Sterilization of Selected Colorants by Cobalt-60

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Abstract □ A series of selected colorants composed of eight FD&C dyes, seven FD&C lakes, and four D&C dyes were subjected to 2.5 Mrads of γ-irradiation from a cobalt-60 source over approximately 10 hr. The soluble colorants so irradiated exhibited no changes when examined by visible, UV, and IR spectrophotometry, nor did they show any measurable radioactivity above that shown before irradiation. Color lakes were examined by IR spectroscopy, and the spectra of the dyes separated from the lakes in the visible and UV regions were obtained. These showed no changes. All colorants were examined by TLC using four different solvent systems, with no additional spots being obtained from the irradiated colorants. All colorants before irradiation were shown to contain microorganisms, and all were shown to be sterile after irradiation by a membrane filter technique or a direct plating method.

Keyphrases □ Colorants, certified dyes and lakes—sterilization by ^{60}Co, determination of possible irradiation-induced degradation □ Dyes, FD&C and D&C—sterilization by ^{60}Co □ Sterilization—FD&C dyes, FD&C lakes, and D&C dyes by γ-irradiation from ^{60}Co □ Irradiation—used to sterilize pharmaceutical colorants

The recent literature reflects a surge of interest in microbial contamination of cosmetics and nonsterile pharmaceuticals prompted by a concern for the safety of the product user. Numerous studies have appeared detailing microbial contamination of these products, both used and unused (1–8), as well as discussing the significance of the contaminating organism (1, 2, 9, 10). Legal aspects of the question were explored (11, 12), and limits on the acceptable (13) number and type of organisms were suggested. The types of microbial tests to be used on raw materials and finished products used topically or orally were outlined (14–17), and possible modifications of the USP microbial limits and tests were proposed previously (18).

Attention was focused on the preservatives used in these products (19–28). Testing methods and programs used to evaluate the effectiveness of preservatives were examined (29–32), as were interactions of the preservatives (33–35). Sources of contamination were indicated (36, 37) to be raw materials, equipment, environment, and personnel. Concern with microbial contamination of raw materials (38–41) led to a discussion of the use of ethylene oxide for sterilization of such materials (42, 43). Certified colorants were reported to show from 10 to 100% of samples contaminated with 10–1000 microorganisms/g (44). Controlling or eliminating troublesome microbes during the manufacturing and packaging operations has also received attention (45–51).

The use of ionizing radiation for killing microbes is widely recognized (52). An electron beam is currently used to sterilize sutures (53), and cobalt-60 (^{60}Co) has been shown to sterilize tubing, gloves (54), and various medical equipment (55) and to inactivate bacterial spores in penicillin (56). Many pharmaceuticals have been reported to be safely sterilized by a 2.5-Mrad dose of ^{60}Co (57). These include multivitamins, ascorbic acid, heparin, pregnenolone, cortisone acetate, potassium penicillin G, chlortetracycline, oxytetracycline, ergonovine maleate, and morphine sulfate. While the dose of ^{60}Co irradiation required for sterilization is still a subject of discussion,

Table I—Selected Water-Soluble Colorants

Colorant	Lot Number	Pure Dye Content, %
FD&C Blue No. 1[a]	Y8839	96
FD&C Blue No. 2[a]	Y7518	90
FD&C Green No. 3[a]	Y8944	89
FD&C Red No. 2[a]	X6945	90
FD&C Red No. 3[a]	X3699	92
FD&C Red No. 40[a]	Y9048	91
FD&C Yellow No. 5[a]	Y9021	91
FD&C Yellow No. 6[a]	Y8743	92
D&C Green No. 5[b]	X0110	85
D&C Yellow No. 10[b]	X0653	92

[a] Warner-Jenkinson Manufacturing Co., St. Louis, Mo. [b] H. Kohnstamm and Co., New York, N.Y.

Table II—Selected Water-Insoluble Colorants

Colorant	Lot Number	Pure Dye Content, %
FD&C Blue No. 1 Lake[a]	W8468	13
FD&C Blue No. 2 Lake[a]	W5126	12
FD&C Red No. 2 Lake[a]	W4868	24
FD&C Red No. 3 Lake[a]	W8904	16
FD&C Red No. 4 Lake[ab]	W6297	35
FD&C Yellow No. 5 Lake[a]	W8447	24
FD&C Yellow No. 6 Lake[a]	W9287	18
D&C Blue No. 6[c]	W9360	95
D&C Yellow No. 11[d]	—	—

[a] Warner-Jenkinson Manufacturing Co., St. Louis, Mo. [b] No longer certified. [c] H. Kohnstamm and Co., New York, N.Y. [d] American Cyanamid Co., New York, N.Y.

Table III—Sterility Test Results of Water-Soluble Colorants

Colorant	Irradiated	Nonirradiated
FD&C Blue No. 1	Sterile	Contaminated
FD&C Blue No. 2	Sterile	Contaminated
FD&C Green No. 3	Sterile	Contaminated
FD&C Red No. 2	Sterile	Contaminated
FD&C Red No. 3	Sterile	Contaminated
FD&C Red No. 40	Sterile	Contaminated
FD&C Yellow No. 5	Sterile	Contaminated
FD&C Yellow No. 6	Sterile	Contaminated
D&C Green No. 5	Sterile	Contaminated
D&C Yellow No. 10	Sterile	Contaminated

Table IV—Sterility Test Results of Water-Insoluble Colorants

Colorant	Filter Method		Direct Plating Method	
	Irradiated	Nonirradiated	Irradiated	Nonirradiated
FD&C Blue No. 1 Lake	Sterile	Contaminated	Sterile	Contaminated
FD&C Blue No. 2 Lake	Sterile	Sterile	Sterile	Contaminated
FD&C Red No. 2 Lake	Sterile	Sterile	Sterile	Contaminated
FD&C Red No. 3 Lake	Sterile	Sterile	Sterile	Contaminated
FD&C Red No. 4 Lake[a]	Sterile	Sterile	Sterile	Contaminated
FD&C Yellow No. 5 Lake	Sterile	Sterile	Sterile	Contaminated
FD&C Yellow No. 6 Lake	Sterile	Contaminated	Sterile	Contaminated
D&C Blue No. 6	Sterile	Contaminated	Sterile	Contaminated
D&C Yellow No. 11	Sterile	Contaminated	Sterile	Sterile

[a] No longer certified.

Table V—Dye Content of Irradiated Water-Soluble Colorants

Colorant	λ_{max}[a], nm	Concentration of Solution[b], mg/100 ml	Absorbance		Pure Dye, %	
			Non-irradiated	Irradiated	Non-irradiated[c]	Irradiated[d]
FD&C Blue No. 1	620	0.2	0.368	0.364	96	95.0
FD&C Blue No. 2	604	1	0.432	0.436	90	90.8
FD&C Green No. 3	618	0.2	0.348	0.349	89	89.3
FD&C Red No. 2	520	1	0.367	0.369	90	90.5
FD&C Red No. 3	524	0.4	0.456	0.452	92	91.2
FD&C Red No. 40	505	1	0.466	0.468	91	91.4
FD&C Yellow No. 5	428	1	0.459	0.458	91	90.8
FD&C Yellow No. 6	480	0.4	0.378	0.377	92	91.8
D&C Green No. 5	602	2	0.398	0.401	85	85.6
D&C Yellow No. 10	410	0.4	0.359	0.360	92	92.3

[a] Uncorrected. [b] Aqueous solution. [c] Labeled strength. [d] No significant difference compared to nonirradiated colorants.

it is generally agreed that 2.5 Mrads is sufficient (58).

Not all pharmaceuticals can be irradiated with no loss of potency. In aqueous solution, ascorbic acid, multivitamins, and heparin show a loss of activity. Insulin and atropine sulfate also suffer severe loss (57). Grosswiner and coworkers (59–61) found that fluorescein and eosin were subject to radiolysis in aqueous solution, and Kimura *et al.* (62) also found eosin to be subject to radiolysis in neutral and alkaline solutions. Prince and Welt (63) irradiated red D&C azo pigment (GBL-70-219) in the dry state and FD&C Red No. 3 in powder and in aqueous solution. They found that γ-irradiation up to 5 Mrads produced no change in the D&C pigment by several types of visual tests using trained observers. Solutions of FD&C Red No. 3 showed marked degradation of colorant at 0.2 Mrad; the same colorant, irradiated at a dose of 0.2–1.5 Mrads in the dry powder state, showed no change as judged by the absorbance of its aqueous solutions. The Food and Drug Administration has data suggesting the partial destruction of FD&C Red No. 3 and FD&C Yellow No. 5 Lake irradiated in the solid state at a 5-Mrad dose[1]. It was decided that in this experiment the selected colorants would be subjected to a 2.5-Mrad dose of γ-radiation from a ^{60}Co source, their microbial contamination would be examined, and changes in their pure dye content would be analyzed.

[1] J. T. Walters, Food and Drug Administration, personal communication, 1973.

EXPERIMENTAL

Materials—The colorants selected for γ-irradiation sterilization and their respective suppliers are given in Tables I and II. The lakes represent those dyes that have been adsorbed from solution onto alumina. Other materials used in this study were as follows: polyethylene vials, 4 dram with snap cap[2]; membrane filter, cellulose ester, 0.45-μm porosity, with hydrophobic edge[3]; Whatman No. 1 filter paper[4]; trypticase soy agar[5]; polysorbate 80[6]; pH 4 buffer solution[7]; TLC plates, silica gel, 0.25 mm thick[8]; and aseptic filtration apparatus[9].

Irradiation—Colorants were placed in polyethylene bottles previously cleaned ultrasonically. The vials were completely filled with colorant to eliminate excessive head space. Some of these vials were then placed into a cardboard mailing tube, 8.0 cm in diameter and 15.5 cm in height, and were subjected to γ-irradiation in this container. The irradiation facility[10] was so constructed that the ^{60}Co source was stored in a water well; when in use, the cobalt was raised toward or above the surface of the water to give the desired rate of irradiation. Since the maximum rate was desired, the cobalt source was raised until it surrounded the cardboard mailing tube. Three batches of colorants were irradiated, two at a dose rate of 2.53×10^5 rads/hr and one at 2.70×10^5 rads/hr. Approximately 10 hr was required to obtain a total applied dose of 2.5 Mrads for each batch.

Sterility Testing—For sterility testing, colorants were separated according to their water solubility. Those regarded as water soluble appear in Table I. A 300-mg portion of each of these colorants was aseptically obtained and dissolved in 30 ml sterile saline solution (0.9% w/v). This solution was vacuum filtered through a sterile, 0.45-μm porosity membrane filter in a sterile fil-

[2] Polyvials, Olympic Plastics, Los Angeles, Calif.
[3] Millipore filter, HAEG047A0, Millipore Corp., Bedford, Mass.
[4] W. and R. Balston, Ltd., England.
[5] Baltimore Biological Laboratory, Baltimore, Md.
[6] Tween 20, ICI America, Inc., Wilmington, Del.
[7] Catalog No. 29905-04, Matheson Scientific, Chicago, Ill.
[8] E. Merck Ag., Darmstadt, Germany.
[9] Sterifil, Millipore Corp., Bedford, Mass.
[10] Research Reactor Facility, University of Missouri, Columbia, Mo.

Table VI—Dye Content of Irradiated Water-Insoluble Colorants

Colorant	λ_{max}[a], nm	Concentration of Solution, mg/100 ml	Absorbance		Pure Dye, %	
			Nonirradiated	Irradiated	Non-irradiated[b]	Irradiated[c]
FD&C Blue No. 1 Lake	624	2	0.314	0.314	13	13.0
FD&C Blue No. 2 Lake	608	5	0.261	0.263	12	12.1
FD&C Red No. 2 Lake	520	2.5	0.317	0.319	24	24.2
FD&C Red No. 3 Lake	485	15[e]	0.381	0.381	16	16.0
FD&C Red No. 4 Lake[d]	500	2	0.383	0.382	35	34.9
FD&C Yellow No. 5 Lake	430	2.5	0.305	0.306	24	24.1
FD&C Yellow No. 6 Lake	480	2.5	0.314	0.318	18	18.2
D&C Blue No. 6	600	5	0.290	0.290	95	95.0
D&C Yellow No. 11	410	0.2[f]	0.267	0.268	—[g]	

[a] Uncorrected. [b] Labeled strength. [c] No significant difference compared to nonirradiated colorants. [d] No longer certified. [e] Ethanol solution. [f] Methanol solution. [g] Not labeled.

Table VII—R_f Values of Components of Water-Soluble Colorants

Colorant	Solvent	Visualization Method		
		Incandescent Light	UV Light	Iodine
FD&C Blue No. 1	A	0.627[a], 0.0	0.627[a], 0.0	0.627[a], 0.0
	B	1.00, 0.600[a]	0.733, 0.600[a]	1.00, 0.733, 0.600[a]
	C	0.379[a], 0.0345, 0.0	0.379[a], 0.0345, 0.0	0.379[a], 0.0345, 0.0
	D	0.128, 0.0567[a], 0.0	0.582, 0.163, 0.128	0.582, 0.163, 0.128
			0.0567[a], 0.0	0.0567[a], 0.0
FD&C Blue No. 2	A	0.797[a], 0.641, 0.0	0.797[a], 0.641, 0.0	0.797[a], 0.641, 0.0
	B	0.0[a]	0.715, 0.0[a]	0.715, 0.0[a]
	C	0.886[a], 0.725, 0.0	0.886[a], 0.725	0.886[a], 0.725, 0.0
	D	0.0[a]	0.324, 0.0[a]	0.324, 0.0[a]
FD&C Red No. 2	A	0.519[a], 0.356	0.519[a], 0.356	0.519[a], 0.356, 0.0741
	B	0.654[a], 0.615	0.654[a]	0.654[a], 0.615
	C	0.174[a]	0.174[a], 0.0	0.174[a], 0.0
	D	0.585[a], 0.363	0.585[a], 0.363	0.585[a], 0.363, 0.0
FD&C Red No. 3	A	0.414[a], 0.223	0.414[a], 0.223	0.414[a], 0.223, 0.0
	B	0.267[a]	0.267[a]	0.345, 0.267[a]
	C	0.388[a], 0.102	0.388[a], 0.133, 0.102	0.388[a], 0.133, 0.102
	D	0.0863[a]	0.0863[a]	0.130, 0.0863[a]
FD&C Red No. 40	A	0.855[a]	0.855[a]	0.855[a], 0.0
	B	0.672[a]	0.672[a]	0.672[a], 0.0
	C	0.419[a]	0.419[a]	0.419[a], 0.0
	D	0.0550[a]	0.587, 0.0734, 0.0550[a]	0.587, 0.0734, 0.0550[a]
FD&C Green No. 3	A	0.831[a]	0.831[a]	0.831[a]
	B	0.833[a]	0.833[a]	0.833[a]
	C	0.0150[a]	0.0150[a]	0.1203, 0.0150[a]
	D	0.527[a]	0.527[a]	0.527[a], 0.0388
FD&C Yellow No. 5	A	0.135[a]	0.460, 0.135[a]	0.460, 0.294, 0.135[a]
	B	0.0678[a]	0.0678[a]	0.153, 0.0678[a]
	C	0.687[a]	0.687[a], 0.084	0.687[a], 0.084
	D	0.783[a]	0.783[a]	0.783[a], 0.444, 0.0
FD&C Yellow No. 6	A	0.258[a]	0.356, 0.258[a]	0.455, 0.356, 0.258[a], 0.0
	B	0.860[a]	0.860[a], 0.174	0.860[a], 0.174
	C	0.712[a]	0.712[a]	0.712[a], 0.0
	D	0.424[a]	0.743, 0.424[a]	0.743, 0.424[a], 0.368, 0.0
D&C Green No. 5	A	0.630[a]	0.630[a], 0.243	0.630[a], 0.243
	B	0.861[a]	0.861[a]	0.861[a], 0.0
	C	0.642[a], 0.585	0.642[a], 0.585	0.642[a], 0.585, 0.100
	D	0.217[a], 0.0	0.269, 0.217[a], 0.0	0.269, 0.217,[a] 0.0
D&C Yellow No. 10	A	0.835[a], 0.671	0.835[a], 0.671	0.835[a], 0.671
	B	0.862[a]	0.862[a], 0.750, 0.0	0.862[a], 0.750, 0.658, 0.0
	C	0.792[a], 0.436, 0.0	0.792[a], 0.436, 0.0	0.792[a], 0.436, 0.0
	D	0.659[a], 0.0	0.659[a], 0.0	0.659[a], 0.477, 0.0

[a] Represents the major component.

tration apparatus. The filter was transferred to a petri dish containing trypticase soy agar and incubated at 28–32° for up to 14 days. The plates were read every 48 hr.

The water-insoluble colorants (Table II) were tested by two methods, one of which was a filtration method. A 300-mg portion of each water-insoluble colorant was aseptically weighed and transferred to 30 ml sterile saline solution (0.9% w/v). This was vacuum filtered through a sterile Whatman No. 1 filter paper in a sterile filtration apparatus, and the insoluble material and the filter were washed with 100 ml of sterile saline solution. The filtrate thus obtained was then filtered through a sterile membrane filter in a second sterile filtration apparatus. The receiver of the first apparatus and the membrane and funnel of the second were rinsed with an additional 100 ml of sterile saline solution. The membrane filter was transferred to a petri dish containing trypticase soy agar and incubated at 28–32° for up to 14 days. The plates were read every 48 hr.

The second method used for water-insoluble colorants was a direct plating method. A 1.8-g sample of the colorant was aseptically weighed and transferred to 30 ml of sterile saline solution.

Table VIII—R_f Values of Components of Water-Insoluble Colorants

Colorant	Solvent	Visualization Method		
		Incandescent Light	UV Light	Iodine
FD&C Blue No. 1 Lake[a]	A	0.546[c]	0.546[c], 0.0987	0.546[c], 0.0987, 0.0
	B	0.517[c]	0.517[c], 0.0464, 0.0	0.702, 0.517[c], 0.0464, 0.0
	C	0.603[c]	0.603[c]	0.851, 0.603[c], 0.0
	D	0.104[c]	0.104[c], 0.0	0.104[c], 0.0
FD&C Blue No. 2 Lake[a]	A	0.231[c]	0.884, 0.231[c], 0.0	0.884, 0.231[c], 0.0
	B	0.459[c]	0.753, 0.459[c], 0.178	0.753, 0.459[c], 0.178, 0.0
	C	0.0[c]	0.707, 0.188, 0.0[c]	0.707, 0.188, 0.0[c]
	D	0.0[c]	0.694, 0.167, 0.0[c]	0.694, 0.167, 0.0[c]
FD&C Red No. 2 Lake[a]	A	0.0816[c]	0.565, 0.0816[c]	0.565, 0.170, 0.0816[c]
	B	0.455[c]	0.455[c], 0.177, 0.0	0.600, 0.455[c], 0.117, 0.0
	C	0.157[c]	0.607, 0.157[c], 0.0857	0.607, 0.157[c], 0.0857
	D	0.129[c]	0.129[c]	0.457, 0.200, 0.129[c], 0.0
FD&C Red No. 3 Lake[a]	A	0.468[c]	0.468[c], 0.0851, 0.0	0.468[c], 0.0851, 0.0
	B	0.299[c]	0.299[c], 0.104, 0.0	0.667, 0.299[c], 0.104, 0.0
	C	0.451[c]	0.451[c], 0.120, 0.0	0.451[c], 0.120, 0.0
	D	0.551[c]	0.551[c], 0.118	0.551[c], 0.118, 0.0
FD&C Red No. 4 Lake[a,b]	A	0.409[c]	0.409[c], 0.0657	0.577, 0.409[c], 0.0657, 0.0
	B	0.452[c]	0.452[c], 0.167, 0.0476	0.452[c], 0.167, 0.0476
	C	0.541[c]	0.541[c], 0.0764, 0.0	0.541[c], 0.0764, 0.0
	D	0.0709[c]	0.0709[c]	0.688, 0.0709[c], 0.0
FD&C Yellow No. 5 Lake[a]	A	0.270[c]	0.688, 0.468, 0.270[c]	0.688, 0.468, 0.270[c], 0.0
	B	0.659[c]	0.659[c], 0.0741, 0.0	0.659[c], 0.0741, 0.0
	C	0.462[c]	0.462[c], 0.0690, 0.0	0.462[c], 0.0690, 0.0
	D	0.0984[c]	0.0984[c], 0.0	0.0984[c], 0.0
FD&C Yellow No. 6 Lake[a]	A	0.512[c]	0.512[c], 0.0	0.512[c], 0.0930, 0.0
	B	0.604[c]	0.604[c], 0.0719, 0.0	0.604[c], 0.0719, 0.0
	C	0.441[c]	0.441[c]	0.441[c], 0.0882
	D	0.0876[c]	0.0876[c], 0.0	0.0876[c], 0.0
D&C Blue No. 6	A	0.0[c]	0.115, 0.0[c]	0.162, 0.115, 0.0[c]
	B	0.0[c]	0.0[c]	0.192, 0.0923, 0.0[c]
	C	0.0[c]	0.116, 0.0[c]	0.116, 0.0[c]
	D	0.0[c]	0.125, 0.0[c]	0.125, 0.0[c]
D&C Yellow No. 11	A	0.694[c], 0.0750	0.694[c]	0.694[c], 0.0750
	B	0.724[c], 0.0414	0.724[c]	0.724[c], 0.0414
	C	0.608[c]	0.608[c], 0.0	0.608[c], 0.0
	D	0.587[c], 0.247	0.587[c], 0.247	0.587[c], 0.247

[a] The dye separated from the lake. [b] No longer certified. [c] Major component.

The tube was shaken to suspend the colorant, and 1- and 5-ml portions of the suspension were placed on trypticase soy agar plates. For the D&C colorants, this procedure was modified by the use of a solution of polysorbate 80 in water (1% w/v) in place of the sterile saline to obtain a more uniform suspension. The plates were incubated at 28–32° and read after 48 hr. If the result was sterile or questionable at the end of the 48-hr period, transfers from both the 1- and 5-ml plates were made by streaking a 1-mm loop across the surface of the original and then across a fresh trypticase soy agar plate. This procedure was repeated five times for each plate, and the loop was flamed between each streak to reduce accidental contamination. The transfer plates and the original plates were incubated at 28–32°, and the plates were read every 48 hr up to 14 days. All manipulations for sterility testing were performed within a laminar flow hood[11].

Spectra—Visible and UV spectra of the colorants were obtained on a spectrophotometer[12] equipped with a linear-log recorder[13]. IR spectra were obtained[14] by a potassium bromide pellet technique. The visible and UV spectra of the dyes separated from the lakes were obtained after the dyes had been removed from the aluminum oxide by sulfuric acid and alcohol (64). Further dilutions were made with water. The same method was used to solubilize D&C Blue No. 6.

TLC—The dyes were spotted on silica gel plates in quantities of 50, 10, and 1 μg. Four different solvent systems were used for development in an ascending method: A, 0.1 *N* hydrochloric acid solution and methanol (1:50); B, 10% (w/v) sodium hydroxide solution and methanol (1:50); C, pH 4 buffer solution and 95% ethanol (1:20); and D, phosphate buffer solution USP, pH 7, and isopropanol (1:10).

Components of the dyes were examined visually, under UV light, after visualization with iodine vapors and ammonium hydroxide solution (5% w/v). Dyes separated from the lakes were spotted from the sulfuric acid–ethanol solution in quantities representing 50, 20, and 2 μg of the lake. Further treatment was the same as for the other dyes.

Residual Radiation—Radiation measurements were collected using a scaler and an ultrathin window tube[15]. The coloring material (0.50 g of the dyes or 2.5 g of the lakes) was placed on a Plexiglas holder 10 mm from the tube window. The counting period was 15 min, and each dye was counted three times before and after irradiation. Samples were also scanned before and after irradiation using a single-channel analyzer[16].

RESULTS AND DISCUSSION

Sterility Tests—It is obvious that the test result "sterile" does not refer to viruses, nor has any special medium or enrichment been employed to produce conditions favorable to growth of certain species. All test results (Tables III and IV) are based on a sample size of 300 mg, as recommended by USP XVIII (65). While results with water-soluble colorants are straightforward, conflicting results obtained for the nonirradiated water-insoluble colorants require some discussion. Before the filtration method was used to test the colorants, *Aspergillus niger* and *Bacillus cereus* were added to portions of the original colorant suspension. It was proved that the microbes could be separated from the insolu-

[11] Primaire, Dexon, Inc., Minneapolis, Minn.
[12] Beckman model DB.
[13] Beckman Industries, Inc., Fullerton, Calif.
[14] Perkin-Elmer model 457 IR spectrophotometer, Norwalk, Conn.
[15] Nuclear-Chicago model 1620A, Nuclear-Chicago, Chicago, Ill.
[16] Nuclear-Chicago model 1810.

ble color by Whatman No. 1 filter paper and washed through to be collected on the membrane filter. Apparently, however, this finding is related to the number of microorganisms present, as evidenced by comparison with test results of the direct plating method.

With the direct plating method, it was usually difficult to read the results; thus, transfer of some original material to fresh plates was made after 48 hr. To reduce errors caused by accidental contamination during the manipulations, transfer plates of the nonirradiated colorants were regarded as having positive growth only if at least four of the five streaks on the plate showed growth. The apparent antimicrobial effect of D&C Yellow No. 11 prevents it from being tested by the direct plating method.

IR Spectra—Examination of the IR spectrum from 250 to 1750 cm^{-1} of each colorant showed no new peaks appearing as a result of γ-irradiation.

Visible and UV Spectra—Examination of the spectrum from 200 to 800 nm of each colorant before and after ^{60}Co radiation showed no discernible differences. Tables V and VI list the wavelengths of the major absorption peaks of the colorants in the visible range and the change in percent of pure dye. The percent pure dye of the irradiated colorants was calculated at the λ_{max} according to the following equation (66):

$$\text{pure dye} = \frac{A_{\text{sample/conc. sample}}}{A_{\text{standard/conc. standard}}} \times \%\ \text{purity of standard} \quad \text{(Eq. 1)}$$

Each absorbance represents the mean of five determinations. The absorbance values were subjected to the t test, which showed no statistically significant differences between the irradiated and nonirradiated colorants.

TLC—The R_f values of the components of the colorants are shown in Tables VII and VIII. There was no discernible difference in the chromatograms of irradiated and nonirradiated colors. The visualization by means of ammonium hydroxide solution is omitted from these tables since it did not visualize any more spots than did iodine vapor.

Residual Radiation—In no case was the radioactivity of the colorant increased by the ^{60}Co irradiation process.

SUMMARY AND CONCLUSION

Selected colorants, 10 water soluble and nine water insoluble including seven lakes, were irradiated by ^{60}Co to a total dose of 2.5 Mrads and the following conclusions were reached:

1. Colorants can be sterilized by 2.5 Mrads of γ-irradiation from ^{60}Co.
2. There is no increase in residual radioactivity.
3. Examination of IR, visible, and UV spectra showed no physical-chemical changes. Therefore, these materials may be regarded as being colorfast to γ-irradiation under these conditions.
4. TLC in four solvent systems showed no difference between irradiated and nonirradiated colorants.

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ACKNOWLEDGMENTS AND ADDRESSES

Received September 24, 1973, from the *School of Pharmacy, University of Missouri-Kansas City, Kansas City, MO 64110*

Accepted for publication January 10, 1974.

Supported in part by a grant from the School of Graduate Studies, University of Missouri-Kansas City, Kansas City, Mo., 1973.

The authors express their appreciation to Jerome W. Kinnison, Warner-Jenkinson Co., St. Louis, Mo., for samples of colorants and valuable suggestions.

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PHARMACEUTICAL ANALYSIS

Analysis of Sodium Levothyroxine or Sodium Liothyronine in Tablets

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Abstract □ A new procedure for the analysis of sodium levothyroxine tablets and sodium liothyronine tablets is proposed. The active ingredient is isolated by partition chromatography. The thyronine is degraded by bromine oxidation, directly converting its iodine content to iodate, which is subsequently reduced by excess iodide and determined spectrophotometrically as the triiodide ion. The method is extremely sensitive and may be applied conveniently to dosage levels as low as 5 μg of sodium liothyronine. A suitable procedure for individual tablet analysis is also presented.

Keyphrases □ Sodium levothyroxine or sodium liothyronine tablets—analysis, partition chromatography and triiodide spectrophotometry □ Levothyroxine or liothyronine tablets—analysis, partition chromatography and triiodide spectrophotometry □ Liothyronine or levothyroxine tablets—analysis, partition chromatography and triiodide spectrophotometry

The USP XVIII (1) monographs for sodium levothyroxine, sodium levothyroxine tablets, sodium liothyronine, and sodium liothyronine tablets incorporate assays that require an alkali fusion of the drugs and subsequent conversion of the alkali iodide thus produced to elemental iodine. The latter is determined titrimetrically with sodium thiosulfate.

This analytical scheme is satisfactory for the drug substances themselves, but difficulties arise when it is applied to the dosage form, particularly those containing less than 100 μg of drug. The assays stipulate that a portion of powdered tablet composite equivalent to 3 mg of sodium levothyroxine or 1 mg of sodium liothyronine be ignited; these amounts theoretically yield 0.09 and 0.03 mEq of iodine, respectively. When these quantities of iodine are titrated with 0.01 *N* sodium thiosulfate, the true end-point is extremely difficult to judge and thus the accuracy of the analysis is doubtful. Furthermore, the great mass of the charge that must be ignited may itself lead to serious errors. The analysis of tablets containing 5 μg of sodium liothyronine would require 200 tablets and about 33 g of potassium carbonate as the fusion mixture. The mass of carbonized material often generated in such an ignition interferes with the quantitative recovery of inorganic iodides produced in the fusion. These difficulties strongly emphasize the need for a more accurate and sensitive assay procedure for tablets of both sodium levothyroxine and sodium liothyronine.

In an investigation of possible new methods, particularly for the assay of sodium levothyroxine, an attempt was made to utilize the reaction between ceric ion and arsenious acid under the powerful catalysis of submicrogram quantities of thyroxine. However, this reaction is subject to so many variables that it was abandoned as an analytical approach. The isolation of thyroxine from tablets by forming an extractable ion-pair with bis(2-ethylhexyl) hydrogen phosphate gave a final sample preparation that could be analyzed by UV spectrophotometry, but the procedure lacked the necessary sensitivity. A colorimetric method utilizing the chromophore produced by oxidizing the product of the reaction between thyroxine and 3-methyl-2-benzothiazolinone hydrazone hydrochloride was successful in determining as little as 80 μg of thyroxine. This procedure also afforded a degree of specificity in that the related compounds, diiodothyronine and diiodotyrosine, did not undergo the reaction and liothyronine yielded a color about one-fifth as intense as that obtained from thyroxine under the same conditions. Unfortunately, erratic results frequently occurred, probably because of variations in tablet excipients, traces of