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ORIGINAL ARTICLE

Modelling aldehyde oxidase activity in aqueous-organic solvent mixtures at various temperatures

A. Jouyban [a,*], M. Dehghany [b], M.R. Rashidi [c], Gh. Dehghan [d], M. Khoubnasabjafari [e]

[a] Drug Applied Research Center and Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz 51656, Iran
[b] Liver and Gastrointestinal Diseases Research Center, Tabriz University of Medical Sciences, Tabriz 51656, Iran
[c] Research Center for Pharmaceutical Nanotechnology, Tabriz University of Medical Sciences, Tabriz 51656, Iran
[d] Faculty of Science, Tabriz University, Tabriz 51664, Iran
[e] Tuberculosis and Lung Disease Research Center, Tabriz University of Medical Sciences, Tabriz 51656, Iran

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Abstract A model is proposed to represent the enzyme activity ratios in water – organic solvent mixtures at various temperatures. The prediction capability of the model was evaluated employing aldehyde oxidase as a model enzyme in seven water – organic solvent mixtures at 25, 35 and 45 °C by using mean percentage deviation (MPD). The MPD obtained for each water – organic solvent mixture was 6.2%. A general model was also proposed employing the Abraham solvent parameters with MPD as 19.5%. The proposed models could be used in industry for speeding up the process designs where water – organic solvent mixtures at various temperatures were employed.

1. Introduction

Water is the unique solvent for biological systems and life of living organisms is lost in the absence of water. Although a number of biochemical reactions are carried out in the non-aqueous parts of the cells, e.g. membranes, aqueous phase plays vital roles in the cell biology. Most of the common organic solvents are toxic and could not be used *in vivo*. However, there are many new applications for enzymatic activity in non-aqueous media (Dordick, 1991) and as an example these media are frequently used in biotechnological reactions in the industry where enzymes are employed in the synthesis of biological products. Conducting some of these reactions in aqueous media are restricted due to low solubility of the substrate or product in water, enzyme recovery from reaction medium, low reaction yield, unfavourable thermodynamic equilibria and enzyme inhibition by the products. Concerning these problems, water – organic solvent mixtures and even non-aqueous medium (Karyakin et al., 1996) could overcome some of these problems and non-aqueous enzymology has a valuable position in biotechnological research.

Addition of organic solvents to the aqueous reaction medium usually decreases the activity of the enzymes (Jouyban

* Corresponding author. Tel.: +98 411 3379323; fax: +98 411 3363231.
E-mail address: ajouyban@hotmail.com (A. Jouyban).
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et al., 2009; Girard and Legoy, 1999; Kermasha et al., 2001) and in some cases it increases enzyme activities (Okazaki et al., 2000; Arroyo et al., 1999). In addition to altering the enzyme activities, the presence of organic solvents could change the stability of the enzymes (Amini et al., 2011), enhance the yield of enzymatic reactions (Tsai et al., 2006), and change enzyme specificity (Schumacher et al., 2006).

In a previous work (Jouyban et al., 2009), a mathematical model was proposed to represent the effect of solvent composition on the enzymatic activity in water – organic solvent mixtures. The applicability of the model on real activity data has been checked using the generated activity of xanthine oxidase in aqueous mixtures of ethanol, 1-propanol, 2-propanol, acetonitrile, dioxane and N,N-dimethylformamide at 25 °C. In practical applications of enzymes in water – organic solvent mixtures, temperature is another parameter to be considered and the trial and error approach is used to find the optimum solvent composition and temperature. Any model representing the simultaneous effects of these variables provides a useful tool for biotechnologists to speed up the process of optimization. The main purpose of this communication is to propose a mathematical model for representing the enzyme activity ratios in mixed solvent systems concerning solvent composition and temperature. To check the accuracy of the model, the activity of aldehyde oxidase (EC 1.2.3.1: aldehyde: oxygen oxidoreductase) in water – organic solvent mixtures at 25, 35 and 45 °C was employed as a model system.

2. Materials and methods

2.1. Chemicals

Phenanthridine was obtained from Sigma–Aldrich (Poole, Dorset, England). All other chemicals were purchased from Merck (Darmstadt, Germany).

2.2. Preparation of partially purified aldehyde oxidase

White New Zealand rabbits weighing 2.0–2.5 kg were obtained from the animal house of Tabriz University of Medical Sciences, Iran. The animals were fed with a standard diet and allowed food and water *ad libitum*. The temperature and humidity were kept at 18 ± 1 °C and 50%, respectively and the lighting cycle was 7:00–19:00 h light and 19:00–7:00 h dark. Animals were handled with human care in accordance with the National Institute of Health guidelines and the study was approved by the local and national ethics committees. The animals were killed between 9:00 and 10:00 am under general anaesthesia; the livers were immediately excised, placed in an ice-cold isotonic potassium chloride solution (1.15% KCl w/v) containing 0.1 mM EDTA and homogenized on ice in a homogenizer fitted with a Teflon pestle. Partially purified enzyme was prepared from the liver homogenate by heat treatment and ammonium sulphate precipitation as described by Johnson et al. (1985).

2.3. Enzyme assay

All spectrophotometric determinations were carried out using a Shimadzu 2550 UV/Vis spectrophotometer. The instrument was connected to a Shimadzu cell temperature control. Aldehyde oxidase activity was measured spectrophotometrically at 322 nm through monitoring the production of phenanthridinone from phenanthridine as the substrate in the presence of molecular oxygen as the electron acceptor (Sorouraddin et al., 2008). Phenanthridine (20 μM) was incubated with the enzyme fraction in Sorenson's phosphate buffer (67 mM, pH 7.4) containing 0.1 mM EDTA and the oxidation rates were measured up to 5 min.

2.4. The assay of aldehyde oxidase activity in organic solvents

The activity of aldehyde oxidase was determined in different water – organic solvent media at 25, 35 and 45 °C as described in Section 2.3. The solutions of organic solvents were prepared in phosphate buffer and their concentration was varied from 0% to a concentration that gave almost complete inhibition of the enzyme activity. The solvents tested were acetonitrile, 1-propanol, 2-propanol, ethanol, methanol, tetrahydrofuran and 1,4-dioxane. The ratio of the measured activity in water – organic solvent mixture to that of the aqueous solution was calculated. The activity was also measured at 25, 35 and 45 °C in the presence of each organic solvent. With all organic solvents, the activity was increased by an increased temperature. Usually, an enzyme activity increases by increasing temperature up to its optimum temperature. Aldehyde oxidase is relatively a thermal stable enzyme, so that the enzyme is treated at 55 °C for 10 min during its purification process (Pirouzpanah et al., 2009). Therefore the enzyme activity was measured up to 45 °C to achieve an enhanced activity without denaturation of the enzyme.

2.5. Computational methods

In an earlier work (Jouyban et al., 2009), a mathematical model was proposed for describing the solvent effects on the residual activity of an enzyme in water – organic solvent mixtures at isothermal condition. On the other hand, temperature is another affecting factor on the enzyme activity (Amini et al., 2011). A model for representing both organic solvent concentration and temperature effects on the activity of an enzyme are more practical and of great importance in the industrial applications. It is obvious that organic solvents and temperature will affect the stability of an enzyme as well and a fine optimization of these parameters is required for a cost-benefit process design. The Jouyban-Acree model (Jouyban, 2008) was employed to represent the effects of organic solvent concentration and temperature on various physico-chemical properties including solubility of drugs and also some other parameters such as acid dissociation constants, electrophoretic mobility in capillary electrophoresis and retention factors in high performance liquid chromatography, the dielectric constants, viscosity, solvatochromic parameter, density, speed of sound, molar volumes, thermodynamic parameters and fluorescent intensity of probes. The general version of the model is:

$$\ln \mathrm{PCP}_{m,T} = f_1 \ln \mathrm{PCP}_{1,T} + f_2 \ln \mathrm{PCP}_{2,T} + f_1 f_2 \sum_{i=0}^{2} \frac{J_i (f_1 - f_2)^i}{T} \quad (1)$$

where $\mathrm{PCP}_{m,T}$, $\mathrm{PCP}_{1,T}$ and $\mathrm{PCP}_{2,T}$ are the numerical values of the physico-chemical property of the mixture and solvents 1 and 2 at temperature T (expressed in K), f_1 and f_2 are the