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A note on the determination of pilocarpine in solutions containing methylcellulose

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Low results obtained by a colorimetric method for the determination of pilocarpine in ophthalmic solutions are attributed to pilocarpine-methylcellulose interaction. Low results were not obtained with a second colorimetric method. Other factors affecting this interaction are discussed.

ANALYSIS of locally manufactured pilocarpine ophthalmic solutions by the procedure of Webb, Kelley & McBay (1952) yielded unusually low results, a phenomenon previously reported by Levine & Horrocks (1960). Examination of the manufacturer's formula suggested methylcellulose 4000 (0·33%) might be responsible, and this was confirmed in preliminary experiments, in which ingredients were omitted one by one.

Application of the Elvidge (1947) method to the same solutions gave total initial concentration of pilocarpine, whether or not methylcellulose was present. Using both the method of Webb & others, and the Elvidge method, a number of ophthalmic preparations of two manufacturers were assayed for pilocarpine content and compared with a set of control solutions of the same concentrations.

Experimental

The solutions were assayed as described by Webb & others (1952)* and by Elvidge (1947), all ophthalmic solutions and control solutions being diluted to contain 2 mg/ml of pilocarpine hydrochloride.

Results and discussion

Calibration curves, constructed using the Webb method, for (i) 1% pilocarpine hydrochloride in water and (ii) 1% pilocarpine hydrochloride in 0·33% methylcellulose 4000 solution, confirmed that the Webb method gave low results in the presence of methylcellulose. Preliminary experiments with the Elvidge procedure showed that the methylcellulose had no effect and a figure representing total initial concentration of pilocarpine hydrochloride was obtained. The methylcellulose must therefore interact with or "bind" a portion of the pilocarpine and the Webb method probably determines only the free or unbound pilocarpine.

The difference in results given by the two methods can be explained on the basis of the chemical reactions involved. Helch's (1902) test, the basis of the Webb method, causes formation of a coloured salt and does not otherwise affect the pilocarpine molecule. If the basic centre of the pilocarpine were involved in the interaction with methylcellulose, the

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* The amount of hydrogen peroxide added is critical (Levine & Horrocks, 1960) and a fresh solution was prepared each day.

relatively mild conditions of the Helch test probably would not affect that amount of the pilocarpine bound to the methylcellulose. The mechanism of the Ekkert (1925) test (the basis of the method described by Elvidge) has not been described but this type of qualitative test usually involves a specific portion of the structure. Also, since the Ekkert test takes place under conditions more likely to reverse any pilocarpine–methylcellulose interactions, total assay is achieved whether the pilocarpine is initially bound or not, or even if it remains bound to the methylcellulose through the part of the molecule remote from the site attacked by the test reagents.

Using the two methods, commercial ophthalmic pilocarpine solutions from two manufacturers and a set of control solutions prepared by ourselves were examined for pilocarpine hydrochloride content. The results are summarized in Table 1. The control solutions contained the stated concentration of pilocarpine hydrochloride in 0·33% methylcellulose solution.

TABLE 1. DETERMINATION OF PILOCARPINE IN SOLUTIONS CONTAINING METHYL CELLULOSE

Source	Labelled strength (%)	Webb method (Free pilocarpine)		Elvidge method (Total pilocarpine)		mg/ml bound
		% labelled strength	mg/ml	% labelled strength	mg/ml	
Manufacturer A	0·5	92·5	4·65	101	5·05	0·4
	1·0	93·8	9·4	103	10·3	0·9
	2·0	95·0	19·0	104	20·8	1·8
	3·0	95·0	28·5	107	32·1	3·6
	4·0	97·5	39·0	102·5	41·0	2·0
Manufacturer B	0·5	45	2·25	103·5	5·18	2·9
	1·0	71	7·1	105	10·5	3·4
	2·0	82	16·4	102·5	20·5	4·1
	3·0	75	22·5	106	31·8	9·3
	4·0	96	38·4	105	42·0	3·6
Control Solutions	0·5	82·5	4·13	99	4·95	0·8
	1·0	91·2	9·1	95	9·5	0·4
	2·0	90·0	18·0	94	18·4	0·4
	3·0	95·0	28·5	98	29·4	0·9
	4·0	99·0	39·6	95·5	38·2	−1·4

The same general trends are evident in the results from all three sets of solutions but there are some well-defined differences. The percentage labelled strength found by the Webb method increases with increase in the strength of the solution. This is to be expected since the *percentage* of pilocarpine bound by a constant amount of methylcellulose must decrease as the total pilocarpine content increases.

Manufacturer A uses a different type of methylcellulose (exact nature unknown to the authors) from manufacturer B and the results demonstrate that much less interaction takes place with the former type. The method of incorporating the methylcellulose may affect the amount of interaction in the final product.

A separate study was made to determine whether the amount of interaction changed with time. Manufacturer B supplied a number of 2%

pilocarpine preparations which had been stored for control purposes and these were again assayed by both methods. The results (Table 2) show that the amount of pilocarpine interacting with the methylcellulose does increase and reaches a constant value after approximately 12 months.

TABLE 2. EFFECT OF TIME ON THE INTERACTION BETWEEN PILOCARPINE HYDROCHLORIDE AND METHYLCELLULOSE IN SOLUTION

Age of solution in months	Webb method (Free pilocarpine)		Elvidge method (Total pilocarpine)		mg/ml bound	$[\alpha]_D^{23}$
	% labelled strength	mg/ml	% labelled strength	mg/ml		
1	82	16·4	102·5	20·5	4·1	+76·6
3	74	14·8	100·5	20·1	5·3	+62·2
8	74	14·8	100	20·0	5·2	not done
12	65	13·0	101	20·2	7·2	+61·4
19	59	11·8	108·5	21·7	9·9	+47·9
55	75	15·0	111·2	22·2	7·2	+51·8
60	70	14·0	106	21·2	7·2	+51·9
63	66	13·2	103	20·6	7·4	+52·4

The specific rotation of each solution in the time experiment was determined and the values are listed in Table 2. This value also reaches a constant value, apparently after a much longer period. The anomalous results for the nineteen month sample can be attributed to an error in manufacture since the viscosity was found to be much higher than that of the other seven solutions. The obviously high methylcellulose content accounts for the high weight per ml bound but the unexpectedly low specific rotation for this solution suggests that methylcellulose may accelerate the conversion of pilocarpine to isopilocarpine. Both assay procedures are non-specific and do not permit differentiation between pilocarpine content and the content of its closely related derivatives which may be formed during storage of solutions.

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References

Ekkert, L. (1925). *Pharm. Zentralhalle Dtl.*, **66**, 36; through *Chem. Abstr.*, (1925), **19**, 1177.

Elvidge, W. F. (1947). *Q. Jl. Pharm. Pharmac.*, **20**, 234–237.

Helch, M. H. (1902). *Pharm. Post*, **35**, 289; through *Chem. ZentBl.*, (1906), **77** (II), 462.

Levine, J. & Horrocks, E. (1960). *J. Ass. off. agric. Chem.*, **43**, 233–234.

Webb, J. W., Kelley, R. S. & McBay, A. J. (1952). *J. Am. pharm. Ass., Sci. Ed.*, **41**, 278–279.