Letters to the Editor

To the Editor:

The recent paper entitled "Influence of Temperature and Hydrophobic Group-Associated Icebergs on the Activation Energy of Drug Decomposition and Its Implication in Drug Shelf-Life Prediction" by A. K. Kishore and J. B. Nagwekar (1) presents a number of specious arguments regarding the role of "icebergs" in the formation of activated complexes in the hydrolysis of aspirin. These arguments are based on changes in enthalpies and entropies of activation with temperature obtained at various pH values and over a 30–70°C temperature range. This letter challenges the conclusions in that paper and the authors' interpretation of the data leading to such conclusions.

Because the lengthy discussion on the role of hydrophobic group-associated icebergs in activation energies rests on the reliability of the activation parameter values reported, these values must first be carefully examined. Of greatest concern is the authors' conclusion that E_a values obtained at 30–40, 45–55, and 60–70°C are significantly different from each other. No description of the tests employed for determining statistical significance of these differences was provided, but it is clear from an examination of the standard deviations of the E_a values in their Table II that some of these apparent E_a values are probably not significantly different at the levels indicated ($P < 0.01$). For example, the paper suggests that E_a values of 19.33 ± 1.17 (SD) and 20.26 ± 0.5 are significantly different, with a $P < 0.01$. Since the value of 20.26 lies within 1 standard deviation of the 19.33 value, and these values appear to be based on single determinations of slope, it is unlikely that these results differ significantly. Most alarming is the fact that there are significant discontinuities in the Arrhenius plots shown in their Figs. 1 and 2 which are not addressed by the authors. Examining their Fig. 2, for example, one observes that the lines through the 60–70°C data and the 45–55°C data do not meet. To connect these two lines, an E_a of three to four times the values reported over these two ranges would be required for the range between 55 and 60°C. Clearly, it would be nonsense to suggest such wild fluctuations in E_a over such a narrow temperature range, but that is what the data in their Fig. 2 imply! A more rational hypothesis is that experimental errors in the data are too large to draw conclusions regarding the changes in E_a or in enthalpy and entropy of activation with temperature. Without significance in these parameters, there is no basis for the conjecture regarding the role of water in the activated complex.

In their section entitled "Diagnostic Test for the Role of Water," the authors state, "If the differences in the Ea values noted in the present study were due to the proposed role the icebergs around the hydrophobic groups play in the formation of the activated complexes, it follows that there should exist a linear relationship between the differential enthalpy of activation ($\Delta\Delta H^*$) and the differential entropy of activation ($\Delta\Delta S^*$), representing the activation parameters due mainly to the net release of water molecules in the formation of the activated complexes." They then show in their Fig. 3 that such a linear relationship exists and argue that this supports a role for icebergs associated with hydrophobic groups in the formation of the activated complex. However, one must be extremely cautious in testing such relationships, as the error in ΔS^* is linearly related to the error in ΔH^* (2). The burden of proof is on the investigators to rule out alternative explanations for linear relationships such as that shown in Fig. 3 of their paper.

Although it may not be central to their conclusions (which we believe to be without foundation), Scheme I in their paper describes a mechanism for the intramolecular nucleophilic catalysis of aspirin hydrolysis that may have been the accepted mechanism prior to 1967 but was cast aside (at least for reactions in water) by Fersht and Kirby in their 1967 paper on the hydrolysis of aspirin entitled "The Hydrolysis of Aspirin. Intramolecular General Base Catalysis of Ester Hydrolysis" (3). The authors fail to cite this paper or any literature on the hydrolysis of aspirin later than 1957.

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To the Editor:

In the above Letter to the Editor, Anderson and Darrington (1) criticize our recent paper (2) and challenged our interpretation of the data and conclusions.

The greatest concern Anderson and Darrington (1) express is that we concluded that the E_a values obtained at 30–40, 45–55, and 60–70°C for each hydrolysis reaction are significantly different from each other, without providing the description of the tests that we employed for determining statistical significance of these differences. The test that we employed to determine if the slope of the plot of log k vs $1/T$ at one temperature range was significantly different from the slope of such a plot at another temperature range for a given mechanism of aspirin hydrolysis was Student's t test (3). For example, we noted that $t = 7.94$ (with df = 35) for the hydronium ion-catalyzed reaction and $t = 3.3$ (with df = 27)

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for the intramolecularly catalyzed reaction, when the slope obtained for the 30–40°C temperature range was compared with the slope obtained for the 45–55°C temperature range, in the case of each reaction. It may be mentioned that $t_\alpha(2),35 = 2.724$ and $t_\alpha(2),27 = 2.771$ were needed for $P < 0.01$ in these cases.

After establishing that the slopes were significantly different from each other for each reaction, we computed the slope values with their standard deviations by linear regression using the Minitab (4) computer program. We also calculated the standard deviation of each slope according to a general procedure described elsewhere (5). The standard deviations of the slopes determined by both approaches were similar. The standard deviations of E_a were calculated by multiplying the standard deviations of the slopes by 1.987 cal/mol, as already reported in our paper. The significance of the differences between the E_a values was determined using Student's t test (6).

Anderson and Darrington (1) consider that the discontinuities in the Arrhenius plots, especially between the 45–55°C and the 60–70°C plots for the hydroxyl ion-catalyzed reaction, are significant, and they suggest that those discontinuities could be due to experimental errors. If we had also determined the reaction rate constants in the 55–60°C temperature range and shown those points in the Arrhenius plots, the discontinuities between the 45–55°C and the 60–70°C Arrhenius plots might not have appeared so conspicuous. As we pointed out on page 735 of our paper (2), when we calculated E_a from rate constants determined in the entire temperature range of 30–70°C, the value of E_a^{30-70} was 8.98 kcal/mol for the hydroxyl ion-catalyzed reaction, and the correlation coefficient for the regression line was >0.99, which most probably would not have been the case if the discontinuities between the Arrhenius plots were truly out of phase. Further, the E_a values determined at three different temperature ranges for each aspirin hydrolysis reaction were significantly different from the respective E_a^{30-70} value calculated for each reaction (2). Since the slope value of each Arrhenius plot was based on at least 15 individual rate constants, with three individual rate constants determined at each temperature, it would tend to minimize the influence of possible errors in the determination of one or two rate constants.

From the section in our paper (2) entitled "Diagnostic Test for the Role of Water," Anderson and Darrington (1) quote only one sentence, and omit the qualifying statements at the beginning and the end of the section. We stated, "A reaction, which involves a single water structure-making or water structure-breaking process, is characterized by a linear relationship between its enthalpy and entropy values. Further, the value of the linear slope, which is referred to as the compensation temperature (T_C), generally lies in the 250–320 K range" (see Ref. 18 in that paper or Ref. 7 in this letter). We further state, "As shown in Fig. 3, the plot of $\Delta\Delta H^*$ vs $\Delta\Delta S^*$ was found to be linear ($r = 0.999$) with T_c equal to 307°K." Thus, our conclusion regarding the role of icebergs associated with hydrophobic groups in the formation of the activated complex was drawn not only because we found a linear relationship between the differential enthalpy ($\Delta\Delta H^*$) and the differential entropy ($\Delta\Delta S^*$) but also because the linear relationship was accompanied by a T_c value of 307°K, which was within the T_c range of 250–320°K (7). Incidentally, there are many reactions that may yield a linear relationship between ΔH^* and ΔS^*, but the slope values of such linear curves will be far different from the T_c range mentioned above if water did not play a direct role in the reactions.

As Anderson and Darrington (1) point out, we indeed failed to notice that the hydrolysis of aspirin at pH 6.2 is due to the intramolecular general base catalysis by the carboxylate group as proposed by Fersht and Kirby (8), and not to the kinetically equivalent mechanism of intramolecular nucleophilic catalysis. I regret this omission and thank them for pointing this out to us. Fortunately, this omission does not seriously affect the interpretation of our results as presented in that paper (2).

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