Determination of Carbachol in Aqueous Solution

RAMONA PUCKETT and RICHARD D. POE

Abstract □ A more specific method of analysis of carbachol (carbamylcholine chloride) in aqueous solution is reported. The procedure requires the chlorination of the free N—H moiety which in turn is utilized to oxidize iodide to iodine. Quantitation is then accomplished by reacting this latter product with starch and measuring the resultant blue color. Limited data are also presented regarding the stability of aqueous solutions of carbachol.

Keyphrases □ Carbachol solutions—analysis □ Colorimetric analysis—spectrophotometer □ Stability—carbachol solutions

Carbachol [(2-hydroxyethyl)trimethylammonium chloride carbamate] has been shown to be useful in the treatment of glaucoma (1). Numerous additional reports have been published regarding its biological activity, including a recent paper by Beasley *et al.* (2). However, few studies concerning its chemical analysis and stability can be found in the literature.

The USP XVII (3) contains a method for the analysis of aqueous solutions of this drug. This method requires precipitation of the carbachol by ammonium reineckate, filtration, washing, redissolving in acetone, and quantification of the resultant colored solution in a suitable spectrophotometer. However, this method has been found to be unsuitable for use in very dilute aqueous solutions.

Connors (4) in his discussion of carbamic acid derivatives, refers to numerous qualitative tests for carbamates and carbachol. However, the two quantitative methods indicated are the reineckate method and a paper chromatography technique. Taylor (5) has also reported a qualitative TLC test for carbachol and other choline derivatives.

Heyndrickx *et al.* (6) have reported a colorimetric method which is sensitive to the unsubstituted amide moiety. However, this method requires that the sample be extracted and dried prior to analysis and further suffers from a critical time dependence. All of the above procedures suffer from either a lack of specificity or from the necessity of tedious time consuming techniques.

Dittert and Higuchi (7) have published a study of the rates of hydrolysis of carbamates. Consideration of these data has prompted the authors to initiate a search for a method more specific than the official reineckate technique. Pryde and Soper (8) have reported a chlorination and estimation method for acetanilide. Ellis and Hetzel (9) have reported an adaptation of this chlorination technique to the determination of the N—H group of meprobamate. This principle and a modification of the technique have been applied to carbachol.

EXPERIMENTAL

Reagents—Carbachol USP XVII Reference Standard; sodium hydroxide, 1 *N;* hydrochloric acid, 0.1 *N* and 3.5 *N;* potassium iodide, 0.3% aqueous; phenol, 0.5% aqueous; amylose indicator (Mallinckrodt), 0.2% aqueous; chlorinating solution: immediately before using, mix equal volumetric quantities of 1 *N* sodium hydroxide and a 1:15 dilution of approximately 5.25% sodium hypochlorite (Clorox or Purex) in 0.1 *N* sodium hydroxide. (It is suggested that the 1:15 dilution stand at room temperature for about 30 min. prior to mixing with the 1 *N* sodium hydroxide.) Make fresh daily.

Standard—Accurately prepare an aqueous solution containing approximately 0.05 mg./ml. carbachol. To prepare the standard curve, use a blank, 2.0, 4.0, and 5.0 ml. of this solution. A standard curve should be prepared with each series of analyses conducted.

Method—The sample for analysis should be diluted to contain approximately 0.10 mg./ml. carbachol. Add the appropriate standards, 2.0 ml. of the sample, and 2.0 ml. of water for a blank, to separate 50-ml. conical flasks. Add 1.0 ml. of 0.1 *N* hydrochloric acid. Mix well. Add 4.0 ml. of chlorinating solution directly onto the liquid in the flasks, preventing, if possible, the adherence of any to the neck or side of flasks. Rinse sides of flasks with water if necessary. Mix well. Let stand 15 min. beginning with the addition of chlorinating solution to the first flask. Add 2.0 ml. of phenol solution, washing the sides of the flasks with the solution. Rinse flasks down with water. Mix well and let stand 5 min. beginning with the addition of phenol to the first flask. Add 2.0 ml. 3.5 *N* hydrochloric acid, washing the flasks' sides upon addition. Rinse flasks sparingly with 0.1 *N* hydrochloric acid to assure complete acidification of all contents. Mix well. Add 1.0 ml. potassium iodide solution; mix. Let stand 5 min. Add 5.0 ml. of indicator. Mix well

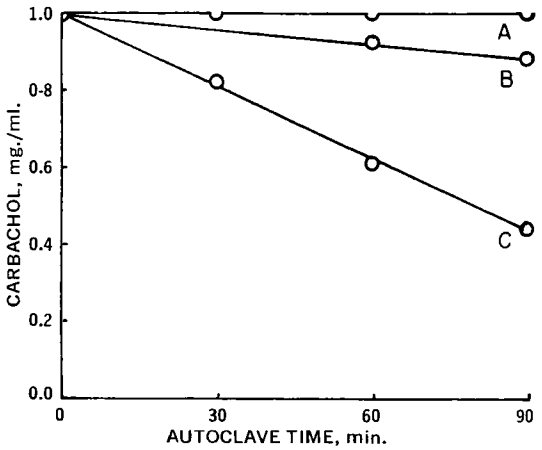

Figure 1—*Comparison of analysis of aqueous carbachol by three methods after prolonged autoclaving. Key:* A, *dipicrylamine technique (10);* B, *ammonium reineckate technique (3);* C, *technique described.*

and transfer to a 50-ml. volumetric flask. Make to volume with water. Mix thoroughly.

Measure the absorbance of the standards and product in a suitable spectrophotometer using 1-cm. cells and the blank as the reference. The maximum absorbance should occur at approximately 590 mμ. Construct a standard curve of absorbance *versus* concentration. Similarly treat the sample and determine its concentration from the curve.

Results—The standard curves obtained were linear in all cases. However, the Beer-Lambert law is not obeyed in each instance. Therefore, the construction of a reference curve for each series of analyses is required. In a series of 65 standard curves of three points each, a representative average absorbance value was found to vary by as much as ±16%. However, replicate (six) analyses of a sample, concomitantly determined with a standard curve gave an average recovery of 101% of theory with a standard deviation of 1.1%. The duplicate analyses of eight random samples by two analysts gave an average deviation between the two sets of data of only ±1.2%. The initial addition of acid may be excluded if the sample is acidic when received. The proposed mechanism (7) for hydrolysis of carbachol includes the formation of an intermediate, carbamic acid. This compound is unstable in acidic solutions, but is somewhat more stable in basic solution and gives a positive reaction to the procedure unless removed prior to analysis. Analysis of a 20-mg./ml. sample of carbachol hydrolyzed in 0.1 *N* sodium hydroxide for 18 hr. at room temperature gave results of 52% of initial. However, analysis of an aliquot of the same sample without prior acidification yielded results of 80% of initial value. Therefore, it appears necessary to ensure that this intermediate has been destroyed prior to initiation of the chlorination procedure.

An alternate chlorinating agent was investigated. The commercially available sodium hypochlorite solutions used produced a blank having a high absorbance. Chlorine water was studied as a possible improved alternate. Chlorine gas was bubbled through about 500 ml. chilled water for about 4 min. The amount of chlorine in the water was estimated at 0.15% using the orthotolidine procedure.

Standard curves were prepared varying conditions such as chlorinating time, volume of chlorine water, volume of phenol, and temperature.

The extent of chlorination of the molecule appeared unchanged between 15 and 20 min. Five milliliters of the chlorine water was adequate to effect sufficient chlorination.

The amount of phenol used is critical. A large excess of phenol will yield a standard curve with considerable negative interference. Using the amount of chlorine water mentioned above, 2 ml. of 0.5% phenol was sufficient to react with the excess chlorine, yet it did not cause negative interference.

In order for the curve to pass through the origin, best results were found when the amount of phenol used was just enough to allow a slight blue color in the blank. No difference was found between chlorination at RT and 40°. Very incomplete chlorination occurred when the reaction flasks were placed in an ice bath. The greatest advantage of the chlorine water over the commercially available

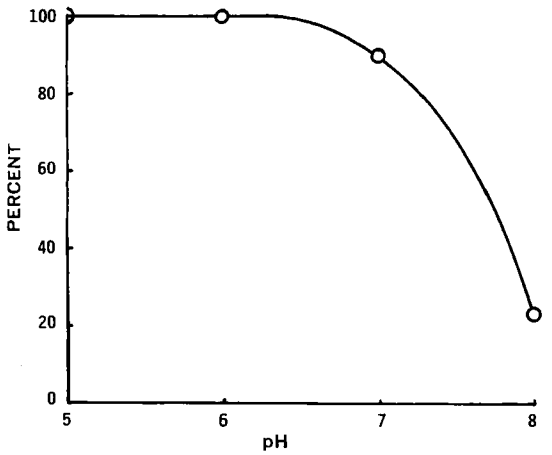

Figure 2—*Comparison of percent of initial dilute carbachol (0.1%) remaining after 30 min. autoclaving at four pH ranges.*

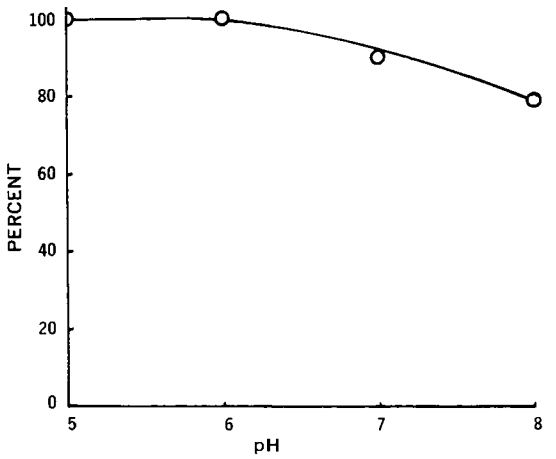

Figure 3—*Comparison of percent of intial carbachol (2.0%) remaining after 30 min. autoclaving at four pH ranges.*

sodium hypochlorite was the lower blank value. Also, absorption per sample concentration is more reproducible with chlorine water.

A disadvantage of the chlorine water is the problem of determining the volume of chlorine water to use for optimum chlorination from batch to batch of the fresh reagent and the chore of balancing the chlorine-to-phenol ratio in the procedure.

Dittert and Higuchi (7) indicate that one of the expected degradation products of carbachol would be choline chloride. The compound does not respond to this procedure and is consequently eliminated as a source of interference. Choline does give a positive response to the reineckate procedure and is therefore a source of positive interference in that technique. Results of analyses of autoclaved samples of carbachol are given in Fig. 1.

The dipicrylamine method is equally sensitive to the quaternary ammonium moiety of both carbachol and its degradation product. The reineckate method reflects some degradation due to the positive interference exhibited by the choline chloride decomposition product. This compound has a lower apparent extinction coefficient than carbachol when treated as in Method B. Based upon these apparent extinction coefficients, and utilizing Method B, a sample of choline chloride representing 100% hydrolysis of an equivalent carbachol sample would be analyzed and reported as 76% of label. Method C would correctly yield results of 0% of label. Method C apparently reflects the true concentration of carbachol remaining.

A brief study was conducted to investigate the apparent stability of autoclaved aqueous carbachol solutions at various pH values. The results are listed in Figs. 2 and 3.

Table I shows data from solutions of 2% carbachol heated at 100° in buffer solutions. This would indicate that, as listed in the Merck Index (11), aqueous solutions may be heated. However, basic solutions tend to be unstable. Additionally, a dilute aqueous solution was reduced to dryness and the reconstituted carbachol analyzed by the reported technique. Results indicated only 55% recovery of the sample. Therefore, any procedure which required isolation by evaporation of the sample prior to analysis could tend to yield low results.

The report of Dittert and Higuchi (7) contains an excellent study of the kinetics of this type of system. Therefore, only a brief preliminary study of comparative pH effects is reported here (Fig. 4).

Summary—A more selective analytical technique is reported for aqueous solutions of carbachol. The method appears suitable for

Table I—Data from 2% Carbachol Solutions Heated at 100° in Buffer

pH	% Remaining after Heating 15 min.
5.0	98
7.0	98
8.0	94
7.2 (aqueous, no buffer)	100

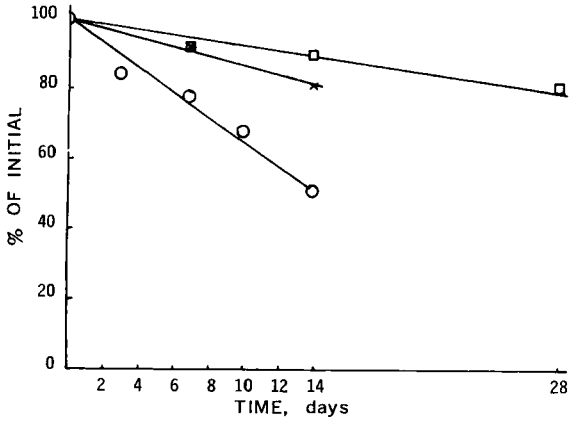

Figure 4—*Comparison of percent of initial carbachol remaining after storage at 60° in three buffer systems (0.1 M phosphate). Key: ○, pH 7.9; ×, pH 7.0; □, pH 6.0.*

use in the presence of possible hydrolysis products and in systems in which the interfering N—H groups may be excluded. The procedure is simple, rapid, and applicable to small or very dilute samples.

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Determination of Calcium in Pharmaceutical Preparations by Atomic Absorption Spectrometry

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Abstract □ Atomic absorption spectrometry is applied to the determination of calcium in pharmaceutical samples using both the direct method and the method of standard additions. Comparison with the standard chelometric method using EDTA and hydroxy naphthol blue indicates that the atomic absorption methods require less time and are equivalent to the chelometric method in precision and accuracy. The majority of interferences are eliminated by the addition of one percent lanthanum to the solutions.

Keyphrases □ Calcium determination—pharmaceuticals □ Lanthanum—assay interference elimination □ Capsule absorption—active components □ Atomic absorption spectroscopy—analysis

Pharmaceutical preparations containing calcium are usually analyzed by the USP (1) or NF (2) methods which involve chelometric titration with the disodium salt of ethylenedinitrilotetraacetic acid (EDTA) using hydroxy naphthol blue as the indicator. Many pharmaceutical preparations, however, contain phosphate and/or organic compounds which interfere with the end point of the chelometric method. The elimination of these interferences increases the time and complexity of the analysis.

This paper reports an atomic absorption spectrometric method for the analysis of calcium in many types of pharmaceutical preparations that is simple and easy to follow and is equivalent to the chelometric method in accuracy and precision. Atomic absorption spectrometry has been used to determine calcium in many types of samples (3–10) but has not been reported for pharmaceutical materials. The majority of interferences are eliminated by the addition of lanthanum to all standards and samples as shown by Yofè and Finkelstein (11) and Williams (12) for flame photometric determination of calcium and by David (3) for atomic absorption spectrometry. Lanthanum is seldom a component and thus is the element of choice for the elimination of phosphate and other interferences. Strontium (3) can also be used for the same purpose.

EXPERIMENTAL

Instrument—Atomic absorption spectrophotometer[1] equipped with a dual element (Ca-Mg) hollow cathode lamp. The photometer was operated with either a standard (single slot) or Boling (three-slot) head of 10-cm. path length at a wavelength of 4227 Å. and a slit width of 1 mm. using an air-acetylene flame with an atomizer flow rate of 2.8 ml./min.

Reagents—Lanthanum oxide, code 528.[2] All other reagents were A.C.S., USP, or NF grade. Deionized water was used for all solutions.

[1] Perkin-Elmer model 303.

[2] American Potash and Chemical Co.