Impact of Light Shielding on Photo-Degradation of Dacarbazine during the Preparation Process

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Dacarbazine (DTIC) is converted to the photo-degradation product 4-diazoimidazole-5-carboxamide (Diazo-IC) by light. Diazo-IC production is often responsible for the pain reactions observed during peripheral intravenous infusion of DTIC in clinical settings. Although light shielding during infusion decreases the photo-degradation of DTIC, its usefulness for the preparation of DTIC has not yet been fully clarified. The aim of this study was to investigate the light conditions during the preparation of DTIC solution in the compounding room from the viewpoint of the production amount of Diazo-IC. DTIC solution was prepared in the compounding room. Various light and temperature conditions and dissolving solutions during the preparation were investigated. The amounts of DTIC and Diazo-IC in solutions were determined using an HPLC coupled to UV detection. The photo-degradation of DTIC was estimated by the amount of Diazo-IC. Diazo-IC production in the dissolving solutions increased in a time-dependent manner at 4 and 25°C under light shielding. Light exposure during the dissolving process did not affect the DTIC and Diazo-IC concentrations. Light shielding during dissolution did not alter the Diazo-IC production until 4 h after dilution. In conclusion, short duration light exposure did not affect Diazo-IC production. These findings suggest that light shielding is not needed in the preparation of DTIC in the compounding room from the viewpoint of Diazo-IC production.

Key words dacarbazine; photo-degradation; preparation; hazardous drug

INTRODUCTION

Dacarbazine (DTIC), an antineoplastic chemotherapy drug, is classified as an alkylating agent. DTIC is commonly used in combination with other chemotherapy drugs for the treatment of metastatic malignant melanomas, Hodgkin lymphoma, and pheochromocytomas.1 DTIC often causes pain reactions such as local pain, a burning sensation, and irritation at the needle site during peripheral intravenous infusion. DTIC dissolved with water is rapidly degraded to various products under light. Recent studies have reported that the pain reactions during DTIC infusion were associated with the production of photo-degradation compounds.5,6

Photo-degradation compounds are produced in DTIC solution by sunlight exposure9 (Fig. 1). DTIC (I) is photodegraded to 5-diazoimidazole-4-carboxamide (Diazo-IC, II) and dimethylamine. Subsequently, Diazo-IC is converted to 4-carbamoylimidazolium-5-olate (III) or 2-azahypoxanthine (IV) under differing light and pH conditions. 4-Carbamoylimidazolium-5-olate (III) coupled with Diazo-IC (II) is transformed to 5-carbamoyl-2-(4-carbamoylimidazol-5-ylazo) imidazolium-5-olate (V). These photo-degradation processes proceed in a time-dependent manner, and shielding DTIC solution from light reduces the progression of photo-degradation. Diazio-IC among the photo-degradation products was shown to be associated with pain reactions at the needle site during peripheral intravenous administration of DTIC and to enhance in a concentration-dependent manner the pain reactions in mice.5 These data indicate that the production of Diazo-IC is responsible for the pain reactions during peripheral intravenous infusion of DTIC.

Several studies have reported the procedure for reducing the pain reaction during peripheral intravenous infusion of DTIC.4–7) The dissolving solution selected and the adjustment of its pH were found to affect the photo-degradation.8) Light shielding infusion bags with a light-blocking sheet reduced the generation of Diazo-IC during peripheral intravenous infusion.5,9 A light-blocking cover with black cotton reduced photo-degradation during peripheral intravenous infusion, compared to a light-blocking sheet.9 Concomitant nonsteroidal anti-inflammatory drug administration also relieved the pain reactions caused by DTIC in mice.5

Earlier studies have suggested that the drip infusion process of DTIC should be shielded from light5,9 and that the administration should be finished as soon as possible. Similarly, DTIC should be prepared rapidly under dark conditions during the preparation process. However, few studies have focused on the preparation process of DTIC. In Japan, healthcare workers such as pharmacists prepare antineoplastic chemotherapy drugs in safety cabinets located in the compounding room of a pharmacy. No light or light shielding during the preparation of DTIC solution is performed as a procedure for reducing photo-degradation in clinical settings. However, the necessity for light shielding during the preparation of DTIC has not yet been fully clarified in clinical settings. The aim of this study was to investigate the impact of light shielding on the photo-degradation of DTIC in the compounding room from the viewpoint of the production of Diazo-IC during the preparation of DTIC solution.

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MATERIALS AND METHODS

Chemicals, Equipment, and Materials  DTIC was purchased from Toronto Research Chemicals (Toronto, Ontario, Canada). Diazoo-IC was obtained from LKT Laboratories, Inc. (St. Paul, MN, U.S.A.). Photo-degradation of DTIC during the preparation was estimated using DTIC pharmaceutical products (Dacarbazine Injection 100, Kyowa Hakko Kirin Co., Ltd., Tokyo, Japan) delivered to Hamamatsu University Hospital (Hamamatsu, Japan) from January to May 2015. The DTIC products containing several lots were purchased from the same wholesaler and stored at the same storage condition (cold storage). The DTIC product was prepared under a fluorescent light in the compounding room of our hospital pharmacy. The illuminance mean level was 1118 lx and the room temperature was 25°C. The preparation was performed using a class I biological safety cabinet (SterilchemGARD® III Advance SG-603 ATX, The Baker Company, Sanford, ME, U.S.A.). Normal saline solution (NSS, Isotonic Sodium Chloride Solution, Fuso Pharmaceutical Industries, Ltd., Osaka, Japan), 5% glucose solution (5%GS, Glucose Injection, Fuso Pharmaceutical Industries, Ltd.), and distilled water (DW, Water for Injection, Fuso Pharmaceutical Industries, Ltd.) were used as dissolving and diluting solutions for preparing the DTIC product. Syringes (Terumo Co., Tokyo, Japan) were used in the preparation and light-blocking sheets were provided by Terumo Co.

Determination of DTIC and Diazoo-IC  All samples were prepared and analyzed immediately after collection under a sodium vapor lamp. Sample solutions (50 µL) were diluted with 450 µL of distilled water for the measurement of DTIC in light shielding microtubes. For the measurement of Diazoo-IC, the samples were directly injected onto the analytic column. The concentrations of DTIC and Diazoo-IC in prepared solutions were determined using a high-performance liquid chromatograph coupled to photodiode array UV detection. Chromatographic separation of each analyte was performed using an octadecylsilane column (TSKgel ODS-100V, 75 mm length × 4.6 mm internal diameter, 3 µm, Tosoh, Tokyo, Japan). The mobile phase was a mixture of 20 mM phosphate buffer (pH 3.0) and acetonitrile (92/8, v/v). The flow rate was 1 mL/min, the column temperature was 40°C, and the auto-injector was set at 4°C. Their calibration curves were linear over the concentration ranges of 10–1000 µg/mL for DTIC (r > 0.999) and 0.5–50 µg/mL for Diazoo-IC (r > 0.999). The lower limits of quantification of DTIC and Diazoo-IC were 10 and 0.5 µg/mL, respectively. The intra-day accuracy and imprecision values for DTIC were 99.2–101.8% and within 3.6%, while their inter-day values were 98.7–103.6% and within 4.4%, respectively. The corresponding values for Diazoo-IC were 99.8–106.8% and within 1.5%, and 101.6–105.7% and within 3.1%, respectively.

Amounts of DTIC and Diazoo-IC in DTIC Products  The DTIC products containing 100 mg of DTIC were dissolved with 10 mL of NSS (n = 15), 5%GS (n = 15), and DW (n = 15) to adjust the solution concentration to 10 mg/mL. The concentrations of DTIC and Diazoo-IC in the solutions were determined immediately after preparation. The amounts of DTIC and Diazoo-IC in DTIC product vials were estimated from these concentrations in the solutions.

Stability of DTIC in Each Dissolving Solution and Temperature Condition  The pharmaceutical products containing 100 mg of DTIC were dissolved with 10 mL of NSS, 5%GS, and DW to adjust the solution concentration to 10 mg/mL. Each dissolving solution in a syringe was covered
with aluminum foil (full light shielding) and then kept for 640 min at 25°C (room temperature) or 4°C. Samples were collected at 0, 160, 320, 480, and 640 min. Observation period for every 160 min was set according to the time difference samplings at each sample.

**Stability of DTIC Solutions under Each Light Condition**

The DTIC products were dissolved under a fluorescent light in a compounding room. The DTIC products in syringes were prepared with 10 mL of NSS, 5%GS, and DW to adjust the solution concentration to 10 mg/mL under three different light conditions, as follows: (1) no protection from light (low level); (2) protection from light (medium level): cabinet-lighting off and using a syringe covered with a light-blocking sheet; and (3) complete protection from cabinet and room lights using a syringe covered by aluminum foil (high level). These solutions were stored for 10 min. Samples were collected at 0, 1, 2, 5, and 10 min.

**Stabilities in Infusion Solutions of DTIC**

The DTIC products were dissolved with 10 mL of NSS, 5%GS, and DW to adjust the solution concentration to 10 mg/mL. DTIC solutions under two light conditions were stored for 10 min. The light conditions were as follows: (a) no protection from light; and (b) protection from cabinet and room lights using aluminum foil. Each solution was then diluted by adding 25 mL of NSS or 5%GS (100 mg/35 mL). These diluted solutions with aluminum foil shielding were stored for 4 h (common administration time) at 25°C (room temperature). Samples were collected at 0, 1, 2, and 4 h after dilution.

**Statistical Analyses**

Statistical analyses were performed using GraphPad Prism software (6.0, GraphPad Software, Inc., San Diego, CA, U.S.A.). The difference in the amounts of DTIC and Diazio-IC in pharmaceutical product estimated from each dissolving solution was determined by the Student’s t-test. The differences in the DTIC and Diazio-IC concentrations in dissolving solutions between temperature conditions or between NSS, 5%GS, and DW were tested using the Student’s t-test or the one-way ANOVA and post-hoc Tukey multiple comparison test. The influence of preparation conditions on the concentrations of DTIC and Diazio-IC in infusion DTIC solutions was tested by one-way ANOVA and the post hoc Tukey multiple comparison test. A p-value <0.05 was considered to indicate statistical significance. All values are expressed as the mean and standard deviation (S.D.) unless otherwise stated.

**RESULTS**

**Amounts of DTIC and Diazio-IC in Pharmaceutical Products**

Figure 2 shows the amounts of Dacarbazine (DTIC) (A) and 4-Diazoimidazole-5-carboxamide (Diazio-IC) (B) in Pharmaceutical Products

NSS, normal saline solution; 5%GS, 5% glucose solution; and DW, distilled water.

**Stability of DTIC Solutions under Each Light Condition**

DTIC solutions were tested by one-way ANOVA and the post hoc Tukey multiple comparison test. A p-value <0.05 was considered to indicate statistical significance. All values are expressed as the mean and standard deviation (S.D.) unless otherwise stated.

**Stabilities of DTIC Solutions under Various Light Conditions**

Table 2 shows the concentration profiles of DTIC and Diazio-IC under three light conditions for 640 min. Differences were observed in the amounts of DTIC and Diazio-IC in pharmaceutical products after dissolving with NSS, 5%GS, or DW. The mean ± S.D. of the amounts of DTIC and Diazio-IC in products were 98.0 ± 1.1 mg per vial and 114 ± 45 μg per vial, respectively.

**Stability Profile of DTIC at Each Temperature and in Dissolving Solution**

Table 1 shows the concentration profiles of DTIC and Diazio-IC in pharmaceutical products. Diazio-IC was detected in all DTIC products. No differences were observed in the amounts of DTIC and Diazio-IC in pharmaceutical products after dissolving with NSS, 5%GS, or DW. The mean ± S.D. of the amounts of DTIC and Diazio-IC in products were 98.0 ± 1.1 mg per vial and 114 ± 45 μg per vial, respectively.

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the DTIC and Diazo-IC concentrations.

**Influence of DTIC Stabilities in Diluted Infusion Solutions on Photo Exposure** Table 3 shows the concentration profiles of DTIC and Diazo-IC in diluted infusion solutions for 4 h after dilution under two light conditions. The rate of change of DTIC concentrations in NSS and 5%GS did not differ between the two light conditions. In contrast, the Diazo-IC concentrations in NSS and 5%GS increased after dilution in a time-dependent manner under the two light conditions. No differences were observed in the rates of change of the Diazo-IC concentrations in infusion solutions between the two light conditions. In the comparison of the dissolving solutions, the rate of change of the Diazo-IC concentration in 5%GS after the dilution was significantly higher than that in NSS at 1, 2, and 4 h.

**DISCUSSION**

This study investigated the optimal light conditions during the dissolving and diluting processes of DTIC in the compounding room from the viewpoint of the amount of Diazo-IC produced. Diaz-o-IC was detected in all measured DTIC pharmaceutical products. There was a time-dependent increase in Diazo-IC concentrations in the dissolving solutions at each temperature under light shielding. Light exposure during the dissolving process did not affect the DTIC and Diazo-IC concentrations in the solutions. In addition, no differences were observed in the DTIC and Diazo-IC concentrations for 4 h in diluted infusion solutions with or without light exposure during the dissolving process. These findings suggest that a short duration exposure to light during the dissolving process does not influence the amount of Diaz'o-IC produced. To the best of our knowledge, this is the first report that has assessed the impact of short duration light exposure on Diaz'o-IC production during the process of dissolving DTIC.

Slight amounts of Diaz'o-IC were found in all solutions immediately after dissolving the DTIC products. Although the amounts of Diaz'o-IC varied among the DTIC products, the dissolving solution selected was not associated with the amount of Diaz'o-IC produced. Since this study samples were prepared under a sodium vapor lamp and the analytes were measured immediately after dissolution, Diaz'o-IC was most likely not produced by photo-degradation. These results suggest that the Diaz'o-IC was present in the vials before dissolution and was produced sometime during the manufacturing, transportation, and/or storage processes. The manufacturer has stated that Diaz'o-IC is a contaminant of DTIC pharmaceutical product.10)

The DTIC concentrations in the dissolving solutions under light shielding did not change for 640 min under any temperature used. In contrast, the Diaz'o-IC concentration in solutions increased and its concentration at 4°C was lower than that at 25°C. Although the present study observed the DTIC stability in dissolving solution for 640 min, no differences were observed in the change rates of DTIC and Diaz'o-IC concentrations. Since only a slight amount of Diaz'o-IC was produced at low temperature, cooling the dissolving solution and the infusion bag may reduce the production of Diaz'o-IC. However, this approach is difficult as it may induce pain due to peripheral vein constriction. The Diaz'o-IC concentration in 5%GS

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**Table 1. Concentration Profiles (%) of Dacarbazine (DTIC) (A) and 4-Diazoimidazole-5-carboxamide (Diazo-IC) (B) after Dissolving DTIC Pharmaceutical Product at Each Temperature and Dissolving Solution**

| Temperature | Dissolving solutions | Times after dissolution (min) | (A) DTIC | (B) Diazo-IC |
|-------------|----------------------|-------------------------------|---------|-------------|
| 25°C        |                      | 0          | 160      | 320         | 480          | 640         |
| NSS         | 100                  | 99.6±0.2  | 99.6±0.1 | 99.3±0.3   | 98.9±0.1    |             |
| 5%GS        | 100                  | 100.1±0.1 | 99.5±0.2 | 99.3±0.3   | 98.5±0.1    |             |
| DW          | 100                  | 99.7±0.2  | 99.7±0.0 | 99.2±0.1   | 98.8±0.2    |             |
| 4°C         |                      |            |          |             |             |             |
| NSS         | 100                  | 100.8±0.1 | 100.8±0.1| 100.5±0.1 | 100.5±0.2   |             |
| 5%GS        | 100                  | 100.8±0.2 | 101.5±0.9| 100.9±0.5  | 98.6±3.3    |             |
| DW          | 100                  | 100.9±1.0 | 100.5±0.5| 100.2±0.5  | 99.9±0.5    |             |

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Data are expressed as mean with standard deviation (S.D.) of percentage of the initial value (n=3). The value of DTIC and Diazo-IC concentrations at 0 min is defined as 100%. NSS, normal saline solution; 5%GS, 5% glucose solution; and DW, distilled water. Student’s t-test, *p* < 0.05 versus NSS.

*DISCUSSION*

This study investigated the optimal light conditions during the dissolving and diluting processes of DTIC in the compounding room from the viewpoint of the amount of Diaz'o-IC produced. Diaz'o-IC was detected in all measured DTIC products. There was a time-dependent increase in Diaz'o-IC concentrations in the dissolving solutions at each temperature under light shielding. Light exposure during the dissolving process did not affect the DTIC and Diaz'o-IC concentrations in the solutions. In addition, no differences were observed in the DTIC and Diaz'o-IC concentrations for 4 h in diluted infusion solutions with or without light exposure during the dissolving process. These findings suggest that a short duration exposure to light during the dissolving process does not influence the amount of Diaz'o-IC produced. To the best of our knowledge, this is the first report that has assessed the impact of short duration light exposure on Diaz'o-IC production during the process of dissolving DTIC.

**Table 3**

| Temperature | Dissolving solutions | Times from dissolution (min) | (A) DTIC | (B) Diazo-IC |
|-------------|----------------------|-------------------------------|---------|-------------|
| 25°C        |                      | 0          | 160      | 320         | 480          | 640         |
| NSS         | 100                  | 170.9±2.0 | 284.6±1.5| 416.7±1.8† | 558.0±3.8†   |             |
| 5%GS        | 100                  | 180.0±0.9 | 295.1±3.0| 430.0±4.3† | 569.2±7.6†   |             |
| DW          | 100                  | 174.4±4.3 | 310.1±4.2| 471.5±3.6  | 646.2±1.1   |             |
| 4°C         |                      |            |          |             |             |             |
| NSS         | 100                  | 189.0±6.3* | 257.3±11.0^ | 306.6±11.6^ | 346.6±14.9^ |             |
| 5%GS        | 100                  | 158.6±2.3* | 203.4±3.8^ | 236.8±5.8^ | 257.8±14.7^*|             |
| DW          | 100                  | 193.7±10.5* | 264.1±17.0^ | 317.7±21.6^ | 357.9±24.2^ |             |

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Data are expressed as mean with standard deviation (S.D.) of percentage of the initial value (n=3). The value of DTIC and Diazo-IC concentrations at 0 min is defined as 100%. NSS, normal saline solution; 5%GS, 5% glucose solution; and DW, distilled water. Student’s t-test, *p* < 0.05 versus 25°C. One-way ANOVA and post hoc Tukey’s multiple comparison test, †*p* < 0.05 versus DW, ‡*p* < 0.05 versus NSS.
was lower than that in the other solutions at 4°C. In contrast, this difference was not observed at 25°C. Although 5%GS may be more stable at 4°C than the other dissolving solutions, it is not suitable for preventing Diazo-IC production from the viewpoint of clinical practice.

The DTIC and Diazo-IC concentrations did not differ between the three light conditions for 10 min during the dissolving process. Our study used 10 min since this is commonly the amount of time needed for the dissolving and diluting process of DTIC injection in clinical settings. Medium and high levels of light shielding did not contribute to the prevention of photo-degradation of DTIC product. Recent reports have demonstrated that light shading during the infusion process reduced the photo-degradation of DTIC.\(^3\)\(^,\)\(^6\)\(^,\)\(^9\) The infusion process of DTIC requires 2–3 h, while the dissolving and diluting processes require a total of 10 min. A short duration of light exposure most likely does not increase the production of Diazo-IC. These results suggest that light shading does not affect the production of Diazo-IC during the dissolving process. In addition, our findings also show that the dissolving solution selected did not alter the DTIC and Diazo-IC concentrations for 10 min in the dissolving process.

Light exposure during the dissolving process did not increase the production of Diazo-IC in infusion bags after dilution. In this study, the stability of DTIC solution in the infusion process after dilution was evaluated. We chose 4 h as the common amount of time from dilution to completion of the DTIC injection infusion in clinical settings. With or without light shielding during the dissolving process did not affect the DTIC and Diazo-IC concentrations in the infusion process for 4 h. In clinical settings, the DTIC solution is commonly protected from light exposure during the infusion process. If DTIC infusion is protected from light exposure during infusion, light shielding during the 10-min dissolving process may not be required. The Diazo-IC concentration in NSS was slightly lower than that in 5%GS for 4 h at room temperature under each light condition. In contrast, Sugihara et al. reported that the alteration from NSS to 5%GS as dissolving solution reduced the pain reaction in two cases.\(^1\)\(^1\) The reduction mechanism of pain reaction using 5%GS as dissolving solution is not clarified. The use of NSS for DTIC injection would be recommended from the viewpoint of Diazo-IC production in clinical practice. Further clinical studies enrolling a large number of patients are needed to determine the suitable

| Dissolving solutions | Light protection level | Times after the dissolution (min) |
|----------------------|-----------------------|----------------------------------|
|                      |                       | 0      | 1      | 2      | 5      | 10     |
| **(A) DTIC**         |                       |       |       |       |       |       |
| NSS                  | Low level             | 100   | 100.0±0.5 | 100.4±0.7 | 100.2±0.9 | 100.2±0.6 |
|                      | Medium level          | 100   | 100.1±0.3 | 100.1±0.3 | 100.2±0.5 | 100.1±0.4 |
|                      | High level            | 100   | 100.5±1.1 | 100.6±1.3 | 100.4±0.9 | 100.5±1.1 |
| 5%GS                 | Low level             | 100   | 100.6±0.3 | 100.8±0.5 | 100.4±0.4 | 100.5±0.4 |
|                      | Medium level          | 100   | 100.2±0.2 | 100.2±0.2 | 100.1±0.1 | 100.4±0.2 |
|                      | High level            | 100   | 99.8±0.0 | 99.9±0.0 | 99.8±0.2 | 100.1±0.3 |
| DW                   | Low level             | 100   | 99.8±0.4 | 99.9±0.3 | 99.8±0.5 | 99.6±0.5 |
|                      | Medium level          | 100   | 100.5±0.4 | 100.0±0.2 | 100.3±0.2 | 100.2±0.2 |
|                      | High level            | 100   | 99.6±0.2 | 99.7±0.6 | 99.3±0.0 | 99.3±0.1 |
| **(B) Diazo-IC**     |                       |       |       |       |       |       |
| NSS                  | Low level             | 100   | 100.2±0.4 | 100.7±0.2 | 101.1±0.3 | 102.1±0.8 |
|                      | Medium level          | 100   | 100.4±0.4 | 100.5±0.2 | 101.3±0.4 | 102.1±0.3 |
|                      | High level            | 100   | 100.8±1.2 | 101.2±1.4 | 101.4±1.2 | 102.0±1.2 |
| 5%GS                 | Low level             | 100   | 100.9±0.3 | 101.3±0.5 | 101.7±0.4 | 102.9±0.4 |
|                      | Medium level          | 100   | 100.4±0.2 | 100.5±0.1 | 101.1±0.3 | 102.2±0.3 |
|                      | High level            | 100   | 100.0±0.1 | 100.1±0.1 | 100.5±0.2 | 101.5±0.6 |
| DW                   | Low level             | 100   | 99.7±1.5 | 100.2±1.4 | 100.4±1.4 | 101.3±1.6 |
|                      | Medium level          | 100   | 100.4±0.6 | 100.2±0.8 | 100.8±0.8 | 102.0±0.6 |
|                      | High level            | 100   | 99.9±0.2 | 100.2±0.2 | 100.3±0.2 | 101.1±0.4 |

Data are expressed as mean with S.D. of percentage of the initial value (n = 5). The value of DTIC and Diazo-IC concentrations at 0 min is defined as 100%. Low level, dissolution was not protected for a light; medium level, dissolution was protected for a light: turning off a light in cabinet and a syringe covered with a light-blocking sheet; and high level, dissolution was protected completely for a light and a syringe covered with an aluminum foil. NSS, normal saline solution; 5%GS, 5% glucose solution; and DW, distilled water. Differences were analyzed using one-way ANOVA and post hoc Tukey’s multiple comparison test.
Third, photo-degradation products other than Diazo-IC were necessary because the production of Diazo-IC increases with time. 2) In the old concentration of Diazo-IC for the pain reaction has not solution and the pain reaction was not evaluated. The threshold between the concentration of Diazo-IC in the infusion process and therefore a reduction in health care costs. may contribute to a decrease in errors during the preparation of DTIC solution. Student’s t-test, # versus Protection at each time after dilution.

Our results suggest that light shielding during the dissolving process is complicated and makes the preparation more difficult. In addition, the use of light shielding materials increases costs. Thus, these procedures and the use of shielding materials can lead to preparation errors. Our results confirm our findings.

In conclusion, short duration light exposure did not affect Diazio-IC production. This finding suggests that light shielding is not needed during the preparation of DTIC in the compounding room from the viewpoint of Diazio-IC production.

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Conflict of Interest The authors declare no conflict of interest.

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