When a promising new compound enters early development, studies are conducted to determine the properties of the drug under various chemical and physical conditions in order to find a formulation suitable for use in clinical trials. Measuring both the chemical and physical stability of the drug candidate is an important part of early phase development since the shelf life of the compound will ultimately depend on the stability of the compound in the formulation. Thus, information gathered in early phase stability screening tests is critical to late phase development scientists responsible for developing the final product formulation.

The most common method for characterizing the stability of a new drug candidate involves preparing solutions under various “stressing” conditions such as low and high pH, and in the presence of oxidizing agents such as hydrogen peroxide (Alsante et al., 2003). Solid samples are stressed at various humidity levels and at elevated temperatures. The solutions are then analyzed by HPLC to determine degradation over time, whereas the solid samples are analyzed by powder X-ray diffraction to determine if significant physical changes occurred, and also by HPLC (following dissolution) to investigate chemical stability.

Drug candidates are by design fairly stable materials. Therefore, stability testing is generally conducted at relatively high temperatures to accelerate degradation, allowing analyses to be completed in a reasonable time. While this can result in errors in relative reactivity, for example due to a change in reaction mechanism or existence of an isokinetic temperature below the stressing temperature, these stability studies are generally conducted with the practical goal of screening compounds for reactivity, and not to obtain exact reaction kinetics.

Microcalorimetry is the technique of choice for characterizing the stability of pharmaceutical compounds (Hansen, 1996; Selzer et al., 1998; Phipps et al., 2000; Skaria et al., 2005) due to the ability to monitor reactions at a variety of temperatures, the high sensitivity of the instruments, and the ability to monitor both chemical and physical processes. If large samples (a gram or more) are available, extremely low (less than 1% per year) degradation rates can be measured in a day or so (Angberg et al., 1995). However, even if only milligram amounts are available, current ultra-sensitive microcalorimeters can rapidly screen relative reactivity, especially if higher temperatures are employed. If the rate and heat of reaction are sufficiently large, microcalorimetry data can be obtained rapidly at a number of temperatures and be used to determine if, and at what temperatures, a change in the mechanism of the degradation reaction takes place. Importantly, microcalorimetry requires essentially no method development: solutions or solids are placed directly into the instrument and change in heat flow with time is measured. Unlike HPLC, relative reactivity data can be obtained without the need to optimize
gradient methods or filter insoluble reaction products that can form as the drug candidate degrades. In fact, microcalorimetry can be used to help optimize the protocol for traditional stability screening of a drug candidate: by providing approximate relative reactivity rates under various stress conditions, predictions can be made on how long the degradation process must proceed before reliable HPLC results can be obtained.

**Instrumentation and practical examples**

The chambers of the TAM are sufficiently large to accept small vials of sample sealed in a second vial containing a saturated salt solution. This simple arrangement makes it possible to study slow chemical and physical processes occurring in a solid sample over a range of controlled temperature and humidity conditions. If humidity initiates fast kinetic effects, reusable ampoules specifically designed to expose solid samples to saturated salt solutions after pre-equilibration in the calorimeter are available for all three models.

In a typical stability screening experiment, a solution, suspension or solid is placed in the microcalorimeter and the thermal activity (heat flow) is monitored. The basic assumption is that the rate of heat production at any given temperature is proportional to the rate of chemical and/or physical processes taking place in the sample. A simple example would be the conversion of A to B:

\[ A \rightarrow B \]

Thermal activity = \( dq/dt = \Delta H (dn/dt) \)

where \( q \) is the heat (e.g., calories or Joules), \( t \) is time in seconds, \( \Delta H \) is the heat of reaction, and \( n \) is the number of moles of B formed. Therefore, the output of the calorimeter is directly proportional to the rate of formation of B at any given time \( t \).

Higher temperatures increase the rate of degradation and thus permit the use of smaller sample sizes. Figure 1 shows data collected from 25 to 90 °C using a MC-DSC. The concentration of the sample (10 mg/mL) was the same as that used for HPLC stability tests. Although there was no sign of thermal activity at either pH 4 or 10 at 25 °C, as the temperature was slowly raised (0.2 °C/min), significant non-zero thermal activity was observed, indicative of chemical reactions occurring in the samples. Under basic conditions the reaction was endothermic, while in acidic conditions the reaction was exothermic. Information such as this is useful when attempting to understand degradative reaction pathways.

In traditional HPLC stability screening studies it is generally assumed that all degradation products absorb UV equally. While this is incorrect, it is often impractical to run preparative HPLC to isolate and determine the extinction coefficients of all the components in a reaction mix. Using a similar assumption (i.e., that the heats of reaction in Figure 1 are the same for both solutions), and remembering that thermal activity is proportional to rate, it can be concluded from Figure 1 that the apparent rate of reaction is significantly faster at pH 10.
than at pH 4 at any given temperature.

Graphing the logarithm of the thermal activity versus the reciprocal temperature results in an Arrhenius plot of the microcalorimetry data (Hansen et al., 1989). Figure 2 shows this plot for the two datasets from Figure 1 and indicates that, at pH 4, there appears to be a single activation energy, while at pH 10 there are at least two reaction pathways with different activation energies. This information can be very useful in designing traditional stability screening studies since the data obtained at pH 10 would indicate the need to qualify results obtained at low rather than high stress temperatures.

**SUMMARY**

Microcalorimetry is a powerful tool that complements traditional approaches for screening the stability of pharmaceutical compounds. For example, the ability to test three pH conditions in a day yields information to guide the design of HPLC-based assays. In many cases fundamental information can also be obtained, such as whether data generated at elevated temperatures can be reliably used to predict relative reactivities at lower temperatures by an Arrhenius extrapolation. Essentially the same methodology is used for all microcalorimetry measurements, making it possible to rapidly determine important stability information very early in drug development. In addition, in late phase development microcalorimetry can be used to quickly determine if proposed processing conditions (for example, milling to decrease particle size) affects the stability of the drug candidate.

**CITED REFERENCES**


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