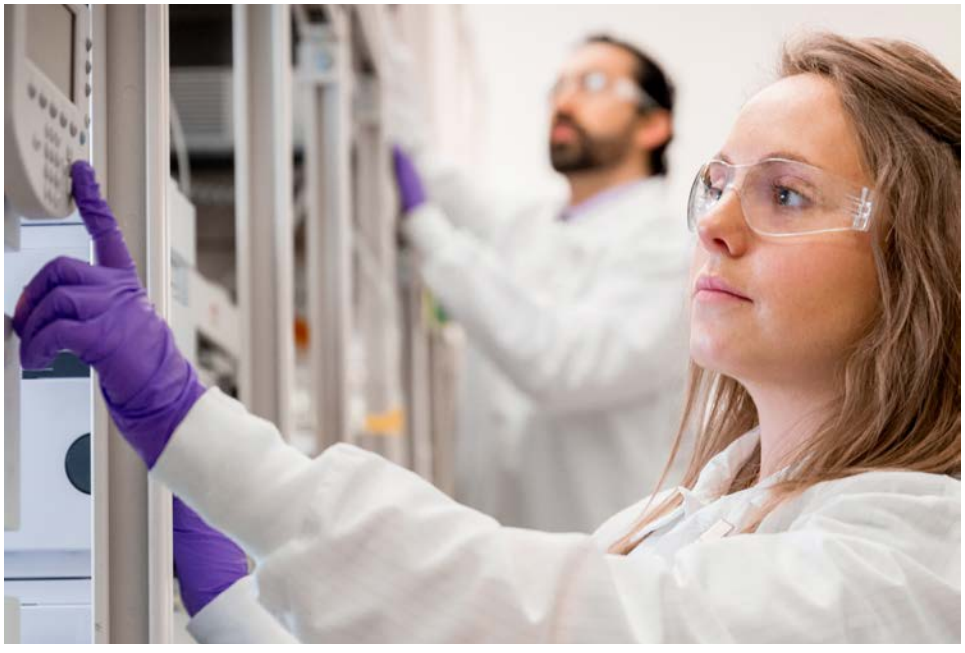

Predicting Extractables and Leachables from Container Stoppers

Data from migration kinetics studies can be used to develop models that predict levels of leachables and extractables at different temperatures and time points.

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Pharmaceutical products are exposed to numerous polymeric materials throughout manufacturing processes and during packaging, when they come into contact with container and closure systems. They can also interact with polymers when the drug is administered.

Compounds that migrate from these different materials into drug products, and can directly reach patients taking the drug, are known as leachables (1). Because leachables may affect drug product safety, efficacy and purity, regulatory authorities require that potential leachables be evaluated appropriately for any new drug application (2).

A controlled extraction study is recommended (1) early in the pharmaceutical development process to provide a thorough understanding of potential leachables from appropriate drug product container/closure system components, manufacturing process components, and/or product administration components. These studies are typically performed by using vigorous techniques (e.g., reflux or Soxhlet extraction), to extract polymeric components with multiple solvents of various polarity, pH, and other variables. The goal is to achieve asymptotic extraction (3).

The controlled extraction study provides the worst-case possible leachables and amounts, but it cannot fully predict the presence of specific leachables and their amounts. A simulation or forced migration study is commonly used to predict more realistic leachables profile and amounts (3,4).

In these studies, the container/closure system is filled with a simulation solvent or the drug product solution, and the solutions are exposed to the polymeric container/closure components so that conditions are similar to what the actual products will experience. Container and closure components are then subjected to accelerated aging conditions, such as higher temperatures, to accelerate the migration of extractables into the simulation solvents or drug product solutions.

A fundamental question for any simulation or forced migration study is what the optimum temperature and duration

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IMAGE COURTESY OF PPD LABORATORIES.

Table I. High-performance liquid chromatography operating parameters. UV is ultraviolet.

Column	XDB-C8, 75 x 4.6 mm, 3.5 µm from Agilent Technology	
Column temperature	60 °C	
Mobile phase A	Water	
Mobile phase B	Acetonitrile	
Flow rate	1.0 mL/min	
Detection	UV @ 220 nm	
Injection volume	5 µL	
Gradient		
Time	Mobile phase A	Mobile phase B
0	75	25
12	10	90
17	10	90
17.5	75	25
21	75	25

should be in order to predict the amount of leachables that would be released by the end of the product's shelf life, or at other specific points (e.g., 24 months at 5 °C or 25 °C).

The authors designed a forced migration study to address that question and determine how the amount of leachables would change with intended temperature changes (e.g., from refrigerated to ambient storage, i.e., 5 °C to 25 °C or vice versa) at the multiple time points (e.g., 0, 1, 3, 6, 12, 18, or 24 months) required for drug product stability studies and at the end of the drug's shelf life.

The research also explored whether the level of leachables could be predicted, using data that had been generated at previous time points.

The resulting extractables/leachables study was designed to establish empirical mathematical models for the projection of extractable and leachables amounts from a container/closure system in pharmaceutical products. A model solvent (i.e., 50:50 isopropanol:water) and a bromobutyl rubber stopper commonly used for biopharmaceutical products packaging, were used in this study. Butylated hydroxytoluene (BHT), a common antioxidant in polymer materials and a well-characterized extractable from container/closure components, was used as the model analyte. Results from this study were used to:

- Determine a relationship between extractable concentration and extraction time
- Determine a relationship between extractable concentration and extraction temperature
- Determine a relationship between extraction temperature and extraction time.

High-performance liquid chromatography (HPLC) was utilized to analyze for the concentration of extracted BHT at various timepoints and temperatures. Extracted BHT amounts, extraction time, and extraction temperature were evaluated and compared against each other. The data were used to develop three empirical equations to predict extractables and leachables amounts at different time points or at different temperatures and to predict the extraction time and temperature required to achieve the same extractables and leachables amounts.

The empirical equations also were compared to the American Society for Testing and Material's ASTM F1980-16 standard model (5), and results were consistent with that model when certain Q_{10} values (i.e., the rate of change resulting from a 10 °C increase in temperature) were applied. This article will describe the methods used, and summarize findings and their implications.

MATERIALS AND METHODS

Three bromobutyl rubber stoppers were immersed in 20 mL of 50:50 isopropanol:water (H_2O) in glass jars with polytetrafluoroethylene (PTFE)-lined caps for a total time of six months, with time points interspersed throughout.

The samples were then statically stored in chambers at various temperatures (i.e., 5 °C, 25 °C, 40 °C, 50 °C, and 60 °C), and were pulled at specific time intervals (i.e., 1, 2, 4, 6, 8, 10, 15, 20, 30, 60, 90, and 180 days). Duplicate preparations were made for each condition. Once pulled from storage conditions, all solutions were transferred to 25-mL volumetric flasks and brought to volume with 50:50 isopropanol: H_2O. Prepared solutions were stored at 5 °C until analysis.

At each time point, the resulting extraction solution was analyzed directly via HPLC using a photodiode array detector (Agilent 1100). HPLC parameters are shown in **Table I.** A BHT standard (Sigma-Aldrich) prepared at a concentration of 10 µg/mL in 50:50 isopropanol:H_2O was used for quantitation.

RESULTS AND DISCUSSION

Correlation of extractable amounts versus extraction time. **Figure 1** shows all extracted BHT amounts plotted against extraction time. As expected, extracted amounts of BHT increased with increasing time. They also increased with temperature, with more BHT extracted at higher temperatures when compared to lower temperatures across the same time intervals.

It was observed that at high temperature, including 50 °C and 60 °C, there was excessive degradation of BHT after 90 days. Because accurate extractable BHT amounts could not be obtained at the six-month time point and thereafter, those data are not included in **Figure 1**.

Data trending analysis shows that extracted BHT amounts and extraction

Figure 1. Butylated hydroxytoluene (BHT) amounts at all temperatures and time points from day 1 to day 180.

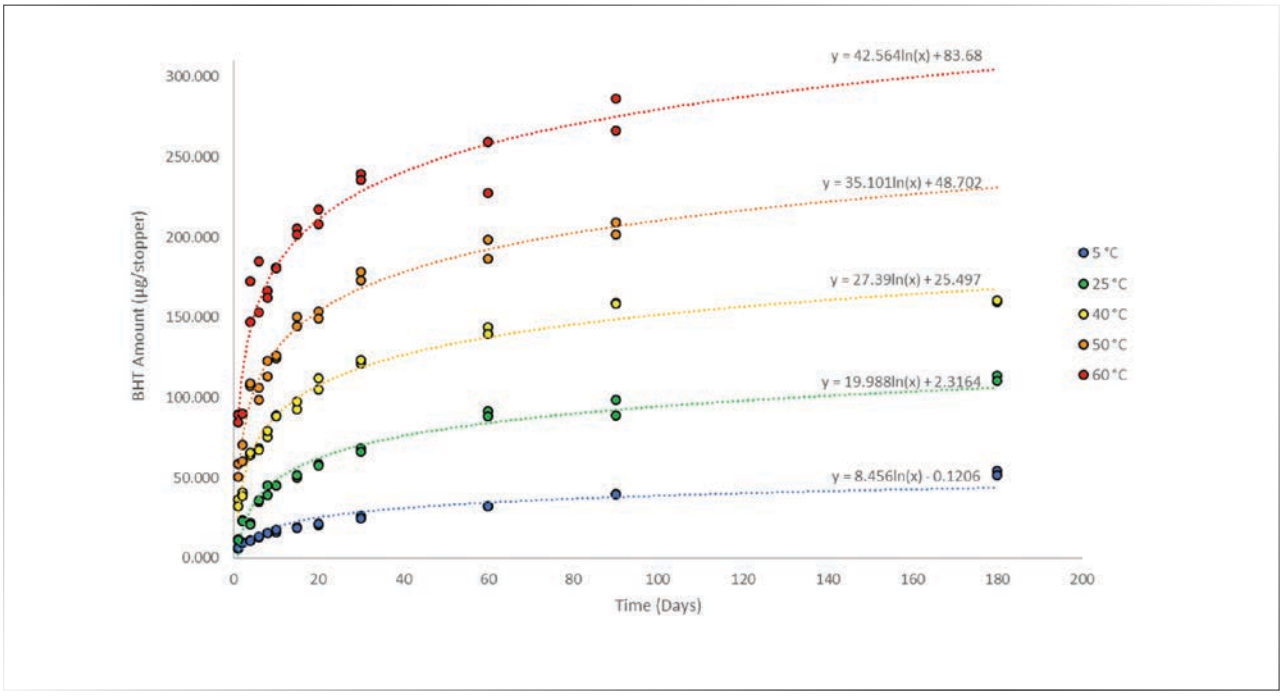


Table II. Correlation of extract butylated hydroxytoluene amounts vs. extraction temperature.

Time	Equation	Equivalent equation
1 day	$C = 4.1336 \times e0.^{0507T}$	$C = 4.1336 \times 1.052^{T}$
2 days	$C = 8.0736 \times e0.^{0407T}$	$C = 8.0736 \times 1.042^{T}$
4 days	$C = 7.5252 \times e0.^{0515T}$	$C = 7.5252 \times 1.053^{T}$
6 wdays	$C = 10.715 \times e^{0.0455T}$	$C = 10.715 \times 1.047^{T}$
8 days	$C = 13.193 \times e^{0.0433T}$	$C = 13.193 \times 1.044^{T}$
10 days	$C = 14.442 \times e^{0.0433T}$	$C = 14.442 \times 1.044^{T}$
15 days	$C = 15.535 \times e^{0.0452T}$	$C = 15.535 \times 1.046^{T}$
20 days	$C = 18.380 \times e^{0.0422T}$	$C = 18.380 \times 1.043^{T}$
30 days	$C = 30.756 \times e^{0.0364T}$	$C = 30.756 \times 1.037^{T}$
General equation	$C = a \times e^{bT}$ a and b are constants, and e is Euler number	$C = a \times dT$ d value variations are very small, and on average d = 1.045, therefore: $C = a \times 1.045^{T}$
Extractables amount projection at the same timepoint:	$C_2/C_1 = e^{b(T_2 - T_1)}$ b = 0.0443	$C_2/C_1 = 1.045^{(T_2 - T_1)}$

time follow a logarithmic correlation. The correlation equations at different temperatures are listed in **Figure 1**. Using these data, **Equation 1** was developed:

$$C=a\times\ln(t)+b \quad \textbf{[Eq. 1]}$$

where C = extractables or leachables concentration or amounts, a and b = constants that can be derived from existing time points data, and t = extraction time.

Based on the equation, theoretically, given two or more timepoints of extractables or leachables data, extractables and leachables amounts for all other time points can be predicted.

Correlation of extractables (amounts versus extraction temperature). **Figure 2** shows the amount of BHT extracted at each interval against each extraction temperature, where each plotted line corresponds to a time interval (in days). As the figure shows, the extracted BHT amounts increased exponentially with extraction temperature. **Table II** summarizes the correlation of the extracted BHT amounts vs. the extraction temperatures at each point.

Using the equation generated from each extraction time point, a generic equation (**Equation 2**) was developed that can be used to predict an extractables/leachables concentration or amount at a given temperature at a specific time interval from the concentration or amount at another temperature:

$$C_2/C_1 = 1.045^{(T_2 - T_1)} \quad \textbf{[Eq. 2]}$$

All figures courtesy of the authors.

Figure 2. Extracted butylated hydroxytoluene (BHT) amounts against extraction temperature for each time interval (from bottom to top: 1, 2, 4,6, 8, 10, 15, 30, 60, and 90 days).

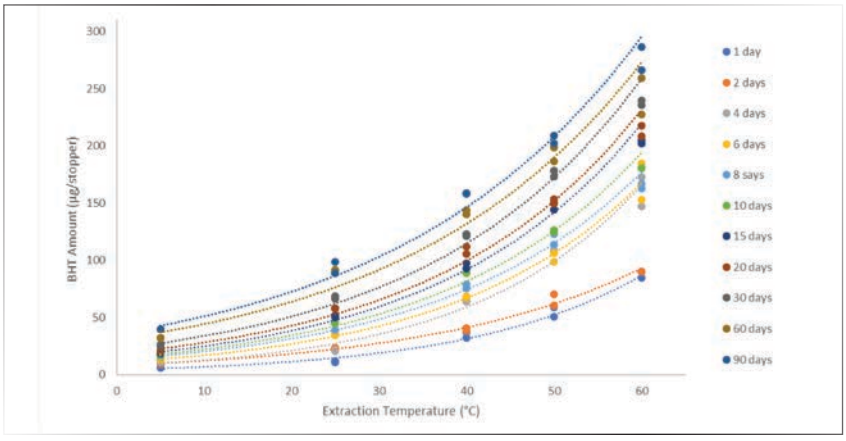
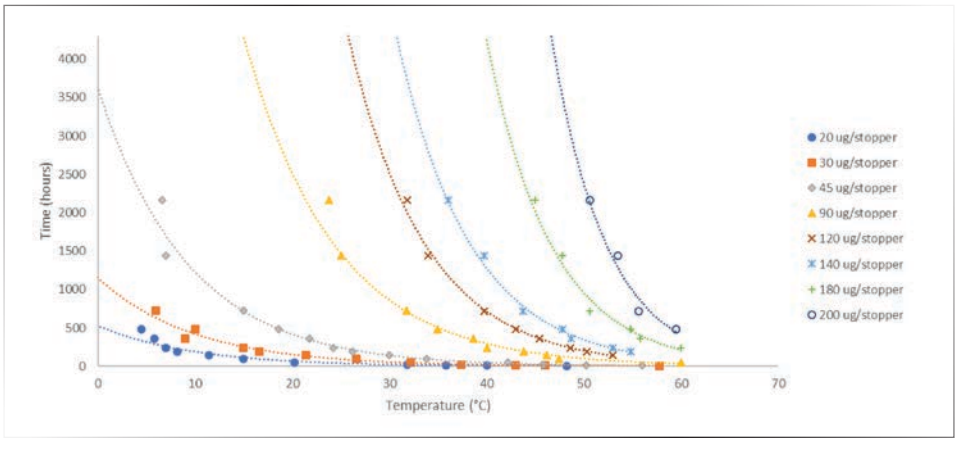


Table III. Extraction time and temperature required to achieve certain extracted butylated hydroxytoluene amounts.

Time (days)	Temperature (°C) (20 µg/stopper)	Temperature (°C) (30 µg/stopper)	Temperature (°C) (45 µg/stopper)	Temperature (°C) (90 µg/stopper)
1	31.8	37.4	45.8	NA
2	20.2	32.2	42.2	60.0
4	15.0	26.6	33.8	47.4
6	11.4	21.4	30.0	46.2
8	8.2	16.6	26.2	43.8
10	7.0	15.0	24.2	40.0
15	5.8	9.0	21.8	38.6
20	4.5	10.0	18.6	35.0
30	NA	6.0	15.0	31.8
60	NA	NA	7.0	25.0
90	NA	NA	6.6	23.8

Figure 3. The amount of time required to achieve a specified extractables amount at a given temperature.



where T_1= reference temperature, T_2= temperature for prediction, C_2 = predicted concentration or amount, and C_1= reference concentration or amount.

CORRELATION OF EXTRACTION TIME VS. TEMPERATURE

Further analysis of the data, as shown in **Figure 2**, led to the development of a model for predicting the extraction temperature and duration of a study at a constant extractable amount of BHT. The extraction temperature data were generated by drawing a constant BHT amount line, such as 20 µg/stopper, 30 µg/stopper, 45 µg/stopper, 90 µg/stopper, etc., across each time interval curve from **Figure 2**. Example extraction temperature data are summarized in **Table III.** The extraction time data were plotted against the extraction temperature at each extracted BHT amount, and the result is shown in **Figure 3**.

Figure 3 highlights the amount of time required to achieve a desired extractables amount at a given temperature. As expected, the amount of time required to achieve a specific extracted BHT concentration decreases with increasing extraction temperature. The correlation of extraction time vs. extraction temperature to achieve specific extracted BHT amounts is shown in **Table IV**.

Using the equations from each dataset, a generic third model was developed that can be used to predict the duration of the study required to achieve a certain amount of an extractable (**Equation 3**) at a given temperature:

$$t_2/t_1 = 1.110^{(T_1 - T_2)} \quad \textbf{[Eq. 3]}$$

Where T_1= reference temperature; T_2= temperature for prediction; t_2 =

Table IV. Correlation of extraction time and extraction temperature.

At amounts, µg/stopper	Equation	Equivalent equation
20	$t = 525.77 \times e^{-0.102T}$	$t = 525.77 \times 1.107^{(-T)}$
30	$t = 1150.3 \times e^{-0.101T}$	$t = 1150.3 \times 1.106^{(-T)}$
45	$t = 3619.0 \times e^{-0.108T}$	$t = 3619.0 \times 1.114^{(-T)}$
90	$t = 21422. \times e^{-0.107T}$	$t = 21422. \times 1.113^{(-T)}$
General equation	$t = a \times e^{-bT}$ a and b are constants, and e is Euler number	$t = m \times n^{(-T)}$ on average n = 1.110
Duration projection for achieving the same extractables amounts:	$t_2/t_1 = e^{b(T_1 - T_2)}$ on average, b = 0.1045	$t_2/t_1 = 1.110^{(T_1 - T_2)}$

predicted duration, and t_1= reference time interval.

DISCUSSION

The ASTM F1980-165 standard was developed as a guide for the accelerated aging of sterile barrier systems for medical devices testing. It had previously been proposed as a way to calculate and justify accelerated migration testing for extractables and leachables studies (6,7). The technique is based on an accelerated aging factor (AAF), which is mathematically expressed in **Equation 4**:

$$AAF = Q_{10}^{[Taa-Tref/10]} \quad \textbf{[Eq. 4]}$$

where Q_{10} = aging factor, Taa = accelerated temperature of contact, and Tref = reference temperature of typical use.

The AAF term is used to calculate the accelerated aging time (taa) as expressed in **Equation 5**:

$$t_{aa} = t_{ref}/AAF = t_{ref}/Q_{10}^{[T_{aa}-T_{ref})/10]} \quad \textbf{[Eq. 5]}$$

where tref is the reference time.

When Q_{10} = 2.85, **Equation 5** converts to the following expression, which is the same as **Equation 3**:

$$t_{aa} = t_{ref}/1.110^{(T_{aa}-T_{ref})} \quad \textbf{[Eq. 3]}$$

This suggests that ASTM F1980-16 is an appropriate model for calculating the accelerated aging time when a suitable Q_{10} value is used. The Q_{10} value was determined to be 2.85 in this study.

The AAF term also may be used to calculate the extractables concentration or amount at accelerated temperature (C_{aa}), as expressed in **Equation 6**:

$$c_{aa} = C_{ref} \times AAF = C_{ref} \times Q_{10}^{[T_{aa}-T_{ref/10}]} \quad \textbf{[Eq. 6]}$$

where C_{ref} is the extractables concentration or amount at reference temperature.

When Q10 = 1.55, **Equation 6** converts to the following expression, which is the same as **Equation 2**.

$$C_{aa} = C_{ref} \times 1.045^{(T_{aa}-T_{ref})} \quad \textbf{[Eq. 2]}$$

This suggests the ASTM F1980-16 is an appropriate model for calculating and predicting extractables and leachables amounts at one temperature from extractables and leachables amounts at another temperature, when a suitable Q_{10} value is used. The Q_{10} value was determined to be 1.55 in this study.

CONCLUSION

Three empirical kinetic models were developed to predict the concentration of an extractable and leachable at a given temperature or time, as well as to predict the duration and temperature of a study given a target concentration. Future work will focus on comparing the models developed here to models generated for different types of materials and extraction solvents.

These efforts will help determine whether these generic models are appropriate for use with different types of polymer materials and target extractables and leachables. Additional studies also will indicate whether these models can be applied with differing extraction solvents and drug product matrixes.

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