ORIGINAL ARTICLES

OPTICAL SPECTROANALYZER WITH EXTENDED DYNAMIC RANGE FOR PHARMACOKINETIC INVESTIGATIONS OF PHOTOSENSITIZERS IN BIOTISSUE

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Abstract

Currently, the most promising method for the study of pharmacokinetics of drugs with fluorescent properties is the spectral-fluorescent method. In this article, we propose an algorithm for expanding the dynamic range of the spectrum analyzer by automatically monitoring the maximum spectral density in the recorded fluorescence spectrum and automatically controlled changes in the accumulation time depending on this value, followed by compensation of the output signal with regard to this change, as well as hardware circuit solutions that allow this algorithm.
Testing of LESA-01-"Biospec" spectrum analyzer, upgraded using the proposed approach, was carried out on photosensitizer dispersions based on tetra-3-phenylthiophthalocyanine hydroxyaluminium of various concentrations (from 0.01 mg/l to 50 mg/l), approximately corresponding to the concentrations realized in the process of studying pharmacokinetics in calibration samples and tissues of experimental animals.
The proposed solutions that implement the algorithm for recording fluorescence spectra with automatic change of accumulation time depending on the signal level, ensured a significant expansion of the dynamic range of the spectrum analyzer (up to 3.5 orders of magnitude) and improved accuracy in pharmacokinetic studies

Keywords: fluorescence diagnostics, pharmacokinetics, spectrum, intensity, exposure.

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ОПТИЧЕСКИЙ СПЕКТРОАНАЛИЗАТОР С РАСШИРЕННЫМ ДИНАМИЧЕСКИМ ДИАПАЗОНОМ ДЛЯ ФАРМАКОКИНЕТИЧЕСКИХ ИССЛЕДОВАНИЙ ФЛУОРЕСЦИРУЮЩИХ ПРЕПАРАТОВ В БИОТКАНЯХ

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Резюме

В настоящее время наиболее перспективным методом для исследования фармакокинетики препаратов, обладающих выраженными флуоресцентными свойствами, является спектрально-флуоресцентный метод. В этой статье мы предлагаем алгоритм расширения динамического диапазона спектроанализатора путем автоматического мониторинга максимального значения спектральной плотности в регистрируемом спектре флуоресценции и автоматического контролируемого изменения времени накопления в зависимости от этого значения с последующей компенсацией выходного сигнала с учетом этого изменения, а также схемные решения, позволяющие реализовать этот алгоритм.
Тестирование спектроанализатора ЛЭСА-01-«Биоспек», модернизированного с использованием предложенного подхода, проводилось на дисперсиях фотосенсибилизатора на основе тетра-3-фенилтиофталоцианина гидроксиалюминия различной концентрации (от 0,01 мг/л до 50 мг/л), примерно соответствующих концентрациям, реализующихся в процессе исследования фармакокинетики в калибровочных образцах и тканях экспериментальных животных. Предложенные решения, реализующие алгоритм регистрации спектров флуоресценции с автоматическим изменением времени накопления в зависимости от уровня сигнала, обеспечили существенное расширение динамического диапазона спектроанализатора (до 3.5 порядков) и повышение точности при фармакокинетических исследованиях.

Ключевые слова: флуоресцентная диагностика, фармакокинетика, спектр, интенсивность, экспозиция.

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Introduction

The study of the pharmacokinetics and biodistribution of a medicinal substance is based on an assessment of its concentration in organs, tissues and biological body fluids at specific points in time after administration [1]. One of the most important requirements for such studies is a wide dynamic range of the measurement method, which must be at least three orders of magnitude.

Currently, the most promising technique for studying the pharmacokinetics of drugs with pronounced fluorescent properties is the spectral-fluorescent method [1-4]. It is widely used to assess the level and selectivity of the accumulation of photosensitizers (PS) intended for photodynamic therapy and fluorescent diagnostics in biological tissues.

The spectroscopic equipment used for such studies usually includes a laser, a polychromator, a fiber-optic probe containing illuminating fibers which deliver excitation radiation to the biological tissue, and receiving optical fibers for delivering fluorescence radiation from the biological tissue to the polychromator input, and a matrix photodetector at the polychromator output, in particular, a charge-coupled device (CCD) or CMOS-ruler («Complementary Metal-Oxide-Semiconductor structure»). The signal recording system from each of the cells of the ruler, proportional to its charge, includes an analog-to-digital converter (ADC), a block of buffer memory and a personal computer (PC) [2, 5]. In a spectral-fluorescent study, the radiation from the laser output is introduced into the light conductor of the optical fiber probe. Coming out of the distal end of the illuminating fiber, this radiation irradiates the biological tissue containing the fluorescent drug, and initiates the fluorescence of its molecules. The intensity of the characteristic fluorescence band of the pharmaceutical agent in the first approximation is proportional to its content in the biotissue. The receiving optical fibers of the fiber-optic probe deliver the fluorescence radiation from the biological tissue to the input of the polychromator, where the spectral decomposition of this radiation takes place, after which the radiation falls on the ruler. The signal from the output of the ruler enters the ADC and the buffer memory unit. The computer uses digital data coming from the output of the buffer memory, corresponding to the intensity of the signal from each cell of the photodetector, and cell numbers of the photodetector, for which a certain wavelength is set according to the calibration results, to form a spectral curve (intensity vs. wavelength) that is displayed on the screen of the computer.

The dynamic range of the spectrum analyzer is mainly determined by the characteristics of the ruler. With high levels of incident light on a cell of the ruler, this cell and the adjacent cells of the ruler may undergo charge saturation; at low levels, the signal associated with the incident light may be hardly distinguishable against the background of hardware noise of the device (first of all, the noise of the ruler). Because of this, the dynamic range of known devices does not normally exceed two orders of magnitude.

This article discusses the possibility of expanding the dynamic range of a spectrum analyzer for pharmacokinetic studies of fluorescent drugs.

Materials and methods

Samples of liposomal dispersions of tetra-3-phenylthiophthalocyanine hydroxyaluminium in distilled water with concentrations of 0.01 mg/ml; 0.05 mg/ml; 0.1 mg/ml; 0.5 mg/ml; 2 mg/ml; 10 mg/ml; 25 mg/ml; 50 mg/ml in Eppendorf tubes were used as test objects. The control sample was an Eppendorf tube with water.

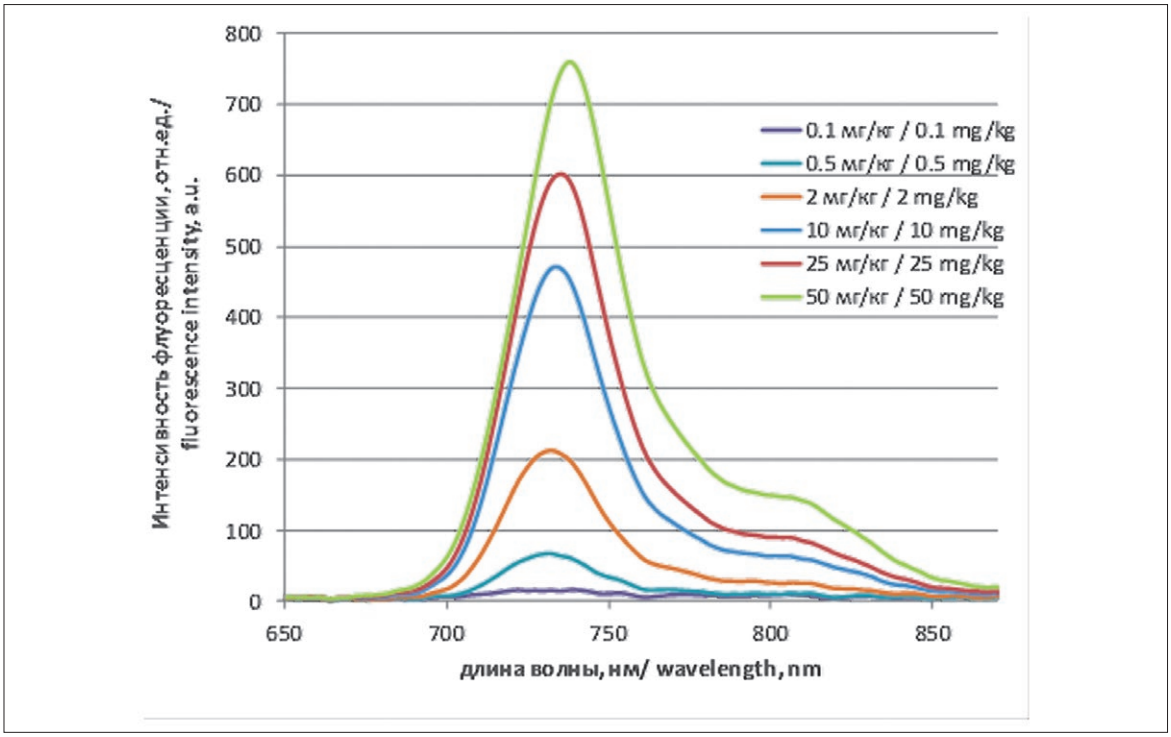
Fluorescence studies were performed with the use of a laser electron spectral analyzer LESA-01-Biospec (OOO «BIOSPEC», Russia).

Results and discussion

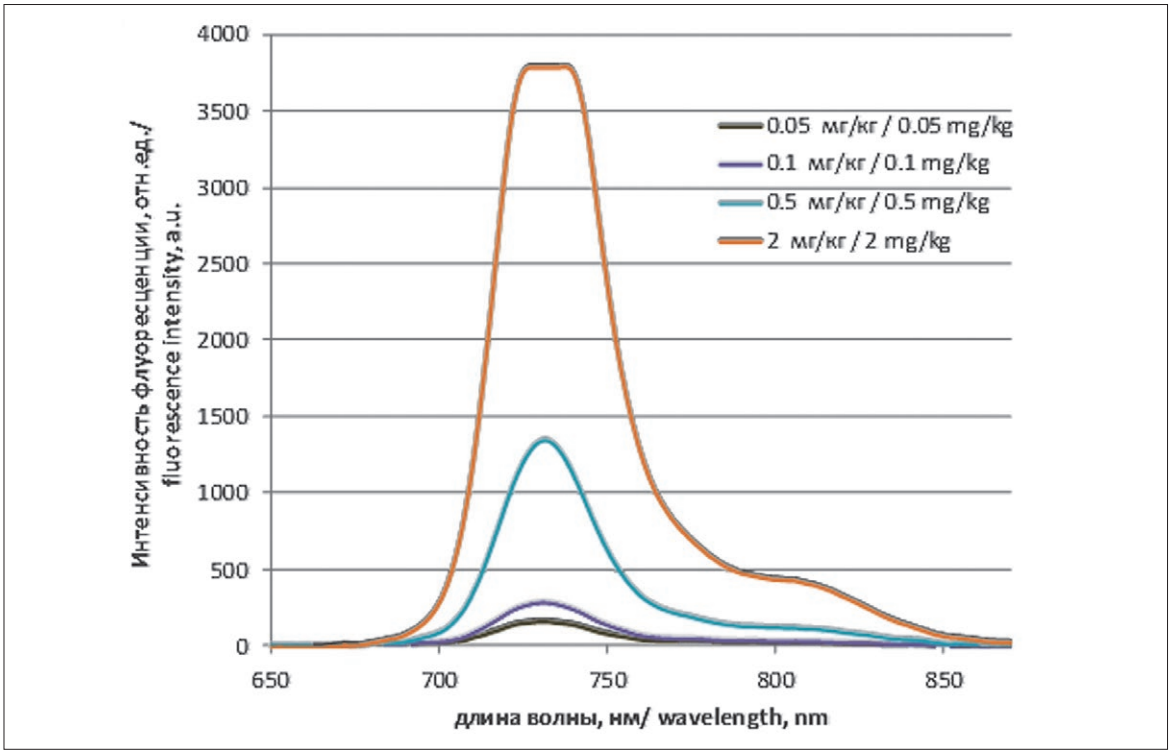
The study of the dependence of the output signal of the spectrum analyzer on the time of accumulation

The results of studies of the spectra of liposomal dispersions of tetra-3-phenylthiophthalocyanine hydroxyaluminium in various concentrations show that for small values of the accumulation time, LESA-01-Biospec spectrum analyzer provides undistorted recording of the signals of the fluorescence of dispersions with a high concentration. However, the fluorescence signals of dispersions with a low concentration are almost indistinguishable against the background of curcuit noise (Fig. 1a).

With high values of accumulation time, the spectrum analyzer performs accurate recordings of fluorescence signals at low PS concentrations. However, when registering fluorescence of PS with a high concentration,



a



b

Рис. 1. Спектры флуоресценции дисперсий ФС разной концентрации при времени накопления:
a – при 12 мс;
b – при 300 мс.
Fig. 1. Fluorescence spectra of PS dispersions of various concentrations with an exposure time:
a – 12 ms;
b – 300 ms

when the intensity of the fluorescence signal is high, the cells of the ruler corresponding to the region of the spectral maximum of fluorescence may undergo charge saturation (Fig. 1b). Therefore, the work of the spectrum analyzer becomes incorrect due to hardware distortion of the spectrum shape.

The expansion of the dynamic range of the intensity of detected fluorescence signals

In order to extend the dynamic range of the fluorescence signal intensity, the following approach has been proposed. The maximum and minimum values of the spectral density are automatically monitored throughout the period of spectral signal registration. If this value falls outside the limits of the specified range, the algorithm of automatic adjustment of the photodetector exposure, processing and recording of the polychromator ruler signals is launched.

Fig. 2 shows an improved block diagram of the recording system of the spectrum analyzer [5] with the following designations: 1 – laser for excitation of fluorescence; 2 – a fiber-optic probe containing light-guiding fibers for delivering excitation radiation to biological tissue and receiving optical fibers for delivering fluorescence radiation from a biological tissue to the input of the polychromator; 3 – biological tissue; 4 – polychromator; 5 – CCD or CMOS ruler at the output of the polychromator; 6 - ADC; 7 - buffer memory unit; 8– comparator; 9 - upper reference signal setter; 10 - comparator; 11 - lower upper reference signal setter; 12 — accumulation time control unit; 13 — accumulation correction unit; 14 - personal computer (PC).

If the signal of all cells of the ruler 5 is less than the voltage of the reference signal supplied to the reference input of the comparator 8 from setting device 9, or more voltage of the reference signal to the reference input of comparator 10 of setting device 11, the set of signals corresponding to the fluorescence spectrum comes unchanged from block 7 of the buffer memory to the input of PC 14, which constructs and displays the spectrum.

If the signal from any of the cells of ruler 5 is greater than the voltage of the reference signal input to the comparator 8 from setting device 9, the command from comparator 8 is sent to the accumulation time control unit 12, which sends a command to ruler 5 to reduce the accumulation time. With a reduced accumulation time, the signal from the output of ruler 5, reduced in proportion to the accumulation time, is fed to the input of the buffer memory unit 6, and from the buffer memory unit, to the signal inputs of the comparators. If, at a reduced accumulation time, the signal from any of the cells of the ruler is less than the voltage of the reference signal input to comparator 8 from setting device 9, the set of signals corresponding to the fluorescence spectrum is fed from the buffer memory unit to accumulation correction unit 13, where it changes (increases) in inverse proportion to accumulation time, and then is sent in the digital form to the PC input for the PC to build and display the spectrum. Similarly, if the signal from any of the cells of the ruler is less than the voltage of the reference signal at the input of comparator 10 from setting device 11, a command is sent from comparator 10 to accumulation time control unit 12, which in turn sends a command to the ruler to increase the accumulation time. With increased accumulation time, the signal from the output of the ruler, increased in proportion to the accumulation time, is fed to the input of the buffer memory block, and from the buffer memory block to the signal inputs of comparators 8 and 10. If, with an increased accumulation time, the signal from all the cells of the ruler is higher than the voltage of the reference signal input to comparator 10 from setting device 11, the set of signals corresponding to the fluorescence spectrum is fed from the buffer memory unit to accumulation correction unit 12, where it decreases in inverse proportion to accumulation time, and then is sent in the digital form to the PC input for the PC to build and display the spectrum.

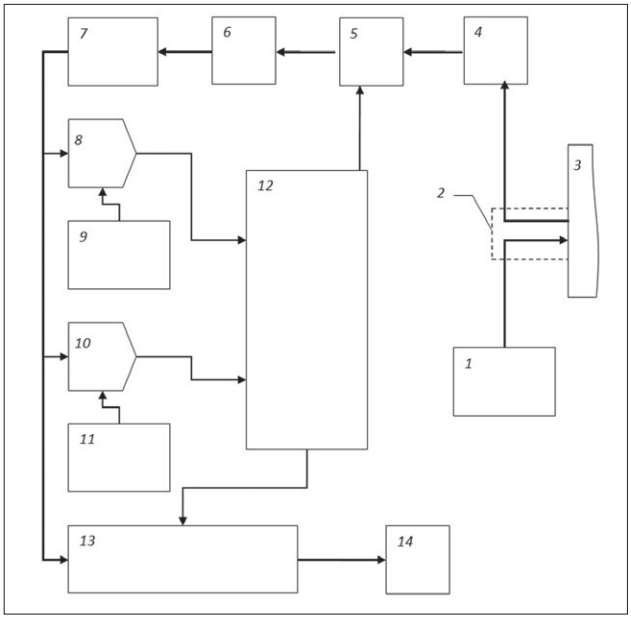


Рис. 2. Блок-схема спектроанализатора для спектрально-флуоресцентных исследований с расширенным динамическим диапазоном интенсивности сигналов флуоресценции
Fig. 2. Block-diagram of the spectra-analyzer for spectral-fluorescent studies with an extended dynamic range of intensity of fluorescence signals

Thus, if the signal value from any cell of the ruler is above the upper limit or below the lower limit of the specified range, the accumulation time of the device decreases (or, accordingly, increases) the set number of times. If the specified exposure adjustment is not sufficient to ensure that the signal is within the per-

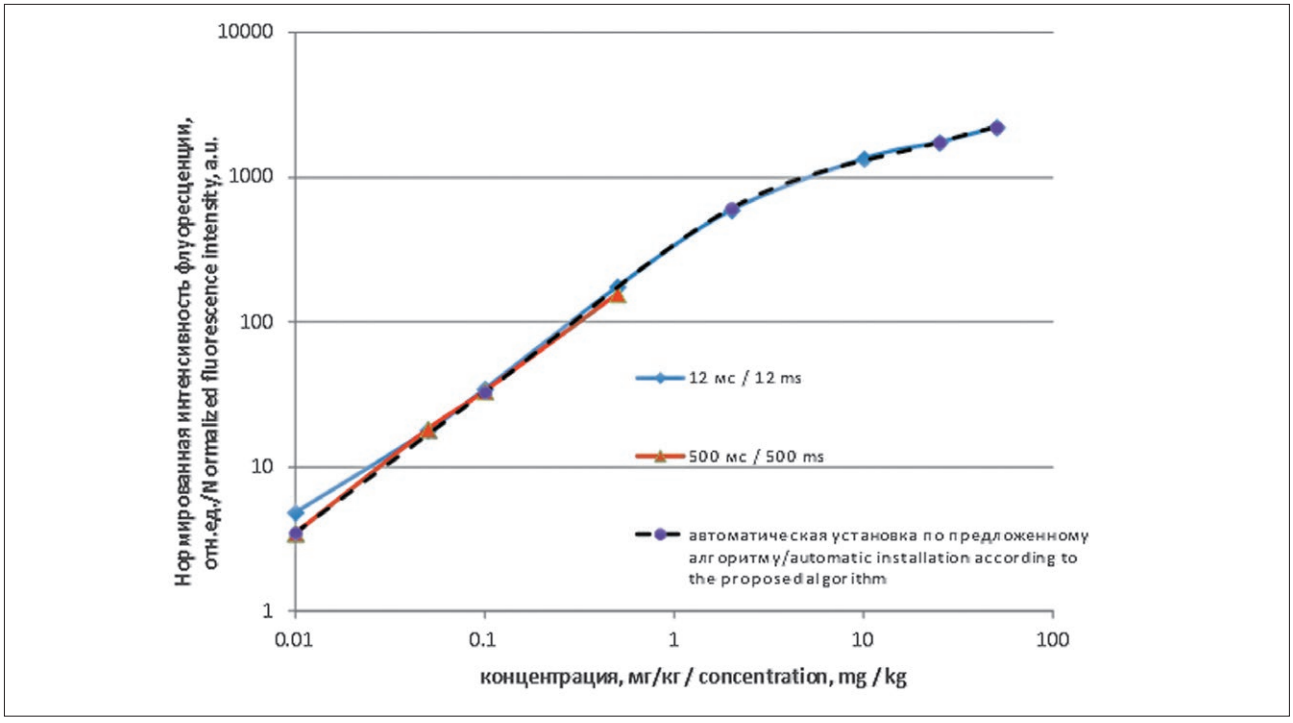


Рис. 3. Зависимость интенсивности флуоресценции ФС от его концентрации при разных временах экспозиции
Fig. 3. Dependence of fluorescence intensity of PS on its concentration at various exposure times

missible range of intensity, exposure adjustment is automatically repeated until the signal falls within this range.

The spectrum analyzer was tested on dispersions of a photosensitizer based on tetra-3-phenylthiophthalocyanine hydroxyalumium of various concentrations (from 0.01 mg/l to 50 mg/l), approximately corresponding to the concentrations used in the process of pharmacokinetics research in calibration samples and tissues of experimental animals.

When registering the fluorescence of PS with a constant small accumulation time, the dependence is distorted in the region of low concentrations, where an additional positive error is added to the signal due to the hardware noise of the spectrum analyzer (Fig. 3, the blue curve). At a constantly high accumulation time (Fig. 3, red curve), an error appears in the fluorescence signal region of dispersions with high concentrations. Moreover, starting with some concentration values, which depend on the accumulation time, signals cannot be recorded due to charge saturation of a part of the ruler.

When the spectrum analyzer is operated according to the proposed registration algorithm (with automatic change of accumulation time depending on the level of the fluorescence signal), the spectral information was recorded at high values of the signal-to-noise ratio and without distortion of the spectrum shape (see the dashed curve).

This made it possible, upon further processing of the signal, to construct the correct (undistorted) dependence of the integral fluorescence intensity of the dispersions of tetra-3-phenylthiophthalocyanine hydroxyaluminium on their concentration in the extended dynamic range of values exceeding 3.5 orders of magnitude (Fig. 3, the dashed curve).

Conclusion

The proposed algorithm for recording fluorescence spectra with an automatic change in the accumulation time depending on the signal level provided a significant expansion of the dynamic range of the spectrum analyzer and an increase in accuracy in pharmacokinetic studies. In addition, in photodynamic therapy, this made it possible to implement a more precise control of PS content in the tumor just before irradiation, during and after irradiation, to optimally choose the time to start irradiation and the whole radiation regime.

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