RAMAN SPECTRAL DETERMINATION OF CHEMICAL REACTION RATE CHARACTERISTICS

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The feasibility of using Raman spectroscopy to determine chemical reaction rates and activation energies has been demonstrated for the saponification of ethyl acetate. The temperature dependence of the reaction rate was found in the range from 15 to 45°C.

Keywords: Raman spectroscopy, chemical reaction rate, chemical kinetics.

Introduction. The determination of chemical reaction rates has remained a fundamental goal of modern chemistry. Special attention has arisen for the determination of the rate characteristics of physicochemical processes on phase separation boundaries, which is exceedingly important in enzymology for the description of enzymatic reactions on the surface of biological membranes. This has led to interest in the development of noninvasive methods for the measurement of chemical reaction rates, which can be applied for compounds in any aggregate state and under close-to-natural conditions.

Many highly sensitive and specific methods are known for measuring chemical reaction rates, in particular, enzymatic reactions [1–3]. In some cases, the specificity a method makes it the preferred technique for determining a certain reagent or product at a given concentration. For example, the solvents and substrates for enzymatic reactions are selected such that they do not absorb light in the spectral region, in which the product absorbs [5]. Chemical reactions rates can also be measured using optical activity and circular dichroism effects [3], often employed in studying proteins [6]. These methods are very convenient and effective but are applicable only for a narrow class of optically active compounds. A universal method, which would permit us to work with any reagents and products, does not yet exist.

Raman spectroscopy provides extensive information, permitting the study of molecular structure and the determination of chemical reaction products. This method is applicable for compounds in any aggregate state and for any pH and temperature. Raman spectra have a well-known fingerprint region, unique for each compound. Analysis of the spectra in this region permits us to determine structural features of the components of enzymatic reactions, in particular, proteins [7, 8], lipids [9], and carbohydrates [10]. Thus, Raman spectroscopy can be suitable for the determination of chemical reaction rates since the spectrum should have a set of spectral components characteristic for each compound.

Despite its common use, Raman spectroscopy has not been employed to determine reaction rates, primarily, due to the low sensitivity of this method. With optimized spectrometers, Raman spectra can be taken for compounds at concentrations of ≥100 mm. Characteristic accumulation times for a high-quality spectrum are tens of minutes, which is unsuitable for describing chemical reactions, and concentrations must be raised to hundreds of millimoles per liter. Secondly, as a rule, Raman spectra have a wideband fluorescence band even in the transparency region of compounds studied. Despite methods to eliminate the background component, the separation of the spectrum into fluorescence and Raman components is a complicated mathematical problem. Modification of Raman spectroscopy may be used to improve its sensitivity, in particular, surface-enhanced Raman scattering (SERS) [11]. However, the use of SERS requires powerful local electric fields, which affect compound reactivity [12]. Furthermore, the SERS spectrum is not a scaled Raman spectrum [13]. Thus, the amplification is greater in the low-frequency region and the amplification itself depends on the orientation of the molecules on the SERS base upon their adsorption.

In the present work, we have demonstrated the feasibility of using Raman spectroscopy for determining chemical reaction rate characteristics.

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Materials and Methods. We studied the alkaline hydrolysis of ethyl acetate, EtOAc, in 100 mM aqueous KOH to give ethanol (C_2H_5OH) and potassium acetate (CH_2CO_2K, KOAc):

$$CH_3COOC_2H_5 + KOH \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C_2H_5OH + CH_3COOK .$$
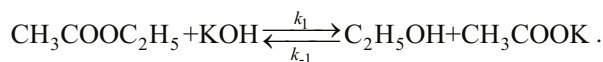
The reaction was initiated by the addition of 10 mL EtOAc into 1 mL aq. KOH at pH 13.5 at a given temperature. Thus, the reagents in the initial reaction mixture (KOH and ethyl acetate) are present in equimolar amounts (100 mM). Immediately after the addition of ethyl acetate to a $10 \times 10 \times 40$ mm quartz cell, the mixture was stirred for 3–5 s using an Amtast Vortex 2 orbital mixer and this cell was placed into the thermostatted object table of the spectrometer on a layer of Arctic MX4 thermal paste. The time interval between the reaction onset and the start of the measurements was 10–20 s. An LOIP LT108 circulation thermostat was used to maintain the temperature in the cell. The cell temperature was varied in the range 15–25ºC.

The measurements were carried out on a Raman spectrometer [14] with excitation by continuous emission from a 1-W Coherent Innova 90 argon laser with $\lambda = 514.5$ nm. The long-term intensity instability was $\leq 0.5\%$. The beam divergence was 1 mrad. The spectral resolution was 1 cm^{-1}. The minimal accumulation time for one spectrum was 20 ms. The measurement time for each spectrum was 3–4 s. The time for measurement of a series of spectra was 20–60 min. An algorithm for the approximation of one spectrum to another with the addition of a polynomial of a given power was used to treat and compare the spectra in each series [15]. This permitted comparative analysis of the spectra without the necessity of eliminating the background signal and carrying out the normalization of each spectrum in a series relative to the intensity of the water deformation band line at 1640 cm^{-1}, which is invariant throughout the reaction. Then, we plotted the change of the integral intensity of a line of the product or reagent over time and carried out an approximation of each curve by solving a system of kinetic equations.

Results and Discussion. Figure 1 shows the Raman spectra of the individual reaction components and the reaction mixture as well as an approximation curve obtained as a linear combination of the spectra of the reaction components. The spectrum of potassium acetate (curve 4) was obtained by subtracting the ethanol spectrum from the spectrum of the reaction mixture 36 h after the reaction onset using our previous technique [14].

The spectrum of the reaction mixture has lines at 636 (EtOAc), 789 (EtOAc), 850 (EtOAc), 880 (C_2H_5OH), 928 (KOAc), 1048 (C_2H_5OH, EtOAc), 1095 (C_2H_5OH, EtOAc), 1118 (C_2H_5OH, EtOAc), 1276 (C_2H_5OH, EtOAc), 1413 (KOAc), 1453 (EtOAc), 1640 (H_2O), and 1737 cm^{-1} (EtOAc). The decreasing and increasing lines (lines of the reagents and products, respectively) are indicated by arrows.

Having measured a series of spectra of the reaction mixture during the reaction at a given temperature, we can obtain a curve for the change in the integral intensity of the selected spectral line over time. An approximation of this curve can be obtained in accord with the following model for this reversible reaction

$$A + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C + D .$$
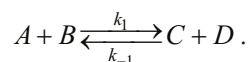
The kinetic equations are as follows:

$$\begin{cases} d[A]/dt = d[B]/dt = -k_1[A][B] + k_{-1}[C][D], \\ d[C]/dt = d[D]/dt = k_1[A][B] - k_{-1}[C][D]. \end{cases}$$

Taking account of the initial conditions $[A]|_{t=0} = a$ and $[B]|_{t=0} = b$, the system reduces to one differential and several algebraic equations:

$$\begin{cases} d[C]/dt = k_1[A][B] - k_{-1}[C]^2, \\ [A] = a - [C], \\ [B] = [A] - a + b, \\ [D] = [C]. \end{cases}$$

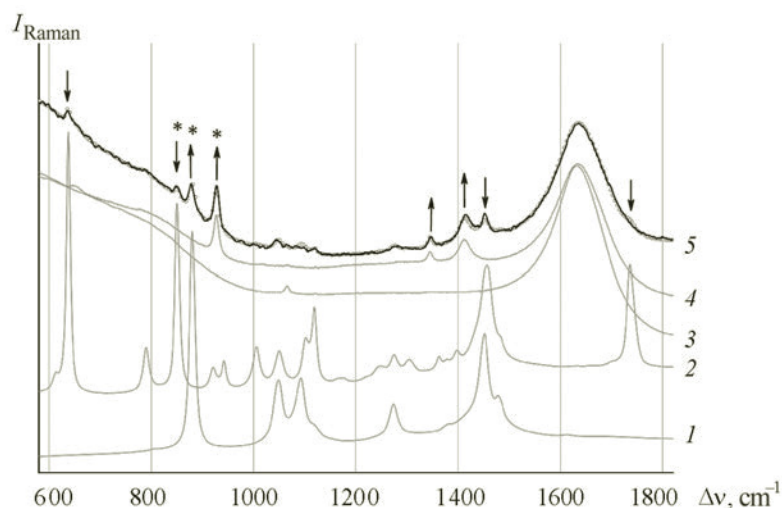The solution of the system for the concentration of product C is as follows

Fig. 1. Raman spectra of C_2H_5OH (1), EtOAc (2), KOH (3), KOAc (4), and the reaction mixture (5). The points give the approximation curve.
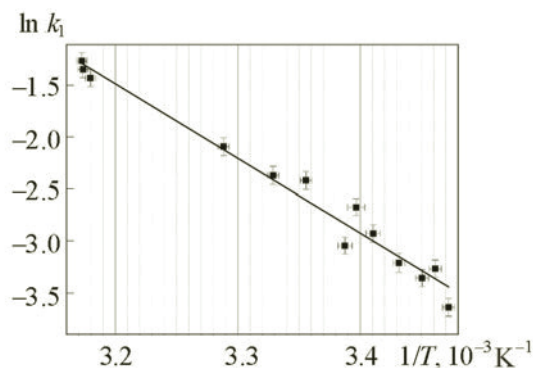

Fig. 2. Dependence of the logarithm of the rate constant for the alkaline hydrolysis of ethyl acetate on inverse temperature and its approximation by a linear function.

$$[C] = \frac{(a+b)k_1}{2(k_1 - k_{-1})} - \frac{\sqrt{k_1}\sqrt{a^2 k_1 + b^2 k_1 - 2ab(k_1 - 2k_{-1})}}{2(k_1 - k_{-1})}$$

$$\times \tanh\left[\frac{1}{2}\sqrt{k_1}\sqrt{a^2 k_1 - 2abk_1 + b^2 k_1 + 4abk_{-1}}\, t + \operatorname{artanh}\left\{\frac{(a+b)\sqrt{k_1}}{\sqrt{a^2 k_1 + b^2 k_1 - 2ab(k_1 - 2k_{-1})}}\right\}\right]$$

and may be used to approximate the experimental data. As a result, we obtain the rate constants for the alkaline hydrolysis of ethyl acetate k_1 (Fig. 2). We note that the value thereby determined is independent of the background signal in the treated spectra. The experimental data are approximated by the Arrhenius function $k_1 = A \exp(E_a/RT)$, where A is the Arrhenius constant, E_a is the activation energy, R is the universal gas constant, and T is the absolute temperature [18]. We obtain activation energy $E_a = 60 \pm 3$ kJ/mole. This result is compared with the literature values in Table 1. The discrepancies between the rate constants and activation energies obtained by different workers may be related to imprecision in measuring the probe volumes, unmonitored delay between the reaction onset and start of measurements, error in determining the rate constant due to deviation of the kinetics from exponential behavior, and nonlinearity of the measurement method used.

TABLE 1. Rate Constants, Activation Energies, Measurement Methods and Reagent Concentrations

Reagent concentrations, mM	Measurement method	T, °C	$1/T$, 10^{-3} K^{-1}	k_1, 10^{-3} M^{-1}·s^{-1}	E_a, kJ/mole	References
NaOH 20 EtOAc 20	Titration	3.6	3.62	23.6	46.8 ± 0.4	[19]
		5.5	3.59	28		
		6	3.58	29.7		
		6.6	3.58	28.8		
		7.2	3.57	32		
		11	3.52	42.7		
		12.4	3.50	46.5		
		12.7	3.50	47.9		
		19.3	3.42	76.1		
		20	3.41	78.9		
		20.4	3.41	79.7		
		20.9	3.40	83.2		
		23.6	3.37	100.2		
		27	3.33	120.7		
		28.4	3.32	133.9		
		30.4	3.30	147.9		
		32.9	3.27	164.5		
		34	3.26	182		
		35	3.25	194.8		
		37.7	3.22	223.5		
NaOH 10 EtOAc 8	Titration	25	3.36	112.5	–	[20]
NaOH 50 EtOAc 50	Volumetric titration	20	3.41	45.3	61.5	[21]
		30	3.30	109		
		40	3.19	229		
		50	3.10	479		
NaOH in excess	High-frequency titration	30	3.30	147 ± 3	–	[22]
NaOH 20 EtOAc 20	Conduction measurement	25	3.36	112	48.4	[23]
NaOH 30 EtOAc 30	UV spectroscopy	30	3.30	144	–	[24]
NaOH 10 EtOAc 10	Titration	30	3.30	122 ± 7	–	[25]
NaOH 200 EtOAc 250	Conduction measurement	15	3.47	63	39.9	[26]
		20	3.41	81		
		25	3.36	109		
NaOH 1000 EtOAc 800	Microcalorimetry	25	3.36	140.1	46.1	[27]

TABLE 1. Continuated

Reagent concentrations, mM	Measurement method	T, °C	$1/T$, 10^{-3} K^{-1}	k_1, 10^{-3} M$^{-1}\cdot$s^{-1}	E_a, kJ/mole	References
NaOH 100 EtOAc 100	Conduction measurement	30	3.30	162 ± 2	41.4 ± 0.6	[28]
		38.7	3.21	266 ± 3		
		40.3	3.19	280 ± 3		
		44.5	3.15	346 ± 3		
		50	3.10	437 ± 5		
		55.5	3.04	603 ± 7		
NaOH 37-40 EtOAc 26-31	Conduction measurement	19.1	3.42	64.7 ± 1.3	48.7	[29]
		21	3.40	77.5 ± 0.5		
		23	3.38	86.7 ± 3.3		
		24	3.37	96.2 ± 1.5		
		25	3.35	100.0 ± 1.7		
		27.1	3.33	115.3 ± 0.3		
		32	3.28	153.3 ± 3.3		
KOH 100 EtOAc 100	Raman spectroscopy	15	3.47	26 ± 2	60 ± 3	Present work
		15.9	3.46	38 ± 3		
		16.8	3.45	35 ± 3		
		18.4	3.43	40 ± 4		
		20.2	3.41	54 ± 5		
		21.4	3.40	69 ± 6		
		22.2	3.39	48 ± 4		
		25	3.36	89 ± 8		
		27.4	3.33	94 ± 8		
		31.1	3.29	124 ± 10		
		41.5	3.18	241 ± 20		
		42.1	3.17	262 ± 21		
		42.2	3.17	284 ± 23		

A feature of Raman spectroscopy as a method for measuring chemical reaction rates is the possibility of simultaneously analyzing several kinetic curves corresponding to different lines of one or several reaction components. The points in Fig. 3 show the kinetic curves for lines at frequencies 850 (EtOAc), 880 (C_2H_5OH), and 928 cm^{-1} (KOAc) (indicated by asterisks in Fig. 1). The intensity of the first line corresponding to the ester reagent decreases, while the intensities of the other two lines, corresponding to the reaction products, increase. Let us modify the initial model in order to simplify the procedure for approximation of the data. Our calculations show that constant k_{-1} is ~300 times less than constant k_1 and we can envisage an irreversible reaction

$$A + B \xrightarrow{\ k\ } C + D.$$
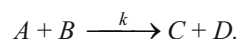
In this case, the system of kinetic equations becomes

$$\begin{cases} d[A]/dt = d[B]/dt = -k[A][B], \\ d[C]/dt = d[D]/dt = k[A][B], \end{cases}$$
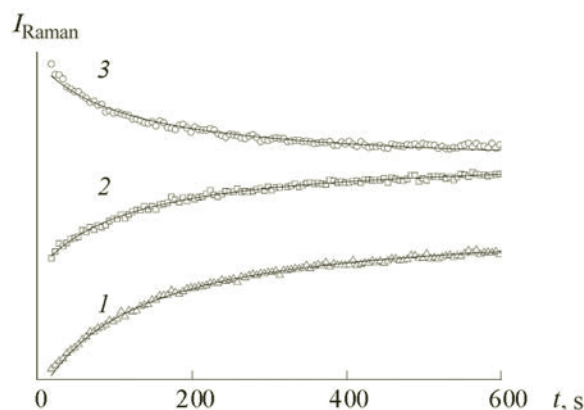
and its solution is as follows:

Fig. 3. Dependence of the intensity of Raman lines at frequencies 928 (1), 880 (2), and 850 cm^{-1} (3) on elapsed time during the alkaline hydrolysis of ethyl acetate at 25°C. The experimental data are given as points, while their approximation is given by lines.

$$\begin{cases} [A] = \dfrac{b-a}{(b/a)e^{kt(b-a)}-1}, \\[2ex] [C] = \dfrac{b(e^{kt(b-a)}-1)}{(b/a)e^{kt(b-a)}-1}. \end{cases}$$

The simultaneous approximation of the kinetic curves for lines 850, 880, and 928 cm^{-1} using this solution leads to rate constant $k = (88 \pm 6)\cdot10^{-3}$ M$^{-1}\cdot$s^{-1} at 25°C.

Conclusions. A method has been presented for measuring chemical reaction rates using Raman spectroscopy. A series of measurements of the Raman spectra of the reaction mixture provided data for calculating the rate of the alkaline hydrolysis of ethyl acetate at different temperatures and for determining the activation energy. This technique permits the simultaneous analysis of several spectral lines corresponding to both reagents and reaction products, which enhances the precision of the rate constant determination. For the problem of determining rate data of reactions, the relatively low sensitivity of Raman spectroscopy is compensated by the unique nature of the spectra of the chemicallypure compounds.

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