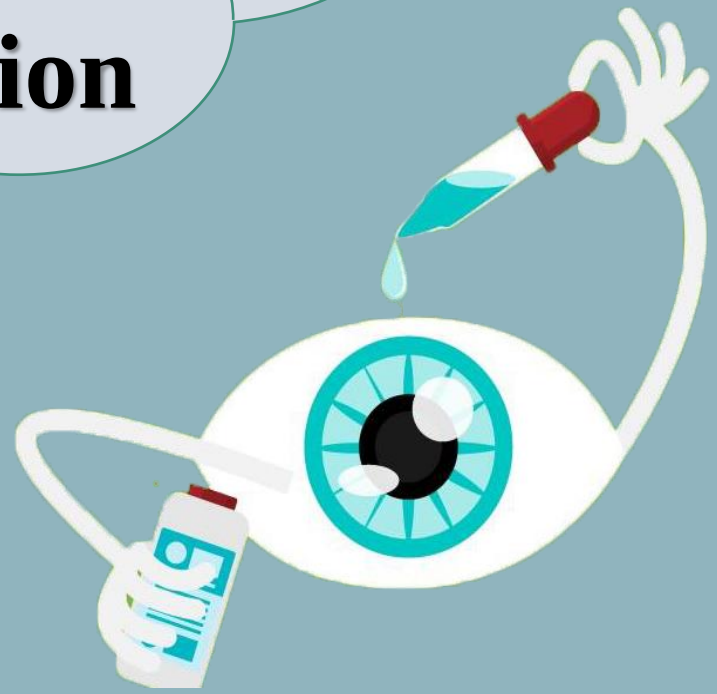
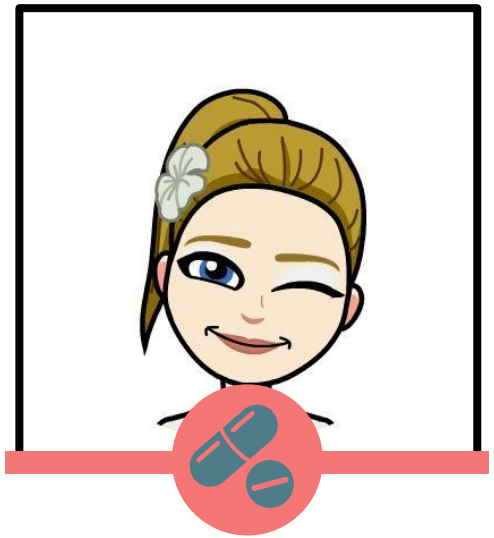
كلية الصيدلة
Faculty of Pharmacy

LIMU

The Physical, Chemical, and Microbiological Stability of Chloramphenicol Ophthalmic Solution





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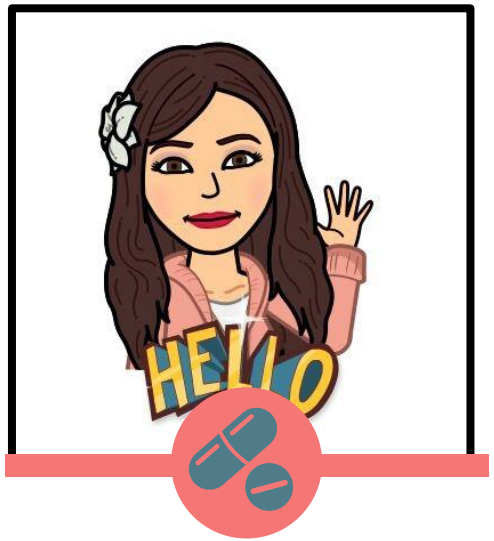

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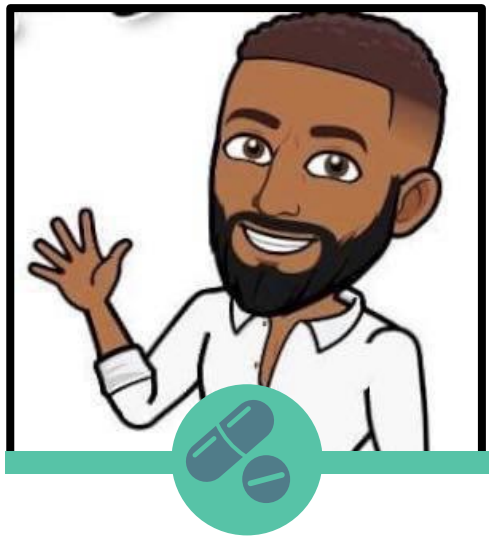

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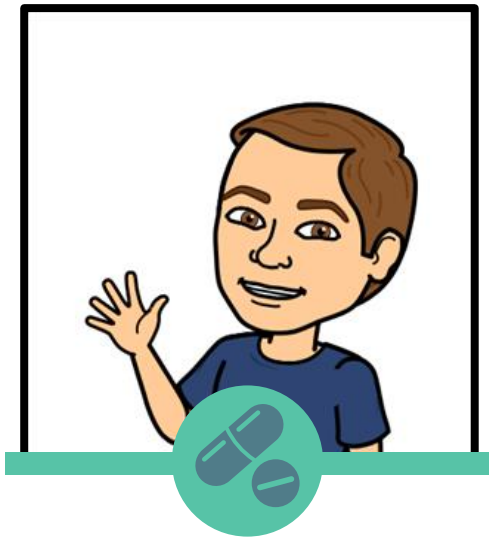

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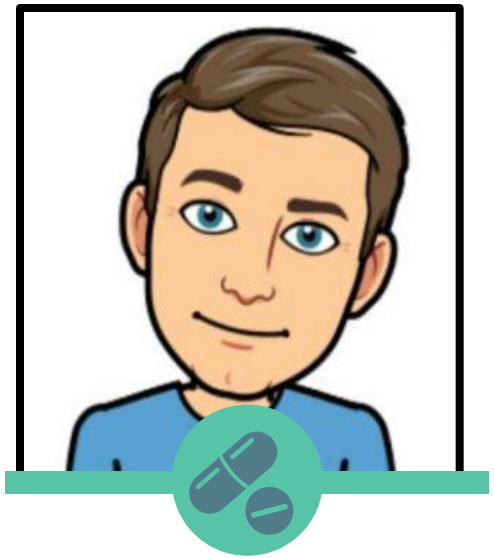

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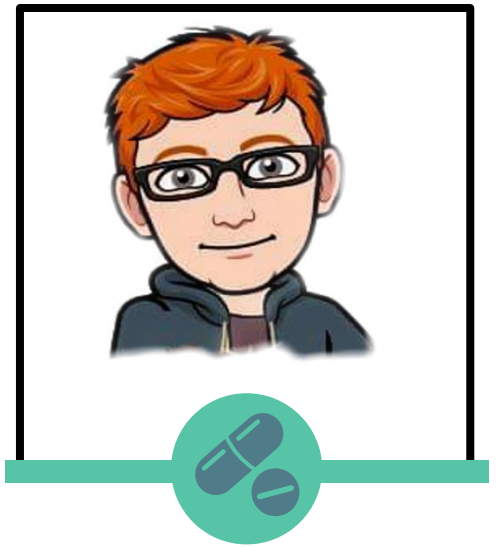

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The physical, chemical, and microbiological stability of chloramphenicol ophthalmic solution

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Introduction

Intended learning outcomes

Different evaluation method of chloramphenicol ophthalmic solution:

❖ Chloramphenicol dilution for diffusion method of assay:

Sample of 5 ml of drug solution was aseptically withdrawn from the container and transferred to 45 ml of sterile water solution to make up a stock solution from which several dilutions were made to produce final concentrations of 500 µg/ml, 50 µg/ml, and 2.5 µg/ml, respectively.

❖ Preparation of media Antibiotic assay by agar medium:

Ready-made medium for antibiotic assay was used for the preparation of the plate , at 121°C for 15 mins at 15 lbs. pressure.

❖ Soya bean casein broth for culturing reference *Escherichia coli* :

Used for measuring the potency assay of CH by autoclaving at 121°C for 15 mins at 15 lbs.

❖ Fluid thioglycolate medium:

Fluid thioglycolate medium is primarily intended for the culture of anaerobic bacteria. However, it is suitable for the culture of both fungi and aerobic bacteria depending on incubation temperature sterilized by autoclaving.

❖ Plate count agar (standard methods agar):

The plate count agar was used for the culturing of *E. coli* ATCC/10536 strain.

❖ Preparation of reference bacterial strain-*Escherichia coli* ATCC/10536:

The USP states that the test microorganism for CH assay is *E. coli* ATCC/10536, using the following procedure:

Reference strain of *Escherichia coli* ATCC No/10536 was provided by the Centre of Drug Control (Tripoli, Libya).

❖ Sample inoculation for cylinder-plate method (Diffusion method):

Samples of 50 ml of media were poured into square-petri dishes and allowed to solidify. The media was then occulated by swab with 0.2-ml of *E. coli* broth. Then, 0.1 ml volume of CH dilutions (as prepared in section 2.2.1) were transferred to the pores. The plates were incubated at 37°C for 24-hrs.

❖ Sterility testing:

Using the direct-inoculation method. Samples of 10 ml of sterile ophthalmic CH solution was aseptically handled and transferred into flasks-containing 100-ml of fluid thioglycolate media. The flasks were then incubated at 25°C and 37°C for 14-days for detection of growth of fungal and bacterial contamination, respectively.

❖ pH measurement:

The USP states that the pH should be in the range 7–7.5 except in case of unbuffered solution in which pH is within the range from 3 to 6 pH (pH meter).

❖ Assay of chloramphenicol content:

The absorbance was measured by spectrophotometrically at 278 nm.

❖ Calibration curve of chloramphenicol in purified water:

Then several samples from this stock solution were withdrawn and completed to a specific volume to produce five different concentrations. The absorbance of samples was then measured spectrophotometrically at 278 nm.

❖ Effect of storage temperature on the stability of chloramphenicol ophthalmic solution:

To evaluate the effect of storage temperature on the stability of CH solution, selected samples were stored at 2 temperatures: cold storage (4°C) and room temperature (25°C). The samples were stored at these conditions for 6 months, through which samples were analyzed every month for antibiotic assay, antibacterial activity, sterility, and pH measurement.

❖ Accelerated stability study:

Samples of CH ophthalmic solution, in its original packaging, were stored at 55°C using an oven (Memmert, Germany) for the period of 3 months and the samples were analyzed every month for antibiotic assay, antibacterial activity, and pH measurement as described above.

❖ Prediction of true shelf life of chloramphenicol ophthalmic solution:

Longland and Rowbotham have suggested that the concept based on Q10 values could be useful for estimating the shelf-life at room temperature of such products.

❖ Statistical analysis:

The analysis was carried out using Excel-2007 software statistical package (Microsoft, USA).

❖ Results:

- **Prediction of "true" shelf-life of chloramphenicol eye-drops:**

In manufacturing

Q_{10}	Temperature (C°)	Days remaining	Expiration date
2	12.6	431 days	April 6, 2016
	20	258 days	October 15, 2015
	27.4	155 days	July 4, 2015
3	12.6C	317 days	December 13, 2015
	20	140 days	June 19, 2015*
	27.4	62 days	April 2, 2015*
4	12.6	255 days	October 12, 2015
	20	91 days	May 1, 2015*
	27.4	33 days	March 4, 2015*

In pharmacy

Q_{10}	Temperature (C°)	Days remaining	Expiration date
2	12.6	360 days	May 26, 2016
	20	215 days	January 31, 2016
	27.4	129 days	October 7, 2015
3	12.6	264 days	February 19, 2015
	20	117 days	September 25, 2015
	27.4	52 days	July 22, 2015
4	12.6	212 days	December 29, 2015
	20	76 days	August 15, 2015
	27.4	27 days	June 28, 2015*

- **Analysis of chloramphenicol concentration during stability study:**

Chemical assay of chloramphenicol during the 6-months stability study

	Conditions	Assay (%)
July	CS	99.74±3.46
	RT	99.88±2.3
August	CS	98.04±1.40
	RT	98.5±1.71
September	CS	98.1±1.96
	RT	98.1±2.53
October	CS	98.18±1.64
	RT	98.84±1.86
November	CS	100.8±1.33
	RT	99.34±1.53
December	CS	100.8±1.45
	RT	100.8±1.96

- **Sterility testing during the 6-months stability study:**

The results indicate that the samples stored at room temperature or cold storage maintained their sterility anywhere the study. Neither bacterial or fungal growth was observed for the time of 6 months.

❖ Accelerated stability study on chloramphenicol eye-drops:

Measurement of zone of inhibition of Chloramphenicol

Concentration	December		January		February	
	4°C	55°C	4°C	55°C	4°C	55°C
5 mg/ml	3.65±0.07	3.65±0.07	3.65±0.07	3.3±0	3.95±0.07	3.5±0.14
500 mg/ml	3.05±0.07	3.05±0.07	3.2±0	2.25±0.07	3.3±0	2.8±0
50 mg/ml	2.35±0.07	2.25±0.07	2.45±0.07	1.15±0.07	2.55±0.07	1.85±0.07
Control	-	-	-	-	-	-
pH	7.42	7.20	7.42	7.10	7.42	6.92

❖ Discussion:

- It is well documented that CH solution should be stored at low temperature to maintain stability.
- We should use the Q10 mathematical model.
- The stability study was carried out for 6 months and included evaluation of sterility, measurement of MIC, and assay of active ingredient concentration.

- **The accelerated stability study was carried out employing an extreme of storage conditions using high temperature to accelerate the rate of change in the dosage form.**

- **The instability was also confirmed by production of color which increases in intensity with the progress of time at elevated temperature and accompanied by a drop in pH.**

❖ Conclusion:

The prediction model of Q10 value should be used with precaution as false conclusions may be derived. Based on the conducted experiments, it can be concluded that CH solution for ophthalmic administration could be stored at room temperature for a period of up to 6 months without any significant change in its activity.

❖ Reference:

Ahmed, W., Basharat, A., Ijaz, M.J., Kiran, A., et al. (2018). The physical, chemical, and microbiological stability of chloramphenicol ophthalmic solution. *Libyan In Med Univ J.* 3(2), pp.42-48.

Thank you