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An Analysis of Vacuum Effects in the Sterilization of Microorganisms†

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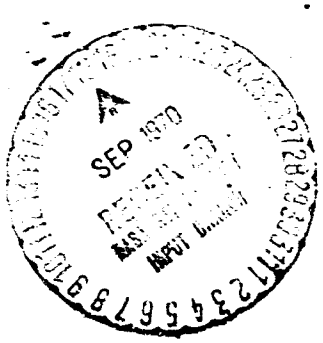
ABSTRACT

By using the thermodynamic formulation of the absolute reaction-rate theory, variations in pressure can be incorporated in a rational model for dry-heat sterilization of microorganisms. Coupling this model to observations, a $\Delta V^{\ddagger}$ value of 15 to 65 liters/mole is estimated for the sterilizing reaction. The theoretical $\Delta V^{\ddagger}$ for DNA is about 7 liters/mole.



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1. Introduction

The effects of vacuum on the sterilization of microorganisms by heat is of interest for planetary quarantine applications. It is not reasonable to assume that the 10^{-17} torr pressure of outer space can be attained in a terrestrial laboratory for the purpose of observing the effect on microorganisms. However, it is possible to carry out an analysis based on observations at greater pressures, which provides the parameters necessary for gaining some insight into the combined effect of vacuum and heat on microorganisms in outer space. In addition, evidence is presented that the sterilization mechanism for B. subtilis by heat is a monomolecular reaction on one or two vital macromolecules. This provides additional evidence to that which we provided (Brannen, to be published) for a thesis by Wax (1963), that in the sterilization of B. subtilis by heat, we are observing the action of heat on DNA in vivo.

2. Analysis and Model

The basic model for this work is the kinetic model discussed by this author (Brannen, 1968). The basic assumption is that, in a dry-heat environment, sterilization results from chemical reactions inside the microorganisms, and these reactions "in the small" have order. The Arrhenius formulation

$$k_r = A \exp(-E/T)$$

was used for the required reaction-rate constants. To investigate the effect of pressure, we shall use the thermodynamic formulation of the absolute reaction-rate theory. Here, k_r, the reaction-rate constant, is given by

$$k_r = \frac{kT}{h} \exp\left[-\frac{\Delta F^{\ddagger}}{RT}\right],$$

where h = Planck's constant, k = Boltzmann's constant, T is the temperature in degrees Kelvin, R is the gas constant, and $\Delta F^{\ddagger}$ is the Gibbs free energy.

A more complete formulation for $\Delta F^{\ddagger}$ is

$$\Delta F^{\ddagger} = \Delta H_0^{\ddagger} - T\Delta S_0^{\ddagger} + p\Delta V^{\ddagger} ,$$

where $\Delta H_0^{\ddagger}$ is the activation enthalpy and $\Delta S_0^{\ddagger}$ is the activation entropy, both at 0 pressure, where p is the pressure and $\Delta V^{\ddagger}$ is the volume change as the reactant goes into the activated state.

Usually, it is sufficient to set $\Delta V^{\ddagger} = 0$, which effectively includes the pressure effect on the reaction-rate constant in $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$. However, data from Dairs, Silverman & Keller (1962) suggests that, in some cases at least, the $p\Delta V^{\ddagger}$ term should not be neglected. For example, they report that, under a pressure of 10^{-8} torr and 60°C, only 40% of a population of B. subtilis survive after 5 days. This translates to 90% destruction in 12.5 days. On the other hand, Silverman (1966) reported a $\Delta H^{\ddagger}$ of 34.6 kcal/mole and a $\Delta S^{\ddagger}$ of 14.65 e.u. for the same organism under what one can presume to be a pressure of 1 atmosphere. Assuming, as did Silverman, 1 molecule being inactivated by a first-order reaction as the sterilization mechanism, and using his values for $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$, it follows that 123 days should be required for the 90% reduction at 60°C. In the same report, Silverman gives $\Delta H^{\ddagger}$ = 25,000 cal/mole and $\Delta S^{\ddagger}$ = -11.1 e.u. for data generated by Koesterer (1964). Using these values for $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ leads to a value of 24.8 days for 90% destruction at 60°C and 1 atmosphere.

From a 60°C, 1-atmosphere, 90% reduction value (D value), and a 60°C 10^{-8} torr D value, reaction-rate constants can be calculated by the formula

$$k_r = -\log_e (0.1)/D .$$

An estimate for $\Delta V^{\ddagger}$ can now be obtained by means of the formula

$$\Delta V^{\ddagger} = \frac{RT}{(p_2 - p_1)} \log_e \left(k_{p_1} / k_{p_2} \right) \qquad (1)$$

where p_1 and p_2 are pressures in atm, R is the gas constant in cc-atm, and k_{p_1} and k_{p_2} are the reaction-rate constants at pressures p_1 and p_2 (Johnson, Eyring & Polissar, 1954, p. 305).

In this manner it was found that, with $\Delta H^{\ddagger}$ = 34.6 kcal/mole and $\Delta S^{\ddagger}$ = 14.65 e.u.,

$$\Delta V^{\ddagger} = 62{,}480.7 \text{ cc/mole} ,$$

while, if $\Delta H^{\ddagger}$ = 25 kcal/mole and $\Delta S^{\ddagger}$ = -11.1 e.u.,

$$\Delta V^{\ddagger} = 18{,}770.1 \text{ cc/mole} .$$

Additional analysis without $\Delta H^{\ddagger}$'s and $\Delta S^{\ddagger}$'s can be carried out via equation (1), data presented by Silverman, Davis, and Keller (1964) and the relationship

$$\log_e \left(k_{p_1} / k_{p_2} \right) = \log_e \left(D_{p_1} / D_{p_2} \right) \qquad (2)$$

where D_p denotes D value at pressure p.

Silverman et al. report that after 5 days at 60°C, they found 72% survival for B. subtilis at 1 atm and 40% survival at 10^{-6} torr. Employing D values implied by these data in equation (1) and the equation (2) relationship yields $\Delta V^{\ddagger} = 28.1$ liters/mole. They also report that after 5 days at 88°C, they found 0.01% B. subtilis survival at 10^{-6} torr and, at 90°C, they found 0.55% survival in one experiment and 0.09% survival in another. Assuming no great difference between 88°C and 90°C as regards survival, these results give $\Delta V^{\ddagger} = 62$ liters/mole for the first experiment and 19.4 liters/mole for the second.

The $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values computed by Silverman for his and Koesterer's data roughly represent the extremes which one sees for B. subtilis. This plus the Silverman, Davis, and Keller results leads to the conclusion that $\Delta V^{\ddagger}$ probably lies between 15 and 65 liters/mole.

It was reported by Silverman, Davis, and Keller that after 5 days at 25°C there were 113% B. subtilis survivors under ultrahigh vacuum. This leads us to conclude that the vacuum effect alone is negligible and that it is the kinetic effect of heat plus vacuum which leads to increased sterilization rates.

The positive value for $\Delta V^{\ddagger}$ indicates that the activated state of the "vital molecule" is less ionized than the unactive state. Since $\Delta V^{\ddagger}$ is generally positive for unimolecular reactions (Johnson, Eyring & Polissar, 1954, p. 306), the positivity of the $\Delta V^{\ddagger}$ computed from the Silverman-Koesterer data is consistent with the assumption of a 1-molecule sterilization mechanism.

Assuming a unimolecular sterilization reaction, it is possible to approximate $\Delta V^{\ddagger}$ for a very wide range of pressures by the formula

$$\Delta V^{\ddagger} = \frac{0.1 b^{\ddagger} V}{\sum b + r_1 + r_2 + 1},$$

where $b^{\ddagger}$ is the length of the bond that is broken, $\sum b$ is the sum of the bond lengths in the chain, V is the molar volume and r_1 and r_2 are the covalent radii of the terminal atoms (Glasstone, Laidler & Eyring, 1941, p. 473). This formula is based on the assumption that the volume change from reactant to activated state is all due to length changes and that the molecular cross section remains constant.

Suppose the "vital" molecule responsible for sterilization is DNA. To compare a $\Delta V^{\ddagger}$ estimate for bond breakage in DNA with the $\Delta V^{\ddagger}$ computed from the Silverman-Koesterer data, we will assume DNA has 20,000 nucleotide pairs (White, Handler & Smith, 1964, p. 173), a molecular weight of 2.5×10^9 (Watson, 1955, p. 85), bond lengths of 3 Å (Pauling, 1960, p. 503), and a specific volume of 0.55 ml/g (Katz & Schachman, 1955).

Under these assumptions, the value of $\Delta V^{\ddagger}$ for DNA is approximately 7 liters/mole. This is in fair agreement with the $\Delta V^{\ddagger}$ computed from Koesterer's data, especially in view of the assumption that $\Delta V^{\ddagger}$ results solely from length changes. Since the volume of a mole of DNA is about 10^6 liters, these $\Delta V^{\ddagger}$'s represent a volume change of approximately 0.003%.

3. Applications

Having an approximation for $\Delta V^{\ddagger}$, pressure is easily incorporated as an environmental parameter in a kinetic sterilization model. One must be careful, however, in using the formula $\Delta F^{\ddagger}/RT = (\Delta H^{\ddagger} - T\Delta S^{\ddagger} + p\Delta V^{\ddagger})/RT$. Since $\Delta V^{\ddagger}$ is in cc/deg mole, $\Delta S^{\ddagger}$ in cal/deg mole, and $\Delta H^{\ddagger}$ in cal/mole, the gas constant R used with $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ should be in cal/deg mole, while with $\Delta V^{\ddagger}$, it should be in cc atm/mole.

It follows that the $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values computed by Silverman, from the point of view expressed here, actually include $p\Delta V^{\ddagger}$. After $\Delta V^{\ddagger}$ has been determined, it is possible to compute corrected values for $\Delta H^{\ddagger}$ assuming $\Delta S^{\ddagger} = \Delta S_0^{\ddagger}$. For the Silverman data,

$$\Delta H_0^{\ddagger} = 33.087 \text{ kcal/mole}$$

and, for the Koesterer data,

$$\Delta H_0^{\ddagger} = 24.545 \text{ kcal/mole} .$$

Using these corrected values for $\Delta H^{\ddagger}$ and the previously determined $\Delta V^{\ddagger}$'s, one is in a position to examine D values as a function of pressure. Table 1 shows comparison of D values computed for Silverman's and Koesterer's data at a temperature of 125°C and for pressures from 1.3×10^{-11} to 4 atm. (It was found in the computation that, with the available computing equipment, a pressure of 1.3×10^{-11} atm = 10^{-8} torr and 0 atm were indistinguishable.)

It is also of interest to examine D values at a low pressure as a function of temperature. Table 2 shows a comparison of calculated D values for Silverman's and Koesterer's data as a function of temperature at 1.3×10^{-11} atm.

4. Conclusions

The primary purpose of this paper is the presentation of a model and an analysis technique for considering pressure as an environmental parameter in dry-heat sterilization. The outcome of such calculations is entirely dependent on the input data, and the data used for this analysis were not generated with this analysis in mind. Consequently, one should hesitate to draw firm conclusions from this analysis. However, the $\Delta V^{\ddagger}$ value of 7 l/mole for DNA is within an order of magnitude of the 15- to 65-l/mole values computed for Silverman's and Koesterer's observations with B. subtilis. Under these conditions, the agreement is good. The fact that the lower $\Delta V^{\ddagger}$ value was obtained for DNA raises the question of what else in the microbe could lead to a greater $\Delta V^{\ddagger}$ value. We then are led to agree with Wax that, in the sterilization of B. subtilis by heat, we are likely observing effects on DNA in vivo.

The input data for this analysis were dry-heat sterilization data. From these, it followed that D values increased with pressure. Within limits, it has been found that this is true in solutions under hydrostatic pressure (Johnson & Zobell, 1949).

An examination of Table 2 suggests that a significant decrease in a spacecraft's bioburden can occur in outer space, provided the temperature can be maintained at above 50°C.

TABLE 1

Calculated 125°C D values as a function of pressure. It is assumed all other environmental parameters are constant.

Pressure (atm)	D values in minutes, based on Silverman's data	Koesterer's data
1.3×10^{-11}	4.3	35.5
0.2	6.3	40.2
0.4	9.2	45.5
0.6	13.5	51.4
0.8	19.8	58.2
1.0	29.0	65.8
1.4	62.3	84.3
2.0	196.4	122.0
2.4	422.3	156.1
3.0	1330.7	226.1
3.4	2860.3	289.3
4.0	9014.1	418.9

TABLE 2

D values calculated for a pressure of 1.3×10^{-11} atm = 10^{-8} torr as a function of temperature. It is assumed all other environmental parameters are constant.

Temperature (deg C)	D values in days, based on Silverman's data	Koesterer's data
0	902,643.0	52,327.0
10	100,920.0	10,227.0
20	13,088.0	2,232.0
30	1,940.0	538.0
40	324.6	142.0
50	60.6	40.6
60	12.5	12.5
70	2.8	4.1
80	0.69	1.45
90	0.18	0.537
100	0.052	0.21

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