Technology of recording spectra of bioactive drugs secondary radiation and mathematical treatment thereof

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Abstract. Technology of recording spectra of secondary radiation of bioactive specimens that makes it possible to perform express analysis of molecular structures of various classes was developed. The FSD Soft software package has been proposed for analyzing fluorescence spectra and mathematical treatment thereof. The software used made it possible to choose the scan mode (discrete or continuous) directly from the application window; to set exposure time for optical sensor; and to average measured spectrum in case of strong noise in measured radiation. Correlation functions have been built for identifying drugs from various manufacturers. Corresponding correlation coefficients have been calculated for analyzed drugs in relation to the standard. It has been shown that the calculated correlation functions make it possible to obtain highly accurate information about conformity of the sample analyzed to the standard.
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Introduction

As of today, the trend of studying secondary radiation spectra from complex molecular compounds has become notable. Experiments are being made in many laboratories, with the purpose to develop methods of substances analysis using secondary radiation methods. In most cases, researchers consider direct use of their research results in relevant industries, such as biomedicine, pharmacology and industrial production. For example, in works [1,2] from laboratory of the Edinburgh School of Engineering, several methods of analyzing substances (biological preparations) are compared – the one using the fiber-optic technology and the one using a vial and a microscope. It was noted that the Raman-scattering spectroscopy becomes an effective means of studying composition and structure of complex substances, including explosives. [3] In order to improve transmission of laser radiation (femto-second laser), it is suggested to create probes with various butt coating [4] for fast scanning of the substance. In works [5,6] it is proposed to use schemes and fiber optic probing to obtain secondary radiation spectra, and efficiency of this method is calculated.

The aim of this work was obtaining, analysis and mathematical processing of spectra for analyzed and reference bioactive drugs on the example of commercially available pharmaceutical products from various manufacturers. The goal was achieved by using the fiber-optic methods [7, 8]. Mathematical processing and analysis of fluorescence spectra were performed using the FSD Soft software package.

Experiment method

The subjects of study were typical pharmaceuticals: citramonum, analgin, aspirin and paracetamol. In works [9, 10], ultraviolet and infrared absorption spectra of a number of pharmaceuticals were studied. The structure of all studied substances contains benzene rings, which fact leads to fundamental electronic absorption in the middle UV band. Accordingly, fluorescence is observed in these substances in the violet-red band when fluorescence is excited by short-wave (266 nm) electromagnetic radiation [11].

The method of recording fluorescence spectra using a "reflection" scheme (Fig. 1) was used. In this scheme, the desired signal is collected from the channel with the substance virtually from the point from which excitation radiation exits from the adjacent lightguide.

Therewith, the source of exciting ultraviolet radiation was the fourth harmonic (266 nm) of a YAG laser generating pulse-periodic radiation with 1064 nm wavelength. Average power of the exciting ultraviolet radiation was 10 mW, the laser radiation pulse repetition frequency was 3000 Hz, and duration was 10ns. The peak density of exciting ultraviolet radiation power on the surface of the drug analyzed was 105 W/cm2. A small amount of the substance to be analyzed in the form of a tablet was placed in a cuvet (14, Figure 1).

Quartz lightguides (10, 11, Figure 1) were used to guide the ultraviolet radiation to the substance and to retract the secondary radiation

arising in the sample to small-size spectral instrument (12, Figure 1).

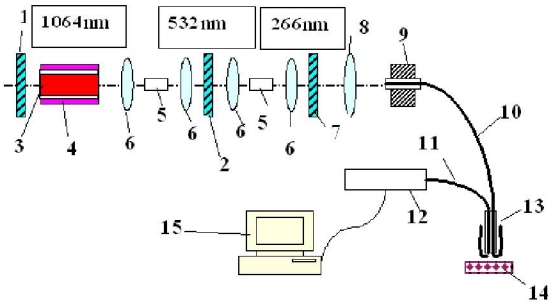

Fig.1. Scheme for recording secondary radiation spectra with single-channel probe for "reflection" bioactive compounds; 1, 2, 7 - mirrors; 3 - active element; 4 - "pumping"; 5 - nonlinear crystal; 6 - lens; 8 - condenser; 9 - fiber lock; 10, 11 - lighguides; 12 - spectrograph; 13 - probe; 14- sample to be analyzed; 15 - computer.

The FSD-8 mini spectrometer was used as the spectral instrument. The FSD Soft software supplied with the mini spectrometer is intended to make the mini spectrometer operate with a computer running on Windows XP, Windows 2000. This software made it possible to choose the scan mode (discrete or continuous) directly from the application window; to set optical sensor exposure time; and to average the measured spectrum in case of strong noise in measured radiation.

The application has two active windows. The first one is for displaying graphs of the spectrum, and the second one - for displaying graphs of integral components of the spectral characteristic. At the very top of the window, a menu bar is located, and below it there is a tool bar with standard buttons: "Open File", "Close file", "Save to file", "Print", "About". In the upper part of the window for displaying graphs of the spectrum, there is a wavelength axis (W) graduated in nanometers (nm); on the left side, there is an intensity axis (I) for the radiation tested. At the bottom there is the monitor of travel and operational control of studied graphs' colors. In the right part of the entire window there is a control panel (CP) for the measurement process that has several controls. Below the panel, there are bookmarks "Measurement" and "Processing".

The spectrometric data obtained in course of the experiment using the software were translated into a tabular form and stored for further processing. Due to high sensitivity of the apparatus, spectra had noisy nature, so noise filtering algorithms were applied, based on linear approximation of the data embedded in the Origin package for numerical data analysis.

In the FSD Soft application, all graphical and mathematical operations are only in EXCEL database format.

Main part

Fluorescence spectra of four pharmaceuticals have been recordednamely: citramonum, analgin, aspirin and paracetamol. [7] After computer processing, the normalized fluorescence spectra of studied aromatic compounds were built. Figures 2 (a) - (d) show normalized fluorescence spectra for aspirin (a), citramonum (b), analgin (c) and paracetamol (d). As it can be seen from this figure, for all analyzed pharmaceuticals, structured fluorescence bands are actually observed in the violet-red region of the spectrum, shape of which does not differ much, at least for citramonum and aspirin, as well as for analgin and paracetamol. Proximity of fluorescence spectra types for citramonum and aspirin is caused by the presence of the same component in them. At the same time, differences in fluorescence spectra of different citramonum surface areas are caused by uneven distribution of phenacetin and caffeine components in it [11]. Broadening of the analgin fluorescence band (see Fig. 2) in comparison to the paracetamol spectrum can be attributed to the more complex molecular structure of analgin.

To determine the quantitative distinction of fluorescent spectra obtained from various pharmaceuticals, correlation functions K_X^A ([lambda]) were built (see Figure 3) using the following relation [11]:

$$K_X^A(\lambda) = 1 - \left| i_X(\lambda) - i_A(\lambda) \right|, \qquad (1)$$

where i_X ([lambda]), i_A ([lambda]) are normalized fluorescence spectra of the analyzed drug (X) and aspirin (A). Correlation spectra were built in the wavelength [delta] [lambda] = 300 -500 nm with intervals of partition [delta] [lambda] i = 0.25 nm.

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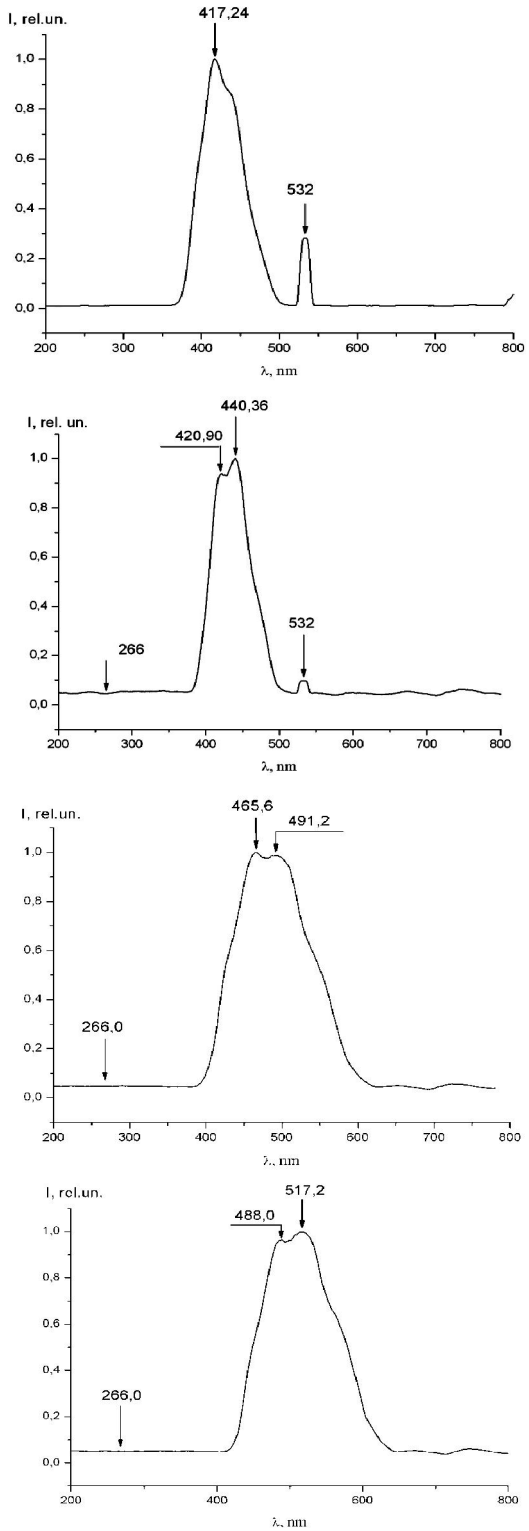
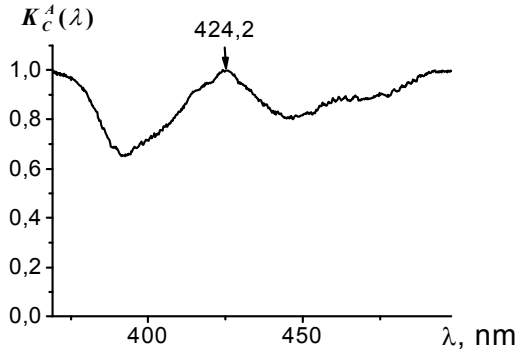
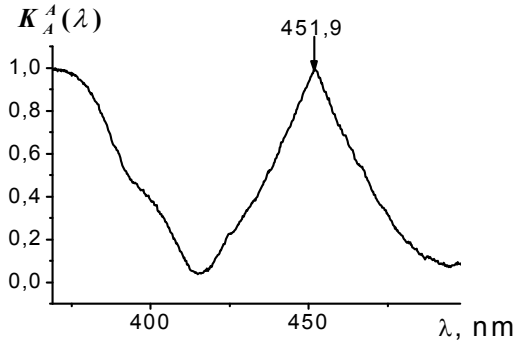
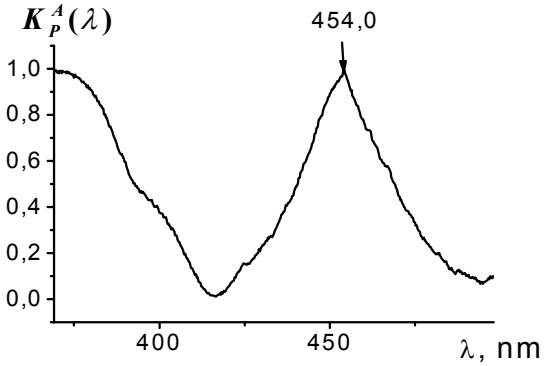


Fig. 2. Normalized spectra of secondary radiation forcitramonum (a), aspirin (b), analgin (c) and paracetamol (d) obtained under excitation by ultraviolet radiation of the fourth harmonic (266.0 nm) of a pulse-periodic YAG laser



$$K_C^A = \frac{1}{N}\sum_{i=1}^{i=N} K_C^A(\lambda_i) = 0.87$$





$$K_A^A = \frac{1}{N}\sum_{i=1}^{i=N} K_C^A(\lambda_i) = 0.47$$

b

$$K_P^A = \frac{1}{N}\sum_{i=1}^{i=N} K_P^A(\lambda_i) = 0.45$$

c

Figure 3. Correlation between the spectra for citramonum (a), analgin (b) and paracetamol (c) compared to the fluorescence spectrum for aspirin

Furthermore, corresponding correlation coefficients K_X^A (shown in Fig.3) were calculated for analyzed drugs as compared to aspirin by the following formula:

$$K_X^A = \frac{1}{N}\sum_{i=1}^{i=N} K_X^A(\lambda_i), \qquad (2)$$

where N is the number of breakdown ranges.

As it can be seen from Figure 3, correlation spectra provide quantitative information about the difference in the fluorescence spectra, which makes it possible to assign the drug analyzed to the desired type, and to monitor quality of commercial pharmaceuticals.

Conclusions

Thus, in this work, on the example of structurally similar pharmaceuticals (citramonum, aspirin, analgin and paracetamol), it was shown that for quantitative nondestructive control of molecular composition and structure of bioactive drugs that contain benzene rings, the method of fluorescence analysis may be effectively used, supplemented by building corresponding correlation functions. The developed method of correlation fluorescent spectroscopy can also be used for quantitative control of compliance for a wide class of bioactive drugs that are luminescent under ultraviolet excitation, spectral characteristics of which have been populated into the database. For mathematical processing and analysis of fluorescence spectra, FSD Soft software package is required.

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