This discussion is supported by determinations of the amount of water in cellulose acetate phthalate films having moisture on one or both sides of the film (Table III). It is apparent that when vp_{distal} is held at zero, the amount of water in the cellulose acetate phthalate film decreases at a faster rate with increasing temperature than when vp_{distal} has a finite value and moisture is present on both sides of the film.

The question as to whether cast films differ from sprayed films of the same polymer system in their water vapor transmission properties is resolved from a consideration of Table I. It is apparent that there is no significant difference between the values of P^0 for each system at the three temperatures studied.

SUMMARY AND CONCLUSIONS

This work demonstrated that significant permeation differences from expected behavior exist with hydrophilic cellulose acetate phthalate films when the atmosphere adjacent to the distal surface of the film is devoid of water vapor. No such effect is observed with lipophilic *n*-butyl methacrylate films. This anomalous effect, which increases with increasing temperature, is due to partial dehydration of the cellulose acetate phthalate film. This factor should be kept in mind when developing realistic testing conditions for evaluating the water vapor permeation properties of free hydrophilic films, especially if extrapolation to coated solid dosage forms is anticipated. The results also show that the method of film preparation, be it casting or spraying, does not affect the permeation characteristics of cellulose acetate phthalate films.

REFERENCES

(1) G. S. Banker, A. Y. Gore, and J. Swarbrick, *J. Pharm. Pharmacol.*, **18,** 457(1966).

(2) J. Swarbrick and A. H. Amann, *ibid.*, **20,** 886(1968).

(3) M. Patel, J. M. Patel, and A. P. Lemberger, *J. Pharm. Sci.*, **53,** 286(1964).

(4) "International Critical Tables, III," McGraw-Hill, New York, N. Y., 1928, pp. 248–292.

(5) P. Meares, "Polymer Structure and Bulk Properties," Van Nostrand, New York, N. Y., 1965, pp. 257–274.

ACKNOWLEDGMENTS AND ADDRESSES

Received February 24, 1972, from the *Pharmaceutics Section, School of Pharmacy, University of Connecticut, Storrs, CT 06268*

Accepted for publication May 19, 1972.

* Present address: Abbott Laboratories, North Chicago, IL 60064

▲ To whom inquiries should be directed. Present address: Sterling Winthrop Research Institute, Rensselaer, NY 12144

Colorimetric Estimation of Cephalexin, Cephaloglycin, and Related Compounds

L. P. MARRELLI

Abstract □ A specific color reaction is described for the colorimetric estimation of cephalosporin derivatives having the following intact side chain in the 7-position: $RCH(NH_2)CO—$, R being a heterocyclic or aromatic ring. The method is applicable for the detection of cephaloglycin (or desacetyl cephaloglycin) or cephalexin in urine. Penicillin analogs respond to the test, but the sensitivity is much less.

Keyphrases □ Cephalexin and related compounds—colorimetric estimation □ Cephaloglycin and related compounds—colorimetric estimation □ Colorimetry—estimation of cephalexin, cephaloglycin, and related compounds

A specific colorimetric test was developed for the determination of cephalosporin derivatives having the following intact side chain in the 7-position: $RCH(NH_2)CO—$, R being a heterocyclic or aromatic ring. The D-phenylglycine derivatives of both 7-aminocephalosporanic acid (cephaloglycin[1]) and 7-aminodesacetoxycephalosporanic acid (cephalexin[2]) responded well (1, 2). These compounds (0.5–1.0 mg./ml. in water) reacted with acetone and sodium hydroxide at 100° to form characteristic red chromophores. Ampicillin[3] responded under the same conditions, but the sensitivity was not as great. Ampicillin must be present at approximately 50 times the concentration of cephalexin to give the same response in the test. Cephalexin and cephaloglycin (desacetyl cephaloglycin present as a degradation product) may be detected in urine at the concentration specified.

EXPERIMENTAL

Reagents—The following were used: acetone, reagent grade; and sodium hydroxide solution, 13.0% w/v.

Recommended Procedure—Transfer 2.0 ml. of aqueous sample (0.5–1.0 mg./ml.) to a test tube. Add 0.5 ml. of sodium hydroxide solution and 0.3 ml. of acetone. Mix and cover tube with a marble. Place in a boiling water bath for 3 min. The formation of a red color indicates the presence of a cephalosporin derivative having the following intact side chain in the 7-position: $RCH(NH_2)CO—$, R being a heterocyclic or aromatic ring. Determine the absorbance of the solution at 520 nm. after standing 3 min.

The sensitivity of this procedure was increased considerably (0.1–0.2 mg./ml.) by initially predegrading the sample under controlled conditions. Buffering the sample solution at pH 7 and allowing it to stand at 70° for 3 hr. were the conditions chosen for predegradation. The extent of color formation was found to be greater

[1] Cephaloglycin is the generic name for 7-(D-α-aminophenylacetamido)cephalosporanic acid.

[2] Cephalexin is the generic name for 7-(D-α-aminophenylacetamido)-desacetoxycephalosporanic acid.

[3] Ampicillin is the generic name for 6-(D-α-aminophenylacetamido)-penicillanic acid.

(1.8 times) if the buffered sample solution was allowed to stand at a lower temperature (25°); however, the time necessary to reach maximum absorbance was 144 hr.

RESULTS AND DISCUSSION

The red chromophore described exhibits a peak at 520 nm. The color is stable for 30 min. and then slowly fades on standing. An effort was made to quantitate the color reaction for cephalexin; however, a nonlinear relationship was observed in the concentration range from 0.25 to 1.0 mg./ml.

Effect of Reagent Concentrations on Color Formation—A series of test tubes, each containing 2.0 ml. of an aqueous solution of cephalexin (1.0 mg./ml.), was prepared. Varying volumes of reagents (±0.1 ml. of that specified in the *Recommended Procedure*) were added to the tubes and the test was completed. No significant difference in the extent of color formation was found in the series of solutions tested.

Effect of Heating Time and Temperature on Color Formation—A series of tubes, each containing 2.0 ml. of a solution of cephalexin (1.0 mg./ml.), was treated with the volume of reagents specified in the *Recommended Procedure* and heated in a boiling water bath for varying periods of time. The heating time that resulted in the greatest extent of color formation was 3 min. A similar series of tubes was heated at varying temperatures for 3 min.; 100° was the temperature that resulted in the greatest extent of color formation in the time specified.

Effect of Predegradation of Compound on Color Formation—Urine samples containing cephalexin were tested by this technique. It became apparent that the color formation was more intense if the solution stood at room temperature for several days prior to testing. Investigation of this phenomenon revealed that the pH of the solution was definitely related to the extent and rate of color formation observed.

The maximum rate and extent of color formation were achieved on testing a solution of cephalexin that had been buffered at pH 7 and allowed to stand at room temperature prior to the test. The time of standing at pH 7 (25°) required to obtain a maximum absorbance in the test was 6 days for cephalexin solutions and 2 days for cephaloglycin solutions. This increase in color formation was concurrent with the degradation of the cephalosporin prior to the test, as evidenced by the decrease in UV absorbance at the nucleus wavelength, approximately 260 nm.

Compounds Tested—Several compounds were tested by the recommended procedure to determine the specificity of the color reaction. The following compounds gave a positive (+) or negative (−) response to the test:

cephalexin	+
cephaloglycin	+
desacetyl cephaloglycin	+
cephaloglycin lactone	+
D-phenylglycine	−
7-aminocephalosporanic acid	−
7-aminodesacetoxycephalosporanic acid	−
sodium cephalothin	−
cephaloridine	−
sodium cephalosporin C	−
ampicillin (25–50 mg./ml.)	+

The sensitivity of the color reaction for cephalosporin derivatives was much greater than that observed for the corresponding penicillin analogs. The failure of the phenylalanyl derivative of 7-aminocephalosporanic acid and the indole derivative of 6-aminopenicillanic acid to respond was puzzling. The necessity of the presence of a heterocyclic or aromatic ring immediately adjacent to the α-amino group was conjectured. This theory could not be fully tested since an appropriate derivative was not found [$RCH(NH_2)CO$—ceph, where R is an aliphatic chain].

REFERENCES

(1) J. L. Spencer, E. H. Flynn, R. W. Roeske, F. Y. Siu, and R. R. Chauvette, *J. Med. Chem.*, **9**, 746(1966).

(2) C. W. Ryan, R. L. Simon, and E. M. Van Heyningen, *ibid.*, **12**, 310(1969).

ACKNOWLEDGMENTS AND ADDRESSES

Received August 6, 1970, from the *Analytical Chemical Development Department, Eli Lilly and Company, Indianapolis, IN 46206*

Accepted for publication May 24, 1972.

The author expresses his indebtedness to Dr. E. H. Flynn, Mr. C. W. Ryan, Dr. W. E. Wright, Dr. H. W. Murphy, and Dr. H. L. Dickison for providing many of the compounds tested.

Influence of Chain Length on Oil–Water Ion-Pair Partitioning Behavior of *p*-Alkylpyridinium Chlorides

K. C. YEH and W. I. HIGUCHI▲

Abstract □ The intrinsic partition coefficients (k_i) of *p*-alkylpyridines and the extraction constants (K_e) of the corresponding ion-pairs of the protonated base chlorides were determined in chloroform–water and octanol–water systems. A linear relationship with unit slope was found between log (k_i) and log (K_e). This result has been explained on the basis that, because of methylene (CH_2) increments occurring distantly from the polar portion of the molecule, their influence upon the oil-water partition coefficient would be expected to be the same for the free base and the ion-pair.

Keyphrases □ *p*-Alkylpyridinium chlorides—influence of chain length on oil-water ion-pair partitioning behavior □ Ion-pair partitioning behavior, oil–water—influence of chain length using *p*-alkylpyridinium chlorides □ Chain length—influence on oil–water ion-pair partitioning behavior of *p*-alkylpyridinium chlorides □ Partition coefficients, *p*-alkylpyridinium chlorides—chloroform–water and octanol-water systems □ Extraction constants, *p*-alkylpyridinium chlorides—chloroform–water and octanol-water systems

There has been increased interest in the understanding of substituent effects upon the oil–water partitioning tendencies of drugs because of the possible correlation of the latter with drug absorption efficiency and biological availability in general. Studies on rat intestinal drug absorption (1, 2) and human buccal absorption