Optica Applicata, Vol. XXVIII, No. 3, 1998

Optical calibration of the picosecond time scale and correlated background elimination in fluorescence dynamics measurements by time-correlated photon counting

K. Dobek, J. Karolczak, D. Komar, J. Kubicki, M. Szymański, T. Wróżowa, M. Ziółek

Institute of Physics, Adam Mickiewicz University, ul. Umultowska 85, 61–614 Poznań, Poland.

A. Maciejewski

Faculty of Chemistry, Apparatus Laboratory, ul. Grunwaldzka 6, 60–780 Poznań, Poland.

A simple, feasible and precise method of calibration of the time scale of a TCSPC laser spectrometer is devised, based on the insertion of an optical delay into one of the start/top channels. The delay can vary from a fews ps to about 1 ns depending on the geometry and refractive index of the medium used, and can be determined with an accuracy better than 1%. A procedure is proposed for eliminating the time-correlated background appearing mainly due to Raman scattering and trace impurities in solvents, which is a serious impediment in TCSPC measurements and analysis of excited state decay in the picosecond time scale. The problem of the correlated background elimination is particularly important for samples of the excited state decay times of single picoseconds, which usually show very weak luminescence.

1. Introduction

Time-correlated single-photon counting (TCSPC) is a highly advanced and precise technique for investigation of luminescence decay dynamics, which over the last three decades has reached a high level of experimental sophistication [1]–[4]. At present, the TCSPC technique can use stable and tunable Ti:Sapphire laser sources of excitation pulses short enough to neglect their width in the time scale of the phenomena studied, fast and reliable high-speed multichannel-plate photomultiplier tubes (MCP-PMT's) [5]–[7], and efficient procedures of data processing allowing elimination of numerous artefacts, adjusted to the possibilities of desk-top computers [8]–[22]. These tools enable analysis of fluorescence decay from the excited states of lifetimes of single picoseconds. The actual limitations of the TCSPC technique follow rather from inadequacies of deactivation models used for description of the real bahaviour of the media studied.

A frequent impediment is the emission inhomogeneity of samples, *i.e.*, the presence of emission from a solute or solvent other than the luminescence examined. In the simplest case, these undesirable emissions can be attributed to trace im-

purities of the substance or solvent used, and since the possibilities of purification are limited, their presence particularly disturbs studies of weak short-lived fluorescence. This becomes especially important in the case of expensive solvents and quenchers of special properties which, for obvious reasons, are used in very small amounts. Organic solvents excited in ultraviolet range in the vicinity of the absorption bands almost always show emission of trace organic impurities, frequently of large molar absorption coefficients. Also, purification of biological samples is often very difficult. Moreover, even in the spectra of absolutely pure samples there is a Raman scattering band present, which is Stokes shifted by a few tens of nanometers with respect to the excitation line. When measurements are carried out in the range of the Raman band, the scattered light behaves like an additional emission lasting as long as the exciting pulse and distorting the emission studied in the instrument response function (IRF) range. The simplest solution is to avoid using the solvents and media which bring additional emission signals, or at least avoid the ranges of emission and excitation in which these signals may cause problems. Unfortunately, this is not always possible and frequently the experimental reasons force us to work in these unfavourable conditions. The problem of unwanted emission most often concerns the sample studied, however, it may also concern the reference samples used in various deconvolution procedures for determination of IRF. The presence of such a disturbing emission time-correlated with the exciting pulse hinders the process of deconvolution, deteriorates the quality of the fit and affects, sometimes significantly, the results of measurements.

In this paper, a simple, precise and efficient method for optical calibration of the time scale in TCSPC experiment is described which ensures avoidance of significant systematic errors appearing when using the electronic methods of calibration. Also, we propose a procedure which permits elimination or considerable reduction of the effect of foreign emissions on results of TCSPC experiment in conditions when use of solvents or quenchers which introduce time-correlated undesirable luminescence cannot be avoided.

2. Experimental system and its optical calibration

In the TCSPC spectrometer used for investigation of luminescence decay of the excited states in the time scale of single picoseconds, the source of exciting pulses was a Spectra-Physics pico/femtosecond laser system pumped with an argon ion BeamLok 2060 laser. In the experiments reported, a Ti:Sapphire Tsunami laser tunable in the range 720 – 1000 nm generated 1 – 2 ps (fwhm) pulses at the repetition rate of 82 MHz with mean power of above 1 W. The pulse selector model 3980-2S reduced repetition rate to the range from 4 MHz to a single shot. GWU-23 PS second and third harmonic generator with the extention for the range 840 – 1000 nm was used for converting the Tsunami laser output to the UV and blue range. The optics for luminescence excitation and collection as well as the systems controlling the measurement and data processing were prepared by Edinburgh Instruments with the use of fast Tennelec timing electronics units: upgraded quad TC 454 constant

fraction discriminator and biased TC 864 TAC as well as Hamamatsu photodetectors: H5783-04 PMT module and R3809U-05 MCP-PMT with appropriate preamplifiers (Hamamatsu). The stop signal was taken from a fast photodiode recording the unused portion of red laser beam of the fundamental frequency transmitted through the harmonics generator.

Calibration of the time scale of the spectrometer, *i.e.*, determination of the time per channel depending on the preset of TAC and the multi-channel analyser (MCA) is usually performed with the use of decay standards — solutions of well determined lifetime or electronic standards: calibrated delay lines and/or generators of delayed pulses, in longer time scales. The first method is relatively inaccurate and includes at least a few significant error-prone moments. Delay lines cannot also be treated as reliable and stable standards because of their limited accuracy of calibration, parameters deteriorating with time, and the fact that for lengths corresponding to times < 1 ns, the delays at joints and connectors become increasingly important. In view of the above, we have decided to use an optical delay as a well-defined and easily reproducible one. In the exciting beam channel there was placed a plane-parallel optical cell filled with pure solvents of well-determined refractive indices at the excitation wavelength λ_{ex} to introduce a calibrated shift of the start signal with respect to the stop signal. For the sake of simplicity the exciting pulse was scattered in a solution of ludox and the position of IRF on the time scale was recorded with

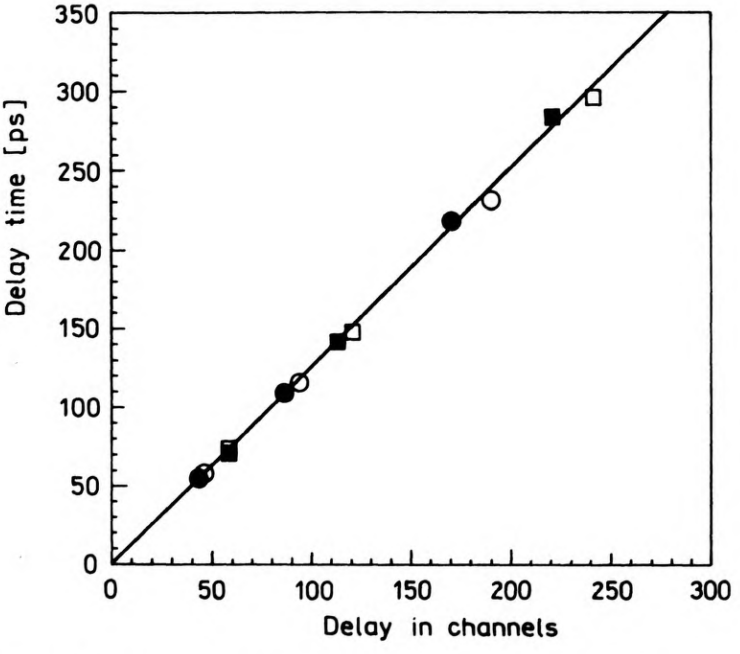

Fig. 1. Calibration of the time scale of TCSPC spectrometer. Time per the multichannel-analyzer channel determined from the slope of the weighted linear regression. ■ — cyclohexane, 750 nm, ● — water, 750 nm, □ — cyclohexane, 400 nm, ○ — water, 400 nm. Slope = (1.25±0.02) ps/ch, intercept ≃ 0 ps, corr. coeff = 0.996)

and without the additional delay introduced by the solvent in the cell of a length of 5, 10 or 20 cm. The solvents were water and cyclohexane of refractive indices equal to 1.3477 and 1.4452 for $\lambda_{ex} = 400$ nm, and 1.3279 and 1.4269 for $\lambda_{ex} = 750$ nm, respectively. The IRF peak was found as the maximum of the Gauss curve fitted to the top of the experimental profile. Figure 1 presents exemplary results of determination of the time per channel in the above described experiment. The procedure allows the time per MCA channel calibration with an accuracy better than actually required.

3. Time-correlated backgroud elimination

The procedures for TCSPC data processing usually include elimination of the background of time-uncorrelated counts homogeneously distributed along the whole scale. In Section 1 we mentioned factors causing the appearance of the background counts time-correlated with the exciting pulse, which can lead to considerable distortion of results and whose elimination is much more difficult. Some of these factors, such as Raman scattering or scattering in the wing of the Rayleigh line cause the appearance of instantaneous emission occurring only in the exciting pulse duration, which modifies the shape of the IRF line used for deconvolution of the decays studied. Other factors, like *e.g.*, real emission from trace impurities present in the solvents or quenchers, cause deformation of the decay curves of the substances studied as a result of introduction of long-lived luminescence.

The way of getting rid of counts time-correlated with the excitation pulse is based on the idea of collecting, under the same experimental conditions and for the same acquisition time, decay curves of emission from a solution studied and also the curves representing the time-correlated background from pure solvents or solvents with quenchers in the same concentrations as used in measurements. Since the rules for the correct use of the TCSPC technique ensure additivity of counts due to different components of the complex emission, the counts due to unwanted emission of the solvents in appropriate channels can be subtracted from those originating from the solutions. When the absorbance of the solution at λ_{ex}, caused by the presence of the solute is not negligibly low, the counts from the solvent should be reduced in proportion to the decrease of the exciting beam intensity in the solution on its way to the part of the solution volume being the source of luminescence measured by the emission detector. The sofrware used by us not only permits a reduction of the number of counts in particular channels in order to account for the solute absorption, but also a shift of the entire solvent emission curve on the time scale (channel shift) by a preset value.

The procedure was verified in our laboratory by analysis of decays of thioketones in H-D isotope substituted solvents, perfluorated or partly fluorated solvents – the latter with the active groups left at selected parts of the molecule, in solutions with quenchers of particular properties, *e.g.*, used for investigation of anisotropy of reactivity. Figure 2 shows an exemplary deconvolution of the emission decay of

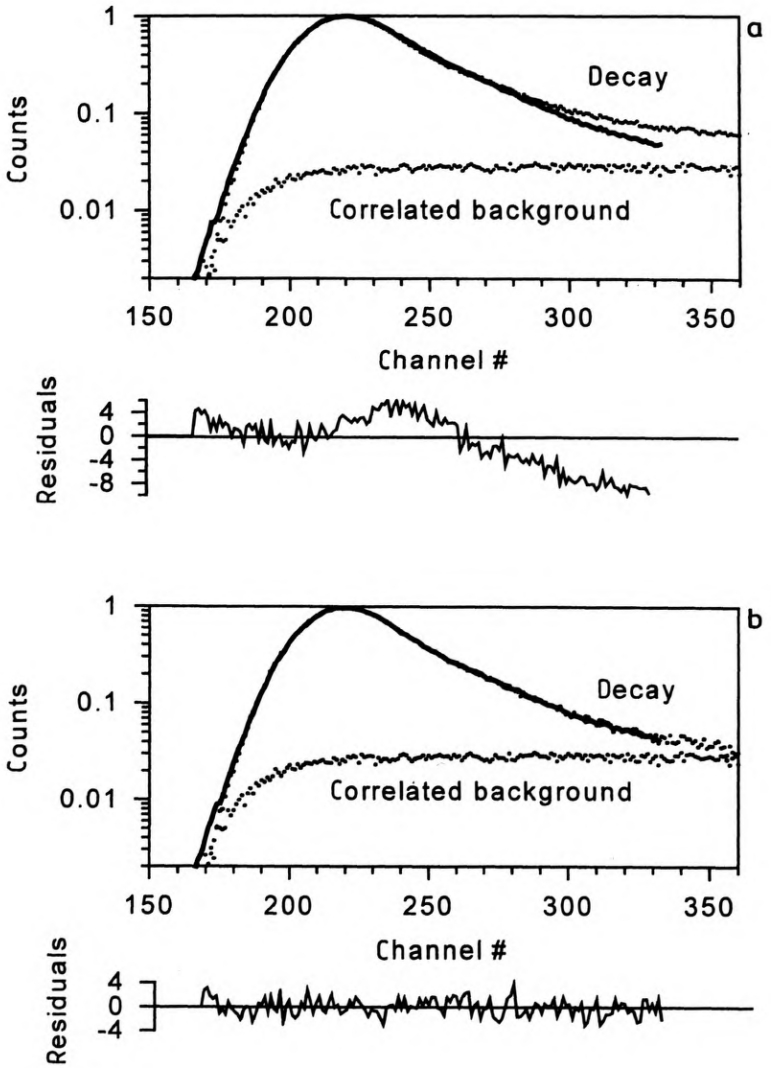

Fig. 2. TCSPC data for benzpyrathione fluorescence in partly deuterated butanol (BuOD) solution. The points represent experimental data and the solid curve is the optimum fitted decay function. The weighted, scaled residuals are plotted over the range of the fit. **a** — results calculated without time-correlated background elimination: $\tau = 7.2$ ps, $\chi^2 = 23.33$, **b** — results obtained after background elimination: $\tau = 6.2$ ps, $\chi^2 = 1.90$

benzpyranthione in deuterated buthyl alcohol performed with the excitation pulse-shape mimic technique [19], using a solution of xanthione in acetonitrile as the reference for IRF determination.

4. Conclusions

The paper presents two practical solutions facilitating the use of TCSPC technique in analysis of deactivation of the luminescence states in the picosecond time scale. The proposed method for calibration of the time scale of the picosecond spectrometer for fluorescence decay time measurements is very simple and meets the requirement of easy reproduction of a precise standard of delay for the time range from extremely short to about 1 ns. When using monochromatic radiation, in this range of times the change in the exciting pulse profile due to group velocity dispersion can be disregarded and the accuracy of time per channel calibration below 1% can be achieved. Such an accuracy cannot be attained with the use of simple electronic standards – delay lines and generators – whose verification in the optical laboratory conditions is very difficult.

The procedure for eliminating the time-correlated emission background proposed in this paper is a practical solution helful in overcoming experimental limitations caused by sometimes very efficient emission from trace impurities in the solvents or quenchers used in the picosecond measurements of fluorescence decay by TCSPC method. In the case of strongly emitting substances of long-lived excited states the emission from trace impurities does not create much problem, but when studying weak emission of decay times of single picoseconds it may considerably distort the signal. The distortions in the course of the signal not only deteriorate the quality of the fit but can also lead to erroneous results. The proposed method for the time-correlated background elimination permits investigation of picosecond decay dynamics even when the luminescence is strongly distorted by unwanted emission from the solvent or when the measurements have to be performed in the region of a strong Raman scattering band.

Acknowledgement – The financial support from the State Committee for Scientific Research (KBN) within Grant No. 2P03B 057 12 is gratefully acknowledged.

References

[1] DEMAS J. N., *Excited State Lifetime Measurements*, Academic Press, New York 1984.
[2] O'CONNOR D. V., PHILIPS D., *Time-Correlated Single Photon Counting*, Academic Press, New York 1984.
[3] LAKOWICZ J. R., *Principles of Fluorescence Spectroscopy*, Plenum Press, New York 1983.
[4] BOENS N., [In] *Luminescence Techniques in Chemical and Biochemical Analysis*, [Ed.] R. G. Baeyens, D. De Keukeleire, K. Korkidis, Marcel Dekker, New York 1991, pp. 21–45.
[5] KUME H., KOYAMA K., NATSUGAWA, SUZUKI S., FATLOWITZ D., Appl. Opt. **27** (1988), 1170.
[6] YAMAZAKI I., TAMAI N., KUME H., TSUCHIYA H., OBA K., Rev. Sci. Instrum. **56** (1985), 1187.
[7] BEBELAAR D., Rev. Sci. Instrum. **57** (1986), 1116.
[8] SPEARS K. G., CRAMER L. E., HOFFLAND L. D., Rev. Sci. Instrum. **49** (1978), 255.
[9] KOESTER V. J., DOWBEN R. M., Rev. Sci. Instrum. **49** (1978), 1186.
[10] MEECH S. R., O'CONNOR D. V., ROBERTS A. J., PHILIPS D., Photochem. Photobiol. **35** (1981), 159.
[11] VAN DEN ZEGEL M., BOENS N., DAEMS D., DE SCHRYVER F. C., Chem. Phys. **101** (1986), 311.
[12] RAYNER D. M., MCKINNON A. E., SZABO A. G., Rev. Sci. Instrum. **48** (1977), 1050.
[13] WAHL PH., AUCHET J. C., DONZEL B., Rev. Sci. Instrum. **45** (1974), 28.

[14] Ross J. B. A., Rousslang K. W., Brand L., Biochemistry **20** (1981), 4261.
[15] Wijnaendts an Resandt R. W., Vogel R. H., Provencher S. W., Rev. Sci. Instrum. 53 (1982), 1392.
[16] Boens N., Ameloot M., Yamazaki I. De Schryver F. C., Chem. Phys. **121** (1988), 73.
[17] Gauduchon P., Wahl P., Biophys. Chem. **8** (1978), 87.
[18] Libertini L. J., Small E. W., Anal. Biochem. **138** (1984), 314.
[19] James D. R., Demmer D. R. M., Verrall R. E., Steer R. P., Rev. Sci. Instrum. **54** (1983), 1121.
[20] Eisenfeld J., Ford C. C., J. Biophys. **26** (1979), 73.
[21] Knutson J. R., Beechem J. M., Brand L., Chem. Phys. Lett. **102** (1983), 501.
[22] Beechem J. M., Ameloot M., Brand L., Anal. Instrum. **14** (1985), 379.

Received July 9, 1998