SUMMER 2013 • Volume 12 / Issue 2 • ISSN 1538-8786

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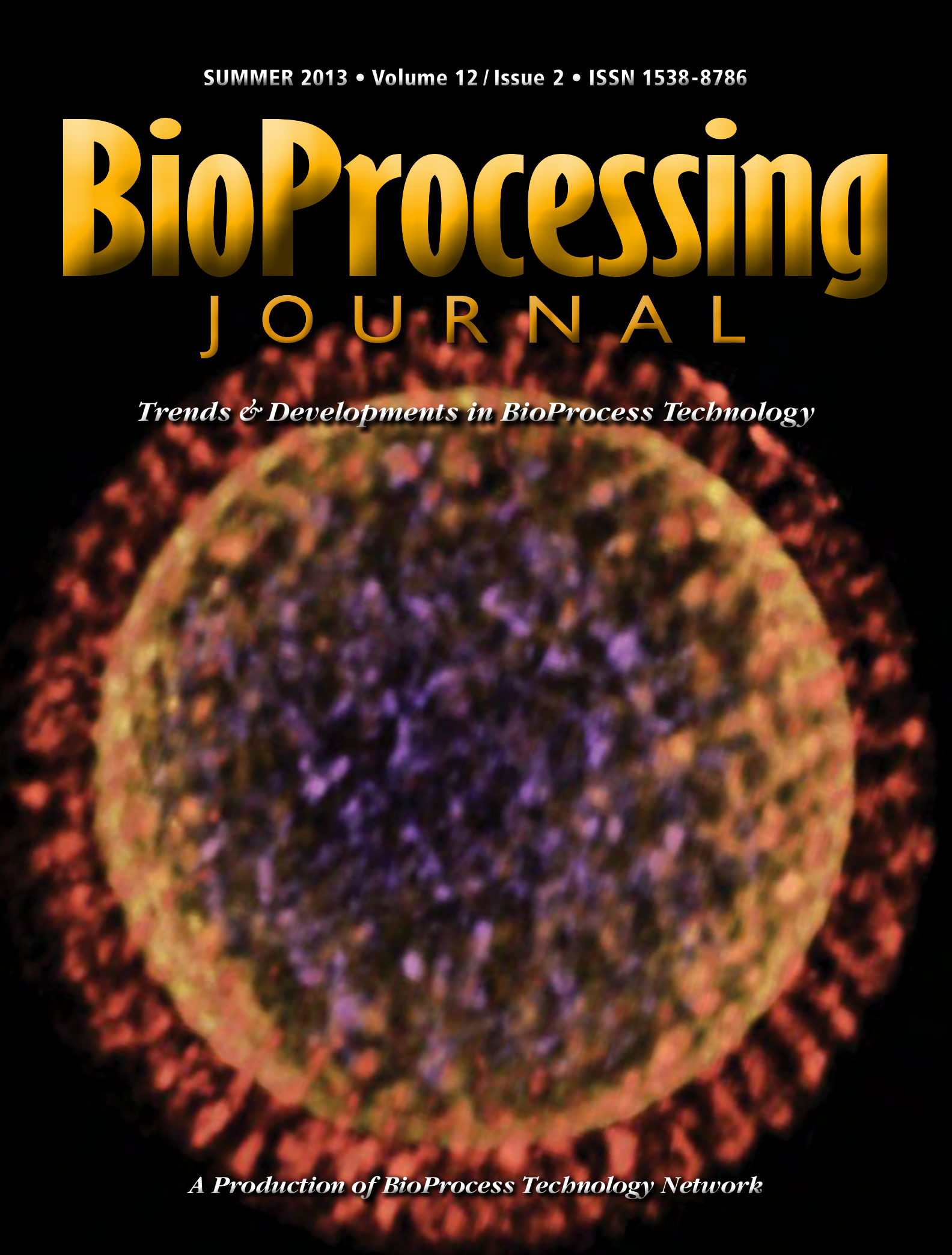

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TRENDS & DEVELOPMENTS IN BIOPROCESS TECHNOLOGY

A Proposed Modeling Approach for Comparing the Heat Inactivation Susceptibility of Viruses

By RAYMOND NIMS and MARK PLAVSIC

Abstract

Heat inactivation is dependent both on temperature and time at temperature, making inter-assay and inter-virus comparisons of heat sensitivity of viruses problematic. Historically, heat inactivation data for pathogens, including viruses, have been evaluated by determining decimal reduction value ([*D*] the time required to inactivate 1 $\log_{10}$ of the organism at a given temperature) and the incremental temperature required to decrease the *D* by 1 $\log_{10}$ (*z*). We recommend the use of a straightforward approach for extrapolating heat inactivation (*i.e.*, inactivation vs. time at fixed temperature) data from measured to non-measured temperatures that is based not on the *z* value, but on a power function fit of the *D* vs. temperature plots.

There needs to have been at least three temperatures evaluated in the inactivation vs. time kinetics studies in order to conduct these modeling analyses. For inter-assay and inter-virus comparisons of heat inactivation sensitivity, we propose the use of two modeled parameters: (1) *temperature required to inactivate 1 $\log_{10}$ of virus in 0.5 minutes;* and (2) *time required for 1 $\log_{10}$ reduction in infectivity at 80°C*. By using both modeled parameters, we have calculated consensus heat inactivation values for two caliciviruses (feline calicivirus and murine norovirus).

Introduction

Heating of viruses, either in the dry state or in solution, represents an important and commonly employed physical inactivation approach. Variations on this theme can include heating at relatively low temperatures for extended durations (*e.g.*, 45–70°C for 15–60 minutes or longer), high-temperature short-time pasteurization ([HTST], heating in solution or in food matrices at temperatures of 72°C [161°F] for 15 seconds), and ultra-high temperature treatment (heating at 138°C [280°F] for a minimum of two seconds). The factors influencing the efficacy of heating as an inactivation approach for viruses primarily include the virus itself, temperature, time at temperature, and the inactivation matrix. In general, heating of viruses dried on surfaces requires greater temperatures for a given inactivation efficacy relative to heating of viruses in solutions.[1] Regardless of the inactivation matrix and virus type, heat inactivation kinetics display an inverse relationship between temperature and time at temperature.

Differences in susceptibility of viruses to heat inactivation can be detected at the strain level, the species level, and the family level. For instance, differences at the strain level have been reported for six feline calicivirus isolates, with $\log_{10}$ reductions ranging from approximately 3.0–7.4 at 52°C, and approximately 5.3–9.0 at 56.9°C.[2] Species-level differences are exemplified by the relatively striking dissimilarity in susceptibilities of two parvovirus species, mouse minute virus and B19.[3] At the family level, the factors that contribute to the differences in susceptibility to heat inactivation are not entirely

IMAGE: Norovirus transmission electron micrograph. *(Provided by CDC/Charles D. Humphrey, PhD, http://phil.cdc.gov/phil/home.asp.)*

clear. As opposed to chemical/disinfectant inactivation approaches, susceptibility of viruses to heat inactivation is apparently not determined by presence or absence of a lipid envelope.[1]

The mechanisms underlying heat inactivation of single-stranded RNA viruses have been discussed by Ginoza *et al*.[4] and Nuanualsuawan and Cliver[5] have addressed the mechanisms for the caliciviruses and picornaviruses in particular. A more recent discussion of mechanisms of heat inactivation of viruses of importance to food protection, including caliciviruses, picornaviruses, and adenoviruses, can be found in Hirneisen *et al*.[6] As might be expected, the primary mechanism of heat inactivation involves denaturation of capsid proteins with eventual loss of genomic material from the leaky capsids.

The temperature kinetics of viral inactivation at fixed time are not commonly evaluated. When an experiment is done in this fashion, however, the results are as displayed in Figure 1. This plot for inactivation of feline calicivirus[7] indicates the absence of much effect until a certain temperature is reached (approximately 50°C in this case), a fairly steep increase in inactivation over the next 10–15°C, followed by a plateau effect. The significance of this type of plot is that if inactivation is examined in the range of temperatures covered by the steep portion of the temperature/inactivation curve, a certain degree of inter-assay variability in results is to be expected. In confirmation of this, survey of the heat inactivation results for caliciviruses[8] indicates a rather high degree of variability in inter-assay results over the temperature range of 50–60°C.

The kinetics of heat inactivation of viruses have more typically been evaluated at varying times at a fixed temperature. Under these conditions, the kinetics often appear to be first-order with respect to time. It is not uncommon, however, for graphs of $\log_{10}$ inactivation vs. time to display departures from first-order kinetics.[9] These departures can involve biphasic kinetics or *n*th order kinetics. Evaluation of viral inactivation vs. time kinetics at fixed temperatures has typically been performed by determining the amount of time required to achieve a 1 $\log_{10}$ inactivation of virus. This yields *D* at that temperature (Figure 2). If inactivation vs. time curves are evaluated at multiple temperatures, *D* values for each temperature may be obtained as shown in Figure 2. Due to the possibility for the departure from first-order kinetics mentioned above, the reporting of heat inactivation results, solely in terms of decimal reduction values at a given temperature, may be of marginal use in predicting the extent of inactivation at times greater than those required for 1 $\log_{10}$ reduction. This problem is resolved when authors report both *D* values and the inactivation vs. time plots from which the *D* values were obtained. When first-order kinetics can be demonstrated at a given temperature over 4 or 5 $\log_{10}$ of viral inactivation, the *D* values can be converted easily to the times required for 2 $\log_{10}$ inactivation ($2 \times D$), 3 $\log_{10}$ inactivation ($3 \times D$), and so on.

Historically, predictions of pathogen inactivation efficacy at temperatures different from those evaluated empirically have involved the use of the *z* value, which is obtained from plots of $\log_{10} D$ vs. temperature.

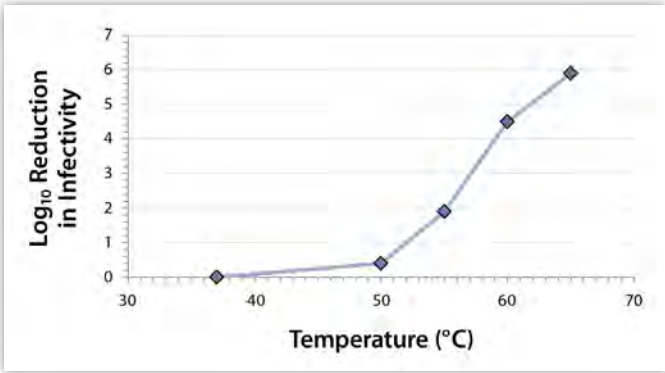

FIGURE 1. Inactivation of feline calicivirus strain F9 infectivity by heating for two minutes at various temperatures in a Dulbecco's phosphate-buffered saline (PBS) matrix (modified from [7]).

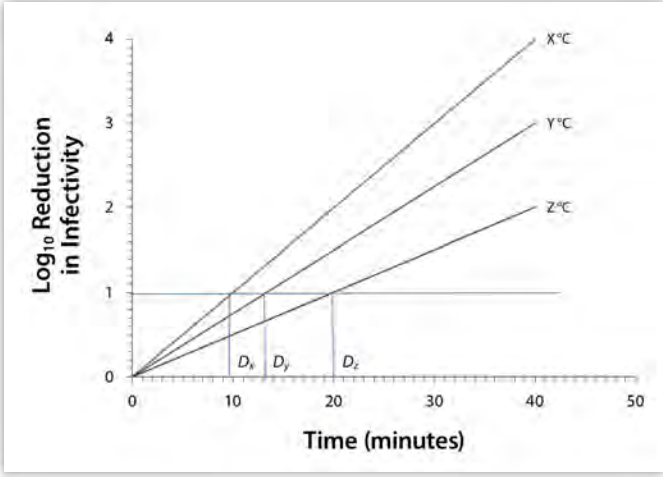

FIGURE 2. Theoretical inactivation vs. time curves for a virus at three temperatures (X°C, Y°C, and Z°C). A graphical representation of the *D* value (decimal reduction value, or the time in minutes for a 1 $\log_{10}$ reduction in viral titer) at each temperature is also shown.

When relatively narrow temperature ranges are covered (or when only three temperatures are included in the analysis), a linear line fit is often found to approximate the data points well, as shown in Figure 3 depicting the heat inactivation of feline calicivirus.[10] The *z* value obtained from this plot can then be used to predict *D* values for other (non-measured) temperatures (*e.g.*, Table 1).

Viral inactivation vs. time kinetics studies have been reported for selected viruses, especially those of concern in the food industry where heat inactivation (*e.g.*, pasteurization) is an important risk mitigation tool. Outside of the food safety arena, however, it has been more typical for investigators to report heat inactivation results at one or two temperatures and time points rather than performing systematic kinetics studies of the types described above. In the latter case and even in those cases where complete kinetics studies have been reported, the results have not typically facilitated comparison of inter-assay studies for a given virus or of inter-virus studies. How, for instance, is one to establish inter-study consensus inactivation results for a given virus when one investigator has examined 15, 30, and 60 minutes heating at 34, 56, and 65°C, while another has evaluated 10, 20, and 40 minutes heating at 50, 70, and 90°C?

In this paper, we discuss an alternative to the use of the *z* value for extrapolating heat inactivation results from reference (measured) temperatures to other (non-measured) temperatures, and propose the use of two modeled heat inactivation parameters for comparing inter-assay and inter-virus results for susceptibility to heat inactivation: (1) *temperature causing a 1 log_{10} reduction in infectivity in 0.5 minutes;* and (2) *time required for 1 log_{10} inactivation at 80°C*.

Methods

The virus heat inactivation literature was surveyed for reports containing sufficient experimental data to allow analysis. As will become apparent in the following discussion of results, sufficient data means inactivation data were obtained from at least three different temperatures with measurements taken at sufficient time at temperature to achieve at least 1 log_{10} inactivation of the virus. For the purpose of this investigation, we analyzed primarily solution inactivation results in order to minimize the impact of matrix (and especially dry heating vs. wet heating) effects on the inter-assay and inter-virus comparisons to be made. As mentioned above, the literature that was found containing the information required to enable the analyses performed was limited primarily to virus families of concern to the food industry (*i.e.*, caliciviruses, picornaviruses, birnaviruses, paramyxoviruses, and orthomyxoviruses).

D values were reported in most of the studies reviewed. In three of the studies, *D* values had to be estimated from examination of the published inactivation vs. time curves. Under the latter conditions, we acknowledge that a certain degree of error was introduced during the *D* value estimation process.

Plots of $\log_{10} D$ vs. temperature were evaluated using the linear regression function of Excel to obtain the linear fit equation:

$$y = mx + b \quad (1)$$

where $y = \log_{10} D$, m = slope, x = temperature, and b = the y-axis intercept. As mentioned above, the *z* value (°C per $\log_{10}$ change in *D*) for a given data set may be obtained from this linear fit equation as:

$$z = \frac{1}{|m|} \quad (2)$$

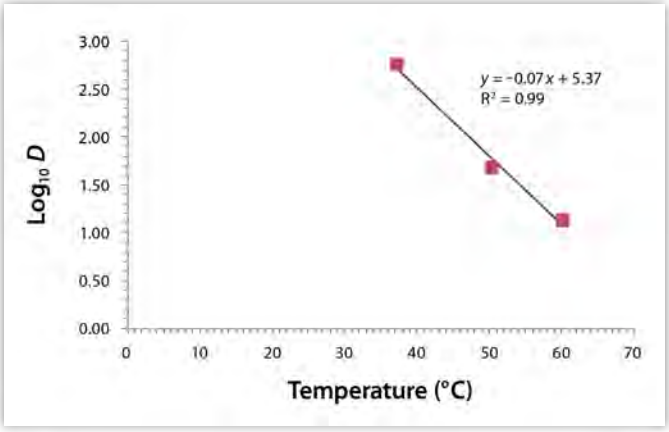

FIGURE 3. Plotting $\log_{10} D$ vs. temperature to obtain a *z* value (14.3°C) for feline calicivirus. (*D* values reported in [10].)

TABLE 1. Use of *z* value (14.3°C) to predict *D* at various temperatures for FeCV.

Temperature (°C)	*D* (minutes)	Measured/ Predicted
35.7	506	Predicted
50.0	50.6	Measured
64.3	5.06	Predicted
78.6	0.506	Predicted

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This z value can then be used to predict D values at other (non-measured) temperatures, using the formula:

$$log_{10} D_{predicted} = log_{10} D_{ref} - \frac{T_{predicted} - T_{ref}}{z} \quad (3)$$

where $T_{predicted}$ is the temperature at which D is to be predicted, and T_{ref} is the temperature at which D_{ref} was actually measured (reference temperature).[11]

Plots of D vs. temperature were evaluated using the power function of Excel to obtain the line fit equation:

$$y = ax^{-b} \quad (4)$$

where $y = D$, $x =$ temperature, and a and b are constants unique to each fit equation. This equation allows one to calculate the D value (time needed to achieve 1 $\log_{10}$ inactivation) at any given temperature. This equation can also be rearranged to solve for temperature as:

$$temperature\ (°C) = \left(\frac{D}{a}\right)^{-\left(\frac{1}{b}\right)} \quad (5)$$

allowing one to calculate the temperature associated with any given D value.

Results and Discussion

For certain physical inactivation approaches that typically display first-order kinetics such as irradiation with ultraviolet light or ionizing radiation, efficacy may be expressed in terms of an inactivation kinetic constant (*e.g.,* $\log_{10}$ inactivation per unit fluence, or the more commonly employed D_{90} which is the radiation dose resulting in 90% inactivation). This enables the direct comparison of inactivation efficacy for viruses within a given matrix. Unfortunately, heat inactivation effectiveness is not so easily addressed. We have been interested in consolidating various published heat inactivation results for non-enveloped viruses from the circovirus,[12] polyomavirus,[13] calicivirus,[8] and picornavirus families into consensus values that would allow for inter-and intra-family comparisons. This effort has been hindered greatly, however, by the fact that published inactivation studies have rarely been performed under the same conditions of temperature and time.

In the context of heat inactivation, the commonly evaluated terms D and z have been proposed to be unique to a given virus. These terms do not lend themselves to inter-virus comparisons, unfortunately. For instance, the term D is applicable only to a given temperature, so unless the viruses to be compared were each tested at the same temperature, the D values are not directly comparable. The z term allows extrapolation of D values from measured temperatures to other temperatures, but viruses having strikingly different sensitivities to heat can have similar z values. Figure 4 displays the z values for feline calicivirus (FeCV, three studies) and another calicivirus, murine norovirus (MNV, three studies). These members of the same virus family should have similar z values and, within experimental error, it could be argued that they do. The parvovirus, mouse minute virus (MMV), is considered to be among the most heat-resistant of the viruses. As shown in Figure 4, MMV was found to have a z value similar in magnitude to that for a heat-sensitive strain of foot and mouth disease virus (FMDV), a picornavirus.

More to the point, when z values for a set of eight viruses from six families were compared to a more definitive measure of heat sensitivity (*the temperature required to inactivate 1 log_{10} of virus in 0.5 minutes*), the resulting correlation coefficient (R) was 0.41 (Figure 5). A similar correlation coefficient

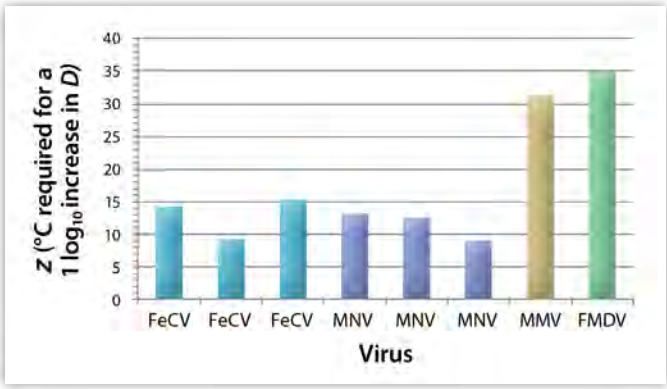

FIGURE 4. z values for two caliciviruses (FeCV, MNV), a parvovirus (MMV), and a picornavirus (FMDV). (Data from Table 3.)

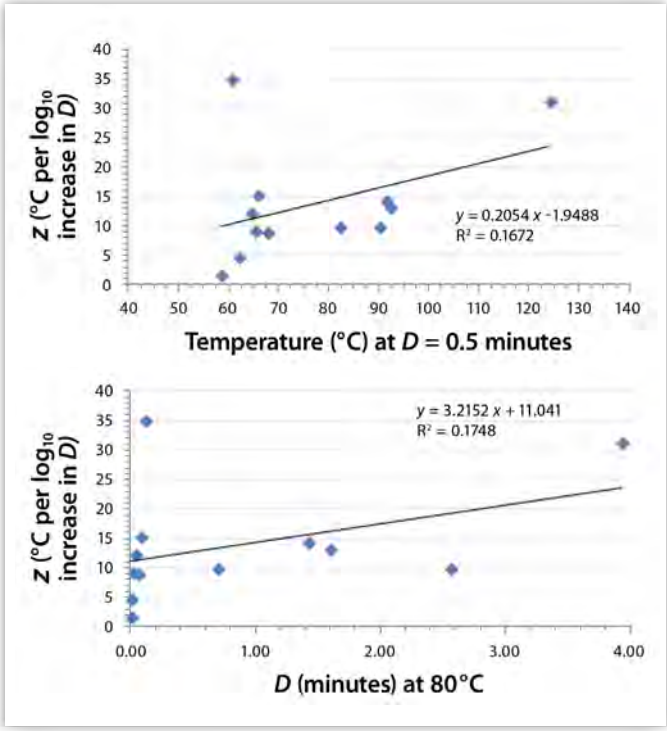

FIGURE 5. Correlation analyses to determine whether z values for viruses are related to the heat inactivation susceptibilities of the viruses. (Data from Table 3.)

(R = 0.42) was obtained when *z* for this set of viruses was compared with another measure of heat sensitivity (*time required for 1 log_{10} reduction in infectivity at 80°C*). These correlation coefficients indicate that, at least for this limited set of viruses, there is little dependency between magnitude of the *z* value and susceptibility to heat inactivation.

It should, therefore, be acknowledged that the *z* value is a reflection of the steepness of the log_{10} *D* vs. temperature curve for a virus, and is not really a measure of the heat sensitivity of the virus. Are there limitations to the use of the *z* value for extrapolating heat inactivation results to non-measured temperatures? Depending upon the linearity of the log_{10} *D* vs. temperature plot, predictions based on the *z* value may be less accurate when extrapolating to temperatures higher than or lower than the range of temperatures from which the *D* values were obtained. As mentioned above, plots of log_{10} *D* vs. temperature appear to be linear in many cases, especially those addressing relatively narrow temperature ranges and only three temperatures. When broader temperature ranges are evaluated in such plots, some deviation from linearity may be observed.

An example of this can be found in a recent report from Kamolsiripichaiporn and coworkers.[14] These authors evaluated the inactivation of a series of FMDVs at 50, 60, 70, 80, 90, and 100°C. Plots of log_{10} *D* vs. temperature over this range exhibited curvature, as shown in Figure 6. Using all data points (50–100°C), a *z* value of 21.8°C was obtained for strain O (isolate OPN). The authors proposed the use of data points from 60–100°C due to this deviation from linearity, although it is evident that even over this narrowed temperature range, some deviation from strict linearity of the plot remains (Figure 6). Use of the *z* value (35°C) obtained from the 60–100°C range to predict *D* at other (non-measured) temperatures is shown in Table 2. The authors stressed[14] that extrapolations of *D* to temperatures outside of the range of experimental temperatures should be avoided due to the deviation from linearity observed in the log_{10}*D* vs. temperature plots.

In the course of our efforts to reduce heat inactivation results for viruses to some common denominator or constant that would facilitate inter-study and inter-virus comparisons, we noted that plots of *D* vs. temperature for viruses have the characteristic appearance shown in Figure 7. The data points are typically approximated best by a line fit that employs the power function (equation 4). In the case shown in Figure 7 for FeCV, the R^2 value for the power function line fit shown was 1.00.

It struck us that this type of plot, which may be referred to as a temperature/time inactivation surface, represents a more direct approach to extrapolating *D* values (and hence, 1 log_{10} inactivation) from measured to non-measured temperatures than is afforded by the *z* value approach. All

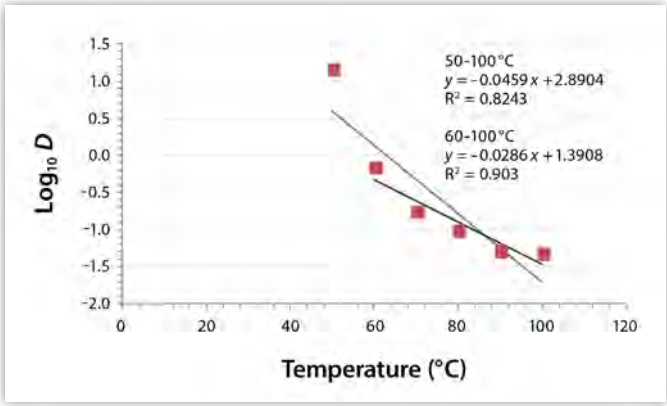

FIGURE 6. A plot of log_{10}*D* vs. temperature for the OPN strain of FMDV. (*D* values reported in [14].)

TABLE 2. Use of *z* value (35.0°C) to predict *D* at various temperatures for OPN strain of FMDV.

Temperature (°C)	*D* (minutes)	Measured/ Predicted
60	0.70	Measured
65	0.50	Predicted
75	0.26	Predicted
85	0.14	Predicted

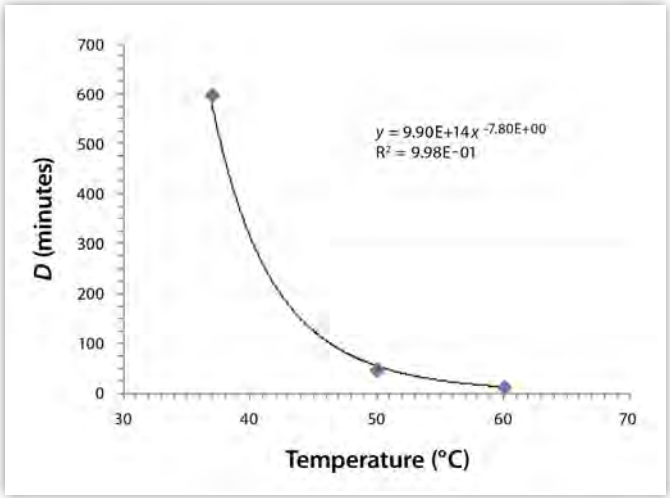

FIGURE 7. The relationship between *D* value and temperature for feline calicivirus. (*D* values reported in [10].) The power function fit line and equation are shown.

points along this surface correspond to 1 $\log_{10}$ inactivation of the virus. By entering temperatures into the power function equation, corresponding *D* values are returned. This process is more straightforward than the use of the *z* value, and is not subject to the subjectivity required during extrapolation using the *z* value approach. For instance, use of the equation 3 for extrapolation based on *z* values requires that the user select one of the measured temperatures to be T_{ref}. Which one of the three or more experimental T values should be used? This subjectivity is eliminated through extrapolation based on the power function equation, which is based on data from all measured temperatures.

Modeling of heat inactivation results (or more specifically, *D* vs. temperature) may be done using either the *z* value approach or the power function approach described above. How closely the two modeling approaches agree with each other and with experimental results is determined by the linearity of the $\log_{10} D$ vs. temperature plot, in the case of the *z* value approach, and the goodness of fit of the power function for the *D* vs. temperature plot in the case of the power function approach. Analysis of the data[10] for feline calicivirus indicated an R^2 of 0.99 for the linear fit in the $\log_{10} D$ vs. temperature plot (Figure 3) and an R^2 of 1.00 for the power function fit in the *D* vs. temperature plot (Figure 7). Not surprisingly then, the modeling of *D* vs. temperature using each approach yields similar curves that each approximate the experimental results closely (Figure 8).

On the other hand, when some deviation from linearity is observed in the $\log_{10} D$ vs. temperature plot, as in the FMDV case shown in Figure 6, the modeling that is done based on the *z* value approach does not show as good an agreement with the modeling done using the power function approach or for that matter with experimental values (Figure 9). Analysis of the data[14] for the OPN isolate of FMDV indicated an R^2 of 0.90 for the linear fit for the $\log_{10} D$ vs. temperature plot over the 60–100°C temperature range (Figure 6) and an R^2 of 0.94 for the power function fit of the *D* vs. temperature plot over the same range.

The evidence described above suggests that the power function approach may represent a more straightforward, less subjective, and perhaps more robust method for extrapolating experimental *D* vs. temperature results to non-measured temperatures than the more commonly employed *z* value approach. Why then has the *z* value historically been used for this purpose? Before curve-fitting software became commonly available, a plot of $\log_{10} D$ vs. temperature could be created on graph paper and the linear line equation could be determined easily from the plot. Once the *z* value was obtained from the slope of this line, extrapolation of *D* values for non-measured temperatures at whole *z* increments from T_{ref} could be performed easily without the benefit of calculators, as shown in Table 1. On the other hand, assigning a best fit power function equation to a *D* vs. temperature plot would be a daunting task without the benefit of curve-fitting software. Modeling *D* vs. temperature surfaces using the power function (equation 4) would also be challenging without the use of a calculator. With such tools now at our disposal, perhaps it is time for the more straightforward power function approach to replace the more dated *z* value

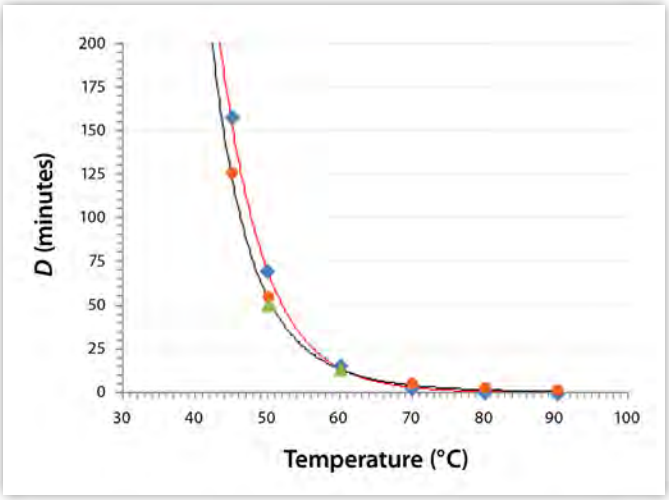

FIGURE 8. Predictive modeling of *D* at various temperatures using the power function approach (—●—) or the *z* value approach using 60°C as T_{ref} (—◆—). Two actual (measured) data points at 50°C and 60°C are shown (—▲—). (*D* values for FeCV reported in [10].)

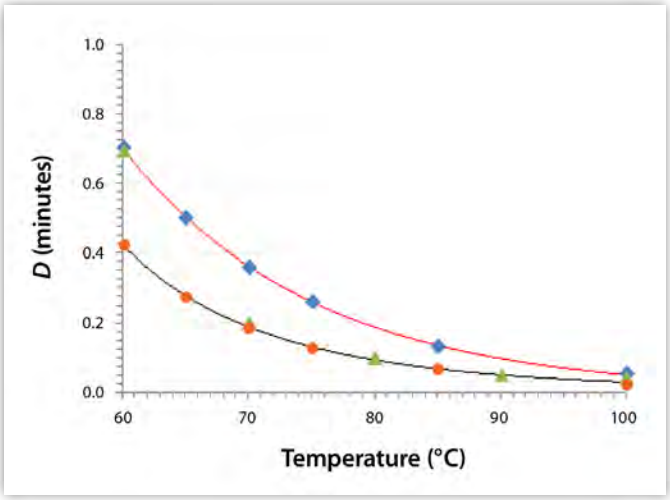

FIGURE 9. Predictive modeling of *D* at various temperatures, using the power function approach (—●—) or the z value approach using 60°C as T_{ref} (—◆—). Five actual (measured) data points at 60, 70, 80, 90, and 100°C are shown (—▲—). (*D* values for FMDV strain OPN reported in [14]).

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approach for this purpose.

In Table 3, we present the analysis of heat inactivation susceptibility for eight viruses from six families. Included in this presentation are the *z* values for each virus, the constants *a* and *b* of the power function approximating the *D* vs. temperature plots for each virus, and the R^2 values for the power function fits for these plots. Also shown are the two modeled heat inactivation parameters for each virus and the reference from which the *D* values underlying the analysis for each study were obtained (or estimated).

We are still searching, though, for a way to more easily compare inter-assay and inter-virus results for viral sensitivity to heat inactivation. We wondered, for instance, whether the constants *a* and *b* of the power function line fit equation for *D* vs. temperature might at all be predictive of heat sensitivity. Taking an approach similar to that described earlier for assessing the use of the *z* value as a predictor of heat sensitivity, we evaluated the association between power function constants *a* and *b* and two measures of the heat sensitivity of the viruses comprising our data set. The resulting R values for constant *a* were 0.30 and 0.22, respectively, and 0.44 and 0.34 for constant *b*, respectively. These correlation coefficients indicate that, at least for this limited set of viruses, there is little dependency between susceptibility to heat inactivation and the magnitude of the *a* and *b* constants of the power function relating *D* to temperature. Like the *z* value, these constants are more indicative of the shape of the *D* vs. temperature curve than they are of viral susceptibility to heat inactivation.

We are left using the two modeled parameters themselves as our best tools for conducting inter-study and inter-virus comparisons of heat inactivation efficacy. That each parameter is a similar reflection of heat inactivation

TABLE 3. Comparison of heat sensitivity of different viruses.

Virus	Family	Matrix	*z* (°C)	Power Function Parameters			Temperature (°C) for *D* = 0.5 min	*D* (minutes) at 80°C	Ref
				a	*b*	R^2			
Mouse Minute Virus	*Parvoviridae*	Growth Medium	31.3†	3.62×10^{9}	4.71	0.98	124	3.94	[15]
Feline Calicivirus	*Caliciviridae*	Phosphate-Buffered Saline*	14.3‡	9.90×10^{14}	7.80	1.00	91	1.42	[10]
		Growth Medium	9.3‡	3.43×10^{28}	15.9	0.94	65	0.019	[16]
		Growth Medium	15.3†	8.21×10^{16}	9.47	0.87	66	0.078	[17]
Murine Norovirus	*Caliciviridae*	Phosphate-Buffered Saline*	13.2‡	5.41×10^{15}	8.16	0.98	92	1.60	[10]
		Growth Medium	12.4‡	2.53×10^{21}	12.0	0.94	64	0.037	[16]
		Phosphate-Buffered Saline*	9.1†	4.79×10^{23}	13.1	0.98	68	0.056	[18]
Foot & Mouth Disease Virus (strain OPN)	*Picornaviridae*	Phosphate-Buffered Saline*	35.0‡	1.24×10^{9}	5.27	0.94	61	0.116	[14]
Hepatitis A Virus	*Picornaviridae*	Phosphate-Buffered Saline*	10.0‡	5.30×10^{25}	13.6	0.96	82	0.695	[10]
Infectious Pancreatic Necrosis Virus	*Birnaviridae*	Peptone Salt Medium*	9.9‡	7.26×10^{26}	13.9	1.00	90	2.56	[19]
Newcastle Disease virus	*Paramyxoviridae*	Fat-Free Egg Product	4.7‡	1.62×10^{50}	28.2	0.97	62	<0.001	[20]
Avian Influenza	*Orthomyxoviridae*	Fat-Free Egg Product	1.8‡	1.53×10^{108}	61.4	0.95	58	<0.001	[20]

* pH 7.0. † *D* values used to calculate the *z* value were estimated from the published inactivation vs. time plots.
‡ *D* values used to calculate the *z* value were reported in the reference.

efficacy is suggested by the correlation analysis of the two parameters (Figure 10) obtained for our limited data set of viruses (data from Table 3). The correlation coefficient (R) for these two modeled parameters was found to be 0.96.

Furthermore, when we examined the two modeled parameters for assessing inter-assay variability in heat inactivation for the caliciviruses, FeCV and MNV ($n = 3$ studies each; data are from Table 3), it became apparent (Table 4) that the results for parameter 1 are much less variable than the results for parameter 2. This suggests that perhaps the former will be more useful for conducting inter-study comparisons although, in the end, both modeled parameters may turn out to have some utility.

Conclusion

We have proposed the use of a straightforward approach for extrapolating heat inactivation data from measured to non-measured temperatures that is based not on the *z* value, but on a power function fit of the *D* vs. temperature plots. In order to conduct these modeling analyses, at least three temperatures must have been evaluated in the inactivation vs. time kinetics studies. For inter-assay and inter-virus comparisons of heat inactivation sensitivity, we propose the use of two modeled parameters: (1) *temperature required to inactivate 1 log_{10} of virus in 0.5 minutes;* and (2) *time required for 1 log_{10} reduction in infectivity at 80°C*. Using the two modeled parameters, we have calculated consensus heat inactivation values for two caliciviruses (FeCV and MNV).

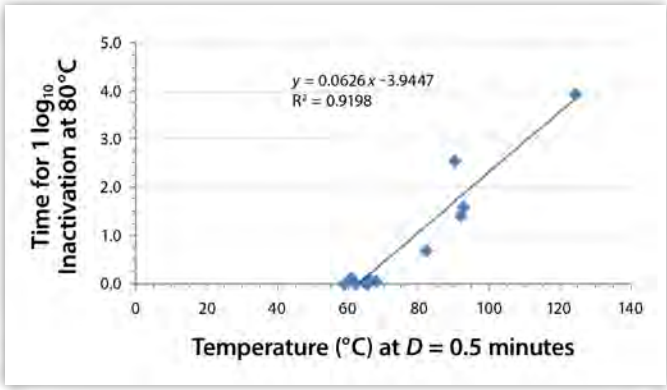

FIGURE 10. Correlation analysis for two modeled parameters proposed for comparing the heat inactivation susceptibilities of viruses. (Data from Table 3.)

TABLE 4. Inter-study variability in results for heat inactivation of two caliciviruses.

Temperature required to inactivate 1 log_{10} of virus in 0.5 minutes		
Statistic	**FeCV**	**MNV**
Mean ($n = 3$)	74.0°C	74.7°C
Standard Deviations	14.7°C	15.1°C
Relative Standard Deviations	20%	20%
Time required for 1 log_{10} reduction in infectivity at 80°C		
Statistic	**FeCV**	**MNV**
Mean ($n = 3$)	0.506 min	0.564 min
Standard Deviations	0.792 min	0.897 min
Relative Standard Deviations	157%	159%

Acknowledgment

The authors thank Dr. Deb Quick for help in rearranging the power function equation to facilitate solving for temperature.

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