Chapter-11

Influence of Inclusion on Photostability
11.1 Photostability of drugs

Stability of drugs towards exposure to light and oxidation in air is a topic of great practical interest but of considerable complexity. The primary focus of any controls instituted for product administration include precautionary product-labeling statement that product efficacy and safety is maintained. The term photostability includes the degradation caused by exposure to light, along with processes such as radical formation, energy transfer and luminescence. The limited part of spectrum associated with photochemistry is the one covering the range from around 290 nm to 800 nm and subdivided into the three regions UVB (290-320 nm), UVA (320-400 nm) and visible light (400-800 nm). UVB is considered to be associated with direct sunlight and UVA with daylight filtered through glass. Since the UV end of the spectrum is responsible for most photochemical reactions, there is no correlation between the visual perception of light intensity and incident radiation that may induce chemical change. The number and the wavelength of incident photons affect the photodegradation rate of drugs. Study of quantitative effect of light is complicated because the wavelength dependence of degradation varies among drug substances and because light sources have different spectral distributions. Thus, for photosensitive reactions, the causative wavelengths for photodegradation may be quite different than those associated with absorbance spectra of the active and must be examined carefully to establish which wavelength of light are pertinent. Understanding the mechanism for photodegradation or other photoinduced changes also help in identification of the causative wavelength (Yoshioka and Stella, 2000). Various types of photodegradation mechanisms and illustrative examples of drugs known to degrade by each type of reaction, are summarized in Table 11.1.

Photodegradation in solution, particularly in aqueous solutions is likely to differ considerably from photodegradation in the solid-state. Secondary reactions of primary photoproducts with the solvent can result in the formation of species that are not possible in the solid state. Moreover, for solid samples, only a limited portion of static sample will actually be exposed to the radiations, in contrast to more uniform conditions existing during thermal treatments (Gross et al, 2004).
Table 11.1 Representative routes of photodegradation of drugs.

<table>
<thead>
<tr>
<th>Type of Reaction</th>
<th>Examples of drugs</th>
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<tbody>
<tr>
<td>N-Dealkylation</td>
<td>chlodiazapoxide, furosemide</td>
</tr>
<tr>
<td>decarboxylation</td>
<td>indomethacin</td>
</tr>
<tr>
<td>dehalogenation</td>
<td>maclofenamic acid</td>
</tr>
<tr>
<td>Dehydrogenation</td>
<td>nifedipine, reserpine, nicardipine</td>
</tr>
<tr>
<td>Dehydroxylation</td>
<td>21-cortisol tert-butylacetate</td>
</tr>
<tr>
<td>Dimerization</td>
<td>primaquine, levonorgestrel,</td>
</tr>
<tr>
<td>Hydrolysis</td>
<td>mefloquine, curcumin</td>
</tr>
<tr>
<td>Isomerization</td>
<td>cefuroxime, digitoxin</td>
</tr>
<tr>
<td>Oxidation</td>
<td>fumagillin, phenothiazines, cianidanol, menadione</td>
</tr>
<tr>
<td>Rearrangement</td>
<td>chlodiazopoxide</td>
</tr>
<tr>
<td>Reduction</td>
<td>reserpine</td>
</tr>
<tr>
<td>Ring dealkylation</td>
<td>chloroquine</td>
</tr>
</tbody>
</table>

Some of the important factors affecting photodegradation of drugs in dosage forms (Thoma, 1996) can be summarized as follows:

- Particle size and surface area
- Crystal structure. Amorphous forms often behave very differently from crystalline forms and different crystalline polymorphs can be expected to behave differently.
- Wavelength, the intensity and time of exposure to the radiation
- Type shape and orientation of the sample holder
- Temperature of photoexposure
- Humidity conditions under which photoexposure is being conducted
- Presence or absence of oxygen in the atmosphere where photoexposure is being carried out
- Transparency of photoproducts to the radiation being used. If the photoproducts are strong absorbers, only limited overall reaction will occur.
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- Presence and nature of excipients present, if any. Only absorbed radiations are known to participate in photodegradation process. Excipient may reduce photodegradation because of dilution and other possible shielding effects. Some excipients may act as photosensitizers by being better absorber of radiation and by transferring energy to drug without degradation themselves.

While thermal degradation is assumed to continue till completion, in the photolysis of solids, reaction may be limited to within a short distance inwards from the irradiated surface. Thus photodegradation is a complex phenomenon exhibiting variations due to number of factors as listed above.

Pharmaceutical product degradation usually results from one of the two scenarios. The first involves direct absorption of light by the active pharmaceutical ingredient and the subsequent conversion to another chemical entity. In this case, the classical first law of photochemistry put forward by Grotthus and Draper is maintained (light must be absorbed to be photochemically active). The second photodegradation pathway results from photosensitization reactions in which one component of the formulation absorbs light and activate another component for subsequent chemical reactions, leading to degradation of the active pharmaceutical ingredient. Both types of photodegradation pathways must be taken into consideration when examining the photosensitivity of formulated pharmaceutical products (Templeton et al, 2005).

Depending upon extent of photoprotection required for a product, a pharmaceutical product photosensitivity classification system has been proposed (Templeton et al, 2005). The system is useful for understanding and managing implications of product photosensitivity during manufacturing, packaging, shelf storage, testing and administration. The classification system (Figure 11.1) delineates products into following three classes:

Class I: Formulated products that do not chemically and/or physically change to any reasonable extent upon exposure to light. Hence these products are the most straightforward to handle from photosensitivity standpoint.

Class II: Products that photodegrade or otherwise exhibit significant change upon direct-light exposure. These formulated products are, however, fully protected from photodriven change when placed into an appropriately protecting immediate package.
Class III: Products that photodegrade or otherwise exhibit significant change upon direct-light exposure. These products may be adversely affected by light even when housed in an immediate package. Hence, these products require protection from light with an additional packaging layer (e.g., a cardboard carton).

**Figure 11.1 Pharmaceutical product photosensitivity classification system (Templeton et al, 2005).**

### 11.2 Photostability studies

The intrinsic photostability characteristics of new drug substances and products should be evaluated to demonstrate that, as appropriate, light exposure does not result in unacceptable change. Photostability testing in the pharmaceutical industry has evolved rapidly, particularly since the May 1997 publication of the ICH Q1B guidance “Photostability Testing of New Drug Substances and Products” in the *Code of Federal Regulations* (ICH, 1997). Although some criticism have been leveled against the document for its perceived shortcoming (Helboe, 1998), Q1B guidelines have provided much-needed input to pharmaceutical applicants about regulatory requirement of photostability testing. Significantly, Q1B alleviates much of the ambiguity around
spectral range and irradiance level requirements, harmonizes global pharmaceutical laboratory practice and provides a sequential approach to follow when examining protective packaging requirements for photosensitive pharmaceutical products.

**Figure 11.2 Photostability study chamber as per Q1B guidelines.**

Normally photostability testing is carried out on a single batch of material by exposing the material in a photostability chamber (Figure 11.2) to light providing an overall illumination of not less than 1.2 million lux hours and an integrated ultraviolet energy of not less than 200 watt hours/square meter. Any light source that is designed to produce an output similar to the D65/ID65 emission standard or a combination of cool white fluorescent lamp and a near UV fluorescent lamp may be utilized. (As per Commision Internationale de L’Eclairage, D65 is equivalent to average outdoor daylight and ID65 an equivalent indoor daylight standard) Appropriate control of temperature is recommended to be maintained to minimize the effect of localized temperature changes or a dark control may be included. The photostability testing may consist of two parts i.e.

*The forced degradation testing*, which may be considered as equivalent to stress studies, are undertaken deliberately to degrade the sample. The purpose is to evaluate the intrinsic characteristics of the material, to allow identification of photodegradants as necessary and to facilitate method development will depend on the photosensitivity of the drug. The exact conditions to be used are not prescribed.
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Confirmatory studies, which may be equated to accelerated/long term testing of parent guideline and are undertaken to establish photostability characteristics under standardized conditions. These provide information necessary for handling, packaging and labeling. Normally, only one batch of drug need to be tested in the development phase, and characteristics confirmed on a single definitive batch. This definitive batch must meet the requirements of the parent guidelines being manufactured to a minimum of pilot scale by the same synthetic route and method of manufacture and process that simulates the final manufacturing procedure.

11.3 Cis-RA-A photosensitive drug

In general, retinoids are unstable compounds, being sensitive to oxygen, heat, and light (Tan et al, 1992; Lucero et al, 1994; Brisaert et al, 1995). Their stability is, therefore, of pharmaceutical interest. Theoretically, upon photo-exposure, each of the double bonds in the conjugated polyene chain portion of the retinoid moiety can undergo isomerization to give both mono-cis or multiple cis isomers but due to steric hinderances, some of these isomers may not be thermodynamically stable at room temperature and therefore tend to isomerize to more stable forms (Motto et al, 1989; Bempong et al, 1995). Cis-RA has three trans and one cis double bonds of which those at C-9, C-11 and C-13 are vulnerable to photoisomerization upon photoexposure. Presumably steric hindrance by the cyclic methyl group prevents significant amounts of 1-cis derivative being formed. (Curley and Floble, 1988). The photostationary mixture consists of a mixture of all possible geometric combinations including the original 13-cis form. Various products detected after prolonged photodegradation of cis-RA and their relative amounts are listed in Table 11.2. The molecular structure of retinoic acid and its various photoisomers is included in Figure 11.3.

Some of the effects of retinoic acid depend on the geometry of polyene chain. For example, 13-cis retinoic acid and all-trans retinoic acid display differential effects on mRNA levels of Retinoic Acid receptors (Haq et al, 1991). In particular, the photoisomerization of retinoids plays an important role in vision (11-cis-retinal isomerization in retinyl opsins) and in some bacterial proton transport (13-cis-retinal isomerization in bacteriorhodopsin) (Bridges, 1984). Also there are differences in the receptor binding of the various isomers (Sundquist et al, 1993). Therefore, the
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development of novel formulations characterized reduced sensitivity towards retinoic acid photoisomerisation seems to be important.

Table 11.2 Photodegradation products and the percentage area for degradation peaks after cis-RA solution and solid samples have been exposed to light and air for a period of 4 and 8 weeks respectively (Bempong et al, 1995)

<table>
<thead>
<tr>
<th>Product</th>
<th>Solution</th>
<th>Solid</th>
</tr>
</thead>
<tbody>
<tr>
<td>13-cis RA</td>
<td>28.14</td>
<td>88.15</td>
</tr>
<tr>
<td>e/z isomer of RA</td>
<td>22.78</td>
<td>0.41</td>
</tr>
<tr>
<td>all-trans RA</td>
<td>29.10</td>
<td>7.6</td>
</tr>
<tr>
<td>e/z isomers of RA</td>
<td>15.64</td>
<td>2.56</td>
</tr>
<tr>
<td>13-cis 5,6-epoxy RA</td>
<td>02.94</td>
<td>0.88</td>
</tr>
<tr>
<td>All trans 5,6-epoxy RA</td>
<td>04.47</td>
<td>0</td>
</tr>
<tr>
<td>e/z isomers of 5,6-epoxy RA</td>
<td>0.64</td>
<td>0</td>
</tr>
</tbody>
</table>

An alcoholic solution of retinoic acid was reported to isomerize or degrade to less than 50% of the initial concentration after in 1 h under natural light. After 24 h, less than 10% was left. When the same alcoholic solution was stored under yellow light, no isomerisation or degradation was observed (Disdier et al, 1996). The stability of retinoids was also investigated in plasma, and it was found that all-trans retinoic acid, 13-cis-retinoic acid and 4-oxo metabolites to be stable for only 3 months when stored at – 20° C. This was extended to 9 months when stored at – 20° C (Wyss, 1995). Similarly, no significant degeneration of retinol and retinyl esters was observed when stored in human plasma at -80° C for 6 months (Wyss, 1995).

In order to study the effect of inclusion of cis-RA in urea on its photoisomerization, photostability studies of both the drug and its urea co-inclusion compound were conducted as per Q1B Guidelines for Photostability Testing.
Fig. 11.3 Various photodegradation products of \textit{cis}-retinoic acid.

- \textit{13-cis} retinoic acid
- \textit{all-trans} retinoic acid
- \textit{9-cis} retinoic acid
- \textit{11-cis} retinoic acid
- \textit{11,13-dicis} retinoic acid
- \textit{9, 13 dicis} retinoic acid
- \textit{all-trans 5,6-epoxy} retinoic acid
- \textit{13-cis 5,6-epoxy} retinoic acid
11.4 Photostability testing of Cis-RA and Urea Inclusion compound

11.4.1 Method
Photodegradation process was performed in Neutronics Photostability study apparatus Model NEC/09RSPS, equipped with cool white fluorescent lamp and near UV fluorescent lamp, option 2 according to the ICH Guidelines Q1B for photostability testing. The apparatus was set up with an electronic device for both irradiation and temperature controlling inside the box. Irradiance power was set to overall illumination of 5.2 Klux/hr and near ultraviolet energy of 1.3 Watt-Hour/m². Temperature and relative humidity inside the chamber were maintained at 25°C and 60% respectively throughout the study.

Weighed quantity of finely powdered cis-RA and of its urea co-inclusion compound (UIOA) were spread as thin layer in a glass petri dish (Ø 10 cm). The petri dishes were placed in photostability chamber sufficiently apart to avoid shadowing and irradiated with both visible and UV lamps. The samples were withdrawn from the chamber after every 24 hrs till 6 days. The withdrawn samples were immediately sealed hermetically in a completely light resistant packaging and stored in refrigerator.

On the day of HPLC analysis, an accurately weighed amount of sample was washed into volumetric flask with mobile phase and suitably diluted to contain a concentration of ~ 20 µg/ml. The content of cis-RA in each sample dilution was analyzed by HPLC method. The complete HPLC analysis was conducted under subdued light.

The complete experiment was conducted in duplicate.

11.4.2 Result and discussions
Fig. 11.4 shows contents of cis-RA left in the drug sample and in its urea co-inclusion compound when exposed simultaneously to both the cool white fluorescent and UV lamp as per Q1B photostability studies guidelines. The bright crystals of cis-RA were found to get converted into sticky lumpy mass on photo-exposure and marginal increase in weight of samples after photo-exposure was noticed. On the other hand, UIOA samples were found to retain their free flowing characteristic even after 144 hr of combined photo-exposure to both UV and visible light.
The plots clearly indicates that drug co-inclusion with RAE in urea appreciably reduces the photodegradation process if compared with that of the pure solid drug. While the sample of pure drug was found to retain only ~29% of residual drug content after irradiance with ~200W/m² of UV and ~750 Klux /hr of visible light exposure, the same amount of photo-exposure resulted in a residual drug concentration of ~ 35 % for the co-inclusion compound of cis-RA.

For a particular solid the appropriate kinetic equation for decomposition is usually obtained by analyzing the experimental decomposition data using the existing kinetic forms to see which one gives the best fit (Ng, 1975). Hence, the experimental data obtained on photostability testing was subjected to various kinetic equations proposed for characterization of decomposition in solid state. The kinetic equations, in their differential and integral forms, along with the correlation coefficient obtained for each equation for both the sample i.e. the drug and its urea co-inclusion compound with urea are listed in Table 11.3. Figures 11.5 -11.9 exhibit the plots for the decomposition data when fitted into different kinetic equations.

Figure 11.4 Figure showing percentage of cis-RA remained undecomposed in sample of pure drug and in the sample of co-inclusion compound of drug upon photoexposure.
Table 11.3 Different kinetic equations used to describe decomposition in solid state, and the fit for the equation for photodecomposition profile of *Cis-RA* and its urea co-inclusion compound.

<table>
<thead>
<tr>
<th>Kinetic Equation</th>
<th>Differential form</th>
<th>Integral form</th>
<th>Fit ( r^2 ) for the equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(^{st}) order</td>
<td>(-dc/dt = kc)</td>
<td>( k = (\ln c_o - \ln c) / t)</td>
<td>Cis-RA 0.9716 0.9777</td>
</tr>
<tr>
<td>2(^{nd}) order</td>
<td>(-dc/dt = kc^2)</td>
<td>( l/c - l/c_o = kt)</td>
<td>UIOA 0.9461 0.9411</td>
</tr>
<tr>
<td>Unimolecular Decay</td>
<td>(da/dt = k (1-\alpha))</td>
<td>(-\ln (1-\alpha) = k t)</td>
<td></td>
</tr>
<tr>
<td>Prout-Tompkins Equation</td>
<td>(da/dt = k\alpha (1-\alpha))</td>
<td>(\ln [\alpha/(1-\alpha)] = k t + C)</td>
<td>Cis-RA 0.9694 0.9424</td>
</tr>
<tr>
<td>Modified Prout-Tompkins Equation</td>
<td>(da/dt = k\alpha^{1-1/k}(1-\alpha)^{1+1/k})</td>
<td>(\ln[\alpha/(1-\alpha)] = k \ln t + C)</td>
<td>UIOA 0.9588 0.9703</td>
</tr>
</tbody>
</table>

\(c\) = conc. of drug at time \(t\); \(c_o\) = initial concentration; \(\alpha\) = fractional decomposition of drug at time \(t\); \(k\) = rate constant

Figure 11.5 Plot between \(\ln\) (percentage drug left) vs time, showing fit for 1\(^{st}\) order kinetic equations to the photodecomposition profile of cis-RA and its urea co-inclusion compound.
Figure 11.6 Plot between Inverse of percentage drug left vs time, showing fit for 2\textsuperscript{nd} order kinetic equations to the photodecomposition profile of cis-RA and its urea co-inclusion compound.

Figure 11.7 Plot between $-\ln (1-\alpha)$ vs time, showing fit for Unimolecular decay kinetic equations to the photodecomposition profile of cis-RA and its urea co-inclusion compound.
Figure 11.8 Plot between $\ln \left( \frac{a}{1-a} \right)$ vs time, showing fit for Prout-Tompkins Equation to the photodecomposition profile of cis-RA and its urea co-inclusion compound.

Figure 11.9 Plot between $\ln \left( \frac{a}{1-a} \right)$ vs ln (time in days), showing fit for the Modified Prout-Tompkins Equation to the photodecomposition profile of cis-RA and its urea co-inclusion compound.
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Retrofit analysis of Table 11.4 and of Figures 11.5 - 11.9 shows that out of all the kinetic equations investigated, the best fit for both the drug sample and its urea co-inclusion compound is obtained with unimolecular decay equation. While the decomposition rate constant for photodecomposition for pure drug was found to be 0.1698 day\(^{-1}\). Co-inclusion of the drug in urea lattice led to a reduction in the value of reaction rate constant to the level of 0.1597 day\(^{-1}\).

The photochemistry within an inclusion complex involve features, which may be quite distinct from those of uncomplexed substances since the interior of the cavity constitutes as isolated environment where the included species are usually present as single molecules restricting the photochemistry to intermolecular events (Glass et al., 2004). In case of cis-RA, photodecomposition has been attributed to mainly isomerization along double bonds in the side chain and to oxidation of the unsaturation within the ring, as exhibited by the types of photodecomposition products isolated from a photodegraded drug sample. Both these events may be considered as individual molecular events, which may explain the photodecomposition profile by unimolecular decay.

Co-inclusion of cis-RA in urea in the presence of suitable RAE is evident as characterized by DSC, FTIR and XRD studies. Thus an included moiety is contained within the narrow, hexagonal, parallel and non-intersecting channels stabilized by hydrogen bonds between urea molecules. The containment of guest moiety within these channels may tend to restrict its molecular motion and also prevent direct availability of incident photons to conjugated double bonds, which provide necessary energy for isomerization. Thus, urea inclusion compound formation tends to reduce photosensitivity of included guest species.

However, inclusion of a bulky guest is known to cause distortions in the vicinity of aromatic ring in the surrounding hexagonal channels formed of urea molecules. These distortions in turn, weaken the intermolecular hydrogen bonds and destabilize the structure. Increasing the proportion of RAE in the co-inclusion compound will reduce frequency of these distortions and may lead to improved stability of the urea lattice as a whole. Thus, though inclusion of cis-RA led to appreciable improvement in photostability, improved photoprotection can be further expected by incorporation of increased relative proportion of RAE in the co-inclusion compound.
11.5 Conclusion

Pure cis-RA drug and its co-inclusion compounds with urea were subjected to photoexposure in photostability testing chamber as per Q1B guidelines for photostability testing. Co-inclusion of drug in urea provided appreciable degree of photoprotection as exhibited by reduction in rate constant from 0.1698 day⁻¹ for pure drug to 0.1597 day⁻¹ for co-inclusion compound. The extent of photoprotection can be further improved by modifying the relative proportion of RAE in the co-inclusion compound of drug in urea.