Available online at www.sciencedirect.com
ScienceDirect
Mendeleev Commun., 1991, **1(2)**, 57–58

Mendeleev Communications

Application of Thermal Lens Spectrometry to the Determination of Theophylline

Andrei G. Abroskin, Yuri A. Barbalat, Tatiana A. Belyaeva, Elena K. Ivanova, Michael A. Proskurnin,* Valentina M. Savostina and Vera A. Filichkina

Department of Analytical Chemistry, Faculty of Chemistry, M. V. Lomonosov Moscow State University, 119899 Moscow, USSR

Thermal lens spectrometry has been applied to the determination of the purine series alkaloid theophylline in the nerve cells of molluscs.

Methods of trace analysis classified as thermal lens spectrometry (TLS) are based upon the change in the optical properties of a sample on absorption of laser energy and quantitative registration of the resulting thermal perturbations. TLS allows one to measure absorptivities of 10^{-7}–10^{-8}, concentrations of 10^{-9} mol dm^{-3} (refs. 1 and 2), to analyse volumes of 10^{-15} dm^3 and to detect 10^2 molecules in this volume.[3]

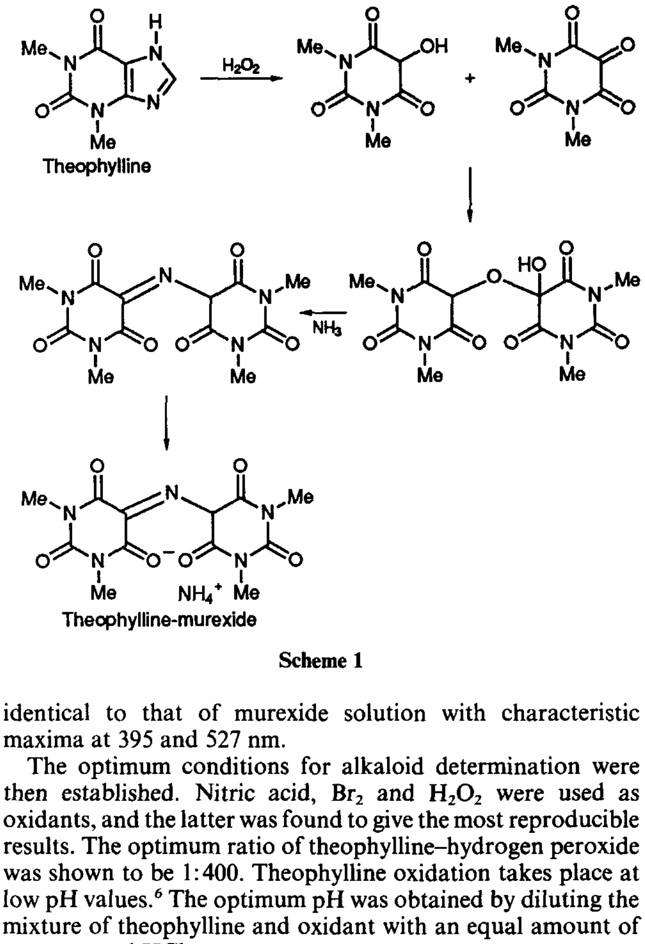
The aim of the work presented was to construct the thermal lens spectrometer and to apply it to a routine for the ultrasensitive photometric determination of theophylline.

The spectrometer was set up with a parallel dual-beam optical configuration.[1,2] A current-regulated pump Ar^+ laser was operated at 488 nm, giving an emitted laser power of about 1 W. An He–Ne laser (20 mW) was exploited as a probe, and photometry cell volumes varied from 0.5 to 2 ml. The scheme was improved by the application of an optical multichannel analyser (OMA-2, EG&G PARC, USA) giving more accurate data acquisition. The data obtained were corrected and smoothed by an FFT-based algorithm on a IBM PC AT computer.[4] Spectrophotometric studies were made on a 'Hitachi-124' spectrometer with 1 cm cells.

Theophylline is an alkaloid of the purine series which is widely used in medicine and biophysics. Its medicinal applications are increasing owing to its stimulating effect on the heart and the central nervous system. An important task at present is the determination of theophylline in biological objects such as nerve cells. There is a limited set of determination methods for purine alkaloids, *e.g.* HPLC with conductometric detectors and some spectrophotometric techniques.[5] However, HPLC demands a high alkaloid concentration that necessitates sophisticated pre-extraction of the probe. Existing spectrophotometric methods do not allow sensitive and selective detection, and the main interfering compounds are substances with amino-groups. Thus we have attempted to work out a selective method for theophylline determination and to use TLS to improve the sensitivity.

The procedure for quantitative determination of theophylline is based on its oxidation to a compound similar to murexide[6] and subsequent measurement of the absorbance of the solution. This reaction is specific only for the purine alkaloids and it therefore avoids interference from all other constituents of nerve cells. It also allows the determination of the total concentration of theophylline and its metabolites (which can also be treated as purine alkaloids).

The compounds obtained by oxidation of theophylline (theophylline–murexide) differ from murexide by either two or four extra symmetrical methyl substituents at the nitrogen atoms (depending on the metabolite). Scheme 1 shows the process. Comparison of the spectral characteristics of murexide and the compounds obtained reveals that the resulting absorption spectrum of aqueous theophylline–murexide solution is identical to that of murexide solution with characteristic maxima at 395 and 527 nm.



Scheme 1

The optimum conditions for alkaloid determination were then established. Nitric acid, Br_2 and H_2O_2 were used as oxidants, and the latter was found to give the most reproducible results. The optimum ratio of theophylline–hydrogen peroxide was shown to be 1:400. Theophylline oxidation takes place at low pH values.[6] The optimum pH was obtained by diluting the mixture of theophylline and oxidant with an equal amount of concentrated HCl.

The colour of the resulting solution is stable for 30 min. In order to increase the colour stability and to enhance the TLS signal a water–acetone solvent (1:100) was used, which gave a colour which was stable for a few weeks. The absorption spectrum of the water–acetone solution reveals maxima at 410 and 480 nm (the latter was used during the spectrophotometric studies). The absorbance of this solution is much higher than that of an aqueous solution with equal reagent concentration. As the amount of water in the solvent increases the absorbance falls and the spectral maxima move to the values of the aqueous solution spectra. The difference between the spectra may be explained in terms of the different solvating abilities of water and acetone. Exchanging water molecules for acetone ones reduces the possibility of hydrogen bonds formation and leads to a shorter conjugated system and a hypsochromic shift.

Mendeleev Commun., 1991, 1(2), 57–58

During the spectrophotometric studies under the above conditions water–acetone mixture alone was used as a blank. Under these conditions the limit of theophylline detection was 0.4 μg ml^{-1}. A line calibration curve was obtained for theophylline concentrations from 0.9 to 230 μg ml^{-1} in the final solution.[7]

The procedure described was used for theophylline determination in the cytoplasm of the mollusc nerve cells without preliminary separation in samples consisting of 10^5–10^6 cells. In the case of smaller samples (10^2–10^3 cells) it is necessary to exclude cell constituents for they may interfere because of the great sensitivity of the thermal lens effect. Thus, theophylline was isolated from cells by a mixture of chloroform and ethanol in the presence of NaCl (as suggested in the literature[8]) and measured in comparison with a cytoplasm extract without alkaloid.

The TLS method may be successfully employed in many other tasks: analysis of organic liquids, solid substances and gases. Its application to organic analysis may be extended by the development of hybrid methods and the TLS spectral range widened by using tunable lasers.

Received in USSR, 29th November 1990
Received in UK, 4th January 1991; Com. 0/05519K

References

1 H. L. Fang and R. L. Swofford, in *Ultrasensitive Laser Spectrophotometry*, ed. D. S. Kliger, Academic Press, New York, 1983, ch. 3, pp. 210–266.
2 N. J. Dovichi, *CRC Crit. Rev. Anal. Chem.*, 1987, 357.
3 N. J. Dovichi, *Prog. Anal. Spectrosc.*, 1988, **11**, 179.
4 A. G. Abroskin, M. A. Proskurnin, Yu. A. Barbalat and E. K. Ivanova, *Zh. Anal. Khim.*, 1991, **46**, 870.
5 J. J. Lauff, *J. Chromatogr.*, 1987, **417**, 99.
6 R. Poludek-Fabine and T. Beirich, *Organicheski Analiz* (Organic Analysis), Khimiya, Leningrad, 1981, p. 182 (in Russian).
7 A. G. Abroskin, T. V. Belyaeva, E. K. Ivanova, S. G. Mayakova and M. A. Proskurnin, *Zh. Anal. Khim.*, 1991, **46**, 1199.
8 E. Naline, *J. Chromatogr.*, 1987, **423**, 179.