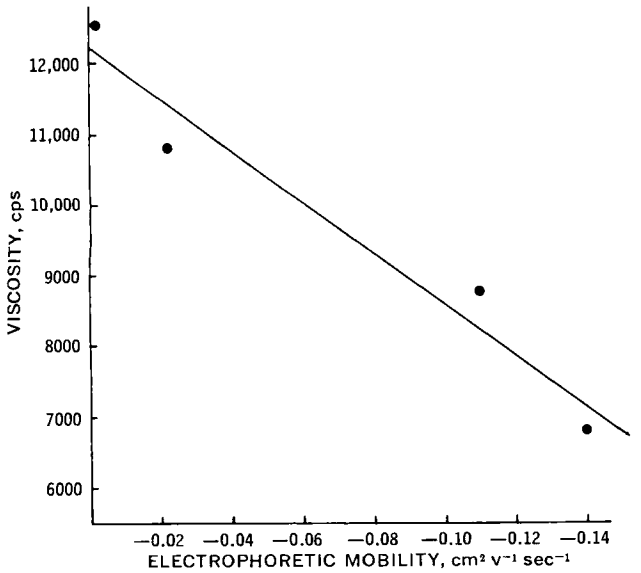

Figure 5—*Relationship between electrophoretic mobility and viscosity.*

creased from −0.140 (cm/sec)/(v/cm) to essentially zero as the gel was washed with 24 volumes of water. This decrease in electrophoretic mobility is due to the removal of water-soluble components as demonstrated by the decrease in specific conductance of the wash water (Fig. 2). The specific conductance reached a low, essentially constant value after 22 volumes of wash water had been eluted.

The loss of the gel's negative electrophoretic mobility during washing suggests that chloride ion present at the time of precipitation may be responsible for the negative surface charge. This is confirmed by determining the concentration of chloride ion in the wash water. Figure 3 shows that chloride ion was present in the wash water in decreasing amounts during washing. Chloride ion was eluted at a constant rate of 0.76 volume^{-1} during the first 8 volumes of wash, during which the chloride-ion content of the wash water decreased from 3.0×10^{-1} to 1.5×10^{-3} *M*. Further elution of chloride ion occurred more slowly as the chloride content of the wash water fell to 1×10^{-4} *M*, the lower limit of sensitivity for the chloride-selective electrode, during the 15th volume of wash. The sum of the chloride ion present in the first 15 volumes of wash water plus the amount present in the initial draining totals 2.11 moles. This amount accounts for virtually all of the 2.25 moles of chloride ion contributed by the aluminum chloride. The removal of chloride ion from the gel was demonstrated further by dissolving 10 g of the gel that had been washed with 24 volumes of water in 1.5 ml of nitric acid and 20 ml of doubly distilled water. No precipitate formed when several drops of 0.1 *N* silver nitrate were added.

The decrease in electrophoretic mobility during washing thus appears to be directly related to the elution of chloride ion. The constant rate of elution suggests that chloride ion is associated with the gel by a single mechansim which allows the chloride ion to be completely eluted.

The viscosity of the gel is also related to the degree of washing. The viscosity increases as the gel is washed, but the viscosity appears to be reaching a limiting value as chloride ion is eluted and the electrophoretic mobility approaches zero (Fig. 4). The increased viscosity is inversely related to electrophoretic mobility (Fig. 5) and may be due to greater interaction occurring between colloidal gel particles possessing low surface charge.

The washing operation is an important part of the preparation of aluminum hydroxide gel because properties such as electrophoretic mobility and viscosity, which are significant in the design of suspension dosage forms, are directly related to washing.

REFERENCES

(1) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970, p. 26.

(2) S. L. Hem, E. J. Russo, S. M. Bahal, and R. S. Levi, *J. Pharm. Sci.*, **59**, 317(1970).

(3) D. Cismaru, J. Iordachescu, G. Georgescu, and V. Cristescu, *Rev. Roum. Chim.*, **10**, 803(1965).

(4) J. A. Lewis and C. A. Taylor, *J. Appl. Chem.*, **8**, 223(1958).

(5) P. H. Hsu and T. F. Bates, *Miner. Mag.*, **33**, 749(1964).

(6) H. Nogami and T. Nagai, *Chem. Pharm. Bull.*, **10**, 741(1962).

ACKNOWLEDGMENTS AND ADDRESSES

Received October 25, 1973, from the *Industrial and Physical Pharmacy Department, Purdue University, West Lafayette, IN 47907*

Accepted for publication December 12, 1973.

[x] To whom inquiries should be directed.

Improvements in the Synthesis of Pentylenetetrazol-10-^{14}C

J. W. DIRKSEN*, G. S. BORN[x], W. V. KESSLER, and S. M. SHAW

Abstract □ Improvements in the synthesis of pentylenetetrazol-10-^{14}C from hydrazoic acid and cyclohexanone-1-^{14}C are reported. Purification was accomplished by crystallization. Chemical purity was determined by GLC and TLC. The radiochemical purity was determined by TLC and autoradiography. The specific activity was found by liquid scintillation counting. A more efficient synthesis was obtained by improvements in the cold bath, the extraction procedures, and the crystallization procedure. The latter change increased the effectiveness of the purification procedure.

Keyphrases □ Pentylenetetrazol, radiolabeled (10-^{14}C)—improvements in synthesis □ Radiolabeled compounds—improved synthesis of pentylenetetrazol-10-^{14}C

Pentylenetetrazol has been synthesized in a number of ways. One of the most productive and yet simple synthesis developed (1) involves reacting cyclohexanone with hydrazoic acid. Modifications of this method have been utilized to produce pentylenetetrazol-5,9-^{14}C (2) and pentylenetetrazol-10-^{14}C (3).

Close examination of the reported syntheses (1–3) revealed large variations in the yields obtained. Ronzio and Murray (2) reported a 22% yield of pentylenetetrazol-5,9-^{14}C. Stiver (4) reported a 47% yield of pentylenetetrazol-10-^{14}C with a radiochemical purity of 94.4% after four sublimations and a 36% yield with a radiochemical purity greater than 99% after thick-layer chromatography. In contrast, Chapman *et al.* (1) reported a 70% yield of pentylenetetrazol after distillation and a 50% yield after crystallization. This paper describes improvements made in the radiolabeled synthesis of Stiver *et al.* (3).

EXPERIMENTAL

Hydrazoic Acid (5)—*Caution:* "Since hydrazoic acid is very poisonous, all reactions involving it should be carried out under a good hood" (6). Sodium azide (16.58 g or 0.255 mole) was placed in a 250-ml, three-necked, round-bottom flask fitted with a mechanical stirrer, thermometer, and dropping funnel. An equal weight of water was added to the sodium azide, forming a paste. To the reaction vessel was added 100 ml of a solvent consisting of benzene–*n*-heptane (85:15). The solution was cooled to 0°, using rapid stirring (650 rpm), in a bath consisting of dry ice-2-ethoxyethanol. While maintaining the desired temperature, 6.78 ml (0.127 mole) of sulfuric acid was added dropwise (1 mole of sulfuric acid for each 2 moles of sodium azide).

When acid addition was complete, stirring was stopped and the resultant semisolid mass was thoroughly broken up. The reaction was then allowed to continue for 3 hr more; the 0° temperature and vigorous stirring were maintained. At the end of 3 hr, most of the organic layer was decanted into a 500-ml erlenmeyer flask. The inorganic salts were filtered from the remaining organic layer using a büchner funnel and water aspirator. The combined organic layer was dried over anhydrous sodium sulfate for 2 hr.

Pentylenetetrazol-10-^{14}C (3)—The dried hydrazoic acid obtained was placed in a 250-ml, three-necked, round-bottom flask fitted with a mechanical stirrer, dropping funnel, and three-way connecting tube. Anhydrous ferric chloride was added (1 g for each gram of ketone) to the reaction flask, and the mixture was cooled to 0° with a dry ice-2-ethoxyethanol bath. Five grams of cyclohexanone containing 0.033 g of cyclohexanone-1-^{14}C[1] (2.36 mCi) and 4.967 g of unlabeled cyclohexanone was dissolved in 50 ml of benzene. This solution was added dropwise to the reaction flask. The reaction was stirred for 4 hr while the temperature was allowed to rise slowly to room temperature.

At the end of the prescribed time, the organic layer was decanted into a 500-ml separator to which was added 35 ml of a 30% sodium hydroxide solution. The separator was shaken well. The lower alkaline aqueous layer was removed and placed in the three-necked reaction flask which still contained the black, tarry pentylenetetrazol–ferric chloride complex. The resulting mixture was stirred for about 2 min, and the aqueous suspension was centrifuged at 3000 rpm for 6 min. The centrifugate was poured into a 150-ml beaker, and the residue from the centrifugation was resuspended in another 35-ml portion of sodium hydroxide. The suspension was shaken on a mixer for 2 min and centrifuged again at 3000 rpm for 6 min. The centrifugate was added to the first centrifugate. A third 35-ml portion of 30% sodium hydroxide was added to the centrifugation residue, and the extraction was completed as described previously. The combined aqueous sodium hydroxide solution was saturated with ammonium sulfate. The undissolved salt was filtered from the alkaline solution using a medium porosity fritted-glass büchner funnel and a filtering flask attached to a water aspirator. The undissolved salt remaining in the büchner funnel was washed with two 20-ml portions of benzene to remove any pentylenetetrazol-10-^{14}C remaining with the salt.

The ammonium sulfate-saturated sodium hydroxide solution, approximately 100 ml, was placed in the 500-ml separator containing the original organic reaction layer decanted earlier. The labeled pentylenetetrazol was extracted from the solution in the separator with six 100-ml portions of benzene. The combined extract was dried for 12 hr over anhydrous sodium sulfate and the benzene was removed under reduced pressure, leaving the impure pentylenetetrazol. An 81.8% chemical yield was obtained.

Purification by Crystallization—The impure product was placed in a 50-ml erlenmeyer flask in a 70° water bath and was dissolved in the smallest possible quantity of absolute ethanol. *n*-Heptane was then added slowly to the point of cloudiness. Absolute ethanol was added dropwise until the cloudiness just disappeared. The warm solution was placed in a refrigerator and allowed to cool. When a temperature of approximately 40° was observed, a small crystal of authentic pentylenetetrazol was placed in the flask to seed the saturated solution, and the container was again placed in the refrigerator for further cooling. Upon further cooling, well-defined crystals of pentylenetetrazol-10-^{14}C were obtained. Crystallization was complete within 12 hr. After purification, a 62.6% chemical yield of pentylenetetrazol-10-^{14}C was obtained. The radiochemical yield was found to be 62.7%.

Radiochemical purity was determined by TLC, autoradiography, and liquid scintillation counting. A study of the TLC separation of pentylenetetrazol, cyclohexanone, cyclohexanone oxime, and ϵ-caprolactam revealed that pentylenetetrazol and cyclohexanone oxime were chromatographically inseparable in the two solvent systems reported previously (3). GLC showed that cyclohexanone oxime, which was present during the synthesis, was removed during the crystallization procedure. The results of radiochemical determinations revealed that the synthesized pentylenetetrazol-10-^{14}C had a radiochemical purity greater than 99.7%. The specific activity was 0.326 mCi/g. The melting point was 57.7–58.9°; the reported melting point is 57–59° (7). A mixed melting-point determination showed no depression.

This procedure was used five times with unlabeled cyclohexanone. The chemical yields of purified pentylenetetrazol ranged from 57.0 to 63.1%.

SUMMARY

The improved synthesis reported in this paper resulted in a 62.6% yield as compared to reported yields for the radiolabeled synthesis of 22% (2) and 36% (3). Specifically, the following improvements were made in the synthetic procedure reported by Stiver *et al.* (3).

1. The cold bath used was dry ice–2-ethoxyethanol as compared to the ice-acetone (not dry ice) bath reported previously. This improvement was needed to maintain the 0° temperature necessary for the reactants in the synthesis.

2. Preliminary syntheses revealed that the reaction was complete in approximately 2 hr and that the longer time periods reported (1, 3) produced no increase in yield. In this study, a 4-hr reaction time was utilized for convenience and to ensure completion of the synthesis.

3. Improved yields were realized by improvements in the extraction of the product from the reaction mixture. Specifically, a study revealed that three sodium hydroxide washes were essential for removal of the product from the pentylenetetrazol–ferric chloride complex. Also, this study revealed that two benzene washes were necessary to remove the product from the undissolved ammonium sulfate salts.

4. Crystallization from a suitable solvent was shown to be superior to sublimation and thick-layer chromatography as a method for purification of pentylenetetrazol-10-^{14}C. A greater yield was achieved with considerably less expenditure of time and no sacrifice in either chemical or radiochemical purity.

REFERENCES

(1) N. B. Chapman, H. McCombie, and B. S. Saunders, *J. Chem. Soc.*, **45**, 929(1945).

(2) A. R. Ronzio and A. Murray, U.S. Atomic Energy Commission Document, AECU 1882, LADC 1087, 1952.

(3) J. F. Stiver, J. B. Data, and J. E. Christian, *J. Label. Compounds*, **6**, 40(1970).

(4) J. F. Stiver, Ph.D. thesis, Purdue University, West Lafayette, Ind., 1970.

(5) L. F. Audrieth and C. F. Gibbs, in "Inorganic Synthesis," vol. I, McGraw-Hill, New York, N.Y., 1939, p. 78.

[1] Amersham/Searle, Arlington Heights, Ill.

(6) H. Wolff, in "Organic Reactions," vol. III, Wiley, New York, N.Y., 1946, p. 327.
(7) "The National Formulary," 11th ed., Mack Publishing Co., Easton, Pa., 1960, p. 267.

ACKNOWLEDGMENTS AND ADDRESSES

Received June 28, 1973, from the *Bionucleonics Department, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907*

Accepted for publication December 18, 1973.

Supported in part by a grant from Philips Roxane Laboratories, Inc., Columbus, OH 43216

* Present address: Nuclear Medicine, Medical Center, University of Nebraska, Omaha, NB 68105

x To whom inquiries should be directed.

Sorption Rates of Water-Soluble Dyes on Soft Gelatin Capsules

HAROLD L. SMITH

Abstract □ A method was developed for determining sorption rates of water-soluble dyes by soft gelatin capsules. This method was used for determining the diffusivities and activation energies of five FD&C water-soluble dyes. The activation energies of these dyes were in either of two groups which had ranges of 25.1–25.7 and 34.8–36.0 kcal/mole.

Keyphrases □ Dyes, water soluble—determination of sorption rates on soft gelatin capsules □ Gelatin capsules, soft—determination of sorption rates of water-soluble dyes □ FD&C water-soluble dyes—determination of sorption rates on soft gelatin capsules □ Surface dyeing—determination of sorption rates of five FD&C water-soluble dyes on soft gelatin capsules

Several FD&C dyes were removed from the Food and Drug Administration's food additives list because they exhibited carcinogenic activity in animals (1). As a result, various methods for reducing the quantity of dyes in pharmaceutical dosage forms have become a subject of considerable interest. For soft shell gelatin capsules, such dye reductions have been achieved by surface dyeing untinted capsules instead of incorporating the dye into the gelatin shell formulation prior to preparing the capsules. The use of surface dyeing methods for this purpose is predicated upon the dyes diffusing into the capsule surface at rates slow enough to obtain a desired color and fast enough to be a reasonable processing step in the manufacture of the dosage form. A survey of the literature reveals that a method suitable for studying dyeing rates of this type of product has not been reported.

The purpose of this article is to describe a method for determining the sorption rates of dyes by intact untinted soft gelatin capsules under conditions similar to those used in production for surface dyeing. Data obtained using this method are presented for five FD&C water-soluble dyes: Yellow No. 5 (Tartrazine), Yellow No. 6 (Sunset Yellow FCF), Red No. 2 (Amaranth), Red No. 4 (Ponceau SX), and Green No. 3 (Fast Green FCF).

Table I—Diffusivities for the Sorption of FD&C Water-Soluble Dyes by Soft Gelatin Capsules

FD&C Dye	Temperature	Diffusivity $\times 10^7$, cm^2 sec^{-1}	Correlation Coefficient
Yellow No. 5	3.8°	1.11	0.994
Yellow No. 5	8.4°	1.79	0.997
Yellow No. 5	12.9°	4.70	0.997
Yellow No. 5	15.6°	6.66	0.994
Yellow No. 5	20.0°	13.6	0.999
Yellow No. 6	6.0°	1.35	0.995
Yellow No. 6	11.2°	2.88	0.999
Yellow No. 6	15.0°	4.75	0.994
Yellow No. 6	20.3°	12.6	0.999
Yellow No. 6	25.0°	24.5	0.999
Red No. 4	4.3°	0.657	0.989
Red No. 4	7.8°	1.28	0.999
Red No. 4	12.4°	2.34	0.995
Red No. 4	16.1°	4.85	0.999
Red No. 4	20.0°	7.70	0.999
Red No. 2	4.0°	0.865	0.998
Red No. 2	7.6°	1.65	0.996
Red No. 2	13.0°	5.94	0.997
Red No. 2	16.2°	12.4	0.999
Red No. 2	20.0°	25.2	0.999
Green No. 3	4.0°	0.323	0.998
Green No. 3	8.3°	0.805	0.995
Green No. 3	12.9°	2.31	0.999
Green No. 3	15.6°	5.05	0.999
Green No. 3	20.0°	10.8	0.999

EXPERIMENTAL

The capsules used were prepared with no fill material (air-fills) on a conventional soft gelatin encapsulating machine. The dried capsules contained approximately 66% gelatin[1] (125 Bloom strength), 30% glycerol, 3% water, and 1% parabens [methylparaben-propylparaben (1:3)]. All dye solutions contained 0.5% of one of the FD&C soluble dyes[2] in 50% (v/v) isopropyl alcohol and water.

Three capsules were mounted near the end of each of five sample holders (8 × 1 × 0.1 cm) with a space of approximately 1.0 cm left between capsules. Each capsule was mounted perpendicular to the holder with a small amount of silicon rubber[3]. The

[1] Vyse and Co., Chicago, Ill.
[2] H. Kohnstamm and Co., Inc., New York, N.Y.
[3] RTV 102 General Electric.