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DETERMINATION OF CARBACHOL OPHTHALMIC SOLUTION BY PROTON MAGNETIC RESONANCE SPECTROSCOPY

by

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ABSTRACT

By using proton magnetic resonance spectroscopy, carbachol can be assayed in commercial ophthalmic solutions with a minimum of steps and reagents, and with a high degree of accuracy. The sample is freeze-dried to a powder which is first mixed with acetamide, the internal standard, and next is dissolved in methanol. An aliquot of the solution is evaporated to dryness, the residue is dissolved in deuterium oxide, the solution is mixed with the reference standard, and the spectrum is recorded. The quantity of carbachol in the dosage form is calculated from the integral value at ca. 3.27 ppm (carbachol) and ca. 2.01 ppm (acetamide). The mean recovery value ± SD for carbachol added to synthetic formulations was 100.0

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± 0.5% (n = 10), CV = 0.5%. The mean assay values for commercial 15 and 30 mg/ml ophthalmic solutions were 100.2 (n = 3) and 99.9 (n = 3)% of declared, respectively. The proposed method precludes interferences by synthetic celluloses, which are added to carbachol ophthalmic solutions to both prolong and enhance pharmacological effects.

INTRODUCTION

Carbachol (choline chloride carbamate) is a cholinergic agent which finds use as a miotic during ocular surgery , and for reducing intraocular pressure in patients with glaucoma who may have become resistant to other cholinergics (1). Carbachol has been analyzed in bulk samples and in dosage forms by colorimetric (2-5), titrimetric (2,5), and infrared spectroscopic (6,7) methods. Colorimetric methods depend on its conversion to a colored soluble reineckate salt (2), to a N-chloro derivative which in turn will oxidize iodide to iodine so as to obtain a blue color with starch (3,5), or to a colored ferric hydroxamate (4). Among the problems that are encountered with these and the titrimetric methods are: lack of sensitivity and selectivity, too many procedural steps, and the need for numerous reagents. Infrared spectroscopic methods, on the other hand, are simpler in design and highly selective, but they demand strict adherence to instrumental details and are too slow for multiple sample analysis. This report describes the use of proton magnetic resonance (PMR) spectroscopy to assay carbachol in commercial ophthalmic solutions with a minimum of steps and reagents. The proposed method will also measure car-

bachol with a high degree of accuracy and precision in the presence of ophthalmic wetting agents such as the synthetic celluloses.

EXPERIMENTAL

Instrumentation - All PMR spectra were recorded on a 90 MHz Varian EM-390 spectrometer (Varian Associates, Palo Alto, CA) at an ambient probe temperature of 35°C, using a sweep time of 5 min and a sweep width of 10 ppm.

Materials - USP carbachol Reference Standard (U.S. Pharmacopeial Convention, Inc., Rockville, MD), acetamide (99+ mol %, Eastman Kodak Co., Rochester, NY), deuterium oxide (D_2O, 99.7 atom % D, Merck & Co., Inc., Rahway, NJ), deuterated sodium 3-trimethyl silylpropionate-2,2,3,3-d_4 (TSP, Merck & Co., Inc.), methanol ACS (Fisher Scientific, Fair Lawn, NJ), carbachol chloride ophthalmic solution, 15 and 30 mg/ml (from commercial sources).

Method - Transfer an accurately measured volume of ophthalmic solution, equivalent to about 100 mg of carbachol, to a freeze drier vacuum jar, freeze the solution at about -40°C, and freeze dry to the solid state at about 10^{-3} Torr. To the residue add about 97 mg of acetamide, accurately weighed, and about 2 ml of methanol, and effect solution with the aid of a vortex mixer. Using a capillary pipet, transfer the methanolic solution to a 25 ml glass-stoppered flask, and evaporate the solvent to dryness with the aid of a stream of dry nitrogen. Dissolve the residue in 1-2 ml of D_2O and, using a capillary pipet, transfer about 0.5 ml of the solution to an analytical PMR tube that contains a few crystals of TSP, mix, and place the PMR tube in the spectrometer. Ob-

tain the spectrum using a spin rate that will not produce interfering spinning side bands in the spectral regions 1.7-2.3 ppm and 3.0-3.6 ppm. Assign all chemical shifts with reference to TSP, taken as 0.0 ppm. Integrate the singlet at about 2.01 ppm and the singlet at about 3.27 ppm at least five times each, and obtain the average values. Calculate the amount of carbachol in the dosage form from: mg/ml = (Au/As) x (EWu/EWs) x (C/V), where Au = the average integral value for the singlet at about 3.27 ppm, due to the 9 N-methyl protons of carbachol; As = the average integral value for the singlet at about 2.01 ppm, due to the methyl protons of acetamide, serving as an internal standard; EWu = the formula weight of carbachol divided by the number of absorbing protons, i.e., 182.65/9 = 20.29; EWs = the formula weight of acetamide divided by the number of absorbing protons, i.e., 59.07/3 = 19.69; C = the weight of acetamide taken for the analysis, mg; and V = the volume of ophthalmic solution taken for the analysis, ml.

RESULTS AND DISCUSSION

Figure 1 is the 90 MHz PMR spectrum of a mixture of carbachol and acetamide in D_2O solution. Carbachol exhibited three resonance signals (labeled a-c) at about 3.27 ppm (singlet), 3.73 ppm (multiplet), and 4.57 ppm (multiplet), which correspond to the $-\overset{+}{N}(CH_3)_3$, $-NCH_2-$, and $-OCH_2-$ protons, respectively. The two multiplets (b and c) are complex and not easily interpreted by a first-order treatment. These multiplets are better described as belonging to an AA'XX' spin system because the chemical shift difference between the two multiplets (Δ ca. 80 Hz) is larger than the vicinal cou-

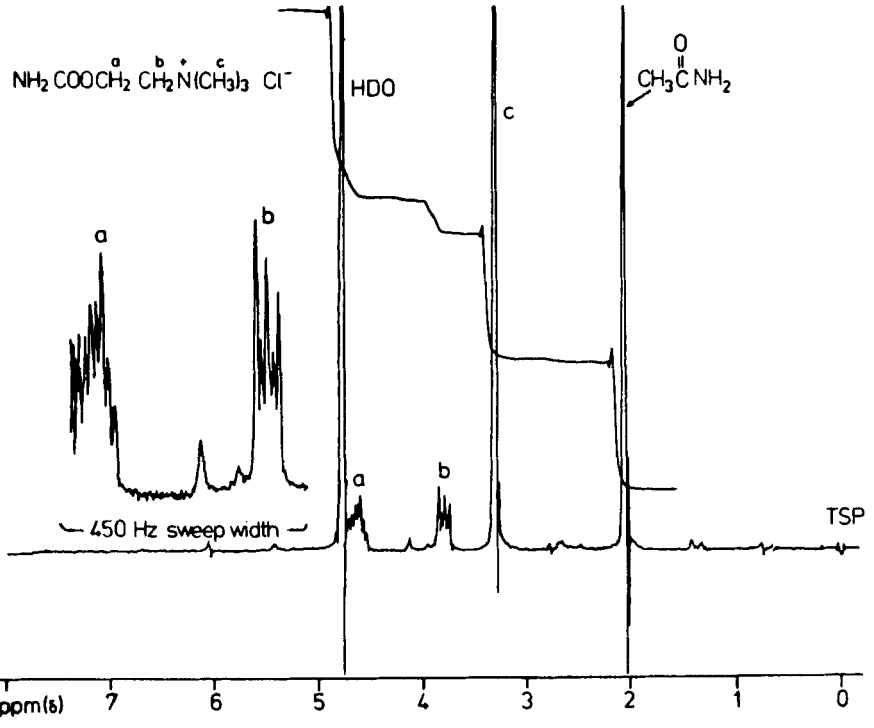

Figure 1. 90 MHz PMR spectrum of a mixture of carbachol and acetamide, the internal standard, in D_2O.

pling constant J_{AX}. The lower field multiplet due to the $-OCH_2-$ protons is broader than the $-NCH_2-$ multiplet because of additional significant coupling between the $-OCH_2-$ protons and the positively charged nitrogen (nuclear spin = 1). Figure 2 shows a 90 MHz expansion of the $-OCH_2-$ and $-NCH_2-$ multiplets of carbachol, together with a diagramatic representation of the AA' portion of their AA'XX' spin system. The distance between the outer lines gives the sum of vicinal coupling constants, N, while the distance between the inner lines gives the approximate magnitude (but not the sign) of the difference between the vicinal coupling constants, L (8). From the values of N (10.5 Hz) and L (5.1 Hz), the coupling constants

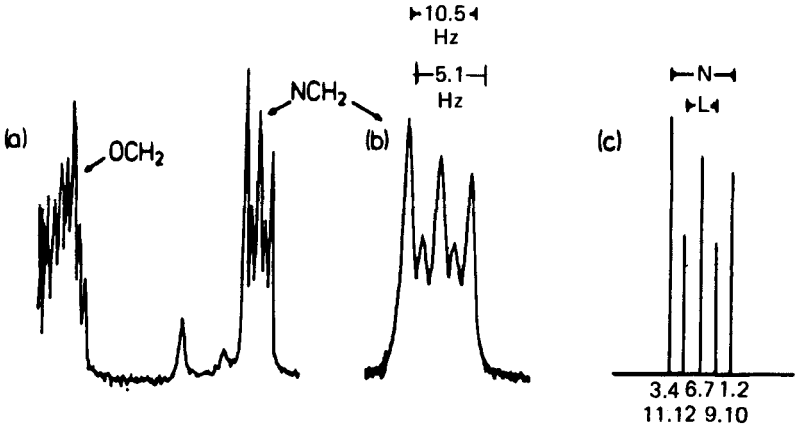

Figure 2. Expanded portion of the 90 MHz PMR spectrum of carbachol in D_2O: (a) $-OCH_2-$ and $-NCH_2-$ signals (sweep width, 450 Hz); (b) $-NCH_2-$ signal (sweep width, 180 Hz); (c) diagrammatic representation of AA' portion of AA'XX' spin system.

J_{AX} and $J_{AX'}$ were calculated to be 7.8 Hz and 2.7 Hz, respectively (8). For quantitative purposes, the integral of the signal representing the $-\overset{+}{N}(CH_3)_3$ protons was compared with that of the $-CH_3$ protons of acetamide.

Standard mixtures containing varying amounts of carbachol and internal standard were prepared in 10 ml of distilled water, freeze dried, and put through the proposed PMR spectroscopic method. The assay of these mixtures yielded the results presented in Table 1. Overall, the mean recovery $\pm$ SD was 100.0 $\pm$ 0.5% (n = 10). The accuracy of the method was found to be independent of the relative proportion of internal standard to drug for the range of proportions shown in the same table. Commercial samples of ophthalmic solution from three manufacturers, and representing two strengths of carbachol, were assayed by the PRM spectroscopic method and found to be

Table 1

Determination of carbachol in standard mixtures by PMR spectroscopy

Mixture No.	Acetamide added, mg	Carbachol		
		Added, mg	Found, mg	Recovery , %
1	100.5	122.0	121.8	99.8
2	89.2	117.9	118.5	100.5
3	98.3	111.7	111.5	99.8
4	90.1	107.9	107.8	99.9
5	97.1	100.2	100.1	99.9
6	89.5	98.1	97.8	99.6
7	97.5	91.0	90.2	99.1
8	90.2	86.6	86.8	100.2
9	100.5	72.3	72.8	100.7
10	89.7	67.8	67.9	100.1
Mean				100.0
SD				0.5
CV,%				0.5

Table 2

Determination of carbachol ophthalmic solutions by PMR spectroscopy

Brand	Amount declared, mg/ml	Amount found, mg/ml	Amount found, %
A	15	14.96	99.7
B	15	15.11	100.7
C	15	14.89	99.3
A	30	29.92	99.7
B	30	30.21	100.7
C	30	30.03	100.1

within the declared amounts (Table 2). The mean assay values for 15 and 30 mg/ml samples were 100.2 (n = 3) and 99.9 (n = 3)% of declared, respectively.

Hydrophilic wetting agents suchs as hydroxypropylcellulose, methylcellulose, and ethylcellulose, which are added to carbachol ophthalmic solutions to prolong as well as to enhance the pharmacological effects of the drug, will produce resonance peaks in the vicinity of those observed for carbachol. This potential interference was readily eliminated by dissolving the mixture of freeze-dried ophthalmic solution and internal standard in anhydrous methanol, where the cellulose remains insoluble as a gelatinous material. In this regard, deuterated methanol would appear more appropriate as a solvent than methanol because its use will preclude the necessity of evaporating the methanolic solution to dryness and of redissolving the resulting residue in D_2O. The longer route was preferred on the premise that D_2O is less costly than deuterated methanol. The trace amounts of HDO that might be present in deuterated water will increase upon exchange with the amino and amide protons of carbachol and acetamide, respectively. During this study, the levels of HDO that were encountered and the intensity of the resulting side bands were not great enough to interfere with the integration and subsequent quantitation of the resonance peaks of carbachol. The addition of a drop of deuterated hydrochloric acid to a solution of carbachol in D_2O will greatly simplify the PMR spectroscopic identification of the drug because the acid will shift the signal of HDO further downfield, thus preventing its overlapping with the $-OCH_2-$ resonance signal.

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