JOURNAL OF ANALYTICAL CHEMISTRY, 2025, Vol. 80, No. 1, pp. 43–51

ARTICLES

LUMINESCENT DETERMINATION OF DOPAMINE USING A CAMERA

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Received June 26, 2024
Revised July 30, 2024
Accepted July 31, 2024

Abstract. It is shown that the interaction of dopamine with fluorescamine can be used as the basis for the luminescent determination of dopamine using a camera, since the product formed as a result of this interaction is characterized by a maximum luminescence in the visible region (485 nm), and an LED emitting light in the near ultraviolet is sufficient to excite luminescence areas (395 nm). The reaction should be carried out at a pH of 8-8.5 in a phosphate buffer solution for 5 minutes, fluorescamine should be introduced into the reaction mixture last. Some analytical characteristics of camera detection are evaluated and compared with the characteristics of a similar dopamine detection using a professional spectrofluorimeter and spectrophotometer. The detection limits of dopamine using a camera, spectrophotometer, and spectrofluorimeter were 1.8, 1.6, and 0.5 μM, with a range of detectable concentrations of 5.4–50 μM, 4.8–100 μM, and 1.5–100 μM, respectively. The presence of widespread inorganic ions, whose content is 10 times higher than the content of dopamine, does not interfere with the determination. The proposed method for determining dopamine can be used to control the quality of medicines.

Keywords: *dopamine, luminescent spectroscopy, color measurement, camera*

DOI: 10.31857/S00444502250105e6

It is of interest to use various household color-recording devices in chemical analysis as an alternative to professional equipment [1, 2]. The use of such widely available devices for analyzing objects characterized by the simplicity of the matrix and high content of analytes is especially promising, since the use of expensive equipment for these purposes is in some cases not justified. Cameras [3, 4], scanners [5], smartphones [6, 7], and monitor calibrators [8-11] have proven themselves well in the analysis of various substances. With the help of such devices, it is possible to determine compounds capable of absorbing or emitting light in the visible region of the spectrum, as well as their derivatives with these properties. Through the use of photometric or luminescent reactions can be carried out by colorimetric determination of biologically active compounds, for example, catecholamines (dopamine, norepinephrine, adrenaline) – chemical mediators and "control" molecules (mediators and neurohormones) in intercellular interactions in humans and animals [12].

It is known that fluorescamine, which does not possess intrinsic luminescence, has proven itself well as a reagent for the luminescent determination of primary amines [13]. There is information about the chromatographic determination of dopamine and norepinephrine by reaction with fluorescamine, the latter being used as a derivatizing agent [14-17]; the use of this reaction for the determination of norepinephrine by luminescent spectroscopy is described [18]. It is known that the resulting product is characterized by the excitation of luminescence in the near UV region (350-400 nm) and luminescence in the visible region of the spectrum, and therefore the use of household color-recording devices for signal registration is promising.

The purpose of this work is to study the possibility of luminescent determination of dopamine by its reaction with fluorescamine using a digital camera.

METHODS AND MATERIALS

Reagents and solutions. The reagents used were dopamine hydrochloride H. D. A. (Sigma-Andrich, USA), fluorescamine H.D. A. (Sigma-Aldrich, Israel), hydrochloric acid reagent grade, acetic acid reagent grade, sodium hydroxide reagent grade, phosphoric acid reagent grade, boric acid reagent grade, KH_2PO_4

reagent grade, Na_2HPO_4 reagent grade, K_2SO_4 reagent grade, NaCl reagent grade, $NaNO_3$ reagent grade, $MgSO_4$ reagent grade, epinephrine AR grade (Sigma-Andrich, USA), acetonitrile (HPLC grade), 1-octanesulfonate sodium AR grade (Sigma-Aldrich, Germany).

A standard 2.5 mM dopamine solution was prepared by dissolving an exact sample of dopamine hydrochloride in deionized water and stored at a temperature of -18 °C.

A working dopamine solution with a concentration of 100 μM was prepared by diluting the initial standard solution with deionized water. A 3.5 mM fluorescamine solution was prepared by dissolving an exact sample in acetonitrile. For the preparation of universal buffer mixtures with pH 2.2, 4.8, 6.9, 7.5, 8.4, 8.8, 9.6, 11.5 to 10 ml of a mixture of 0.04 M phosphoric, acetic and boric acids using an automatic pipette of variable volume Discovery Comfort 100-1000 μl (HTL, Poland) was added 1.00, 3.25, 5.00, 5.50, 6.25, 6.50, 7.50, 8.75 ml of 0.2 M NaOH solution. To prepare a borate buffer solution with a pH of 8.5 to 6.7 ml of 0.05 M sodium tetraborate solution (12.367 g H_3BO_3, 100 ml of 1 M NaOH solution in 1 liter), 3.3 ml 0.1 M HCl was added. To prepare a phosphate buffer solution with a pH of 8.5, 1/150.6 ml of an M solution of Na_2HPO_4 was added to 19.4 ml of an 1/15M solution of KH_2PO_4.

Equipment. The luminescence spectra of the analyzed solutions were recorded on a fluorometer Fluorat-02-Panorama (Lumex, Russia). The absorption spectra were measured on an SF-104 spectrophotometer (Aquilon, Russia). The pH values were determined on a pH meter-ionomer Expert 001 (Econix-expert, Russia). Deionized water was obtained using the Millipore Simplicity purification system (Millipore, Germany).

Photographs of solutions of the fluorescent product of the interaction of dopamine and fluorescamine were obtained using a Canon EOS 550D camera (Canon, Japan) equipped with a Canon EF-S 18-55 mm lens (Canon, Japan), shutter speed 0.5 s, ISO 800, f 4.5. A LUF-11 ultraviolet lamp equipped with three LEDs (LuazON, Russia) was used to excite the luminescence of the samples. Using the GIMP GIMP 2.10.36 program (GIMP, USA), the averaged color coordinates of the samples in the RGB system were extracted from the obtained photographs for areas measuring 200x200 pixels.

When analyzing a real object, a Tsvetyauza liquid chromatograph (NPO Khimavtomatika, Russia) with an amperometric detector (E = 1.2 V), an Eclipse XDB-C18 column (Adgilent, USA), and a mobile phase of acetonitrile (10%) acidified with 0.1% phosphoric acid were used to protonate catecholamine with an additive of 0.3 mM 1- sodium octanesulfonate as an ion-paired reagent [19], the sample was injected using a dispenser loop (20 μl), the flow rate was 0.4 ml/min.

Experimental methodology. The experiment involved the reaction of dopamine with fluorescamine in the presence of a specific buffer solution. Fluorescamine was administered last. 5 minutes after mixing the reagents, the analyzed solution was placed in a quartz cuvette (l = 1 cm) and the luminescence spectrum was recorded using a Fluorat-02-Panorama spectrofluorimeter, the absorption spectrum using an SF-104 spectrophotometer, or photographs of the solutions were obtained using a Canon camera. Photographing was carried out in the absence of extraneous light sources, the cuvette was placed on a sheet of black matte paper, and an ultraviolet lamp was placed at a distance of 5 cm from the cuvette. The received data was exported to Microsoft Excel.

To analyze the drug, the test solution was pre-diluted with deionized water to a concentration of 1000 μM, assuming that the content of the active substance in the drug corresponds to the declared one (40 mg / ml in terms of dopamine hydrochloride). During the fluorimetric determination, an aliquot of the resulting solution was introduced into the reaction mixture, creating a dopamine concentration of 25 μM. For chromatographic analysis, the solution was diluted to an estimated dopamine content of 5 μM and injected into a chromatograph. To obtain the results of the analysis, a calibration dependence was used, built in the range of 1-10 μM.

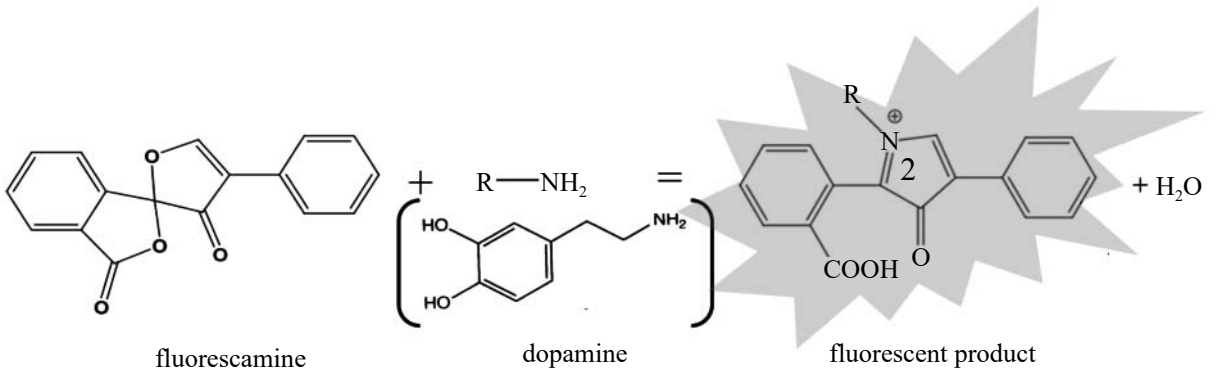
DISCUSSION OF RESULTS

The determination of dopamine is based on its reaction with fluorescamine to form a fluorescent product, the luminescence intensity of which serves as an analytical signal (Scheme 1).

To select the conditions for the determination of dopamine, a 3D luminescence spectrum of the dopamine-fluorescamine interaction product was preliminarily obtained, and the effect of pH, the order of reagent administration, reaction time, and fluorescamine concentration on the luminescence intensity was studied.

Figure 1 shows the normalized 3D luminescence spectrum of the dopamine-fluorescamine reaction product recorded on a spectrofluorimeter. The maximum luminescence excitation is observed at 395 nm, the maximum luminescence is at 485 nm, and no other pronounced spectral features have been detected in the studied wavelength range up to 220 nm. The luminescence intensity at 485 nm was used as an analytical signal for measurements using a spectrofluorimeter.

Effect of pH and order of reagent administration. To study the effect of pH on luminescence intensity, solutions with constant concentrations of dopamine and fluorescamine with pH values ranging from 2 to 11.5 were prepared. The required pH values were created using universal buffer mixtures. Figure 2 shows the dependences of luminescence intensity on pH for cases



Scheme 1. Interaction of dopamine and fluorescamine.

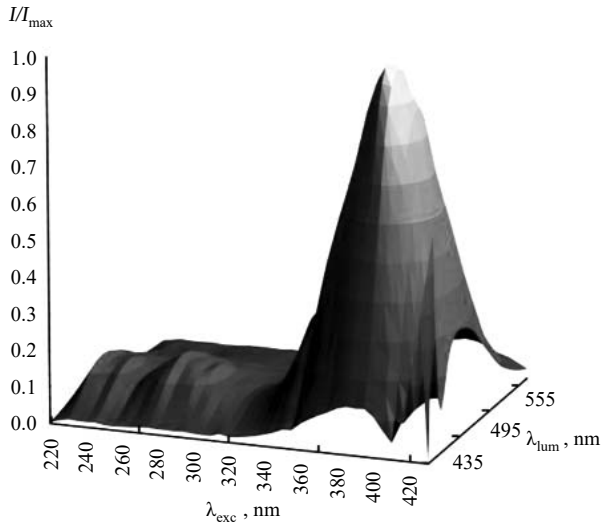


Fig. 1. Normalized 3D luminescence spectrum of the dopamine-fluorescamine reaction product. $c_{dopamine}$ = 25 μM, $c_{fluorescamine}$ = 200 μM, pH 8.5, V = 5.0 ml).

when fluorescamine (curve *1*) and dopamine (curve *2*) were introduced into the reaction mixture last. It can be seen that in both cases the luminescence intensity is maximal during the reaction at pH 8-8.5. This is due to the fact that in a highly alkaline medium, the product of the reaction of an amine with fluorescamine is in a hydroxylated form, and in a strongly acidic form it is in a lactone form, which have a non–planar structure and do not luminesce [13]. In addition, a decrease in the intensity of luminescence in an acidic environment may be associated with a decrease in the concentration of the luminescent product due to protonation of dopamine and a decrease in its nucleophilic activity. At a pH of 8-8.5, dopamine is present mainly in an uncharged form, and the product of its reaction with fluorexamine is in the form of a rigid planar cation with luminescence.

When fluorescamine is last introduced into the reaction mixture, the luminescence intensity is significantly higher than when dopamine is last introduced. Probably, if fluorescamine is introduced first, then even before the interaction with dopamine begins, it will undergo hydrolysis or react with the components of the buffer solution, i.e. its concentration will decrease and, as a result, the amount of the target product will decrease. Dopamine does not change in a short period of time, so it should be administered earlier than fluorescamine.

The effect of the buffer solution composition on the luminescence intensity was studied. For this purpose, a universal buffer mixture, borate and phosphate buffer solutions with a pH value of 8.5 were prepared. In Fig. 3a, the luminescence intensity values in the corresponding buffer solutions are presented as a histogram. As you can see,

The luminescence intensity is maximal in a phosphate buffer solution. This is probably due to the fact that the matrix of this buffer solution has the simplest composition, in which the side reactions of fluorescamine with the components of the solution occur to the least extent, as well as luminescence quenching.

Varying the volume of the buffer mixture from 0.5 to 2 ml has no noticeable effect on the luminescence intensity. To reduce the effect of foreign components on the determination, it is advisable to use as small a volume of the buffer mixture as possible, capable of maintaining a set pH value, therefore, in further experiments 0.5 ml of phosphate buffer solution was introduced into the reaction mixture.

The effect of the time elapsed after the start of the reaction on the luminescence intensity is illustrated in Fig. 3b. Measurements were carried out on a spectrofluorimeter at certain intervals after the start of the reaction between dopamine and fluorescamine. The interaction occurs in less than 1 minute, and during the first 5 minutes after mixing the reagents, a decrease in luminescence is observed, probably due to the formation of unstable luminescent fluorescamine products with foreign components of the mixture, which disintegrate within 5 minutes. Then, for 1 hour, the luminescence intensity of the product practically does not change.

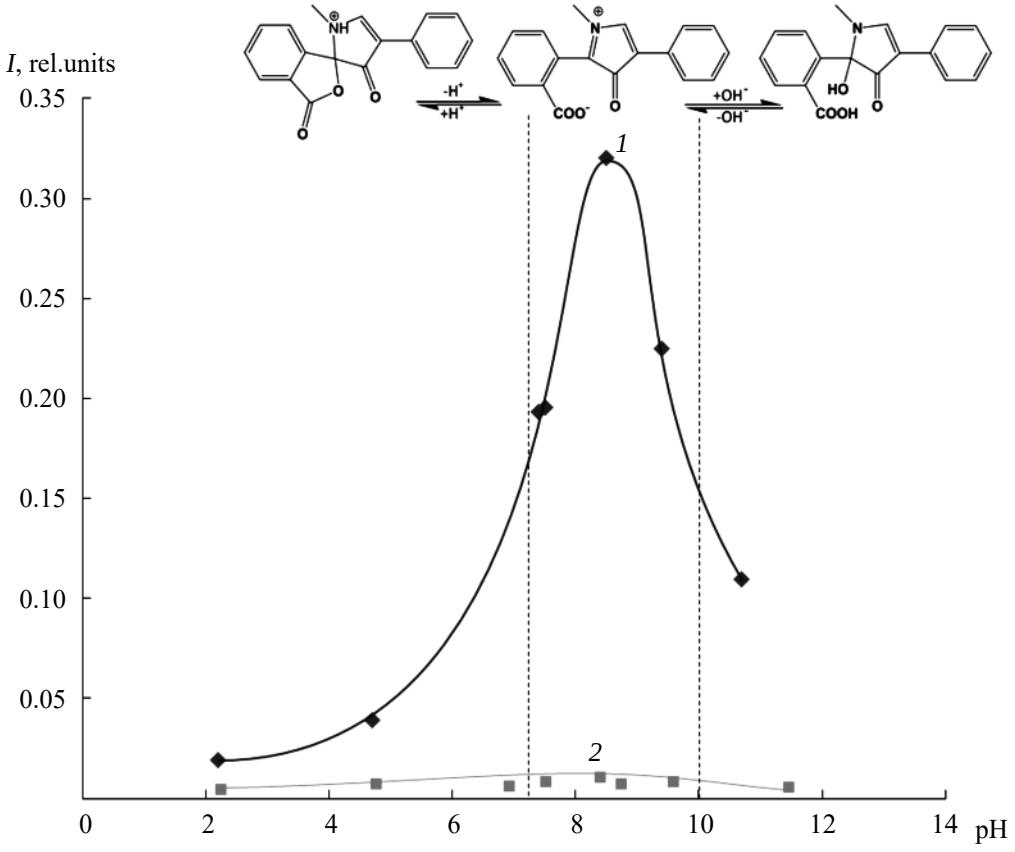


Fig. 2. Dependence of the luminescence intensity on the pH of the solution. $c_{dopamine}$ = 25 μM, $c_{fluorexamine}$ = 200 μM, 2.0 ml of universal buffer mixture, V = 5.0 ml, last of all (*1*) fluorescamine, (*2*) dopamine were administered.

Further measurements were carried out 5 minutes after mixing the reagents.

The **effect of the fluorescamine content** on the completeness of the reaction was studied. Figure 3b shows the dependence of the luminescence intensity on the concentration of the reagent. With an increase in the concentration of fluorescamine in the reaction mixture from 50 to 1000 μM, the fluorescence intensity of the product increases, with a further increase in the fluorescamine content, no noticeable increase in fluorescence is observed. To determine dopamine, you can choose a fluorescamine concentration of 200 μM, and to increase the intensity of the recorded signal and, probably, higher concentrations of fluorescamine can be used to increase the sensitivity of determination.

Determination of dopamine by reaction with fluorescamine. Under the selected conditions for dopamine determination, luminescence spectra were obtained using a spectrofluorimeter for solutions with fluorescamine concentrations of 200 and 1000 μM, as well as light absorption spectra measured on a spectrophotometer (Fig. 4a–b) for solutions with different concentrations of dopamine (from 1 to 100 μM) and the analytical signal dependences on dopamine concentration were constructed. In the case of luminescence measurement on a spectrofluorimeter, the luminescence intensity at 485 nm was used as the analytical signal; in the case of spectrophotometric analysis, the analytical signal was the optical density at 395 nm (the maximum absorption of the reaction product). The calibration dependences obtained using a spectrofluorimeter and a spectrophotometer are linear in the selected range of dopamine concentrations, the dependence equations and the values of the determination coefficients are given in Table 1. Since, as noted above, the introduction of a larger amount of fluorescamine into the reaction mixture allows for a higher luminescence intensity, it is advisable to study the effect of fluorescamine concentration on the sensitivity of the determination. The initial section of the calibration dependence with the addition of 1000 μM of fluorescamine is indeed characterized by a large tilt angle tangent. In the case of spectrophotometric analysis, as can be seen from the spectra, the maximum absorption of the reaction product falls on the shoulder of the maximum absorption of fluorescamine, the background signal in this case is high and it can be expected that the reproducibility of the analysis in this case will be low. For this reason, spectrophotometry is not recommended to determine dopamine under these conditions.

When dopamine interacts with fluorescamine, a product with luminescence in the visible region of the spectrum is formed, therefore it is advisable to evaluate the possibility of using colorimetry to determine dopamine. One of the options for colorometric analysis is to study the dependence of the color coordinate values obtained from photographs of samples on the concentration of the analyte. To perform colorometric analysis, photographs

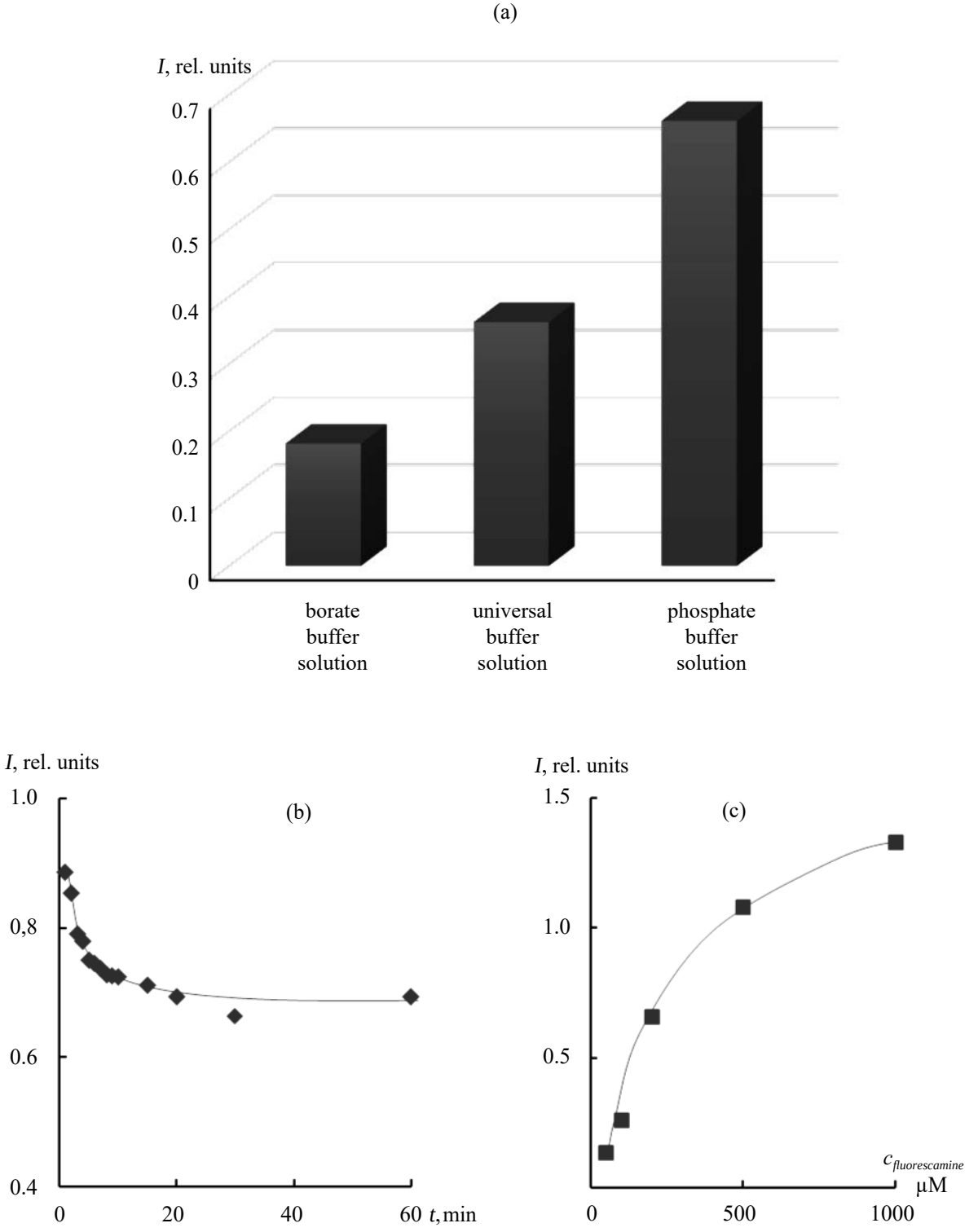


Fig. 3. The effect of (a) the nature of the buffer solution, (b) the interaction time, and (c) the fluorescamine concentration on the luminescence intensity of the dopamine-fluorescamine reaction product. c_{dopamine} = 25 μM; $c_{\text{fluorexamine}}$ = 200 μM (a), (b); 0.5 ml buffer solution with pH 8.5; phosphate buffer solution (b), (c); V = 5.0 ml; fluorescamine was administered last.

Table 1. Analytical characteristics of various methods for determining dopamine ($n = 5$, $P = 0.95$)

Recording device	Calibration equation dependencies	R^2	c_{min}, μM	RDC, μM
Spectrofluorimeter (200 μM of fluorescamine)	$y = 0.0254x + 0.06$	0.9965	0.5	1.5–100
Spectrofluorimeter (1000 μM of fluorescamine)	$y = 0.101x + 0.067$	0.9991	0.5	1.5–10
Spectrophotometer	$y = 0.0025x + 0.116$	0.9909	1.6	4.8–100
Photo camera	$y = 186(1 - e^{-0.055x})$	0.9951	1.8	5.4–50

Note: RDC is the range of detectable contents.

of calibration solutions were obtained when irradiated with an ultraviolet lamp (Fig. 5a), and color coordinates in RGB, CMYK, and Lab systems were extracted from the photographs. It was found that the values of the color coordinate along the green channel (G) in the RGB system, the brightness L and the b coordinates in the Lab system increase monotonously with increasing dopamine content, the values of the M coordinate in the CMYK system and the a coordinate in the Lab system decrease monotonously – the use of these coordinates as an analytical signal can be used as the basis for determining dopamine using a camera. For the remaining coordinates, there is no correlation with the dopamine content in these color spaces. In order to select the most appropriate signal for determining dopamine, the ratio of the signal at a dopamine content of 10 μM (minus the control experiment) to the background signal (standard deviation of the control experiment) was estimated, calculated using different color coordinates ($n = 5$) as an analytical signal:

Color coordinate	G	M	L	a	b
Signal/Background	12	9	8	8	9

As you can see, the greatest ratio is achieved when using the coordinate G. In addition, the RGB color coordinate system is the most common and is usually used by default in various color-recording devices. In this case, it is advisable to use the G coordinate as an analytical signal. Figure 5b shows the calibration dependence obtained with the camera, using the analytical signal the color coordinate G minus the control experiment signal. The obtained dependence is linear in the concentration range of 0-15 μM; at high concentrations, a deviation from linearity is observed. In the concentration range of 0-100 μM, the obtained dependence can be approximated using a function of the form $y = a(1 - e^{-(bx)})$, where y is the color coordinate G, x is the concentration of dopamine, μM, a, b are constants. This function corresponds to the dependence of the luminescence intensity on the concentration of the substance being determined at high values of the optical density of the analyzed solutions: $I = I_0\varphi_к(1 - 10^{-\varepsilon lc})$ at $\varepsilon lc > 0.05$, where I is the luminescence intensity of the analyzed solution, I_0 is the intensity of the radiation source, $\varphi_к$ is the luminescence quantum yield, ε is the molar absorption coefficient, l is the optical path length, c is the concentration of the luminescent substance. The

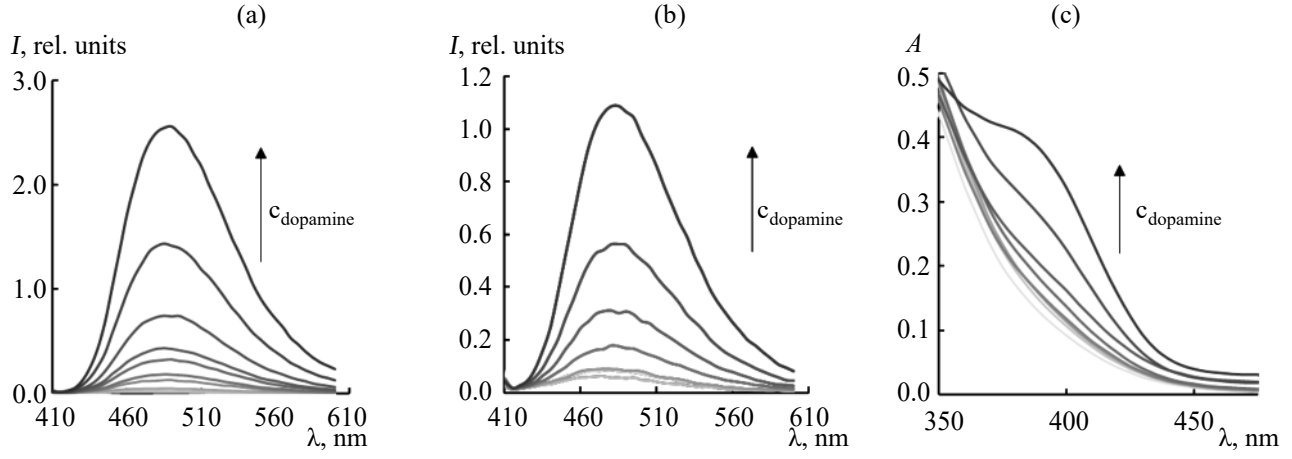


Fig. 4. Spectra of (a), (b) luminescence (c) and absorption of dopamine solutions after interaction with fluorescamine. (a), (c) 0—100, (b) 0—10 μM of dopamine; (a), (c) 200, (b) 1000 μM of fluorescamine; 0.5 ml of phosphate buffer solution with pH 8.5; $t = 5$ min; $V = 5.0$ ml; fluorescamine was administered last.

fact that for the same concentration range, the calibration dependence obtained with a fluorimeter is approximated by a linear function, and with an exponential function with a camera, can be explained by the exposure time that is too high for these conditions (0.5 s) and, accordingly, a long accumulation of the signal: the value of the G coordinate approaches its maximum value (255). For measuring solutions with high concentrations of reagents, it is recommended to reduce the exposure time (1/10–1/15 s for 25-100 μM of solutions), and to increase it (0.8 s) for dilute 0-10 μM of solutions.

Analytical characteristics of dopamine determination. Table 1 presents some analytical characteristics of the proposed methods for determining dopamine. The lowest detection limit, as expected, is characterized by a professional spectrofluorimeter, while the introduction of more fluorescamine into the reaction mixture does not

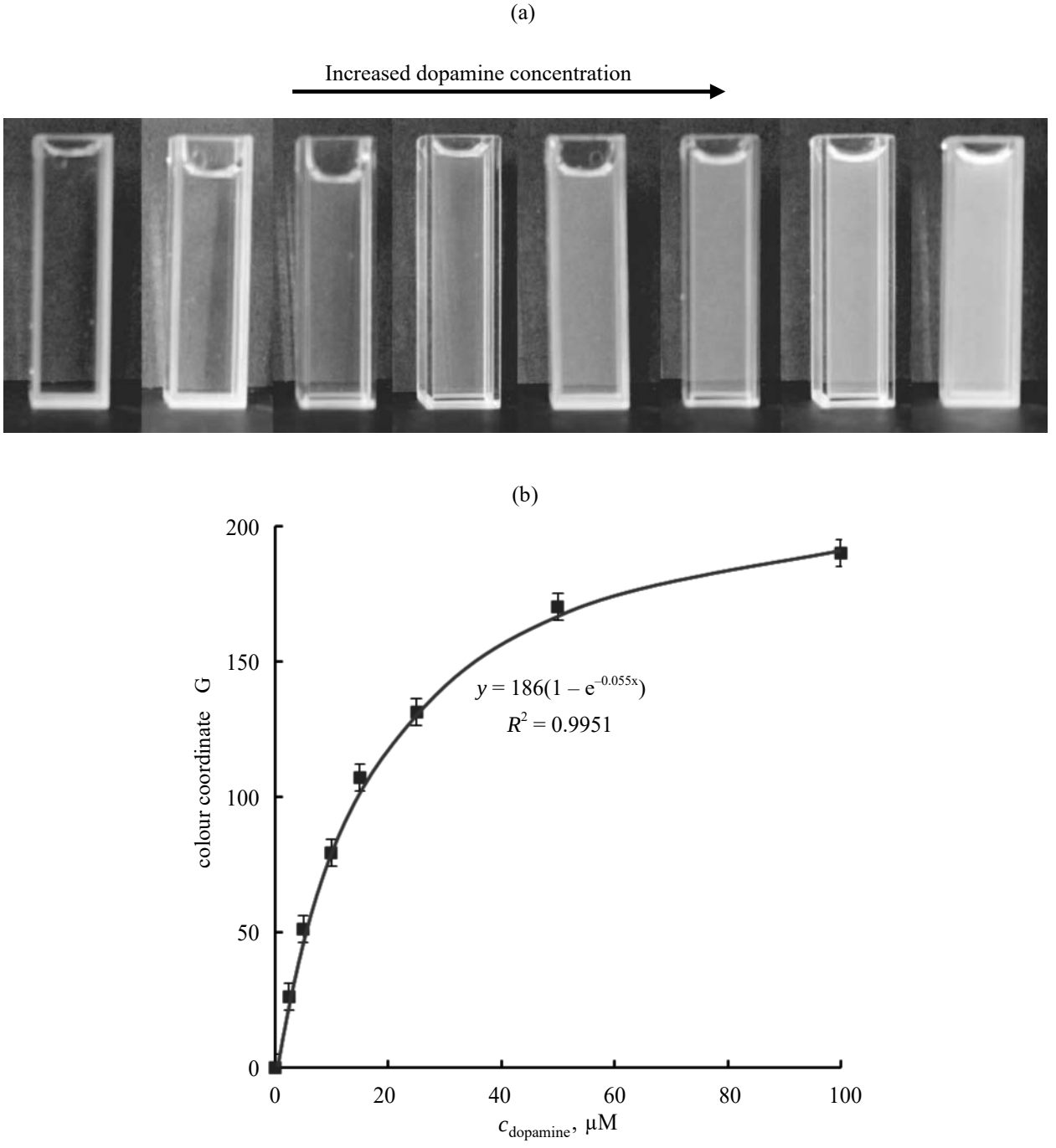


Fig. 5. Photographs of calibration (a) solutions of the dopamine-fluorescamine interaction product and (b) the calibration dependence for the determination of dopamine obtained using a camera. 0, 2.5, 5, 10, 15, 25, 50, 100 μM of dopamine; 200 μM of fluorescamine; 0.5 ml of phosphate buffer solution with pH 8.5; $t = 5$ min; $V = 5.0$ ml; fluorescamine was administered last.

lead to a decrease in the detection limit due to an increase in the standard deviation of the control experiment, i.e. the use of higher concentrations of fluorescamine is impractical. Comparable limits of detection are achieved using a spectrophotometer and a camera. A camera is a more compact, mobile, and inexpensive instrument, so it can be recommended for field measurements, unlike professional devices.

The influence of the most common inorganic ions on the luminescence intensity was studied. The criterion for the interfering effect was the deviation of the detected dopamine content from the administered one by more than 5%. It was found that Na^+, K^+, Cl^-, NO_3^-, SO_4^{2-}, Mg^{2+} ions do not interfere with the determination of dopamine at a dopamine: foreign ion ratio of 1: 10. The presence of comparable amounts of epinephrine, a catecholamine containing a secondary amino group, also does not interfere with the determination.

Analysis of a real object – the medical drug Dopamine-Ferein (PJSC Britsalov-A, Russia; active ingredient – dopamine hydrochloride, 40 mg/ml) for dopamine content. The data obtained using a camera, a spectrofluorimeter, and the HPLC method are shown in Table 2. The results obtained by different methods are in good agreement with each other, which indicates the correctness of the definition.

Table 2. The results of the determination of dopamine in the medical drug Dopamine- Ferein (PJSC Britsalov-A, Russia) by various methods ($n = 3$, $P = 0.95$)

Method	Content of dopamine in preparation, mg/ml	s_r
Luminescent spectroscopy		
spectrofluorimeter	47 ± 3	0.02
photo camera	45 ± 7	0.06
HPLC	47.8 ± 0.3	0.003

* * *

Thus, the interaction of dopamine with fluorescamine is the basis for the luminescent determination of dopamine. When using a spectrofluorimeter, the luminescence of the samples was excited at 395 nm, and the measurement was carried out at a luminescence wavelength of 485 nm. The resulting product has luminescence in the visible region of the spectrum, therefore, colorometric determination of dopamine using a camera is possible when the luminescence is excited by an external UV source. The value of the G coordinate in the RGB system was used as an analytical signal. A spectrophotometric determination of dopamine by reactions of dopamine with fluorescamine is also possible. The detection limits of dopamine using a spectrofluorimeter, spectrophotometer, and camera are 0.5, 1.6, and 1.8 μM, ranges of defined contents − 1.5–100, 4.8–100, 5.4–50 μM, respectively. The determination is not hindered by the presence of the most common inorganic ions, whose content is 10 times higher than the content of dopamine, and comparable amounts of adrenaline. The proposed method for determining dopamine can be used for drug analysis.

FUNDING

The work was carried out with the financial support of the Russian Science Foundation (Grant No. 24-23-20004).

ACKNOWLEDGEMENTS

The study was conducted within the framework of the Development Program of the Interdisciplinary Scientific and Educational School of Moscow University "The future of the planet and global environmental changes".

CONFLICT OF INTERESTS

The authors of this paper declare that they have no conflict of interest.

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