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Accelerated aging: Prediction of chemical stability of pharmaceuticals

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Abstract

Methods of rapidly and accurately assessing the chemical stability of pharmaceutical dosage forms are reviewed with respect to the major degradation mechanisms generally observed in pharmaceutical development. Methods are discussed, with the appropriate caveats, for accelerated aging of liquid and solid dosage forms, including small and large molecule active pharmaceutical ingredients. In particular, this review covers general thermal methods, as well as accelerated aging methods appropriate to oxidation, hydrolysis, reaction with reactive excipient impurities, photolysis and protein denaturation.

Keywords: Drug stability; Accelerated aging; Hydrolysis; Oxidation; Shelf-life

1. Introduction

In the development of pharmaceutical dosage forms, one of the persistent challenges is assuring acceptable stability. While classically stability refers to the ability to withstand loss of a chemical due to decomposition, in the pharmaceutical world, the term "stability" more often refers to the storage time allowed before any degradation product in the dosage form achieves a sufficient level to represent a risk to the patient. Based on this time, the expiration date (shelf-life) of a product is determined. The allowable level of any given impurity will depend on the dose and likelihood of toxicity; however, for most drugs, the allowable levels of a single impurity permissible without explicit toxicological clinical testing are generally well less than 1% based on the drug. The International Council of Harmonization (ICH) specifies the amount of impurities allowed to form during product storage (ICH, 2003).

The amounts permitted are based on the total daily intake of the drug. The amount of impurity allowed is described as a reporting, identification, or qualification threshold. A reporting threshold is defined as the level that must be reported to regulatory agencies to alert them of the presence of the impurity. An identification threshold is defined as the level that requires chemical identification of the substance. Finally, the qualification

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Table 1
Total amount of degradants allowed for new drug products based on regulatory guidelines (International Council of Harmonization, 2003) (TDI = total daily intake)

Type of threshold	Maximum daily dose	Threshold
Reporting	≤1 g	0.1% TDI
	>1 g	0.05% TDI
Identification	<1 mg	1.0% TDI or 5 μg (lower of two)
	1 mg–10 mg	0.5% TDI or 20 μg (lower of two)
	10 mg–2 g	0.2% TDI or 2 mg (lower of two)
	>2 g	0.10%
Qualification	<10 mg	1.0% TDI or 50 μg (lower of two)
	10 mg–100 mg	0.5% TDI or 200 μg (lower of two)
	100 mg–2 g	0.2% TDI or 3 mg (lower of two)
	>2 g	0.15% TDI

threshold is the level that must be tested in toxicology studies to ensure the safety of the compound. These are defined as a percent of the drug total daily intake or an absolute mass amount, whichever is lower. Table 1 describes the specific levels of impurities allowed for each threshold.

One of the consequences of using product formation rather than drug loss to determine stability is that the precision of measurements is inherently higher for low conversions. This is due to the fact that it is generally harder to detect a small change in a large number than the same absolute change in a small number because the relative change in values is much greater in the latter case. For example, for a product formed during a degradation study going from 0.05 to 0.50% is a 1000% increase in the level of the material, while decreasing the initial amount of drug by the same amount, i.e., from 100.00 to 99.50%, is a decrease of only 0.5%.

The advent of more sensitive analytical methods in pharmaceutical development has resulted in a concomitant decrease in the allowable levels of impurities and degradants in dosage forms. In the development of a pharmaceutical product, speed to market has a great influence on the profitability of that product. Formation of a degradant at levels significantly below 1% of the drug during an anticipated shelf-life (usually two to three years) may result in significant product introduction delays, especially if such instability is uncovered late in the development process. Once clinical trials have begun, a change in formulation may necessitate additional clinical trials to assure bioequivalence of the formulations. Consequently, there is a strong incentive to predict any instability in pharmaceutical formulations as early as possible in the development process, thereby enabling remedies to be applied. It is important that such methods be effective enough to predict even slow rates of degradant formation, yet remain accurate enough that relevant degradation problems are addressed.

In this review, we examine a number of methods in use for accelerating chemical degradation with a special emphasis on recent advances in the field. Other reviews previously published largely predate the advent of highly sensitive assays (Pope, 1980a, 1980b; Parikh, 1981; Stewart and Tucker, 1984a, 1984b, 1985a, 1985b; Witthaus, 1981; Meunier, 1981). More recent reviews have addressed accelerated stability testing in biological products, hence this field is only briefly mentioned in the present review (Franks, 1994; Young, 1990). Most of the previously reviewed work involved loss of potency of drug rather than formation of low levels of degradant products. Current formulation development and expiry determination is more often concerned with the formation of specific degradants (Darrington and Jiao, 2004). Though the basic science behind current methodology has not changed significantly, the methods available for predicting stability have evolved over the years as will be discussed in this review.

Another type of stability important for pharmaceutical dosage forms is physical stability. In this review, physical stability is only discussed with respect to its effects on chemical stability; however, accelerated aging for prediction of physical stability has been reviewed in the literature (Stewart and Tucker, 1985c).

In assessing the stability of drugs, the use of multiple methods can help in determining the mechanism of degradation. Learning the degradation mechanism can, in turn, be helpful in the design of stabilization methods or, potentially in redesign of the active pharmaceutical ingredient (API).

In this review, we discuss various options for accelerated study of product formation from drug chemical degradation. These options are not necessarily the same as those used for regulatory testing to set expiry dating. Instead, this review focuses on options for getting an early stability assessment and providing some mechanistic understanding to help in taking remedial actions to rapidly develop a prototype commercial dosage form. This manuscript first describes the general use of thermal acceleration methods, which are broadly applicable to many degradation mechanisms. Some of the specific major degradation mechanisms common with active pharmaceutical ingredients are then discussed in greater detail. Since the type of accelerated aging used will often depend on the specific dosage form, these are separated out for each mechanism as appropriate. Finally, photostability and estimation of stability in packaged products are reviewed.

2. Thermal methodologies

Accelerated aging traditionally involves use of temperature increases to speed reactions. The process of estimating ambient stability involves estimating the reaction rate at different temperatures, and then extrapolating to the desired temperature. Both the process determining the reaction rate at a single temperature and the process for extrapolating to a desired temperature are discussed separately below.

2.1. Estimating reaction rates

Chemical stability is generally expressed in terms of a rate constant, k, representing either product formation or drug degradation. In general, these two rates will not be the same except in the special circumstance of a one step reaction where the drug irreversibly degrades to a single product. In current pharmaceutical testing programs, the rate of formation of individual products, independent of whether they are primary or secondary decomposition products of the drug, is the determining factor in setting shelf-life expiry. While drugs commonly decompose to give multiple products, the shelf-life is rarely determined by the overall drug decomposition rate (i.e., the sum of these individual rates), but rather by the rate of formation of individual products (as determined by their toxicity limits). The time dependence of drug degradation will vary, depending on whether or not the drug itself is involved in the rate-determining step of its degradation (zero order if not, first order for most reactions that do depend on the drug concentration). The form of the rate equation is shown below for zero and first order reactions based on formation of product (D_0 is initial drug concentration, P_t is the product concentration at time t; P_0, the initial product concentration is assumed to be zero):

$$\text{zero order}: \quad P_t = kt \tag{1}$$

$$\text{first order}: \quad \ln\left(1 - \frac{P_t}{D_0}\right) = -kt \tag{2}$$

For zero-order reactions (i.e., zero-order in drug), the reaction rate is independent of the drug concentration, while for first order reactions, the rate depends linearly on drug concentration. The logarithmic term of Eq. (2) can be expressed as a series expansion with little error for low conversions; i.e., for total drug conversions of less than 2% as is common in pharmaceutical stability programs, it is possible to ignore higher order terms in the expansion to give the following:

$$\ln(1 - P_t D_0) \approx -P_t D_0 \tag{3}$$

Substituting Eq. (3) into Eq. (2), and assigning k' to equal the rate constant divided by the initial drug concentration gives Eq. (4).

$$\begin{aligned} -P_t D_0 &= -kt \\ P_t &= \left(\frac{k}{D_0}\right) t \\ P_t &= k' t \end{aligned} \tag{4}$$

Comparison between Eqs. (1) and (4) shows that for low conversions typical in pharmaceutical stability programs, both zero and first order processes can be treated as following zero-order kinetics. For first order processes, this is accomplished using the initial linear portion of the data to provide an estimate of the reaction rate. Though this zero-order approximation provides a rate of reaction for the initial product formation, for reactions that are indeed first order in the

drug, the zero-order rate constant determined by initial rates will depend on the initial drug concentration. With a zero-order reaction, however, this rate constant will be independent of the initial concentration. Using this distinction can be helpful in elucidating the mechanism of a degradation process. This is most useful in solution, where drug concentrations can be varied to determine the reaction dependency.

Obtaining rate constants based on experimental data at a given temperature is most often accomplished by fitting the data using linear least squares methods. This method can provide acceptable rate constants, especially in liquid dosage forms. With solid dosage forms, better predictability is reported using median-based robust regression methods, especially when there are outliers (Cabiglioli et al., 1996). This is partially achieved by taking replicates into account. It has also been noted that since least square fitting (linear regression) generally relates the drug concentration at a given time to the initial concentration of drug, the error associated with the initial drug concentration gets multiple weightings. Fitting of data using nonlinear regression, factoring in confidence intervals, has been proposed to better account for the errors associated with the initial drug concentration (Ebel et al., 1989). Confidence intervals become important since there is a coupling of the uncertainty of the prediction due to both fitting for the rate constants at each temperature and fitting of the temperature dependence for extrapolation to storage temperatures.

2.2. Temperature dependence

Reaction rates will follow Arrhenius kinetics (Eq. (5)) for the majority of products generated by drug degradation. Arrhenius kinetics is a linear dependence of the natural logarithm of the reaction rate, k, versus the reciprocal of the absolute temperature T (R is the gas constant and A is an indication of the entropy of activation for the process).

$$\ln k = \ln A - \frac{E_a}{RT} \tag{5}$$

This can also be expressed as:

$$k_T = k' \exp\left[\frac{E_a}{R(1/T' - 1/T)}\right] \tag{6}$$

Here, k_T is the degradation rate at temperature T, and k' is the degradation rate at temperature T' (usually the desired temperature for prediction). The rate constant equals the amount of product formed per unit time, assuming low conversions.

In practice, many systems do not show Arrhenius behavior, at least over a wide temperature range. The following are a list of some of the major reasons for non-linearity in Arrhenius plots:

1. Phase transitions: If a phase change (e.g., melt, glass transition, vaporization) occurs in the temperature range studied, there can be a discontinuity (see for example Duddu and DalMonte, 1997; Jans-Frontini and Mielck, 1996; Duddu and Weller, 1996). Similarly, as temperature shifts, the solubility of a drug or reactive species in a solvent or excipient can change. For example, the solubility of oxygen in excipients (solvents) tends to decrease with increasing temperature. This will also be true with volatile reactive species. In suspensions, changes in the amount of material dissolved in the solvent can have dramatic effects on reaction rates.
2. pH shifts: As temperature changes, the pH of a solution can change even with buffers present (Bates, 1962). In fact, even with an adequate buffer capacity, the pH of a buffer itself can change as a result of a change in pK_a values for the buffer with temperature as well as shifts in the autoprotolysis constant K_w (Connors, 1982). This shift can alter the rate of reaction in a non-Arrhenius fashion.
3. Uncontrolled relative humidity: Relative humidity changes as a function of temperature can significantly impact the linearity of Arrhenius plots for solid dosage forms. This is further discussed in Section 2.4.
4. Complex reaction mechanisms: For multi-step reaction pathways, the overall reaction rate is dependent directly on the rate-determining step as well as indirectly on earlier steps. As temperature changes, different activation energies (slopes) and pre-exponential terms (intercepts) for these steps can lead to non-Arrhenius behavior of product formation, even if each individual rate constant shows Arrhenius behavior. For example, in the case of recombinant bovine granulocyte-colony stimulating factor, a reversible equilibrium between the native protein and an intermediate state is established,

followed by irreversible aggregation (Roberts, 2003). Temperature changes affect the equilibrium (i.e., the rate constants for the forward and backwards reactions) with different Arrhenius parameters than that for the rate-determining step. The consequence is an overall non-Arrhenius temperature behavior for product formation, though the individual steps appear to behave in an Arrhenius fashion. In the extreme case, a switch in the rate-determining step or shift of reaction pathway to the product can occur. In the case of multiple pathways to a single product, where one pathway dominates at low temperature while another dominates at high temperature, the predictions based on the high temperature behavior will always underestimate the instability, since the high temperature slope will be steeper than the low temperature slope. Since the failure in this case is for an underestimate of a stability issue, this possibility can be a very significant issue in predicting shelf-life. For this reason it is advisable to always maintain a low temperature (∼25 °C) sample to ensure a complete understanding of the temperature dependent profile.

5. Change in Arrhenius parameters with temperature. Although the Arrhenius parameters are generally assumed to be temperature independent over the narrow temperature ranges typically used in accelerated aging studies, they will in fact vary with temperature based on any heat capacity change on going to the activated complex (Vyazovkin, 1999; Wold and Exner, 1973). This factor should be considered especially when using a wide temperature range in accelerated aging studies.

Characteristically, some systems show abrupt changes in their Arrhenius curves at specific temperatures (at a phase transition, for example), while others show more continuous curvature; for example for complex reactions with different temperature dependence for different steps. In the former case, data above the critical temperature for the discontinuity is essentially useless for predictions of lower temperatures and should not be included in extrapolations. For most phase transitions, the curvature in the Arrhenius plot gives higher than expected reaction rates at high temperatures, while most complex reaction mechanisms give lower than expected reaction rates at high temperature.

To take into account the non-linear Arrhenius behavior, in many cases, it is possible to use the modified Arrhenius relationship shown in Eq. (7) (IUPAC, 1996) to better fit the data:

$$k = AT^n \, \mathrm{e}^{-E_\mathrm{a}/RT} \tag{7}$$

where A, n and E_a are parameters determined using nonlinear fitting programs ($0 < n < 1$). This equation can be mathematically simplified to give Eq. (8), which is generally easier to use fitting programs to solve:

$$\ln k = \alpha - \beta \ln m - \gamma m \tag{8}$$

Here, m equals $1/T$, and α, β and γ are fitted parameters based on a linear least square fit to the experimental data ($0 < \beta < 1$). It should be noted that for kinetics that indeed follow the Arrhenius equation, β goes to zero, and a linear relation is determined between $\ln k$ and $1/T$. Use of this equation allows for better general predictions of room-temperature stability (Herberger et al., 1987) since some level of curvature in the Arrhenius plot can be accounted for. As would be expected, the number of temperature points must be sufficient to allow fitting to Eq. (8) (greater than three). Other modified Arrhenius relationships have also been used successfully with pharmaceutical systems to improve predictions (Ertel and Carstensen, 1990).

2.3. Non-isothermal accelerated aging

Traditionally, accelerated pharmaceutical stability studies are carried out at a set of fixed temperatures. The data provided by this isothermal method can then be used in an Arrhenius (Eq. (6)) or modified Arrhenius (Eq. (8)) fashion to predict ambient stability. Because the number of temperatures generally used in pharmaceutical investigations is small, there can be considerable error associated with the temperature extrapolations. To counter this, long aging times are often employed, especially at temperatures close to ambient. By lowering the error bars associated with the lower temperature degradation rates, the confidence intervals for the final extrapolated ambient rates are reduced. Unfortunately, the time involved in such predictions can be considerable. To narrow the confidence intervals in the same aging time, non-isothermal methods (Zhan et al., 1997; Yoshioka et al., 1987; Tucker, 1985; Crespo and Alvarez, 1985; Hempenstall et al., 1983;

Tucker and Owen, 1982), where samples are placed in temperature ramping ovens and pulled at various time points have been suggested. Conceptually, the tightest confidence intervals for the final prediction occur with more data under the lowest temperature conditions such that extrapolation is minimized. At higher temperatures, the conversion percentage as a function of time increases such that there are tighter degradant level confidence intervals; however, the extrapolation distance is greater, effectively expanding those confidence intervals based on the extrapolation distance. To more evenly balance the effects of rates and confidence intervals, it has been shown that an exponential heating process, which increases the time a sample spends in the low temperature range relative to the higher temperatures, provides greater accuracy and precision for room-temperature extrapolations than other non-isothermal heating protocols (Zhan et al., 1997). The general form of this temperature profile is shown in Eq. (9),

$$T = T_0 - 10 \ln \left[\frac{1 - t/t_{\text{final}}(1 - d^{-\Delta T/10})}{\ln d} \right] \quad (9)$$

where T is the temperature at time t, T_0 the initial temperature, t_{final} the experiment duration, ΔT the temperature range of the experiment and d is a factor between 1 and 4. The d-factor is based on the activation energy for the process, with higher d-values indicated for higher activation energies. If the activation energy is not known, exponential heating can still provide a reasonable process for conducting accelerated aging. One can assume a value of 2 for d (i.e., doubling the reaction rate with every 10 °C increase in temperature). The data from an exponential, non-isothermal heating experiment can then be analyzed replacing temperature with an integral of the temperature profile with respect to time. Arrhenius kinetics, for example, can be expressed as shown in Eqs. (10) and (11) (assuming zero order kinetics and a d-factor for the heating program of 2).

$$\Delta c = k' \int_0^t \exp \left[\lambda \left(\frac{1}{T'} - \frac{1}{x(t)} \right) \right] \mathrm{d}t \quad (10)$$

$$x(t) = T' - 14.43 \ln \left[1 - \frac{t}{t_{\text{final}}(1 - 2^{-\Delta T/10})} \right] \quad (11)$$

where t is the time for the individual pull points, k' is the rate constant at temperature T' (k' is determined as part of the fitting program) and λ is a fitted parameter equal to E_a/R. Solving this equation requires assigning a temperature for which a rate is desired (e.g., 25 °C), assuming a value for λ, numerically solving the integral (e.g., by Simpson's integration) at each time point, then performing a linear, least squares fit of the data (i.e., Δc versus the integral). This is iterated over values of λ until the best correlation is achieved.

Non-isothermal aging allows for samples to experience effectively many temperature/time points from a single oven by pulling samples at multiple time points during a heat cycle. The disadvantage of this approach is that an oven is dedicated to a single experimental set for the duration of a program. This in turn requires a multitude of ovens for multiple concurrent stability programs.

2.4. Humidity

Humidity can have a significant effect on solid drug substances or drug products, even for reactions which themselves do not involve water. Among the effects are changes in the drug form (such as hydrate formation) and plasticization (dissolution) of drug or excipients. Plasticization, where water acts to lower the glass transition of a material, can lead to a significant increase in mobility and corresponding reactivity in solid dosage forms. The role of moisture in causing physical changes (as well as its role as a reactive species) are related to the water activity of the system rather than the moisture content of either the dosage form or the surrounding air. Water activity is a thermodynamic term referring to the equilibrium relative humidity (ERH) over a sample. At equilibrium, the water activity of a sample is equal to the ERH of the air surrounding it. ERH represents the moisture content relative to the saturated moisture content at that temperature, which is defined as ERH = 100% (water activity = 1). As one increases temperature, the amount of water in the air for a given relative humidity increases; however, the activity of the water, that is its ability to affect reactivity, permeation and plasticization, depends only on this relative humidity, not the absolute humidity. Though accelerated aging predictions will generally correlate significantly better using ERH (water activity) than

with total water content in the dosage form or absolute humidity in the environment (Heidemann and Jarosz, 1991), correlations are sometimes seen between water content and drug stability using the Carstensen equation (see for example Matsunaga et al., 1993). In this report, a plot of the logarithm of the degradation rate versus the logarithm of the water content is linear with the slope being an interaction term between water content and reaction rate (Carstensen et al., 1966). In reality, drug product water content will directly correlate with the degradation rate only when the water content correlates directly with the water activity. This lack of correlation can be seen, for example, with a lyophile of vecuronium bromide where the degradation rate correlated with the water activity but only roughly with water content (especially when comparing different formulations) (Vromans and Schalks, 1994). Since water activity is in principle feasible to measure, this would be the preferred method; especially until a correlation between water activity and water content is established.

For solid samples open to the environment (e.g., open bottles in stability chambers), the ERH will equal the RH of the chamber. For packaged systems, transport of moisture to the dosage form (or formulation) can be rate limiting. Water activity in the package will depend on the amount of initial water associated with each component, the initial packaging relative humidity, the relative vapor sorptions of the drug and various excipients, as predicted using the SDMT model (Zografi et al., 1988; Kontny, 1988), the water permeability of the package and the RH of the external environment. With more hygroscopic excipients, equilibrium is often reached more slowly; that is, the water activity can be lower within the package than with less hygroscopic excipients present. This can result in the counterintuitive situation where more hygroscopic excipients stabilize moisture-sensitive drugs (Heidemann and Jarosz, 1991). In contrast, some excipients can bring moisture into closed packages thereby increasing the water activity (relative humidity) and decreasing the drug stability (Patel et al., 1988). When doing accelerated aging predictions in such water-transport limited systems, it is important to understand how the water activity varies with different conditions. For example, at higher temperatures in closed systems, water activity can increase due to water desorption from drug or excipients, or decrease due to the lower activity of a fixed concentration of water as temperature increases (decreased relative humidity for the same absolute humidity). One method of studying the change in water activity in closed systems, as a function of temperature is to use small, self-contained, battery operated humidity meters (such as those produced by Microtek Corporation). These meters can be packaged into bottles with a dosage form, stored over extended periods, and then removed. Upon removal, the thermal and humidity history can be downloaded directly onto a computer.

Even in liquid dosage forms, water activity can affect reaction kinetics. The water activity of aqueous solutions is affected by the overall concentration of species in solution. Since this water activity can change with temperature (based on solubility changes), reaction rates can be affected in a non-Arrhenius fashion. Though not reported in the pharmaceutical literature, we would suggest that it should be possible, by measuring water activity as a function of temperature, to explicitly correct for the water activity changes in accelerated aging studies.

In conducting accelerated aging studies on solid samples, it is important to know how high in relative humidity one should go before the rates become non-predictive for extrapolation to relevant relative humidities. This can be done by first determining the critical relative humidity (CRH) of a system (Kontny and Zografi, 1995), that is, the relative humidity above which moisture will start to dissolve some of the components in the formulation (deliquesce). Since ambient storage of formulations and dosage forms is typically below their CRH values, accelerated aging studies above this value will often not extrapolate to product storage conditions. This is the case because below the CRH, the water activity in the sample will equilibrate to the water activity in the air surrounding the sample, while above the CRH, the sample will pick up water to dissolve material until the solution activity can match the surroundings. For conditions above the CRH, water vapor and heat transport can be rate-limiting processes rather than the reaction kinetics themselves (Kontny and Zografi, 1985).

The CRH of a sample can be measured using commercial instrumentation such as AquaLab® water activity meters (available from Decagon Devices Inc., Pullman, WA). Another method of determining the CRH for a drug or formulation is to prepare a saturated aqueous slurry of the material. The ERH of a closed

system over the slurry will equal the CRH. When using a formulation, as opposed to a drug alone, care must be taken that no single component is below its saturation point. A technique that provides similar values but can be carried out with smaller sample volumes and shorter run times is isothermal microcalorimetry (Jacobsen et al., 1997). Another indirect technique especially useful for mixtures of solids involves extrapolating the water sorption rate of the material as a function of high relative humidities back to a zero sorption rate (Kontny and Zografi, 1985). Any of these techniques can also be applied to determine the CRH at different temperatures. It should be noted that solubility changes as a function of temperature can lead to significant changes in the CRH value of a system as the temperature changes.

Solid formulations can be homogeneous (for example, some lyophiles), or more commonly, heterogeneous. When all components of a formulation interact only minimally, one would anticipate that the highest relative humidity appropriate for use in accelerated aging would be the CRH equal to that of the lowest value for the individual components or even just that for the drug alone. As an aid to determining the maximum predictive storage humidity for accelerated aging of solid dosage forms, the CRH values for a number of common pharmaceutical excipients are listed in Table 2. For chemically interacting components, CRH values can be estimated as the product of the individual CRH values (expressed as fractions) (Ross, 1975).

Once the CRH is determined, various humidities below the CRH can be examined for their effect on the drug degradant formation rate. Knowing the sensitivity of a system to the RH can help in making accurate predictions to ambient conditions. For example, a highly moisture sensitive drug product can show very different 20 °C degradation rates at 20% RH versus 60% RH. Knowing this sensitivity can also help in the design of packaging or justify the use of desiccants (Badawy et al., 2001). Allowing free equilibration of a formulation to a range of controlled RH environments permits the determination of the RH sensitivity. Salt slurries can be used to provide a range of humidities as listed in Table 3, or one can use commercially available humidity controlled chambers.

Even below the CRH for a bulk drug or excipient, moisture can affect (partially dissolve) the surface of particles, presumably due to the presence of high-energy molecules at the surface (Kontny et al., 1987).

Table 2
Critical relative humidity values for a number of common excipients (Waterman and MacDonald, unpublished data)

Excipient	CRH (%) at 20 °C	CRH (%) at 40 °C
Dextrose	100	88
Fructose	72	64
Lactose	100	100
Mannitol	100	100
Sorbitol	80	69
Sucrose	86	83
Xylitol	91	73
Ascorbic acid	100	98
Fumaric acid	100	100
Tartaric acid	84.5	78
Calcium chloride	29	21
Potassium chloride	84	82
Potassium sulfate	97	97
Sodium chloride	75	75
Sodium citrate	60.5	78
Hydroxypropylcellulose	100	100
Polyethylene glycol (3350)	94	85
Polyvinylpyrollidone	100	100
Hydroxypropylmethyl cellulose	100	100
Polyethylene oxide	100	96
Sodium carboxymethylcellulose	84	83.5
Hydroxyethyl cellulose	93	91
Pluronic F127	96	99
Pluronic F87	88	92.5

The importance of this effect on a given system will depend on a number of properties of the material including solubility, surface area and presence of defects and amorphous regions. This effect can account for some highly humidity dependent processes even for samples studied below their CRH values.

For a range of solid formulations below the CRH, the effect of relative humidity on the Arrhenius behav-

Table 3
Salts for control of relative humidity in accelerated aging studies (Marsh, 1987; ASTM, 1991; Greenspan, 1977)

Salt	% Relative humidity over slurry at indicated temperature				
	20 °C	30 °C	40 °C	50 °C	70 °C
LiCl	11.3	11.3	11.2	11.1	10.8
$MgCl_2$	33.1	32.4	31.6	30.5	27.8
K_2CO_3	43.2	43.2			
$Mg(NO_3)_2$	54.4	51.4	48.4	45.4	
NaCl	75.3	75.1			
KCl	85.1	83.6	82.3	81.2	79.2
K_2SO_4	97.9	97.0	96.4	95.8	

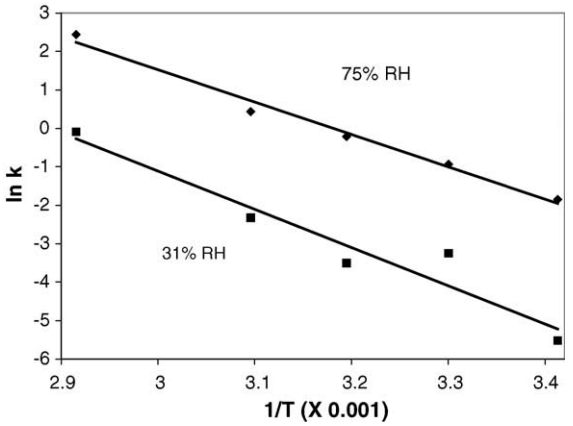

Fig. 1. Rate of formation of dehydroascorbic acid (k) from commercial Vitamin C tablets as a function of temperature and relative humidity (Waterman, 2004).

ior for the kinetics of degradant formation is largely on the intercept rather than the slope (Waterman, 2004). This can be seen in Figs. 1 and 2 for an oxidation and a hydrolysis, respectively. That the relative humidity effects are largely on the intercepts suggests that the relative humidity, at least in a number of cases, does not change the degradation mechanisms, but rather affects the collision frequency. The collision frequency in turn is dependent on the mobility of the species involved. This mobility dependence results in a linear relationship between the relative humidity

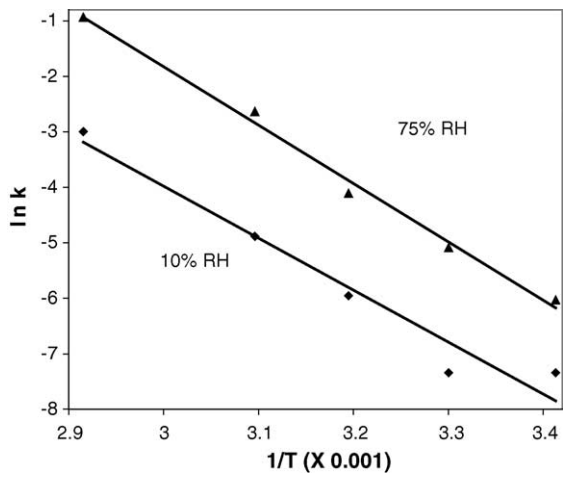

Fig. 2. Rate of formation of salicylic acid (k) from commercial aspirin tablets as a function of temperature and relative humidity (Waterman, 2004).

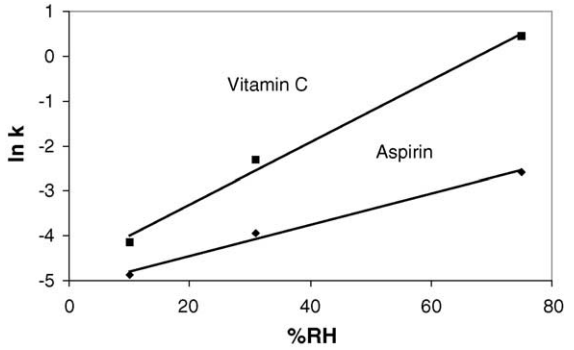

Fig. 3. Rates of product formation for Vitamin C and aspirin degradation at 50 °C as a function of relative humidity (Waterman, 2004).

and the logarithm of the degradation rate (product formation rate) (Waterman, 2004). This can be seen in a number of cases as shown in Figs. 3 and 4, as well as other literature examples (Tripet and Kesselring, 1975; Plotkowiak, 1989; Hladon and Cwiertnia, 1999). The effect of relative humidity on reaction rates can differ significantly from this general pattern when moisture induces physical changes in a system (Shalaev and Zografi, 1996). For example, in lyophilized formulations of methylprednisolone, moisture induced plasticization of amorphous drug (decreasing the glass transition temperature) was found to increase the molecular mobility and thereby the hydrolysis rate for the drug (Herman et al., 1994). Moisture induced plasticization of polymeric excipients can also lead to phase transitions at elevated temperature conditions.

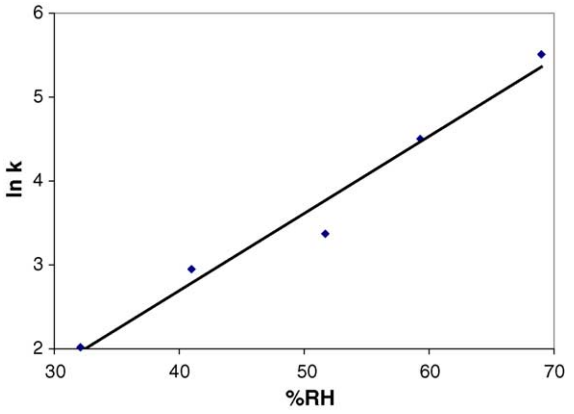

Fig. 4. Hydrolysis of nitrazepam as a function of relative humidity (Genton and Kesselring, 1977).

Such transitions can provide non-predictive stability in extrapolations to lower temperatures. For example, elevated temperature aging combined with high humidity resulted in exceeding the glass transition temperature of the excipient polyvinylpyrrolidone in a tablet formulation, which did not transfer to conditions below that temperature/humidity, and were therefore non-predictive (Fitzpatrick et al., 2002). In some cases the mobility increase due to moisture and temperature can allow a drug to react with an excipient that it otherwise might remain separated from and unreactive. For example, reaction of ibuprofen with bases in the solid state was found to be highly moisture sensitive (Byrn et al., 2001). In each of these cases, high humidity conditions caused an overestimate of the rate of degradant formation from the rate actually observed at ambient humidities.

When accelerated conditions of humidity and temperature are used with drugs, formulations and dosage forms, caution must be observed that there are no form changes across the conditions used. In some cases, differential scanning calorimetry (DSC) or other calorimetric techniques can be useful to study temperature effects, though coupling humidity effects may require specialized instrumentation. For the drug, hydration or dehydration can result in the formation of new crystal structures or loss of crystallinity, both of which can result in a change in stability. Importantly, these changes may only occur under accelerated conditions and therefore be non-predictive of issues under ambient conditions. For example, cefixime trihydrate loses its water of crystallization, first to give a disordered crystal, then to give amorphous material upon further dehydration (Kitamura et al., 1990). These changes result in greater hydrolysis rates under low humidity, where drug converts to a less stable amorphous form, than at high humidity, where crystals remain intact.

While in the preceding sections, general methods for accelerated aging were reviewed, in the following sections, more specific discussion is included about a number of common pharmaceutical degradation mechanisms.

3. Hydrolysis

Hydrolytic reactions are among the most common processes for drug degradation (Waterman et al., 2002a). In addition to rate dependencies on the temperature and moisture, hydrolysis rates can depend on the concentration of catalytic species, usually acids or bases. With many hydrolytic reactions, such as those involving esters and aryl carbamates, the reactions are reversible, i.e., degradation products can react to reform the drug (March, 1992). The result is that at high drug conversions, the reaction rate may slow due to accumulation of product; i.e., the back reaction becomes significant as the products accumulate in an approach to equilibrium. It is therefore generally advisable, for purposes of predicting drug stability, to only carry reactions out to low conversions (formation of less than 10% of a degradant, or more preferably, less than 2% of a degradant). Unfortunately, even with low drug conversions, the reaction may still approach an equilibrium value; that is, a 10% conversion could represent complete reaction. Although in theory one could measure the forward and backward reaction rate constants, in practice, most often conversions are limited to levels consistent with the expiry-limiting degradant level (generally below 1%). In solid systems, local high product concentrations can result in product accumulation effects even with low overall conversions. Without the diluting effect of the solvent, the equilibrium position of a hydrolysis can shift to the starting material. The overall result can be that a hydrolysis that goes to high conversions in solution (where the drug product is dilute and shifts the equilibrium to the product direction) goes only to low conversions in the solid (where the high product concentrations shift the equilibrium towards the reactants). Under these conditions, it may be necessary to use a more complex kinetic model than the zero-order approximation discussed for low conversions. More specifically, under conditions where the back reaction becomes significant, the back reaction's dependence will be second order (i.e., first order in each product), while the forward reaction is only first order in drug. This second order back reaction explains the high dependence of the equilibrium position (which represents the competition between the two rates) on the drug concentration. An exception to this is with internally cleaved hydrolytic reactions as seen with lactones and lactams. With these species, the extent of reaction remains the same in solid and solution, assuming similar effective pH environments.

3.1. Aqueous solutions

For many aqueous drug solutions, pH-stability profiles are generated to determine the pH of maximum stability (Carstensen, 2000). Using a pH where a drug is unstable to predict quantitatively how fast a degradant will form at a pH where the drug is more stable requires fitting the pH profile to a typically parabolic curve shape, then extrapolating to the desired pH. In some cases, such an analysis can provide an understanding of mechanistic factors involved in the degradant formation. For example, 4-dedimethylaminosancycline was studied over a pH range of 2–10, with such results able to be rationalized in terms of the degradation of various ionized forms and base catalysis (Pinsuwan et al., 1999). Because of the sensitivity to catalysis, however, pH rate profiles can involve discontinuities, which can lead to erroneous predictions (Mirrlees and Taylor, 1994). In addition, since many aqueous drug solutions are buffered, intentionally changing the pH for accelerated aging will require exceeding a buffer capacity, which increases ionic strength, another factor in the rate of some drug degradation.

Products of hydrolysis can sometimes affect the hydrolysis rate itself by catalyzing further drug degradation, usually in unbuffered systems. For example, ester drugs can hydrolyze to give acidic products, thereby lowering the pH of the solution once the buffer capacity is exceeded. This was seen, for example with the drug lonapalene (Powell et al., 1988). In this case, the initial pH dropped from 6–7 to 4–5 during the course of the reaction. Since we are generally most interested in the formation of drug degradants, it is possible to minimize the effect of such pH changes by carrying out accelerated aging only to produce only low levels of such products.

For reversible drug hydrolysis, re-equilibration between the drug and its hydrolyzed form can result from changes to the system external to the drug itself, such as a change in pH or co-solvent ratio (by evaporation, for example). For example, equilibria between lactone and ring-opened acid moieties on drugs depend heavily on pH and the presence of co-solvents as is observed with canrenoic acid (Garrett and Won, 1971) and atorvastatin (Kearney et al., 1993). This thermodynamic control of drug degradant formation is different from irreversible reactivity controlled by catalysis, at least in some cases. For example, shifts in pH with temperature may result in a product formation, which will disappear when the temperature is reduced. The analytical results can therefore depend on whether samples are monitored in situ or worked up back at ambient temperatures.

In some cases the drug itself can act as a catalyst in its own decomposition such that the reaction kinetics will depend on the drug concentration. This has been observed with ceftazidime, which acts as a general base in catalyzing its own hydrolysis (Fubara and Notari, 1998). This example emphasizes both the importance of using drug concentrations similar to those of the ultimate drug product and again of carrying out reactions to only low conversions.

Although the solution pH generally has a significant impact on drug hydrolyses, one should also note that general acid or base catalysis (buffer catalysis) can also be significant (Carstensen, 2000). Generally, the implications of this are that buffer concentrations must be carefully controlled during accelerated aging studies to assure predictive results, though mathematical models have been developed that can help interpret pH and buffer catalysis for stability (Can der Houwen et al., 1994). Such models can allow specifications to be set on catalysts levels for such products.

Temperature changes during accelerated aging can alter solution pH, leading to non-Arrhenius behavior (Newton and Miller, 1987). It is therefore useful to measure the solution pH over the desired temperature range to assure the pH remains constant. Alternatively, one could use a computer model that simulates observed changes in pH with temperature based on enthalpies for dissociation (Kipp and Schuck, 1995). Without pH shifts, high temperature accelerated aging has been successful as seen, for example, with indomethacin and its glycolamide ester (Arrhenius hydrolysis up to 70 °C in various buffer solutions (Chiba et al., 1992; Kahns et al., 1989)) and adenosine triphosphate, ATP (Arrhenius hydrolysis up to 80 °C (Seki and Hayashi, 1982)).

When carrying out accelerated aging processes in solution, it is important to distinguish between degradation products that remain in solution and those that precipitate from the solution, at least for reversible processes. In the former case, the kinetics are relatively straightforward; however, in the latter case, matters can become quite complex. For example, if the degradant

solubility depends on the temperature, accelerated aging by elevating temperature may not be predictive. Temperature increases that increase drug degradant solubility may not adequately account for the increased driving force associated with drug degradant precipitation. Consequently, this case could involve an underrepresentation of the actual ambient rate of formation of a drug degradant.

Although accelerated hydrolysis in solution generally entails using HPLC analysis to study the rate of product formation, it is also possible to study the overall reaction rate using microcalorimetry (Angberg et al., 1990). Since the heat flow is proportional to the rate of a reaction and the heat evolved in the process (assuming an exothermic reaction), this technique can provide a rapid assessment of the reaction kinetics. However, since multiple reaction pathways still give a single heating curve which can be difficult to deconvolute, use of microcalorimetry is best suited to drug degradation processes that go through a single pathway. Increasing the drug concentration increases the heat generated, and thus the sensitivity. One can measure the heat flow at a given time, or use the total amount of heat evolved during a fixed time interval. This method was used in model studies with aspirin (Beezer et al., 1999) and meclofenoxate hydrochloride (Otsuka et al., 1994), where the hydrolysis was successfully measured as a function of pH and temperature.

3.2. Non-aqueous solutions

For some non-aqueous solution formulations, water can theoretically be a limiting reactant. As such, one would anticipate that addition of water can be an effective means of accelerating such hydrolytic reactions. To use this as a predictive tool, it is first important to establish the moisture content (or range of moisture contents) expected in the system when prepared in a process similar to the one anticipated for commercial production of the dosage form. The second step is to determine the maximum level of water the solvent system can contain before phase separation occurs. This level can range from very low for oils to completely miscible for alcohols and glycols. To measure water solubility in oils, Karl Fischer techniques can be used on the solvent after stirring with excess water then separating the oil layer out (Acker and Frediani, 1945). It should be noted that the presence of solutes in the solution may alter the saturated moisture content. Intentional addition of water to non-aqueous drug solutions will generally accelerate hydrolytic reactions linearly with the concentration of unbound water in solution. To use this method, one can measure stability with two or more levels of water up to the saturation level of water in the solvent then extrapolate back to the moisture level present in the system without added water. Obviously, if there is no water in the native system, hydrolytic reactions are not possible and adding water will not represent a valid prediction method. One must be careful not to add more water than is phase-compatible with the solvent system since that water will not be reactive in a linear fashion as a function of overall water concentration. It should also be possible, in principle, to couple the thermal methods with added moisture using a linear relationship for the water concentration and a logarithmic (Arrhenius) relationship for the temperature. This may be especially appealing for systems where the amount of water in the solvent can be increased at higher temperatures.

3.3. Aqueous and non-aqueous suspensions and emulsions

For drug suspensions and emulsions, the situation is complicated by the nature of a two-phase (solid drug and solution or drug in oil solution and aqueous suspending fluid) system. In many cases, the hydrolysis rate of the drug in aqueous solution will be orders of magnitude higher than that in the solid or oil phase. For this reason, the amount of drug in aqueous solution can be the major factor in determining the rate of hydrolysis. For example, for the diterpenoid forskolin, the drug partitions partially into the oil phase in an oil-in-water emulsion, thereby providing corresponding stabilization in direct relation to its partition ratio (Yamamura et al., 1991). Acceleration of drug instability to hydrolysis for such systems requires separating effects of any changed conditions into increases in the aqueous solubility of the drug versus acceleration of the reaction kinetics. For example, an increase in temperature may lead to an increase in drug solubility, which in turn can lead to a greater drug conversion rate (even with the same rate constant for the hydrolysis). Solubility will generally vary according to either (or both) the van't

Hoff equation (Eq. (12)):

$$\ln \mathrm{Sol}_T = -\frac{\Delta H}{RT} + \text{constant} \quad (12)$$

or the Hildebrand equation (Eq. (13)):

$$\ln \mathrm{Sol}_T = \left(-\frac{\Delta H}{RT_\mathrm{m}}\right) \ln T + \text{constant} \quad (13)$$

where Sol_T is the solubility at temperature T, ΔH the heat of fusion, T_m the melting temperature and R is the gas constant (see for example Yu et al., 1994). By plotting either $\ln \mathrm{Sol}_T$ versus $1/T$ or versus $\ln T$, a line should be obtained which allows the temperature effects of solubility to be explicitly accounted for at any temperature (Tingstad et al., 1973). In some cases, the ideal solubility relationship described in Eqs. (12) and (13) does not apply due either to multiple melting temperatures of the solid or changes in heat capacities with temperature (e.g., Prankerd and McKeown, 1990; Grant et al., 1984). For systems showing temperature dependent solubility, this factor should be explicitly accounted for in accelerated aging studies. For oil-in-water emulsions, the partitioning between the water and oil phases will depend on the relative solubility of the drug in each phase, and how this changes with temperature.

For suspensions in non-aqueous media, many of the considerations discussed for non-aqueous solutions should also apply. For example, any added water used for accelerating the reaction should remain in the solvent phase. In addition, added moisture can affect the drug solubility in addition to accelerating hydrolysis reactions. By measuring the drug solubility in the solvent as a function of water concentration, it is in theory possible to separate the terms and provide a better prediction of "native" system stability.

3.4. Solids

A rapid prediction of ambient rates of hydrolysis for susceptible drugs in the solid-state is one of the more challenging problems in pharmaceutical science. Since the stability of moisture-sensitive drugs depends both directly and indirectly on the moisture content, hydrolytic reactions can involve a complex combination of rates (and temperature dependencies). The result of this complex dependence is that it can be difficult to a priori deconvolute humidity effects. A nonlinear model has been developed for determining the kinetic parameters of decomposition as a function of both temperature and relative humidity, below the CRH (Yoshioka and Carstensen, 1990). Deconvoluting humidity and temperature can also be accomplished by varying each independently.

Attempts have been made to use drug slurries to accelerate the reaction rate of solid-state hydrolyses; however, slurries can be problematic for prediction of solid-state stability due to pH effects, which may dominate in solution, while in contrast, mobility often dominates in the solid-state (Po et al., 1983). Also, as indicated before, the drug solubility itself may be a dominant factor in slurries (suspensions). Although this method can be applied to gain some mechanistic insight into a drug hydrolysis, it is not easily used for quantitative predictions of drug stability.

4. Oxidation

Oxidative degradation of pharmaceuticals can broadly be divided into two types: reaction with molecular oxygen, and reaction with other oxidizing agents present in the formulation. Though these can in general be distinguished by the dependence of the degradation on the presence of oxygen, in some cases oxidizing agents are generated from oxygen-derived decomposition of excipients. For oxidations derived from excipient impurities (e.g., peroxides), accelerated stability follows the pattern discussed in Section 5.

Accelerated aging of oxygen-dependent drug decomposition can be complicated due to the nature of the reaction itself. At most oxygen concentrations, the rate-limiting step in the kinetics of drug oxidation is the oxygen-independent initiation process (often associated with impurities) (Waterman et al., 2002b). Only when oxygen concentrations are sufficiently low, or the initiation rate is increased (by increasing temperature or exposure to light, for appropriate systems) does oxygen concentration affect degradation rates. What makes thermal methods of accelerated aging complicated is the fact that oxygen solubility drops with increasing temperature in most systems. For example, Fig. 5 shows the saturated oxygen concentration in water as a function of temperature. As can be seen, a drop in the saturated oxygen concentration in water drops by about a factor of two in going from 25 °C to 70 °C. The

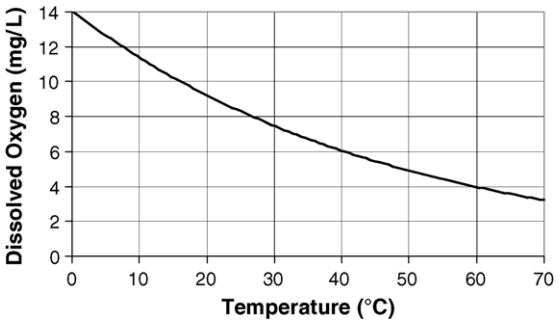

Fig. 5. Effect of temperature on oxygen solubility in water (generated by extrapolation of data from Vesilind, 1996).

presence of most solutes lowers the solubility still more (Franchini et al., 1993). Based on Henry's Law we expect the solubility of oxygen in liquid and solid excipients to similarly decrease with increasing temperature. In cases where the oxygen concentration is still sufficient that the addition of oxygen is not rate-limiting, the kinetics should still follow Arrhenius behavior. For example, the oxidation of 2-mercaptobenzothiazole to its disulfide was studied in buffered solutions up to 90 °C. Arrhenius extrapolation to 25 °C gave a rate constant of $1.35 \times 10^{-5}\,h^{-1}$, which compares well with the observed rate of $1.05 \times 10^{-5}\,h^{-1}$ (Kottke et al., 1984). In cases where oxygen addition becomes rate-limiting, the temperature effect can become distinctly non-Arrhenius. For example, in the degradation of a sulfide-dicarboxylic acid in aqueous solution, the reaction mechanism switches from an oxidative to a non-oxidative degradation pathway between 60 °C and 90 °C (Franchini and Carstensen, 1994). This change is attributed to the decrease in oxygen concentration as the temperature increases. Although it should be possible to correct for the loss of oxygen in a solvent by increasing the total pressure on the system such that the partial oxygen pressure and oxygen solubility remain stable (or at least sufficiently high that the rate-determining step is not oxygen addition), this has yet to be demonstrated in pharmaceutical systems.

The shelf-life of oxidizable drugs can be extended by use of antioxidants. Since many antioxidants are themselves consumed as they act to stabilize the drug, the shelf-life of the drug will depend on the time before the antioxidant is depleted. The overall result for the kinetics is an induction time where there is little to no drug degradant formation, then more rapid degradant formation once the antioxidant is consumed. The induction time and the drug oxidation following the induction period are both theoretically amenable to Arrhenius analysis, but generally with different activation parameters. For accelerated aging, therefore, sufficient time points need to be sampled such that both the induction time and the rate after the induction can be determined. This process was used successfully for example in an accelerated aging study of morphine solutions (Gleditsch and Waaler, 2001).

Since the rates of most pharmaceutical oxidations depend heavily on the rate of initiation, one way of accelerating oxidation is to add known concentrations of standard initiators and extrapolate to zero initiator concentration (Boccardi, 1994). This method was applied to a number of drugs using azobisisobutyronitrile (AIBN). This method is best suited to solutions or lyophiles, since in most heterogeneous solid dosage forms, it is difficult to assure the initiator is in the same phase as the drug.

Another method that has recently been reported to very rapidly assess the stability of drugs in the solid state was the use of nonisothermal differential scanning calorimetry (DSC) (Simon et al., 2004). In this method, the onset temperature for oxidation during DSC was found to correlate with the room temperature stability for a limited set of compounds. Because of the assumptions involved in this method of accelerated aging, it is most useful for rank ordering stabilities, especially of similar drugs or formulations of a single drug.

Oxidation in tablet dosage forms could in theory depend on the tablet hardness or on the presence of coatings since either of these could affect the oxygen penetration rates. In reality, the penetration rate for oxygen through tablets (Felton and Timmins, 2003) or through pharmaceutical coatings is so fast as to make permeation rates unlikely to be rate limiting even as oxidation rates (and oxygen consumption rates) increase with temperature. With hard-shell gelatin and cellulosic capsules, the oxygen permeation rates are reported to be sufficiently slow (Felton et al., 2002) that oxygen depletion inside capsules is possible such that accelerated aging predictions can switch as a function of temperature from oxidation-rate limited to oxygen permeation-rate limited. The result of this switch can be a deviation from linearity in the

Arrhenius plot with high temperature extrapolations underestimating the drug degradant formation rate.

5. Reaction with excipients

Reactivity of drugs with excipients (including co-solvents, sugars or stabilizers) often involve reaction of nucleophilic drugs (e.g., amines, sulfides and phenols) with electrophilic excipients (e.g., esters), or electrophilic drugs (e.g., carboxylic acids, esters, amides and alkyl halides) with nucleophilic excipients (e.g., alcohols). Assuming the reactive excipient is present in molar excess, many of the reactions with drugs will depend linearly on the excipient concentration (pseudo-first order kinetics). In solutions, this can be used to accurately accelerate such reactions by extrapolating reaction rates as a function of the reactive excipient concentration back to the planned formulation excipient concentration.

One of the most common reactions observed between drugs and excipients is the reaction of primary and secondary amine drugs with reducing carbohydrate excipients. Reducing carbohydrates include lactose, fructose, dextrose, glucose and maltose. Non-reducing carbohydrates include mannitol, sucrose and trehalose. This reaction is known as the Maillard reaction and is often observed as a brown color formation in dosage forms (Kumar and Banker, 1994). Although salts of amine drugs would be expected to be less reactive than free amine bases, there is generally sufficient proton exchange, most likely through trace moisture, to initiate the reaction. For example, fluoxetine hydrochloride was found to react in the Maillard reaction (with an Amadori rearrangement) with a number of reducing sugars with the reaction accelerated by moisture (Wirth et al., 1998). Accelerated aging studies of Maillard reactions are often complicated by secondary decomposition of drug-carbohydrate adducts, often to multiple products. The shelf-life can also be limited by the color formation itself rather than the absolute amount of any product formed in the degradation. When the intermediate adduct is detectable, it is sometimes possible to characterize the drug degradation process by following both loss of starting drug, and formation of adducts as a function of time and temperature. From the rate constants and their respective activation parameters, it is sometimes possible to predict room temperature rates for degradant formation. In the case of fluoxitine and lactose, this type of analysis was able to predict the time to reach 0.1% total degradants at room temperature based on extrapolation from temperatures of 75–95 °C (Wirth et al., 1998). When the intermediate is not detectable, one may be forced to follow loss of starting drug, a less sensitive indication of stability as discussed in the introduction. In some cases, the formation of the brown color itself can be used as an assay for degradation (using reflection spectroscopy for solids) (see for example Pellerin et al., 1971).

Excipient impurities and degradants, can react either directly with drugs or act as catalysts for other drug degradation processes, e.g., hydrolysis or oxidation (Waterman et al., 2003). A characteristic of drug degradation by an excipient impurity (generally a low molecular weight electrophile (Waterman et al., 2003)) is a limited extent of reaction based on the impurity level. This generally manifests itself as a rapid reaction rate that depends on the ratio of drug to excipient (lower drug concentration leads to a greater extent of reaction) followed by slower degradation (often by a different pathway). Even in the early stages of formulation development, examining drug stability at several ratios of drug to excipient can provide clues as to the sensitivity of a drug to excipient impurity levels. What makes this problem particularly insidious is that the excipient impurity level may vary from lot to lot or vendor to vendor. In particular, peroxides (especially hydrogen peroxide (Huang et al., 2003)), small aldehydes (especially formaldehyde, as seen for example with polysorbate 80 (Chafetz et al., 1984)) and small carboxylic acids (especially formic acid and formate ion) can be present in many excipients at levels up to several hundred parts per million. Because of the low molecular weight of these reactive impurities, for low dose formulations, unacceptable degradation levels of drug can occur. For example, formaldehyde in polysorbate 80 and PEG 300 were recently shown to react with an experimental drug in a parenteral formulation (Nassar et al., 2004). Caution must also be observed to not over-exaggerate a problem associated with excipient impurities. This can easily happen because of rapid reaction of a drug with an impurity thereby extrapolating to a long-term unacceptable formulation, when in reality, the impurity may be consumed after only a low extent of reaction resulting in adequate long-term stability.

Reaction of drugs with excipient degradation products can in many ways mimic the reaction of drugs with excipient impurities; however, while in the latter case the impurity level will limit the extent of reaction, drug reaction in the former case will continue. Often, a reactive excipient degradant will show a biphasic kinetic plot of drug degradation as a function of time: an initial rapid reaction will occur as accumulated excipient degradant reacts with drug followed by a slower process limited by the rate of the excipient degradant formation. These processes will show a drug to excipient ratio dependence (lower drug concentration gives faster reaction). Because of the biphasic kinetics, the shelf-life of the drug (based on allowable levels of drug degradation or of formation of a degradant) can easily be underestimated. To prevent this, it is desirable, whenever possible, to carry out accelerated aging studies under conditions that produce drug degradation or individual degradant formation to a greater extent than the allowable levels. One should especially use caution when formulating with such excipients as polyethers and polyvinylpyrrolidone. For example, peroxide degradants of polyethylene glycols were found to react with a steroid in a topical formulation (McGinity et al., 1975). In this case, removal of the peroxides led to a decrease in initial reaction, though peroxides eventually reformed. Similarly, peroxides in povidone reacted with raloxifene to generate an *N*-oxide product (Hartauer et al., 2000). Removal of peroxide from the excipient stabilized the drug, but peroxides eventually reform from oxidation of the povidone, depending on storage conditions.

In some cases, the drug reactivity with an excipient degradant can depend on the loss of an excipient stabilizer. In the case of polyethylene glycol, the rate of peroxide formation resulting from an oxidation process depends on the level of antioxidants present (added by the manufacturer), as shown for example in a study on extruded excipient (Crowley et al., 2002). Careful monitoring of the antioxidant level, which varies from lot to lot and depends on the manufacturer, is crucial to successful prediction of ambient stability when using such excipients. In addition, such common antioxidants as butylated hydroxytoluene (BHT) can sublime out of a formulation under accelerated aging (or processing conditions) (Dow Chemical, 2003). When antioxidants are consumed or lost due to sublimation or evaporation, the rate of excipient degradation, and hence drug degradation, can accelerate dramatically. The result from processes that have a limiting amount of a stabilizer is that initial drug degradant formation will be slow, and then increase as the stabilizer is consumed. If the stabilizer loss occurs at a phase transition, a non-Arrhenius temperature dependence can result; i.e., rapid degradation of the drug above the phase transition. The above pitfalls emphasize the general advantages of understanding a drug's degradation mechanism when estimating the shelf-life of that drug in a formulation.

The most common catalytic impurities in excipients are transition metals, acids or bases (Waterman et al., 2003). Metal contaminants are often associated with oxidation reactions (Waterman et al., 2002b; Hovorka and Schöneich, 2001). Acid and base impurities are often associated with hydrolyses (Waterman et al., 2002a) and cyclizations (e.g., lactone or lactam formation). In solution, metal-catalyzed processes can be accelerated by addition of known concentrations of the appropriate metal salts (often copper or iron) and extrapolating the rate of drug degradation back to measured or anticipated metal concentrations as was done in the recent study of a drug candidate (Hong et al., 2004). In the solid state, this is more difficult since the added metal salts are not necessarily in the same chemical phase as the reactive excipient and therefore are not necessarily representative of how the catalyst will affect a drug in the corresponding dosage form.

6. Accelerated aging of protein pharmaceuticals

Whereas with small molecules, the rate of formation of reaction products is the most sensitive indication of drug stability, with proteins, degradation to inactive protein does not always yield isolable reaction products. Consequently, proteins are often assayed on the basis of activity or structure changes (e.g., unfolding or aggregating). Chemical purity is usually assessed using SDS-PAGE or capillary electrophoresis (Wiltfang et al., 1991; Hutterer and Dolnik, 2003). Structural information is often evaluated using circular dichroism, and the extent of folding is assessed using microcalorimetry (Cai and Dass, 2003; Keiderling and Xu, 2002; Kelly and Price, 2000; Boye et al., 1997).

Chemical degradation of proteins is often preceded by a physical change in secondary, tertiary or even quaternary structure. Some temperature-dependent structural changes correspond to phase transitions, potentially detectable using DSC (Lyubarev and Kurganov, 2001; Shnyrov and Zhadan, 2000; Cooper et al., 2001; Lopez and Makhatadze, 2002). Since it can be difficult to extrapolate to low temperatures using data above a phase transition, phase transition temperatures will often represent the upper limit of usable temperatures for accelerated aging. Even below a phase transition, the complexity of the structure of proteins means that many degradation pathways are often present which in some cases can result in deviations from Arrhenius behavior. In spite of this, Arrhenius behavior has been observed in both solid and solution formulations of proteins (Yoshioka et al., 1994).

Some non-Arrhenius behavior can be accounted for by the observation that the most common degradation mechanism for proteins involves equilibration of the native protein with an aggregation-prone intermediate (usually a denatured form of the protein) followed by irreversible protein degradation (aggregation) (Roberts, 2003). In this scenario, the overall protein degradation rate depends on both the equilibrium constant for formation of the aggregation-prone state and the rate of irreversible aggregation. Since both the equilibrium constant and the aggregation rate constant can change as a function of temperature, the overall kinetics will not necessarily follow the Arrhenius relation. The temperature effects on the equilibrium constant have successfully been estimated using the heat capacities and entropies associated with the denaturation based on the individual residues (Ganesh et al., 1999). With estimates of the temperature dependence of the equilibrium constant between the native protein and the aggregation-prone state, the overall kinetics for the protein degradation have been successfully modeled and used to predict ambient stability.

7. Photochemical degradation

Light exposure can induce chemical degradation in susceptible molecules (Tonnesen, 1996). For light to induce a chemical reaction, the light must be absorbed. Since transmission of ambient light (solar, incandescent or fluorescent) through glass is minimal or nil at wavelengths shorter than about 320 nm, one generally is most concerned with drug chromophores having relatively long ultraviolet to visible absorptions. Although a discussion of the various mechanisms involved in photochemical reactions is beyond the scope of the present review, such reactions can broadly be divided into processes that depend on oxygen (photooxidations) and those independent of oxygen (such as dehydrogenations, rearrangements and dimerizations). The degree to which accelerated studies can successful predict ambient photostability depends largely on the extent of the system reciprocity with respect to the exposure. Ideal reciprocity is seen when light given in short, high intensity exposure, or long duration, low intensity exposure, or even pulsed exposures, gives the same amount of photodegradation if the same number of photons are absorbed. In such a situation, a plot of the log of the radiant light intensity versus the log of the degradant formation rate is a linear relationship. For systems that are reciprocal, accelerated aging can be accomplished by using high intensity exposures. Fortunately, such reciprocity is common in pharmaceutical systems. Reciprocity was seen, for example, with nifedipine in solution (Zhao et al., 2003) and in the solid state (Teraoka et al., 1999), and with tretinoi tocoferil in the solid state (Teraoka et al., 2001). However, it is important to recognize when high intensity will not be predictive of ambient exposure conditions, i.e., when there is high intensity reciprocity failure. Although not often recognized in the pharmaceutical literature, such reciprocity failure is well documented in other fields (Kinameri et al., 1981). The major reasons for such reciprocity failures are as follows:

1. Depletion of a diffusible species: When high intensity light strikes a sample, it is possible to consume a reactive species locally such that diffusion of that species becomes rate limiting. The result is that the degradation kinetics at high intensity are less than proportional to the amount of light absorbed, i.e., more light exposure does not lead to more reaction. A particular example of this is with oxidation where local oxygen depletion can result in lower than proportional degradation. A variation on this is the local depletion of a quenching species. In this case, the high intensity response will be greater than would be observed at low intensities (for the same amount of absorbed quanta) due to the high-

intensity light locally overwhelming the quenching species.

2. Accumulation of diffusible reaction products: If degradants cannot diffuse at the rate they are formed, their presence can affect the drug photoreactivity. Ambient exposure conditions can allow for diffusion, while high exposure conditions may not. The diffusing species can act to increase or decrease the reactivity of the drug such that the accelerated conditions can either over or under predict the ambient light stability.
3. Generation of increased temperatures: When materials absorb light, much of that energy is generally converted to heat. With the low intensity exposure common under ambient conditions, the temperature rise will be low due to convective and radiative heat loss. However, with higher intensity exposure, temperatures can increase significantly. This temperature increase can in turn lead to a rate increase beyond that due to the photochemical process itself. This problem is especially challenging in packaged systems (e.g., glass bottles) where convective and radiative heat loss in minimized. Temperature monitoring can at least assure that any temperature increase in minimal. It is important, however, that the temperature monitoring be of the sample and not of a control that does not absorb the same amount of light since the latter will not show the same degree of temperature rise. When possible, circulating air around samples can help. Another factor that can help is to use long-wavelength cutoff filters, which can eliminate infrared emissions. This helps minimize temperature increases while still allowing for accelerated photostability since little photochemistry occurs at long wavelengths (low energy), but absorption (especially in the infrared) is common and causes temperature increases.

For most pharmaceutical systems, the key to accelerated photostability predictions is to know the amount of degradant formed for a given total exposure. Since the exact exposure of a sample will depend on the specific light source and the distance the sample is placed from the light, it is often useful to determine the total integrated amount of incident light using a chemical actinometer. In chemical actinometers, a solution of a compound that degrades with a known quantum yield (moles of product per mole of light absorbed) is used to calibrate a particular exposure chamber. Several such actinometers have been reported in the pharmaceutical literature (Piechocki and Wolters, 1993; Baertschi, 1997; Favaro, 1998; Bovina et al., 1998; Allen et al., 2000). Electronic photometers can be useful for determining light intensity at a given time; however, due to fluctuations in light intensities, integration of the light intensity over the exposure time is needed to determine the actual exposure of samples in light chambers.

The photostability of a drug can be heavily influenced by excipients (Thoma and Kuebler, 1997) and manufacturing processes (Aman and Thoma, 2002a). In particular, titanium dioxide particles can catalyze the photodecomposition of drugs. The degradation rates for drugs are expected to depend on the intimacy of contact between the titanium dioxide and the drug, a factor that can lead to variability in predictions. Photocatalysis by titanium dioxide has also been shown to be moisture sensitive (Kakinoki et al., 2004).

The absorption wavelength of a drug can change with its environment. This can be particularly pronounced when looking at a drug in solution versus the solid state, but can be observed even when changing solvents. For this reason, changes in formulations may cause changes in the apparent stability with no change in mechanism.

In some cases, photolytic products can themselves absorb light and undergo photochemical transformations. As discussed with thermal processes, these secondary processes can confuse the low-level degradation picture important in most pharmaceutical stability programs. It is therefore generally useful to avoid high conversions.

One of the challenges in determining photostability of solid drug products is that the light penetration depth is often limited by the absorption of the drug and excipients. The result is that there can be high exposure on the surface of a dosage form, with little exposure at the center. If the light bleaches the drug as it degrades, greater penetration can continue as the reaction proceeds. In contrast, if the drug degradant absorbs strongly, it can limit the penetration depth for photoreaction. For example, the photodegradation of nifedipine in tablets occurred to depths between 360 μm and 880 μm, depending on drug loading (higher penetration with lower loading) (Aman and Thoma, 2003a).

Photodegradation on solids can lead to discoloration at the surface with little to no measurable bulk change

of the drug. Since appearance change can be a factor in setting drug expiry, color changes can in some cases be a more sensitive assay than bulk methods (such as HPLC). Additionally, since the surface exposure is important with photolytic processes, the dosage form size (surface area to volume ratio) can impact the overall degradation rate (smaller dosage forms showing greater instability on a percentage basis). This factor makes it important that the size and shape of a dosage form that is tested be representative of the intended final dosage form.

For tablets, film coatings can provide a significant reduction in the exposure of the tablet core to light. The effects of opacifying agent level and coating thickness of film coats on photosensitive drug cores have been characterized in terms of a contrast ratio (Bechard et al., 1992). It was found that good light protection is afforded with contrast ratios greater than 98%, which was achieved with a coating of 29.5% titanium dioxide with a thickness of 145 μm. Care must be taken, however, that variations in film coating do not lead to a reduction in the opacity of a coating, and thereby fail to provide the desired protection. In accelerated photostability studies, it can be useful to use film coated tablets at the thinnest coating level likely to be seen in production in order to anticipate whether this would affect the exposure limits of the dosage form.

Ambient light conditions are difficult to define since this will depend on storage conditions. To get a rough idea of this exposure, consider a 100 W light source at a distance of 1 m from a sample with the light on 24 h per day. The overall exposure would then be:

$$\text{exposure} = \frac{100\,\text{W}}{4\pi(1\,\text{m})^2} \times 365\,\text{days year} \times 24\,\text{h/day}$$

$$= 70\,\text{kWh/(m}^2\,\text{year)}$$

Light sources, including direct and indirect (indoors) sunlight, fluorescent lights and incandescent lights have only a fraction of light in the most damaging ultraviolet region. For this reason, the ICH standard for UV exposure is 200 Wh/m^2 (International Conference of Harmonization, 1997; Drew, 1998; Thatcher et al., 2001a, 2001b; Aman and Thoma, 2003b). For visible light, exposure sources are rated in terms of their lux values. Lux represents light flux corrected for the eyes' response. One lux is equivalent to 1.46 mW/m^2 at 555 nm. Using the above approximation, a year's light exposure would then equal 48 million lux-hours; however, since the fraction of the light likely to cause harm is significantly less than this, the standard exposure requirements set by the ICH are 1.2 million lux-hours. With commercial light chambers, it is possible to achieve the ICH exposure conditions for both ultraviolet and visible exposure in less than a week. A number of lamps have been used in pharmaceutical testing (Piechocki, 1998; Boxhammer and Willwoldt, 1998; Matsuo et al., 1996). Often the desired exposure can be achieved by a combination of an artificial daylight fluorescent lamp with an ultraviolet emitting lamp (typically, a xenon arc lamp).

8. Prediction of stability in packaged product

In many cases, drug product stability studies must include the packaging to provide an assessment of the shelf-life of the product as used. Two major factors dominate considerations on predicting the effect of packaging on drug products: (1) leachable (or volatile) impurities that affect the drug product, and (2) permeability of the packaging largely to moisture and oxygen.

8.1. Leachable and extractable substances

Leachable chemicals in packaging are generally of greater concern for liquid dosage forms than for solids, due to the direct liquid contact with the packaging materials (Jenke, 2002). Of particular concern are extractable materials in elastomers used for stoppers and seals for packaging (Groeger and Compton, 1996). Independent of the specific nature of various leachable materials in bottles and stoppers and the specific drug formulation, the rate of leaching is generally temperature dependent. Leachable and extractable materials can be undesirable for a number of reasons: (1) these materials can affect the chemical stability of the drug substance either as reactants, as catalysts or by affecting the pH of a drug solution and thereby affecting the drug stability; (2) they can themselves be toxic; or (3) they can induce physical changes in the pharmaceutical formulation (e.g., cause precipitation). Predicting packaged product stability using elevated temperatures faces the usual challenges of non-Arrhenius behavior for complex reaction pathways, as well as specific factors affecting the chemical leaching process. In

many cases, the degradant from the reaction of the drug with a leachable material is different from other drug degradants. In this case, it is possible to follow the formation of the appropriate degradant(s) as an indication of the packaging sensitivity. The leaching process generally follows the Stokes–Einstein relationship for diffusional processes; that is, it depends linearly on temperature and inversely on viscosity. Viscosity, in turn, is exponentially dependent on temperature. In the absence of a phase transition, one would therefore anticipate the temperature dependence of leaching to follow the Arrhenius or modified Arrhenius relationship shown in Eq. (8). At phase transitions (for example, the T_g of a plastic), the viscosity of the packaging material, and hence its permeability, can change dramatically. For this reason, care must be taken in accelerated aging studies to use temperatures below such transitions to predict the ambient behavior.

8.2. Moisture and oxygen permeability

While glass and foil packaging show virtually no permeation of moisture or oxygen, many pharmaceutical packaging systems (e.g., plastic bottles, plastic blisters, etc.) show significant permeability. In accelerated aging, the functional dependence of the permeability with temperature can sometimes complicate predictions for ambient conditions. The permeability to moisture and oxygen of most packaging plastics follows the Arrhenius equation; thus, whether such permeability affects the predictions of ambient drug stability depends on the relative activation energetics of the permeability versus that of the chemical degradation, as well as how dependent the chemical degradation is on the moisture or oxygen level surrounding the dosage form.

Oxygen permeability for pharmaceutically acceptable plastic packaging is high; however, the activation energies are low. For example, polyethylene, a common bottle plastic, shows an activation energy for oxygen permeation of less than 1 kcal/mol (Gajdos et al., 2001). While the permeability of packaging plastics to oxygen increases with temperature, the rate of increase will often be lower than the rate of increase of a drug oxidation rate. This remains an issue only if oxygen concentration is depleted at high temperatures. With the high permeability of most packaging plastics to oxygen, this is unlikely to be a real issue in accelerated aging studies.

With moisture transfer through packaging, the situation can be quite a bit more complicated. The moisture content of a drug product varies with the relative humidity surrounding it and the residual moisture of the sample when packaged. Samples stored at humidities above their CRH values will continue to pick up moisture, while below the CRH, the moisture content in the samples will eventually equilibrate to that relative humidity. When studying stability of moisture sensitive products in packaging, samples can both gain or lose moisture through the packaging depending on the relative humidities of the external environment and the environment surrounding the dosage form. For this reason, it is important that any higher temperature studies done to predict ambient stability be done at a constant relative humidity. The permeability of plastic bottles shows temperature dependencies that follow Arrhenius behavior in the temperature ranges used in most accelerated aging studies. For example, for high density polyethylene (HDPE), the activation energy for moisture permeability was found to be about 10 kcal/mol (Morillon et al., 2000). In some cases, this temperature dependence can dominate over the degradation reaction kinetics, at least at higher temperatures. The absolute permeability depends on the difference in relative humidity inside and outside a bottle. A new model has been proposed for predicting the moisture uptake by solid dosage forms packaged in plastic (Chen and Li, 2003).

8.3. Photolysis in packaged products

Since light protection by packaging can partially or completely eliminate exposure of a dosage form to light, testing photostability of drug products in packaging is essential to understand the stability of a product under real-world conditions. ICH guidelines therefore suggest that drug products be tested not only under direct exposure, but also in their immediate packaging (i.e., blister packs, bottles, vials or any packaging that is in direct contact with the drug product) and in the marketing packaging (i.e., the box or package the drug product is sold in) (International Conference of Harmonization, 1997). Obviously, if the drug product itself withstands the light exposure, then there is no need to test under packaging conditions. Similarly, if

the immediate packaging protects the drug product, there is no need for testing in the marketing package. Clear blister packaging of different colors has been studied for its effectiveness at protecting light sensitive drugs (Aman and Thoma, 2002b). With glass and plastic bottles, light transmission can vary significantly even for bottles of similar description (e.g., amber glass bottles) (Baumgartner et al., 1995; Beyrich and Tibussek, 1981; Krogerus et al., 1970). For pharmaceutical products that require light protection from the packaging, it is important that the accelerated aging be conducted with the actual packaging or that the specific transmission spectrum be measured of the packaging to assure that any packaging changes do not result in greater light transmission.

9. Concluding remarks

Assuring adequate product stability remains one of the primary challenges in the development of pharmaceuticals. The present review provides a comprehensive survey of advances in the field since the last major reviews in the 1980s. In particular, with the advancement of analytical chemistry, drug stability is more commonly associated with formation of low levels of degradants rather than loss of drug potency. This has resulted in some factors actually becoming easier to address (less relevance for reaction order), while other factors have become more complex (multiple reaction products with overlapping mechanisms). The added measurement sensitivity has led to tighter regulatory requirements for allowable degradant levels. The need for rapid drug development has increased the need for accelerated stability measurements that are predictive of actual storage conditions. When stability concerns arise, rapid assessments of improvements can help with formulation and packaging improvements. Mechanistic insights, as described in this review, can often lead to remedies for stability problems. In addition, these methods will help prevent unexpected instabilities due to changes in processing, drug substance or excipient purity during commercialization of a drug product. We anticipate future advances in the field of accelerated aging based on new technologies and better use of current theories.

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