Kinetics and Thermodynamics of Enzymatic Reactions from Spectrophotometric in Situ Measurements

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Enzymes are proteins acting as biocatalysers in the metabolic networks of the living systems. Enzymatic reactions can be widely described both kinetically and thermodynamically as a function of environmental conditions. Calorimetry has shown to be quite appropriate for the analysis of these properties although it generally requires complementary spectroscopic analysis to detail the initial and final states of the system. Therefore, using a spectroscopic technique in situ coupled to calorimetry could be a very useful tool to obtain conclusive results about the physical chemical properties of chemical reactions. Spectrophotometry can be regarded as a technique to be explored since biochemicals are frequently quantified by absorbance measurements in the ultraviolet-visible region of radiation. Kinetics and thermodynamics of enzymatic reactions are proposed to be studied in this work by a novel experimental technique using in situ spectrophotometric measurements with an optic fibre. This non-invasive optic probe allows analyzing in situ the reaction evolution and the equilibrium at controlled conditions of mixing and temperature. The first results of this research are shown here for two enzymatic reactions found in glycolysis, a ubiquitous metabolic pathway in nature which recovers some biochemical energy by starting the conversion of the monosaccharide glucose in living cells. Hexokinase (E.C. 2.7.1.1) and pyruvate kinase (E.C. 2.7.1.40) are, respectively, the first and the last enzymes of glycolysis catalysing phosphate transfers. Both enzymatic reactions are studied in this work and their enthalpies and Gibbs energies are derived as a function of pH, ionic strength and temperature.