

Formulation and Stability Studies of Chloramphenicol as Ophthalmic Eye Drop

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Abstract

The chloramphenicol is D+threo (-) 2,2 dichloro-N- β -hydroxy - α (hydroxyl methyl)-p-nitrophenyl acetamide , a bacteriosttic and highly effective antibacterial against gr (+) and gr (-) ocular pathogenic bacteria causing conjunctivitis or corneal ulcers. The ophthalmic preparations of chloramphenicol are produced in different formulation due to its lower stability . Therefore , the present study introduce the know-how for the production of cholramphenicol sterile ophthalmic solution. The ophthalmic preparations of chloramphenicol 0.4% are not produced in Syria as well as the actice agent . Therefore , the prepared report produces the know – how for the production and study the stability for sterile solution. chloramphenicol for ophthalmic solution is a sterile , dry mixture with borax buffer .The active ingredient is chloramphenicol at concentration (40mg / ml) was prepared under aseptic conditions. The fait yellow color ophthalmic solution , clear , sterile , isotonic with 306 mOsm / Kg , the pH measured at 6.51. This solution did not cause any eye irritation in rabbits after daily application for four consecutive days . Finally , the stability of the prepared chloramphenicol 0.4 % eye drops was studied at 4 , 25 , 40 and 50C° for 90 days in glass amber containers and calculated the expiration date of the preparation on was approximately two years .

دراسة ثباتية تركيبة محلول كلوروفينكول العيني

فارس العباجي فاضل البندر نهى الديب زينة جعفر

المستخلص

الكلوروفينكول هو دى-(+) اريثرو (-) 2,2 ثنائي كلور(ن) بيتا هيدروكسي , الفا - (هيدروكسي مثيل) بارا نايتروفنيل استاميد , يعمل على تثبيط نمو البكتريا الموجبة (+) و السالبة (-) الكرام , والبكتريا المرضية التي تسبب تقرحات حرقية العين. توجد عدة انواع من كلوروفينكول كقطرات للعين , وتختلف من حيث الثباتية , وعلية تم تحضير ودراسة تركيبة محلول كلوروفينكول للعيون معقم , يكون تركيز المادة الفعالة (40ملغم / مل) في ظروف معقمه , ويكون المحلول رائق , معقم , ذات توتر ازموزي 306 ملي ازمول/كغم عند دالة حموضة 6,51 . لم يسبب المحلول المحضراي تخدش في عيون الارانب لدى استخدامه اربع مرات يوميا . واخيرا فان ثباتية مستحضر قطرات العيون كلوروفينكول 0.4% درست في درجات حرارية مختلفة 4 , 25 , 40 , 50 مئوية لمدة 90 يوم في قناتي زجاجية قاتمة اللون وتم احتساب تاريخ نافذية المحلول لمدة لا تتجاوز السنتين .

Introduction

Chloramphenicol is a potent inhibitor of microbial **protein** synthesis. It binds reversibly to the 50S subunit of the bacterial ribosome. It inhibits the peptidyl transferase step of protein synthesis. Chloramphenicol is a bacteriostatic broad spectrum antibiotic that is active against both aerobic and anaerobic gram positive and gram negative organisms. It is also active against rickettsiae but not chlamydiae [1]. Chloramphenicol is occasionally used topically in the treatment of eye infections because of its wide antibacterial spectrum and its penetration of ocular tissues and the aqueous humor [2]. The chemical stability of drug is of great importance since it becomes less effective as it undergoes degradation. Also drug decomposition may yield toxic by products that are harmful to the patient. Microbiological instability of a sterile drug product could also be hazardous [3]. The aim of paper is to study the stability of chloramphenicol 0.4% eye drop at different temperatures for 90 days in glass amber containers and calculate the expiration date of these eye drops.

Materials and Methods

Chloramphenicol powder form All ingredients are from /Merck/. Chloramphenicol from / Jiangxi Dongtai Science & Technology Co./ [China]. All solvents were of analar grade and used as received. The prepared chloramphenicol solution was examined for pH 6.51 (Philips, UK), osmolality using an automatic freezing point depression osmometer (Osmomat T 030, Germany), the concentration of the active ingredient chloramphenicol

using high performance liquid chromatography with uv detector HPLC (Hitachi). UV Detector: L-2455, pump: L-2130, and the wave length λ_{max} measurement using Cary UV-VIS, V-630. Chloramphenicol (0.4% as a base) ophthalmic solution was prepared under aseptic conditions as follows in table 1

Table (1): Amount of ingredients found in the prepared ophthalmic chloramphenicol solution

CHLORAMPHENICOL	0.04 g
PROPYLENE GLYCOL	1.0 g
POLYETHYLENE GLYCOL 1500	1.0 g
EDTA	0.01 g
CETRIMIDE	0.01 g
SODIUM CHLORIDE	0.08 g
BORIC ACID	0.02 g
BORAX	0.005 g
DISTILLED WATER	Until 10 mL

The process of formulation was done by three stages;

Stage 1:

- One gram of each of the ingredients (PROPYLENE GLYCOL WITH POLYETHYLENE GLYCOL 1500) are mixed stir until homogenous.
- Raise the temperature about 70°C for the mixture.
- Add the active ingredient (CHLORAMPHENICOL) and stir until dissolve and homogenous.

Stage 2:

- A (0.01 g) of the agent (EDTA) was added to
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- quantity of Distilled water and stir until dissolve.
- Add preservative agent (CETRIMIDE) and stir until dissolve.
- Add the isotonic agent (SODIUM CHLORIDE) and stir until dissolve.
- Add the buffering agent (BORIC ACID) and stir until dissolve.
- Adjust the pH with (BORAX) at (pH=6.6) [the limits of pH (6.0-7.5)].

Stage 3:

- Add the mixture from Stage 1 into the solution of stage 2 and stir until homogenous.
- Complete the volume to the mark with Distilled water.
- Filter the solution and fill in polyethylene bottles.

All ingredients in table I are mixed, filter using 0.2µm filter membrane, then measure the pH and osmolality. Pack the solution in small dropper glass amber container, sterilized. Then the content of the solution can be examined by HPLC techniques at $\lambda_{max} = 277$ (nm). The chromatographic procedure may be carried out using a column 0.25 m long and 4.6 mm in internal diameter packed with *octadecylsilyl silica gel for chromatography R* (5 µm). The mobile phase at a flow rate of 1.0 ml/min a mixture of 5 volumes of a 20 g/l solution of *phosphoric acid R*, 40 volumes of *methanol R* and 55 volumes of *water R* respectively. The absorbance of the

solution was measured at 277 nm by using uv/vis spectrophotometer detector.

Method of analysis

Standard preparation: Dissolve quarterly 200 mg chloramphenicol in 50 ml-volumetric flask with water, transfer 5ml to 100ml -volumetric flask and complete the volume with water, then transfer 5 ml of this solution to 50 ml-volumetric flask and complete the volume with water. **Assay the preparation:** Transfer 5 ml of eye drops preparation to 100 ml -volumetric flask and complete the volume with water, then transfer 5 ml of this solution to 50 ml-volumetric flask and complete the volume with water.

$$\text{Percentage Of Active Ing. \%} = \frac{\text{Avg}(ABS)_{\text{pro}}}{\text{Avg}(ABS)_{\text{ref}}} \times \frac{W_{\text{ref}}}{W_{\text{pro}}} \times \frac{\text{Dilution pro}}{\text{Dilution ref}} \times \frac{\text{Dilution Factor pro}}{\text{Dilution Factor ref}}$$

$$\times \frac{\text{Dose}}{\text{Active Ing/Dose}} \times \text{Potency} = 101.03 \%$$

Stability Study of the eye preparation drops were incubated at 4, 25, 40 and 50C °.for 3 months, and the samples were removed later at 30, 60 and 90 days following incubation. All experiments were carried out in duplicate and the mean of the results was used. The rate constants of decay were determined by plotting the log chloramphenicol concentration against time, and the energy of activation (E) was estimated from the Arrhenius equation [3&4].

$$\text{Log K} = \text{log A} - E / 2.303 \text{ RT}$$

Where K is the rate constant of the reaction, A the frequency factor, R the gas constant (1.9872) and T the absolute

temperature (K), and 2.303 was constant for natural log. The method of shelf-life prediction based on three months accelerated stability data was utilized. A graphic technique was employed to predict the degradation that may occur over prolonged storage at 4, 25, 40, & 50 C°

Results

The design of the formula, stability studies of chloramphenicol 0.4% eye drop should be based on knowledge of the behavior and the properties shown in table 2:

Table (2):- Summary of physical and chemical properties of Chloramphenicol 0.4 % eye drop

Test	Results
Color	colorless liquid
Clearance	Free from particles
TLC (W:B:M:A)(5:5:5:0.4) V/V %	R _f = 0.7
pH	6.51
Osmolality	306 mOsmol
Content(HPLC)	101.03%
Pharmacological test on Mice & Rabbits	No any redness on both eye applied three times daily for four consecutive
Sterility	(-)ve , the solution is sterile
W:B:M:A =Water:Butanol:Methanol:Acetic acid	
(-)ve =Negative test.	

The accelerated stability studies should be conducted on the eye drop packed in a glass amber closed container [5], the time and the concentration of the active

ingredients studies at four different temperatures for three months. The results were shown in table 3

Table(3):- Time and concentrations for Chloramphenicol 0.4 % eye drops at different temperatures.

Time (Days)	Conc. at 4 C°	pH	Conc. at 25 C°	pH	Conc. at 40 C°	pH	Conc. at 50 C°	pH
30	101.03	6.47	101.03	6.47	100.09	6.42	99.85	6.42
60	101.025	6.42.	101.01	6.22	98.11	6.01	97.44	5.6
90	100.6	6.12	99.1	5.99	97.53	4.88	96.21	2.71

The rate constants of decay was determined for 0.4 % chloramphenicol eye drop using Arrhenius equation . The

rate constant of the reaction will be calculated as shown in table 4 .

Table(4):- Degradation rate constants (K) of chloramphenicol 0.4% eye drops at four different temperatures.

Temperature (Kelvin °)	K (month ⁻¹)
277	$5.9 * 10^{-5}$
298	$1.66 * 10^{-4}$
313	$6.31 * 10^{-4}$
333	$1.98 * 10^{-3}$

Discussion

Chloramphenicol, is one of the most chemically stable antibiotics in common use. It has good stability at room temperature (25 C°) in the pH range 6.51- 2.71. [6]. Chloramphenicol is administered into the eye to treat a type of eye infection called bacterial conjunctivitis, which can be caused by various types of bacteria. Putting the medicine directly into the eye allows the chloramphenicol to act directly on the bacteria that are causing different infections [7]. The eye drop preparation of chloramphenicol 0.4 % used in Syria for the first time, the preparation of this kind and the stability studies should be done on this new formulated eye drops, and known to be very active agents against different types of microorganisms. At this time, only 0.4 % eye drop formulation of chloramphenicol solution have been prepared and tested for their physical, chemical and pharmacological activity as shown in table 1. The solution is clear liquid, each ml contains 40 mg of chloramphenicol ml equivalent to (0.4 %). The best preservative used is the breakdown that may occurs over prolonged periods of storage at normal shelf conditions. chloramphenicol concentration in this preparation was

cetramide with other inactive ingredients as shown in table 1. The solution should be filtered under aseptic condition using 0.2 µm filter, and the pH adjust at 6.51, and the solution should be isotonic for ophthalmic purposes. The testing for stability of drugs contain all appropriate, physical, chemical and biological attribute validated stability including analytical procedures should be applied. Table 3 shows the change in pH degrees for hydrolysis of chloramphenicol at different temperatures. This means that the degradation of chloramphenicol is linearly dependent on buffer concentration (borax), the primary pathway for the degradation of chloramphenicol is the hydrolysis of the amide linkage, forming the corresponding amine and dichloroacetic acid (Scheme 1) [8].

Stability studies were conducted on chloramphenicol 0.4% eye drops incubated at 4, 25, 40 and 50 C°, and 90 days following incubation. The accelerated studies at different temperatures were employed to predict

measured by the procedures provided high performance liquid chromatography techniques for assaying component. The rate constants of decay was determined

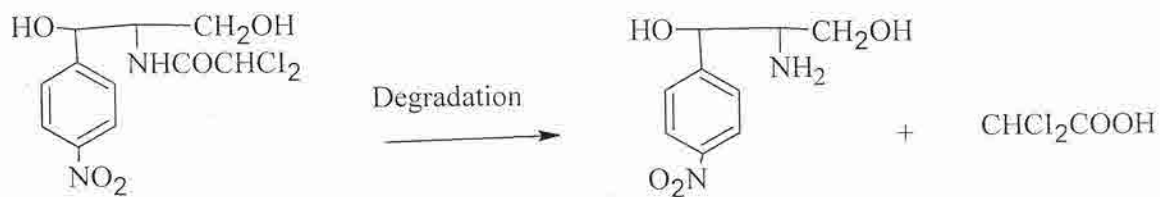
by plotting the log concentration of chloramphenicol concentrations against time using Arrhenius equation [5]. The Arrhenius plot showed the degradation of chloramphenicol 0.4% solution according to first order kinetics. Besides, the physical and chemical stability during storage period of the solution was monitored. The degradation rate constants (K) were calculated from slopes of the straight lines. These degradation rate constants are presented in table 4. To determine the shelf life [6], (t_{10} %) Arrhenius plots were constructed to predict the degradation rate constants at room temperature (K_{25}) for chloramphenicol eye drops and was found to equal $0.00166 \text{ month}^{-1}$, and the predicted shelf life was 1.8 year. The estimated shelf life is two years of the product ensured extended clinical efficacy under normal storage

conditions. However clinical trial of the product is lacking, through it was found to be clinically effective in treating conjunctivitis in mice and rabbits. Further no eye irritation was detected in rabbits used for this purpose [9]. The photodegradation of the chloramphenicol indicate that solutions should be protected from light, even at ordinary temperature. The glass amber color containers were found to afford the best light protected in various dispensing containers that were tested [10]. At ordinary temperatures, chloramphenicol possesses unusual stability over a wide pH range. At the same time, it is susceptible to general acid-base catalyst due to borax / boric acid buffer at pH 6.47 has been recommended for dispensing chloramphenicol 0.4% solution, and keep the containers should be kept in dark and cold conditions.

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Scheme 1 : Pathway of chloramphenicol degradation