IR Spectrophotometric Assay of Carbachol Solutions

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Abstract □ Carbachol ophthalmic solutions can be assayed by evaporating a measured volume, dissolving the residue in methanol, and scanning the carbonyl stretching frequency in an IR spectrophotometer using a cell with calcium fluoride windows. Methylcellulose and other formulation vehicle components do not interfere. The method is stability indicating with respect to hydrolysis. It affords a recovery of 99.5 ± 0.51% (*RSD*).

Keyphrases □ Carbachol—IR spectrophotometric analysis, commercial ophthalmic preparations □ IR spectrophotometry—analysis, carbachol, commercial ophthalmic preparations □ Cholinergics, ophthalmic—carbachol, IR spectrophotometric analysis, commercial preparations

Carbachol, the carbamoyl ester of choline, has been shown to be stable in aqueous solution at pH values lower than 7. A kinetic study of its hydrolysis was reported in which choline separated from carbachol by TLC was determined photometrically as its dipicrylamine complex (1). USP XVIII (2) used a reinecke salt colorimetric method for the assay of carbachol ophthalmic solution; however, the method does not discriminate between carbachol and choline. It was replaced in the First Supplement (3) by a stability-indicating method, apparently based on the work of Puckett and Poe (4). The procedure involves formation of the *N*-chloro derivative of carbachol by reaction with alkaline hypochlorite, destruction of excess hypochlorite with phenol, and oxidation of iodide by the *N*-chloro derivative to iodine, which is determined as its amylose complex. Erratic results with the method led to the development of the simple and highly selective IR spectrophotometric method described here.

EXPERIMENTAL

Pipet a volume of carbachol ophthalmic solution[1], equivalent to about 90 mg of carbachol, into a 250-ml round-bottom flask. Add about 10 ml of methanol and evaporate the solution to dryness in a rotary evaporation apparatus[2], using reduced pressure and a water bath temperature of 50°. Add exactly 10.0 ml of methanol to the flask, stopper, and swirl to dissolve the carbachol. (Methylcellulose, in the test preparations used in this work, forms a thin film which does not dissolve.)

Fill a 0.1-mm path length IR cell, equipped with calcium fluoride windows, with the solution. Record its spectrum in the carbonyl region (1970–1550 cm^{-1}) three times, using a suitable double-beam IR spectrophotometer[3] and a matched cell filled with methanol in the reference beam. Concomitantly scan the carbonyl region of a methanol solution of carbachol USP reference standard containing a known concentration of about 9 mg/ml. Draw a horizontal straight line from the shoulder at about 1980 cm^{-1} and draw a straight line from the absorption band maximum at 1720 cm^{-1} perpendicular to the first line and intercepting it. The length of this line, in the units of the recorder chart paper, is the percent transmittance from which absorbance is calculated from the relation $A = \log_{10}(1/T)$.

Calculate the concentration, in percent (w/v), of carbachol in the ophthalmic solution from the formula $C/V(A_U/A_S)$, where C is the concentration, in milligrams per milliliter, of carbachol in the standard solution; V is the volume, in milliliters, of carbachol ophthalmic solution taken for assay; and A_U and A_S are the average absorbances determined for the assay preparation and the standard solution, respectively.

[1] Carbacel 1.5 and 3%, Softcon Products Division, Warner-Lambert Co., Morris Plains, NJ 07950.

[2] Buchler flash evaporator.

[3] Perkin-Elmer model 621 ratio-recording double-beam IR spectrophotometer.

Table I—Recovery of Carbachol Added to 1.5 and 3.0% Formulation Vehicles

Vehicle	Amount Added, mg	Amount Found, mg	Percent Recovered
1.5%	39.1	39.0	99.7
	78.2	78.0	99.7
	156.4	155.0	99.1
3.0%	94.9	94.4	99.5
	189.9	189.0	99.5
	379.8	380.0	100.0

RESULTS AND DISCUSSION

The feasibility of the method was first investigated using ordinary sodium chloride cells; however, these cells are fogged by methanol and require polishing after only a few uses. Calcium fluoride windows are impervious to methanol. Methanol was chosen as the solvent because of its excellent solvent powers for carbachol and its transparency in the spectral area of interest.

Precision and Recovery—The vehicles for the 1.5 and 3.0% test preparations differ in that the latter contains a borate buffer and sodium chloride as well as methylcellulose and benzalkonium chloride. However, both vehicles gave IR spectra indistinguishable from a methanol blank when tested in the procedure. Carbachol was added in three concentrations to each vehicle. Average recovery (Table I) for the six trials was 99.6% with a relative standard deviation of ±0.51%.

Standard solutions containing 3.91, 7.82, and 15.64 mg of carbachol/ml in methanol provided absorbances of 0.140, 0.280, and 0.572, respectively. These data afford a straight-line graph that intercepts the origin.

Selectivity—None of the constituents of the ophthalmic solution vehicles used interfered in the assay. Other commercially available carbachol eye solutions list 1.4% polyvinyl alcohol and edetate disodium as vehicle components. Since polyvinyl alcohol contains acetate ester groups to some extent and edetate disodium has carboxyl functions, solutions of 1.4% polyvinyl alcohol and 0.1% edetate were assayed by the proposed method. Neither interfered in the assay; both gave spectra identical with the blank in the spectral region used for analysis.

The validity of the assay as a stability-indicating method was verified by heating 5 ml of water containing 100 mg of carbachol and 4 ml of 1 *N* sodium hydroxide in a sealed ampul at 105° overnight to saponify the carbamate. Assay of this solution by the proposed method showed no carbonyl band.

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