CHAPTER II

MATERIALS AND METHOD

MATERIALS

1. Chemicals for formulations

1. Chloramphenicol powder lot no. 43749800 (Boehringer Manhamm, Germany)

2. Boric acid lot no. 547K1033265 (Merck, Germany)

3. Borax lot no. 310K2147303 (Merck, Germany)

4. Phenylmercuric acetate Lot no. 3201427 (Merck, Germany)

5. Polyethyleneglycol 400, Batch no. 42-0385 (Lutrol E 400, BASF, Germany)

6. Polyethyleneglycol 1500, Lot no. 512911 (Nippon oil & Fats, Japan)

7. Polyethyleneglycol 4000, Lot no. 53109 (Nippon oil & Fats, Japan)

8. Polyethyleneglycol 6000, Lot no. 501959 (Nippon oil & Fats, Japan)

9. Polyethyleneglycol 20000, Lot no. 6230879 (Merck, Germany)

10. Hydroxypropyl Methylcellulose 4000, Lot no. MM

84031601 E (Methocel E 4 M, The Dow chemical company, U.S.A.)

11. Polyvinylpyrrolidone K90 , Batch no. 82-4195 (Kollidon 90,BASF, Germany)

12. Poloxamer$_{407}$ Lot no.783 0022 (Lutrol FC 127,BASF,Germany)

2. Chemicals for High Performance Liquid Chromatography

1. Methanol HPLC grade; U.V. cut - off 205 nm., Lot no. 631093 (J.T. Baker, U.S.A.)

2. Propylparaben Lot no. 3048 (Ueno. Fine Chemicals industry, Japan)

3. Distilled water

APPARATUS

1. Analytical Balance (Type L-200 SM, Shimadzu, Japan)

2. pH Meter (digital pH / millivolt meter 611, Orion research, Inc. Cambridge, Mass. U.S.A.)

3. Osmometer (Model 3D2, Advanced instruments Inc., Needham Heights Massachusetts., U.S.A.)

4. Membrane filter apparatus and 0.2 µ membrane filter

5. Laminar air flow (Type Nr. UW Z48, Holten, Denmark)

6. Incubator (WCL - IST, Laboratory Thermal equipment Ltd.)

Incubator (INA - 305 series, Gallenkamp, England)

Incubator (Model 4, Precision Scientific co.)

Incubator (Model 6, Precision Scientific co.)

7. Viscometers (Ostwald - Cannon - Fenske)

8. Specific gravity bottles (Pycnometer) 10 ml

9. High Performance Liquid Chromatography (LC - 3A,

Shimadzu, Japan)

- Detector, UV 254 nm. (Shimadzu, Japan)
- Recorder (Chromatopac C-RIA, Shimadzu, Japan)
- Column (Zorbax ODS, 5 um, 25 cm X 4.6 mm., Du. Pont P.N.850952 - 702)
- Precolumn (Reverse phase ,Zorbax ODS , Du.Pont)

METHOD

1.Formulation Preparation

1.1 Chloramphenical Eye Drops BPC 1973 was prepared by formulation and method shown below (3).

Chloramphenical	0.5	g
Phenylmercuric Acetate (PMA)	0.002	g
Borax	0.3	g
Boric acid	1.5	g
Purificd water to	100	ml

Boric acid , borax , and phenylmercuric salt were dissolved in 90 ml of purified water with the aid of heat. The temperature of the solution was adjusted to 60°C and maintained at 60°C until CPC was added and dissolved. The solution was cooled and the sufficient purified water was added to produce the required volume. Then, the solution was sterilized by filtration , and was transferred by means of aseptic technique to sterile ampules,which were then closed so as to exclude micro-organisms.

1.2 The other formulations as shown below were prepared by the same method but some water portions were replaced by

HPMC, PVP, PEG, PF_{407} and some formulations were adjusted to pH=6.0 by adjusting the concentration of boric acid and borax (Appendix A).

Ingredient (g)	BPC 1973	BPC 1973 adjust to pH=6.0
CPC	0.5	0.5
Boric acid	1.5	adjust to
Borax	0.3	pH = 6.0
PMA	0.002	0.002
H_2O to	100 ml	100 ml

Ingredient (g)	0.1% HPMC	0.4% HPMC	0.8% HPMC
CPC	0.5	0.5	0.5
Boric acid	1.5	1.5	1.5
Borax	0.3	0.3	0.3
PMA	0.002	0.002	0.002
HPMC	0.1	0.4	0.8
H_2O to	100 ml	100 ml	100 ml

Ingredient (g)	1% PVP	4% PVP	8% PVP
CPC	0.5	0.5	0.5
Boric acid	1.5	1.5	1.5
Borax	0.3	0.3	0.3
PMA	0.002	0.002	0.002
PVP	1	4	8
H_2O to	100 ml	100 ml	100 ml

Ingredient(g)	10%PEG_{400}	15%PEG_{400}	20%PEG_{400}	25%PEG_{400}
CPC	0.5	0.5	0.5	0.5
Boric acid	1.5	1.5	1.5	1.5
Borax	0.3	0.3	0.3	0.3
PMA	0.002	0.002	0.002	0.002
PEG_{400}	10	15	20	25
H_2O to	100 ml	100 ml	100 ml	100 ml

Ingredient(g)	10%PEG_{1500}	15%PEG_{1500}	20%PEG_{1500}	25%PEG_{1500}
CPC	0.5	0.5	0.5	0.5
Boric acid	1.5	1.5	1.5	1.5
Borax	0.3	0.3	0.3	0.3
PMA	0.002	0.002	0.002	0.002
PEG_{1500}	10	15	20	25
H_2O to	100 ml	100 ml	100 ml	100 ml

Ingredient(g)	10%PEG_{4000}	15%PEG_{4000}	20%PEG_{4000}	25%PEG_{4000}
CPC	0.5	0.5	0.5	0.5
Boric acid	1.5	1.5	1.5	1.5
Borax	0.3	0.3	0.3	0.3
PMA	0.002	0.002	0.002	0.002
PEG_{4000}	10	15	20	25
H_2O to	100 ml	100 ml	100 ml	100 ml

Ingre-dient(g)	10% PEG_{6000}	15% PEG_{6000}	20% PEG_{6000}	20% PEG_{6000} pH=6.0	25% PEG_{6000}	25% PEG_{6000} pH=6.0	30% PEG_{6000}	40% PEG_{6000}
CPC	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Boric acid	1.5	1.5	1.5	adjust	1.5	adjust	1.5	1.5
Borax	0.3	0.3	0.3	pH=6.0	0.3	pH=6.0	0.3	0.3
PMA	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
PEG_{6000}	10	15	20	20	25	25	30	40
H_2O to	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml

Ingredient(g)	10% PEG_{20000}	15% PEG_{20000}	20% PEG_{20000}	25% PEG_{20000}	30% PEG_{20000}	40% PEG_{20000}
CPC	0.5	0.5	0.5	0.5	0.5	0.5
Boric acid	1.5	1.5	1.5	1.5	1.5	1.5
Borax	0.3	0.3	0.3	0.3	0.3	0.3
PMA	0.002	0.002	0.002	0.002	0.002	0.002
PEG_{20000}	10	15	20	25	30	40
H_2O to	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml

Ingredient (g)	5% PF_{407}	10% PF_{407}	15% PF_{407}
CPC	0.5	0.5	0.5
Boric acid	1.5	1.5	1.5
Borax	0.3	0.3	0.3
PMA	0.002	0.002	0.002
PF_{407}	5	10	15
H_2O to	100 ml	100 ml	100 ml

2. Tonicity Measurement

2.1 The tonicity of each formulation was measured by an osmometer. The osmolality was recorded in milliosmoles/kg (Appendix B).

2.2 Formulations having feasible value of osmolality were selected in order to determine the stability at 60°C.

3. Stability Determination at 60°C

3.1 The feasible formulations from 2.2 were incubated at 60°C in an incubator and sampling at predetermined times. Then the remained concentration of CPC of each sample was analysed (Appendix C).

3.2 The remained concentrations of CPC were plotted against times and the ln's of the remained concentrations were also plotted against times to determine the order of reaction rate (5,46,47).

3.3 The remained concentrations versus times and the ln's of the remained concentrations versus times were treated by simple linear regression statistical technique. The order of reaction rate was determined (48) and the rate constant (k) of each formulation was compared.

3.4 The formulations of improving stability were selected.

4. Physical Properties Determinations

All formulations incubated at 60°C were measured of their physical properties pertaining pH,color,viscosity. The measurements were as follow

4.1 pH was recorded before and after being incubated at 60°C.

4.2 Color change was recored before and after being incubated at 60°C.

4.3 Viscosity was measured at 25°C by a glass capillary viscometer * (Ostwald Viscometer).

5. Stability at 40°,50°,55°C

5.1 The formulations of improving stability from 3.4 were incubated at 40°, 50°, 55°C and sampling at predetermined times. The remained concentration of CPC in each sample was analysed (Appendix C).

5.2 The remained concentrations were plotted against times. The ln's of the remained concentrations against times were also plotted to determine the order of reaction rate (5,46,47).

5.3 The remained concentrations versus times and the ln's of the remained concentrations versus times (of each temperature) were treated by simple linear regression statistical technique (48). Then the order of reaction rate and the rate constant (k) of each formulation at 40°, 50°, 55°C were calculated.

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* The glass capillary viscometer was chosen because PVP, PF_{407} were Newtonian flow and HPMC, PEG were recommended to use glass capillary by ASTM (49,50,51,52).

6. Determination for Arrhenius Equations: Plotting & Calculating

The rate constants (k) of each formulation at 40°, 50° , 55°C from 5.3 and 60° C from 3.2 were taken into natural logarithm (ln k) . The ln k, reciprocal of temperature in degree kelvin (1/T ,kelvin) were plotted for Arrhenius plot. The simple linear regression was calculated. The Arrhenius equations were achieved (48,49 Appendix D).

7. Calculation for heat of activation

The slope taken from Arrhenius equation was used in order to calculate the heat of activation by the method in Appendix D .

8. Calculated stability at 25° C and 8°C

8.1 Degradation rate constants at 25° C (k_{25}) and 8° C (k_8)

From Arrhenius equation, the natural logarithms of degradation rate constants at 25°C ($\ln k_{25}$) and 8°C ($\ln k_8$) were calculated (Appendix D). Then the $\ln k_{25}$ and $\ln k_8$ were converted to k_{25} and k_8.

8.2 Shelf life calculation

Since the content of CPC eye drops BPC 1973 is limited between 90 - 110 % labelled amount , shelf - life calculation according to BPC 1973 or BP 1980 will be the duration that the concentration drops from 110 % to 90 % labelled amount. On the other hand, the shelf - life for the concentration changes from 130 - 90 % labelled amount is accepted by the USP XXI. The calculation was shown in Appendix D.