Thermal Lens Spectrometric Detection and Characterization of Fatty Acids

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Summary

Photothermal spectrometric techniques such as thermal lens spectrometry (TLS) are characterized by high sensitivity, which enables measurements of absorbances as low as 10^{-7}. In this work the potentiality of TLS to measure small absorbances of liquid samples at CO (5–7 μm) and CO_2 (9–11 μm) laser wavelengths was investigated. CO_2 laser was applied for excitation of characteristic vibrational transitions of trans-unsaturated fatty acids, while the CO laser was used for excitation of the carbonyl group in oleic acid. Results were compared to those obtained on an FT-IR spectrometer. For trans-unsaturated fatty acids and oleic acid the limits of detection were improved by a factor of 220, compared to the IR transmission and FT-IR method, respectively. Tunability of the CO laser enabled recording of the first TLS spectra of carbonyl compounds in liquid samples. In addition to chemical characterization the TLS technique enables measurements of some physical properties of the sample. This was successfully exploited to measure thermal conductivities of fatty acids, which were not reported to date.

Keywords: fatty acids, thermal lens spectrometry, IR absorption spectra, thermal conductivity

Introduction

Content of *trans*-unsaturated fatty acids (TFA) is an important criterion for qualitative classification of edible oil, fat and related products. For concentration levels of a few percentages or higher, the determination of TFA by IR spectrometry is usually based on measurement of transmission of IR radiation at 965 cm^{-1} absorption band (1,2), characteristic of isolated double bonds in long chain unsaturated fatty acids, esters and glycerides. Lower concentration levels are, however, unaccessible by IR spectrometry. Gas chromatography (GC), after transformation of fatty acids (FA) into volatile esters (3), is used for concentrations down to a few tenths of percentage. Often, even the sensitivity of GC technique is not sufficient. Therefore, research was conducted to develop a more sensitive method, based on thermal lens spectrometry (TLS), for determination of TFA.

TLS is known as a very sensitive analytical tool and substantial progress recently made in the area of TLS is well documented in several books and review articles (4–6). Most of its applications are however related to detection of metal ion species in solutions, detection of amino acids and other bioorganic molecules, and detection of some gaseous compounds. Very few reports dealing with application of TLS for food industry (7,8) can, however, be found in literature. This is somewhat surprising because there are several problems of quality control in food industry, which are demanding sensitive analytical techniques for detection of minute amounts of diverse compounds. One reason that TLS has not been applied more extensively in this area is probably the fact that most compounds of interest do not absorb the visible light considerably. This is actually the range of wavelengths where TLS was most frequently used because of the availability of many lasers emitting visible light. Efforts, described in this contribution, have therefore been made to perform measurements of liquid samples in the infrared (IR) spectral range.

In addition to qualitative classification, information on thermal properties such as thermal diffusivity and thermal conductivity of foodstuffs is often desired because they are very important for the stability of products. Accurate values of these properties are needed to assure the most appropriate processing, packaging and storage of foodstuffs. It is therefore quite surprising that values on thermal conductivities of many compounds, including fatty acids, are only accurate to within ±15% or not available at all. This is probably associated with the extreme difficulty of obtaining reliable values on thermal conductivities of fatty acids by generally available techniques for measurement of thermal conductivity.

Recently TLS was successfully applied to measurements of thermal conductivities of different liquids (9). TLS is therefore not only a highly sensitive analytical technique, but can also be used to obtain data on thermal properties of samples. In this work TLS was exploited to measure thermal conductivities of fatty acids.

Experimental

TLS measurements in the IR can be done by using thermal lens spectrometers similar to those for measurements in the visible spectral range. It should however be pointed out that dual beam instruments are preferred for the IR range because the sensitivity of TLS technique is inversely proportional to the probe laser wavelength (10). In a single beam experiment the same laser serves as an excitation source and as a probe. Therefore, it can be readily calculated that a dual beam thermal lens instrument, using a CO_2 laser for excitation (9–11 μm) and a He-Ne laser (632.8 nm) as a probe, would produce almost 20 times better sensitivity compared to a single beam instrument incorporating a CO_2 laser (CO_2 laser beam sizes in the sample are assumed to be equal in both cases). It was indeed for the reason of higher sensitivity that a dual beam instrument was used to perform measurements described in the following sections. The experimental set-up, which has been described in detail elsewhere (7,8) is designed so that, depending on the wavelength of interest, either CO_2 or CO laser could be used for excitation of samples.

By cooling the CO laser to –42 °C it was possible to tune its emission to about 46 lines between 1703 cm^{-1} and 1914 cm^{-1}. The lines are separated by approximately 4 cm^{-1}.

A single beam instrument incorporating a He-Ne laser, was however preferred for determination of thermal properties of samples. This is because of the ease of operation and generally much higher stability of single beam compared to dual beam instruments.

By using water as a reference sample with very well defined thermal properties it was possible to obtain thermal conductivity values for fatty acids with the accuracy of ±5% or better. The addition of an absorbing compound (azulene, disulfine blue) to oleic acid and water (transparent at 632.8 nm) was needed to induce thermal lens signals. Concentrations about 10^{-8} M to 10^{-9} M were sufficient. Therefore, the presence of absorbing additives did not affect the thermal conductivity of investigated specimen.

It stems from signal's dependence on thermooptical properties of the sample (4,10) that thermal diffusivity

($D = k/\rho c_p$; k is thermal conductivity, ρ is density, and c_p is sample's specific heat capacity at constant pressure) of the sample can be deduced from the magnitude of thermal lens effect and the characteristic time constant ($t_c = r^2/4D$; r is laser beam radius in the sample).

Differently from dual beam measurements, where lock-in detection was used to obtain thermal lens signals, the so called kinetic approach was required to obtain the time constant of the thermal lens effect. This was realised by curve fitting of TLS signals to equations describing the time dependence of the thermal lens signal (9,10). The time constant was deduced as one of the constant parameters in curve fitting procedure (9). Once the time constants for fatty acid and water were known, the thermal conductivity of the fatty acid was calculated according to:

$$k_{(f)} = \frac{k_{(w)} \, \rho_{(f)} \, c_{\mu(f)} \, t_{c(w)}}{\rho_{(w)} \, c_{\mu(w)} \, t_{c(f)}}$$

where subscript (f) refers to fatty acid and (w) to water.

Results and Discussion

TLS detection of trans-unsaturated fatty acids at CO_2 laser wavelength

Calibration curves, obtained by measuring TLS signals of methyl-elaidate (t-octadecenoic acid methyl ester) in carbon-disulphide (CS_2), displayed good linearity ($R = 0.998$) over 0.06–0.6% concentration range. Based on the slope of the calibration curve and signal to noise ratio of three, the limit of detection (LOD) of the technique was calculated to be 0.002% of methyl elaidate in CS_2. The performance of the thermal lens technique was tested by analysing three different commercial brands of margarine (Goudas Glory, Bona and Dietella). Dried margarine samples of 10–100 mg were simply dissolved in about 3 mL of CS_2 and injected into the sample cell. The results obtained are shown in Table 1.

Table 1. Content of TFA in various margarine samples as determined by TLS (Results are calculated for dried samples)

Margarine	Content of trans double bonds/(mol/g)	w (trans-unsaturated FA)/% (C16:1 + C18:1)
Brand 1	$(7.4\pm0.1)\times10^{-4}$	19.9±0.3
Brand 2	$(2.2\pm0.2)\times10^{-4}$	6.0±0.6
Brand 3	$(2.51\pm0.03)\times10^{-4}$	6.8±0.2

For trans-unsaturated FA present predominantly as octadecenoic (C18:1) and hexadecenoic (C16:1) acids (as it is usually the case) at 1:1 ratio, one can calculate the results given Table 1. The lack of knowing the actual ratio between C18:1 and C16:1 acids can result in 5% error at maximum, calculated on the basis of molecular weights of C18:1 and C16:1 fatty acids.

By the measurement of absorbance via thermal lens effects the sensitivity of TFA determination was significantly improved compared to IR technique. Considering few tenths of % as the LOD obtained by IR transmittance measurement, an improvement of over two orders of magnitude was achieved. This agrees well with the

enhancement $E = 220$ of thermal lens technique calculated from thermo-optical properties of CS_2, for 50 mW power (P) and probe laser wavelength $\lambda = 632.8$ nm ($E = 0.52 \, P(dn/dT)/\lambda k$; dn/dT is temperature coefficient of refractive index) (*10*).

TLS measurements in liquids at CO laser wavelengths

CO laser emits in the 5–7 μm range, which enables excitation of carbonyl group containing compounds such as fatty acids, glycerides and aldehydes. The absorption cross section for the C=O stretching is higher compared to the absorption cross sections for C-H bending in the case of TFA and O-H out of plane bending in the case of the FFA. Therefore, when the selectivity is not a problem (after chromatographic separation), measurement at this absorption band should provide better sensitivity. It should however be pointed out that the position of the carbonyl band is very much solvent and concentration dependent in the case of fatty acids. At higher concentrations fatty acids are mostly present as dimers and the C=O absorption band appears at lower wavenumbers. When the concentration of fatty acids decreases, the number of monomers in the solution increases, and the position of the C=O absorption band shifts towards higher wavenumbers. A similar trend can be observed for FFA absorption spectra in liquid (dimers) and in gaseous phase (monomers). Conventional IR transmission method is not sensitive enough to measure the position of absorption maximum for monomeric fatty acids in solution. Application of TLS for measurements of fatty acids in low concentration range should therefore represent an advantage over transmission method not only in terms of sensitivity but also because it enables optimisation of the measurement with respect to the wavelength of absorption maximum. This later advantage stems from line tunability of CO lasers, which actually enabled measurements of the first TLS absorption spectra in the 5–7 μm spectral range.

The advantage of using TLS technique to obtain TLS spectra of C=O group containing compounds is evident in the case of oleic acid. For easier comparison of TLS and FT-IR techniques, the same concentration of oleic acid (1 mg/mL) in chloroform was used in both cases. The peak around 1730 cm^{-1}, assigned to the C=O stretch of monomeric FA, is clearly pronounced in the TLS spectrum (Fig. 1) despite the presence of some dimers at this concentration. Diversely from this, the two peaks cannot be accurately identified on the FT-IR spectrum (Fig. 2), because of the relatively high noise. In fact, the concentration used is already close to the limit of detection, which was estimated to be 0.3 mg/mL for FT-IR.

As mentioned earlier, the position of the carbonyl peak changes due to the solvent effect. This effect was also observed in the TLS absorption spectrum of oleic acid measured in carbon tetrachloride (CCl_4). As shown in Fig. 3 the peak (1708 cm^{-1}) is shifted towards lower wavenumbers compared to chloroform. This agrees well with the expected effect of nonpolar solvents on the position of the carbonyl band in the IR spectrum (*11*).

As demonstrated previously, the TLS technique has the potential to detect carbonyl group containing compounds at concentrations that are not accessible by con-
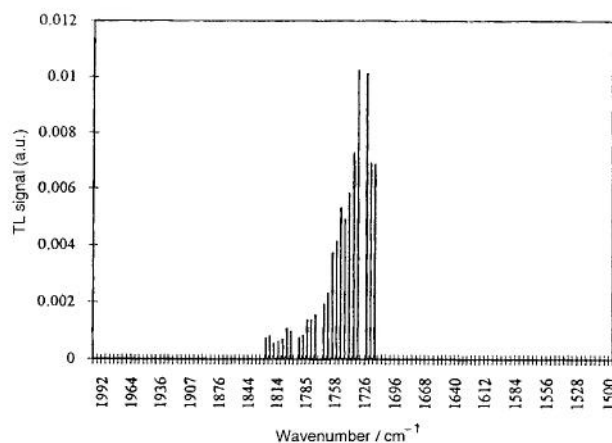
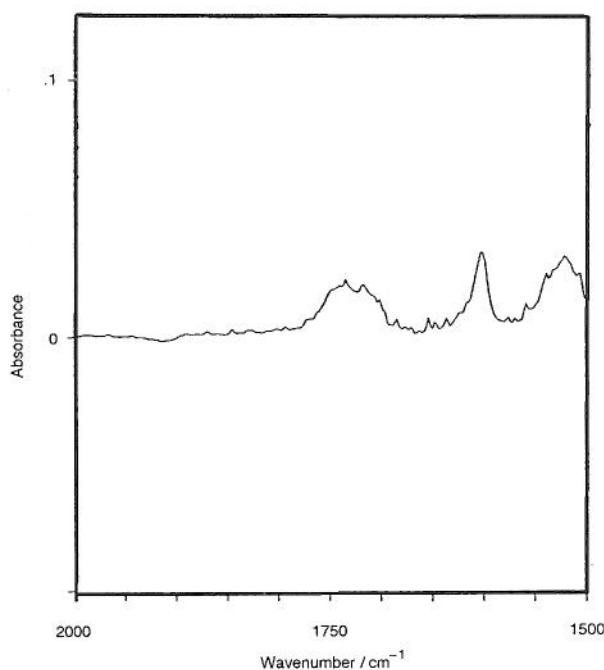Fig. 1. TLS spectrum of oleic acid in $CHCl_3$ ($\gamma = 1$ mg/mL)

Fig. 2. FT-IR spectrum of oleic acid in $CHCl_3$ ($\gamma = 1$ mg/mL)

ventional IR transmission techniques. This was also confirmed by quantitative determinations of oleic acid in chloroform and in carbon tetrachloride.

As shown in Fig. 4, a linear TLS response over a wide range of concentrations (20–450 μg/mL for oleic acid in $CHCl_3$) was obtained. Furthermore the CO laser exhibits much better stability compared to CO_2 laser, and the TLS signals can be measured with a relative error of less than 1%. Improvements in LOD of over two orders of magnitude compared to FT-IR were obtained as a result of low standard deviation. Calibration curves were in general prepared only for the concentration range lower than the LOD for oleic acid obtainable by the FT-IR technique (LOD = 300 μg/mL). It is evident from Fig. 4 that the background thermal lens signal in chloroform is about five times higher compared to the background signal in carbon tetrachloride. Consequently the LOD in
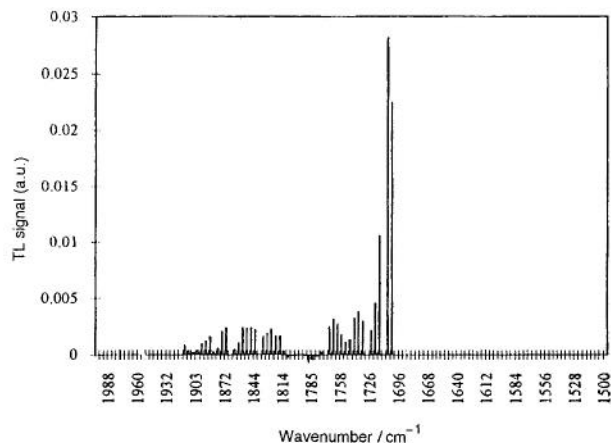
Fig. 3. TLS spectrum of oleic acid in CCl$_4$ ($\gamma = 1$ mg/mL)

carbon tetrachloride (LOD = 1.4 µg/mL) is lower than in the chloroform (LOD = 2.3 µg/mL), but the improvement in LOD is only 1.6 fold. This is partly due to the lower power at the 1708 cm^{-1} line used for measurements in carbon tetrachloride (about five times lower power compared to 1735 cm^{-1} line) and partly to the different power stability at different lines. The 1708 cm^{-1} line was used because of the already mentioned shift in the absorption maximum.
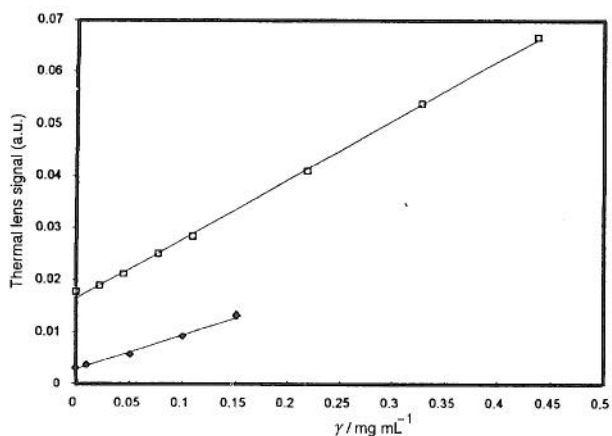

Fig. 4. Calibration curve for oleic acid in CHCl$_3$ (□) and in CCl$_4$ (◆)

Further improvements in LOD of the technique are expected by using the differential experimental configuration (12) which can eliminate up to 95% of the background signal.

Measurement of thermal properties by TLS

Thermal conductivity of oleic acid was measured following the curve fitting procedure, described in the experimental section, to verify the accuracy of the technique. A typical set of time dependent TLS signals used for curve fitting is shown in Fig. 5.
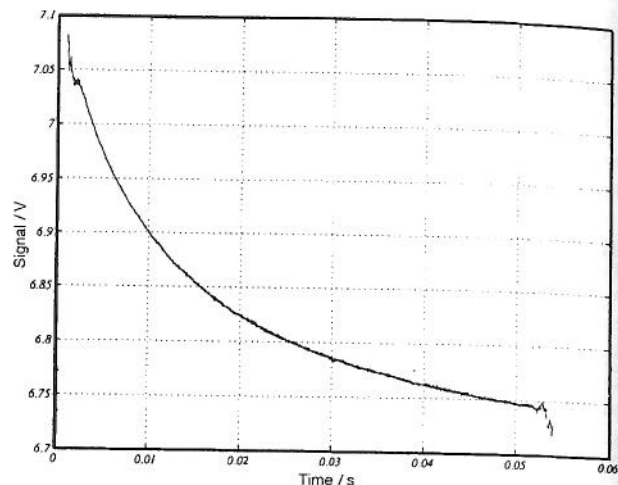

Fig. 5. Beam centre intensity changes during a TLS experiment performed on a sample of oleic acid to determine its thermal conductivity

At 20 °C a k value of (0.18±0.01) W m^{-1} K^{-1} was obtained for oleic acid. There are no literature data available on thermal conductivity of oleic acid. However, the value of 0.18 W m^{-1} K^{-1} is in excellent agreement with the value of 0.18 W m^{-1} K^{-1}, which can be calculated from the value of thermal diffusivity of oleic acid 10.3·10^{-8} m^2 s^{-1}, measured by the photopyroelectric technique (13), and values of $c_p = 1.961$ J g^{-1} K^{-1} and $\rho = 0.8905$ g cm^{-1} from literature (14). Thermal conductivity values for fatty acids such as butanoic acid, hexanoic acid and others were also measured. The results show now significant difference from the value for oleic acid. Such behaviour can be predicted from the general trend of increasing thermal conductivity with increasing chain length of aliphatic hydrocarbons and from the trend of decreasing thermal conductivity with the decreasing polarity of solvents. Since the polarity of fatty acids decreases with increasing chain length, the two effects cancel each other. This results in actually no or very small difference in thermal conductivity of fatty acids. Such small differences could not be observed with thermal lens technique, which enabled measurements with an experimental error of about 5%.

Conclusions

Thermal lens spectrometry was demonstrated to be a highly sensitive technique for detection of *trans*-unsaturated fatty acids and oleic acid, providing limits of detection over 200 times lower than conventional IR transmission technique or FT-IR. In addition, the first TLS absorption spectra of liquid samples in the CO laser spectral region were recorded. 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. This is however not always possible and the applicability of TLS for routine analysis of realistic samples is still limited. Therefore, our research is currently focused onto the development of suitable separation

schemes (HPLC) which, in combination with TLS detection, would provide high selectivity and sensitivity for analysis of different foodstuffs and agricultural products. Besides the high sensitivity of TLS its capability of measuring thermal properties of fatty acids was demonstrated and its accuracy confirmed by measurements with other photothermal techniques.

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List of symbols:

D	thermal diffusivity
k	thermal conductivity
ρ	density
c_p	specific heat capacity at constant pressure
t_c	time constant
r	laser beam radius
w	mass fraction
γ	mass concentration
λ	probe laser wavelength
dn/dT	temperature coefficient of refractive index
P	laser power

Spektrometrija s termičnimi lečami kot metoda za detekcijo in karakterizacijo maščobnih kislin

Povzetek

V delu je predstavljena uporabnost spektrometrije s termičnimi lečami za merjenje nizkih koncentracij trans nenasičenih maščobnih kislin in oleinske kisline v območju emisijskih spektrov CO (5–7 µm) in CO_2 (9–11 µm) laserjev. Dosežene spodnje meje detekcije so za faktor 220 nižje v primerjavi z FT-IR spektrometrijo. Obenem smo uspeli posneti prve TLS spektre tekočih vzorcev v IR območju. Metodo smo uporabili tudi za merjenje toplotnih prevodnosti maščobnih kislin, ki doslej še niso bile izmerjene.