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**Journal of King Saud University –
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ORIGINAL ARTICLE

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

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Received 1 October 2012; accepted 11 February 2013

Available online 27 February 2013

KEYWORDS

Enzyme activity;
 Jouyban-Acree model;
 Solvent effects;
 Temperature effects;
 Abraham solvent parameters

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.

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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

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Peer review under responsibility of King Saud University.



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. Alde-

hyde 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 (Pirou-zpanah 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:

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

where $\text{PCP}_{m,T}$, $\text{PCP}_{1,T}$ and $\text{PCP}_{2,T}$ 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

fractions of solvents 1 and 2, respectively and J_i represents the model constants. Eq. (1) could be modified as:

$$\ln V_{m,T} = f_1 \ln V_{1,T} + f_2 \ln V_{2,T} + f_1 f_2 \sum_{i=0}^2 \frac{M_i(f_1 - f_2)^i}{T} \quad (2)$$

in which $V_{m,T}$, $V_{1,T}$ and $V_{2,T}$ are the initial rates in water – organic mixtures, in mono-solvents 1 and 2, respectively, and M_i is the model constants. For representing the enzyme activity ratios in water – organic solvent mixtures ($\alpha_{m,T}$) at various temperatures, since $(\ln \alpha_{1,T} = \ln \frac{V_{1,T}}{V_{1,T}} = \ln 1 = 0)$, therefore the first term ($f_1 \ln \alpha_{1,T}$ or $f_1 \ln V_{1,T}$) could be eliminated from the model, on the other hand, numerical values of $V_{2,T}$ could not be measured for most of enzymes due to deactivation of the enzyme and $\ln \alpha_{2,T}$ is not a defined value. By further assuming the Arrhenius relationship to be valid, $\ln \alpha_{2,T} = A_1 + \frac{A_2}{T}$ if no enzyme deactivation occurs (in our experiments up to $f_2 = 0.05$ to 0.2 for different organic solvents), one could replace $f_2 \ln \alpha_{2,T}$ term with a function of temperature (i.e. $A_1 f_2 + \frac{A_2 f_2}{T}$), then Eq. (2) is simplified to:

$$\ln \alpha_{m,T} = A_1 f_2 + \frac{A_2 f_2}{T} + f_1 f_2 \sum_{i=0}^2 \frac{M_i(f_1 - f_2)^i}{T} \quad (3)$$

in which A_1 , A_2 and M_i are the model constants.

The mean percentage deviation (MPD) values have been calculated using the following equation as an accuracy criterion:

$$MPD = \frac{100}{N} \sum \left| \frac{(\alpha_m)_{calculated} - (\alpha_m)_{observed}}{(\alpha_m)_{observed}} \right| \quad (4)$$

where N is the number of data points in each set.

3. Results and discussion

The enzyme activity ratios of aldehyde oxidase in water – organic solvent mixtures at 25, 35 and 45 °C are reported in Table 1. Each datum is mean value of at least three repetitive experimental measurements. The activity at a given temperature was decreased with an increase in the concentration of the cosolvent and 1,4-dioxane is the best tolerated cosolvent in which the activity ratio in 5 v/v% at 25 °C is ~74% and the enzyme is still active in 20 v/v% with the activity ratio of 25%. Methanol is the most active inhibitor of aldehyde oxidase among investigated cosolvents. By addition of 0.5 v/v% of methanol, the activity of the enzyme is reduced to 62% of initial activity and the activity ratio is 12% after addition of 5 v/v% of methanol. The activity ratio of the enzyme in 2-propanol at 25 °C (at $f_2 = 0.10$, 29%) is less than the corresponding value for 1-propanol (i.e. 31%) and the observed pattern is

Table 1 Experimental activity ratios of aldehyde oxidase in various water – organic solvent mixtures at 25, 35 and 45 °C.

f_2	25	35	45	25	35	45
<i>Dioxane</i>			<i>Acetonitrile</i>			
0.000	1.00	1.00	1.00	1.00	1.00	1.00
0.050	0.74	0.60	0.45	0.58	0.67	0.73
0.100	0.48	0.39	–	0.33	0.41	0.58
0.150	0.41	0.22	0.17	0.11	0.18	0.22
0.200	0.25	0.14	0.10			
<i>1-Propanol</i>			<i>2-Propanol</i>			
0.000	1.00	1.00	1.00	1.00	1.00	1.00
0.005	0.81	–	–	0.78	0.75	
0.015	0.55	0.52	0.50	0.75	0.70	0.67
0.025	0.51	0.47	0.41	0.63	0.63	0.60
0.050	0.34	0.31	–	0.45	0.43	0.40
0.100	0.31	0.17		0.29	0.26	
0.150				0.24		
<i>Methanol</i>			<i>Tetrahydrofuran</i>			
0.000	1.00	1.00	1.00	1.00	1.00	1.00
0.005	0.62	0.65	0.74	0.79	0.59	
0.010	–	0.46	0.60	0.64	0.45	0.52
0.015	0.27	0.38	0.52	0.55	0.38	0.34
0.020	0.21	0.32	0.39	0.34	0.35	0.30
0.025	0.18	0.26	0.35	0.28	0.28	0.23
0.050	0.12	0.15		0.13		
0.100				0.06		
<i>Ethanol</i>						
0.000	1.00	1.00	1.00			
0.005	0.66	0.67	0.61			
0.010	0.54	0.51	–			
0.015	0.53	0.42	0.30			
0.020	0.52	–				
0.025	0.31	0.20				
0.050	0.26	–				

Table 2 The Abraham parameters of the solvents used in this work taken from Stovall et al. (2005).

Solvent	<i>c</i>	<i>e</i>	<i>s</i>	<i>a</i>	<i>b</i>	<i>v</i>
1-Propanol	0.15	0.44	-1.10	0.39	-3.89	4.036
2-Propanol	0.06	0.32	-1.02	0.45	-3.82	4.067
Acetonitrile	0.41	0.08	0.33	-1.57	-4.39	3.364
1,4-Dioxane	0.10	0.35	-0.08	-0.56	-4.83	4.172
Ethanol	0.21	0.41	-0.96	0.19	-3.65	3.928
Methanol	0.33	0.30	-0.67	0.08	-3.39	3.512
Tetrahydrofuran	0.21	0.37	-0.39	-0.24	-4.93	4.447

different from xanthine oxidase where the activity ratios were 49% and 28%, respectively (Jouyban et al., 2009).

Eq. (3) was fitted to each set of activity ratio data of aldehyde oxidase in water-organic solvents at three investigated temperatures, the model parameters were computed and listed in Table 3. The calculated model constants of M_1 and M_2 are not statistically significant ($p > 0.05$). Concerning the basic theory of the model proposed by Acree (1992), in the area of solute solubility in binary solvents, the constants represent the possible two-body and three-body interactions in the solution. The dominant interactions in the investigated solvent systems include the interactions of enzyme-solvent 1, enzyme-solvent 2, substrate-solvent 1, substrate-solvent 2, product-solvent 1, product-solvent 2, solvent 1-solvent 2 and enzyme-substrate. The generally observed pattern for the investigated solvent systems is the decreasing pattern and the extent of the reduction in the enzyme activity could be represented by M_0 terms. Since enzyme, substrate and product are the same for all solvent systems and the only variable is the nature of the organic solvent, this could be represented using solvent descriptors (see Table 2) as discussed in the following section. The back-calculated (fitted) activity ratios using the computed model constants were compared with the corresponding experimental values, the MPDs were computed and listed in Table 3. The lowest MPD was observed for 2-propanol (4.4%) and the highest value was observed for tetrahydrofuran (7.7%). The overall MPD was 6.2% for this analysis.

As discussed above, the model constants of Eq. (3) are functions of the possible interactions between enzyme, substrate, product, water and organic solvent. In the investigated systems, the enzyme, substrate, product and water are the same, so it is possible to correlate the variations of the activity of the enzyme in various water – organic solvent mixtures with the nature of the organic solvents. Among various available

parameters representing the physico-chemical properties of the solvents, Abraham solvation parameters are the most accurate and popular descriptors. These parameters were used to correlate various biological properties (as examples see Hoover et al., 2005; Abraham et al., 2008). The applicability of these descriptors for representing the interactions in the investigated systems, are studied in this work. By combining these parameters with the statistically significant constants of Eq. (3), i.e. A_1 , A_2 and M_0 , the model could be written as:

$$\ln \alpha_{m,T} = f_2[A_{1,1}c + A_{1,2}e + A_{1,3}s + A_{1,4}a + A_{1,5}b + A_{1,6}v] + \frac{f_2}{T}[A_{2,1}c + A_{2,2}e + A_{2,3}s + A_{2,4}a + A_{2,5}b + A_{2,6}v] + \frac{f_1f_2}{T}[M_{0,1}c + M_{0,2}e + M_{0,3}s + M_{0,4}a + M_{0,5}b + M_{0,6}v] \quad (5)$$

in which c , e , s , a , b , and v are the solvent coefficients of Abraham solvation model calculated using experimental solubility data. The numerical values of these parameters for the common solvents were computed and could be found from the literature (Stovall et al., 2005).

All activity data points of aldehyde oxidase in water – organic solvent mixtures at various temperatures ($N = 110$) are fitted to Eq. (5) and the model constants with the probability of more than 0.05 are excluded from the model. The obtained model is:

$$\ln \alpha_{m,T} = f_2[-167.992e] + \frac{f_2}{T}[-20255.875s + 17887.814a - 5848.179b] + \frac{f_1f_2}{T}[-48594.221c + 54500.334e - 38627.191a + -9984.753v] \quad (6)$$

Eq. (6) correlates the data with respect to the variables of solvent composition, temperature and the Abraham solvent parameters with the correlation coefficient of 0.966 and the F value of 179. The correlation is statistically significant with $p < 0.0005$. The back-calculated activity data are compared with the corresponding experimental values and the MPD values are computed for each organic solvent (for details see Table 3). The maximum MPD is obtained for tetrahydrofuran (32.9%), the minimum value is observed for 1-propanol (11.1%) and the overall MPD is 19.5%. Although this version of the model produced relatively a higher prediction error, it is possible to employ it for prediction purposes for the activity of aldehyde oxidase in other water – organic solvent mixtures at various temperatures. This could be evaluated by one solvent

Table 3 The constants of the proposed model for different water – organic solvent mixtures, the mean percentage deviations (MPD) for fitted data (Eq. 3) and the MPD for general model (Eq. 6).

Organic solvent	A_1	A_2	M_0^1	N	Eq. (3)	Eq. (6)
Dioxane	-90.588	25564.25	-738.098	14	5.9	11.3
Acetonitrile	65.706	-37374.5	15874.99	12	6.0	14.2
Methanol	316.287	149712.1	-271199	19	6.7	20.5
Ethanol	-632.635	343252.3	-171239	15	7.1	32.2
1-Propanol	-174.125	102807.4	-60448.8	14	5.7	11.1
2-Propanol	-44.735	28240.8	-20819.1	17	4.4	14.1
Tetrahydrofuran	-254.397	139822.6	-80367.7	19	7.7	32.9
					6.2	19.5

¹ M_1 and M_2 constants are not statistically significant.

leave-out cross validation method, i.e. excluding one solvent data from training process, and then prediction of its activity ratios by the trained model. The obtained MPDs for predicted activity ratios for tetrahydrofuran and 1-propanol sets are 49.9 and 17.8%, respectively. The only required data for this prediction is the Abraham solvent parameters which are available for most of commonly used organic solvents. This could speed up the process of optimization of the enzyme action in water – organic solvent mixtures at different temperatures which is carried out by the trial and error approach in the biotechnological industry. It should be added that Eq. (6) is only valid for aldehyde oxidase activity in aqueous-organic solvent mixtures and the model should be trained for other enzymes using the experimental data.

In conclusion, the model possesses good capability for describing the activity of aldehyde oxidase in the water-organic solvent mixtures at various temperatures investigated in this work. The combined version of the model with the Abraham solvent parameters is a predictive model and could be used to predict the activity of aldehyde oxidase in other water – organic solvent mixtures at various temperatures which are required in many industrial processes. The proposed model could be considered as an empirical expression from theoretical point of view, however, it provides a predictive tool after training for a given enzyme. These predictions are highly in demand in the biotechnological process designs.

Acknowledgment

Authors would like to thank the reviewers of this manuscript for their constructive and helpful comments.

References

- Abraham, M.H., Acree Jr., W.E., Mintz, C., Payne, S., 2008. Effect of anesthetic structure on inhalation anesthesia: implications for the mechanism. *J. Pharm. Sci.* 97, 2373–2384.
- Acree Jr., W.E., 1992. Mathematical representation of thermodynamic properties. Part II. Derivation of the combined nearly ideal binary solvent (NIBS)/Redlich-Kister mathematical representation from a two-body and three-body interactional mixing model. *Thermochim. Acta* 198, 71–79.
- Amini, K., Sorouraddin, M.H., Rashidi, M.R., 2011. Activity and stability of rat liver xanthine oxidase in the presence of pyridine. *Can. J. Chem.* 89, 1–7.
- Arroyo, M., Torres, R., de la Mata, I., Castillon, M.P., Acebal, C., 1999. Interaction of penicillin V acylase with organic solvents: catalytic activity modulation on the hydrolysis of penicillin V. *Enzyme Microbiol. Technol.* 25, 378–383.
- Dordick, J.S., 1991. Non-aqueous enzymology. *Cur. Opin. Biotechnol.* 2, 401–407.
- Girard, E., Legoy, M.D., 1999. Activity and stability of dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F in the presence of organic solvents. *Enzyme Microbiol. Technol.* 24, 425–432.
- Hoover, K.R., Acree Jr., W.E., Abraham, M.H., 2005. Chemical toxicity correlations for several fish species based on the Abraham solvation parameter model. *Chem. Res. Toxicol.* 18, 1497–1505.
- Johnson, C., Stubble-Beedham, C., Stell, J.G.P., 1985. Hydralazine: a potent inhibitor of aldehyde oxidase activity in vitro and in vivo. *Biochem. Pharmacol.* 34, 4251–4256.
- Jouyban, A., 2008. Review of the cosolvency models for predicting solubility of drugs in water-cosolvent mixtures. *J. Pharm. Pharm. Sci.* 11, 32–58.
- Jouyban, A., Taherzadeh, F., Sorouraddin, M.H., Rashidi, M.R., 2009. Mathematical representation of xanthine oxidase activity in hydro-organic solvent mixtures. *Bioresour. Technol.* 100, 6635–6638.
- Karyakin, A.A., Lukachova, L.V., Gladilin, A.K., Levashov, A.V., 1996. Improvement of electrochemical biosensors using enzyme immobilization from water – organic mixtures with a high content of organic solvent. *Anal. Chem.* 68, 4335–4341.
- Kermasha, S., Bao, H., Bisakowski, B., 2001. Biocatalysis of tyrosinase using catechin as substrate in selected organic solvent media. *J. Mol. Catal. B: Enzymatic* 11, 929–938.
- Okazaki, S., Goto, M., Furusaki, S., 2000. Surfactant–protease complex as a novel biocatalyst for peptide synthesis in hydrophilic organic solvents. *Enzyme Microbiol. Technol.* 26, 159–164.
- Pirouzpanah, S., Hanaee, J., Razavieh, S.V., Rashidi, M.R., 2009. Inhibitory effects of flavonoids on aldehyde oxidase activity. *J. Enzyme Inhib. Med. Chem.* 24, 14–21.
- Schumacher, J., Eckstein, M., Kragl, U., 2006. Influence of water-miscible organic solvents on kinetics and enantioselectivity of the (R)-specific alcohol dehydrogenase from *Lactobacillus brevis*. *Biotechnol. J.* 1, 574–581.
- Sorouraddin, M.-H., Fooladi, E., Naseri, A., Rashidi, M.-R., 2008. A novel spectrophotometric method for determination of kinetic constants of aldehyde oxidase using multivariate calibration method. *J. Biochem. Biophys. Methods* 70, 999–1005.
- Stovall, D.M., Acree Jr., W.E., Abraham, M.H., 2005. Solubility of 9-fluorenone, thianthrene and xanthene in organic solvents. *Fluid Phase Equilib.* 232, 113–121.
- Tsai, S.W., Chen, C.C., Yang, H.S., Ng, I.S., Chen, T.L., 2006. Implication of substrate-assisted catalysis on improving lipase activity or enantioselectivity in organic solvents. *Biochim. Biophys. Acta* 1764, 1424–1428.