Drug Development and Industrial Pharmacy, 31:1001–1007, 2005

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040500306260

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Reversible Z-E Isomerism and Pharmaceutical Implications for SU5416

Anand Sistla
Pfizer Inc., 10777 Science Center Drive, San Diego, California, USA

Narmada Shenoy
AraVasc Inc., Mountain View, California, USA

ABSTRACT SU5416 (Z-isomer), the first in its class of angiogenesis inhibitors, in solution converts to the E-isomer following light exposure and reverts to the Z-isomer in the dark. Kinetics of this Z-E isomerism in pharmaceutical media is reported. Analytical solutions need light protection at 5°C to maintain integrity. While E-isomer in light-exposed product increased to 0.9% in 24 hours, light-protected product showed no change (25°C, 18 months). Infusate studies indicated that <1.9% E-isomer will be dosed to patients and would likely convert to the Z-isomer, following administration. This report implies Z-E isomerism in SU5416 is controllable with no limitations towards ensuring pharmaceutical product quality.

KEYWORDS SU5416, Z-E isomerism, Photo-isomerism, Thermal reversion, Pharmaceutical implications

INTRODUCTION

SU5416, the first in its class of anti-angiogenic agents, is a small synthetic organic molecule that specifically inhibits VEGF-mediated signaling through Flk-1. SU5416 inhibits the growth of solid tumors by preventing the formation of blood vessels, thus starving the cells (Fong et al., 1999). The structure of the molecule is given in Fig. 1.

SU5416 has an exocyclic alkenyl group and is capable of showing Z-E isomerism. In the solid state SU5416 exists as the Z isomer, which is the stable form of this isomer. Preliminary studies indicate that in solution SU5416 showed the presence of an unstable E isomer when exposed to light. Similar photo induced isomerism of molecules with double bonds has been reported in literature (Becker et al., 1986; Kropp, 1979; Nakagawa & Sigal, 1970; Saltiel et al., 1998; Turro, 1969; Zhang et al., 2001) and have shown to result in the formation of the less stable isomer (Whitesides et al., 1969; Zhang et al., 2001). When these light-exposed solutions of SU5416 were placed in the dark, the E isomer was observed to revert back to the Z isomer, which is consistent with other molecules containing C=C or N=N bonds (Abdel-Mottaleb et al., 1983; Benniston & Harriman, 1994; Bramham & Samuel, 1989; Clarke et al., 1991). In previous studies for 2-oxo-3-indolinylidene derivatives, molecules similar to

Address correspondence to Anand Sistla, 10777 Science Center Drive, San Diego, CA 92121, USA; E-mail: Anand.Sistla@Pfizer.com

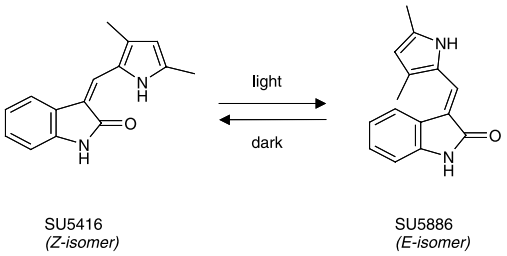

FIGURE 1 Z-E Isomers of SU5416.

SU5416, only thermal isomerism has been reported and light induced isomerism has not been studied (Autrey & Tahk, 1967; Morales-Rios et al., 1992). However, such isomerism via light induced forward (formation) reaction and thermal reversion has been reported for alkenes (Abdel-Mottaleb et al., 1983; Benniston & Harriman, 1994; Bramham & Samuel, 1989) and hydrazones (Clarke et al., 1991). Though Z-E isomerism and mechanisms have been discussed thoroughly in the reported studies, the studies were conducted with molecules different from SU5416. In addition, most studies were conducted in organic solvents and none have focused on pharmaceutically relevant media and pharmaceutical product implications.

The objective of this study was to understand and minimize the implications of Z-E isomerism of SU5416, thus allowing for product development and acceptability. SU5416 is an intravenous solution concentrate, which is diluted with saline prior to administration; therefore light exposure may lead to the formation of the E isomer in a typical clinical environment. Consequently, determination of the inter-conversion kinetics is important to assure the integrity of drug product concentrate and the infusate in a clinical setting. To ensure the integrity of the analytical methods used to determine the inter-conversion kinetics, studies were conducted to determine the inter-conversion kinetics in analytical media. In addition, results indicating the potential behavior of the isomers *in vivo* have also been presented.

Kinetic studies reported in this study were used to determine, set, and meet specifications towards quality assurance of the drug product and infusate.

EXPERIMENTAL

Chemical

SU5416 was prepared by the Chemistry Department (SUGEN, Pfizer Inc., South San Francisco, CA). Cremophor® EL was obtained from BASF Corporation (Florham Park, NJ) and 0.45% w/v Sodium Chloride Injection, USP was obtained from B. Braun Medical Inc. (Bethlehem, PA). Polyethylene Glycol 400, benzyl alcohol, ethanol, and potassium phosphate monobasic were obtained from Spectrum Quality Products (Gardena, CA). Triethylamine, methanol, and tetrahydrofuran were obtained from Fisher Scientific (Fairlawn, NJ).

Kinetics of Z-E Isomerism

Kinetics of inter-conversion of the E-Z isomers was studied in analytical and pharmaceutically relevant media (drug product concentrate and the infusate solution prepared by a 1:2 dilution of the concentrate with 0.45% Sodium Chloride Injection, USP).

Analytical Solutions of the Drug Product

Analytical solutions were prepared by diluting the drug product concentrate (1:9) with mobile phase. The resulting solution contained approximately 0.45 mg/mL SU5416 and was filled into High Performance Liquid Chromatography (HPLC) vials. These vials were then exposed to light at ambient temperature (18–22°C) and samples obtained at various time points for analysis by HPLC. Following 23 hours exposure to light, the vials were protected from light and stored at 25, 40, or 60°C. At various time points, samples were withdrawn and analyzed by HPLC as described in "Analytical Method."

Drug Product

The drug product is a non-aqueous formulation consisting of SU5416 at 4.5 mg/ml, polyethylene glycol 400, Cremophor® EL, benzyl alcohol, and ethanol. Following preparation, the drug product was filled in clear glass Type I glass vials and stoppered with Teflon coated gray butyl rubber stoppers. These vials were then exposed to light at ambient temperature (18–22°C) and samples obtained at various time points for analysis by HPLC. Following exposure to light, the vials were stored in the dark at 25°C. At various time points, samples were withdrawn and diluted (1:9) with the mobile phase and analyzed by HPLC as described in "Analytical Method."

Drug Product Infusate

The drug product infusate was prepared in non PVC infusion bags by diluting (1:2) with 0.45% Sodium Chloride Injection, USP to obtain a concentration of 1.5 mg/mL SU5416. These bags were then exposed to light (lab light) at ambient temperature (18–22°C). At various time points, samples were withdrawn and analyzed by HPLC. After approximately 28 hours of exposure to light, the samples were filtered to remove any particulate matter and stored in the dark at 25°C. At various time points, samples were taken and analyzed by HPLC without dilution as described in "Analytical Method."

Effect of Plasma Incubation on Z-E Isomerism

Drug product infusate at 0.375 or 1.5 mg/ml was diluted 1:5 with plasma and stored in the dark at 37°C in tubes. At time zero and various intervals, samples were withdrawn, the protein precipitated by 1:9 dilution with methanol, followed by centrifugation and HPLC analysis. Dilution was made with phosphate buffered saline and analyzed similarly.

Analytical Method

The analytical system consisted of a HP1090 HPLC (Agilent Technologies, Palo Alto, CA) with a C18 column (Hypersil ODS, Agilent Technologies, Palo Alto, CA, 5 μm, 4.6×200 mm). The mobile phase consisted of methanol:tetrahydrofuran:35mM potassium phosphate monobasic buffer containing 0.1% triethylamine (40:20:40) with an injection volume of 10 μl and detection was at either 425 or 275 nm. The flow rate was 1.2 ml/min. The retention time of the E and Z isomers of SU5416 were 3.4 and 7.1 min, respectively. The autosampler was maintained at 5°C and all samples were injected immediately.

Data Analysis

Percent E isomer was calculated as the % area of E isomer to the total area of the isomers at the detection wavelength. The equation used in the calculation of the rate constants for formation of E isomer is defined as:

$$(E_{eq} - E_t) = (E_{eq} - E_0)e^{-kt}$$

where E_{eq} is the % area of the E isomer at equilibrium, E_t is the % area of the E isomer at time t, and E_0 is the % area of the E isomer at time zero. Correspondingly, for the disappearance of the E isomer, the equation is given as:

$$(E_t - E_{eq}) = (E_0 - E_{eq})e^{-kt}$$

RESULTS AND DISCUSSION

The pharmaceutical implications of any phenomenon affecting the drug molecule can be divided into analytical, product, and biological implications.

Analytical Implications of Z-E Isomerism

The kinetics of the Z-E isomerism in the analytical solutions was studied to understand and set the methods for analysis of drug product samples from various experiments. The results from the light exposure study for the analytical solutions are shown in Fig. 2. The results indicated that formation of E isomer does not reach completion with 23% formed after 13 days. This lack of completion is attributed to the presence of a reverse E-Z transformation in addition to the light-induced Z-E reaction. The observed rate constant for formation of E isomer was 0.15 hr^{-1} in the analytical solutions with a calculated equilibrium value of 21%. The studies showed that approximately 3% of the E isomer is

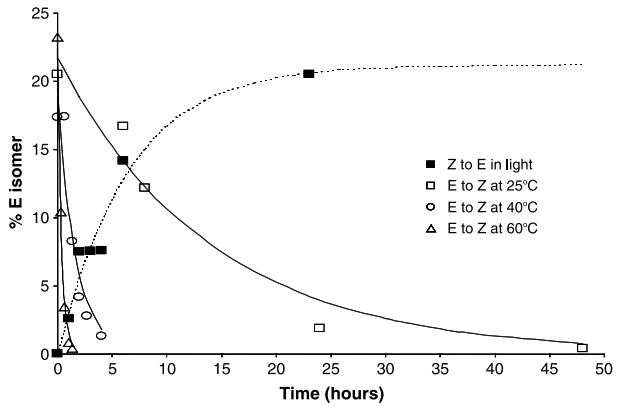

FIGURE 2 **Z-E Isomerism in Analytical Solutions of the Clinical Drug Product. "Z-E in Light" Represents Samples Exposed to Fluorescent Light at RT in a Laboratory Setting. "E to Z" Represents Analytical Solution Pre-Exposed to Fluorescent Light for 23 Hours and then Protected from Light.**

TABLE 1 Rate Constants of Conversion of the E Isomer to the Z Isomer in the Dark at Different Temperatures

Sample description	Estimated rate constant (hr^{-1}) (% E isomer range)		
Temperatures	25°C (0 to 48 hrs)	40°C (0 to 4 hrs)	60°C (0 to 1.33 hrs)
Drug product in mobile phase	0.07 (20.5 to 0.5)	0.71 (17.4 to 1.3)	2.7 (23.2 to 0.4)

formed within one hour of light exposure underscoring the importance of light protection to prevent formation of the E isomer during sample preparation and analysis.

While light protection minimizes the photo-induced formation of the E isomer, storage in the dark can result in a decrease in the E isomer already formed. To determine the reversion kinetics, the analytical solution of SU5416 was first exposed to light for 23 hours to attain equilibrium. This solution was then protected from light at different temperatures to study the E to Z isomer conversion. Results from these studies are shown in Fig. 2 and the calculated rate constants are given in Table 1. The E isomer was observed to revert to the Z isomer following storage in the dark with an increase in the reconversion rate at higher temperatures (lnK vs. $1/T$: $r^2=0.96$). This observation indicates that the reversion of E to Z isomer is a thermal reversion. Since the lowest feasible storage temperature for the analytical solutions is 5°C, the rate constant at 5°C was extrapolated to be 0.007 hr^{-1} using the Arrhenius plot. This reversion rate was considered acceptable to preserve the integrity of any light-exposed formulation samples and samples were stored at 5°C (auto sampler) for <2 hours prior to injection of a particular sample. These precautions were maintained during the analysis of samples generated from the drug product and infusate studies.

Thus, light exposure results in the formation of the unstable isomer (E isomer) followed by thermal reverse isomerism, which results in the reversion to the stable isomer (Z isomer) and is consistent with previous studies investigating Z-E transformations (Abdel-Mottaleb et al., 1983; Benniston & Harriman, 1994; Bramham & Samuel, 1989).

Product Implications of Z-E Isomerism

The next step was to understand the implications of the Z-E isomerism on the drug product and final clinical product. Z-E isomerism kinetics in the drug product concentrate was carried out simulating conditions in a typical clinical setting. A graphical representation of the light induced formation of E isomer and thermal reversibility in the drug product concentrate is shown in Fig. 3. Levels of the E isomer in the drug product concentrate increased to 15.4% after 12 weeks of fluorescent light exposure in a typical clinical setting without reaching complete conversion. This formation of the E isomer followed a first order kinetics with an observed rate constant of 0.002 hr^{-1} with approximately 0.9% of the E isomer formed after 24 hours, whereas the level of E isomer was observed to remain constant (<0.3%) in the drug product formulations, not exposed to light (Fig. 3). Consequently, as in case of analytical solutions, light protection is required to prevent or minimize formation of the E isomer in the drug product. Since in the clinic the product needs to be visualized prior to dilution to ensure absence of particulate matter, the product concentrate could not be stored in light-protected primary containers such as amber colored glass vials to enable light protection. Therefore,

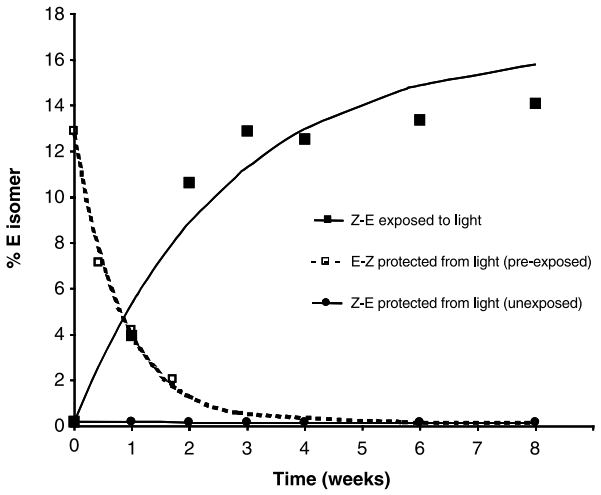

FIGURE 3 Z-E Isomerism in the Drug Product Concentrate. "Z-E Exposed to Light" Represents Samples Exposed to Fluorescent Light. "E-Z Protected from Light (Pre-Exposed)" Represents Samples Pre-Exposed to Fluorescent Light and then Protected from Light. "Z-E Protected from Light (Unexposed)" Represents Samples, Which were Never Exposed to Light Following Preparation.

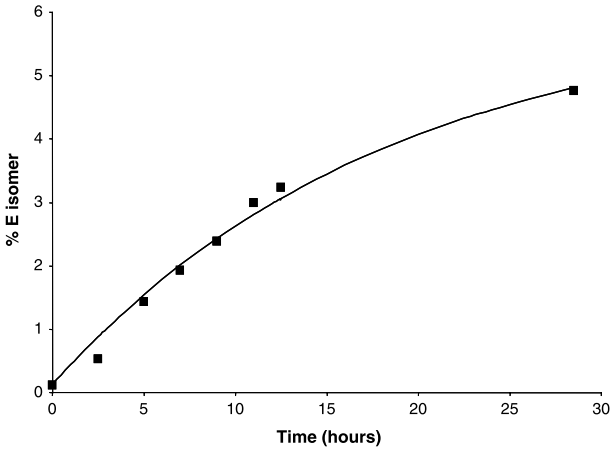

FIGURE 4 **Formation of E Isomer in Drug Product Infusate.**

secondary containers such as chipboard boxes contained within cardboard boxes were used to afford light protection of the drug product concentrate. Experience with clinical supplies stored for 18 months show no increase in the E isomer (<0.3% E isomer) compared to the initial values indicating that this approach is feasible.

Furthermore, when the light exposed samples were stored in the dark at 25°C, the E isomer in the drug product converted back to the Z isomer with an estimated rate constant of 0.007 hr^{-1} (Fig. 3). This observation indicates that drug product concentrate accidentally exposed to light for short periods of time can be transferred to light-protected conditions to decrease the levels of the E isomer formed and presents a potential approach in maintaining the shelf life of the drug product.

Since in the clinic the drug product concentrate is diluted 1:2 with 0.45% saline prior to administration and administered within 6 h of dilution to patients, Z-E transformations were also studied in the infusate (diluted drug product concentrate). The infusate is prepared in non-PVC clear intravenous bags to allow observation and ensure absence of particulate matter in the infusate, therefore not light protected. Kinetic studies were carried out to simulate these conditions and the results are shown in Fig. 4. Following exposure of the infusate to fluorescent light under simulated clinical conditions, the E isomer increased to 4.5% (over 24 hrs) at an observed rate constant of 0.05 hr^{-1} at room temperature. However, since the formulation is administered within 6 h of dilution, the patients are expected to receive <2% of the E isomer.

Results also indicate that thermal reversion of the E isomer to the Z isomer in the dark is observed in the infusate. However, the limited physical stability and post-dilution storage of the solution precludes any utility towards maintaining or increasing stability of the drug product infusate.

These studies allowed for reconciliation of the E isomer administered in various studies required to support and set the clinical product specifications.

Biological Implications of Z-E Isomerism

Considering the thermal reversion of the E isomer in various media, plasma dilution studies were carried out to attempt to predict the fate of the E isomer following administration to patients. The results from the study are shown in Fig. 5 and given in Table 2. The half-life of the conversion of E to the Z isomer in plasma was estimated to be 23 min. These studies show that any E isomer present is decreased when incubated with plasma or phosphate buffer at 37°C. Therefore, E isomer formed following exposure to light during the administration may convert to the Z isomer in the plasma. The effect of other constituents of the blood not present in plasma is unknown.

Effect of Media on Kinetics of Z-E Isomerism

Though exposed to the similar intensity of light, a significant difference was seen in the observed rates of formation between the infusate and drug product concentrate (Table 3). The formation of E isomer in

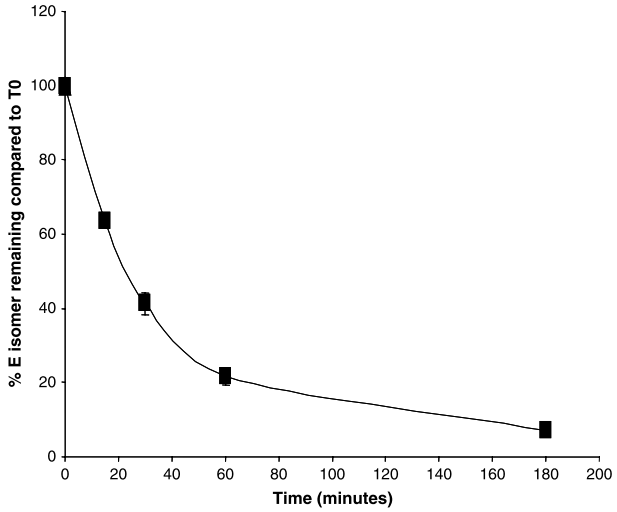

FIGURE 5 **Time-Related Effect of Plasma Incubation at 37°C on % E Isomer of SU5416.**

TABLE 2 Concentration-Related Effect of Plasma Incubation on %E Isomer of SU5416

Concentration of diluted drug product (mg/ml)	Medium	% E isomer at t_{zero}	% E isomer after 30 min incubation	%E isomer remaining compared to t_{zero}
1.5	Plasma at 37°	3.7	1.7	47
	PBS at 37°	3.9	2.7	70
0.38	Plasma at 37°*	4.1±0.2	1.5±0	37±2
	PBS at 37°	4.1	1.8	44

Results are mean ($N=2$).
*±SD ($N=3$).

light-exposed solutions follows the order analytical solutions>infusate>drug product concentrate and is the reverse of the drug concentration. Previous studies in this laboratory (unpublished data) have indicated a similar concentration effect following light exposure of methanol solutions of SU5416 at different concentrations. This difference may be attributed to a decrease in light intensity following higher degree of absorption at the external surfaces in comparison to the internal region of the more concentrated solutions. However, the decreased intensity of light entering concentrated solutions does not explain a similar increase in isomerism rates during the thermal reversion, where the experiments are carried out in the dark at the same temperature for all the solutions. Therefore, additional factors exist that affect the isomerism rates and were attributed to differences in polarity and viscosity of the media.

An increase in the polarity of the media resulted in increased isomerization rates for SU5416. The polarity of the media in this report follows the order plasma>infusate solutions>drug product concentrate, which follows from the water content of 90, 33, and 0%, respectively. Previous reports (Belsky et al., 1977; Benniston & Harriman, 1994; Pappalardo et al., 1993) have indicated that isomerism occurs following a reduction in the rotational barriers of the C=C bond brought about by delocalization of electrons in the double bond. Hydrogen bonding, which increases with increasing polarity of the media, results in increased electron delocalization throughout the molecule (Benniston & Harriman, 1994) and thus, higher isomerization rates (Benniston & Harriman, 1994; Gille et al., 1999; Pappalardo et al., 1993). A similar mechanism is proposed for isomerization rates in SU5416 and is attributed to the ability of carbonyl group in SU5416 to hydrogen bond and cause delocalization of electrons at the exocyclic double bond. Accordingly, the isomerism rates are higher in media that exhibit higher polarity.

In addition, the decrease in isomerism rates in both the light induced and the thermal isomerism can also be attributed to the differences in the viscosities of the media. The viscosity of the media follows the order analytical solutions < infusate < drug product concentrate, which is the inverse of the isomerism rates either in light or dark. The result is consistent with previous studies where an increase in the viscosity has shown a decrease in the isomerism rates of C=C bonds in bis-oxonols (Benniston & Harriman, 1994) and N=N bonds in azo benzene dyes (Gille et al., 1999; Wang & Knoll, 2001). Higher viscosities result in higher friction between the molecules and the media and, consequently, lower rotation and isomerization rates (Gille et al., 1999). In their study, Benniston & Harriman (1994), studied the effects of viscosity of various protic (alkanols ranging from methanol, ethanol to glycerol) and aprotic (DMSO, DMF, acetone, ethers) solvents on light induced and thermal isomerism rates in bis-oxonols. An inversely linear

TABLE 3 Summary of the Observed Rate Constants (hr^{-1}) of Formation and Reversion of the E Isomer in the Drug Product Related Solutions

Sample	Analytical solutions	Drug product concentrate	Infusate	Plasma
SU5416 concentration in sample (mg/ml)	0.45	4.5	1.5	1.5
Formation of E isomer in light (hr^{-1})	0.15	0.002	0.05	—
Reversion of E isomer in dark (hr^{-1})	0.07	0.007	0.05	1.8

relationship between light induced rates and viscosity was observed in both protic and aprotic solvents, especially at ambient temperature. However, in the same study, the authors (Benniston & Harriman, 1994) reported that though a similar inverse relation was found between the thermal isomerism rates and viscosity, a higher scatter was observed indicating that thermal isomerism is less sensitive to viscosity effects. The exact reasons remain unknown. Therefore, the differences in rates for light induced formation of the E isomer are attributed to the effect of concentration, polarity, and viscosity, whereas the thermal dark reversion is considered to be mainly dependent on the polarity of media.

CONCLUSIONS

SU5416 in solutions undergoes photo induced formation of the unstable E isomer, which then undergoes thermal reversion and converts to the Z isomer in the dark. The photo-induced and thermal isomerism rates are dependent on concentration, polarity, and viscosity of the media. Light and thermal protection (5°C) are required to maintain the integrity of the analytical samples. SU5416 drug product has been successfully stored for 18 months in accordance with the recommended storage conditions (15–30°C, protected from light) without detectable loss of SU5416 and with less than 0.3% of the E isomer. In case of accidental exposure to light, the drug product could be transferred to the dark to revert the E isomer to the Z isomer. Given the conversion rate in light exposed clinical infusate, $\leq$2% E isomer would be administered to the patients. Preliminary ex-vivo incubation studies of an isomeric mixture of SU5416 in human plasma suggest that the E isomer would convert to the Z isomer in the human body. This report implies that though Z-E isomerism is observed in SU5416, the phenomenon is controllable and does not present limitations towards ensuring pharmaceutical product quality.

ACKNOWLEDGMENTS

The authors would like to acknowledge the contributions of Weilin Yang, Kumuda Shenoy, and Tony Luu, and also acknowledge Dave Johnson for his input towards the preparation of this report.

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