CHAPTER I

INTRODUCTION

PROBLEM

Chloramphenicol eye drop is a preparation of broad spectrum antibiotic, effective against a wide range of gram-negative and gram-positive and is popularly prescribed in eye infections. It is now listed in the National List of Essential Drugs (1).

Chloramphenicol (CPC) in aqueous solution is rapidly degraded, therefore, both CPC eye drops BPC 1973 (2) and BP 1980 (3) were specified the concentration of CPC, its degradation products and shelf-life. The major degradation product , 2 - amino - 1 - p - nitrophenylpropane - 1 , 3 - diol is not more than 5.0 % of content of CPC. When stored at a temperature of 2° to 8°C , it may be expected to retain its potency for eighteen months from the date of preparation. When stored at a temperature not exceeding 25° C, it may be expected to retain its potency for four months from the date of preparation.

CPC eye drops are well distributed in Thailand. Most manufactures restricted the expiration date about two years from the date of preparation; however, their shelf - lives are doubted.

There was a report of the content of both drug and its

degradation product of CPC eye drops from eight different manufactures before their date of expiration. It was found that only one preparation has the content of drug more than 90.0% label amount and the degradation products were more than 5.0% of content of CPC (4).

Suwanna Laungchonlatan (5) studied the stability of CPC in seven marketted eye drops by accelerated thermodegradation process. It was found that the shelf- life of CPC eye drops at room temperature (33° C) was about 2.5 months which was shorter than the shelf-life specified by BPC 1973 as 4 months at 25° C.

It could be seen that the problem of the stability of CPC eye drops occurred. The actual shelf-lives were too short and the quality of all marketed products were lower than the standard which may cause side effect, or resistance to patient.

THE PURPOSES OF THIS STUDY

1. To Improve the stability of chloramphenicol eye drops via vehicle compositions. In addition, the formulations were adjusted to meet the requirement of eye drops such as pH, viscosity, color change and tonicity.

2. To study the effect of viscosity - inducing substances, cosolvent and surfactants on stability, viscosity, pH, color change and tonicity of preparations.

3. To present the theoretical basis and model for accelerated stability testing and estimating the shelf - life of products.

REVIEW LITERATURE

1. General Properties of CPC

Description

1.1 Nomenclature (6)

1.1.1 Chemical Names

D (-) - threo - 2 - Dichloroacetamido - 1 - p - nitrophenyl 1 - 1 , 3 - propanediol.

1.1.2 Generic Names (6)

Chloramphenicol

1.1.3 Trade Names (7)

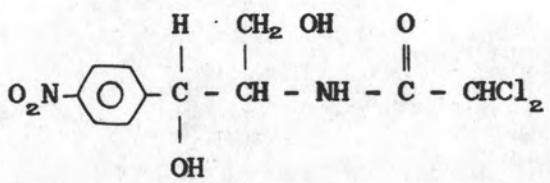
Chloromycetin (The Merck Index lists 45 other trade names.

1.2 Formulae (6)

1.2.1 Empirical

$C_{11} H_{12} Cl_2 N_2 O_5$

1.2.2 Structural



1.3 Molecular Weight (6)

323.13, 322.01

1.4 Appearance, Color, Odor and Taste (6)

Fine white to greyish white or yellowish white crystals, needles or elongated plates from water or ethylene dichloride with

very bitter taste.

2. Physical Properties

2.1 Melting Point

150.5° - 151.5°

2.2 Solubility

2.2.1 Single solvents

Soluble (25°) in water: 2.5 mg/ml, in propylene glycol: 150.8 mg/ml, very soluble in methanol, ethanol, butanol, ethyl acetate, acetone. Fairly soluble in ether, insoluble in benzene, petroleum ether, vegetable oils. Solubility in 50 % acetamide solution is 5 %. Aqueous solutions are neutral. Neutral and acid solutions are stable on heating (6).

2.2.2 In mixed solvents or as a result of complexation

The solubility profiles for CPC in several aqueous solvent mixtures were determined by Negoro and Associates (7).

Kostenbauder(8) also determined the solubility of this antibiotic in aqueous solutions of N, N, N', N' - tetramethylphthalamides.

The solubility of CPC in water was increased with the addition of benzalkonium chloride. The two substances had a synergistic action in vitro against Pseudomonas aeruginosa (9).

Aqueous solvents containing 5% Tween 20-80 increase the water solubility of CPC approximately 3 fold. The solubility of CPC in serum and urine is approximately the same as it is in water (7).

The solubility of CPC in water is increased by addition of borax (10). This solubility increase is explained on the basis of the

formation of a 1:2 complex between the borate ion and the antibiotic (7). Thus , a 0.3% (0.0078 molar) borax was used in CPC eye drops BPC 1973 (0.5% CPC). It was used as a buffer and to increase solubility.

2.3 pH Effect

Since CPC is an essentially neutral compound, changes in pH (over the pH region 3 to 9) do not result in significant changes in solubility. The solubility of the antibiotic is increased in the presence of strong acid (11) due to protonation of the weakly basic amido nitrogen.

3. Methods of Analysis

3.1 Identification Tests (6)

There are many tests to identify CPC, see reference (6).

3.2 Quantitative Analysis

3.2.1 Biological Methods

Two biological methods determining CPC were reported; bioassay (6,12) and enzymatic assay (6,13,14,15).

3.2.2 Chemicals Methods (6)

There are titrimetric methods, polarographic methods, colorimetric methods, ultraviolet spectrophotometric methods, infrared spectrophotometric methods, proton magnetic resonance spectrometric methods and mass spectrometric methods.

3.2.3 Chromatographic Methods (6)

There are thin layer, paper and column chromatography, gas chromatographic methods, high performance liquid chromatography(HPLC).

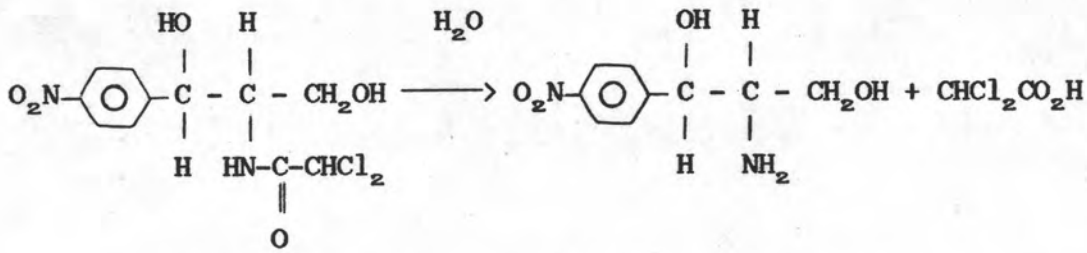
Recently, Suwanna Laungchonlatan (5) assayed CPC using HPLC with column (Zorbax ODS 5 um, Dupont 15 cm x 4.6 mm), mobile phase (mathanol - water 60:40), detector (UV at 254).

4. Stability in solution

4.1 Hydrolysis

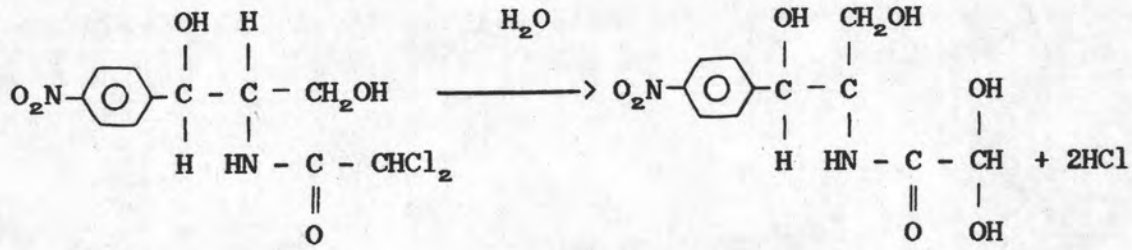
The stability of CPC in aqueous solution is governed by the rate at which hydrolytic processes occur. The two primary routes of decomposition have been determined (7,11,16,17) to be

a) Amide hydrolysis with the formation of 1 - (p-nitrophenyl) - 2 - amino - 1, 3 - propanediol

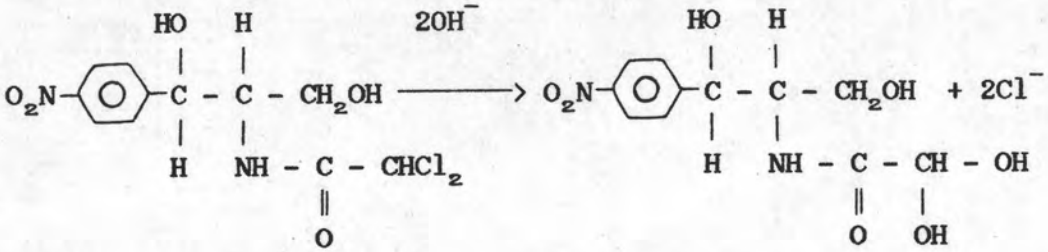


The hydrolytic cleavage of the amide linkage (16) is the major cause of CPC breakdown and is the only significant route of degradation in solutions below pH 7. The rate of amide hydrolysis is independent on pH over the pH region 2 to 6 and independent of the ionic strength of the medium. Studies involving phosphate, acetate, and citrate buffers indicate that the amide hydrolysis is general acid-base catalyzed (7,17).

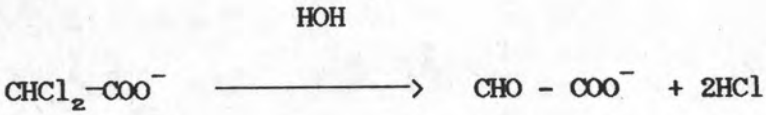
b) Hydrolysis of covalent chloride of the dichloroacetamide moiety



The hydrolysis of covalent - bound chlorine is insignificant at pH values below 6 but increase dramatically as pH increases. This increase is attributed to hydroxyl ion catalysis(16).



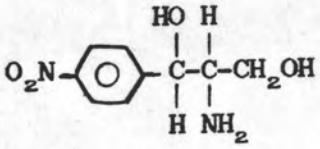
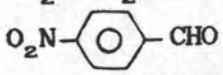
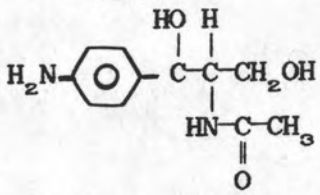
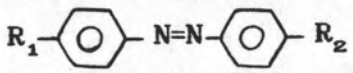
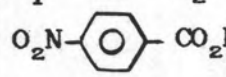
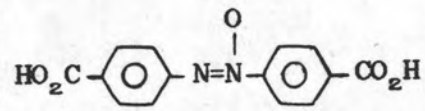
Numerous secondary reactions can occur which give rise to a variety of decomposition products. Among these secondary reactions are those associated with subsequent hydrolysis of dichloroacetic acid and oxidation-reduction reactions which involve the nitro group as oxidant and the side chain (particularly the aminodiol side chain of the primary hydrolysis product) as reductant (7).



Products isolated from partially or completely decomposed CPC solutions exposed to a variety of conditions are given in Table 1 (7).

The presence of borate buffers has been shown to increase the aqueous stability of CPC (7). Heward and associates (18) studied the stability of the borate-buffered CPC eye drops BPC 1968 (19). The

Table 1 Decomposition Products of CPC.

No.	Compound	Environmental Conditions
1.		Acidic or basic aqueous solution.
2.	Cl_2CHCO_2H	Acidic or basic aqueous solution.
3.		Aqueous solution, ambient temperature.
4.		Aqueous solution, ambient temperature.
		
5.	$R_1 = R_2 = CH_2OH$	Aqueous alkaline solution, high temperature.
6.	$R_1 = R_2 = CO_2H$	
7.	$R_1 = R_2 = CHO$	
8.	$R_1 = R_2 = OH$	
9.	$R_1 = CO_2H$; $R_2 = OH$	
10.	$R_1 = CO_2H$; $R_2 = CH_2OH$	
11.	$R_1 = OH$ $R_2 = CH_2OH$	
12.	$R_1 = OH$ $R_2 = CHO$	
13.		Aqueous solution after exposure to light.
14.		Aqueous solution after exposure to light.
15.	HCl	Aqueous solution; high temperature.

results obtained that calculated $t_{10\%}$ at 30°C is 38 days and at 20° C is 4 months. James and Leach (10,20) suggested that complexation between the antibiotic and borate ion is responsible for the increased stability of CPC in this buffer system.

4.2 Oxidation and Reduction (21)

Incubated aqueous solutions of CPC at various pH values (1-14) yielded detectable amounts of p-nitrobenzaldehyde (an oxidation product) and arylamine (a reduction product). Identical degradation products were also found in certain dosage forms (creams and capsules), although they were not found in ophthalmic ointment.

4.3 Photodegradation

Shin (22) reported that, when CPC aqueous solution was explosed to sunlight, uv light, tungsten light photodegradation occured. The chemical reaction of photodegradation may be oxidation, reduction or condensation and a lot of degradation products were formed, such as hydrochloric acid, p - nitrobenzaldehyde, p - nitrobenzoic acid , 4 , 4 ' - azoxy - benzoic acid and p - aminophenyl - 2 - acetamido - 1 , 3 - propandiol.

In 1982 Mubarak et al, (23) identified photochemical decomposition of CPC in Clark Lubs borate buffer pH 7.8, otherwise he reported that photodegradation products of CPC in other solvents such as ethanol, benzene, ethanolamine and acetic acid were shown as the same as in water. The more degradation produced the more yellow substances formed, the concentration of yellow color indicated the degree of degradation.

4.4 In the presence of microorganism (7)

Smith studied the decomposition of CPC in the presence of various micro organisms. Various routes by which CPC could undergo degradation was defined.

4.5 Drug Kinetics

4.5.1 Reactions and Rate Equations (24)

The primary pathway for the degradation of CPC is the hydrolysis of the amide linkage, forming the corresponding amine and dichloroacetic acid (see 4.1)

The observed rate constant represents the sum of a number of terms. For an aqueous solution, k_{obs} the observed rate constant, may be given as

$$k_{obs} = k_{H2O} + k_H[H^+] + k_{OH}[OH^-] + k_{HB}[HB] + k_B[B]$$

where k_{H2O}, k_H, k_{OH}, k_{HB} and k_B are constants for the uncatalyzed, hydrogen-ion-catalyzed, hydroxide-ion-catalyzed, general acid-, and general base-catalyzed reactions, respectively.

Degradation via dehalogenation of CPC plays an insignificant part in the total degradative picture, at least below pH 7.

4.5.2 pH - Rate Profile (24)

Figure 1 is the log k, pH plot for the hydrolysis of CPC at 91.3°C in perchloric acid solutions. Specific acid catalysis is seen below pH 2, and the curve levels off as pH approaches 2 In universal buffer (containing citric, phosphoric, boric, and

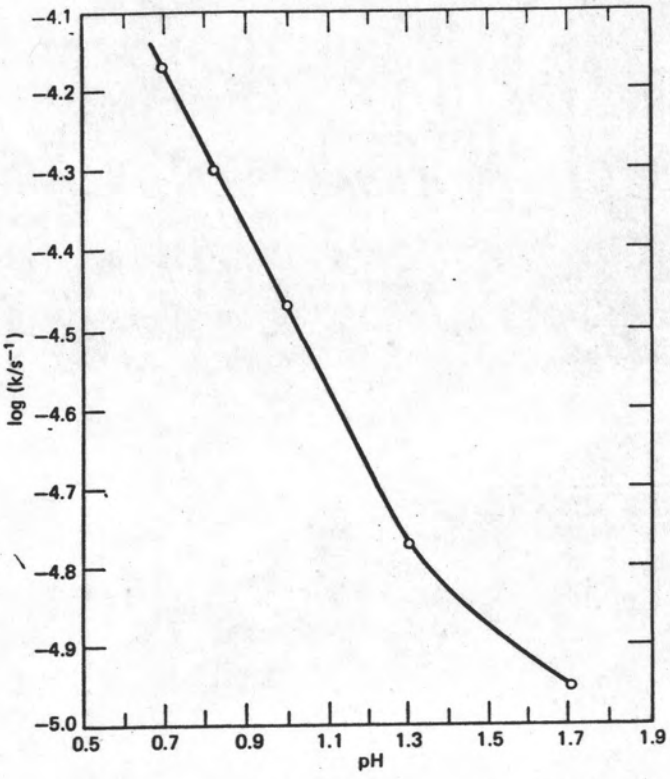


FIGURE 1. CHLORAMPHENICOL. Logarithmic k, pH plot for hydrolysis of chloramphenicol at 91.3°C in perchloric acid solutions.

hydrochloric acids and sodium hydroxide), and at 80° C, hydrolysis of CPC is independent on pH between 2 and 7. The rate constant in this pH range is 6.3×10^{-6} sec^{-1}.

4.5.3 Activation Energy (24)

The Arrhenius plot for the hydrolysis at pH 6, corresponding mainly to the uncatalyzed (water) reaction, is shown in Figure 2. The activation energy at this pH is 24 k.cal/mol (11,24). At 25° C and pH 6 the calculated rate constant is 7.5×10^{-9} sec^{-1}, giving a half-life of almost 3 yr (11,24).

4.5.4 Other Data

The rate of degradation of CPC is linearly dependent on buffer concentration, the buffer species acting as general acids and bases. The rate of degradation in phosphate buffer was shown to be independent of ionic strength, and unaffected by the concentration of dihydrogen phosphate ion; thus the catalytic activity was attributed to the monohydrogen phosphate ion acting as a general base. Acetate buffers also act as catalysts for CPC degradation, the catalytic activity being almost entirely associated with the acidic component of the buffer (11).

4.6 Stability Summary (24)

The major cause of the degradation of CPC in aqueous media can be attributed to the hydrolytic cleavage of the amide linkage. It shows good stability at room temperature in the pH range from 2 to 7. The maximum stability is at pH 6. At 25° C and pH 6 it has a half-life of almost 3 years.

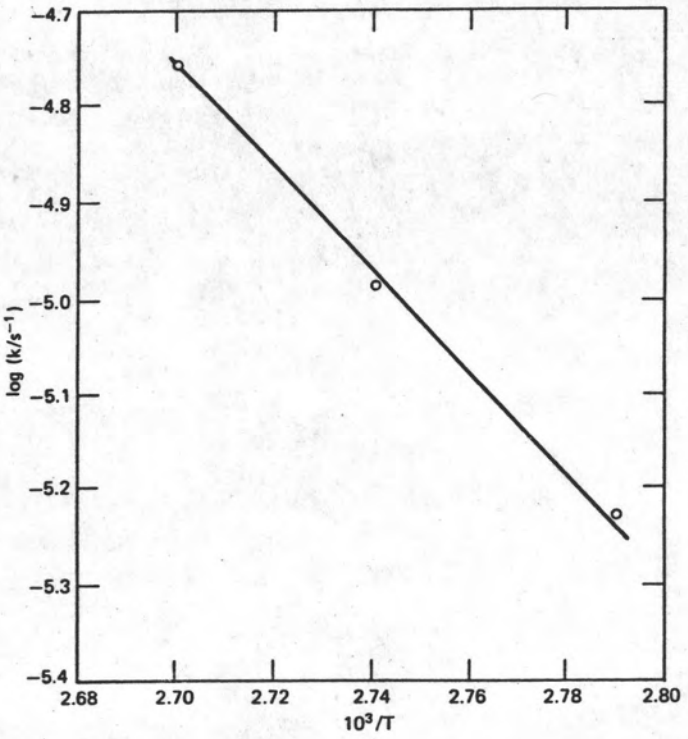


FIGURE 2. CHLORAMPHENICOL. Arrhenius plot for hydrolysis of chloramphenicol at pH 6.

The degradation rate is first-order with respect to the drug and is independent of ionic strength of the medium

The hydrolysis is generlly acid-base catalyzed, but in the pH range 2 to 7 the rate is substantially independent of pH. Catalyzing species are the general acids and bases present in the buffer used; specifically, monohydrogen phosphate ion, undissociated acetic and mono-and dihydrogen citrate ions catalyze degradation. But borate buffers increase stability.

Below pH 2 specific hydrogen - ion - catalyzed hydrolysis plays a major role in the degradation of CPC.

The drug is highly unstable in alkaline media, and the reaction shows both general and specific base catalysis.

Below pH = 2	pH = 2-7	Above pH = 7
- H^+ catalyzed	-amide hydrolysis -pH = 2-7, pH independent, good stability and maximum at pH = 6 -Ionic strength independent -general acid-base catalyzed -borate buffer increase stability	- OH^- catalyzed -general and specific base catalysis -Chloride hydrolysis increases

Photodegradation of CPC in aqueous solution at ordinary temperatures has also been reported and in the presence of microorganism decomposition was found.

4.7 Stabilization Methods

Because of major decomposition of CPC in solution is hydrolysis, the stabilization is to intended decrease hydrolysis. The general stabilization methods are the following (25).

4.7.1 Limit acess of the drug to water (25)

In the case of CPC eye drops, the dosage form can be change to CPC for ophthalmic solution. This dosage form is dry mixture of CPC with or without one or more suitable buffers, diluents, and preservatives (26). It is more stable than CPC eye drops, however CPC is difficult to dissolve (1:400 in water) to get the therapeutic concentration of 0.5%. In addition, it is inconvenient to use.

The method to decrease hydrolysis of CPC eye drops is limiting the amount of water by replacing with another solvents such as polyethylen glycol.

4.7.2 Control pH and buffer

Control of pH offers a means for controlling stability of drugs in aqueous solutions. In order to select the optimum pH, it will usually be necessary to have available the experimental pH - rate curve (pH - rate profiles), which will show at a glance the pH for maximum stability (25).

Buffer system is important for stabilization because it controls changing of pH and pH is importance in degradation. Some

buffer systems catalyze the reaction so the formulator will choose the best one and the best concentration.

The optimum pH of CPC eye drops is between 2-7 and maximum stability being at pH=6 (11,24). A borax - boric acid buffer at pH 6 has been recommended for dispensing CPC in eye drops solutions (24).

4.7.3 Lowering the temperature

Stability can always be increased by lowering the temperature, but the formulation should not be frozen unless prior experimentation has shown that freezing is not detrimental (25).

In the case of CPC eye drops BP 1980 (3), when stored at a temperature of 2°C to 8°C , they may be expected to retain their potency for eighteen months from the date of preparation. When stored at a temperature not exceeding 25°C they may be expected to retain their potency for four months from the date of preparation. CPC eye drops are more stable in the cool place than in the room temperature.

4.7.4 Changes in solvent composition of formulation

Changes in solvent composition can be significantly decrease rate effects. Esters will hydrolyse in water more rapidly than in another low dielectric constant solvents. If the suitable solvent is chosen, the preparation can be stabilized (27).

For example, CPC eye drops is aqueous solution with buffers and preservative, the shelf-life is about 2.5 months at room temperature which is shorter than the shelf-life of CPC ear drops (CPC in PEG_{400}) as 3.45 months at the same temperature (5).

Alteration of the dielectric constant by the addition of

non-aqueous solvents such as alcohol, glycerin or propylene glycol may in many cases reduce hydrolysis (28).

4.7.5 Adding a compound that would form a complex with the drug (28,29)

For CPC eye drops; James and Leach (10,20) suggested that complexation between the antibiotic and borate ion was responsible for the increased stability of CPC in this buffer system.

4.7.6 Solubilisation of a drug by surfactants

Solubilisation of a drug by surfactants in many cases protects against hydrolysis, the presence of surfactants in micellar form has a modifying effect on the rate of hydrolysis of drugs. The magnitude of the effect depends on the difference in the rate constant when drug is in aqueous solution and when it is solubilised within the micelle, and also on the extent of solubilisation (28).

4.7.7 Increasing viscosity of the vehicle

Increasing viscosity of the vehicle may decrease rate of reaction because of decreasing in the collision frequencies.

The theory of reaction rates (30) stated that when 2 kinds of reactants chemically react, a new product is obtained. The rate of a chemical reaction is related to the absolute temperature by the Arrhenius equation.

$$k = PZe^{-Ea/RT}$$

Z is the collision frequency and P is the deviation between experimental and collision theory

If the medium is high viscosity, collision frequency (Z) is

expected to decrease, thus rate constant (k) is decreased then the chemical reaction rate is decreased.

The effect of viscosity on the stability of drugs have been several reported, for example, Giral (31) hinted that the viscosity of a pharmaceutical vehicle such as simple syrup might be a protective factor in stabilizing ascorbic acid. Moreover, Rolland I. Poust and John L. Colaizzi (32) explained that the decrease in oxidation rate of ascorbic acid may be due to a decrease in the collision frequencies caused by the increasing size of the micelles (Polysorbate 80).

4.7.8 Modifying chemical structure using appropiate substituents

This method has been suggested for drugs for which such a modification does not reduce therapeutic efficiency.

A number of reports in the literatures showed that certain substituents added to the alkyl or acyl chain of aliphatic of aromatic esters or to the benzene ring of aromatic esters cause a decrease in the hydrolytic rate. This may be attributed to a stearic and/or polar effect of the substituent group (29).

4.7.9 Salts and esters

Another technique that is sometimes employed to increase the stability of pharmaceuticals undergoing degradation through ester hydrolysis is to reduce their solubility by forming less soluble salts or esters of drug. Usually, only the fraction of the drug that is in solution undergoes hydrolytic degradation. Garrett, in his study with acyl-salicylates, found that a compound that shows rapid hydrolysis in

solution may be made to exhibit better stability than a more stable analog by reducing its solubility (29).

5. Chloramphenicol Eye Drops

5.1 Chloramphenicol eye drops in pharmacopeia

In this review literature, it was stated only the new volume of pharmacopeia. They are

- Chloramphenicol Eye-drops BPC 1973 (2)
- Chloramphenicol Eye Drops BP 1980 (3)
- Chloramphenicol Ophthalmic Solution USP XXI (26)
- Chloramphenicol for Ophthalmic Solution USP XXI (26)

They were compared in Table 2

6. Requirement of Eye Drops

Definition : Eye drops are sterile solutions or suspensions of one or more medicaments intend for instillation into the conjunctival sac for diagnostic or therapeutic purposes (33).

Requirements which must be considered in the preparation and in the control of ophthalmic products are (34)

- Sterility
- Clearity
- pH
- Buffer
- Tonicity
- Preservatives
- Viscosity
- Additives

Table 2 Comparison of CPC eye drops in Pharmacopeia.

	Chloramphenicol Eye-drops BPC 1973	Chloramphenicol Eye-drops BPC 1980	Chloramphenicol Ophthalmic Solution USP XXI	Chloramphenicol for Ophthal-mic Solution USP XXI
1.Formulation	Chloramphenicol 0.5 g Phenylmercuric Acetate or Nitrate 0.002 g Borax 0.3 g Boric Acid 1.5 g Purified Water to 100 ml	The formulation was not stated, it was deseribed that, Chloramphenicol Eye Drops are sterile solution in Purified Water of Chloramphenicol, containing suitable buffering agents and 0.002 per cent w/v of either phenylmercuric acetate or Phenylmercuric Nitrate.	The formulation was not stated, it was described that, Chloramphenicol Ophthalmic Solution is a sterile buffered solution of Chloramphenicol.	The formulation was not stated it was described that Chloramphenicol for Ophthalmic Solution is a sterile, dry mixture of Chloramphenicol with or without one or more suitable buffers, diluent, and preservatives.
2. How to prepare	Dissolve the boric acid, the borax, and the phenyl mercuric salt in 90 ml of the purified water with the aid of heat; adjust the temperature of the solution to 60° , add the chloramphenicol,and maintain the temperature at 60° until the chloram-phenicol is dissolved. Cool the solution, and sufficient purified water to produce the required volume, and mix. Then, either (i) sterilise the solution by filtration, and transfer by menans of an aseptic technique to sterile containers, which			

Table 2 (cont.)

	Chloramphenicol Eye-drops BPC 1973	Chloramphenicol Eye-drops BPC 1980	Chloramphenicol Ophthalmic Solution USP XXI	Chloramphenicol for Ophthal-mic Solution USP XXI
	are then closed so as to exclude micro-organisms (Method B) or (ii) clarify the solution by filtration, transfer it to the final containers, which are then closed to exclude microorganisms, and sterilise it by maintaining at 98° to 100° for thirty minutes (Method C).			
3.Strength	0.5% w/v	0.5% w/v	-	-
4.Content	0.45-0.55% w/v	90-110% of the prescribed or stated amount.	90-130% of the labeled amount.	90-130% of the labeled amount.
5.Limitation of 2-Amino-1-(4-nitro - phenyl) propane-1,3-diol	Not more than 5.0% of con-tent of chloramphenicol.	Not more than 5.0% of con-tent of chloramphenicol	-	-
6.pH		7.0-7.5	7.0-7.5	7.1-7.5
7.Storage	It should be protected from light. When stored at a temperature of 2° to 8°, it may be expected to retain its potency for eighteen months form the date of preparation. When stored at a	Chloramphenicol Eye Drops should be protected from light. When stored at a temperature of 2° to 8°, they may be expected to retain their potency for eighteen months from the date of preparation. When	Preserve in tight containers.	Preserve in tight containers.

Table 2 (cont.)

	Chloramphenicol Eye-drops BPC 1973	Chloramphenicol Eye-drops BPC 1980	Chloramphenicol Ophthalmic Solution USP XXI	Chloramphenicol for Ophthal-mic Solution USP XXI
	temperature not exceeding 25°, it may be expected to retain its potency for four months from the date of preparation.	stored at a temperature not exceeding 25° they may be expected to retain their potency for four months form the date of preparation.		

- Packaging

- Stability

6.1 Sterility

Sterility is the most important requirement of all in eye drops. Eye drops may be prepared and sterilized by filtration, moist heat, dry heat sterilization and gas sterilization (36,43).

6.2 Clearity

Ophthalmic solutions are free from foreign particles and clearity is normally achieved by filtration. The degree of clearity can be estimated by the use of light - scattering devices such as the Coleman Nephelo-Colorimeter (34,35).

6.3 pH and Buffer

The pH value for tears is approximately 7.4 (with normal individual variations from 5.2 to 8.35 , with the usual range lying between 7.3 and 7.7). Small temporary changes in the pH of their tears are exhibited by all individuals; these minor variations do not give rise to symptoms. An acceptable range for eye drops which will not harm the epithelium is pH 5.0 to pH 9.0 (36). It will not harm the epithelium because the application of a solution to the eye stimulates the flow of tears and the rapid neutralization of any excess hydrogen or hydroxyl ions with in the buffer capacity of the tears (35,36,37).

Product pH and the buffer required to establish pH are a part of product design and are usual essentials for product stability. A properly formulated eye product should include a buffer with a

capacity sufficient to maintain product pH during the proposed shelf-life. At the same time, the capacity should be minimized in order to permit the tear fluid to readjust to pH 7.4 following instillation of the product into the eye (36).

6.4 Tonicity

Tonicity refers to the osmotic pressure exerted by a solution from the solutes or dissolved solids present. Tear fluid and other body fluids exert an osmotic pressure equal to that of normal saline or 0.9% sodium chloride solution. A solution with a greater amount of solutes than tear fluid has a greater osmotic pressure and is called "hypertonic." Conversely, a solution with less solute has a lower osmotic pressure and is "hypotonic" (38).

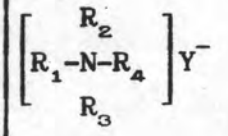
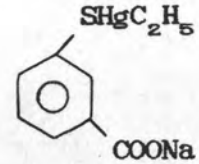
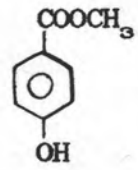
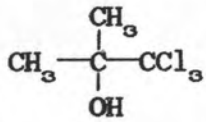
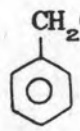
In actuality the eye is much more tolerant of tonicity variations than was at one time suggested without great discomfort. Range of tonicity equivalent to sodium chloride was 0.5% - 2.0% sodium chloride (35,38,39,40).

6.5 Preservatives

Ophthalmic solutions may be packaged in multiple-dose containers, so it must contain a preservative to prevent the growth of, or to destroy, microorganisms accidentally introduced when the container is opened during use. The properties of chemical compounds used as preservatives in ophthalmic solutions are summerized in Table 3 (34).

Many preservative substances may be inactivated by binding with other formulation additives, in particular with macromolecular

Table 3 Ophthalmic Preservatives

Type	Typical structure	Concentration range	Incompatibilities	Remarks
Quaternary ammonium compounds		0.004%-0.02% 0.01% Most Common	Soaps Anionic materials Salicylates Nitrates	Benzalkonium chloride is the single most frequently used ophthalmic preservative. EDTA increases effectiveness.
Organic mercurials	 (Thimerosal)	0.001%-0.01%	Certain halides with phenyl-mercuric acetate	Typically used as substitute for benzalkonium where latter is incompatible.
Parahydroxy benzoates		Maximum 0.01%	Adsorption by macromolecules	Infrequently used; activity limited to bacteriostasis.
Chloro-butanol		0.5%	Stability is pH-dependent; activity concentration is near solubility maximum	Will diffuse through low-density polyethylen container.
Aromatic alcohols		0.5%-0.9%	Low solubility in water	As above; occasionally used in combination with other preservatives.

compounds. Studies have shown that inactivation by various substances is selective and generalizations are difficult to make. For example, chlorobutanol and benzyl alcohol are inactivated by polysorbate 80 and polyvinylpyrrolidone, but not by methylcellulose. Cetylpyridinium chloride is inactivated by methylcellulose; benzalkonium chloride is not (34).

6.6 Viscosity

The USP permits the use of viscosity increasing agents to prolong contact time in the eye and thus enchance drug absorption and activity (35).

R.D. Schoenwald and J.J. Boltralik compared the bioavailability of steroids formulated as high-viscosity gels (carbopol 940) and reference aqueous preparations. Concentrations of steroids gel were found to be approximately four times larger than those of the reference preparation. The gel vehicle is well retained in the eye and responsible for the large increase in bioavailability (41).

Suitable thickening agents such as (35,36,39,40)

- cellulose derivative : methylcellulose, hydroxymethyl cellulose, hydroxypropyl methylcellulose, hydroxyethylcellulose
- polyvinyl alcohol

The thickened ophthalmic solution must be free from visible particles.

6.7 Additives

The use of various additives in ophthalmic solutions is

permissible , however the choices are few in number. An antioxidant, specifically sodium bisulfite or metabisulfite is permitted in concentrations up to 0.3% particularly in solutions containing epinephrine salts. The antioxidant acts in this case as a stabilizer to minimize oxidation of epinephrine.

The use of surfactants in ophthalmic preparations is similarly restricted. Nonionic surfactants, the least toxic class of such compounds, are used in low concentrations particularly in steroid suspensions and as aids in achieving solution clarity. Surfactants may rarely be used as cosolvents to increase solubility.

The use of surfactants particularly in any significant concentration should be tempered by recognition of the sorption characteristics of these compounds. Nonionic surfactants in particular may react by adsorption with antimicrobial preservatives compounds and inactivate much of the preservative system (35).

6.8 Packaging

Containers for eye drops are specified in BP 1980 (42) and USP XXI (43). The other interested details of packaging are summerized in Ref 38, 40, 43.

6.9 Stability

Chemical stability influences the mode of sterilization of eyedrops, for example, some drugs are thermolabile (affected by heat) and cannot be autoclaved at 115°C, but may be heated with a bactericide at 98°-100°C, or sterilized by (bacterial) filtration, for example, amethocaine and physostigmine eyedrops may be sterilized

by heating with a bactericide at 98°-100°C or by filtration. Some ophthalmic drugs are affected by light, and their solutions must be kept in amber bottles, for example, amethocaine, sulphacetamide, physostigmine and adrenaline. Oxygen (from the air) affects physostigmine and adrenaline, and these both require an antoxidant (sodium metabisulphite) in the formula of their eyedrops (36).

THE BASIS CONCEPT FOR IMPROVING STABILITY OF CHLORAMPHENICOL EYE DROPS VIA VEHICLE COMPOSITIONS

1. Decreasing Hydrolysis

The major degradation of CPC is hydrolysis, thus the basis for improving stability is to decrease the hydrolysis. The stabilizations via vehicle compositions were conducted to improve the stability of Chloramphenicol Eye Drops BP 1973 as standard formulation.

1.1 Optimum pH

The maximum stability at pH = 6.0 was reported (11,24) therefore the eye drops were adjusted to pH = 6.0 and compared to the standard formulation.

1.2 Optimum Buffer System and Concentration

Since other buffers catalyse hydrolysis, borate - boric buffer was chosen. In addition, the complexation between the antibiotic and borate ion was responsible for the increased stability of CPC. The concentration of buffer in the eye drops was equal to the concentration of the standard formulation.

1.3 Increasing Viscosity.

Both hydroxypropylmethylcellulose (HPMC) and polyvinyl pyrrolidone (PVP) were chosen to increase the viscosity. HPMC was selected because it produced clearer solution than methylcellulose (40). Various concentrations were formulated.

1.4 Partial Replacement of Water with a Cosolvent of Low Dielectric Constant.

Different molecular weights of PEG were expected to decrease hydrolysis by partial replacement of water. In addition high concentration of PEG increased the viscosity. The effect of both molecular weight and concentration of PEG were investigated.

1.5 Solubilization by Surfactants.

Poloxamer$_{407}$ (PF_{407}) nonionic surfactant was chosen, because it was normally incorporated in ophthalmic products (44). It produced clear solution. No irritation occurred when directly applied to the eye of rabbit in 5% and 10% concentrations (45).

2. Screening the Formulations Suitable for Eye Drops.

Tonicity, pH, viscosity and color change were measured to screen the formulations suitable for eye drops.

3. Stability Testing.

The accelerated thermodegradation process was applied for stability testing. The solutions of CPC were accelerated by heat at 40° , 50° ,55° and 60°C. The natural logarithms of the degradation rate constants (ln k) of all temperatures were plotted against the recipocal of temperature in degree kelvin (1/T) to obtain Arrhenius relationship. The predictation of stability at shelf and refigerator temperature were to extrapolate the relationship and calculated from their k values.

4. Analysis of CPC.

The content of intact CPC in each formulation was assayed using High Performance Liquid Chromatography.