyedrops after stored 0; 7; 21; and 30 days unsealed with various storage condition: under the sunlight, room temperature, and refrigerated. Data obtained was analyzed statistically using one-way ANOVA with 95% CI by SPSS for windows software. The result showed that the concentration of chloramphenicol eyedrop before stored was to meet the requirements which were 95.96%. The result of chloramphenicol concentration in eyedrop after stored 30 days in 3 storage condition was reduced significantly. While sunlight and storage temperature did not affect the reduction of chloramphenicol concentration significantly.

1. Introduction
Chloramphenicol is a broad spectrum antibiotic that acts by inhibiting protein synthesis. Chloramphenicol having a bacterostatic activity against almost all gram positive and negative bacteria, and against Spirochaeta, Chlamydia trachomatis and Mycoplasma. Chloramphenicol having a good bactericidal activity against Str. pneumonia, Neiss. meningitides, and H. Influenzae [1].

One of chloramphenicol dosage form available in market is eyedrops, in 0.25–1% concentration. Eyedrops is a sterile solution which combined and packaged for eyes purposes. Eyedrops have to be sterile, isohydric, and isotonic. This dosage form could not be used six weeks after being unsealed [2].

Drug storage affect drug stability utmost. Drug stability could be seen by concentration reduction during storage [3]. Drugs have to be stored in room temperature and avoid direct sunlight to ensure drug stability [4]. Temperature, light, moist and air condition are factors that induced drug degradation. Although reduction 10% of prior concentration of active ingredients is acceptable [5].

There are some theories stated about relation of drug stability to temperature, light and length of storage. Thus, it need to be studied about the effect of temperature and length of storage to chloramphenicol concentration in eyedrops.

2. Experimental
2.1. Materials
Equipments used were UV-Vis Spectrophotometer (Genesys 10S UV-Vis), analytical balance (Ohaus), micropipet (Joanlab), and glassware. Materials used were chloramphenicol standard, chloramphenicol eyedrops brand X, aquadest and ethanol.
2.2. Methods

2.2.1. Standard Solution. Standard solution was made by reconciling 100 mg chloramphenicol in 10 mL ethanol then diluted in aquadest until reached the concentration of 1000 ppm. From chloramphenicol 1000 ppm solution, was taken 0.1; 0.5; 1; 1.5; 2; 2.5; 3 mL and added to aquadest until 100 mL to generate standard solution of 1, 5, 10, 15, 20, 25, and 30 ppm chloramphenicol.

2.2.2. Sample Treatment. Samples were tested by stored in refrigerator temperature (3-7°C), room temperature (27-30°C), and exposed to direct sunlight. The drugs were unsealed before stored. Concentration of chloramphenicol in eyedrops then analyzed at day 0; 7; 21; and 30 after storage.

2.2.3. Determination of Chloramphenicol Concentration in Sample. As much as 5 mL samples were added by 25 mL aquadest. The mixture was taken 1 mL and added by aquadest until 100 mL. The solutions were measured its absorbance using spectrophotometer at 278 nm wavelength.

2.3. Data Analysis
Absorbance of samples were analyzed by linear regression until obtained chloramphenicol concentration in eyedrops in various storage condition. The data then analyzed by one way Anova to know the effect of temperature and length of storage to chloramphenicol concentration in eyedrops.

3. Results and Discussion

3.1. Determination of Maximum Wavelength
The result of 10 ppm chloramphenicol standard solution scanning in figure 1 showed that maximum wavelength is 278 nm. Based on other research, maximum wavelength of chloramphenicol is 278 nm [6].

![Figure 1. Maximum wavelength of chloramphenicol](image)

3.2. Determination of Chloramphenicol Standard Curve
Standard curve equation obtained was $y = 0.0441x + 0.0115$ and the correlation coefficient ($r$) was 0.9999. Based on requirement, $r > 0.997$ showed that the curve is having a good linearity [7]. This can be concluded that standard curve equation obtained was having a good linearity. The chloramphenicol standard curve could be seen in figure 2.
3.3. Determination of Chloramphenicol Concentration in Sample

The result of chloramphenicol concentration in eyedrops determination after storage showed that there were reductions of chloramphenicol concentration both in refrigerator temperature, in room temperature, and exposed to direct sunlight. The prior concentration of chloramphenicol in eyedrops was 95.96%. This is in accordance with the requirements stated in USP standard that the concentration of chloramphenicol in eyedrops is not less than 90% and not over 130%. In addition, the deadline for using sterile preparation after unsealed is 28 days [8]. At 30 days length of storage after unsealed in refrigerator temperature, room temperature, and direct sunlight exposure, the chloramphenicol concentration in eyedrops were above 90%. This showed that chloramphenicol eyedrops brand X is fulfill the standard and still be able to use until 30 days after unsealing. Chloramphenicol concentration in various storage condition could be seen in figure 3.

During storage, concentration of chloramphenicol in brand X eyedrops were reduced. The longer the storage period, the lesser concentration of chloramphenicol found in eyedrops. This might be resulted from hydrolysis reaction. This reaction occurred because chloramphenicol in watery media will be degraded by hydrolysis breakdown in amides circle and forming appropriate amides and dichloracetic acid. Reaction rate is occurred under the first order and independent from ionic power of the media [9]. The hydrolysis reaction could be seen in figure 4.
Besides, chloramphenicol in solution also sensitive to photodegradation reaction. Chloramphenicol under direct sunlight slowly changed into yellow color and formed yellowish orange residu. In this photodegradation reaction, chloramphenicol will changed into 4-nitrobenzaldehyde, 4-nitrobenzoic acid, and 4,4'-azoxybenzoic acid through oxidation, reduction, and condensation reaction [9].

Storage in room temperature resulted in relatively lower concentration of chloramphenicol than in direct sunlight exposure and refrigerator temperature. This might be caused by moist which commonly found higher in room temperature. Factors inducing degradation reaction of a drug i.e. temperature, light, moist, and air condition. Esters and amides compounds like chloramphenicol is easily hydrolized in a presence of moisture [10].

Storage in refrigerator temperature resulting the least reduction of chloramphenicol concentration compared to direct sunlight exposure and in room temperature storage. This showed that chloramphenicol in eyedrops will be most stable if stored in refrigerator temperature. The temperature of refrigerator used in this study is 3–7 °C, and included into cold temperature. The lower the temperature, the slower the reaction rate will be. Reaction rate is linear to number of collision per unit of time.

Result of statistic analysis using SPSS showed that there was a correlation between length of storage, light, and temperature to chloramphenicol concentration. Based on normality test using Kolmogorov-smirnov, the data were distributed normally by sig. 2 tailed value was 0.482. The value of p > 0.05 showed that data were distributed normally.

Statistical analysis by one way anova resulted that significance value of length of storage, light, and temperature to chloramphenicol concentration were 0.000; 0.925; dan 0.140. Based on these result, significance value of length of storage was < 0.05. Thus, can be conclude that length of storage can affect the reduction of chloramphenicol concentration. While sunlight and temperature were not affecting chloramphenicol concentration significance.

Insignificance effect of light to chloramphenicol concentration might be caused by the package of eyedrops that are white colored and not translucent so the effect of sunlight will be smaller, and the incidence of photodegradation reaction also being lower.

4. Conclusion
Based on chloramphenicol concentration analysis in eyedrops for 30 days storage in 3 various condition which are exposed directly to sunlight, room temperature, and refrigerator temperature using UV Vis Spectrophotometer, could be concluded that length of storage having a significant effect to chloramphenicol concentration reduction, while lights and storage temperature were not having a significant effect to chloramphenicol concentration reduction.

Acknowledgement
The authors acknowledge to Sebelas Maret University which given the facilities for the research and publication.

References
[1] Rahal JJ 1979 Antimicrob Agents Chemother 16(1) 13.
[2] Virani S, Rewri P and Vyas A 2016 Del J Ophtalmol 26(3) 176.
[3] Bajaj S, Singla D and Sahuja N 2012 J. Appl. Pharm. Sci. 2(3) 129.
[4] Shafaat K, Hussain A, Kumar B, Hasan R, Prabhat P and Yadav VK 2013 World J. Pharm. Pharm. Sci. 2(5) 2499.
[5] Cantrell L, Suchard JR, Wu A and Gerona RR 2012 Arch Intern Med. 172(21) 1685.
[6] Karthikeyan S 2011 Arch. Phy. Res. 2(4) 72.
[7] Chan CC, Lam H, Lee YC and Zhang XM 2004 Analytical Method Validation and Instrument Performance Verification (New Jersey: John Wiley & Sons, Inc.)
[8] US Pharmacopeial Convention, Inc. 2007 United States Pharmacopeia 30 (Rockville, MD: US Pharmacopeial Convention, Inc.)
[9] Ali SL 1978 J. Chromatogr. A 154(1) 99.
[10] Honmane SM, Dange YD, Osmani RAM and Jadge DR 2017 Asian J. Pharm. 11(3) 479.