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# Comparison of optoacoustic and photothermal-lens determination of lipopolysaccharides

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ABSTRACT

As a primary aim, several approaches to photothermal (thermal-lens) and optoacoustic determination of exogenous pyrogens (lipopolysaccharides) based on photometric procedures for their determination at the microgram level and below are compared. The limits of detection of lipopolysaccharides by thermal-lens spectrometry and optoacoustic spectroscopy are at a level of 2–100 ng/mL, and the conditions of optoacoustic and photothermal procedures are the same. Optoacoustic spectroscopy is advantageous in determining suspensions, while thermal lensing is superior in determining lipopolysaccharides from homogenous aqueous solutions. As a secondary aim, photometric procedures for lipopolysaccharides by the formation of their ion pairs with several dyes and by the reaction of 2-keto-3-deoxyoctulosonic acid as a part of a lipopolysaccharide molecule with thiobarbituric acid are optimized. In the case of the 2-keto-3-deoxyoctulosonic acid reaction, the sampling stage time is decreased twofold, and the possibility of substitution of the toxic metaarsenite for sulfite with better sensitivity is shown.



## 1. Introduction

Optoacoustic (OA) and photothermal (PT) spectroscopies are actively used in chemistry as most sensitive techniques of molecular absorption spectroscopy [1,2]. Recently, the progress in laser technologies has provided a sound basis for compact optical schemes and commercial applications of these methods [3–5]. However, despite a vigorous discussion of their advantages in chemical analysis, their joint use is still rather limited, although the methods, having many similarities, are complementary rather than competing. This situation is characteristic for analytical chemistry because OA/PT spectroscopies show spectral nonselectivity.

Apart from technical reasons, there seems to be a problem of methodology: contrary to PT spectroscopy, OA measurements are not very often used in chemical analysis of condensed samples, and the methodology of OA analytical measurements are not so developed as in photothermal spectroscopy [2,6]. Photometric determination can be substantially improved using PT spectroscopy [1]. As the most widespread PT method, thermal-lens spectrometry (TLS) provides the determination of various substances at the submicrogram level [1,2]. To the contrary, signal processing and data handing are more advanced in optoacoustics, and the joint studies involving both methods can provide a new quality of analysis and a mutual enhancement.

Previously, we showed that the use of TLS enhances the sensitivity of the determination of lipopolysaccharides (LPS) as the most infamous class of pyrogenic substances because it combines high instrumental sensitivity and the selectivity of chemical methods for LPS for their determination at the submicrogram level [7,8] and also calculated figures of merit for OA determination of LPS showing possible high sensitivity of such an application [9,10]. Hence, LPS seem to be a very good candidate for a comparison study of OA/PT spectroscopies. Thus, the aim of this work was to optimize the conditions for OA/PT determination of LPS by applying several photometric-reagent systems for sensitivity enhancement to the submicrogram level and to compare both methods from the viewpoint of sensitivity and methodology.

We selected three possible approaches to the determination of LPS, which can be distinguished by the size of the test molecules. The first approach is based on the decomposition of an LPS molecule to the three key parts: a polysaccharide, a lipid, and 2-keto-3-deoxyoctulosonic acid (KDO) following by the reaction with one of these parts [11–13]. In this study, we selected KDO as a characteristic part of LPS core only, which provides sensitive determination. The second approach is based on the fact that OA/PT signals depend on the existence of heterogeneity in the samples [14–17]. Large LPS molecules make it possible to see their contribution to OA/PT signals directly, without adding any chemical reagents to the test

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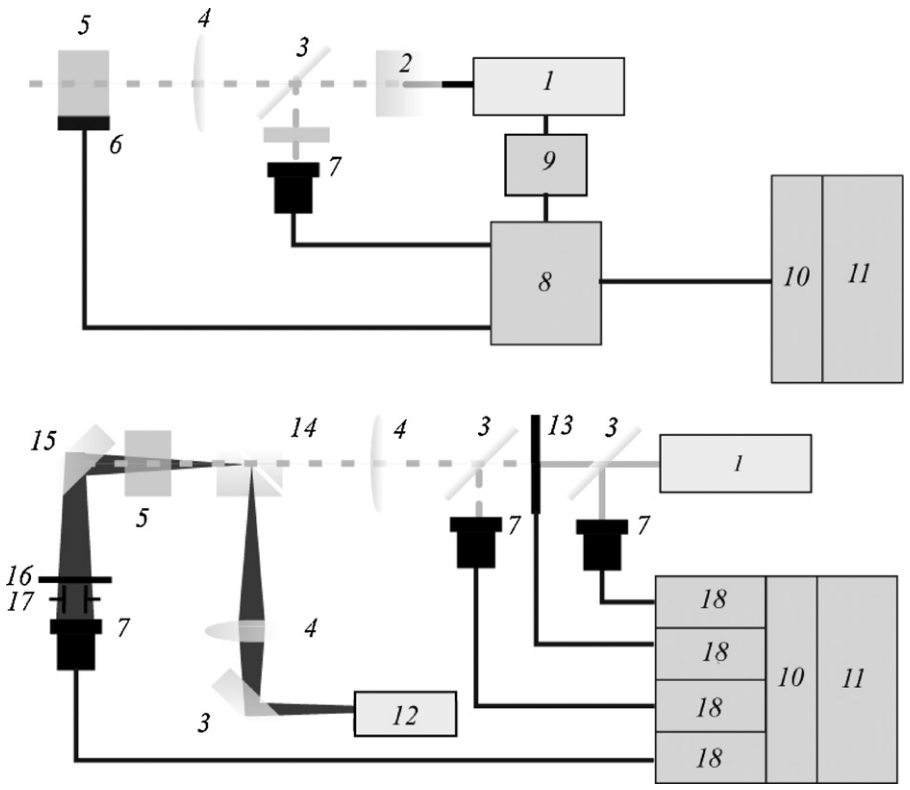


**Fig. 1.** The schematics of (a) optoacoustic spectrometer and (b) coaxial dual-beam thermal-lens spectrometer: *1*, excitation laser; *2*, frequency doubler; *3*, mirror; *4*, focusing quartz lens; *5*, sample quvette; *6*, piezotransducer; *7*, an L-3DP3C Panasonic photodiode; *8*, digital oscilloscope; *9*, pulse generator; *10*, synchronization interface; *11*, a PC; *12*, probe laser; *13*, a mechanical chopper; *14*, a Glan prism; *15*, a ZR-88 type dichroic mirror; *16*, a passband filter; *17*, a pinhole; *18*, an analog signal amplifier and current-to-voltage converter.

samples. Finally, the third approach combines the chemical modification of the first approach while keeping the heterogeneity of the test sample, advantageous for OA/PT measurements, of the second approach. It is ion-pair formation of LPS molecules with dyes [18]. In this study, we used the reactions with the polysaccharide part of LPS molecule with cationic dyes of quinaldine and triphenylmethane series due to their sensitivity [19–21].

## 2. Experimental

### 2.1. Apparatus

#### 2.1.1. Optoacoustic setup

Setup schematics is shown in Fig. 1a. A $Nd^{3+}$-YAG laser of the LTI type (Polyus Productions, Moscow, Russia) was used for the excitation of the sample in an optoacoustic Brodnikovsky-type cuvette cell (radius, 1 cm; Fig. 2) [17]. The second harmonics, $\lambda_e$ = 532 nm, was selected to bring together the conditions of OA and PT experiments. The laser pulse energy was within the range 10–15 mJ and the pulse repetition rate was 1 Hz. The laser beam passed through the center of the cuvette, thus forming a pencil-shaped thermoacoustic array. The distance from the array to a PZT-19 piezoelectric transducer (Russia) was 15 mm (sensitivity, 20 μV/Pa; frequency band, 1 MHz). The energy was measured with a photodiode pre-calibrated in the necessary energy range with an IMO-2M laser power/energy meter (Russia). The signal was registered with a C9-8 digital oscilloscope (Russia), the energy measurements were synchronized with the signal measurements with the same oscilloscope using a G5-60 pulse generator (Russia). Generated pulse parameters are 5 V, 20 μs, 1 Hz.

#### 2.1.2. Thermal-lens setup

A dual-laser coaxial-beam thermal-lens spectrometer with a single-channel detection system is used Fig. 1b [22]. It is set up with a coaxial dual-beam optical configuration. An excitation $Ar^+$ laser (Innova 90-6, Coherent, Palo Alto, CA 94303, USA) is used. A change in intensity in the center of the He–Ne probe laser beam (SP 106-1, Eugene, OR 97402, USA) is measured with a single L-3DP3C photodiode detector. The selection of the parameters of the instrument (linear dynamic range, optical-scheme design, instrumental sensitivity, etc.) is discussed elsewhere [22,23]. The data are gathered and handled online by the software written in-house [22]. The

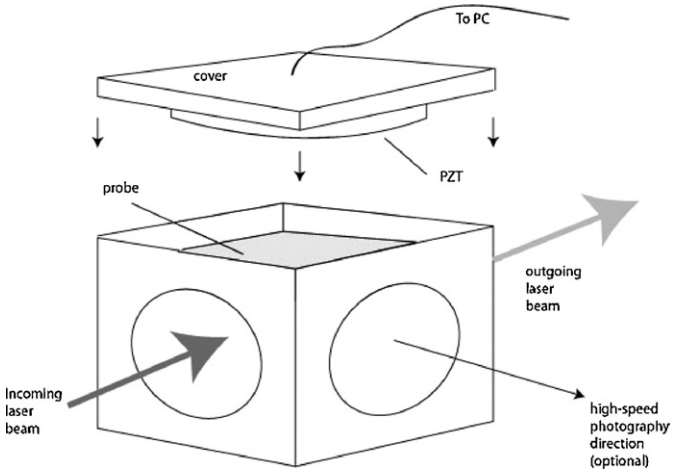


**Fig. 2.** The optoacoustic cuvette cell. See text for details.