A NOTE ON THE STABILITY OF OPHTHALMIC SOLUTIONS CONTAINING PILOCARPINE HYDROCHLORIDE ALONE AND WITH ESERINE

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The stability of pilocarpine hydrochloride in ophthalmic solutions after heating and storage, and the influence of light and of two types of containers on stability, have been investigated. Pilocarpine was assayed by a modification of a published method. In ophthalmic solutions sterilised by heating to 100° for 30 min. it is stable for at least one year at 15–25° in the dark in bottles of good quality glass covered with a nylon cap. The stability of ophthalmic solutions containing pilocarpine and eserine during storage and after different methods of sterilisation was also investigated. This solution can be heated at 100° for 15 min. and, stored in suitable bottles, it is stable for at least 18 months.

ACCORDING to the Swedish Pharmacopoeia, solutions of pilocarpine hydrochloride shall be sterilised by heating at 100° for 1 hr. *The Extra Pharmacopoeia* (1958) recommends sterilisation by autoclaving. According to Riegelman and Vaughan (1958), the substance withstood 24 hr. of autoclaving (120°) with approximately 5 per cent destruction. No reports have been published on its stability in solutions upon protracted storage after sterilisation by heating or by autoclaving.

Eserine is unstable; its greatest stability was found by Hellberg (1949b) to be at pH values of 6 or lower. A 0·4 per cent solution of eserine salicylate in 2 per cent boric acid was stable for 100 days at 25°. Hellberg also considered that eserine solutions could not be sterilised by heating, whatever the pH value. Schradie and Miller (1959) have shown that it is possible to apply a solution with a pH of about 3 without damaging the cornea, if the solution does not have an unduly high buffer capacity. As Hellberg (1949b) indicated that the stability of unheated solutions of eserine might be greater at lower pH values, stability in the pH range 3·5–4·0 has been studied.

EXPERIMENTAL

Effect of Light on the Stability of Pilocarpine Solutions

Two glass-stoppered volumetric flasks were filled with an 0·2 per cent aqueous solution of pilocarpine hydrochloride. One was stored in daylight and the other in the dark for 21 months at 15–25°, after which the pilocarpine content of each was determined.

Effect of Heating on the Stability of Pilocarpine Solutions

An ophthalmic solution of pilocarpine hydrochloride prepared according to the Swedish Pharmacopoeia (Ed. XI), (aqueous solution of 2 per cent pilocarpine hydrochloride with sodium chloride 0·4, and phenyl mercuric nitrate 0·001 per cent) was dispensed in glass ampoules or in

the containers described by Linde (1957). These were of good quality glass with a cap of a polyamide, related to nylon, the top of which is shaped as a tube suitable for giving drops. The cap is closed with a stopper permanently connected to the container.*

Half the containers and ampoules were autoclaved at 110° for 30 min. (Swedish Pharmacopoeia method) and stored in the dark at room temperature for 12 months. The rest were similarly stored without autoclaving.

Pilocarpine content and pH as well as the optical rotation of the solutions were determined before and after heating and storage.

Stability of Pilocarpine and Eserine Solutions

It has been considered possible to stabilise eserine solutions with a reducing agent but, as noted by Hellberg (1949a) and verified by us, the frequently used sodium bisulphite ($NaHSO_3$) causes a low pH, hence it was replaced by ascorbic acid. The ophthalmic solution studied had the composition: pilocarpine hydrochloride, 2; eserine salicylate, 0·2; sodium chloride, 0·1; ascorbic acid, 0·1 g.; sterilised water to 100 ml. It was dispensed in ampoules and the containers described by Linde (1957) and was sterilised by filtration or heating at 100° for 15 min. The content of pilocarpine and eserine was determined immediately and 18 months after sterilisation.

The storage conditions were: (i) in a refrigerator at 4–6°; (ii) in a dark room at 12–15°; (iii) protected from light in a place where the temperature varied with the outdoor temperature from about −10 to +30°.

Assay of Pilocarpine

Pilocarpine can be determined photometrically as the perchromate, a blue compound formed by treating a slightly acid solution of pilocarpine with potassium dichromate and hydrogen peroxide. The method adopted was a modification of that of Levine and Horrocks (1960).

The sample (2·00 ml., equivalent to about 4 mg. of pilocarpine hydrochloride) is pipetted into a 125 ml. separating funnel. 20 per cent acetic acid AR (1·0 ml.), chloroform AR (10 ml.) and 5 per cent potassium chromate AR (1 ml.) is added and then, rapidly, 3 per cent hydrogen peroxide AR (2·0 ml.) by syringe. The funnel is immediately shaken vigorously for 90 sec. The chloroform phase is filtered into a 25 ml. volumetric flask. The extraction is repeated with chloroform (10 and then 5 ml.) and the filter is washed with small portions of chloroform, which are added to the combined chloroform extracts until the flask is made up to the mark. The solutions are protected from light before measuring. The extinction is determined within 20 min. at 560 mμ and the corresponding amount of pilocarpine read from a standard curve prepared from pilocapine solutions of known content. A straight line passing through the origin was obtained within the sample range 0·2–0·7 mg. of pilocarpine.

This procedure is also suitable in presence of eserine.

* "Sonyl" bottle.

Assay of Eserine

Eserine was determined by a modification of a method described by Hellberg (1949a).

The sample (4·00 ml. containing about 8 mg. of eserine salicylate and 80 mg. of pilocarpine hydrochloride) is pipetted into a separating funnel. Sodium carbonate (M, 1 ml.) is added and the solutions rapidly extracted with peroxide-free ether (4 × 40 ml.). The ether phases are siphoned from the funnel, combined, dried with anhydrous sodium sulphate and filtered. Sulphuric acid (0·1N, 10·0 ml.) is added and the ether is evaporated under vacuum in a rotating evaporator in a water-bath at 35°. The remaining solution is transferred to a 25 ml. volumetric flask and water is added to the mark. 10·00 ml. is pipetted into a 50·0 ml. volumetric flask, sodium hydroxide (M, 1·9 ml.) is added, and after standing for 15 min. the mixture is gently shaken mechanically about 80 times/min. Potassium dihydrogen phosphate, (0·1M, 20 ml.) is then added, the solutions made up to the mark and the extinction determined at 500 mμ. The corresponding amount of eserine is read from a standard curve prepared by the same procedure. A straight line passing through origin is obtained within the concentration range of 1–4 mg./ml. of eserine salicylate.

Results and Discussion

A 0·2 per cent aqueous solution of pilocarpine hydrochloride showed no loss after 21 months in the dark and only 5 per cent loss after the same period in the light. The results of the other stability experiments are shown in Tables I and II. The optical rotation was unchanged after heating and storage for 12 months, indicating no transformation to isopilocarpine. Thus pilocarpine withstands heating at 110° for 30 min. and is stable for at least one year when stored at room temperature in the dark, even after being subjected to heat treatment.

TABLE I

STABILITY OF PILOCARPINE HYDROCHLORIDE AFTER HEATING AT 110° FOR 30 MIN.

Solution	Residual per cent pilocarpine hydrochloride		pH values of solutions			
	After storage for 12 months without previous heating	After heating and storage for 12 months	Before heating	Immediately after heating	After storage for 12 months without previous heating	After heating and storage for 12 months
Oculoguttae pilocarpini (Ph.S. Ed XI) stored in bottles ..	97·0	97·0	4·2	3·5	3·4	3·5
Oculoguttae pilocarpini (Ph.S. Ed XI) stored in ampoules ..	97·0	97·0	4·2	3·7	3·5	3·5

According to Riegelman and Vaughan (1958), a solution of eserine salicylate containing sodium bisulphite and a small amount of citric acid can be sterilised by autoclaving. This could not be verified in the present investigation. Heating to 120° for 20 min. caused 25 per cent loss of

eserine. The only possible method proved to be heating to 100° for 15 min. A preservative, e.g. phenylmercuric nitrate, should therefore be added. The sterility of the solution should be checked by a bacteriological test such as prescribed in the Swedish Pharmacopoeia for aseptically prepared solutions.

TABLE II

STABILITY OF PILOCARPINE AND ESERINE IN OPHTHALMIC SOLUTIONS STERILISED BY FILTRATION OR BY HEATING TO 100° FOR 15 MIN. AND STORED FOR 18 MONTHS UNDER DIFFERENT CONDITIONS

Storage conditions			pH values			Residual per cent eserine salicylate after 18 months	Residual per cent pilocarpine hydrochloride after 18 months
Temp. °C	Type of containers	Sterilising methods*	Before heating	Immediately after heating	After 18 months		
4–6	Bottle	A	—	—	3·4	98	101
		B	3·5	3·5	3·4	97	—
12–15	Ampoules	A	—	—	3·3	100	101
		B	3·5	3·5	3·3	100	102
	Bottle	A	—	—	3·5	101	—
		B	3·5	3·4	3·4	98	—
−10 to +30	Ampoules	A	—	—	3·2	100	103
		B	3·5	3·5	3·2	100	—
	Bottle	A	—	—	3·2	99	—
		B	3·5	3·4	3·2	100	101

A = filtration.
B = heating to 100° for 15 min.

As seen from Table II, the solution can be heated at 100° for 15 min. Stored in the containers described by Linde (1957), it is stable for at least 18 months even under greatly varying temperature conditions.

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