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Thermal Lens Detection in Microfluidic Chips

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Abstract—Main possibilities of thermal lens microscopy, a highly sensitive method of molecular absorption spectroscopy, in chemical analysis in microfluidic chips are shown.

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INTRODUCTION

One of the modern tendencies in the development of analytical chemistry is miniaturization of instruments and devices for chemical analysis and integration of all the analysis steps (sampling, sample preparation, separation, preconcentration, analytical reaction, and measurement of the analytical signal) in a single small supercompact device, viz. a chemical microchip with linear dimensions of channels of 10 μm–1 nm [1–10]. These devices are referred to as microfluidic systems, micro-Total Analysis System (μTAS), integrated microanalysis systems, or lab–on–a–chip. At present these systems aid to solve many problems of analytical chemistry, biochemistry, synthetic chemistry, and bioengineering [1–10]. The use of miniaturized integrated systems in synthesis and analysis makes it possible to reduce the volumes of reagents, solvents, and wastes to 0.1–1 μl and reduce analysis times by several orders of magnitude compared to traditional analytical instruments [1, 4, 5]. The principal characteristic features of microfluidic systems are unique mass-transfer, heat-transfer, and separation conditions in microchannels [6, 9]. Sometimes microfluidic systems also integrate detectors, but normally the microchip itself only accommodates a detection (commonly optic) zone and an interface with an external detector [11–13].

The choice of a detection technique is one of the key issues that need to be addressed when passing from classical to microfluidic analysis: Small sample volumes and low target concentrations impose higher demands on detection sensitivity and instrumentation. As the fluid volumes in microfluidic systems usually vary from a few to tens of nanoliters, quite sensitive methods are required to detect a single or few molecules of the target compounds, when it comes to detection in microchips. Analytical responses in microchannels are most commonly measured by optical methods. Since the depth of a channel is 1–100 μm on the average, the optical path is fairly short, and traditional absorption spectroscopy is hardly suitable here. Even if the molar absorptivity of the target compound is higher than 10^4, the optical paths as short as 10 μm–1 mm allow only high concentrations to be detected. Thus, the most widely used technique for trace analysis in microfluidic systems is laser-induced fluorescence, but it is only suitable for fluorescing compounds and their derivatives. Preliminary derivatization reactions are not infrequently too complicated, and, therefore, a highly sensitive and versatile optical detection technique is required. These requirements are met by thermooptical spectroscopy [12, 14, 15], which can thus be considered as a promising detection technique for microfluidic systems.

Thermooptical Spectroscopy. Thermal Lens Spectrometry and Thermal Lens Microscopy

Laser thermooptical (photothermal) spectroscopy is classed with molecular absorption spectroscopy, and, therefore, is suitable for a broad range of compounds.

Thermooptical spectroscopy is based on photoinduced changes in the thermal state of a sample upon exposure of the latter to electromagnetic radiation [14]. The most common thermooptical effect is a thermal lens effect which can be characterized as the thermally

† Deceased.

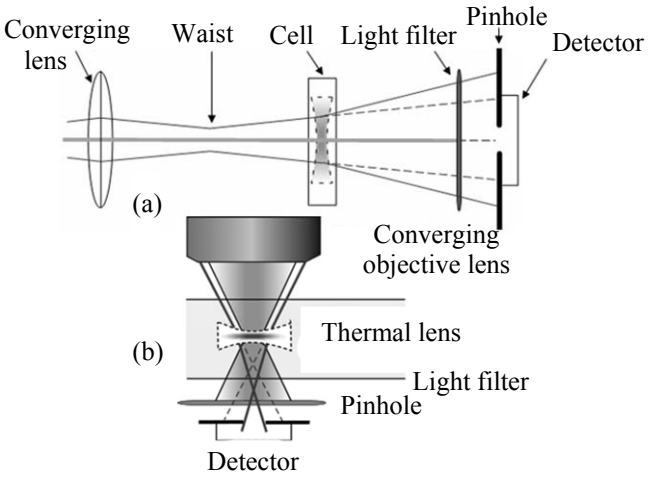

Fig. 1. Scheme of formation of a thermal lens signal in cells (a) and (b) microfluidic chips [36].

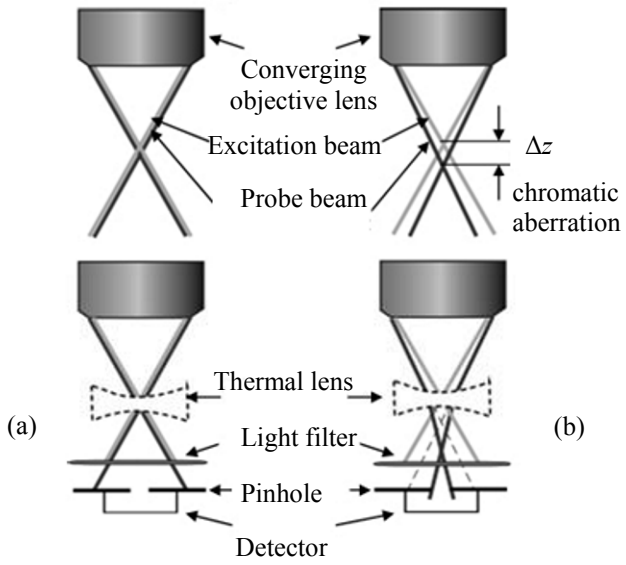

Fig. 2. Scheme of beam paths and signal detection zones in thermal lens microscopy (a) in the presence of chromatic aberration in the objective lens and (b) in the presence of the aberration Δz.

induced change of the refractive index [14–22]. This effect forms the basis of thermal lens spectrometry. When an absorbing medium is exposed to a laser beam (the radiation intensity profile has a Gaussian shape), local heating gives rise to a temperature profile in the medium, and, therewith, the hottest zone is observed in the beam center [20, 21]. Elevated temperature affects the refractive index, and the refractive index distribution corresponds to the radiation energy distribution. As a result, an optical element develops in the medium. This element is similar to a diverging lens and is referred to as the thermal lens [20]. The thermal lens functions as any optical diverging lens: It causes laser beam divergence, i.e. a person looking at the display placed on the beam path will observe beam blooming [20] (Fig. 1). When laser beams are directed coaxially, a spherical lens is formed.

The signal in thermal lens spectrometer is measured as the power of laser radiation passed through a pinhole placed at a certain distance from the cell with a sample (Fig. 1). The formed lens defocuses laser radiation, and the laser power in the beam center decreases [14].

In the practice of thermal lens spectrometry, development has been given to two-laser optical schemes, when a high-power laser beam (excitation) forms a thermal lens in the analyzed medium (and is responsible for a high sensitivity of the measurements), after which it is cut-off by a filter [20, 21]. The signal is a change in the divergence of the second, probe beam of a low-power but stable laser, which ensures high-precision measurements. Along with excitation and probe lasers, the measurement scheme include a beam focusing and convergence system, an object to be analyzed (a cell with a sample, as well as a capillary or a surface), a photodetector, a synchronization system, and a reference channel detector [14]. The system for synchronization of the start of thermal lens formation and the start of signal measurement comprises, as a rule, an electromechanical shutter (to modulate continuous excitation laser beam), control block, and photodiode [20]. The reference a signal serves for registering the current power of the excitation beam and signal conditioning for higher precision measurements.

Thermal lens spectrometry makes it possible to operate with microquantities of substances in any aggregation states and measure absorbances of down to 10^{-8} and concentrations of about 10^{-12}–10^{-10} M [20, 21]. Thermal lens spectrometry is 2–4 orders of magnitude more sensitive than spectrophotometry [20]. Owing to the fact that a laser beam can be focused to the size of flow-through capillaries, this method has found wide application in microfluidics [23]. Its advantages also include simple instrumentation, which provides compact instruments [24] and use of thermal

lens spectrometers for detection in capillary electrophoresis [24–29] and HPLC [20, 22, 30].

Regardless of all the above-mentioned advantages of thermal lens spectrometry, it still has found limited application in microfluidic systems [16, 31, 32]. The most widely used is thermal lens microscopy which is better compatible with planar microfluidic techniques [14, 16]. Even though these two techniques have much in common in signal formation, theoretical basis, and instrumentation features, they are not identical and each occupies its own niche. Let us consider thermal lens microscopy in more detail.

This method was specially developed for measuring signals in microspace. Laser radiation is focused and directed to the sample by means of an optical microscope (Fig. 2). Thus, the microscope is additionally introduced to the optical scheme of a thermal lens spectrometer. The principal distinctive feature of thermal lens microscopy consists in the configuration of laser beam in the sample. If in a dual-beam thermal lens spectrometry the sample cell is located after the waist of the excitation and probe beams, and the probe beam diverges after passing through the thermal lens region [33], in thermal lens microscopy both beams are drawn together by the objective lens and focused into the channel (planar cell) microspace. In the absence of objective aberrations, the waists of the excitation (starting point of thermal lens formation) and probe laser beams coincide (Fig. 2a). Therefore, the thermal lens formation does not cause divergence of the probe beam.

In microscopic objectives, chromatic aberration (Δz) is completely compensated, so that the image quality is not deteriorated. Since for the excitation laser beam the refractive index is higher than for the probe laser, the former beam is focused 2 μm ahead of the latter. Such a configuration results in that the thermal lens "elongates" the focal distance of the objective, the probe laser beam converges (Fig. 2b), and the resulting signal is enhanced [34]. At present thermal lens microscopy makes use of standard compensated objectives, and the distance between the beam focuses is set by means of dual-lens beam expanders [35].

Thus, the main characteristics that distinguish thermal lens microscopy from thermal lens spectrometry are the following: (1) fine focus adjustment due to the focusing of the laser beams into a microchannel (by the sharpness of microchannel image in the microscope objective); (2) locality of photothermal effects due to a shortened optical path length (by 2–3 orders of magnitude); (3) possibility of both horizontal and vertical scanning due to reduced cross-section radii of laser beams; (4) signal caused by the converging effect of the thermal lens due to the focal point of the probe beam focus is located behind that of the excitation beam.

Optical Scheme of a Thermal Lens Microscope

The scheme of a thermal lens microscope is shown in Fig. 3 [34]. The excitation beam is modulated by a mechanical shutter. The modulation frequency and change in the excitation beam power are detected at equal intervals with a lock-in amplifier. The probe laser beam passes through a pinhole and a neutral light filter to attenuate its power. Both beams pass through dual-lens expanders for precise tuning of the distance between beam waists in the microchip channel, as well as for adjusting beam sizes in the sample. Dichroic mirrors are used to redirect the beams and direct them coaxially through an optical microscope objective. Sample image can be observed visually or on a computer display via a CCD camera through the second objective lens. The sample (microcell or microfluidic chip) are placed in an optical table whose position can be varied in three dimensions. After passing through the sample, the probe beam passes through a converging lens and then is separated from the excitation beam by means of narrow-band optical and interference filters. Eventually, having passed through a pinhole, the information-bearing probe beam goes to a photodiode detector coupled with a lock-in amplifier. The signal from the amplifier is transferred to the ADC board of a computer.

Desktop Thermal Lens Microscope

The implementation of such advanced scientific technical solutions as integrated microanalytical systems and thermooptical spectroscopy resulted in the development of the first commercially available photothermal instrument, an ITLM–10 thermal lens microscope (Institute of Microchemical Technology, Japan) [34], which is specially designed for quantitative analysis in microfluidic systems. The instrument dimensions are 650×280×550 mm, weight 2.5 kg. This desktop thermal lens microscope offers such advantages as enhanced sensitivity, wide range of applications, as well as convenience and compactness. It is finding extending application as a detector for

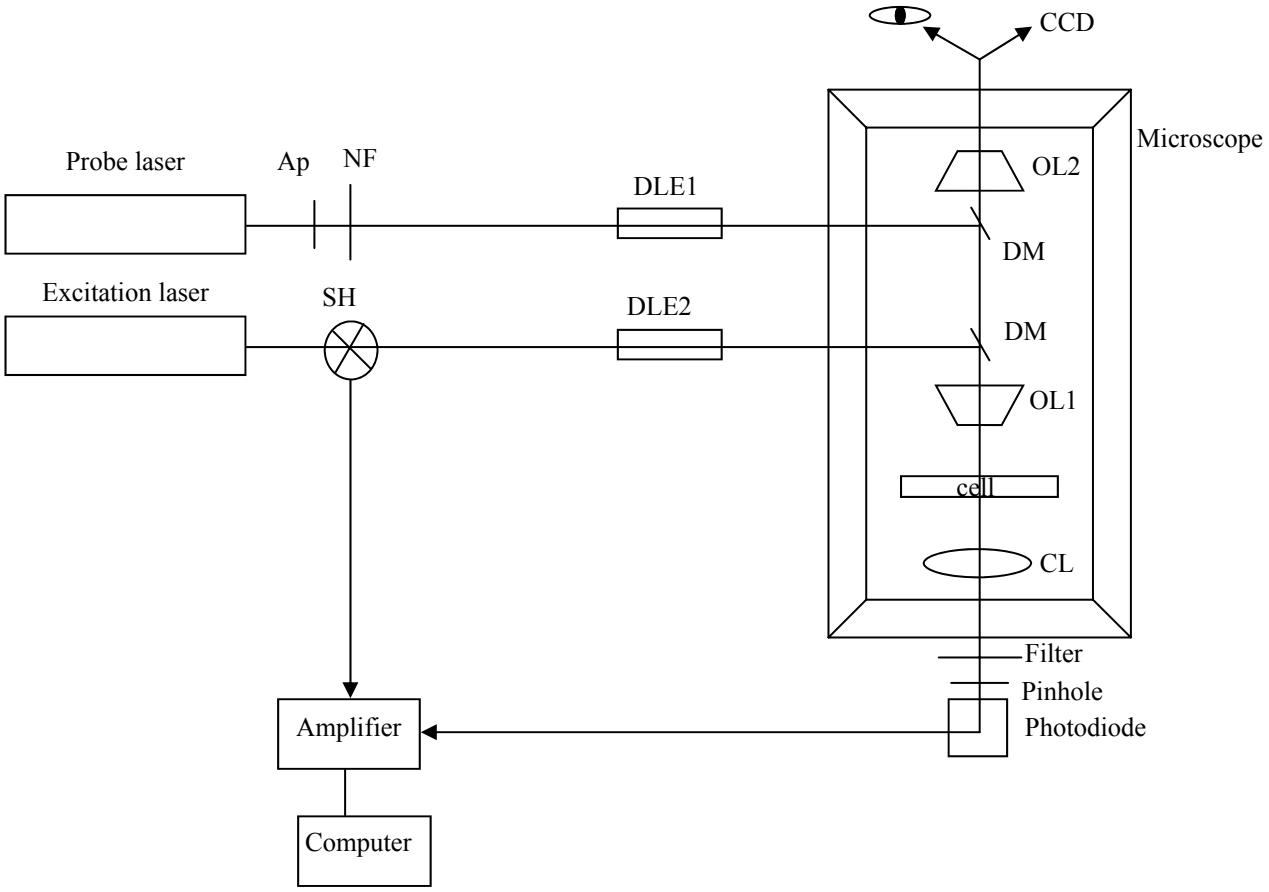

Fig. 3. Thermal lens microscope: (Ap) aperture, (NF) neutral filter, (SH) shutter, (DLE1, DLE2) dual-lens expanders, (DM) dichroic mirrors, (OL1, OL2) objective lenses, (CL) converging lens, and (CCD) digital camera.

microfluidic analytical systems [33, 35] and is actively working up the market of analytical instruments in Japan, USA, and Europe.

Application of Thermal Lens Microscopy

Thermal lens microscopy makes it possible to study reactions in small-volume reactors ($\leq$ 1 μm^3) with nanogram reagent quantities and also to count single molecules [34–39]. It can be integrated with continuous-flow microanalysis and microchromatography [35]. Thermal lens microscopy is used for studying reactions in microfluidic systems [40, 41], quality assessment of microchannels and microflows [42], and detection in combination with various separation techniques [43–46].

The present level of development of thermal lens microscopy can be demonstrated by the following applications of microanalytical systems integrated with this technique: (1) analytical chemistry (immunoenzyme assays, capillary electrophoresis, flow injection, extraction processes, and enzymatic catalysis); (2) synthetic chemistry (study of transfer through a liquid–liquid interface, study of the effect of thermal, electrical, and magnetic fields on reactions in microchannels, and polymerization in microchips); and (3) cellular biochemistry research.

Chemical Analytical Tasks

The main applications fields of thermal lens microscopy in the microchip implementation include environmental analysis, clinical diagnostics, and biochemical and cell analysis.

One of the analytical applications is selective quantitative analysis of potassium and sodium ions [35, 40, 41]. The analysis is performed in the following way. A two-phase system, where the aqueous phase

contains ions to be analyzed, and the organic phase consists of several consecutively injected solutions of ionophores selectively reacting with the analyzed ions and containing a selective lipophilic acid–base indicator, is injected into a microchannel (in the cited work, the reagents for potassium and sodium ions were valinomycin and dibenzo-16-crown-5 derivatives, respectively). The ionophores react with the analyzed ions, thus extracting them into the organic phase; this affects the absorbance of the indicator, which is detected by a thermal lens microscope [43].

Thermal lens microscopy is not only a highly sensitive, but also a highly rapid technique, and this sets it apart from optrode detectors (fiber-optic sensors) [35]. The fast operation speed of this technique is due to short ion diffusion times in microchannels and, as a result, fast detector response (total analysis time 8 s [47]), whereas the slow ion diffusion in an optrode membrane leads to a fairly slow response. Furthermore, micro measurements allow the quantities of high-cost reagents to be reduced considerably. In particular, the minimum volumes of organic reagent solutions in the cited work were 0.5 μl, which allowed determination of subnanogram quantities of alkali metals. The sensitivity of the photothermal the determination of sodium and potassium is higher by an order of magnitude compared to the optrode determination [4].

Thermal lens microscopy was also used for the microflow-injection analysis of L-ascorbic and dehydroascorbic acids by Fe(III) reduction in the presence of 1,10-phenanthroline [47], as well as L-adrenaline and other catecholamines by their oxidation with sodium periodate [48]. The detection limit of ascorbic acid is 1×10^{-7} M (absolute amount 3×10^{-20} mol), which is lower by at least 1.5 orders of magnitude compared to the detection limit of this compound by capillary electrophoresis with electrochemical detection [41], and higher sensitivity than the known pharmaceutical procedure [49]. The described procedure was used for ascorbic acid determination in urine and pharmaceutical preparations [50]. The sensitivity of catecholamine determination was 10^{-6} g/l, which makes the proposed method of micro-flow-injection analysis with photothermal detection feasible for clinical applications. This method was used to determine L-adrenaline, noradrenaline, dopamine, and L-DOPA in injection solutions [48].

Over the past years much attention has been focused on the improvement of the analytical procedures and instruments. Among the principal approaches to the development of microfluidic systems modification and modernization of microchips and improvement of detection techniques are worth mentioning. The first approach involves the modification of microchip surface and shape and depth of microchannels, as well as the choice of microchip materials. Depending on the task to be solved, different types of microchips are applied. Straight and Y-shaped microchips are generally used for analysis. If separation is required along with analysis, microchips with a more complex geometry are used.

New and more and more complex-shaped microchips are being developed for simultaneous analysis of several substances [41]. As an example, we can mention a system that, along with multicomponent analysis, is capable of performing up to 20 micro operations, including mixing, reaction, extraction, detection, etc. [35, 51]. This system represents a 3D microchannel network constructed of three glass microchips made by superimposing them over each other and then by thermal welding. This system was capable of determining Co^{2+} and Fe^{2+} ions simultaneously in two different solutions. The analysis involved five consecutive stages: mixing of the sample solution with a chelating reagent; mixing and the chelation reaction; addition of the second reagent (extractant for Fe^{2+} and acid for Co^{2+}); extraction of Fe^{2+} or decomposition and protonation of Co^{2+}; and detection by thermal lens microscopy. The detection limits of Fe^{2+} and Co^{2+} were 8×10^{-7} and 2×10^{-7} M, respectively.

Of particular interest is a new on-chip liquid–liquid extraction technique [46]. The extraction is performed in a so-called circular microchip, where hemispherical various-depth microchannels form a series of concentric circles joined together (Fig. 4). One part of channels has a hydrophilic surface and the other part has a hydrophilic surface, and, therefore, separated phases are held each in their own channels. A deep channel with a hydrophilic surface accommodates the aqueous phase with the compound to be extracted, and a shallow channel with a hydrophobic surface accommodates the organic phase. The extractant was Methyl Red, a strongly absorbing compound which ensures high-sensitivity thermal lens microscopy detection. The new shape of microchannels allowed sample volumes to be reduced to nanoliters.

In general, the modification of chemical microchips is aimed at extending their analytical potential (qualitative and quantitative analysis, separation, extraction, etc.). State-of-the-art microfluidic technology makes feasible the task of miniaturization of analytical instruments, including the fabrication of microchips with nanochannels.

Another line in the development of microfluidic systems is the improvement of detection techniques, in particular, development of new combined techniques. Note that research effort is not only focused on the use of thermal lens microscopy as a detection technique. Over the past years many new detection techniques (including those based on thermal lens microscopy) have been developed with the aim to improve and facilitate analytical procedures. However, the benefits offered by thermal lens microscopy set it apart from other techniques. Combining microfluidic systems and photothermal detection ensures much lower detection limits and higher sensitivity and selectivity. As mentioned above, at present thermal lens microscopy allows the detection of single particles. Kikutani et al. [52] succeeded to develop a method for fixing gold nanoparticles inside a microfluidic channel for their subsequent quantitative determination.

Among further advances of thermal lens microscopy we would like to mention the measurement of flow rates [53], combination with HPLC [54, 55] and capillary electrophoresis [40], extension of the measurement range to the UV region [56], and combination with various detectors (for example, fluorescence detector [40]). These works can be exemplified by the development of a new detection technique by combining thermal lens microscopy and circular dichroism [57]. A device has been created, which generated radiation with a periodically varied circular polarization direction and thus opened the way to the detection of chiral molecules. This version of thermal lens microscopy provides a sensitivity more than 250-fold higher than circular-dichroism spectrophotometry. Moreover, shifting the excitation beam wavelength from the visible to UV spectral range (which is practiced with non-fluorescing non-labeled molecules [58]) not only favors better analysis performance, but also extends the range of analytes [40].

Other Applications of Thermooptical Spectroscopy

Among biological applications of photothermal spectroscopy in microfluidics we would like to dwell on a system for cell research, which comprises a scanning thermal lens microscope [55]. This microdevice for *in vivo* cell research is suitable for non-fluorescing samples and requires no markers. Its spatial resolution is 1 μm, and the declared detection limit (cytochromes in mitochondria) is 10^{-22} mol [59].

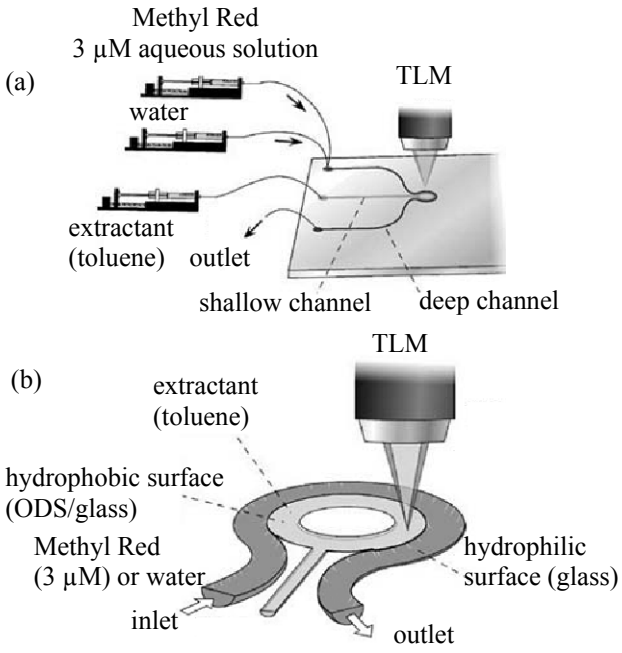

Fig. 4. Experimental device for liquid−liquid extraction: (a) with microsyringes connected to the microchip and (b) general view of a circular microchannel. (TLM) Thermal lens microscope and (ODS) octadecylsilane.

The potential of thermal lens spectrometry integrated with microfluidic chips is well illustrated by the research on the molecular transport in a three-phase continuous-flow water–cyclohexane–water system (the thickness of the organic phase in a microchannel was 64 μm) [59]. Partition parameters of Methyl Red in this system were determined [45]. It was shown that such experiments are only accomplishable in integrated microanalytical systems (the organic phase here functions as a membrane), and, moreover, thermal lens spectroscopy makes it possible to work with non-fluorescing compounds. A model of molecular transport through a liquid membrane in microflows was suggested, which can be useful in biochemical research [45].

Combining photothermal spectroscopy with integrated microanalytical systems provides microflow synthesis with real-time detection of the resulting products [45]. To this end, a two-phase reaction mixture

is used, the synthesis occurs under continuous-flow conditions at the interface to form colored products, and the products that have passed into the organic phase are detected. The reported approach combines a high reaction selectivity with a high detection sensitivity, it was recommended for azo coupling [36] and alkylation reactions [39].

CONCLUSIONS

The enthusiasm for the record sensitivity of thermal lens detection in microfluidic systems, is more and more giving way to the research on chemical processes in new conditions and to development of new procedures. Taking into account the vigorous progress of photothermal instrumentation, it is safe to state that, with time, thermooptical spectroscopy will be gaining more and more importance in the microanalytical chemistry.

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REFERENCES

1. Bruin, G.J.M., *Electrophoresis*, 2000, vol. 21, no. 18, pp. 3931–3951.
2. Maims, C., Hulme, J., Fielden, P.R., and Goddard, N.J., *Sens. Actuators B: Chem*., 2001, vol. 77, no. 3, pp. 671–678.
3. Kutter, J.P., *Trends Anal. Chem*., 2000, vol. 19, no. 6, pp. 352–363.
4. Yi, C.Q., Li, C.W., Ji, S.L., and Yang, M.S., *Anal. Chim. Acta*, 2006, vol. 560, nos. 1–2, pp. 1–23.
5. Min, J.H. and Baeumner, A., *J. Ind. Eng. Chem*., 2003, vol. 9, no. 1, pp. 1–8.
6. Mogensen, K.B., Klank, H., and Kutter, J.P., *Electrophoresis*, 2004, vol. 25, nos. 21–22, pp. 3498–3512.
7. Minteer ,S.D., *Microfluidic Techniques: Reviews and Protocols*, Minteer Shelley, D., Ed., Totowa, N.J.: Humana, 2006.
8. Tokeshi, M., Kikutani, Y., Hibara, A., Sato, K., Hisamoto, H., and Kitamori, T., *Electrophoresis*, 2003, vol. 24, no. 21, pp. 3583–3594.
9. Li, P.C.H., *Microfluidic Lab-on-a-Chip for Chemical and Biological Analysis and Discovery*, Boca Raton: CRC, 2006.
10. Belen'kii, B.G., Komyak, N.I., Kurochkin, V.E., Evstrapov, A.A., and Sukhanov, V.L., *Nauch. Priborostr*., 2000, vol. 10, no. 2, pp. 3–13.
11. Sanders, G.H.W. and Manz, A., *Trends Anal. Chem*., 2000, vol. 19, no. 6, pp. 364–378.
12. Shimizu, H., Mawatari, K., and Kitamori, T., *Anal. Chem*., 2010, vol. 82, no. 17, pp. 7479–7484.
13. Shimizu, H., Mawatari, K., and Kitamori, T., *Ibid*., 2009, vol. 81, no. 23, pp. 9802–9806.
14. Bialkowski, S.E., *Photothermal Spectroscopy Methods for Chemical Analysis*, New York: Wiley–Interscience, 1996.
15. Dovichi, N.J. and Harris, J.M., *Anal. Chem*., 1979, vol. 51, no. 6, pp. 728–731.
16. Navas, M.J. and Jimenez, A.M., Crit. Rev. *Anal. Chem*., 2003, vol. 33, no. 2, pp. 77–88.
17. Dovichi, N.J. and Harris, J.M., *Anal. Chem*., 1981, vol. 53, no. 1, pp. 106–109.
18. Leach, R.A. and Harris, J.M., *Anal. Chim. Acta*, 1984, vol. 164, pp. 91–101.
19. Proskurnin, M.A., Abroskin, A.G., and Radushkevich, D.Yu., *Zh. Anal. Khim*., 1999, vol. 54, no. 1, pp. 91–97.
20. Proskurnin, M.A. and Kononets, M.Yu., *Usp. Khim*., 2004, vol. 73, no. 12, pp. 1235–1268.
21. Snook, R.D. and Lowe, R.D., *Analyst*, 1995, vol. 120, no. 8, pp. 2051–2068.
22. Bendrysheva, S.N., Proskurnin, M.A., Pyell, U., and Faubel, W., *Anal. Bioanal. Chem*., 2006, vol. 385, no. 8, pp. 1492–1503.
23. Smirnova, A., Proskurnin, M.A., Bendrysheva, S.N., Nedosekin, D.A., Hibara, A., and Kitamori, T., *Electrophoresis*, 2008, vol. 29, no. 13, pp. 2741–2753.
24. Shen, J. and Snook, R.D., *J. Appl. Phys*., 1993, vol. 73, no. 10, pp. 5286–5288.
25. Mandelis, A., *Anal. Sci*., 1990, vol. 6, no. 4, pp. 491–503.
26. Martin-Biosca, Y., Roze, C., and Ramis-Ramos, G., *Israel J. Chem*., 1998, vol. 38, no. 3, pp. 169–174.
27. Shen, J., Soroka, A.J., and Snook, R.D., *J. Appl. Phys*., 1995, vol. 78, no. 2, pp. 700–708.
28. Shen, J., Baesso, M.L., and Snook, R.D., *Ibid*., 1994, vol. 75, no. 8, pp. 3738–3748.
29. Smirnova, A., Shimura, K., Hibara, A., Proskurnin, M.A., and Kitamori, T., *J. Sep. Sci*., 2008, vol. 31, no. 5, pp. 904–908.
30. Faubel, W., Heissler, S., Pyell, U., and Ragozina, N., *Rev. Sci. Instrum*., 2003, vol. 74, no. 1, pp. 491–494.
31. Rosenzweig, Z. and Yeung, E.S., *Appl. Spectrosc*., 1993, vol. 47, no. 8, pp. 1175–1179.
32. Logar, J.K. and Franko, M., *Rev. Sci. Instrum*., 2003, vol. 74, no. 1, pp. 300–302.
33. Hiki, S., Tokeshi, M., Hibara, A., and Kitamori, T., *Bunseki Kagaku*, 2003, vol. 52, no. 8, pp. 569–574.
34. Proskurnin, M.A., Slyadnev, M.N., Tokeshi, M., Kitamori, T., *Anal. Chim. Acta*, 2003, vol. 480, no. 1, pp. 79–95.

35. Kitamori, T., Tokeshi, M., Hibara, A., and Sato, K., *Anal. Chem*., 2004, vol. 76, no. 3, pp. 52a–60a.
36. Ueno, M., Hisamoto, H., Kitamori, T., and Kobayashi, S., *Chem. Commun*., 2003, 8, pp. 936–937.
37. Uchiyama, K., Hibara, A., Sato, K., Hisamoto, H., Tokeshi, M., and Kitamori, T., *Electrophoresis*, 2003, vol. 24, no. 1-2, pp. 179–184.
38. Tamaki, E., Hibara, A., Tokeshi, M., and Kitamori, T. *J. Chromatogr. A,* 2003, vol. 987, nos. 1–2, pp. 197–204.
39. Smirnova, A., Mawatari, K., Hibara, A., Proskurnin, M.A., and Kitamori, T., *Anal. Chim. Acta*, 2006, vol. 558, nos. 1–2, pp. 69–74.
40. Hiki, S., Mawatari, K., Hibara, A., Tokeshi, M., and Kitamori, T., *Anal. Chem*., 2006, vol. 78, no. 8, pp. 2859–2863.
41. Sorouraddin, H.M., Hibara, A., and Kitamori, T., *Fresenius J. Anal. Chem*., 2001, vol. 371, no. 2, pp. 91–96.
42. Sato, K., Tokeshi, M., Kitamori, T., and Sawada, T., *Anal. Sci*., 1999, vol. 15, no. 7, pp. 641–645.
43. Hisamoto, H., Horiuchi, T., Tokeshi, M., Hibara, A., and Kitamori, T., *Anal. Chem*., 2001, vol. 73, no. 6, pp. 1382–1386.
44. Hibara, A., Nonaka, M., Tokeshi, M., and Kitamori, T., *J. Am. Chem. Soc*., 2003, vol. 125, no. 49, pp. 14954–14955.
45. Surmeian, M., Slyadnev, M.N., Hisamoto, H., Hibara, A., Uchiyama, K., and Kitamori, T., *Anal. Chem*., 2002, vol. 74, no. 9, pp. 2014–2020.
46. Kikutani, Y., Hisamoto, H., Tokeshi, M., and Kitamori, T., *Lab. Chip*, 2004, vol. 4, no. 4, pp. 328–332.
47. Hisamoto, H., Sato, S., Sato, K., Tsubuku M., Siswanta, D., Shichi, Y., Koike, Y., and Suzuki, K., *Anal. Chim. Acta*, 1999, vol. 396, no. 2-3, pp. 131–141.
48. Sorouraddin, H.M., Hibara, A., Proskurnin, M.A., and Kitamori, T., *Anal. Sci*., 2000, vol. 16, no. 10, pp. 1033–1037.
49. Backofen, U., Matysik, F.M., and Lunte, C.E., *Anal. Chem*., 2002, vol. 74, no. 16, pp. 4054–4059.
50. *British Pharmacopeia*, London: HM Stationary Office, 1998.
51. Smirnova, A., Shimura, K., Hibara, A., Proskurnin, M.A., and Kitamori, T., *Anal. Sci*., 2007, vol. 23, no. 1, pp. 103–107.
52. Kikutani, Y., Mawatari, K., Hibara, A., and Kitamori, T., *Microchim. Acta*, 2009, vol. 164, nos. 3–4, pp. 241–247.
53. Mawatari, K., Tokeshi, M., and Kitamori, T., *Anal. Sci*., 2006, vol. 22, no. 5, pp. 781–784.
54. Kikutani, Y., Mawatari, K., Katayama, K., Tokeshi, M., Fukuzawa, T., Kitaoki, M., and Kitamori, T., *Sens. Actuators B: Chem*., 2008, vol. 133, no. 1, pp. 91–96.
55. Mawatari. K., Kubota. S., and Kitamori, T., *Anal. Bioanal. Chem*., 2008, vol. 391, no. 7, pp. 2521–2526.
56. Uchiyama, K., Tokeshi, M., Kikutani, Y., Hattori, A., and Kitamori, T., *Anal. Sci*., 2005, vol. 21, no. 1, pp. 49–52.
57. Yamauchi, M., Tokeshi, M., Yamaguchi, J., Fukuzawa, T., Hattori, A., Hibara, A., and Kitamori, T., *J. Chromatogr. A,* 2006, vol. 1106, nos. 1–2, pp. 89–93.
58. Yamauchi, M., Mawatari, K., Hibara, A., Tokeshi, M., and Kitamori, T., *Anal. Chem*., 2006, vol. 78, no. 8, pp. 2646–2650.
59. Tamaki, E., Sato, K., Tokeshi, M., Sato, K., Aihara, M., and Kitamori, T., *Ibid*., 2002, vol. 74, no. 7, pp. 1560–1564.