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Oxidative Degradation of Hydrocortisone in Presence of Attapulgite

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Received November 1, 1979, from the **Department of Agronomy and the* ‡*Department of Industrial and Physical Pharmacy, Purdue University, West Lafayette, IN 47907.* Accepted for publication March 12, 1980.

Abstract □ Degradation of hydrocortisone in attapulgite suspensions was monitored by high-pressure liquid chromatography and UV spectrophotometry. The rate of oxidative degradation of hydrocortisone was accelerated significantly in the presence of attapulgite. In addition, degradation appeared to be composed of two apparent first-order reactions rather than the single apparent first-order degradation reaction observed for hydrocortisone solutions. However, the same degradation products were obtained in both hydrocortisone solutions and attapulgite suspensions, indicating that interaction with attapulgite did not alter the degradation pathway. Kinetic and adsorption studies suggested that hydrocortisone is adsorbed weakly by attapulgite and undergoes oxidative degradation, which is catalyzed by adsorbed iron oxides or hydroxides as well as by structural ferric iron at the clay surface. Since clay minerals generally contain surface ferric iron, the potential for accelerating the oxidative degradation of drugs should be considered whenever clays and drugs are combined.

Keyphrases □ Attapulgite—effect on hydrocortisone degradation, kinetic and adsorption studies □ Hydrocortisone—oxidative degradation in attapulgite suspensions □ High-pressure liquid chromatography—monitoring of degradation of hydrocortisone in attapulgite suspensions □ Adsorption—hydrocortisone onto attapulgite, oxidative degradation of hydrocortisone

A recent study of the interaction of montmorillonite with digoxin revealed that the clay surface, through its ability to concentrate both digoxin and protons, accelerated the acid-catalyzed hydrolysis of digoxin and suggested that other neutral drugs which are degraded by acid-catalyzed hydrolysis may be affected similarly by interaction with a clay (1). Since oxidation also is a major mechanism of drug degradation, a study was undertaken to determine if interaction with a clay surface could result in accelerated oxidative degradation.

BACKGROUND

Montmorillonite, a member of the smectite group of clays, promotes the oxidation of a number of organic compounds, including the conversion of pyrogallol to quinones of poorly defined structure (2), of dihydroxyquinones to *p*-benzoquinone (3), and of benzidine to a blue monovalent semiquinone (4). In addition, hectorite, a clay belonging to the same structural group as montmorillonite, catalyzes the oxidation of benzidine (5, 6). Surface-adsorbed contaminants or structural ferric iron at the clay surface have been suggested as being responsible for the oxidation of organic materials by these clays.

Attapulgite was chosen for this study because: (*a*) it belongs to the fibrous group of minerals whose effect on oxidative degradation has not been studied extensively, (*b*) the oxidizing action of Fe^{3+} in attapulgite has been demonstrated (7), and (*c*) it is used in pharmaceuticals as a GI adsorbent (8) or excipient. Hydrocortisone was chosen as the model drug because it is known to degrade by oxidation, it may be coadministered orally with a clay-containing pharmaceutical, and it is used in topical dosage forms that also may contain a clay.

Degradation of the C-17 dihydroxyacetone side chain of corticosteroids has been studied extensively (9–13). Transformations and elimination of the side chain occur in both the presence and the absence of oxygen (12). However, autoxidation appears to be the major mechanism of degradation of corticosteroids in pharmaceutical dosage forms (12).

Factors influencing the degradation of prednisolone in aqueous solution were investigated, and trace metals present as contaminants in the buffer reagents were indicated as the cause of accelerated degradation (11).

EXPERIMENTAL

Materials—All chemicals were official or reagent grade. Attapulgite was obtained commercially[1]. X-ray diffraction of the clay sample confirmed that attapulgite was the major mineral but that a small amount of quartz also was present.

The effect of surface ferric iron was studied by treating the attapulgite by the citrate–dithionite procedure (14), which extracts nonstructural iron from the clay surface. The iron extracted was quantified by the *o*-phenanthroline method (15), and the total iron content was determined by the hydrofluoric acid dissolution procedure (16).

Hydrocortisone Assay—A high-pressure liquid chromatographic (HPLC) method, which was recommended for the analysis of hydrocortisone tablets (17), was modified slightly for this study. The liquid chromatograph[2] was equipped with a UV detector operating at 254 nm and a 20-μl injector loop[3]. A commercially packed octadecylsilane[4] column was used with acetonitrile–water (35:65) as the mobile phase. The operating parameters were: flow rate, 1 ml/min; pressure, 1000–1200 psi; temperature, ambient; and UV attenuator, 0.02 aufs. Linear calibration curves were used to quantify hydrocortisone, while the relative concentration of the observed degradation products was characterized by peak heights.

Changes in the A-ring of hydrocortisone were monitored by UV spectrometry at 254 nm.

Self-supporting films were prepared for IR analysis[5] by pipetting appropriate volumes of either the attapulgite or the hydrocortisone–atta-

[1] Pharmabsorb, colloidal, Engelhard Minerals and Chemicals Corp., Menlo Park, N.J.
[2] Model ALC 202, Waters Associates, Framingham, Mass.
[3] Rheodyne, Berkeley, Calif.
[4] Partisil-10 ODS, Whatman Inc., Clifton, N.J.
[5] Model 180, Perkin-Elmer Corp., Norwalk, Conn.

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Table I—Stability of Hydrocortisone in Presence of Attapulgite

Temperature	Aqueous Solution at pH 8.4, k_1, hr^{-1}	Attapulgite Suspension[a] at pH 8.4		Dithionite-Citrate-Treated Attapulgite Suspension[a] at pH 8.4	
		k_1, hr^{-1}	k_2, hr^{-1}	k_1, hr^{-1}	k_2, hr^{-1}
23°	—[b]	20.2×10^{-3}	4.4×10^{-3}	8.10×10^{-3}	2.3×10^{-3}
38°	—[b]	25.7×10^{-3}	8.3×10^{-3}	—	—
50°	0.8×10^{-3}	67.0×10^{-3}	14.3×10^{-3}	—	—
69°	1.5×10^{-3}	93.6×10^{-3}	22.2×10^{-3}	—	—

[a] For comparative purposes, the complex degradation pattern of hydrocortisone in attapulgite suspension was treated as consisting of an initial first-order phase characterized by k_1, which was followed by a slower, first-order phase characterized by k_2. [b] No measurable degradation of hydrocortisone occurred in 180 hr.

pulgite suspension onto polyethylene terephthalate[6] and air drying them at room temperature (18).

Hydrocortisone was prepared for IR analysis as a potassium bromide pellet.

Kinetic Studies—The attapulgite concentration was selected as representative of the range of clay usually used in pharmaceuticals; the hydrocortisone concentration was below the solubility limit, 280 μg/ml at 25° (19), to ensure complete solubility during the kinetic studies. Thus, 360 mg of attapulgite was mixed with 30 ml of an aqueous solution of hydrocortisone (200 μg/ml) in a 50-ml stoppered centrifuge tube. The samples were aged in a constant-temperature shaker bath at 23, 38, 50, or 69°. At appropriate intervals, aliquots were centrifuged at 6000 rpm, and the supernate was filtered[7] and analyzed by HPLC.

The pH of each suspension was measured and found to be 8.4 at each sampling interval.

Aqueous solutions of hydrocortisone (200 μg/ml), adjusted to pH 8.4 with sodium hydroxide, were aged with the drug–clay suspensions and served as controls.

The supernate from a suspension of 360 mg of attapulgite in 30 ml of water was filtered and used to prepare a hydrocortisone solution (200 μg/ml). The stability of hydrocortisone in this solution was monitored during aging at 23°.

A hydrocortisone–attapulgite suspension was monitored for 1 week while aging at 23°. After 1 week, the solid phase (attapulgite) was separated by centrifugation and washed three times with distilled water. The washed attapulgite was used to prepare a fresh hydrocortisone–attapulgite suspension whose stability at 23° was monitored as described.

RESULTS AND DISCUSSION

Hydrocortisone exhibited very slow degradation in aqueous solution at pH 8.4, which is consistent with previously reported stability results (20). A hydrocortisone solution at pH 8.4 was monitored every 2 days for 42 days while aging at 69°. During this time, the hydrocortisone concentration decreased from 200 to 33 μg/ml. A plot of log hydrocortisone concentration *versus* time gave a straight line ($r = 0.981$), indicating that degradation of hydrocortisone in solution at pH 8.4 occurs by apparent first-order kinetics (Table I). Earlier reports (9–12) suggested that hydrocortisone degrades by apparent first-order kinetics, although Pitman *et al.* (20) used the tetrazolium assay and observed complex kinetics for the degradation of hydrocortisone in 0.05 *M* NaOH.

Hydrocortisone degraded rapidly in the presence of attapulgite at pH 8.4. For example, the hydrocortisone content of an attapulgite suspension at pH 8.4 decreased from 200 to 100 μg/ml after aging at 23° for ~2 days (Fig. 1). During the same period, the hydrocortisone content of an aqueous solution at pH 8.4 and 23° remained at 200 μg/ml (Table I). The relationship between the hydrocortisone concentration or the logarithm of the hydrocortisone concentration and time was not linear at any temperature studied. To characterize the hydrocortisone degradation rate for comparative purposes, the degradation profile (Fig. 1) was treated as consisting of an initial first-order phase, characterized by k_1 which was followed by a slower, first-order phase characterized by k_2 (Table I).

This approach appears to be justified since both k_1 and k_2 followed the Arrhenius equation and the activation energy for the degradation of hydrocortisone was the same regardless of whether it was calculated from k_1 or k_2 (*i.e.*, 7.13 and 7.16 kcal/mole, respectively).

When the solubility of hydrocortisone was monitored by UV spectrophotometry, no change in absorbance at 254 nm was observed in the control solutions. Similarly, except for a small initial decrease, no change in absorbance at 254 nm was observed when the hydrocortisone–attapulgite suspensions were aged at 23, 38, 50, and 69° (Fig. 1). These results indicate that the degradation of hydrocortisone in the control solutions and the attapulgite suspensions occurred by oxidation of the 17-dihydroxyacetone side chain rather than by photolytic decomposition of the A-ring. The initial decrease in UV absorbance at 254 nm in the presence of attapulgite may be due to a small degree of adsorption of hydrocortisone by the clay.

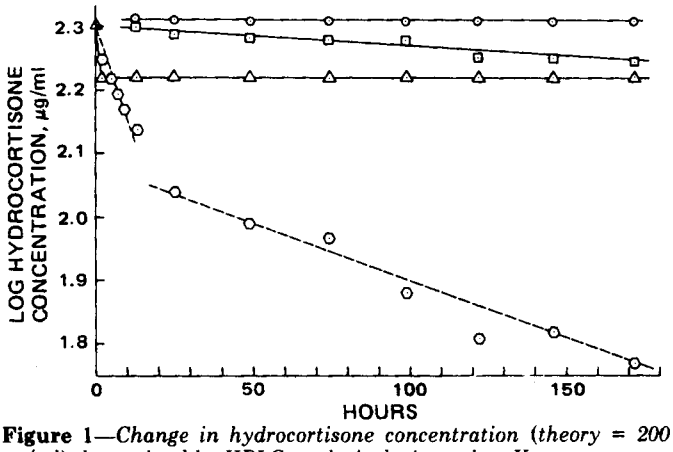

Figure 1—*Change in hydrocortisone concentration (theory = 200 μg/ml) determined by HPLC analysis during aging. Key: □, aqueous solution at pH 8.4 and 50°; ○, solution in supernate from attapulgite suspension at 38°; ○, attapulgite suspension at pH 8.4 and 23°; and △, hydrocortisone concentration of attapulgite suspension at pH 8.4 and 23° determined by UV absorbance at 254 nm.*

The same degradation pathway of hydrocortisone was observed in both the presence and the absence of attapulgite. Other investigators (9, 12) reported two degradation products for prednisolone: a small amount of a neutral keto product and a larger quantity of acidic material. Olson (21) also reported two peaks in addition to hydrocortisone during the HPLC analysis of hydrocortisone in alkaline media.

Figure 2A shows the three peaks observed in the high-pressure liquid chromatograms at 23, 38, and 50°. The peak having a retention time of

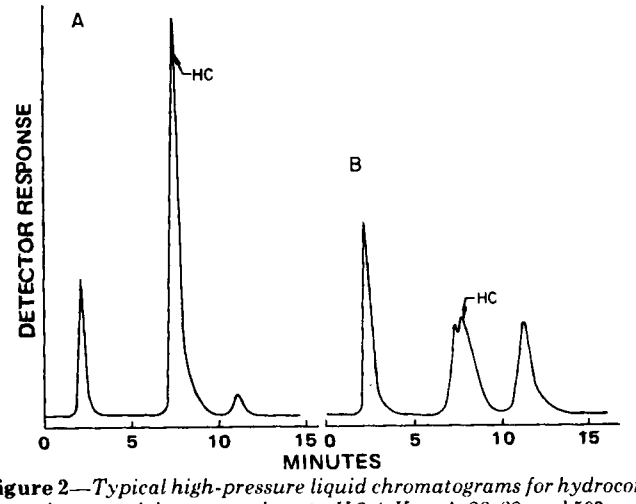

Figure 2—*Typical high-pressure liquid chromatograms for hydrocortisone in attapulgite suspension at pH 8.4. Key: A, 23, 38, and 50°; and B, 69°; HC represents the hydrocortisone peak.*

[6] Mylar.
[7] No. 42, Whatman Inc., Clifton, N.J.

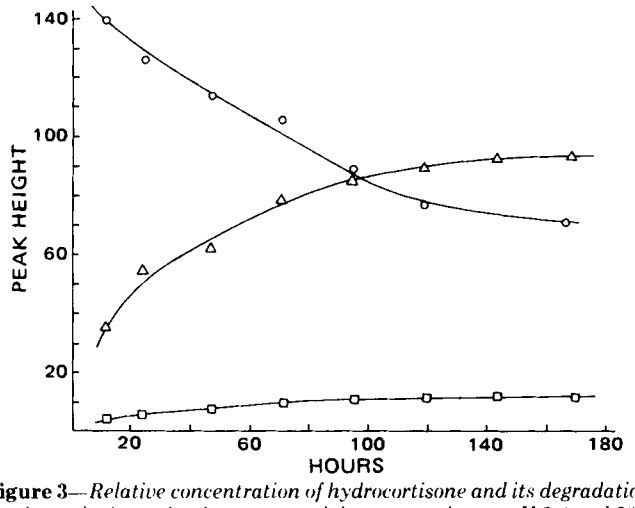

Figure 3—*Relative concentration of hydrocortisone and its degradation products during aging in an attapulgite suspension at pH 8.4 and 23°. Key: ○, hydrocortisone; △, acidic degradation product with retention time of 2.5 min; and □, neutral degradation product with retention time of 11 min.*

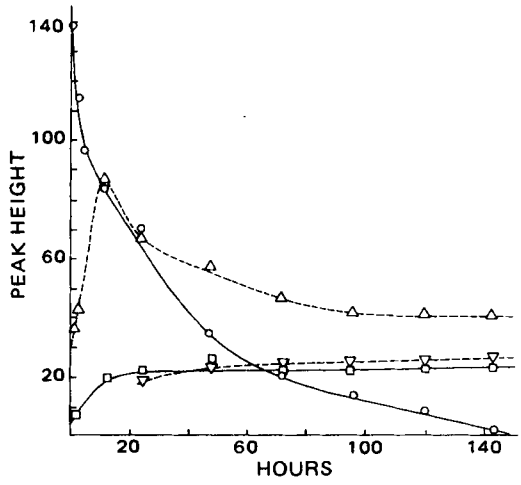

Figure 4—*Relative concentration of hydrocortisone and its degradation products during aging in an attapulgite suspension at pH 8.4 and 69°. Key: ○, hydrocortisone; △, acidic degradation product with retention time of 2.5 min; □, neutral degradation product with retention time of 11 min; and ▽, unknown degradation product with retention time of 7 min.*

8 min corresponded to the hydrocortisone reference standard. No attempt was made to identify the other two peaks. However, the degradation profile seen in Fig. 3 suggests that compounds with retention times of 2.5 and 11 min arise directly from hydrocortisone and that the former compound is the acidic product while the latter compound is the neutral product noted in previous studies. Similar degradation profiles were obtained at 38 and 50°.

A third degradation product having a retention time of 7 min was observed at 69° (Fig. 2B). This compound did not appear until the hydrocortisone was substantially degraded. The third degradation product began to appear after the degradation product with a retention time of 2.5 min (acidic product) reached its maximum concentration (Fig. 4). This type of degradation profile suggests that the third degradation product arises from further decomposition of the acidic product.

The increased degradation rate of hydrocortisone in an attapulgite suspension is not due to any soluble impurity or extractable component of attapulgite since virtually no degradation of hydrocortisone occurred in a solution prepared using the supernate from an attapulgite suspension (Fig. 1). Thus, the accelerated degradation of hydrocortisone in attapulgite suspensions requires contact with the clay surface.

It was hypothesized that oxidation of the C-17 side chain was the mechanism responsible for the accelerated degradation and that the source of the oxidizing action occurring at the surface of attapulgite was ferric iron. Surface ferric iron in attapulgite, as in other clay minerals, may exist either as surface-adsorbed iron oxides or hydroxides or as structural ferric iron, which is located mainly in the octahedral sites of attapulgite. To verify this hypothesis, a portion of attapulgite was treated by the citrate–dithionite method to extract nonstructural ferric iron. The amount of ferric iron extracted was 0.62 mg/g, which represents 2.3% of the total iron (26.6 mg/g) in the sample. Hydrocortisone was much more stable in a suspension of the treated attapulgite, although the degradation was substantially greater than that occurring in aqueous hydrocortisone solutions. The degradation profile did not fit either zero- or first-order kinetics; for comparison, it also was treated as consisting of two first-order phases (Table I). The twofold reduction in the degradation rate of hydrocortisone when surface ferric iron was removed from the attapulgite indicates that nonstructural ferric iron catalyzes the degradation of hydrocortisone. However, structural ferric iron probably also is involved in the catalysis since accelerated degradation occurred in the presence of treated attapulgite.

The IR spectrum of the hydrocortisone–attapulgite suspension did not show any evidence of adsorption of hydrocortisone. However, the small initial decrease in the hydrocortisone content of the supernate of a hydrocortisone–attapulgite suspension (Fig. 1) suggests that hydrocortisone is adsorbed weakly by attapulgite and undergoes oxidative degradation, which is catalyzed by both nonstructural and structural ferric iron at the clay surface.

The catalytic potential of attapulgite appears to be readily regenerated. The degradation rate of hydrocortisone in an attapulgite suspension was determined at 23° (Table I). The attapulgite was recovered and, after washing, was used to prepare a fresh hydrocortisone–attapulgite suspension. The rate profile and k_1 and k_2 values were identical to the results obtained for the original suspension.

It is hypothesized that the two-phase degradation reaction of hydrocortisone observed in the presence of attapulgite may be an indication that two kinds of sites for iron exist in attapulgite. As the oxidizing potential of the more accessible sites is exhausted, the degradation rate decreases.

The rate of diffusion of oxygen to the iron sites may be the rate-limiting factor. In this case, the rapid, initial degradation of hydrocortisone causes the oxidative potential of the iron sites to be exhausted. The regeneration of the sites is limited by the diffusion of oxygen to the sites, and a slower second phase is observed.

A third possible mechanism that may produce the two-phase degradation profile could occur if the hydrocortisone degradation products are adsorbed more strongly by attapulgite than hydrocortisone and preempt the catalytic sites on attapulgite. The two phases also may arise if both an oxidative and a nonoxidative degradation reaction occurs at the C-17 side chain.

Since clay minerals generally contain surface ferric iron, the potential for accelerating the oxidative degradation of drugs should be considered whenever clays and drugs are combined.

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Quantitative Semiautomated Colorimetric Determination of Thyroid (Iodine) in Thyroid Tablets

E. D. McGARY

Received October 1, 1979, from the *Food and Drug Administration, Department of Health, Education, and Welfare, Kansas City, MO 64106.* Accepted for publication March 21, 1980.

Abstract □ A faster, simpler, more sensitive, and semiautomated method was developed for the analysis of thyroid in individual thyroid tablets and composite samples. The sample is combusted in a Schoniger flask in an oxygen atmosphere, and the liberated iodine is trapped in a dilute sodium hydroxide solution. The solution is acidified with sulfuric acid (1:1), and the iodine is determined colorimetrically using a suitable spectrophotometer equipped with an automated analyzer. The proposed methodology was applied to tablets containing 32.4–324 mg of thyroid. Recoveries ranged from 90.0 to 125.0% of the amount of iodine added as potassium iodide. The proposed method is sensitive to 2.5 ng of iodine/ml.

Keyphrases □ Thyroid—semiautomated colorimetric analysis in individual tablets □ Iodine—semiautomated colorimetric analysis in individual thyroid tablets □ Colorimetry—analysis, thyroid in individual thyroid tablets

This paper describes a simple method for the determination of thyroid in individual thyroid tablets and composite samples. The method is fast and reliable. It permits more samples to be analyzed in an equivalent amount of time than previously reported methodology (1–3) and gives comparable results when applied to individual thyroid tablets and composite samples.

BACKGROUND

The USP XIX procedure for content uniformity (1) employs an empty sample wrapper, which is combusted and used as the blank preparation. When these sample wrappers are used for the oxygen flask combustion in the developed procedure, high and erratic blanks are recorded. Experiments (4) indicated that the high blanks are due to the black dye incorporated in the sample wrappers and can be minimized by washing the sample wrappers several times with distilled water and drying before use. For reliable results, the blank must have <3 ng of iodine/ml for this procedure.

In the procedure developed by Graham (2), iodine is determined spectrophotometrically as the triiodide ion, following the oxygen flask ignition of Moody *et al.* (3), from a sample equivalent to 30 mg of thyroid. The USP assay (1) utilizes essentially the same chemical transformation but requires a sample equivalent to 1 g of thyroid and a final thiosulfate titration. Both procedures give comparable results.

Luchtefeld (5) developed a semiautomated procedure, which was based on the kinetic procedure of Sandell and Kolthoff (6, 7) for the determination of iodine in the Total Diet market basket. By combining the procedures and making selected modifications, a method has been developed that is faster, simpler, more sensitive, and capable of being automated.

EXPERIMENTAL

Apparatus—The automated analyzer[1] system consisted of a water bath maintained at 40°, heating coils [6.1-m (20-ft) inner coil and 12.2-m (40-ft) outer coil of 1.6-mm i.d. glass tubing], tubing S[2] and T[3], a spectrophotometer[4] (gears must be adjusted to permit chart scan with stationary wavelength at 405 nm), and 10-mm flowcells. The schematic diagram in Fig. 1 shows the coils, tubing, and fittings.

The combustion system utilizes sample wrappers[5] (which must be washed prior to use), sample carriers[6], stoppers[7], 1-liter Schoniger oxygen combustion flasks[8], clamps[9], and an oxygen flask igniter[10].

Sample Wrapper Preparation—The sample wrappers were placed in a beaker and covered with distilled water. The beaker was placed in a sonic vibrator for 10–15 min, and the water was discarded. The procedure was repeated five times to decrease the amount of iodides present in the sample wrappers. Then the wrappers were placed on a paper towel and allowed to dry at room temperature.

Reagents—*Ceric Ammonium Nitrate Solution*—Ceric ammonium nitrate, 8.66 g, was dissolved in 100 ml of water in a 2-liter flask, 56.8 ml of sulfuric acid (1:1) was added, and the solution was cooled and diluted to 2 liters with water.

Arsenous Acid Solution—Arsenic trioxide, 15 g, was dissolved in 500 ml of water (made basic with 10 ml of 50% NaOH) by heating on a steam bath. The solution was cooled, 56.8 ml of sulfuric acid (1:1) was added, and the solution was cooled and diluted to 2 liters with water.

Sodium Sulfate Solution—To prepare the sodium sulfate solution, 60 ml of 50% NaOH was transferred to a 2-liter flask with 1 liter of water. Sulfuric acid (1:1), 100 ml, was added cautiously. The solution was cooled and diluted to 2 liters with water.

Sodium Chloride Solution—The sodium chloride solution was a 0.5% aqueous solution.

Standard Preparation—To prepare the standards, 0.1308 g of certified ACS grade potassium iodide[11] was dissolved in water, and the solution was diluted to 1 liter to give a concentration of 0.1 mg of iodine/ml. This solution was diluted with the sodium sulfate solution to give a concentration of 0.1 μg of iodine/ml. Standards containing 2.5, 5.0, 10.0, 15.0, 20.0, and 25 ng of iodine/ml were prepared from the resulting solution and the sodium sulfate solution. These solutions are used for the standard curve and should be prepared fresh daily.

[1] AutoAnalyzer, liquid sampler 1, and proportioning pump 1, Technicon Instruments, Tarrytown, N.Y.
[2] Solvaflex. Technicon Instruments, Tarrytown, N.Y.
[3] Tygon, Plastics and Synthetics Division, Norton Co., Tallmadge, Ohio.
[4] Model DK-2A, Beckman Instruments, Fullerton, Calif.
[5] Block, Catalog No. 6414-F70, Arthur H. Thomas Co., Philadelphia, Pa.
[6] Ogg, Catalog No. 6514-F45, Arthur H. Thomas Co., Philadelphia, Pa.
[7] Thomas-Ogg, Catalog No. 6514-F45, Arthur H. Thomas Co., Philadelphia, Pa.
[8] Catalog No. 4980, Arthur H. Thomas Co., Philadelphia, Pa.
[9] Thomas-Ogg, No. 35, Arthur H. Thomas Co., Philadelphia, Pa.
[10] Thomas-Ogg, model 11, Arthur H. Thomas Co., Philadelphia, Pa.
[11] Fisher Scientific Co., Pittsburgh, Pa.

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