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# Thermodynamics of the hydrolysis reactions of nitriles

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## Abstract

Microcalorimetry and high-performance liquid chromatography (h.p.l.c) have been used to conduct a thermodynamic investigation of the following nitrilase catalyzed reactions: (1) benzonitrile(aq) + $2H_2O(l)$ = benzoic acid(aq) + ammonia(aq), (2) benzylcyanide(aq) + $2H_2O(l)$ = benzeneacetic acid(aq) + ammonia(aq), (3) 3-phenylpropionitrile(aq) + $2H_2O(l)$ = 3-phenylpropanoic acid(aq) + ammonia(aq), (4) 4-phenylbutyonitrile(aq) + $2H_2O(l)$ = 4-phenylbutyric acid(aq) + ammonia(aq), (5) α-methylbenzyl cyanide(aq) + $2H_2O(l)$ = α-methylbenzene acetic acid(aq) + ammonia(aq), and (6) 3-indoleacetonitrile(aq) + $2H_2O(l)$ = indole-3-acetic acid(aq) + ammonia(aq). The equilibrium measurements showed that these reactions proceeded to completion. Thus, it was possible to set only lower limits for the values of the apparent equilibrium constants $K'$. However, it was possible to obtain precise values of the calorimetrically determined molar enthalpies of reaction $\Delta_r H_m$(cal). These values were then used in conjunction with an equilibrium model to calculate values of the standard molar enthalpies for chemical reference reactions that correspond to the above overall biochemical reactions.

*Keywords:* Ammonia; Apparent equilibrium constant; Benzeneacetic acid; Benzoic acid; Benzonitrile; Benzylcyanide; Enthalpy; Entropy; Gibbs free energy; Indole-3-acetic acid; 3-Indoleacetonitrile; α-Methylbenzeneacetic acid; α-Methylbenzyl cyanide; 4-Phenylbutyonitrile; 4-Phenylbutyric acid; 3-Phenylpropanoic acid; 3-Phenylpropionitrile

## 1. Introduction

The nitrilase enzymes catalyze the direct hydrolysis of organic nitriles to the corresponding carboxylic acids, which are potentially useful as intermediates in the agricultural and pharmaceutical industries. The use of nitrilase enzymes has attracted substantial interest in the biotechnology community [1–4] because conventional chemical methods for nitrile hydrolysis entail the use of severe conditions such as the use of concentrated acid or base and high temperatures. Such harsh conditions are generally not useful when sensitive complex molecules, or chiral compounds are involved. Most importantly, the chemical methods do not permit asymmetric synthesis. In contrast, the nitrilase-catalyzed reactions proceed under mild conditions and produce a high yield of a stereospecific product. The importance of nitrilase enzymes has led recently to the development of a library consisting of over 200 new forms of this enzyme [3] (see Figure 1).

Having an active catalyst is central to making the desired bioprocess possible. However, a proper engineering of the process requires thermodynamic information in order to optimize the product yield and to obtain a correct heat balance in a bioreactor. Interestingly, there do not appear to be any thermodynamic results in the literature for the hydrolysis of nitriles. Accordingly, we have performed a thermodynamic investigation of several representative nitrilase-catalyzed reactions:

$$\text{benzonitrile(aq)} + 2H_2O(l) = \text{benzoic acid(aq)} + \text{ammonia(aq)}, \qquad (1)$$

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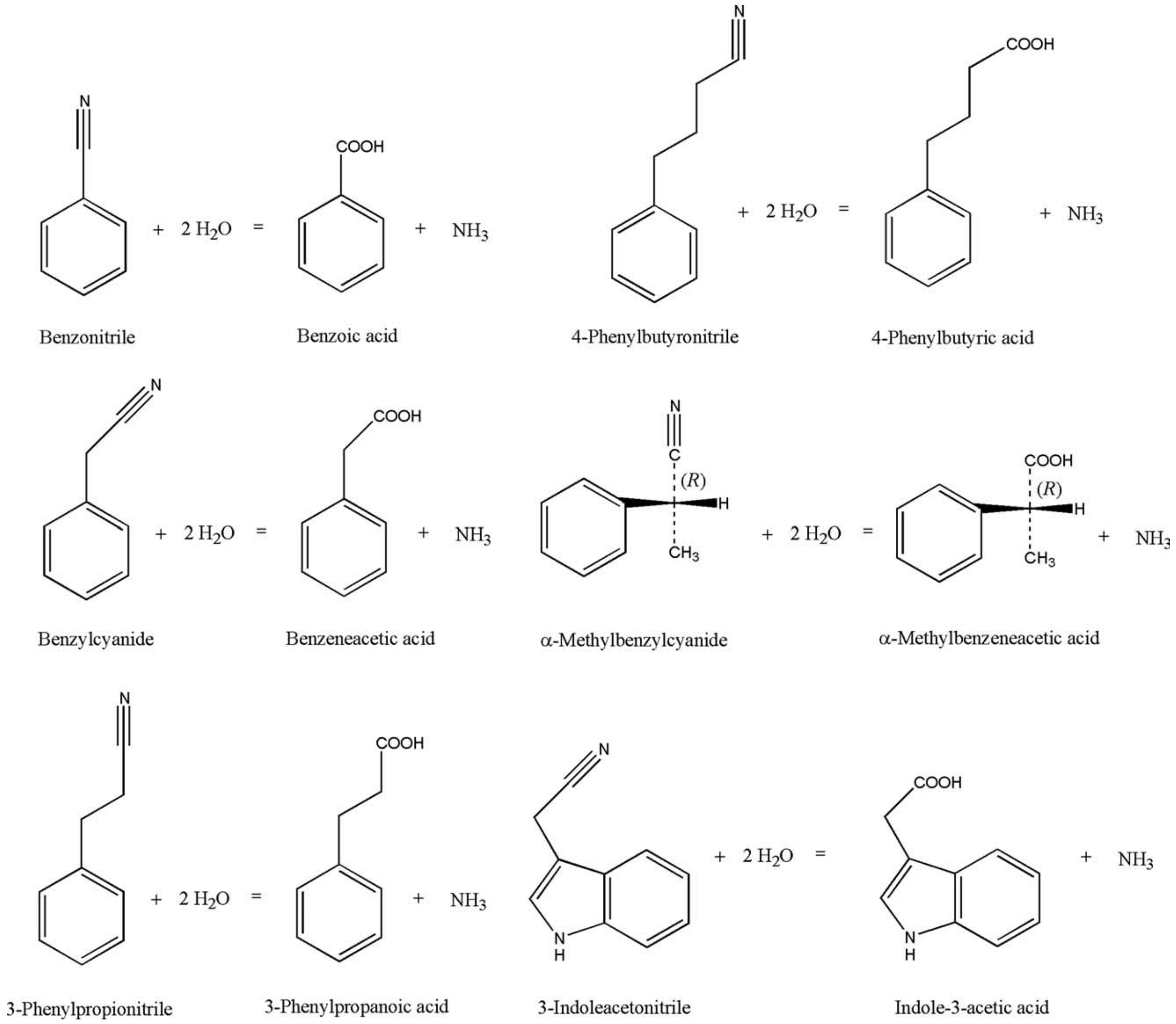

FIGURE 1. The structures of the substances and the reactions studied herein.

benzylcyanide(aq) + $2H_2O$(l) =

benzeneacetic acid(aq) + ammonia(aq), (2)

3-phenylpropionitrile(aq) + $2H_2O$(l) =

3-phenylpropanoic acid(aq) + ammonia(aq), (3)

4-phenylbutyonitrile(aq) + $2H_2O$(l) =

4-phenylbutyric acid(aq) + ammonia(aq), (4)

α-methylbenzyl cyanide(aq) + $2H_2O$(l) =

α-methylbenzene acetic acid(aq) + ammonia(aq), (5)

3-indoleacetonitrile(aq) + $2H_2O$(l) =

indole-3-acetic acid(aq) + ammonia(aq). (6)

## 2. Experimental

### 2.1. Materials

Pertinent information on the substances used in this study is given in table 1.[1] The purities of the nitriles were assessed by using a Hewlett Packard 5890 g.c. equipped with a flame ionization detector and a fused silica Phenomenex ZB-FFAP capillary column (30 m long, 0.53 mm i.d.). The injector and detector temperatures were (523 and 540) K, respectively. The head pressure

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[1] Certain commercial equipment, or instruments, or materials are identified in this paper to specify the experimental procedures adequately. Such identification is not intended to imply recommendation, or endorsement by the National Institute of Standards and Technology (NIST), nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose.