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# RAMAN SPECTROSCOPY IN THE STUDY OF ENZYME KINETICS

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# How to measure reaction rate?

reaction rate = rate of changes in reagent (product) concentration

| Existing optical methods | Disadvantages |
|---|---|
| PHOTOMETRY | Overlapping absorption bands |
| POLARIMETRY | Optically active substrate or product |
| FLUORIMETRY | Overlapping absorption bands and concentrational limitations |

- Each method is efficient in the measurements of concentration of a certain reaction component, but no universal method is known at the moment.
- In physics, the most informative method of studying the structure of molecules (enzymes) is Raman spectroscopy.
- Previously, we have shown the possibility to use Raman spectroscopy to measure the rates of chemical reactions [1]. In this work, **we show that Raman spectroscopy data can be used for calculation of chemical reaction rates and catalytic activity of α-chymotrypsin**

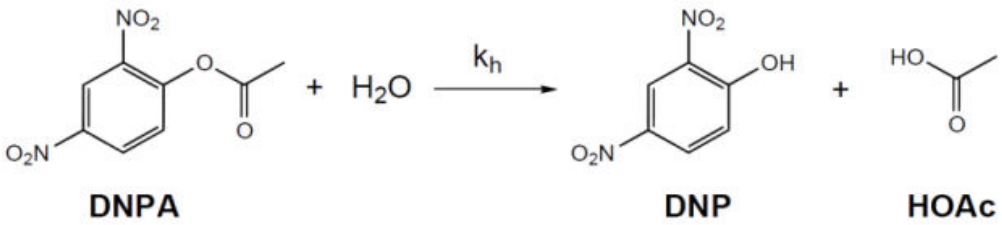
[1] Balakhnina I. A., Brandt N. N., Mankova A. A., Chikishev A. Yu, Shpachenko I. G. "Raman spectral determination of chemical reaction rate characteristics", Journal of Applied Spectroscopy, 84(4), 650-656 (2017).

# Chemical reaction of hydrolysis of 2,4-dinitrophenyl acetate (DNPA) catalyzed by α-chymotrypsin

in absence of enzyme

The product concentration (DNP or HOAc) increases:

$$[P] = [DNPA]_0 \left(1 - e^{-k_h t}\right)$$




in presence of enzyme

Michaelis-Menten kinetics, the rate $v$ of product formation is described by:

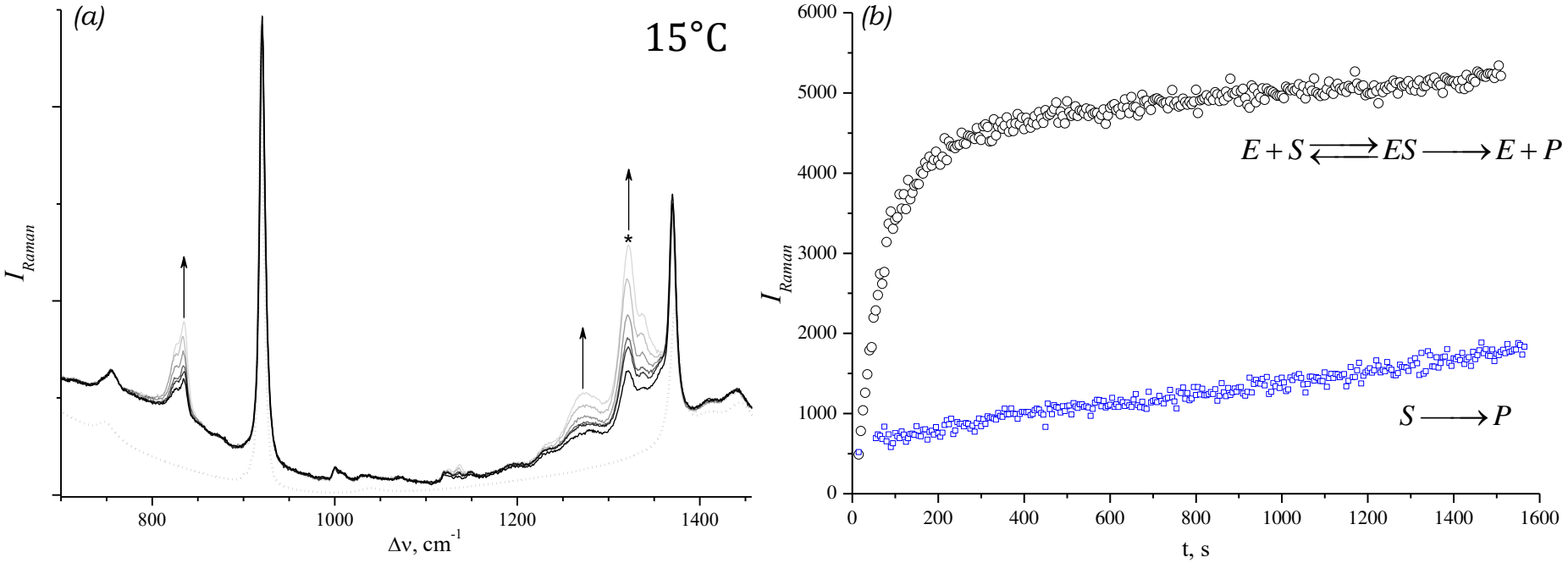
$$v = \frac{v_{\max}}{1 + K_M / [DNPA]}$$

The concentration of the product [P] is set implicitly:

$$[P] - K_M \ln\left(1 - \frac{[P]}{[DNPA]_0}\right) = v_{\max} t$$

[P] – product concentration, $[DNPA]_0$ – initial concentration of DNPA, $K_M$ – Michaelis rate constant

# Raman spectroscopy of hydrolysis of 2,4-dinitrophenyl acetate (DNPA) catalyzed by α-chymotrypsin




(a) Several spectra from the Raman spectra series of spontaneous hydrolysis of DNPA in the range 4-314 minutes at 15°C (lines) and spectra of 75 mM DNPA in acetonitrile (dots);
(b) The intensity of Raman band at 1318 cm$^{-1}$ for enzymatic hydrolysis (black circles) and spontaneous hydrolysis (blue squares) DNPA at 15 °C.

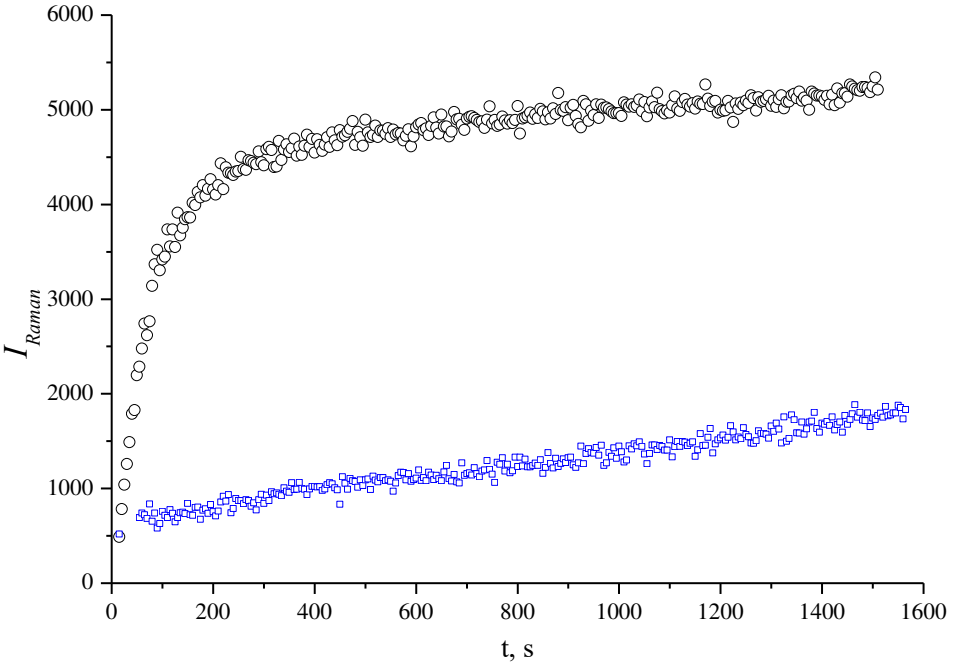
For each experiment a series of 200-2000 spectra is measured. Each spectrum contains a broadband fluorescence background. For a correct comparison of the spectra in the series they are leaded to a same background [2]. After processing integral signal (area) in the range of the analyzed Raman product band is calculated and time dependence of the intensity of a single Raman band is plotted.

[2] Brandt N.N., Chikishev A.Yu., Chulichkov A.I., Ignatiev P.A., Lebedenko S.I. "A method of comparing Raman spectra", Laser Phys., 14 (11), 1386–1392 (2004).

E – enzyme, S – substrate, ES – complex, P - product

# Raman spectroscopy in the study of enzyme kinetics

Figure shows time dependence of the intensity of the Raman band at 1318 $cm^{-1}$ for enzymatic and spontaneous hydrolysis of DNPA. In the curve for enzymatic reaction there are rapid (0-300 s) and slow (after 300 s) stages. In the first stage there is a rapid transformation of substrate into product, the substrate concentration decreases sharply. Reducing the amount of substrate means decrease of enzymatic reaction rate. It is important to note that the catalytic and spontaneous hydrolysis processes occur simultaneously and this is two independent processes. So on a slow phase in enzymatic reaction curve (black circles) is mainly spontaneous hydrolysis. At the initial stage it is possible to calculate Michaelis constant ($K_M$) for enzymatic hydrolysis DPNA involving α-chymotrypsin. For spontaneous hydrolysis $k_h$ is calculated. For substrate DNPA the constants values are the same order of magnitude with those in the literature [3-7].




| | | |
|---|---|---|
| 15°C, pH 7,8 | $K_M$, M | $(39.55\pm0.01)10^{-4}$ |
| | $k_h$, $s^{-1}$ | $(3.1\pm0.1)10^{-5}$ |

Raman spectroscopy data can be used in calculation of chemical reaction rates in the presence and in absence of enzyme. The method is promising for the measurements of enzymatic activity and can be used to estimate the functional activity level.


[3] Klausen J., Meier M.A., Schwarzenbach R.P. "Assessing the Fate of Organic Contaminants in Aquatic Environments: Mechanism and Kinetics of Hydrolysis of a Carboxylic Ester", J. Chem. Ed., 74(12), 1440-1444 (1997).
[4] Mabey W., Mill T.J. "Critical Review of Hydrolysis of Organic Compounds in Water Under Environmental Conditions", Phys. Chem. Ref. Data, **7**, 383–415 (1978).
[5] Levashov A.V., Ryabov A.D. "Introduction to the Kinetics of Homogeneous Reactions. Hydrolysis of 2,4-Dinitrophenyl Acetate Catalyzed by Imidazole: A Simple Laboratory Experiment", *Biochemical Education,* 14(1), 34-36 (1986).
[6] Bender M.L., Kezdy F.J., Wedler F.C. "alpha-Chymotrypsin: Enzyme concentration and kinetics", *J. Chem. Educ.,* 44 (2), 84 (1967).
[7] Mathews C.K., van Holde K.E., Ahern K.G. *Biochemistry (3 ed.)* (Prentice Hall, 1999).