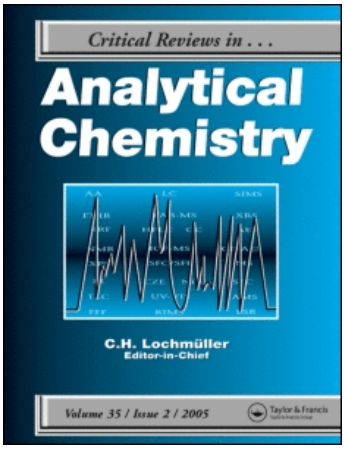

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Thermal Lens Spectrometry as Analytical Tool

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ABSTRACT: Thermal lens spectrometry as an analytical technique is summarized. Specific applications to the determination of metal ions, pesticides, fatty acids, carotenoids, and some interesting biomolecules as nucleotides or hepatitis B antigen are described. The possibilities and limitations of this TLS detection system is evaluated.

KEY WORDS: thermal lens spectrometric, metal ions analysis, pesticides analysis, fatty acids analysis, carotenoids analysis.

I. INTRODUCTION

Photothermal spectroscopy is a group of high-sensitivity methods based on a photoinduced change in the thermal state of the sample. Light energy absorbed and not lost by subsequent emission results in sample heating,[1] and this heating results in a temperature change. The thermally perturbed sample can act as a lens. Light transmitted through an aperture placed beyond the photothermal lens will vary the strength of the lens. Photothermal methods based on measurement of the strength of this lens are called photothermal lensing spectroscopy. The principle of the thermal lens effect has been described previously,[2,3] and the procedure is simple. An excitation light beam passes through the sample that contains the analyte, the light is tuned to an absorption line of this, and the energy is absorbed by the analyte. The molecules are excited into vibrational, rotational, or electronic states; the excited molecules lose the energy in the form of heat through nonradiative relaxation processes, the heat generated equal to the excitation energy. The heating of the sample causes a change of the refractive index, detecting this fact by convergence or divergence of a probe laser beam when it passes through to the sample. Under equilibrium conditions, the temperature change is related to the refractive index. Measurements of the change in convergence or divergence of a laser beam after the formation of the thermal lens allows determination of the absorbances of 10^{-7} to 10^{-6}, which correspond to analyte concentrations of 10^{-11} to 10^{-10} *M*. Thus, thermal lens spectrometry is more sensitive than conventional spectrophotometry.

Thermal lensing was reported first by Gordon et al.[4] when they observed transient power and beam divergence changes in the output of a helium-neon laser after placing transparent samples in the laser cavity. The first dual-beam experiment was conducted by Grabiner et al.[5] in 1972 marking the first activity in trace analysis in gases. Thermal lens spectroscopy appeared as an analytical tool 25 years ago, and it has some problems that were caused by sophisticated laser equipment, lack of serial devices, high requirements for the personnel and competition with other highly sensitive methods. However, despite these problems thermal lens spectrometry has consolidated its positions, prov-

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ing its viability. The method is more sensitive that conventional transmission spectrometry[6] because the photothermal effect amplifies the measured optical signal (relative change in the beam intensity). The enhancement depends on the thermal and optical properties of the sample and the properties of the pump and probe laser beam. The TLS has numerous advantages such the high sensitivity presented and is a nondestructive procedure that provides analysis of biological objects, remote analysis, and on-line determination in the flow and can be presented as an analytical method with unique properties.[7]

There are some research groups that in recent years have carried out numerous research publications[8,9,10] in this field as analytical tools, and they have developed applications in organic or inorganic analysis combining thermal lens spectrometry (TLS) with chromatographic or electrophoretic techniques. In this review we have tried to explain the analytical applications of thermal lens spectrometry appeared in the bibliography of the last 5 years, including inorganic and organic analysis. A compendious of some analytical applications are summarized in Tables 1, 2, 3, and 4.

II. DETERMINATION OF METAL IONS

A. Determination of Chromium

The determination of chromium, especially Cr(III) and Cr(VI) in environmental and biological systems, is currently of considerable interest because the essentiality or the toxicity of chromium compounds, including humans, depends on its oxidation state. Cr(III) is considered as essential in mammals, and Cr (VI) is toxic because of its oxidizing capability and adverse impact on lung, liver, and kidney. The applicability of thermal lens spectrometry (TLS) for quantification and routine determination of hexavalent chromium was investigated by Sikovec et al.[11] using a collinear dual beam thermal lens spectrometer. The authors have validated the technique by comparison between TLS results on realistic samples with results obtained by atomic absorption spectrometry (AAS), and by the determination of Cr(VI) in standard reference materials (SRM). The results have demonstrated that the TLS is a reliable and accurate analytical technique for the determination of Cr(VI) with a detection limit of 0, 1 $\mu g\ l^{-1}$, confirming its high degree of sensitivity. Sikovec et al.[12] have verified the reliability of TLS by determining hexavalent chromium in extracts of CCA-treated building timbers and comparing the results to those obtained by some other techniques, such AAS and spectrophotometry of the diphenylcarbazide chromophore (SPEC). The investigations have revealed that SPEC was not suitable for the determination of hexavalent chromium in CCA-treated building timbers because the turbidity of the alkaline extracts interfered with conventional measurements of absorbance, and the photothermal effects were less affected by light scattering from turbidity. The use of TLS is validated by the good agreement between results obtained by TLS and those obtained by AAS. Because the Cr(VI) enters in natural waters mainly through effluents from electroplating and tanning industries, from dyeing and from metallurgy, there is currently considerable interest in the determination of chromium species in natural waters due to their influence on aquatic animals and man. Thermal lens spectrometry (TLS) has been applied for the determination of hexavalent chromium in drinking water by Sikovec et al.[13] The authors have demonstrated that TLS is a simple, sensitive, and reliable method, and it offers several advantages over the presently used methods for Cr(VI) determination (spectrophotometry). These include higher sensitivity, lower limits of detection

TABLE 1
Determination of Metal Ions

ION	SAMPLE	METHOD	DETECTION LIMIT	REFERENCE
Cr(VI)	Standard reference water samples	TLS	0,1 $\mu g\ l^{-1}$	11
Cr(VI)	Extracts of CCA-treated building timbers	TLS	0,2 $\mu g\ l^{-1}$	12
Cr (VI)	Drinking water	Ion Chromatographic-TLS	0,1 $\mu g\ l^{-1}$	13
Cr (VI)	Water based solution	Ion Chromatographic-TLS	0,1 $\mu g\ l^{-1}$	14
Cr (III)			10 $\mu g\ l^{-1}$	
Fe (III)	Synthetic sample	Ion Chromatographic-TLS	25 $\mu g\ l^{-1}$	15
Fe (II)	Synthetic and real samples		5 $\mu g\ l^{-1}$	
Fe (II)	Real water samples	High performance capillary electrophoresis-TLS	2 $\mu g\ l^{-1}$	16
Fe (II) or (III)	Calf serum	TLS	4 $\mu g\ l^{-1}$	17
Fe (II)	Aqueous solutions	Thermal lensing	0,56 $\mu g\ l^{-1}$	18
Some heavy metal ions	Standard solutions	Ion chromatography-TLS	$\approx$ 20 $\mu g\ l^{-1}$	19
Some transition metals		Flow Injection-TLS	10^{-8} to 10^{-7} M	20
Rh (III)	Catalyst samples	TLS	4 $\mu g\ l^{-1}$	21
Co (II)	Synthetic sample	HPLC-TLS	1,77 $\mu g\ l^{-1}$	22
Ni (II)			5,87 $\mu g\ l^{-1}$	
Pt (IV)	Alloy and catalyst samples	TLS without pre-separation	2 $\mu g\ l^{-1}$	23
Pd (II)			2 $\mu g\ l^{-1}$	

TABLE 2
Determination of Pesticides

ANALITE	SAMPLE	METHOD	DETECTION LIMIT	REFERENCE
4,6-dinitrophenol	Synthetic samples	Capillary electrophoresis and thermal lens detector		5
2-methyl-4,6-dinitrophenol			23 μg l^{-1}	
2-sec-butyl-4,6-dinitrophenol				
2-ter-butyl-4,6-dinitrophenol				
2,4-dinitrophenol	Synthetic samples	Reversed phase liquid chromatography and TLS	1,5 μg l^{-1}	26
4,6-dinitro-o-cresol			0,7 μg l^{-1}	
2-sec-butyl-4,6-dinitrophenol			3 μg l^{-1}	
2-ter-butyl-4,6-dinitrophenol			3 μg l^{-1}	
Paraoxon	Tap water and fruit juice	Flow injection analysis with photothermal detector	0,2 μg l^{-1}	25
Chlorpyrifos			4 mg l^{-1}	
Diazinon			2 mg l^{-1}	
Carbaryl			70 μg l^{-1}	
Carbofuran			1 μg l^{-1}	

(0.1 μg l^{-1}, which is 10 times lower compared with SPEC) and the fact that TLS is much less affected by light scattering in the sample compared to spectrophotometry. In the same way, the effect of methanol, acetone and acetonitrile on the sensitivity, selectivity, and detection limits of the determination of chromium species by ion chromatography was investigated by Sikovec et al.[14] The authors have demonstrated that the addition of acetone in the batch mode determination of Cr(VI) improves the sensitivity of the TLS method. Similarly, the addition of organic solvents to the eluent and post-column reagent enhances the TLS signal and the sensitivity of detection in ion chromatography. Among the solvents used (methanol, acetone, and acetonitrile), enhancement was the highest in the case of acetonitrile addition. The sensitivity of the technique was improved up to three times compared with IC-TLS determination of chromium species in water-based solutions.

B. Determination of Iron

The determination of iron is very important for environmental and biological studies because of the influence of its chemical

TABLE 3
Determination of Carotenoid

ANALYTE	SAMPLE	METHOD	DETECTION LIMIT	REFERENCE
α-carotene	Blood plasma	HPLC and TLS	85 ng l^{-1}	27
Trans-β-carotene			100 ng l^{-1}	
β carotene	Fish oils	Isocratic HPLC and TLS	0,58 μg l^{-1}	28
Trans-β-carotene	Rat liver	HPLC-TLS	0,39 μg l^{-1}	29
	Beef liver		0,49 μg l^{-1}	
Trans-β-carotene	Vegetable oils	Isocratic non-aqueous reversed phase HPLC and TLS	0,5 μg l^{-1}	30
Cis-β-carotene			O,95 μg l^{-1}	
Trans-β-carotene	Synthetic sample	Supercritical fluid and TLS	5 x 10^{-6} absorbance units	31
Peridinin	Commercially available sample		0,29 μg l^{-1}	6
19'-hexano-yloxy-flucoxanthin			O,34 μg l^{-1}	
Zeaxanthin			0.38 μg l^{-1}	
Sudan I			0,36 μg l^{-1}	
Lycopene			0.28 μg l^{-1}	
β-carotene			0,24 μg l^{-1}	

forms on the bioavailability of iron and physicochemical and toxicological properties of the other trace elements. The low concentration of iron present in a natural medium necessitates the use of a sensitive procedure for its determination. Divjak et al.[15] have studied the application of various detection techniques (UV-Vis spectrophotometry, TLS, and amperometric) with different metallochromic post-column reagents for IC determination of Fe(III) and Fe(II). The lowest detection limits for Fe(III) and Fe(II) were obtained by using TLS detection and ascorbic acid and 1,10-phenanthroline as post-column reagents (25 μg l^{-1} and 5 μg l^{-1}, respectively). All three detection systems were tested for the detection of Fe(III) and Fe(II) in synthetic samples and Fe(III) in real samples, in the presence of high concentrations of Cu(II) and Mn(II). Electrochemi-

TABLE 4
Determination of Biomolecules

ANALYTE	SAMPLE	TECHNIQUE	DETECTION LIMIT	REFERENCE
Adenosine	Synthetic	Near-infrared thermal lens spectrometer	$4{,}9 \times 10^{-3}$ M	36
Cytidine			$3{,}4 \times 10^{-3}$ M	
Guanosine			$3{,}9 \times 10^{-3}$ M	
Thymidine			$3{,}1 \times 10^{-3}$ M	
Dabsylated amino acids	Syntetic	Capillary electrophoresis-TLS	$\approx 1{,}8 \times 10^{-7}$ M	37
Hepatitis B surface antigen	Human serum	Enzyme immunoassay-TLS	0, 15 μg l^{-1}	38

cal detection was found to be the most suitable detection system due to the possibility of elimination of Cu(II) interference by post-column copper masking. Natural iron concentrations in real water samples have been determined by thermal lensing as a high-performance capillary electrophoresis detector by Seibel and Faubel.[16] 1.10-phenanthroline was used as a chromogenic reagent, and interferences by other cations can be neglected by a combination of this photometric technique with high performance capillary electrophoresis. Laser-induced thermal lens spectroscopy is demonstrated as a new technique for the measurement of small absorbances in the picoliter detection volume of a capillary. A limit of detection of 36 nmol/l was achieved. In the same way, thermal lens spectrometry has been used for the determination of iron in calf serum by Legeai and Georges.[17] The method is based on dissociation of Fe(III) from proteins, reduction of Fe(III) to Fe(II), and formation of a colored complex between Fe(II) and bathophenanthroline. The method is reliable, sensitive, and reproducible. The limit of detection for iron is 4 ppb, the relative standard deviation is around 2%, and the volume of serum sample necessary for an analysis can be reduced to less than 100 μl. Chernysh et al.[18] have studied the peculiarities of analytical reactions of iron (II) with 1.10-phenantroline at the nanogram level. The stability constant for iron (II) chelate with 1,10-phenanthroline was determined, log $\beta_3 = 21.3 \pm 0.1$. The conditions for the determination of iron(II) with 1.10-phenanthroline by thermal lensing were reconsidered, changes in the conditions at the nanogram level improved both the selectivity and sensitivity of determination. The limits of detection and quantification of iron (II) at 488 nm (excitation beam power 140 mW) are 1×10^{-9} and 6×10^{-9} *M,* respectively; the reproducibility RSD for the range $n \times 10^{-8}$ – $n \times 10^{-6}$ *M* is 2 to 5%.

C. Determination of Other Metals

There is a continuing interest toward developing and evaluating highly sensitive analytical techniques for determining the concentration of trace of heavy metals in

environmental and biological samples. These metals can be essential or toxic for the organism. Sikovec et al.[19] have evaluated the applicability and limitations of dual-beam thermal lens spectrometry (TLS) detection in ion chromatography separations of different heavy metal ions (Cu^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, Fe^{2+}, and Fe^{3+}) and they have investigated the influence of experimental parameters such as excitation laser power on the TLS signal. As a result, in the case of Cu^{2+} and Co^{2+} TLS detection at 514.5 nm was found to be advantageous compared with UV-visible detection at 520 nm (λ_{max} = 520 nm), in the case of Zn^{2+} and Ni^{2+} the detection limits of the two techniques were comparable. A detection limit of 20 ng/ml was obtained for the determination of Fe^{3+} and should be approximately the same for Fe^{2+}. The authors have demonstrated that TLS is a simple and sensitive technique for the online detection of several metal ions after IC separation. In the same way, Proskurnin et al.[20] have employed the TLS as a highly sensitive detector for liquid chromatography and flow analysis. The authors made thermal lens determinations of a wide range of transition metals by a flow injection mode. The conditions for the flow determination of Al(III), Bi(III), Cd(II), Co(II), Cr(III), Cu(II), Fe(III), Mn(II), Nd(III), Ni(II), Pb(II), Pr(III), and Zn(II) by reaction with xylenol orange in aqueous solutions at pH 4.4 and the determination of Cd(II), Co(II), Cu(II), Fe(II), Ni(II), Pb(II), and Zn(II) by reaction with 4-(2-thiazolylazo)resorcinol in water-ethanol mixtures (5:1) at pH 5.0 were proposed. The limits of detection obtained were $n \times 10^{-8}$ – $n \times 10^{-7}$ *M*, the linearity ranges in the calibration graphs were three orders of magnitude, and the relative standard deviation was of 3 to 7%. A highly sensitive method for the determination of rhodium based on the color reaction between rhodium and the new chromogenic reagent 5-bromo-(2-thiazolylazo)-5-diethylaminobenzooic acid (5-Br-TADEB) has been developed by Zhang et al.[21] In optimal conditions, rhodium reacts with 5-Br-TADEB to form a 1:2 stable blue complex. The detection limit and linear range of rhodium (III) are 0.004 μg/ml and 0.01 to 0.08 μg/ml, respectively. The method has also been applied to determine rhodium in catalyst samples with satisfactory results. The application of HPLC and thermal lens spectrometry as a high-sensitive detection method increases the detection limits compared with conventional spectrometry. Dzyabchenko et al.[22] have developed a method by conjunction of HPLC and TLS for determination Co and Ni in the form of their presynthesized chelates with 4-(2-pyridylazo)resorcinol. The limits of detection obtained were 3×10^{-8} and 1×10^{-7} *M,* respectively, and the authors have demonstrated that spectrophotometric data are less efficient than the case of thermal lensing. A selective and sensitive method for determination of platinum and palladium (II) in an aqueous solution simultaneously by laser thermal lens spectrometry are developed by Zhang and Yan.[23] The platinum (IV) and palladium (II) can react with 2-(3,5-diclhloropyridylazo)-5-dimethylaminooamiline to form stable complexes in strong acidic medium, which exhibit absorption maximum at 634 nm and 623 nm, respectively. However, the reaction temperature is different to form the complexes of platinum and palladium. The palladium complex can be formed at room temperature, while the platinum complex can be formed only after being heated in a boiling water bath for 80 min. Based on this temperature difference of complex reactions, the authors have proposed their method, and it was applied to the determination of platinum and palladium in alloy and catalyst samples with detection limits of 2 μg/l. Proskurnin et al.[24] have selected the conditions for thermal lens determination of copper(I) with 2.9-dimethyl-1,10-phenanthroline (neocuproine) and they have estimated the stability constants of chelate ($\log \beta_2 = 16.2 \pm 0.2$). The detection limit found were 1.91 μg l^{-1}, and the results con-

firm that thermal lensing is applicable for trace determination of copper (I).

III. DETERMINATION OF PESTICIDES

Pesticides are one of the principal classes of environmental pollutants, which are widely used throughout the world. Due to high toxicity of pesticides and the possibility of their accumulation in body tissues, they can be the cause of serious diseases.[25] Therefore, development of devices for fast, inexpensive, on-line, and field determination of pesticide levels is of great importance. The multianalyte assay of pesticides in different samples requires complex and expensive techniques like gas chromatography or high-performance liquid chromatography, and cannot be done easily outside the laboratory. In addition, these methods are time consuming because of sample preparation and the need for preconcentration. There are a lot of analytical methods for pesticide identification and quantification, but it was demonstrated that photothermal spectrometric methods due to their high sensitivity enable measurements of very low absorbances and therefore could be applied to improve the sensitivity of such a biosensor without application of a complex substrate.

The characteristic and the performance of a thermal lens detector were studied in a capillary electrophoresis system by Seidel et al.[5] for the determination of pesticides (4,6-dinitrophenol, 2-methyl-4,6-dinitrophenol, 2-sec-butyl-4,6-dinitrophenol and 2-ter-butyl-4,6-dinitrophenol) with high sensitivity. The detection volume was in the order of 75 nl when a 75 mm capillary was employed. The change in intensity of the probe beam was detected by a photodiode behind a pinhole, which was protected with different band-pass interference filters. The performance of the detector in capillary electrophoresis was assessed with various types of capillaries and compared with a conventional UV-absorption detector and the limit of detection obtained is at least one order of magnitude better than it is with the absorption detector. The same investigation group have studied[26] a reversed phase liquid chromatography and it was applied to the separation of pesticide, with a near field thermal lens device as detector. The authors have investigated the separation of 2,4-dinitrophenol, 4,6-dinitro-*o*-cresol, dinoseb, and dinoterb. A continuous wave argon ion laser was taken as excitation light source at a wavelength of 364 nm. The sensitivity of the thermal lensing device was compared with a conventional UV-detector. Using gradient separation technique, the detection limits of the collinear near field thermal lensing device are two to three times better than for the UV-detector.

The determination of organophosphate (paraoxon, chlorpyrifos, diazinon) and carbamate (carbaryl, carbofuran) pesticides in spiked drinking water and fruit juices without any pretreatment steps was carried out by Pogacnik and Franko[25] using a photothermal biosensor. The biosensor consists of a cartridge containing immobilized enzyme acetylcholinesterase placed in a flow-injection analysis manifold and a photothermal detector based on thermal lens spectrometry. The determination of pesticide in a single sample can be accomplished in 15 min. The authors have obtained a LOD of 1.5, 2.8, and 4 ng/ml paraoxon in tap water, orange juice and apple juice, respectively. Furthermore, the application of TLS detection was demonstrated to be advantageous compared with spectrophotometric detection and resulted in almost a five-time improvement in LOD.

IV. DETERMINATION OF CAROTENOIDS

As a precursor of vitamin A, trans-β-carotene is essential to human health. In recent years, carotenoids have attracted considerable attention due to their possible role

in the prevention of some degenerative diseases such as cancer and cataract formation. In addition, it has been observed that compounds from the group of carotenoids, abundant primarily in fresh fruit and vegetables, act as a natural lipid-soluble antioxidants. The levels of trans-β-carotene and other carotenoids in blood plasma of different individuals are dependent on nutritional habits and other activities. Measurements of low concentrations are essential when investigating the effects of external factors and nutrition on the carotenoids levels in the human body and its health. Franko et al.[27] have validated the TLS as a detection technique in HPLC analysis of trans-β-carotene and other carotenoids in blood plasma by comparison with HPLC-UV-vis analysis. The results demonstrated good agreement with the target values for carotenoids in an in-laboratory control sample and confirmed the accuracy of the HPLC-TLS technique. The advantages of TLS compared with UV-vis detection include higher sensitivity and about a 100 times lower LOD for carotenoids in blood plasma. In the same way, Luterotti et al.[28] have demonstrated that TLS combined with efficient isocratic HPLC separation provides an excellent analytical means to profile and quantify carotenes in fish body oils; Marine organisms are very rich carotenoids sources, and possibly also in vegetable oils. The analyte could be determined reliably and precisely in the presence of a complex matrix including other fat-soluble vitamins, such as retinyl palmitate and propionate, cholecalciferol, ergocalciferol, and DL-α-tocopheryl acetate, polyunsatured fatty acids, sterols, and other pigments by matrix-matched calibration in cod liver oil. Highly sensitive TLS detection enabled the reliable quantification of ultratraces of β-carotene in fish body oils with a linearity range of 1 to 120 ng ml^{-1} and LOD of 0.58 ng ml^{-1}. So, HPLC-TLS was demonstrated as a method capable of selectively profiling carotenoids in animal livers and suitable for ultrasensitive determination of trans-β-carotene in the same tissues. Due to high sensitivity of the method, there is no need for enrichment of liver extracts. Luterotti et al.[29] have determined trans-β-carotene in rat and beef livers obtaining by calibration in the mobile phase a linearity range of 1 to 130 ng ml^{-1}, favorable detection limits (0.39 and 0.49 ng ml^{-1}), precision of determination (2.8 to 7.2%) and selectivity evidenced in the presence of multifold molar excess of vitamins A and E, cholesterol and lecithin confirm the suitability of the method. The applicability of HPLC-TLS is not restricted solely to the liver, but it is most likely useful for the analysis of other tissues as well. As such, the HPLC-TLS method may assist in solving many problems in the fields of basic and applied biochemistry, epidemiological studies, nutrition, and veterinary.

From the nutritional and quality control points of view, the separation, identification and quantification of trans/cis isomers of β-carotene in edible oils is an important analytical task. Luterotti et al.[30] have developed an isocratic nonaqueous reversed phase (HPLC) procedure combined with highly sensitive thermal lens detection for fast separation and simultaneous assaying of trans-β-carotene (TBC) and *cis*-β-carotene (CBC) isomers in vegetable oils (olive, safflower, sesame, wheat germ, and linseed) using minimum sample pretreatment. The results demonstrate that together with trans isomer variable portions of *cis*-β-carotene are present in these oils. A coupling of a thermal lens spectrometer with a commercially available supercritical fluid extractor using a high-pressure flow cell interface is proposed by Amador-Hernandez[31] for the sensitive real time detection and monitoring of interesting compound such trans-β-carotene. Under optimum experimental conditions, the relative TLS signal area showed a linear relationship with the concentration of trans-β-carotene from 1.5×10^{-6} *M* to 8×10^{-8} *M* in the supercritical phase. The absorbance at

the detection limit, calculated as three times the background noise, corresponded to 5×10^{-6} absorbance units. Thus, the authors have demonstrated the viability of on-line detection for supercritical fluid extraction with a pulse thermal lens spectrometer. Logar et al.[6] have investigated the possibilities of improving the performance of TLS detection and to analyze the changes of thermo-optical parameters during the gradient elution HPLC. The authors have selected pigments (13 carotenoids and two chlorophylls) as model compounds, LODs for gradient separation with TLS detection are 1.3- to 10-fold lower than for the UV-Vis detection.

V. DETERMINATION OF FATTY ACIDS

The content of trans-unsaturated fatty acids (TFA) is an important criterion for qualitative classification of edible oil, fat, and related products. Franko et al.[32] have developed a sensitive method for the determination of trans-unsaturated fatty acids and oleic acid based on thermal lens spectrometry, providing limits of detection over 200 times lower than conventional IR transmission techniques. CO_2 laser (9 to 11 μm) was applied for the excitation of characteristic vibrational transition of TFA, while the CO laser (5 to 7 μm) was used for the excitation of the carbonyl group in oleic acid. The capability of recording the absorption spectra of investigated samples gives the TLS technique additional selectivity, because the effects of interfering compounds can be, in principle, avoided by tuning the laser to an interference free excitation line. Amador-Hernandez et al.[33] have coupled a supercritical fluid extractor with a thermal lens spectrometer pumped by a commercially available pulsed laser operating in the NIR region (1064 nm), and provided with a high-pressure flow cell interface. Four groups of organic compounds (fatty acids, fatty acid esters, phenolic derivatives, and compounds such as benzoic acid and some surfactants) were considered for the study of their TLS signal at 1064 nm according to their chemical structure. The advantages and limitations of the hyphenated SFE-TLS technique were discussed, and the possibility of on-line detection in SFE with a pulse thermal lens spectrometer was demonstrated. Franko et al.[34] have determined fatty acids (linoleic and oleic acids) with a novel TLS detector that operates at CO laser wavelengths and HPLC technique and tested against the refractive index detector (RID).

The HPLC/IR/TLS detection scheme has demonstrated sensitivity comparable to the RID detector and substantial improvement in selectivity because it is capable of discriminating between fatty acids and interfering compounds like octanol, decanol, and other compounds that do not absorb in the carbonyl band region. Since the oxidative breakdown causes degradation of edible oils, there is a need for optical and thermal techniques capable of characterizing these products. Bicanic et al.[35] have examined the potential of hyphenated HPLC-dual beam thermal lens spectrometry for monitoring changes in safflower oil (moderate stability) subjected to a 10-h long thermal treatment.

VI. DETERMINATION OF BIOMOLECULES

A spectrometer that is based on the use of the thermal lens effect for sensitive measurements of absorption in the near-IR region has been developed by Baptista and Tran,[36] and it was applied in the sensitive determination of nucleotides (adenosine, cytidine, guanosine, and thymidine). The limits of detection were determined for all four nucleotides to assess the relative sensitivity of thermal lens and absorption techniques. The sensitivity of this instrument is ~3.3 times higher than those of the conventional absorption techniques, and

the results obtained have demonstrated that this TL instrument is particularly suited as a detector for small-volume sample measurements, especially for capillary electrophoresis, the technique often used for the separation of nucleotides.

Amino acids are one of the most important classes in biological chemistry. At the present, there is a large interest in sequence determination of minute quantities of proteins. A miniaturized fiber optic thermal lens detector in combination with capillary electrophoresis is described by Seidel and Faubel[37] and applied to monitoring mixtures of amino acids (arginine, histidine, leucine, alanine, glycine, and glutamic acid) labeled with 4-dimethylazobenzene-4-sulfonyl-chloride as absorbance reagent. The addition of electroosmotic flow modifier improves the separation efficiency and the signal-to-noise ratio of the detector head. The LOD with a signal-to-noise ratio of 3:1 was about 1.8×10^{-7} *M*. The thermal lens detector system in combination with CE allow treatment of microliter volumes of amino acids.

A sensitive enzyme immunoassay based on the use of crossed-beam thermal lens detector is described by Zhou et al.[38] to determine hepatitis B surface antigen in human serum, obtaining a background reduction. The enzyme substrate of the horseradish peroxidase that was employed was 3,3'-5-5'-tetramethylbenzidine and a detection limit of 0.15 ng/ml was obtained. The method was compared with a commercially available ELISA method and the results confirmed that the proposed procedure was more sensitive than the ELISA method.

CONCLUSIONS

In this article we have reviewed the analytical applications of thermal lens spectrometry that have appeared in the bibliography in recent years, including determination of metal ions, pesticides, fatty acids, β-carotene, and some interesting biomolecules. The results obtained in the determinations reviewed confirm the TLS as a highly sensitive technique and this high sensitivity permits the use as a biosensor. The possibility of using previously a separation technique as HPLC or capillary electrophoresis, for example, enables us to achieve a sensitive and selective procedure. The above discussion shows the reader that TLS can be an alternative to routine analysis, providing excellent tool in analytical chemistry.

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