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Kinetics of Light Induced Degradation of Aqueous Solution of Chloramphenicol

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Abstract: Aqueous solution of chloramphenicol, plain and ophthalmic preparations were exposed to sunlight, ultraviolet radiation at 365 mm wavelength and red light for varying length of time. The kinetics of decomposition was studied using TLC techniques and U.V. Spectrophotometric methods of analysis of chloramphenicol and decomposing products. The rate of decomposition followed first rate reaction and the K value obtained were 3.386 x 10^{-2} h^{-1}, 3.149 x 10^{-2} h^{-1} and 0.0659 x 10^{-2} h^{-1} in sunlight, ultraviolet radiation and red-light, respectively. The respective half-life ($t_{½}$) of the decomposition was 20.47 h, 22.0 h and 1051.59 h. The average K value for the ophthalmic chloramphenicol preparations were 3.291 x 10^{-2} h^{-1} and 3.540 x 10^{-1} h^{-1} in sunlight and ultraviolet radiation respectively. The stability of chloramphenicol aqueous solution is established in the presence of red light.

Key words: Degradation, aqueos soluction, chloramphenicol

INTRODUCTION

Some pharmaceutical preparations exhibit chemical and physical instabilities leading to decomposition, degradation and deterioration of the formulation or chemical compound. Hence loss of therapeutic benefit of the drug. Such instabilities are either produced or catalysed by environmental factors-such as humidity, heat and light, through chemical process of hydrolysis, oxidation and photolysis[1].

Chemical stability studies may be conducted under normal storage or experimental operation conditions, or under exaggerated conditions (accelerated stability testing) and the result extrapolated to normal conditions[2,3]. The procedure usually involves the monitoring of the rate of decomposition of the parent compound or rate of formation of the degradation/ decomposition products[4].

Chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$) is a broad spectrum antibiotic with wide range of clinical application for systemic and topical use Lawrence and Korolkovas[5,6] Chloramphenicol eye drop is an aqueous sterile solution containing buffers, preservatives,-M-cresol, phenylmecuric benzokonium chloride and isotonicity agents[5].

Commercial chloramphenicol opthalmic preparations are packed in different types of containers, ranging from plain glass, transparent plastic, coloured plastic to amber coloured bottles. All such opthalmic preparation have air space above the solutions. Chloramphenicol aqueous solution (pH 5.4) at room temperature (29°C-30°C) degrades gradually in the presence of sunlight. The chemistry of the photodegradation products, however, suggested that under the influence of light and water, the drug undergo oxidation, reduction and condensation reactions.

The photolysis degradation products have been isolated and identified[7,8]. The degradation of chloramphenicol is therefore of mixed chemical pathways and mechanism, but initiated by light. The rate of decomposition of chloramphenicol may therefore be dependent on the source of light and the light intensity.

Knowledge of the kinetics of decomposition of chloramphenicol is essential in order to be able to access the storage conditions of the formulated aqueous solution and eye drops. The present study reports the kinetics of light influenced decomposition of chloramphenicol as aqueous solution and opthalmic formulations.

MATERIALS AND METHODS

Chloramphenicol eye drops of varying brands, Beltacol®, Optachlor®, Francol®, Elcee® MCA® were bought from pharmacy shops in Jos. Chloramphenicol authentic

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sample was obtained from Europharm Jos. Ethanol 96% (BDH chemical limited), Isopropanol, methanol, (Daykson chemical industries, Lagos, Nigeria), chloroform (May and Baker limited Dugenham, England) silica gel H254 (F.Woelm ICN Pharm. Gonbit and Co. W.Germany) were used for the analysis. A fixed wavelength 254 nm and 365 nm UV lamp; (Eagle Scientific Nottingham England) and red coloured bulb (Philips) purchased from an electrical shop in Jos, were used for the degradation process. A double beam spectrophotometer 200-20 (Hitachi Lted, Tokyo, Japan) was used to take spectrophotometric readings.

Pretreatment of chloramphenicol solutions: A 5 mL sample of the different test samples (commercial chloramphenicol eye drops coded A,B,C,D,E) and aqueous solution of the authentic sample were measured into sets of different conical flask. The samples were then exposed to different light sources-sunlight, ultraviolet light (365 nm) and red light at room temperature (29-31°C). Samples (100 μL) were drawn at 1, 2, 3, 4, 5, 6, 12, 24 and 168 h for analysis.

Qualitative analysis of chloramphenicol and degraded products: The solutions for exposure to light were contained in conical flasks, amber coloured bottle and quatz cuvet. In one set, the solution filled the containers to the brim (no space left for air) and in another set the containers were half filled.

Analytical Thin Layer Chromatographic (TLC) plates were spotted with the chloramphenicol solutions, reference sample in water and eye drops before and after exposure to sunlight, red bulb and UV lamp (365 nm) for varying length of time. The stationary phase was silica gel HF 254.

Two different mobile phases;

- Chloroform: Isopropanol (80:20) and
- Chloroform: Carbontetrachloride (2:1) were used in developing the plates.

After developing, the plates were air dried at room temperature for about 10 min, separated spots were identified by viewing under ultraviolet light at 254 nm. Rf values for chloramphenicol and degraded products were calculated from the ratio of the distance moved by sample to the distance of the solvent front.

Quantitative estimation of residual chloramphenicol: Residual chloramphenicol was estimated using modified method of Shih[8]. Preparative TLC chromatographic plates were spotted with 100 μL of the various samples, using a 100 μL capacity capillary pipette. The plates were developed for 30 min using the chloroform: Isopropanol (80:20) mobile phase. The plates were air-dried and the chloramphenicol spots identified under UV light and scraped off into a test tube. 10ml of ethanol (96%) was added to the test tube shaken and allowed to stay in the dark over night. The ethanolic solution was centrifuged at 2000 g for 5 mins. The supernatant was decanted and the residual chloramphenicol estimated using a double beam ultraviolet spectrophotometer at wavelength 278 nm.

Concentration of chloramphenicol was estimated from the calibration curve and the percentage drug remaining (residual chloramphenicol) was calculated from the formula

$$\frac{\text{Concentration obtained}}{\text{Expected concentration}} \times 100$$

The rate of decomposition K was calculated from the slope of the plot of residual concentration of chloramphenicol against time on a semi log (3-circles) graph sheet. Half life of the composition was calculated from the relationship $t_{½} = 0.693/K$. First order rate of decomposition was assumed.

RESULTS AND DISCUSSION

Qualitative assay: The colourless chloramphenicol aqueous solution (reference sample and chloramphenicol eye drops) changed to orange yellow colour solution, with precipitation on exposure to sunlight and UV light in the presence of air. The colour change was light cream with red light and in containers filled to the brim.

Chromatographic TLC analysis of the exposed chloramphenicol solution showed two degraded products with Rf values of 0.19 and 0.12 with mobile phase, Chloroform: Isopropanol (80: 20) and Rf values 0.17, 0.61 with mobile phase, Chloroform: Carbon tetrachloride (2:1).

This supports the finding of Shih[1] who also reported two degraded products on exposure of chloramphenicol solution to light. There was no evidence of degradation in the reference sample solution.

Quantitative assay/kinetics: Tables 1, 2 and 3 show the effect of sunlight, ultraviolet light (365 nm) and red light on chloramphenicol aqueous solution samples, reference sample and eye drops in the presence of air, respectively.

Table 1: The effect of direct sunlight (Temp. 32-35°) on the percentage drug content of chloramphenicol in aqueous solution samples, reference sample and eye drops

	Percentage drug content (%)							
Time (h)	0	1	2	3	6	12	24	168
Ref	100	45	23.0	22.3	16.6	12.1	7.5	0.5
A	65	43	40.0	34.0	26.8	20.8	14.8	3.0
B	31	28.6	23.0	19.0	18.0	4.0	4.0	1.2
C	80	70	43.0	42.0	28.0	12.0	5.0	2.4
D	95	84	44.6	42.0	31.0	22.0	12.0	0
E	60	44.4	40.0	33.2	20.0	5.8	2.2	0

Table 2: The effect of U.V light 365nm (Temp. 30°C-31°) on the percentage drug content of chloramphenicol in aqueous solution, reference sample and eye drops

	Percentage drug content (%)							
Time (hr)	0	1	2	3	6	12	24	168
Ref	100	90	84	84	75	47	31	2.2
A	65	-	40	36	25	24	7	4
B	31	30	27	20	17	14	6.2	0
C	80	70	46	43.4	29.4	16	6.2	0
D	95	95	92	80	60	38	19	1.2
E	60	42	40	36.4	24	20	5.8	0

Table 3: The effect of red light (Temperature 30°C-31°C) on the percentage drug content of chloramphenicol aqueous solutions, reference sample and eye drops

	Percentage drug content (%)							
Time (hr)	0	1	2	3	6	12	24	168
Ref	100	100	100	100	91	90	89.4	87.6

Table 4: Rate constant (K) of decomposition and half-life ($t_{½}$) of chloramphenicol in aqueous solution under sunlight, UV light and red light

	Percentage drug content (%)					
	Sunlight		UV light		Red light	
Sample	K x $10^{-2}h^{-1}$	t½ (hr)	K x $10^{-2}h^{-1}$	t½ (hr)	K x $10^{-2}h^{-1}$	t½ (hr)
Ref	3.386	20.47	3.149	22.00	0.0659	1052.2
A	3.518	19.7	3.758	18.44	-	-
B	2.747	25.23	3.568	19.42	-	-
C	3.390	20.43	3.753	18.47	-	-
D	2.919	23.74	3.220	21.53	-	-
E	3.880	17.86	3.403	20.36	-	-

The percentage drug contents of the samples just before exposure were, 100% reference sample (Rs); A 65, B 31, C 80, D 95 and E 60%. The BP (1993) however stipulated a percentage drug content of 90-110% for chloramphenicol eye drops[9].

The percentage drug content of chloramphenicol in all the samples decrease with time of exposure. The first order kinetics was assumed for the rate of photo degradation in this study (Fig. 1).

The reaction is first order with respect to either the UV source or the chloramphenicol eye drops. Step I is the slkowest step (rate determining step) when the reaction is first order with respect to UV source while step 2 is the slowest step (rate determining step) when it is first order with respect to chloramphenicol where the decomposition of the photo excited chloraphnenicol leads to formation of a free radical. This step is immediately followed by an α-elimination of a hydrogen radical to form a very reactive specie (a carbene). The caebene readily adds hydrogen molecule from the atmosphere to form the methyl.

Step 2 and 3 could be concerted i.e both reaction taken place at the same time leading to the formation of carbene and Hcl.

Photochemical reactions are mainly free radical processes and in this study, chloride ions are the most labile to photodegradation, hence elemental analysis of the samples before and after exposure confirms the presence of chloride ion in all samples before exposure and absence after exposure which also confirms that photo chemical reaction had taken place.

Table 2 showed the rate constant K for the degradation, irrespective of the mechanisms involved in the degradation. The rate constants of the authentic reference sample are 3.386 x $10^{-2}h^{-1}$, 3.149 x $10^{-2}h^{-1}$ and 0.0659 x $10^{-2}h^{-1}$ in sunlight, ultraviolet light (365nm) and red light, respectively with half-lives of degradation as 20.47 h, 22 h and 1052.2 h, respectively.

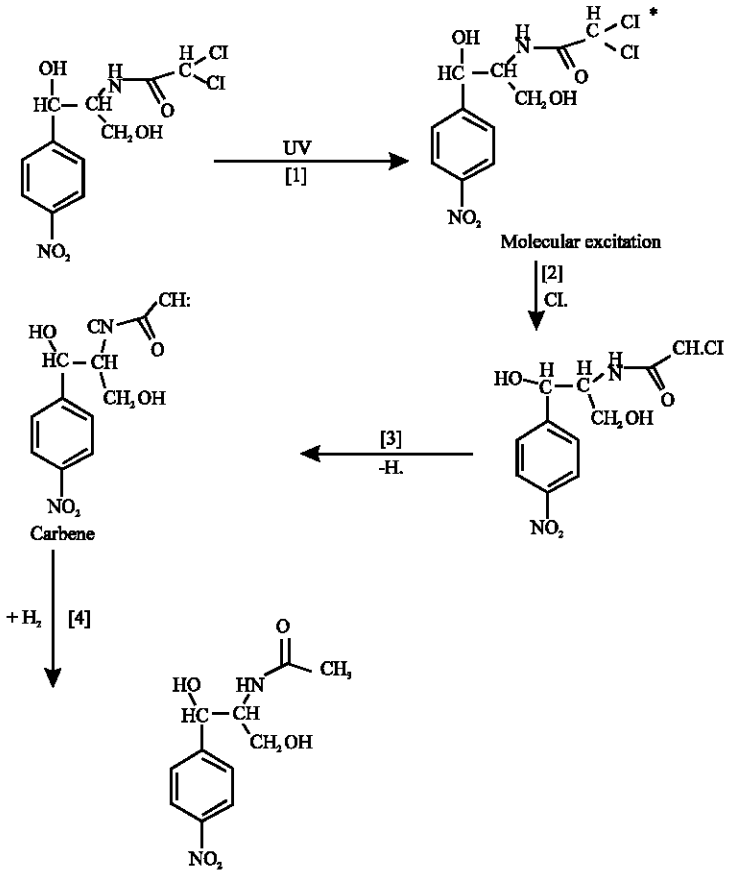

Fig. 1: Photodegradation of chloramphenicol

The chloramphenicol eye drop samples had K values of between 3.880 to 2.747 x $10^{-2}h^{-1}$and 3.758 to 3.149 x $10^{-2}h^{-1}$.

Excipients in the chloramphenicol eye drops did not show any particular effect on stability of the chloramphenicol. The K values of sample in direct sunlight did not differ significantly from those obtained with ultraviolet light. The K, half-life value obtained in this study may infer that chloramphenicol is about 50 times as stable in red light than in sunlight or ultraviolet light. The K values of chloramphenicol eye drops subjected to sunlight or ultraviolet light in the presence of air at room temperature (29°C-31°C) confirms the light susceptibility of chloramphenicol aqueous formulations. While K values and half life value in red light showed that chloramphenicol is more stable in the presence of red light.

CONCLUSION

In conclusion, this study further confirms the suitability of coloured and amber coloured-bottles for dispensing chloramphenicol over colourless bottles and also in airtight containers.

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