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A note on the stability of actinomycin D

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Visible absorption spectra and an agar-diffusion microbiological assay with *Staphylococcus aureus* were used to examine the effects of temperature, time, and pH on the stability of actinomycin D in distilled water, Clark and Lubs buffer solutions, and plasma. The most stable storage pH range for actinomycin D in buffer solution is 5 to 7. Actinomycin D in distilled water and stored 5° C (refrigeration) preserved its stability for at least 150 days. Except in buffer at pH 5, the antibiotic is appreciably degraded by autoclaving.

THE antibiotic actinomycin D, a dipeptide derivative of the chromophore 2-amino-4,5-dimethylphenoxazin-3-one-1,8-carboxylic acid (Bullock & Johnson, 1957; Johnson, 1960) has a broad spectrum of biological activity as evidenced by bacteriostatic action against gram-positive bacteria and some fungi (Brockman, 1954; Foley, 1955; Pugh, Kutz & Waksman, 1956; Slotnick, 1957), cytotoxicity produced in many mammalian lines in cell culture (Eagle & Foley, 1956; Goldstein, Slotnick, Hillman & Gallagher, 1959), and antineoplastic activity against experimental tumours in rodents (see, for example, Garatti, Costa, Murelli, Palma & Vegeto, 1956; Sugiura, Stock, Reilly & Schmid, 1958).

Although chemical and microbiological changes of actinomycin D have been described (Bullock & Johnson, 1957; Brockman, 1960; Johnson, 1960; Katz, 1960), to date there is no information on the stability of this agent under usual laboratory storage conditions. The purpose of this study was to investigate the effect of varied pH and temperature levels over extended periods of time on the stability of actinomycin D in aqueous solution.

Experimental

Actinomycin D concentrated stock solutions, 300 μg/ml, were prepared from dried powder‡ and refrigerated sterile distilled water. Subsequent dilutions were prepared at pH 1, 3, 5, 7, 9, 11, such that the final dilution contained 30 μg/ml. Clark and Lubs buffers (Remington, 1961) were used except for the solutions at pH 1·0 and 11·0, for which 0·1N hydrochloric acid and 0·1N sodium hydroxide were used respectively.

Solutions were stored at −10°, 5° (refrigeration), 25° (room temperature) and 37° and were assayed at zero, 6, 14, 29, 55 and 150 days, by spectrophotometric and microbiological methods. In addition, solutions of actinomycin D at each pH level were autoclaved for 20 min at 15 pounds pressure and assayed.

SPECTROPHOTOMETRIC ASSAY

The spectral range observed was over 500–360 mμ, curves at zero time for each solution of actinomycin D were used as a point of reference.

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MICROBIOLOGICAL ASSAY

A standard agar diffusion paper-disc assay using *Staphylococcus aureus* was made on each solution. An actinomycin D solution of 30 μg/ml in sterile distilled water (pH 7·0) was used as control for each assay. The percentage inhibition was based on the comparison of zones of inhibition of the test samples to the standard solution.

STABILITY OF ACTINOMYCIN D IN PLASMA

Plasma solutions of actinomycin D (30 μg/ml) were prepared aseptically from fresh sterile human plasma. These solutions were held at 37° and assayed at zero time, 7 and 14 days, microbiologically and spectrophotometrically.

Tables 1 and 2 list the result of the spectrophotometric and microbiological assays.

TABLE 1. SPECTROPHOTOMETRIC ASSAY OF SOLUTIONS OF ACTINOMYCIN D

pH	Temp. °C	6 days	14 days	29 days	55 days	150 days
1	−10	*0·89	0·87	0·95	S	S
	5	S	S	S	S	S
	25	S	S	S	S	S
	37	S	S	S	S	—
3	−10	0·95	—	0·95	0·85	0·85
	5	0·97	0·95	0·93	0·85	S
	25	0·87	0·83	S	S	S
	37	0·83	S	S	S	—
5	−10	0·98	0·95	0·96	0·88	0·88
	5	0·98	0·98	0·96	0·96	0·91
	25	0·98	0·98	0·96	0·91	0·77
	37	0·98	0·97	0·93	0·91	—
7	−10	0·92	0·92	0·92	0·80	0·82
	5	1·0	1·0	0·97	0·95	0·92
	25	0·97	0·97	0·95	0·95	0·85
	37	0·95	0·92	0·90	0·87	—
9	−10	0·95	0·90	—	0·70	0·82
	5	0·78	0·70	0·61	0·42	S
	25	0·20	S	S	S	S
	37	S	S	S	S	S
11	−10	0·71	S	S	S	S
	5	S	S	S	S	S
	25	S	S	S	S	S
	37	S	S	S	S	S
Distilled water	5	1·0	1·0	1·0	1·0	0·95

* Values in table are fractional representations of the optical density maximum of the sample as compared to the zero time standard which was taken as unity.
S indicates shifts in spectral curves which did not allow comparison with standard maximum absorbance readings.
— Indicates data could not be recorded.

EFFECT OF pH

The most stable storage pH range between 5 and 7. As pH level was increased above 7 there was a more rapid alteration in spectra and more rapid decrease in microbiological activity than when pH level was decreased below 5.

Solutions of actinomycin D changed from a dark golden brown colour at pH 1 to colourless at pH 11.

TABLE 2. MICROBIOLOGICAL ASSAY OF SOLUTIONS OF ACTINOMYCIN D

pH	Temp. °C	6 days	14 days	29 days	55 days	150 days
1	−10	*1·0	1·0	0·95	0·93	0·72
	5	1·0	0·82	0·76	0·65	—
	25	0·79	—	—	—	—
	37	—	—	—	—	—
3	−10	1·0	1·0	0·95	0·95	0·94
	5	1·0	1·0	0·93	0·98	0·69
	25	0·95	0·85	0·65	—	—
	37	0·87	0·70	—	—	—
5	−10	1·0	0·95	0·98	1·0	0·96
	5	1·0	1·0	0·98	0·93	0·84
	25	1·0	1·0	0·95	0·88	0·81
	37	1·0	1·0	0·91	0·88	—
7	−10	1·0	0·98	1·0	1·0	0·98
	5	1·0	1·0	0·98	0·94	0·88
	25	1·0	1·0	0·95	0·88	0·83
	37	1·0	1·0	0·93	0·88	—
9	−10	1·0	1·0	0·98	0·95	0·94
	5	1·0	0·95	0·88	0·81	—
	25	0·87	0·70	—	—	—
	37	—	—	—	—	—
11	−10	0·67	0·67	—	0·67	—
	5	—	—	—	—	—
	25	—	—	—	—	—
	37	—	—	—	—	—
Distilled water	5	1·0	1·0	1·0	1·0	1·0

* Zones of inhibition, measured in mm, have been converted to a decimal basis with inhibition at zero time an arbitrary 1·0. All other readings are compared to fractional inhibition of control.
— Indicates data could not be recorded.

EFFECT OF TEMPERATURE

With temperature rise, solutions buffered at pH 7 and stored at −10° showed a significant spectral alteration after 7 days while buffered solutions stored at 5° were stable for the same period of time. On the other hand, this was not observed with unbuffered aqueous solutions of actinomycin D treated in the same manner. We have no explanation for this phenomenon.

Solutions of actinomycin D which were autoclaved, degraded significantly except at pH 5·0. At this pH, spectra and microbiological activity remained similar to the standard (Table 3).

TABLE 3. SPECTROPHOTOMETRIC AND MICROBIOLOGICAL ASSAY OF SOLUTIONS OF ACTINOMYCIN D AFTER AUTOCLAVING

Buffer solution pH	After autoclaving	
	Spectrophotometric	Microbiological
1	0·89	—
3	0·83	0·89
5	0·95	1·00
7	0·73	0·80
9	0·00	—
11	0·00	—
In water about pH 7	0·75	0·76

All solutions before autoclaving were assayed microbiologically and spectrophotometrically and given values of 1·0. Cf. Tables 1 and 2.

STORAGE IN AQUEOUS SYSTEM

Solutions of actinomycin D (30 μg/ml) in distilled water at pH 7·0 at 5° exhibited the greatest storage stability (Tables 1 and 2).

Plasma solutions of the antibiotic held at 37° were stable for 7 days. After this period of time, the plasma became cloudy and further spectral analysis was not feasible.

Conclusion

For the preservation of stability of actinomycin D in solution, it is suggested that actinomycin D be solubilised in refrigerated sterile distilled water and stored at refrigeration temperature. The suggested time of storage for optimal stability would be not more than 120 days.

Solutions of actinomycin D should not be sterilised by autoclaving.

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