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Determination of Diacetyl with Spectrophotometry and Thermal-Lens Spectrometry

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Abstract—The conditions for the spectrophotometric and thermal-lens determination of diacetyl with creatine and 2-naphthol are proposed. The obtained value of the detection limit for spectrophotometry (at 527 nm), which amounts to 10 ng/mL, is fivefold lower than the existing values of the spectrophotometric determination of diacetyl. The conditions for the thermal-lens determination of diacetyl (λ = 514.5 nm, strength of the inducing radiation: 40 mW) based on the unmodified procedure of spectrophotometric determination were proposed. Along with a fivefold (down to 2 ng/mL) decrease in the detection limit, which is comparable with that for the determination of diacetyl by means of gas chromatography with mass spectrometric detection (detection limit of 0.7 ng/mL), thermal-lens determination is characterized by the enhancement of other performance parameters of the determination. It was shown that, contrary to the case when gas chromatography is used, ethanol does not interfere with both the spectrophotometric and the thermal-lens determination of acetyl.

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Diacetyl and other vicinal diketones are found in such diverse food products as beer, wines, cheeses, etc., and are desirable to determine diacetyl at the nanogram level. Unfortunately, existing spectrophotometric [1–5], voltamperometric [6, 7], and chromatographic methods [8–10] are not sufficient for that. The present work is devoted to the optimization of the conditions for the spectrophotometric determination of diacetyl in order to enhance its sensitivity, and also to study the possibility of using thermal-lens spectrometry [11], a modern high sensitive method of molecular absorption spectroscopy, for the additional enhancing of the determination of diacetyl.

EXPERIMENTAL

Apparatus. All spectrophotometric measurements were performed on a Shimadzu UV mini 1240 spectrophotometer (wavelength range of 190–700 nm). Thermal-lens measurements were performed on a dual-laser parallel-beam thermal-lens spectrometer [12]. A thermal-lens was excited in a plastic cell (optical path length of 1 cm, volume of 4 mL) by irradiation with an Innova 90-6 argon ion laser (Coherent, United States, TEM_{00} mode, with wavelength λ = 488.0 and 514.5 nm). As a probe laser, we used an SP-106-1 He–Ne laser (Spectra Physics, United States) with λ = 632.8 nm (TEM_{00} mode, 10 mW). The inoLab pH level 1 universal ion meter with a glass indicator electrode and a silver chloride reference electrode were used for measuring pH values with a precision of ±0.05 pH units. Sampling was carried out using a LabMate dispenser (HTL, Poland), and dispensers of various volume: 2–20 μL (0.01 μL sampling accuracy), 20–200 μL (0.1 μL sampling accuracy), and 1–5 mL (0.005 mL sampling accuracy). A Smart InterAB gas chromatograph (Agilent Technologies, Russia) with a 5973 Network mass spectroscopy detector was used for chromatography mass spectrometry determination. An HP-5 column (95% siloxane and 5% phenyl methyl siloxane) was used. The column parameters were as follows: 30 m length, 250 μm diameter, and 0.25 μm thickness of the solid phase. Helium with the flow rate of 1 mL/min was used as the mobile phase. The volume of the injected sample was 1 μL (Agilent Technologies 7683B Series injector)

Measurement data processing. Thermal-lens measurements consist in a series of excitation laser on-off cycles (thermal-lens formation-dissipation) producing a series of signals θ, which can be determined using the equation:

$$\theta = \frac{1}{B}\left(1 + \sqrt{\frac{I_{off} - I_{on}}{I_{on}}}\right), \quad (1)$$

where I_{off} and I_{on} are the intensities in the center of the probe beam without a thermal-lens (the inducing laser is switched-off) and at a completely developed thermal-lens (the inducing laser is switched-on), respectively, and the geometric factor B for the measurement scheme used herein amounts to 0.396 [12]. The analytical signal is converted to absorbance according to the equation:

$$A = \frac{\theta}{2.303 E_0 P_e}, \quad (2)$$

where P_e is the power of the laser beam with wavelength λ_e, exciting the thermal-lens [11], E_0 is the enhancement factor of the thermooptical measurement sensitivity for the thermooptical measurements (an increase in the sensitivity in comparison with the conventional spectrophotometry for an excitation beam power of 1 mW),

$$E_0 = \frac{dn/dT}{k\lambda_p}, \quad (3)$$

where dn/dT is the temperature gradient of the refractive index, k is the thermal conductivity coefficient of the medium, and λ_p is the wavelength of the thermal-lens probe beam. The theoretically expected increase in the sensitivity of the thermal-lens measurements against that of spectrophotometry was determined using the equation

$$\theta/A = 2.303 E_0 P_e \frac{\varepsilon_{\lambda_{TL}}}{\varepsilon_{\lambda_{SP}}}, \quad (4)$$

where $\varepsilon_{\lambda_{TL}}$ and $\varepsilon_{\lambda_{SP}}$ are the molar absorption coefficients for the reaction product at the wavelengths at which the thermal-lens measurements and spectrophotometric analysis were performed, respectively.

Chemicals and solvents. The following chemicals were used: diacetyl (97%, $d = 0.981$, Aldrich, chemically pure), 2-naphtol (Aldrich, chemically pure), creatine (Weider, pure), NaOH (Merck, chemically pure); H_2SO_4 (chemically pure), and acetonitrile (LiChrosolv, high-purity grade, for gradient elusion chromatography, Merck). The solutions of diacetyl and the compound being photometrically analyzed were prepared immediately before the experiment. Distilled water and acetonitrile were used to prepare the diacetyl solution and for chromatography-mass spectrometry determination, respectively. Laboratory glassware was soaked in potassium bichromate and washed with distilled water.

The process solutions were prepared by diluting a 97% chemical solution: 100 μL of the solution was diluted to 10 mL with water, the resulting diacetyl concentration was 0.01 mL/mL. The process 2-naphtol solutions (0.26 M in 0.8 M NaOH) were prepared by dissolving 0.71 g of 2-naphtol and 0.61 g of NaOH with 18.7 mL of water. The process creatine solutions were prepared by dissolving the excess of creatine in distilled water until the saturated solution was obtained, followed by its filtration through a porous filter (0.45 μm pore diameter).

Procedure 1 (Chromatography-mass spectrometry determination of diacetyl)**.** A diacetyl solution was prepared as follows: 0.7 μL of diacetyl (Sigma) was dissolved in 99.3 mL of acetonitrile. Then 1 μL of this solution was injected into the chromatography mass spectrometer. The registration time for the chromatogram was 2.5 min. The mass spectrum for a peak corresponding to diacetyl ($t_R = 2.0$ min) was obtained.

Procedure 2 (The reference procedure for the spectrophotometric determination of diacetyl)**.** The reaction mixture was prepared by sequentially introducing the process solutions of 2-naphtol (150 μL), creatine (350 μL), diacetyl (the final concentration in mixture 0.01–0.20 μg/mL), and distilled water to 10 mL. The mixture was held for 40 min, then the absorption spectra of the resulting product within the wavelength range of 400–600 (190–700) nm was registered. The photometric determination was performed at a wavelength of 527 nm, which corresponds to the maximum absorption band in the visible region of the spectrum.

Procedure 3 (The modified procedure of the spectrophotometric determination of diacetyl)**.** The reaction mixture was prepared by sequentially introducing the process solutions of 2-naphtol (50 μL), creatine (100 μL), diacetyl (the final concentration in mixture 0.001–0.300 μg/mL), and distilled water to 10 mL. After 40 min, the photometric determination was performed at a wavelength of 527 nm.

Procedure 4 (The thermal-lens determination of the presynthesized reaction product)**.** Solutions were prepared with reference to procedure 3, then bidistilled water was added so that the product concentration amounted to $0.1–1.2 \times 10^{-4}$ μg/mL (in terms of diacetyl). The resulting solutions were registered on the thermal-lens spectrometer at a wavelength of 514.5 nm. The measurements of the signal for the background solution and distilled water were carried out in the same way (160 μL and 350 μL of the process solutions of 2-naphtol and creatine, respectively, were introduced to 10 mL of the resulting solution with no diacetyl present).

Procedure 5 (The procedure for the thermal-lens determination of diacetyl)**.** The reaction mixture was prepared by sequentially introducing the process solutions of 2-naphtol (50 μL), creatine (100 μL), diacetyl (the final concentration in mixture 2×10^{-4}–0.02 μg/mL), and distilled water to 10 mL. After 40 min, thermal-lens determination was performed at 514.5 nm. The background solution was prepared in the same way (50 μL of 2-naphtol process solution and 100 μL of creatine process solution with no diacetyl added). The thermal-lens measurements were performed at a wavelength of 514.5 nm, 40 min after introducing the components.

RESULTS AND DISCUSSION

The existing methods of the spectrophotometric determination of diacetyl do not possess sufficient sensibility and selectivity [1–4]. The most sensible of them is based on a photometric reaction with creatine in the presence of naphthols, resulting in the formation of a

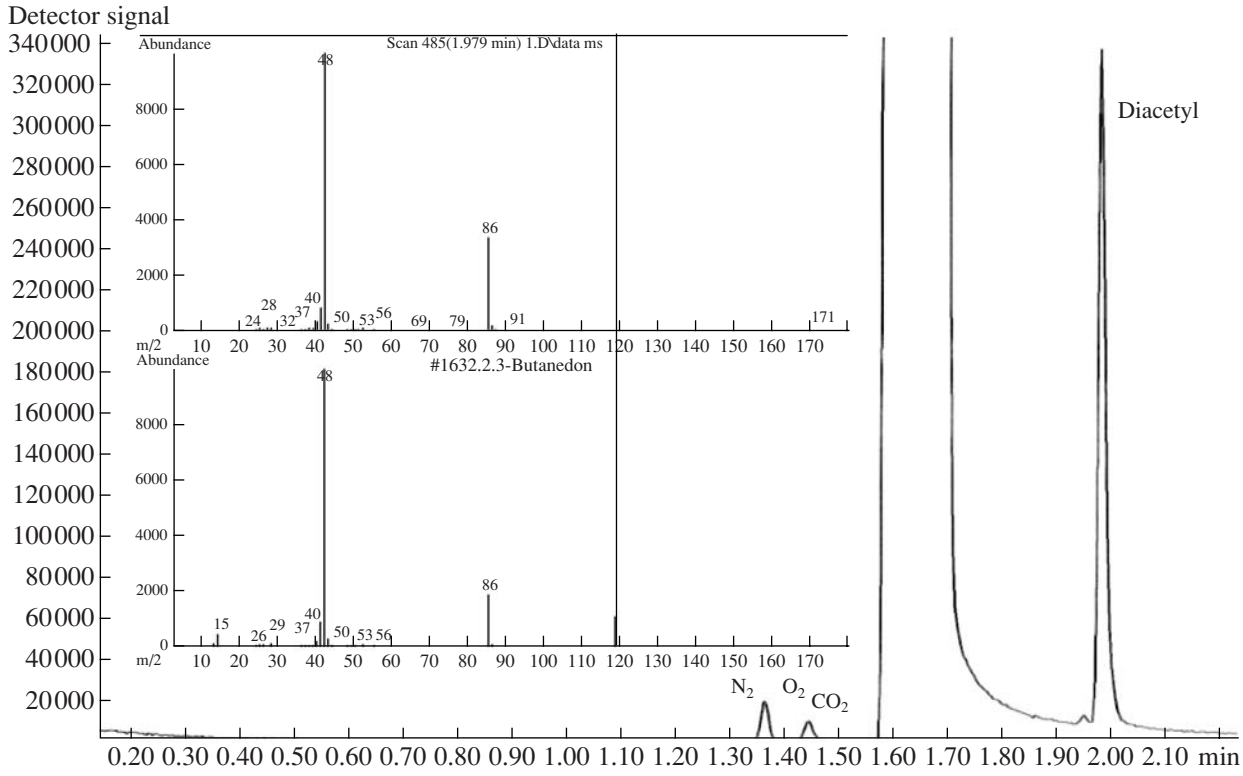

Fig. 1. GC-MS Chromatogram of diacetyl determination (70 μg/mL), retention time t_R = 2.00 min. Insertion: mass spectra of diacetyl, corresponding to the peak with t_R = 2.00 min. The top spectrum represents the analyzed substance, the bottom spectrum represents literature data.

product of undeclared composition [1]. Spectrophotometric and thermal-lens methods for detecting small concentrations of diacetyl in solutions using 2-naphtol are of interest. A less expensive chemical, 2-naphtol can participate in the photometric reaction under consideration, although it has not yet been investigated. Hereby, we needed to select the conditions of the spectrophotometric determination of diacetyl, adapt them for the thermal-lens measurements, and compare the data obtained with the results of chromatography methods using spectrophotometric and mass-spectrometric detection.

Chromatography-mass spectrometry determination of diacetyl. The peaks obtained according to procedure 1 were identified as belonging to acetonitrile and diacetyl (Fig. 1). Intense ion signals with the charge to mass ratio of 86 and 43 Da can be seen in the mass spectrum of the chromatographic peak with t_R = 2 min (Fig. 1, insertion) which correspond to diacetyl and acetyl, the most easily obtained fragmentary ion. A comparison of the spectra obtained with the standard mass spectra from the apparatus database showed a good agreement with the spectrum of diacetyl. According to the results obtained, an estimation of the detection limit for diacetyl using the gas chromatography/mass spectrometry method was made. It amounted to 1.7 ng/mL, which is approximately twofold lower than is claimed in literature [10]. Thus, chromatography-mass spectrometry allows for the determination of diacetyl in microamounts, however, ethanol impedes the determination through its close retention time. For this reason, the identification of diacetyl based on its mass spectrum under such conditions is almost impossible, which narrows the range of objects subjected to analysis.

Spectrophotometric determination of diacetyl. The reference procedure for the determination of diacetyl (procedure 2) is based on data [1]. Under the substitution of 1-naphtol for 2-naphtol, absorbance appears to be higher for all investigated concentrations, while the signal for control experiment amounts to 0.3. Absorption spectra of the reaction mixtures demonstrated that a deviation from Beer's law is observed for solutions where diacetyl concentration is higher than 0.1 μg/mL due to the lack of photometric reagents. For this reason, a small excess of 2-naphtol and creatine against [1] was added, and the photometric measurements were performed at a wavelength of 525 nm, which corresponds to the absorption band maximum.

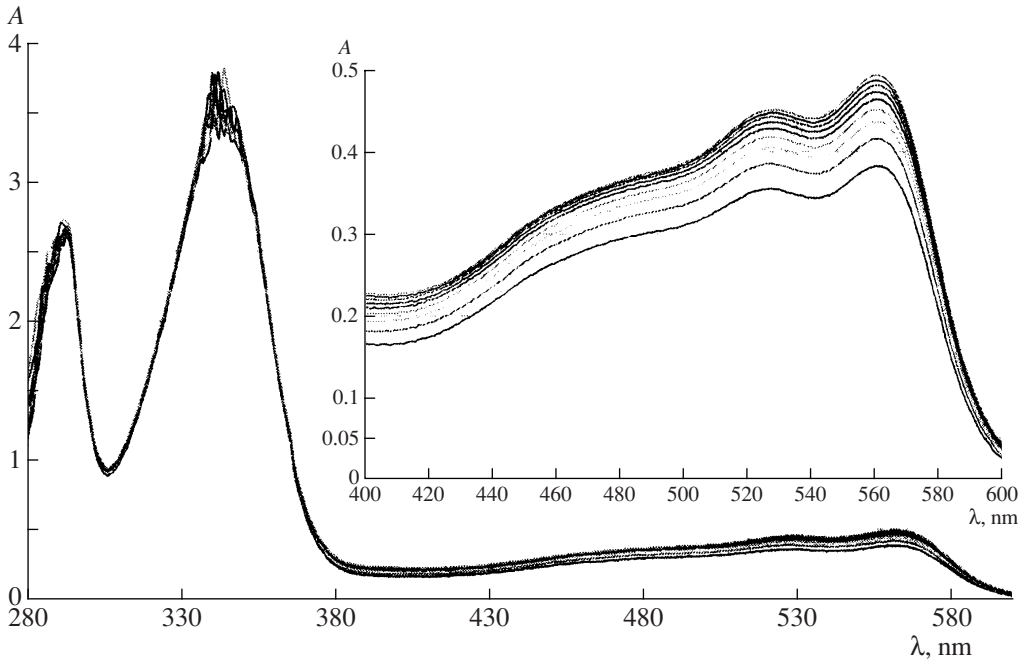

Fig. 2. Dependence of absorption spectrum for the aqueous solution of product resulting from the reaction between diacetyl, creatine, and alkaline solution of 2-naphthol (diacetyl concentration is 0.03 μg/mL, procedure 3) against reaction time, optical path length l = 1 cm (insertion: 400 to 600 nm range, scaled).

The calibrating dependence is described by the equation:

$$A_{525} = (30.0 \pm 0.1)c + (0.30 \pm 0.03)$$
$$(n = 7; \quad P = 0.95; \quad r = 0.98), \qquad (5)$$

where c is the diacetyl concentration (μg/mL). The detection limit for diacetyl c_{min} amounts to 30 ng/mL.

Such insufficient characteristics of the determination of diacetyl caused the need for changing the reference procedure. The absorption spectra of the reaction products against the pH value of the solution, which was controlled by adding diluted H_2SO_4 and NaOH to the reaction mixture, were investigated. It was shown that the optimum pH value is 8.5–10.0. Kinetics of the photometric reaction under consideration was investigated. It was established that it is optimal to register spectra 35–40 min after the introduction of the components, which corresponds to almost a total completion of the formation of colored reaction products (Fig. 2). It can be seen from Fig. 2 that the colored reaction product forming has two absorption maxima at 527 and 563 nm. However, despite the fact that the second product has a higher value of the molar absorption coefficient, it is characterized by a lower linearity of the calibrating dependence, notably, in the region of low diacetyl concentration. All subsequent spectrophotometric measurements were performed at 527 nm. The calibrating dependence is described by the equation:

$$A_{527} = (31.1 \pm 0.1)c + (0.18 \pm 0.02)$$
$$(n = 6; \quad P = 0.95; \quad r = 0.9330), \qquad (6)$$

where c is the diacetyl concentration (μg/mL). The detection limit for diacetyl amounts to 10 ng/mL, the lower bound is 40 ng/mL, the relative standard deviation in the range of diacetyl concentrations from 50 to 300 ng/mL range does not exceed 0.2. The detection limit obtained is fivefold lower than that for the existing spectrophotometric technique using 1-naphtol [1]. The influence of ethanol in a concentration of 4–6 vol % on the metrological characteristics of the spectrophotometric determination of diacetyl is negligible. Good metrological characteristics enable adapting the present procedure for thermal-lens measurements.

Thermal-lens determination of diacetyl. The product of interaction of diacetyl with 2-naphtol and creatine is stable against laser irradiation (a change in absorbance is not observed both when laser irradiation is applied, and is not). For the thermal-lens measuring, a wavelength of 514.5 nm, which is the closest to the absorption band maximum, chosen for the spectrophotometric determination of diacetyl, was selected. The graduating dependence for the presynthesized product

of the diacetyl reaction with 2-naphtol and creatine in alkaline medium (procedure 4) is described by the equation (strength of the inducing radiation being 40 mW):

$$\theta_{514.5} = (210 \pm 1)c + (0.069 \pm 0.005) \quad (n = 6; \quad P = 0.95; \quad r = 0.9330), \tag{7}$$

where c is the diacetyl concentration (μg/mL). The detection limit for the presynthesized product, determined using thermal-lens spectrometry, is 0.3 ng/mL, which is twice as low as the detection limit achieved by means of the gas chromatography-mass spectroscopy method. According to all of the facts mentioned and considering the optimum conditions for spectrophotometric determination of diacetyl, we performed the thermal-lens determination of diacetyl using the selected reaction (procedure 5). Each solution was registered for 3–4 min in order to minimize the effect of short-period noise of the thermal-lens spectrometer [11, 12]. The graduating dependence is described by the equation (strength of the inducing radiation being 40 mW):

$$\theta_{514.5} = (205 \pm 5)c + (0.12 \pm 0.03) \quad (n = 8; \quad P = 0.95; \quad r = 0.9950), \tag{8}$$

where c is the diacetyl concentration (μg/mL). Therefore, the sensitivity coefficients for the presynthesized product, and the procedure of determining diacetyl differ negligibly. The comparison of data on the thermal-lens and spectrophotometric determinations of diacetyl allows for making the following conclusions. First, the theoretical increase in the sensitivity of the thermal-lens measurements (514.5 nm), compared to spectrophotometry (527 nm), according to equation (4) is 2.303×42 mW $\times$ 0.11 1/mV $\times$ 0.60 = 6.36, while the experimental ratio between the sensitivity coefficients for the thermal-lens spectroscopy and spectrophotometry (equations (7) and (5)) provides the value $205/31.1 = 6.6 \pm 0.3$ ($n = 12$, $P = 0.95$); i.e., a small distinction between the theoretical sensitivity and that of the experimental thermal-lens measurements occurs.

The fact that the ratio between the sensitivity coefficients (6.6) and the detection limits for diacetyl (10/2 = 5) are roughly the same is very important, as it proves a slight change in the reaction conditions (between procedures 3 and 5). Another advantage of the conditions of the thermal-lens determination is that it is characterized by the lowest absolute value of a free term for the graduating equations (5) and (7), and the best correlation coefficient of the graduating dependence (0.9950/0.9390 = 10%).

Overall, the selected conditions for the photometric determination of diacetyl are suitable for determining trace amounts of it, moreover, the effect of the disturbing factors is sufficiently small, and conditions of the thermal-lens determination are characterized by high accuracy and sensitivity. The comparison of the detection limits for the presynthesized product and for the photometric reaction demonstrates that the sensitivity of the thermal-lens determination potentially may be substantially lowered through the delicate optimization of the reaction conditions, however, this was beyond the scope of our study.

In contrast to chromatographic determination, in the case of using thermal-lens spectrometry for the determination of diacetyl, the ethanol traces do not have an impeding effect due to a negligible change in the thermooptical properties of the medium. In the case when the determination is performed in solution containing 4–6 vol % of ethanol, the increase of the thermal-lens signal by 25–30% as a result of the enhancement of thermooptical properties of the medium needs to be considered [13, 14]. On the one hand, such an increase of the signal can be neglected near the detection limit, as the determination will be mainly assessed by the signal fluctuations of the control experiment, which will not cause a large inaccuracy of determination. On the other hand, this effect could be used to enhance the sensitivity of the thermal-lens determination, if the solutions containing corresponding amounts of ethanol were used as the reference samples, on which the further investigations can be based.

Gas chromatography-mass spectrometry (SC-MS) and thermal-lens spectrometry (TLS) methods are comparable in terms of detections limits (0.7 and 2.0 ng/mL, respectively), although in TLS the detection limit can be additionally lowered due the optimization of the conditions of the photometric reaction and varying the composition of the reaction medium, and also due to increasing the laser radiation power [2]. Moreover, the cost of the determination according to the proposed procedure with a small amount of comparatively inexpensive chemicals used appears to be significantly lower, which can be considered to be an important advantage of the photometric determination of diacetyl. Finally, although in our work we used sufficiently long reaction times in order to increase the completeness degree, 50% product yield requires only 20–30 s; i.e., the thermal-lens determination of diacetyl can be optimized to be performed in a flow, e.g., in a micro-flow injection analysis or in microfluid chips.

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