



# Thermochemical investigations of hydrolysis of *p*-nitrophenyl acetate in water–acetonitrile mixtures

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## Abstract

The  $\alpha$ -chymotrypsin-catalyzed hydrolysis of the *p*-nitrophenyl acetate in the solvent mixtures containing from 1.6 up to 10% (v/v) acetonitrile in the presence of aqueous Tris buffer at pH 8.0 was investigated at 298 K by use of an isoperibolic batch calorimeter. A special experimental arrangement of the reaction components for the investigation of the hydrolytically instable substrate was used. Furthermore, the release of *p*-nitrophenol was recorded with an UV–vis-spectrophotometer under comparable conditions. The calorimetric curves consist of two parts. The first part is strongly rising and finished by a break point in the  $\Delta T$  (time) curve. This first step is dominated by the enzyme-catalyzed reaction. After the break point a slow non-enzymatic process determines the course of the calorimetric curve. The molar enthalpy changes of overall reaction (ester hydrolysis and buffer protonation) of  $-100 \pm 8$ ,  $-106 \pm 5$  and  $-102 \pm 5$  kJ/mol were evaluated by a combination of the results from the spectrophotometric and calorimetric data for 1.6, 4.0 and 10.0% acetonitrile mixtures, respectively. The obtained results indicate that the enzyme-catalyzed hydrolysis is suitable for quantitative determination of the hydrophobic ester *p*-nitrophenyl acetate in water–acetonitrile mixtures using calorimetric detection.

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## 1. Introduction

The use of enzyme-catalyzed reactions in water–organic mixtures is highly promising for basic research and biotechnology [1–4]. The application of enzymes in biosensors in organic media is also a topic of continuing interest [5,6]. Such systems have a number of advantages compared to enzymes in aqueous solutions. The most important advantages are the higher solubility of hydrophobic substances (fats, oils, steroids, esters), and the solvent effect on the enzyme-catalyzed reactions [7–10]. Hence the analysis of the thermodynamic and kinetic aspects of biocatalysis in water–organic mixtures appears important.

Since practically all chemical reactions have a nonzero enthalpy change, the heat power accompanying the enzyme-catalyzed reactions in various water–organic mixtures might be a very informative property of the intermolecular processes influencing the activity of enzymes at such unusual conditions. Calorimetry is a reliable method to determine quantitatively this thermokinetic property of a reaction and to obtain information about the reaction rate [11–13]. There are a number of reports on the enthalpy of enzyme-catalyzed hydrolytic reactions in water at different pH value and buffer system [11,14–19]. However, information on the enzymatic reaction enthalpy in the presence of significant concentrations of organic solvents is rather limited. For example, at present time there are only two reports on calorimetric studies of the reactions catalyzed by the archetypical hydrolase  $\alpha$ -chymotrypsin (in 3% (v/v) [17] and 3–7% (v/v) [16] ethanol). Experimental data for the enthalpy of enzyme-catalyzed

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