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Appendix A

Preparation of Formulation

A.1 The formulations of CPC eye drops pH=6.0

Three CPC eye drops formulations were adjusted to pH = 6.0, they were BPC 1973 , 20% PEG₆₀₀₀ , and 25% PEG₆₀₀₀. Since PEG₆₀₀₀ did not change the pH of the formulations , the calculation would be applied only to adjust CPC eye drops BPC 1973 to pH = 6.0. The others two formulations were prepared by the same method and some water portions were replaced by PEG₆₀₀₀.

The objective of this experiment was to compare stability at pH = 6.0 and pH = 7.0, thus the total molarity of buffer was constant in both pH = 6.0 and 7.0. If the total molarity of buffer was not constant , the ionic strength might affect to the degradation rate constants, so the effect of pH could not be compared.

CPC eye drops BPC 1973 contained boric acid 1.5g/100ml and borax 0.3g/100ml , molecular weight of boric acid = 61.83 and borax = 381.4

$$\text{molarity of boric acid} = 15/(61.83) = 2.4260 \times 10^{-1} \text{ mole/1000ml}$$

$$\text{molarity of borax} = 3/(381.4) = 7.8658 \times 10^{-3} \text{ mole/1000ml}$$

$$\text{Total molarity} = (2.4260 \times 10^{-1}) + (7.8658 \times 10^{-3})$$

$$= 2.5047 \times 10^{-1} \text{ mole/1000ml}$$

$$\text{pH} = P_{ka} + \log [\text{Salt}]/[\text{Acid}] \quad (\text{eq.3})$$

If [Salt] and [Acid] in CPC Eye Drops BPC 1973 were replaced in above the equation.

$$\begin{aligned}
 \text{pH} &= 9.24 + \log [7.8658 \times 10^{-3}] / [2.4260 \times 10^{-1}] \\
 &= 9.24 + \log 0.0324 \\
 &= 9.24 - 1.489 \\
 &= 7.7508
 \end{aligned}$$

From calculation, pH of CPC eye drops BPC 1973 was 7.75 but actual pH from measurement was 7.0, thus it could not prepare the formulations adjusted to pH = 6.0 by calculation concentration of boric acid and borax by the equation 3.

From trial and error, if the pH of the equation 3 was 6.8, the actual pH of formulations was 6.0 measuring by pH meter.

The concentrations of boric acid and borax were calculated by the same equation.

$$\begin{aligned}
 \text{pH} &= \text{p}K_a + \log[\text{Salt}]/[\text{Acid}] \\
 6.8 &= 9.24 + \log[\text{Salt}]/[\text{Acid}] \\
 \log[\text{Salt}]/[\text{Acid}] &= -2.44 \\
 [\text{Salt}]/[\text{Acid}] &= 3.6308 \times 10^{-3} \\
 [\text{Salt}] &= [\text{Acid}] [3.6308 \times 10^{-3}] \\
 [\text{Salt}] + [\text{Acid}] &= 0.25047 \\
 [\text{Acid}] [3.6308 \times 10^{-3}] + [\text{Acid}] &= 0.25047 \\
 [1.0036308] [\text{Acid}] &= 0.25047 \\
 [\text{Acid}] &= 0.24956 \quad \text{mole/1000ml} \\
 \text{Boric acid} &= 0.24956 \times 61.83 \\
 &= \underline{15.4303} \quad \text{g/1000ml}
 \end{aligned}$$

$$\begin{aligned}
 [\text{Salt}] &= 0.25047 - 0.24956 \\
 &= 9.1 \times 10^{-4} \text{ mole/1000ml} \\
 \text{Borax} &= 9.1 \times 10^{-4} \times 381.4 \\
 &= \underline{0.3471} \text{ g/1000ml}
 \end{aligned}$$

Thus the formulation for CPC eye drops BPC adjusted to pH = 6.0 was

CPC	= 0.5	g
Boric acid	= 1.5430	g
Borax	= 0.0347	g
PMA	= 0.002	g
H ₂ O to	= 100	ml

A.2 Preparation of formulations containing HPMC

To prepare 100 ml of CPC eye drops, 30 ml of water was used to prepare a solution of HPMC and 60 ml was used to dissolve CPC, boric acid, borax and PMA.

A solution of HPMC was prepared by disperse powder of HPMC in 10 ml of hot water (80-90°C) with agitation until the particles of HPMC were thoroughly wetted and evenly dispersed. The remainder of water (20 ml) was added as cold water or ice, while maintaining agitation. The solution was cool in the refrigerator about 2 hours. The remainder of water (60 ml) was used to dissolved boric acid, borax and PMA with the aid of heat at 60°C. The CPC was then added and dissolved. After cooling the solution to room temperature, the solution of HPMC was added with agitation until the homogeneous mixture appeared. Then the volume was adjusted with water (literature

of Dow Chemical U.S.A., and Ref.72)

A.3 Preparation of formulation containing PVP and PEG

Boric acid , borax , PMA and CPC were dissolved into 70 ml of water as previously described. Then PVP or PEG was dissolved in the warm solution agitation until clear solution occurred.

A.4 Preparation of formulation containing PF₄₀₇

Boric acid , borax , PMA and CPC were dissolved into 80 ml of water as previously described. Then PF₄₀₇ was dissolved in the solution with agitation. The solution was then stored into the refrigerator over night.

All solutions were adjusted to 100 ml with water, sterilized by membrane filtration and filled into sterile ampules by aseptic technique.

Appendix B

Measurement of Tonicity

Tonicity is expressed by comparing with sodium chloride concentration. The acceptable range is 0.5%-2% sodium chloride solution (35,38,39,40).

It is a property of system that is not easily quantified, in normally it is expressed in the term of osmotic pressure. In the same way it is difficult to directly measure osmotic pressure. Other properties of solutions that depend on the concentration of particles (colligative properties) are usually measured and the osmolarity calculated. The properties usually measured are depression of the freezing point and the difference in temperature between a solution and pure solvent at the same vapor pressure. The measurements initially yield values in osmolality. This concentration is expressed in unit of milliosmoles of solute per kilogram of solvent (mOs/kg)

The below equations shows the relationship between osmolality and freezing point depression (73).

For dilute solution or ideal solution

$$\text{Freezing-point depression (ideal) in } ^\circ\text{C} = T_{fd} = 1.86 \times m_2 v \quad (\text{eq.4})$$

m_2 = molality of the solute (number of undissociated solute per 1000 g water)

v = number of particles into which the solute dissociates at complete dissociation.

$$\text{Ideal osmolality} = m_2 v \quad (\text{eq.5})$$

From equation 4

$$\text{Ideal osmolality} = T_{1d} / 1.86 \quad (\text{eq.6})$$

For real solution

$$\text{real osmolality} = \text{ideal osmolality} \times g \quad (\text{eq.7})$$

$$g \text{ (osmotic coefficient)} = T / T_{1d} = T / 1.86 m_2 v \quad (\text{eq.8})$$

$$T = \text{real freezing - point depression}$$

Replace (7) with (6) and (8)

$$\text{real osmolality} = (T_{1d} / 1.86) \times (T / T_{1d}) \quad (\text{eq.9})$$

$$\underline{\text{real osmolality}} = T / 1.86 \quad (\text{eq.10})$$

The equation 10 shows the relationship between freezing - point depression and osmolality. If the freezing point is known the osmolality will be calculated.

The freezing - point depression was measured by osmometer. The recorded freezing point was converted to a measurement in terms of milliosmolality (milliosmoles per kg, mOs/kg). All of formulations in this experiment were measured tonicity by osmometer and were compared with the acceptable tonicity range of the eyes (0.5% - 2% sodium chloride solution).

Since it is more practical to compare the tonicity in terms of milliosmolality, the acceptable tonicity range (0.5% - 2% sodium chloride solution) will be converted in terms of milliosmolality.

There are two methods to convert to milliosmoles/kg

1. The solutions of 0.5% and 2% are prepared measured osmolality by osmometer.
2. The osmolality is calculated by the equations and table in Ref. 72.

By the second method , the exceptable tonicity range (0.5% - 2.0% sodium chloride) is 160-630 milliosmoles/kg.

Appendix C

Analysis of CPC

1. Reagent

1.1 Mobile phase , methanol HPLC grade : H₂O (60:40)

1.2 Internal standard , propylparaben 0.4 mg/ml

1.3 Standard CPC solution contains CPC 0.375 mg/ml and propylparaben 0.08 mg/ml

2. Operating Condition of HPLC

Apparatus : HPLC LC - 3A , Shimadzu , Japan

Column : Zorbax ODS, 5 μ m, 25 cm x 4.6 mm ,
Du.Pont, P.N. 850952-702

Precolumn : Reverse phase , Zorbax ODS , Du.
Pont.

Mobile phase : Methanol : H₂O (60:40)

U.V. detector: 254 nm.

Flow rate : 1.0 ml/min.

Attenuation : 2⁵ mV/full scale

Pressure : 200 kg/cm²

Speed : 2 mm/min

Injection volume: 10 μ l

3. Standard CPC

3.1. Inject 10 μ l standard CPC solution into HPLC column 2 times at the beginning, 2 times at the middle and 2 times at the end of running.

3.2. Record the ratio of area under the peak of CPC and internal standard.

3.3. Calculate mean value, this mean value was used to calculate the concentration of samples.

4. Determination of CPC in Samples

4.1. Measure specific gravity of each formulations.

4.2. At suitable times, each formulations were taken out from incubator for two ampules, each ampules was analysed as below 4.3 - 4.6

4.3. Accurately weigh volume metric flask 10 ml

4.4. Fill it with sample about 1 ml and weight it again, the different weight is the weight of sample.

4.5. Fill 2 ml of internal standard and adjust volume with mobile phase and shake it well.

4.6. Inject about 10 μ l into HPLC cloumn 2 times, record chromatograms and ratio of area under the peak of CPC and internal standard.

4.7. Calculate mean value of ratio and then calculate concentration of each ampules.

4.8. From concentration of each ampules calculate mean concentration.

5. Calculation

A = mean ratio of peak area of CPC and of internal standard in standard CPC solution [1.3],[3.3].

B = mean ratio of peak area of CPC and of internal standard in sample solution [4.5],[4.7].

C = weight of sample

D = specific gravity of sample

If ratio = A ,it contains CPC = 0.375 mg/ml [1.3]

sample ratio = B ,it contains CPC = 0.375 x(B/A)mg/ml

Weight of sample = C ,specific gravity = D , so volume of sample = C/D ml

In 10 ml of sample solution in 10 ml volume metric flask contains CPC

$$= 0.375 \times (B/A) \times 10$$

$$= 3.75 \times (B/A) \text{ mg}$$

3.75 x (B/A) mg was picked from (C/D) ml so,

$$C/D \text{ ml of sample contains CPC} = 3.75 \times (B/A) \text{ mg}$$

$$1 \text{ ml of sample contains CPC} = 3.75 \times (B/A) \times (D/C) \text{ mg/ml}$$

$\text{Concentration of samples} = 3.75 \times (B/A) \times (D/C) \text{ mg/ml}$
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Appendix D

Calculation

D.1 Arrhenius relationship

The rate constant of a chemical reaction can be empirically related to the absolute temperature by Arrhenius equation (5).

$$k = Ae^{-E_a/RT} \quad (\text{eq.11})$$

$$\ln k = \ln A - (E_a/R) \cdot (1/T)$$

k = rate constant

A = constant

E_a = activation energy or heat of activation

R = gas constant (1.987 cal / mole / degree)

T = absolute temperature (degree kelvin = °C+273)

D.2 Estimation of rate constant at 25°C and 8°C

From the Arrhenius equation

$$\ln k = \ln A - (E_a/R) \cdot (1/T)$$

$\ln k_{25}$ was calculated by replacing T with $1/273+25$ and $\ln k_8$ was calculated by replacing T with $1/273+8$. The interval of $\ln k_{25}$ and $\ln k_8$ were calculated by statistical technique.

k_{25} , k_8 and the interval of them were found by taking off the natural logarithm.

D.3 Calculation of shelf-life (47)

In this experiment, the shelf-life at room temperature was

25°C and shelf-life in refrigerator was 8°C.

The content of CPC eye drops BPC 1973 and BP 1980 was limited between 90-110% labelled amount, the shelf-life according to BPC 1973 and BP 1980 will be the duration that the concentration drops from 110% to 90% labelled amount. The calculation from first order degradation reaction was the following.

$$\ln C_t = \ln C_0 - kt \quad (\text{eg.12})$$

$$\ln C_t - \ln C_0 = -kt$$

$$\ln (90) - \ln(110) = -kt$$

$$\ln 90/110 = -kt$$

$$\ln 0.81818 = -kt$$

$$t_{110-90} = 0.2007/k$$

The shelf-life according to BPC 1973 and BP 1980 were calculated by replacing k with k_{25} , k_8 and the interval of them.

On the other hand, the shelf-life according to USP XXI will be the duration that the concentration drops from 130% to 90% the equation was

$$t_{130-90} = 0.3677/k$$

The shelf-life according to USP XXI were calculated by the same method.

**VITA**

Miss Siriwan Ruengsawad was born on January 9, 1958. She got her degree in Bachelor of Science in Pharmacy in 1981 from Faculty of Pharmaceutical science, Chulalongkorn University.