Photodeactivation of Ethyl Violet: A Potential Hazard of Sodasorb®

J. Jeff Andrews, M.D., Robert V. Johnston, Jr., M.D.,* David E. Bee, Ph.D.,† James F. Arens, M.D.‡*

Breathing circuit cannisters containing functional CO_2 absorbent are critical to prevent rebreathing CO_2 during general anesthesia using closed or semiclosed breathing systems. Ethyl violet is the indicator dye added to Sodasorb® to indicate impending exhaustion of the absorbent. A case of CO_2 rebreathing due to failure of ethyl violet indicator in exhausted Sodasorb® was encountered. Laboratory investigation demonstrated that dye failure could result from photodeactivation caused by fluorescent lights. Using a fixed intensity fluorescent light source and quantitative spectrophotometric analysis, a highly significant dose–response relationship was demonstrated between duration of light exposure and the decrease in ethyl violet concentration. After 24 h of fluorescent light exposure with a received flux density of 46 nwatts/cm^2 at 254 nm, the concentration of functional ethyl violet remaining in pulverized Sodasorb® was 16% of the baseline value. Furthermore, using multiple light sources of various intensities, the greater the intensity of light, the more rapid the rate of decline of the ethyl violet concentration. It is recommended to minimize the problem by using ultraviolet filters and incorporating additional ethyl violet in Sodasorb®. Finally, ethyl violet undergoes temporal deactivation after a Sodasorb® container is opened, even if it is stored in the dark. (Key words: Carbon dioxide, absorption: indicators, ethyl violet. Light, fluorescent: photodeactivation.)

ETHYL VIOLET is a pH indicator used to assess the functional integrity of the CO_2 absorbent, Sodasorb® [Sodasorb Manual of Carbon Dioxide Absorption, Dewey and Almy Chemical Division, Lexington, Massachusetts, W. R. Grace and Company, 1986]. Ethyl violet normally changes from colorless to violet when the pH of the absorbent decreases as a result of sufficient absorption of CO_2.[1,2] Color change of absorbent is one signal that the absorbent is exhausted and must be changed.[3] Recently, we encountered a case that caused us to question the reliability of this indicator.

Shortly after induction of general anesthesia for an emergency cesarean section, rebreathing was diagnosed by capnography. Preoperatively, the anesthesia machine and circle breathing circuit were checked, and they appeared to be in good working order. The Ohmeda GMS Absorber contained bulk Sodasorb®, which was white. Immediately postoperatively, the absorber was disassembled and carefully examined. There was a thin layer of snow white granules, approximately one granule in depth, around the periphery of the cannister. All granules were violet inside this thin white ring. A violet streak was present up and down the peripheral granules in an area of the cannister that was not exposed to light. The obstetric suite was unique because, unlike other operating rooms in our institution with 14-foot ceilings, it had a low 10-foot ceiling. The entire room was painted bright white. More importantly, it was equipped with 65 40-inch, 40-watt Cool White® fluorescent bulbs (72 yards total).

Initially, channeling within the absorber was suspected as the reason for rebreathing of CO_2, but two clues led us to suspect that light was responsible for the problem. The first was the unique color configuration of the granules within the absorber. Presumably, the granules exposed to the light were white, whereas those protected from the light were violet. Second, the light intensity in our obstetric suite was twice that in our other operating rooms (table 1). To prove our hypothesis that fluorescent light causes photodeactivation of ethyl violet, the following laboratory investigation was performed.

Materials and Methods

Three general experimental approaches were used. Study 1 exposed intact Sodasorb® granules to a fluorescent light source for 2 days. The granules then were exposed to carbon dioxide and evaluated for visual signs of photodeactivation. Study 2 used pulverized Sodasorb®, and the powder was exposed to a fixed intensity fluorescent light source. Samples of the powder were solubilized and acidified at serial time intervals using a mixture of glacial acetic acid and water. The available functional concentration of ethyl violet indicator was quantified using spectrophotometric absorption at a 595 nm wavelength, the absorption maximum of ethyl violet. Study 3 exposed Sodasorb® powder to various different intensities of fluorescent light. Additionally, an ultraviolet filter was placed between the most intense light source and the granules in one portion of the study. At serial time intervals, quantitative spectrophotometric analysis was performed on

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* Assistant Professor of Anesthesiology.

† Associate Professor of Preventive Medicine & Community Health.

‡ Professor and Chairman of Anesthesiology; Rebecca Terry White Chair in Anesthesiology.

·TABLE 1. Light Sources and Intensities

Light Source	Light Intensity (nanowatts/cm²)*
22 Operating rooms†	6.8 ± 1
Pain clinic†	8
Obstetric suite†	14
Study 1 and 2	46
Study 3	
Intensity group	
D	61
C	81
B (UV filter)	83
A	124

* Light intensity was measured with a radiant incidence meter tuned to 254 nm. This device measures received flux density in units of nanowatts per square centimeter.

† Intensities measured in duplicate 36 inches from the floor at the absorber.

solubilized and acidified samples to determine relative ethyl violet concentrations. Light intensity was measured with a radiant incidence meter tuned to 254 nm, the primary mercury line spectrum wavelength. This device measures received flux density in units of nwatts/cm².

STUDY 1: OBSERVATION OF INTACT GRANULES

Five grams of Sodasorb® granules obtained from a 5-gallon pail (Lot AV09-B720-14, 16 months prior to expiration) were placed in each of two uncovered 3.5-inch petri dishes. One dish was placed in the dark and the other was exposed 8 inches below a 48-inch two-tube shop light fitted with General Electric 40-watt, daylight fluorescent bulbs. The light intensity measured at the level of the granules was 46 nwatts/cm². The light-exposed granules were agitated at 8-h intervals. After 48 h granules from the control group and from the light-exposed group were subjected for 30 min to CO_2 from a CO_2 E-cylinder. All granules were observed for color change. The light-exposed granules were further evaluated by cutting single granules in half and observing the interior of the granule for color change (fig. 1).

STUDY 2: QUANTITATIVE ANALYSIS WITH SINGLE LIGHT INTENSITY

Results from Study 1 indicated that photodeactivation of ethyl violet was a surface phenomenon. Therefore, the granules were pulverized to a fine powder with a particle size smaller than the thickness of the thin, white outer core shown in figure 1. A mixture of glacial acetic acid and water was used to acidify and solubilize the pulverized absorbent. Acetic acid is a relatively weak acid with a pK_a less than the pH at which ethyl violet becomes violet (fig. 2). Thus, when added to Sodasorb®, functional ethyl violet changed from colorless to violet. The amount of func-

tional ethyl violet in the acidified and solubilized Sodasorb® was determined using quantitative spectrophotometric analysis.

A sample of bulk Sodasorb® absorbent (Lot AV09-B720-14, 15.5 months prior to expiration) was ground into a fine powder in a darkened room. The powder was sifted through a 64-mesh sieve to eliminate larger granules. Exactly 1 g of powder was placed into each of 50 3.5-inch petri dishes. Two dishes were used to determine baseline ethyl violet concentrations. Twenty-four covered petri dishes were placed in a dark drawer to serve as controls. The remaining 24 uncovered petri dishes were arranged in two rows, 8 inches below a 48-inch two-tube, fluorescent shop light that was turned off. The type of bulbs were General Electric, 40-watt daylight.

At time zero the fluorescent light was turned on, and in a separate darkened room, baseline ethyl violet concentrations were determined. This was accomplished by pipetting a solution of 5 ml sterile water and 5 ml glacial acetic acid into each of the two baseline dishes. The dishes were agitated by hand at 5-min intervals for 40 min until all the Sodasorb® powder dissolved. Aliquots of the solution from each dish were then pipetted into Fisher polystyrene "ulta-vu" microcuvettes. Absorbance was measured immediately at a wavelength of 595 nm. Absolute ethyl violet concentrations were determined using the Bier Lambert law and published molar extinction coefficient.

Every 2 h the water/glacial acetic acid mixture was added to two petri dishes from the dark drawer and to two light-exposed dishes. This terminated the time interval for those plates. In a darkened room duplicate spec-

FIG. 1. Light-exposed Sodasorb® granule. This snow white granule was cut in half to reveal a thin white peripheral layer surrounding a deep violet central core.

FIG. 2. Ethyl violet. The critical pH of ethyl violet is 10.3 [The Sodasorb Manual of Carbon Dioxide Absorption, Dewey and Almy Chemical Division, Lexington, Massachusetts, W. R. Grace and Company, 1986]. The pH of fresh absorbent exceeds this critical pH, and the dye exists in its colorless form (A). When acetic acid is added to Sodasorb®, the pH decreases below 10.3, and functional ethyl violet changes to its violet form (B) via hydroxyl elimination. The quantity of the violet form (B) was determined by spectrophotometric analysis at a wavelength of 595 nm, the absorption maximum of ethyl violet.
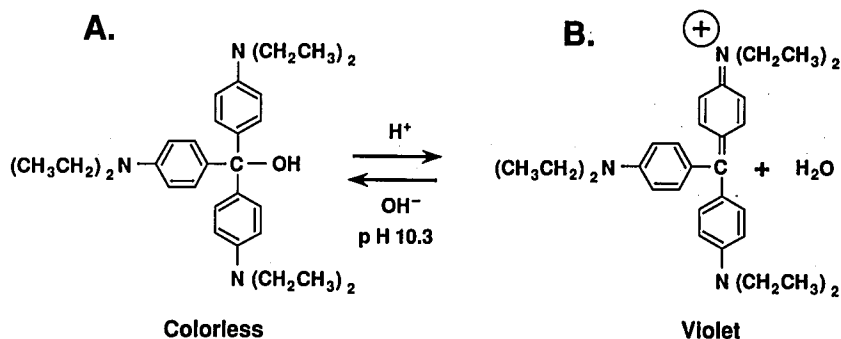
trophotometric measurements of samples from each plate were performed as described above. All dishes, both light exposed and non–light-exposed, were agitated by hand at 1-h intervals prior to determination of ethyl violet concentration. The temperature of the room remained constant at 72° F during all procedures.

STUDY 3: QUANTITATIVE ANALYSIS WITH MULTIPLE LIGHT INTENSITIES

Study 3 was performed 10 weeks after Study 2 using Sodasorb® from the same 5-gallon pail. The experimental technique was similar to Study 2 except that multiple light intensities were used. Exactly 1 g of powdered Sodasorb® was placed into each of 102 petri dishes in a darkened room. Three dishes were used to determine baseline ethyl violet concentrations. Fifteen covered petri dishes were placed in a dark drawer to serve as controls (group E). Twenty-one petri dishes were placed under each of four light sources that were turned off.

Each light source consisted of three, 48-inch two-tube fluorescent shop lights, with new General Electric, 40-watt "Cool White" bulbs. Chain length adjustments were made to obtain four different but uniform intensities. Two light sources, A and B, were adjusted to generate an intensity of 124 nwatts/cm². A Rosco® ultraviolet filter transmitting less than 10% of the ultraviolet light at wavelengths less than 390 nm was placed beneath light source B. The light intensity at 254 nm decreased to 83 nwatts/cm² with addition of the filter. The other two sources, C and D, were adjusted to generate light intensities of 81 and 61 nwatts/cm², respectively. Twenty-one petri dish locations having a uniform light intensity were determined for each of the four sources. An outline of a petri dish was drawn on the counter top when the intensity was appropriate. Thus, under a given light source, the light intensity was uniform at every petri dish location.

At time zero the lights were turned on, and baseline ethyl violet concentrations were determined as described in Study 2. Then, serial spectrophotometric measurements were performed on samples from each light source

and from the dark drawer at times 2, 4, 6, 8, 10, 12, 16, 20, and 23 h. All dishes were agitated by hand at 1-h intervals and moved to random template locations with the same intensity. This maximized light exposure and helped assure uniform light intensity levels within each group. The room temperature was constant at 72° F throughout Study 3.

STATISTICAL METHODOLOGY

The experimental design for the single light intensity study is analogous to a two-factor, factorial arrangement of treatments with factors defined as light intensity at two levels (dark, light) and exposure time at 12 levels (2, 4, ··· 24 h). This design defines 24 combinations of intensity and time that were assigned to 48 experimental units (petri dishes) and, in the analysis of variance context, permits the test of significance of intensity differences, time differences, and the intensity by time interaction with concentration as the outcome variable. Intensity simple effects (dark vs. light) were tested at each level of exposure time.

The multiple light intensity study has the same design structure as the single light study but with five intensity levels or conditions: dark, 61, 81, 124, and 83 nwatts/cm² (124 nwatts/cm² source + UV filter) and nine exposure time levels (2, 4, ··· 12, 16, 20, and 23 h). Fisher's protected least significant difference test was used to separate mean concentration values for each level of exposure time. Pairwise comparisons were made only after the simultaneous eqality of intensity means was rejected.

Duplicate measures for each petri dish were obtained in addition to multiple experimental units per treatment combination. These multiple observations permit an assessment of the relative importance of within dish variability compared to dish-to-dish variation using a variance component analysis.

Results

Photodeactivation of ethyl violet was confirmed not only by visual inspection of intact granules, but also by
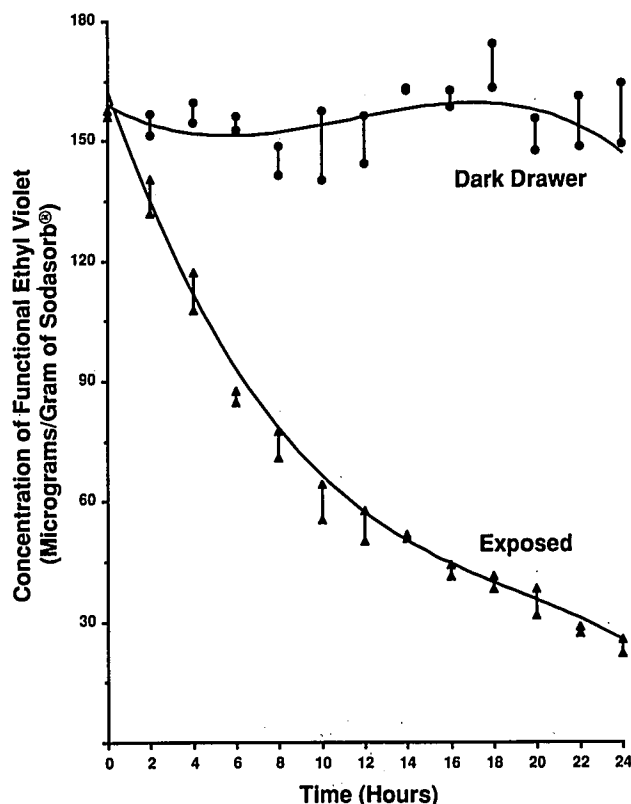
FIG. 3. Quantitative spectrophotometric analysis of ethyl violet photodeactivation using a single light intensity. The ethyl violet concentration of the control group did not change with time. In contrast, the light-exposed dishes exhibited a continual decrease in ethyl violet across time. Each point represents the average of paired ethyl violet concentrations determined from a single petri dish. The light intensity measured at the level of the Sodasorb® was 46 nwatts/cm^2.

quantitative spectrophotometric analysis. There was a direct relationship between degree of photodeactivation and duration of exposure to light. There was a direct relationship between light intensity and photodeactivation. Addition of a UV filter attenuated photodeactivation.

STUDY 1: OBSERVATION OF INTACT GRANULES

The control Sodasorb® granules from the dark drawer turned violet immediately when exposed to CO_2. The light-exposed Sodasorb® granules, however, remained white despite CO_2 exposure. When these granules were cut in half, each had a thin, white outer layer that surrounded a dark violet central core (fig. 1).

STUDY 2: QUANTITATIVE ANALYSIS WITH SINGLE LIGHT INTENSITY

The concentration of ethyl violet for control dishes in the dark drawer remained stable across all 12 measurement periods (fig. 3). In contrast, light exposed dishes

exhibited a continual decrease in concentration means across time with a significant mean separation at each measurement period beginning at 2 h ($P = 0.0042$). The T50 concentration, defined as the time at which the remaining concentration is 50% of the baseline, was 8 h. At 24 h the concentration had decreased to 16% of the baseline value.

STUDY 3: QUANTITATIVE ANALYSIS WITH MULTIPLE LIGHT INTENSITIES

The variance component study for intensity groups A through E indicated excellent stability across all measurement periods for both within-dish and dish-to-dish measurements. Group A exhibited the greatest amount of relative variation with a coefficient of variation (CV = 100 SD/mean) of 1.8% for within-dish error and 8.3% for dish-to-dish variation.

The ethyl violet concentration of the petri dishes in the dark drawer group (E) did not appreciably change with time (ANOVA) (fig. 4). Each of the other groups exhibited a dose–response relationship between duration of exposure and the concentration of functional ethyl vi-
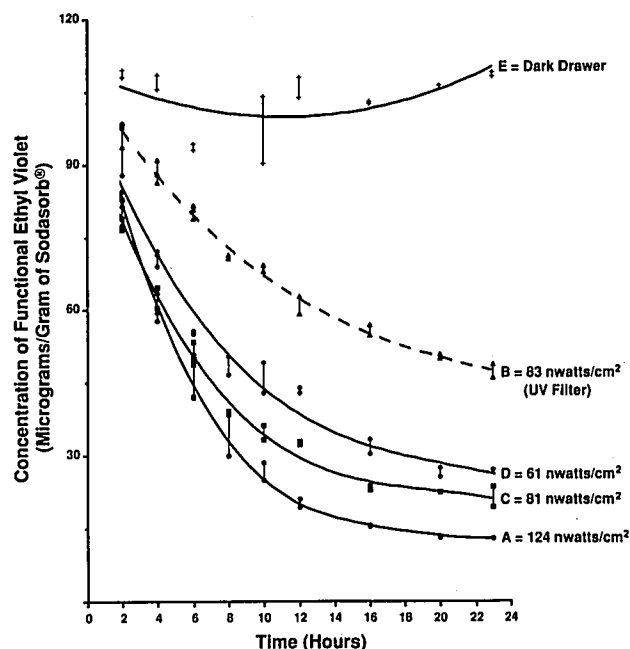

FIG. 4. Quantitative spectrophotometric analysis of ethyl violet photodeactivation using multiple light intensities. The ethyl violet concentration of the control group did not change with time. Each of the other groups A, B, C, and D exhibited a dose–response relationship between duration of exposure and the concentration of ethyl violet. The greater the light intensity, the more rapid the rate of decline of ethyl violet concentration. Addition of a UV filter minimized photodeactivation. Each point represents the average of paired ethyl violet concentrations determined from a single petri dish. Light intensity was measured with a radiant incidence meter tuned to 254 nm.

olet. The greater the intensity of light, the more rapid the rate of decline of the ethyl violet concentration. Mean separation tests (table 2) show that the mean concentration of each of the light-exposed groups is significantly lower than the mean of the dark drawer group (E), regardless of measurement period. Also, means for groups A, C, and D are significantly lower than the mean for group B.

The T50 concentrations for group A (124 nwatts/cm²), Group C (81 nwatts/cm²), and group D (61 nwatts/cm²) were 5, 6, and 8 h, respectively. The T50 concentration for group B (124 nwatts/cm² source + UV filter) was 19 h. This represented almost a fourfold (19/5) increase in the longevity of the powdered granules.

STUDY 2 *VERSUS* STUDY 3

A 10-week time interval existed between Studies 2 and 3, but both studies were performed using Sodasorb® from the same pail. The pail was stored in the dark between the studies. The baseline concentration of functional ethyl violet spontaneously decreased from 154 (Study 2) to 89 (Study 3) micrograms per gram of Sodasorb®. This temporal deactivation represented a 42% decrease in the baseline value.

Discussion

Ethyl violet (basic violet 4), a substituted triphenylmethane dye, is a substituted triphenylcarbonium-ion salt (fig. 2). It is closely related to gentian violet (methyl violet) and is a member of the pararosaniline dye family. Advantages of the basic dyes include extreme brillance of shade, high color value, and relative low cost. However, they demonstrate only poor to fair fastness to light.[4]

TABLE 2. Selected Sodasorb® Mean Separation Tests
by Fisher's Least Significant Difference (LSD)

Exposure Time (h)	Light Intensity Groups*				
	A	C	D	B	E
2	79.4 a	77.7 a	88.1 b	94.7 c	106.4 d
4	58.9 a	60.6 a	69.3 b	86.6 c	104.8 d
6	43.8 a	49.9 b	54.2 b	78.7 c	91.8 d
8	33.5 a	38.2 a	47.6 b	69.4 c	
20	12.9 a	22.0 b	26.0 b	49.5 c	104.2 d

Mean concentrations are presented as micrograms functional ethyl violet per gram Sodasorb®. Means underscored by different lower case letters are significantly different at the 0.05 level by the LSD test.

* Intensity groups: A = 124 nwatts/cm²; C = 81 nwatts/cm²; D = 61 nwatts/cm²; B = 83 nwatts/cm² (124 nwatts/cm² source + UV filter); E = dark drawer.

The absorption spectrum of ethyl violet has a large peak at 595 nm in the yellow-orange range; thus, the dye transmits the reciprocal violet. There are additional absorption peaks in the ultraviolet range at 307, 253, and 209 nm, as expected for any aromatic compound. The molar extinction coefficients (E) are as follows: E 595 = 83800; E 307 = 18900; E 253 = 12600; E 209 = 37100 (concentration = 0.01002 g/l H_2O) [Wantz R: Letter Aldrich Chemical Company, Milwaukee, Wisconsin, March 31, 1988].

Fluorescent tubes are high voltage, gas discharge devices that contain trace amounts of mercury vapor. Inside a fluorescent tube most of the radiation lies in the ultraviolet range, and 85% of the rays are at 253.7 nm. The intense ultraviolet radiation can be utilized to produce sterilizing lamps, but it is harmful to human beings and causes rapid and severe corneal burns. Therefore, in fluorescent lighting this hard UV radiation is softened with phosphors and blocked by the glass used for the lamp tube. Nevertheless, fluorescent lamps still emit the radiation of mercury vapor line spectrum at 254, 303, 313, 334, and 366 nm. [Westinghouse Guide to Fluorescent Lamps, Bloomfield, New Jersey, Westinghouse Electric Corporation, 1979]. The first three peaks of mercury vapor correlate strikingly with the absorption spectrum of ethyl violet.

We believe photodeactivation of ethyl violet was responsible for the case of CO_2 rebreathing described earlier. Factors that exacerbate photodeactivation include prolonged exposure of the granules to light and high intensity light. The light intensity measured at 254 nm in the obstetric suite was twice that in other operating rooms (table 1). After the study we discovered another cannister of Sodasorb® that had undergone photodeactivation. It was located on an infrequently used anesthesia machine in our pain clinic. The light intensity in the pain clinic, measured at 254 nm, was higher than in our other operating rooms (8 vs. 6.8 ± 1 nwatts/cm², table 1).

The portion of the light spectrum responsible for photodeactivation appears to be UV light. In Study 3 insertion of the UV filter caused the light intensity measured at 254 nm to decrease from 124 nwatts/cm² (group A) to 83 nwatts/cm² (group B). This intensity was almost identical to group C (81 nwatts/cm²). Nevertheless, insertion of the UV filter offered additional protection against photodeactivation as can be ascertained from figure 4, curve B versus C. The T50 concentration for group B was 19 h, whereas that for group C was 6 h. Therefore, in addition to the 254 nm wavelength, other UV wavelengths are probably responsible for photodeactivation of ethyl violet.

Ethyl violet undergoes temporal deactivation. Even though Study 3 was performed only 10 weeks after Study 2, the baseline concentration of functional ethyl violet

spontaneously decreased from 154 to 89 $\mu g/g$ of Sodasorb®.

As a result of this study, we recommend the following to minimize the risk of photodeactivation of ethyl violet:

1. The operator should replace the absorbent when it turns violet or after a prescribed time interval. The practice of replacing CO_2 absorbent based solely on a color change is not always the best approach because white granules do not guarantee fresh absorbent. After the thin outer layer of a single granule of Sodasorb® undergoes photodeactivation, the clinical usefulness of ethyl violet as an indicator is abolished because the granule appears white regardless of its CO_2 absorptive ability. This more reliable approach is to combine the color change with a time schedule.

2. Photodeactivation is more likely to occur in operating rooms (OR) that are brightly lighted or in those that utilize UV germicidal bulbs. Intensely lighted OR are the trend, and many existing OR contain germicidal UV bulbs. End-tidal CO_2 monitoring may be more beneficial than color change of absorbent to diagnose rebreathing in those operating rooms.

3. Bulk absorbent should be used as soon as possible after the pail is opened because of temporal deactivation. It is important to recognize that the expiration date on the pail refers to an unopened one.

4. Addition of more ethyl violet to Sodasorb® would prolong the exposure time necessary to achieve the same absolute level of photodeactivation at a given light intensity. A fivefold increase in ethyl violet concentration from 0.02% dry weight to 0.1% weight would help minimize photodeactivation.

5. The use of UV filters in cannister housings will prolong the life of ethyl violet up to fourfold.

6. A different indicator dye that is not as sensitive to light could be used. Clayton yellow indicator dye is described by the manufacturer as light-sensitive. Ethyl orange is a known irritant. Mimosa Z is not known to undergo photodeactivation. However, because it changes from red to white on acidification, photodeactivation would not result in failure to recognize nonfunctional absorbent. Other indicator dyes remain to be studied.

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