Although the results shown in Fig. 2 and Table IV clearly show that the presence of caffeine and acetaminophen in equimolar amounts has a significant effect on the dissolution rate of acetaminophen (and caffeine), they still left some question as to whether or not the presence of smaller ratios of caffeine to acetaminophen, such as are found in many commercial analgesic preparations (2), would have any significant physical effects on the release of acetaminophen from such tablets. Therefore, tablets containing one part anhydrous caffeine (30 mg.) and 10 parts acetaminophen (300 mg.) were prepared. The dissolution behavior of such tablets was then compared to pure acetaminophen tablets (300 mg.). The tablets containing the mixture began to disintegrate within 2 min. and dissolved completely within 50 min. The pure acetaminophen tablets, on the other hand, dissolved without disintegration and required more than 100 min. for complete dissolution.

Whether or not these results can be extrapolated to commercial tablets that contain various additives such as binders and diluents is doubtful. However the results do suggest that caffeine has a substantial effect on the release of acetaminophen from tablets. Therefore, in any formulation attempts or changes, the possible effects of the presence of caffeine in such a system should be considered.

The dissolution rates of theophylline and acetaminophen from various systems are shown in Fig. 5. It is obvious that in these systems the dissolution rates of acetaminophen and theophylline from the same tablet are not as similar as in the acetaminophen–caffeine systems. Furthermore, there is no significant difference between the anhydrous complex and the equimolar physical mixture which, in this case, was prepared by blending of powders. The initial rate of dissolution for theophylline from the physical mixture and the pure theophylline tablet should be noted. The theophylline dissolves approximately 100% faster from the physical mixture than from pure theophylline tablets. This increase in dissolution rate, together with the neutral to slightly acid pH of the resulting solution, suggests that the anhydrous complex may have some utility in the formulation of more rapidly dissolving oral dosage forms of theophylline which could replace, in some cases, the highly alkaline theophylline salts presently employed.

REFERENCES

(1) "Advances in Analytical Chemistry and Instrumentation," vol. 4, C. N. Reilley, Ed., Interscience, New York, N. Y., 1965, pp. 117–212.

(2) "Handbook of Non-Prescription Drugs," 1971 ed., G. B. Griffenhagen and L. L. Hawkins, Eds., American Pharmaceutical Association, Washington, D. C., 1971, pp. 44, 45.

(3) "The Merck Index," 8th ed., Merck and Co., Inc., Rahway, N. J., 1968.

(4) K. A. Connors, "A Textbook of Pharmaceutical Analyses," Wiley, New York, N. Y., 1967, pp. 166–168.

(5) R. A. Robinson and R. H. Stokes, "Electrolyte Solutions," 2nd ed., Butterworths, London, England, 1959, p. 510.

(6) J. W. Poole and C. K. Bahal, *J. Pharm. Sci.*, **57,** 1945(1968).

(7) E. Shefter and T. Higuchi, *ibid.*, **52,** 781(1963).

ACKNOWLEDGMENTS AND ADDRESSES

Received March 6, 1972, from the *School of Pharmacy, University of Kansas, Lawrence, KS 66044*

Accepted for publication May 16, 1972.

Presented to the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, Houston meeting, April 1972.

Abstracted in part from a dissertation submitted by Y. P. Chow to the University of Kansas in partial fulfillment of the M.S. degree requirements.

Supported in part by the Warner-Lambert Pharmaceutical Co., Morris Plains, N. J.

▲ To whom inquiries should be directed.

DRUG STANDARDS

Desorption of Belladonna Alkaloids from Antacids for Analysis

V. DAS GUPTA▲ and K. L. EULER

Abstract □ The desorption of belladonna alkaloids from antacids for analysis was investigated. The antacids studied were dried aluminum hydroxide gel, calcium carbonate, magnesium carbonate, magnesium oxide, and magnesium trisilicate. It appears that the alkaloids are strongly adsorbed only on the surface of magnesium trisilicate. The complete desorption of alkaloids requires boiling the complex with concentrated hydrochloric acid and then using a dye method for the analysis.

Keyphrases □ Belladonna alkaloids—desorption from antacids for analysis □ Desorption, belladonna alkaloids—from antacids, analysis □ Antacids—adsorption of belladonna alkaloids, desorption for analysis □ Magnesium trisilicate—adsorption of belladonna alkaloids, desorption for analysis

The pharmaceutical industry is marketing some liquid antacid preparations in combination with belladonna alkaloids. One typical product of this nature was brought to the authors' notice for the development of an assay procedure for *l*-hyoscyamine. During the investigations, *l*-hyoscyamine was found to be strongly adsorbed onto the surface of one or more of the ingredients. An effective method was required to desorb the alkaloids for analysis. The purpose of this paper is to report the details of these investigations.

EXPERIMENTAL

Reagents and Chemicals—All of the reagents, chemicals, and materials used were either USP, NF, or ACS grade. Bromthymol blue was used as supplied[1] without further purification.

Preparation of Solutions—A bromthymol solution (1×10^{-4} *M*) was prepared in a phosphate buffer (0.05 *M*) solution of pH 7.0 (±0.1)[2].

Simulated Gastric Juice—A solution of simulated gastric juice was prepared according to the directions in the USP (1).

Preparation of Typical Antacid Preparation (Preparation A)—The following were used:

magnesium trisilicate	50 g.
dried aluminum hydroxide gel	25 g.
l-hyoscyamine	12.5 mg.
glycerin	20 ml.
glucose	15 g.
sodium alginate (low viscosity[3])	5 g.
sodium saccharin	375 mg.
chlorophyll (85% concentrate)	47 mg.
sodium benzoate	500 mg.
ethyl alcohol	26.3 ml.
spearmint oil	187.5 mg.
distilled water *q.s.* to	500 ml.

Manufacturing Procedure—*Step 1*—Add sodium alginate to glycerin and stir well using a mortar and pestle until smooth. Dissolve sodium saccharin and sodium benzoate in 96 ml. of distilled water and mix with the above mixture gradually with stirring. Dilute glucose with 64 ml. of water and add to the mixture with stirring.

Step 2—Add spearmint oil and *l*-hyoscyamine to alcohol and mix with Step 1 with stirring. Stir the mixture for 30 min. and then add magnesium trisilicate and dried aluminum hydroxide gel slowly with stirring. Stir until smooth (about 30–60 min.).

Step 3—Dissolve chlorophyll concentrate in 3 ml. of water, add to Step 2, *q.s.* with distilled water, and stir for about 30 min.

Separate batches of the preparation without magnesium trisilicate and dried aluminum hydroxide gel (Preparation B) and without sodium alginate (Preparation C) were also prepared.

New batches of Preparations A, B, and C were prepared with all three belladonna alkaloids (labeled Preparations D, E, and F, respectively) in the following concentrations (alkaloidal salts were dissolved in water with sodium benzoate):

l-hyoscyamine	12.5 mg./500 ml.
atropine sulfate	12.5 mg./500 ml.
scopolamine hydrobromide	1.25 mg./500 ml.

Suspensions of Antacids in Distilled Water—The suspensions (10% w/v) of the following antacids were prepared in distilled water by simply stirring the antacids with distilled water in a beaker. The belladonna alkaloids were dissolved in distilled water, and their final concentrations in the preparations were the same as reported previously.

Antacid	Preparation Labeled as
magnesium trisilicate	G
dried aluminum hydroxide gel	H
magnesium carbonate	I
magnesium oxide	J
calcium carbonate	K

New batches of Preparations G through K were prepared in which a part of the water was replaced with 0.2 *M* hydrochloric acid in order to adjust the pH to approximately 7. These preparations were labeled as L, M, N, O, P, and Q, respectively.

Assay Procedure—The following assay procedure was tried on all preparations, A through Q.

Procedure 1—Dilute 25 ml. of the preparation to 100 ml. with distilled water. Take 5 ml. of the diluted suspension and add 5.0 ml. of the dye solution and 10.0 ml. of chloroform in a 125-ml. separator. Shake it for 1 min. and then follow the same procedure as reported earlier (2) under *Assay Procedure for Ephedrine Sulfate* starting with: "The phases were allowed . . . ." The assay readings were compared with standard readings to obtain the percent of either *l*-hyoscyamine or all of the three belladonna alkaloids contained in each preparation. The results are presented in Table I.

Since the recovery of belladonna alkaloids from Preparations A, C, D, F, G, and L (all containing magnesium trisilicate) was very poor (less than 12%), with the above reported procedure, the following procedures were tried only on these preparations.

Procedure 2—Take 25 ml. of the preparation, add 50 ml. of 0.1 *N* hydrochloric acid, heat to 37°, and stir continuously using a magnetic stirrer with a heater to maintain the temperature for 4 hr. Cool the mixture to room temperature, adjust the pH to approximately 7 using 1 *M* sodium hydroxide solution, and bring the volume to 100 ml. with distilled water. Take 5 ml. of the diluted suspension and assay according to Procedure 1. The results are presented in Table II.

Procedure 3—This was the same as Procedure 2 except that 50 ml. of simulated gastric juice was substituted for 50 ml. of 0.1 *N* hydrochloric acid. The results are presented in Table II.

Procedure 4—This was the same as Procedure 2 except that the preparation was mixed with 1 *M* hydrochloric acid and boiled for 1 min. instead of stirring for 4 hr. at 37°. The results are presented in Table II.

Procedure 5—Take 12.5 ml. of the suspension, mix it with 75 ml. of chloroform, and stir for 1 hr. at 37°. Allow it to cool and collect the chloroform layer. Add more chloroform in small quantities (about 5 ml.) to the suspension and collect the chloroform layer until the total volume is 100 ml. To assay for the alkaloids, take 5.0 ml. of distilled water, mix with 5.0 ml. of the dye solution in a 125-ml. separator, add 10.0 ml. of the chloroform solution, shake the mixture for 1 min., and let the chloroform layer separate. Measure the absorbance of the clear chloroform layer according to Procedure 1. The results are presented in Table II.

Procedure 6—This was the same as Procedure 5 except that boiling chloroform was used for the extraction of the alkaloids. The time allowed for shaking was 1 min. The results are presented in Table II.

Table I—Recovery of *l*-Hyoscyamine or All of the Three Belladonna Alkaloids Using Procedure 1

Preparation	Recovery
A	Very poor[a]
B	Complete[b]
C	Very poor
D	Very poor
E	Complete
F	Very poor
G	Very poor
H	Complete
I	Complete
J	Fair[c]
K	Complete
L	Very poor
M	Complete
N	Complete
O	Complete
P	Complete
Q	Complete

[a] Less than 12%. [b] More than 95%. [c] Approximately 84%.

Table II—Recovery of *l*-Hyoscyamine or All of the Three Belladonna Alkaloids Using Procedures 2 through 7

Preparation	Recovery Using Procedure 2 through 6	Recovery Using Procedure 7
A	Very poor[a]	Complete[b]
C	Very poor	Complete
D	Very poor	Complete
F	Very poor	Complete
G	Very poor	Complete
L	Very poor	Complete

[a] Less than 12%. [b] More than 95%.

[1] Eastman Organic Chemicals.
[2] All pH adjustments in these studies were made using a Beckman Zeromatic pH meter.
[3] Kelco Co., Clark, N. J.

Table III—Assay Results on Belladonna Alkaloids on Preparation D Using Procedure 7

Assay	Recovery, %
1	99.7
2	100.3
3	101.2
4	99.2
Average	100.1
Average deviation	±0.65

Procedure 7—Take 25 ml. of the suspension, add 12.5 ml. of concentrated hydrochloric acid, and heat to boiling on a hot plate. Cool, filter, wash the residue with 3 × 10-ml. portions of distilled water, and combine with the filtrate. Adjust the pH of the filtrate to approximately 7.0 with 3.5 *N* sodium hydroxide solution and bring the volume to 100 ml. Take a 5-ml. quantity of this solution and assay according to Procedure 1. The results are presented in Table II.

Preparation D was assayed four times using Procedure 7. The results are presented in Table III.

DISCUSSION

The results of these investigations (Tables I and II) indicate that all three belladonna alkaloids are strongly adsorbed on the surface of magnesium trisilicate. It was impossible to desorb and determine the alkaloids quantitatively with 0.1 *N* hydrochloric acid or other modified procedures (see Procedures 1 through 6). The only method that made them completely available for determination was boiling with concentrated hydrochloric acid. After desorption, the alkaloids could be easily determined using a dye method. As far as various antacids are concerned, the results of these investigations do not indicate adsorption of alkaloids on the surface of aluminum hydroxide gel as reported by Grote and Woods (3). On the other hand, the results are in agreement with those of Blaug and Gross (4) who reported strong adsorption of anticholinergic drugs on the surface of magnesium trisilicate. The method (Procedure 7) is recommended for complete desorption of belladonna alkaloids for analysis. The average assay result on Preparation D was 100.1 ± 0.65 (Table III).

REFERENCES

(1) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970, p. 1027.

(2) V. D. Gupta and J. B. Reed, Jr., *Amer. J. Hosp. Pharm.*, **26**, 286(1971).

(3) I. W. Grote and M. Woods, *J. Amer. Pharm. Ass., Sci. Ed.*, **42**, 319(1953).

(4) S. M. Blaug and M. R. Gross, *J. Pharm. Sci.*, **54**, 289(1965).

ACKNOWLEDGMENTS AND ADDRESSES

Received November 3, 1971, from the *College of Pharmacy, University of Houston, Houston, TX 77004*

Accepted for publication April 14, 1972.

Supported by Paul B. Elder Co., Bryan, Ohio, and H. W. Nelson Co., Detroit, Mich.

▲ To whom inquiries should be directed.

Quantitative Determination of D-Dopa Present in Levodopa Samples▲

G. COPPI, A. VIDI, and G. BONARDI

Abstract ☐ A method for the assay of D-dopa present in L-dopa samples is reported. The method is based on the ability of an L-amino acid decarboxylase, present in a *Streptococcus faecalis* suspension, to convert quantitatively L-dopa to dopamine while D-dopa remains unchanged. The latter is separated from dopamine by an ion-exchange resin and subsequently assayed according to a fluorometric method. Contaminant amounts of D-dopa present in L-dopa samples can be detected.

Keyphrases ☐ D-Dopa—analysis in levodopa formulations, levodopa biotransformation to dopamine, separation, fluorometry ☐ Levodopa biotransformation to dopamine—separation, fluorometric analysis of D-dopa ☐ Dopamine, biotransformation product of levodopa—analysis of D-dopa in levodopa formulations ☐ L-Amino acid decarboxylase transformation of levodopa to dopamine—analysis of D-dopa in levodopa formulations ☐ Column chromatography—separation of D-dopa and dopamine ☐ Fluorometry—analysis, D-dopa

The presence of D-dopa in levodopa (L-dopa) samples, always possible as a result of the synthetic process, is undesirable in the therapy of Parkinson's disease because it has been well established that the D-enantiomer of β-(3,4-dihydroxyphenyl)-α-alanine is biologically inactive and displays toxic side effects (1–3). As a consequence, the quantitative determination of D-dopa in L-dopa samples is of considerable importance. At the moment, a quantitative separation of the two isomers is possible only by liquid chromatography methods (4).

The present authors studied a specific method for determining the contaminant amounts of D-dopa present in L-dopa pharmaceutical preparations. This method is based on the ability of an L-amino acid decarboxylase, present in a *Streptococcus faecalis* suspension, to convert L-dopa into dopamine while D-dopa remains unchanged.