

Comment on Effect of Heating on the Stability of Quinolones in Milk

In a recent paper published in this Journal, Roca et al. (1) examined the effect of different processing temperatures on the stability of five structurally homologous quinolones in milk. The paper reported that all of the quinolones examined were highly resistant to the heat treatments used in milk processing, and the authors concluded that the high stability of the compounds could cause a significant risk to human health because the residues of these antibiotics can be present in milk even after heat treatment, due to their high thermostability.

Whereas the authors clearly showed that the quinolones found in milk are very thermostable on the basis of their measurements of activation energy and half-life of thermal degradation at a given temperature, we found the kinetic results could be further analyzed to draw a thermodynamic basis of the high thermostability of the compounds. This approach has been found to be valuable in the study of molecular stability. For example, a recent paper (2) applied thermodynamic analysis to the examination of the stability of anthocyanin in blueberry juice during thermal treatment as an attempt to understand the degradation process. In this paper we show our thermodynamic analysis of quinolone degradation kinetics reported by Roca et al. (1) to elucidate the thermodynamic basis of the high thermostability of the structurally homologous quinolones in milk.

Using the kinetic data from the Roca paper (1) as a base, we first obtained the rate constants (k) using eq 1, where $t_{1/2}$ is the given half-life for each molecule/temperature pair as the degradation was shown to follow first-order kinetics. The half-life values ($t_{1/2}$) come from Table 3 of ref 1.

$$k = \frac{\ln 2}{t_{1/2}} \quad (1)$$

Once the k value was obtained, a rearranged version of the Eyring equation, eq 2, was applied to yield the standard molar Gibbs free energy of activation ($\Delta G^{\circ\ddagger}$) at temperature T

$$\Delta G^{\circ\ddagger} = -RT \ln \frac{kh}{k_B T} \quad (2)$$

where R is the gas constant, h is Planck's constant, and k_B is the Boltzmann constant (3). The resulting values of $\Delta G^{\circ\ddagger}$ as a function of temperature are shown in Figure 1A. As can be seen in Figure 1A, the linear plots are clearly defined, allowing us to be confident in the derivation of the thermodynamic variables such as standard molar enthalpy ($\Delta H^{\circ\ddagger}$) and entropy ($\Delta S^{\circ\ddagger}$) of activation using eq 3 as the slope and the y-axis intercept of the linear regression in Figure 1A represent $-\Delta S^{\circ\ddagger}$ and $\Delta H^{\circ\ddagger}$, respectively.

$$\Delta G^{\circ\ddagger} = \Delta H^{\circ\ddagger} - T\Delta S^{\circ\ddagger} \quad (3)$$

Because the basic structures of the five compounds are homologous, we speculated that a correlation existed between $\Delta H^{\circ\ddagger}$ and $\Delta S^{\circ\ddagger}$ throughout the molecules. To examine the correlation, $\Delta H^{\circ\ddagger}$ versus $\Delta S^{\circ\ddagger}$ was plotted (Figure 1B). A linear regression line in Figure 1B shows a clear relationship between the two thermodynamic

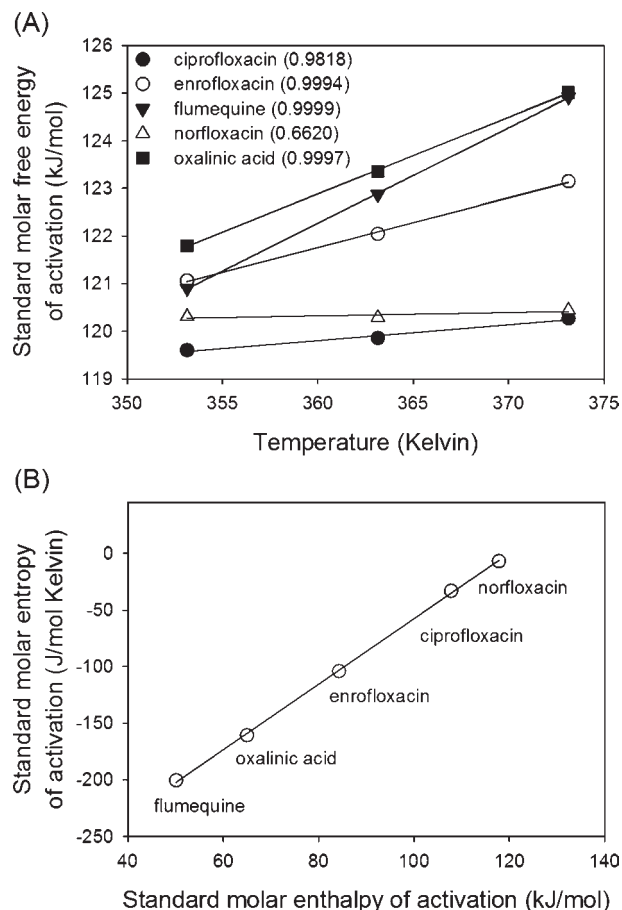


Figure 1. (A) Temperature-dependent standard molar free energy of activation of thermal degradation for five quinolones. A simple linear equation was fit to the data to obtain standard molar enthalpy and entropy of activation. R^2 of the fitting is shown in the figure legend. (B) Enthalpy and entropy of activation of thermal degradation for five quinolones. A simple linear equation ($y = ax + b$) was fit to the data. The fitting parameters a and b and their standard errors are 2.89 ± 0.03 and -346.9 ± 3.0 , respectively. R^2 of the fitting is 0.9996. Graph preparation and fitting was performed using SigmaPlot (version 11, Systat Software Inc., San Jose, CA).

parameters and suggests that the stability of these molecules is not driven solely by enthalpy or by entropy, but by a combination of both that varies greatly in proportion yet remains near constant in total value.

On the basis of the results, thermodynamic characteristics of the thermal degradation of quinolones can be deduced. First, the thermal degradation process is kinetically unfavorable in terms of both enthalpy and entropy throughout all of the quinolone compounds. Second, although all of the antibiotics exhibit high thermal stability, each compound has a different thermodynamic basis for the stability. For example, the stability of norfloxacin is

mainly attributed to its high value of $\Delta H^{\circ\ddagger}$, whereas the smallest value of $\Delta S^{\circ\ddagger}$ is the major reason for the stability of flumequine. Third, although the values of $\Delta H^{\circ\ddagger}$ and $\Delta S^{\circ\ddagger}$ of each quinolone vary widely throughout the antibiotics examined, the value of $\Delta G^{\circ\ddagger}$ shows a relatively small variation. The $\Delta H^{\circ\ddagger}$ values for the quinolones ranged from 50.1 to 117.8 kJ/mol and the $\Delta S^{\circ\ddagger}$ values ranged from -7.0 to -200.4 J/(mol Kelvin), whereas the $\Delta G^{\circ\ddagger}$ values are from 119.9 to 123.4 kJ/mol at 90 °C. This indicates that the structural variation in each quinolone compound can cause a significant change in either $\Delta H^{\circ\ddagger}$ and $\Delta S^{\circ\ddagger}$ in the thermal degradation, but there is no significant change in $\Delta G^{\circ\ddagger}$ due to the compensatory behavior of $\Delta H^{\circ\ddagger}$ and $\Delta S^{\circ\ddagger}$. This phenomenon, called enthalpy–entropy compensation, is often observed in chemical and biological systems, for example, thermal degradation of chlorophyll (4) and molecular interactions (5–7). According to our analysis, it is apparent that enthalpy–entropy compensation is responsible for the relatively small variation in $\Delta G^{\circ\ddagger}$ of the thermal degradation of the antibiotics.

In conclusion, our thermodynamic analysis indicates that the thermal stability of the quinolones is determined by the combination of enthalpy and entropy and that the contributions of each thermodynamic parameter depend on the structure of quinolones. Although there can be a wide variation in the values of enthalpy and entropy of activation in the thermal degradation of quinolones, the free energy of activation is relatively constant due to enthalpy–entropy compensation. Recently, several additional derivatives of quinolones were reported (8–10). It will be interesting to verify if their thermal stability is also described by enthalpy–entropy compensation.

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