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Determination of Kinetic Parameters and Metal Ions in Urea-Urease System Based on the Biochemical Reaction Heat Induced Laser Beam Deflection

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Abstract: A new analytical method for the determination of urea-urease system based on biochemical reaction heat induced laser beam deflection is presented in this paper. With the method, the Michaelis constant (K_m) of urease and apparent inhibition constant (K_i) of some metal ion inhibitors were measured respectively. This method was also used for the quantitative determination of metal ions with satisfactory result.

Keywords: Urease, kinetic parameter, metal ions determination, laser beam deflection.

Reaction heat induced laser beam deflection is a new analytical method which came in to being in 1993[1], by probing the gradient of the refraction index which was induced by reaction heat with a laser probe beam, one can relate the laser beam deflection to the concentration of the sample.

In this paper, the method was used for the study of the kinetic process for urea-urease system. It is well known that urease catalyzes the hydrolysis of urea as follows:

$$NH_2CONH_2+H_2O \xrightarrow{\text{Urease}} CO_2+2NH_3+Q$$

The process is accompanied by large reaction heat, which will lead to the deflection of the probe laser beam. Meanwhile, the process could be inhibited by some metal ion in a non-competitive mechanism[3]. Under certain conditions, the deflection signal of the probe laser beam is inversely proportional to the concentration of the metal ion inhibitor. The kinetic parameters and some metal ions can be determined by the method of biochemical reaction heat induced laser beam deflection.

Experimental

The diagram of the experimental setup and the procedure in this experiment has been presented in the previous paper[2]. A self-made dual-layer reaction and measurement cell was used in the experiment. Having filled 3ml heat-transfer medium (CCl_4) into the outside cell, a certain volume of urea and urease solution was injected into inside cell

with a microinjector. The reaction heat was transferred from the bottom of inside cell (made of a piece of Pt) to the heat-transfer medium in the outside cell. A 0.25 W TEMoo He-Ne laser was used to provide the probe laser beam. Having been focused by a lens, the probe beam passed through the heat-transfer medium in outside cell and was deflected because of the reaction heat given out by the biochemical reaction in the inside cell. The deflection signal of the probe beam was detected by a position detector consisted of a pinhole and a photodiode. A double trace oscilloscope and a recorder were used to display and record the deflection signal.

All chemicals were analytical grade and prepared according to reference[4]. Solutions were prepared using distilled water.

Results and Discussion

Determination of Michaelis constant (K_m) and apparent inhibition constant (K_i)

The Michaelis constant of urease was determined to be 3.31×10^{-3}mol.L^{-1} by the method of biochemical reaction heat induced laser beam deflection (on the basis of the Lineweaver-Burk method[5].), where the value was in good agreement with the value (3.41×10^{-3}mol.L^{-1}) obtained by spectrophotometry[4].

Table 1. Apparent inhibition constant (K_i) of some metal ions

Metal Ions	$K_i \times 10^{-5}$(L. Mol^{-1})	$K_I \times 10^{-5}$(L. Mol^{-1})*	RSD%
Ag(I)	16.13	16.25	-0.74
Hg(II)	5.74	5.68	+1.06
Cu(II)	1.10	1.19	-7.56
Cd(II)	0.92	0.90	+2.22
Co(II)	0.89	0.87	+2.30
Pb(II)	0.45	0.44	+2.27
Ni(II)	0.37	0.38	-2.63

*Determined by spectrophotometric method according to reference[4] .

The apparent inhibition constant of seven metal ions was determined respectively by the method on the basis of the Dixon method[6]. The results are shown in **Table 1**. As can be seen, the decreasing order of toxicity of the seven metal ions is: Ag(I)>Hg(II)>Cu(II)>Cd(II)>Co(II)>Pb(II)>Ni(II). The order agrees very well with the determination result [4].

Determination of metal ions

It is fairly well known that the activity of an enzyme in the reaction has a great dependence on the concentration of the hydrogen ion. The enzyme catalytic system should be buffered at a certain pH so that the enzyme activity could be kept at maximum value. The determination limitation of the metal ion inhibitor was relatively high in the

buffered system [7, 8] since the buffer reagents always have an action on metal ion and form complexes with it. The difference of buffer and non-buffer system was investigated in the experiment. The curve of deflection signal ~ time is shown in **Figure 1**, from which one can draw the conclusion that the peak value of the deflection signal in the non-buffered system was slightly lower than that in buffered system. It means that the urease activity was reduced slightly in non-buffered system. However, the total features of the two curves were similar, which means that non-buffered system has no serious effect on urease activity, so one can detect the trace metal ion inhibitor in non-buffered system. The experiment of quantitative determination of metal ions has been carried out under the condition $C_{urea} >> C_{urease} >> C_i$ (The C_{urea} , C_{urease} and C_i represent the concentration of urea, urease and each metal ion being determined, respectively.). The determination results are shown in **Table 2.** It is shown that those metal ions with larger apparent inhibition constant (K_i) exhibit higher sensitivity, hence it can be determined in smaller quantity.

Figure 1. The difference of buffer and non-buffer system
(1. Buffer system; 2. Non-buffer system)

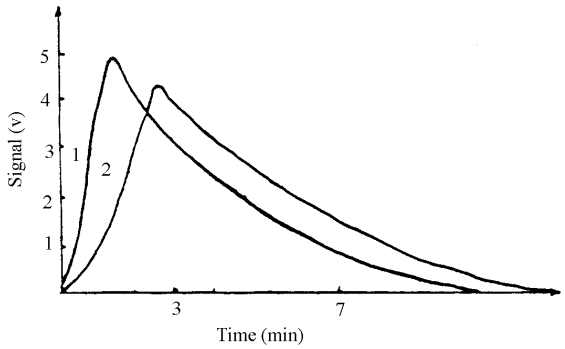

Table 2. Determination result of metal ions

Metal Ions	Linear range (mol/L)	Linear equation	Correlation coefficient(r)
Ag(I)	1×10^{-8}~1×10^{-6}	$1/S=0.1828+9.9487\times10^{5}C$	0.9987
Hg(II)	1×10^{-7}~1×10^{-5}	$1/S=0.1184+1.0811\times10^{5}C$	0.9963
Cu(II)	1×10^{-6}~1×10^{-4}	$1/S=0.1679+1.8423\times10^{4}C$	0.9981
Cd(II)	1×10^{-6}~1×10^{-4}	$1/S=0.1635+1.5002\times10^{4}C$	0.9968
Co(II)	1×10^{-6}~1×10^{-4}	$1/S=0.1664+1.4971\times10^{4}C$	0.9991
Pd(II)	1×10^{-6}~1×10^{-4}	$1/S=0.1827+8.3025\times10^{3}C$	0.9947
Ni(II)	1×10^{-6}~1×10^{-4}	$1/S=0.1749+6.5608\times10^{3}C$	0.9987

The experiment result shows that the method of the biochemical reaction heat induced laser beam deflection can be used not only as an analytical method but also applied to the kinetic study of the biochemical process.

References

1. X. Z Wu, Hiroak Shindoh, Masaaki Yamada,Toshiyuki Hobo, *Anal.Chem*., **1993**, *65*, 834.
2. X.Y. Zhu, H.T. Yan, *Chem. J. Chin. Univ.*, **1995**, *10*, 1535.
3. W.H.R. Shaw, D.N. Raval, *J. Amer. Chem. Soc.*, **1961**, *83*, 3184.
4. J. H. Xing, *Biochemical Analytial Method of Plant*, Science Publishing House, **1981**, P106.
5. H. B. Lineweaver., *J. Amer. Chem. Soc*., **1934**, *56*, 658.
6. M. Dixon , *Biochem. J.*, **1953**, *55*, 170.
7. B. Kratochvil , S.L. Boyer, G.P. Hicks, *Anal. Chem*.,**1967**, *39*, 45.
8. P. Banm , R. Czok, *Biochem. J*., **1959**, *322*, 121.

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