7 Nanosecond Time-resolved Emission Spectroscopy

7.1 Introduction

In the block diagram of a simple SPC instrument, shown previously in Fig. 2.1, F1 and F2 denote filters. If the intensity in the light source is high enough these filters may be replaced by scanning monochromators, at which stage the instrument, since it incorporates a light source, detector and recorder (usually an MCA), can function as a standard spectrofluorimeter. The simplest configuration is one where the TAC is bypassed and the signals from the discriminator on the emission channel (CFTD) are routed, possibly after amplification, direct to the MCA. With the MCA operated in multi-channel scaling mode and the channel advance synchronized with the wavelength drive of one of the monochromators the resulting histogram of counts in the MCA channels represents the fluorescence excitation or fluorescence spectrum of the sample. This type of instrument is a simple photon counting fluorimeter (Arecchi *et al.*, 1966), and is rather insensitive since the intensity in the pulsed flash lamp is orders of magnitude less than in a conventional arc lamp (see Section 3.3.4). An elegant instrument of this type, employing a high intensity source, has been described by Tuan and Wild (1973, 1974).

If the pulsed source is retained but the TAC reintroduced to correlate the fluorescence photons with the excitation events (that is, the electronic components and settings are the same as for decay curve measurements except for the mode of operation of the MCA), a fluorescence spectrum considerably more revealing than a normal spectrum can be collected. This arises from the fact that the amplitudes of the TAC output pulses are proportional to the times of occurrence of the corresponding fluorescence photons. Selection of TAC pulses according to voltage, which is easy to accomplish, is a selection of fluorescence events according to time of occurrence after excitation. Fluorescence spectra recorded, or constructed, by resolving, with respect to wavelength or energy, single photon pulses that have been selected from a limited time interval, are referred to as time-resolved emission spectra or simply TRES. We give TRES the symbol $S(\lambda,t_k)$.

The relationship between the normal fluorescence spectrum, $F(\lambda)$, the

decay functions at a fixed wavelength, $G(\lambda_e,t)$, and the time-resolved spectra can be deduced from the diagram in Fig. 7.1, which is an effort to represent the three-dimensional surface of the intensity at all wavelengths and times during the fluorescence decay. For the moment we specify any point on this surface by $I_n(\lambda_j,t_k)$. It can be seen that any plane parallel to the I_n plane represents the emission spectrum at a particular time (the TRES). Therefore the digital decay function at the wavelength λ_j is made up of the points

$$I_n(\lambda_j,t_1), I_n(\lambda_j,t_2), \ldots I_n(\lambda_j,t_k)\ldots$$
$$\equiv G(\lambda_j,t) \tag{7.1}$$

the TRES (in digital form) at time t_k is made up of

$$I_n(\lambda_1,t_k), I_n(\lambda_2,t_k), \ldots I_n(\lambda_j,t_k)\ldots$$
$$\equiv S(\lambda,t_k) \tag{7.2}$$

and the total emission spectrum (in digital form) is made up of the points

$$\int_0^\infty I_n(\lambda_1,t)\,dt, \int_0^\infty I_n(\lambda_2,t)\,dt, \ldots \int_0^\infty I_n(\lambda_j,t)\,dt, \ldots$$
$$\equiv F(\lambda) \tag{7.3}$$

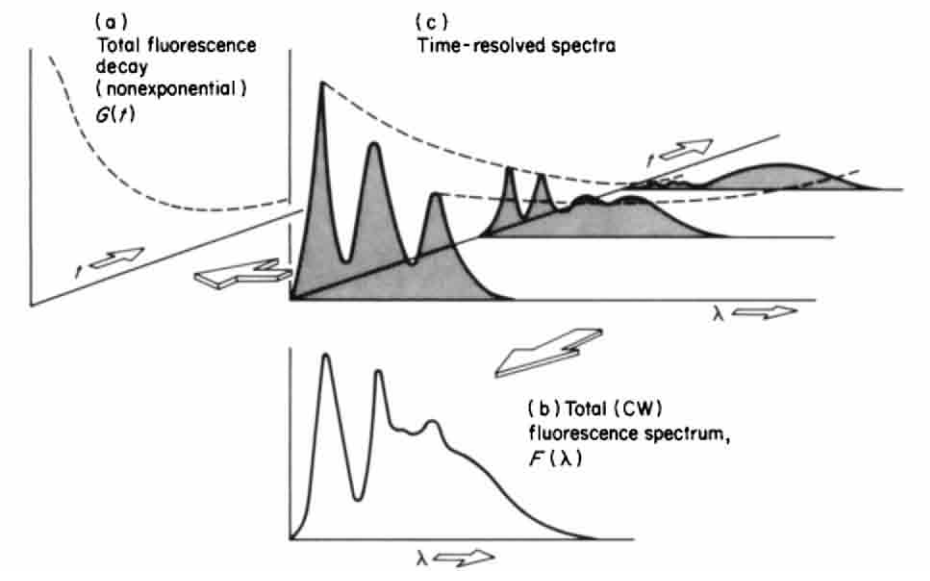

Figure 7.1 Representation of emission intensity distribution as a function of time and wavelength (after Beavan *et al.*, 1979).

It will be seen that the intensity function $I_n(\lambda,t)$ is merely a dummy form of $G(\lambda,t)$ and that

$$F(\lambda) \sim \int_0^\infty G(\lambda_1,t)\,\mathrm{d}t, \int_0^\infty G(\lambda_2,t)\,\mathrm{d}t, \ldots$$

$$\int_0^\infty G(\lambda_j,t)\,\mathrm{d}t \tag{7.4}$$

and

$$S(\lambda,t_k) \sim G(\lambda_1,t_k), G(\lambda_2,t_k), \ldots G(\lambda_j,t) \tag{7.5}$$

In the time-resolved spectral measurement we have outlined, the TRES are made up of points not on the deconvolved functions $G(\lambda,t)$ but on the convolved decay curves, $I(\lambda,t)$. The question of convolution is an important one in the consideration of TRES and we shall return to it in the following sections.

7.2 Applications and Limitations

Time-resolved emission spectroscopy can yield both qualitative and quantitative information about many photophysical processes that cannot be gleaned from normal fluorescence spectra of even extremely high optical resolution. One such process is a simple exciplex equilibrium, in which an excited precursor is quenched reversibly by a ground state molecule to form an excited state complex, precursor and complex having different decay times. If photons emitted only at long times are wavelength-resolved the resulting spectrum will have a much reduced and perhaps negligible contribution from the fluorescence of the short-lived species. Similarly, excited state molecules having two fluorescing configurations of differing decay times are ideal candidates for time-resolved spectral studies. However, if the interconversion between the different emitting species in both of these molecular systems is uncomplicated, it can be studied easily by means of conventional decay time measurements. Time-resolved spectra may be helpful, and even necessary, in characterizing the process under investigation, but perhaps most of the quantitative information will be deduced from decay curve analysis. It is in their application to excited state phenomena that admit of no simple kinetic analysis or that yield decay curves the lifetimes of which can be associated with no simple process or group of processes that time-resolved spectra have most value.

One such phenomenon is the much studied solvent relaxation round an excited state fluorophore. Time-dependent shifts in the fluorescence spectrum may accompany the interactions between solvent and excited solute, the

decay of which will not be interpretable in terms of a simple exponential model if the timescale on which the solvent relaxation occurs is comparable to the normal lifetime of the solute (Bakshiev, 1965). An understanding of the interactions occurring can be attained by means of time-resolved spectra and can serve as a basis for study of, for instance, the effect of the local environment in biological molecules on the spectral characteristics of fluorescent probes (Cockle and Szabo, 1981).

For example, 8-anilino-1-naphthalene sulphonate (ANS) has been much used as a fluorescent probe in biological systems since it exhibits different fluorescence characteristics when bound to proteins, lipids or cellular membranes. This is illustrated by the fluorescence spectra of ANS shown in Fig. 7.2 which reveal a clear shift in emission maximum for the probe bound to a lipid and to the model protein, bovine serum albumin (BSA) (Lee *et al.*, 1975).

It would be useful if in a real membrane with both protein and lipid components, the spectral differences seen in Fig. 7.2 could be distinguished, thus allowing protein sites and lipid sites to be discriminated. However, the spectral differences are small and the actual fluorescence of ANS under continuous illumination in a real membrane, such as the cilia of a small organism *Tetrahymena pyroformis*, is a single irresolvable peak (Fig. 7.3).

If recourse is made to time-resolved fluorescence spectroscopy, however, the fact that the decay time of lipid-bound ANS is relatively short (*c.* 4 ns), whereas that of protein bound ANS is long (*c.* 16 ns) permits the desired spectral resolution, as shown in Fig. 7.4. Here the early "gated" spectrum resembles the spectrum of ANS bound to the model lipid in Fig. 7.2, and the "late-gated" spectrum is very similar to the ANS bound to BSA. The added dimension of time-resolution thus permits the two classes of site to be

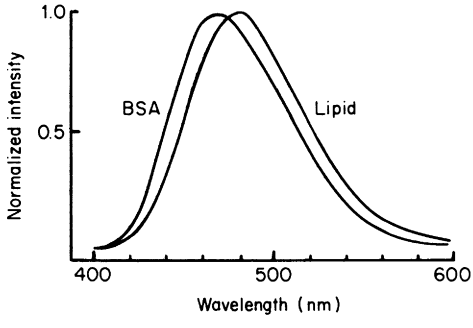

Figure 7.2 Total fluorescence emission spectra for ANS bound to liposomes of egg yolk phostatidylcholine (LIPID) and bovine serum albumin (BSA) (after Lee *et al.*, 1975).

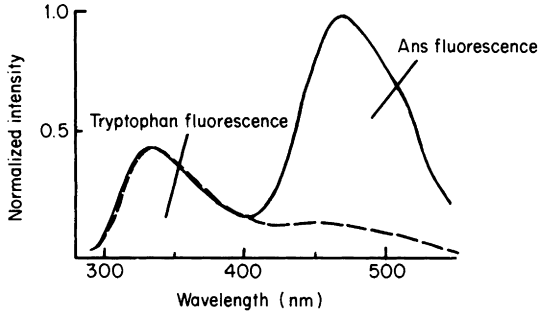

Figure 7.3 Total fluorescence emission spectra for ANS bound to microsomal membranes, -----. Fluorescence from blank microsome sample, ——. Fluorescence from microsomes plus ANS (1.7×10^{-5} M) (after Lee *et al.*, 1975).

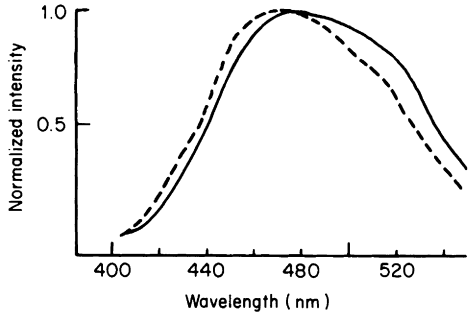

Figure 7.4 Time-resolved fluorescence emission spectra for ANS bound to microsomal membranes. ——, Early gated spectrum; -----, late gated spectrum (after Lee *et al.*, 1975).

distinguished. Use can then be made of the spectral discrimination in biochemical studies on membranes.

As a further example of the use of TRES, we turn to the field of synthetic polymers. There is now increasing evidence that, owing to the variety of excimer conformations that pendant aromatic groups in polymer chains can assume, fluorescence decays in such systems must be described by multi-exponential decay laws (Roberts *et al.*, 1981; Hoyle and Guillet, 1979). Although models involving three exponential components have gained some acceptance it is conceivable that fluorescence decays in polymeric systems are really much more complex. Again, here, time-resolved spectra are invaluable.

For example in the synthetic copolymer poly(vinylnaphthalene)-poly(methylmethacrylate) the decay of the fluorescence at any wavelength

requires a minimum of three exponential components for successful fitting. However, the time-resolved spectra shown in Fig. 7.5 (Roberts *et al.*, 1981) can all be synthesized by summing only two components, suitably weighted. This reveals that the origin of the complexity in fluorescence decay lies in the kinetics of interconversion of these two fluorescent species and their ability for participation in energy migration, rather than the existence of multiple overlapping emitting species.

Even in one-component molecular systems departures from simple kinetics may be observed. Isolated aromatic molecules in the gas phase excited to truly single vibronic levels may undergo intramolecular vibrational redistribution in competition with short-lived fluorescence (Lambert *et al.*, 1981; Cureton *et al.*, 1981) and furnish intriguing examples of multiexponential decay behaviour. There has long been considerable experimental and theoretical interest in this topic, which is now being enhanced with the advent of picosecond time resolution in single photon counting apparatus. There is no doubt that TRES resolved on a picosecond time scale can provide a long-sought experimental tool in the investigation of intra molecular vibrational redistribution (Moore *et al.*, 1983).

As a final example of an area where TRES could provide vital insight into excited state processes we would cite the transient effect associated with concentration gradients in excited state diffusion-controlling reactions. Because of this effect the quenching rate "constant" is time dependent and the kinetic equations cannot be solved analytically without assumptions that may not be valid in all time regions (Nemzek and Ware, 1975). When reversible chemical reaction, as in exciplex systems, occurs on the same timescale as diffusion the kinetics take on a further level of complexity (Andre *et al.*, 1979). Conventional decay curves from such systems have decay functions, $G(t)$, that cannot be described by a simple functional form and that produce highly "distorted" decay data especially at short times (Hui and Ware, 1976). Time and wavelength resolution of the fluorescence emitted from such systems offers an effective means of monitoring the behaviour of the molecules step by step during the time evolution of the reaction.

Clearly time-resolved spectroscopy can be of great value in the understanding of many photophysical processes that attract much current interest. In addition it can serve as a background (scattered light) suppressor in analytical fluorescence techniques (Haugen and Lytle, 1981). It may therefore be wondered why the technique has not to date been implemented more widely. One of the reasons lies in the inherent intractability of the processes referred to, involving as they do fluorescence, sometimes on very short timescales, from molecules reacting in a little understood way to stimuli that may themselves be difficult to characterize properly. Of more importance, however, are the intensity problems posed by the measurement, having their

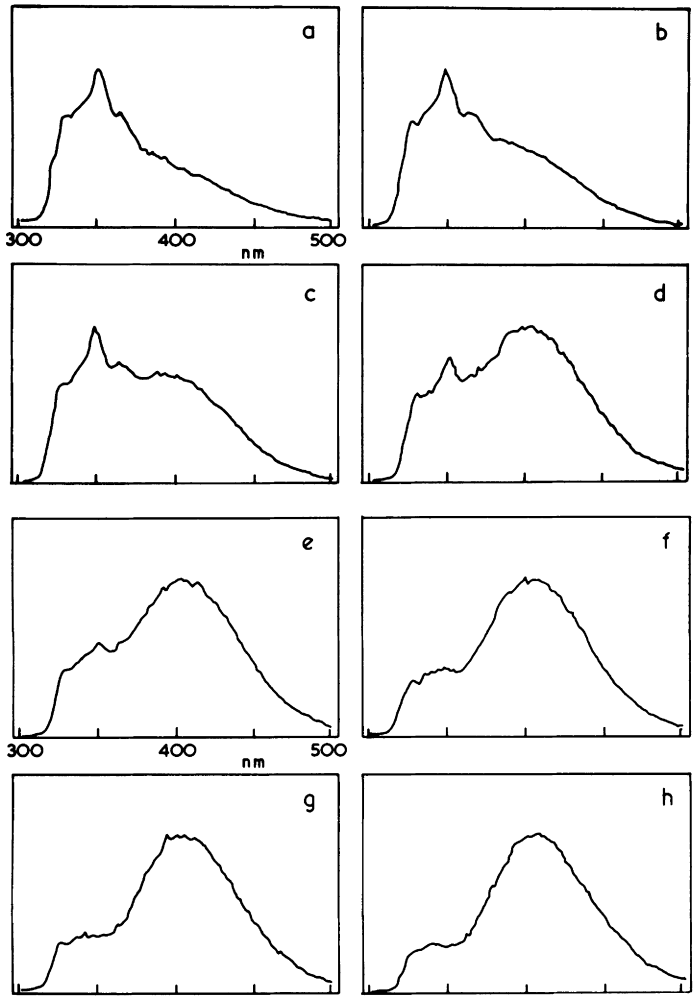

Figure 7.5 Time-resolved fluorescence of poly(vinylnaphthalene)-poly(methylmethacrylate) copolymer. Delays Δt (a) 0 ns, (b) 3.2 ns, (c) 6.4 ns, (d) 12.8 ns, (e) 19.2 ns, (f) 25.6 ns, (g) 32.0 ns and (h) 38.0 ns (after Roberts *et al.*, 1981).

source in the necessarily weak output of conventional nanosecond flash lamps especially if exciting radiation is isolated with a monochromator. In many applications highly monochromatic exciting light is a strict requirement of the investigation, in which case the intensity losses in the two monochromators may be so great as to preclude the measurement of accurate time-resolved spectra. Finally, it will be realized that the spectra recorded in the manner we have outlined are distorted by convolution with the exciting

pulse and the detector response and may, depending on the timescale of interest, be of limited quantitative value. Nevertheless the ability of TRES to provide a qualitative picture of a process can often indicate the most effective means of proceeding with further investigation. Moreover, with the advent of picosecond lasers and detectors convolution effects in time-correlated measurements are being confined to ever shortening times with the consequence that TRES undistorted on a nanosecond timescale can now be applied to an increasing number of short-time phenomena. In addition, monochromatic laser lines of relatively high intensity now render the recording of TRES a matter of some ease. We describe the details of the measurement in Section 7.4.

If the time resolution of the SPC instrument is not high enough or if time-resolved spectra are required for kinetic analysis of picosecond events, a procedure has been developed whereby TRES can be constructed from decay functions from which distortions due to the finite width of the pumping pulse and detector response have been removed. This procedure is described in Section 7.5.

7.3 Other Methods of Spectral Time Resolution

Before discussing spectral time resolution on present day SPC instruments we will briefly mention some other techniques by which spectra resulting from short time fluorescence have been measured. In one of these methods large amounts of a non-specific quencher (such as oxygen) are added to the sample under investigation. If both long-lived and short-lived species are present, proportionately more of the fluorescence from the long-lived species will be quenched and the measured spectrum will result from short-lived unquenched fluorescence. Although the introduction of a high concentration of fluorescence quencher might be totally inappropriate in many studies, this method has some advantages and has been implemented, for example, in a series of elegant experiments concerned with vibrational redistribution in the gas phase (Dolson *et al.*, 1981). In some spectral measurements the dispersed light from a grating is allowed to impinge on a diode array operated by an optical multichannel analyser (OMA). This method is usually employed in conjunction with a pulsed light source operated at low repetition rate. A suitable gating pulse can be delivered to the OMA at any time after the light pulse, in which case the data collection will take place only in a time interval determined by the gating pulse (O'Connor *et al.*, 1983). An extremely sophisticated time gating technique has been described by Moore *et al.*, (1983), and entails down conversion of fluorescence by non-linear mixing with a picosecond laser pulse, the gating time being set by the width of this

pulse. Finally, we mention a technique which was the direct precursor of the method to be described in the following section and employed gating of the fluorescence photomultiplier with a travelling wave gate circuit (Ware *et al.*, 1968; Chakrabarti and Ware, 1971; Egawa *et al.*, 1971). With the advent of TAC based lifetime instruments TRES measured by the travelling wave technique have become less common.

7.4 Direct Recording of TRES

Time-resolved emission spectroscopy with the single photon technique was apparently first performed by Ware and co-workers (1971). The principle of the method is depicted in Fig. 7.6. Output pulses from the TAC, of maximum amplitude, say, 10 V, are routed to a single channel analyser which processes pulses falling within a certain voltage range. A single channel analyser (SCA)

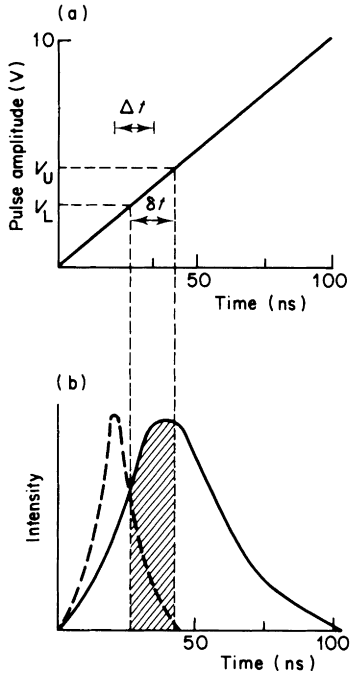

Figure 7.6 Schematic representation of selection of time window by voltage discriminators. (a) TAC voltage ramp (TAC range 100 ns). V_L, level of lower level discriminator; V_U, level of upper level discriminator; Δt, delay time; δt, gate width. (b) Hypothetical pump pulse (- - - - -) and convolved decay curve (——). Photons in time window (hatched area) selected for spectral analysis.

is essentially a timing discriminator with two variable levels V_L and V_U. Input pulses amplitudes lower than V_L and higher than V_U are rejected. The SCA may be a separate electronic component but is usually incorporated in the multichannel analyser. The output pulses from the SCA are routed to the analogue-to-digital converter and thence to the storage channels. In Fig. 7.6 the voltage selection of TAC conversions is shown as time selection in the decay curve. We shall refer to the time, Δt, corresponding to the TAC pulse amplitude $[V_L + (V_U - V_L)/2]$ as the delay time and the time window, δt, corresponding to the voltage range $V_U - V_L$ as the gate width. Usually an accuracy in Δt and δt corresponding to the width of one MCA channel can be attained.

In order to choose the discriminator levels the decay curve is first collected in the normal way with the MCA in pulse height analysis mode. During this collection the lower and upper level discriminators are adjusted until the decay is building up only in channels corresponding to the time window of interest. The MCA channels are then cleared, the MODE switch turned to multichannel scaling and MCA collection started synchronously with the monochromator wavelength drive. Spectral resolution is governed not only by the normal slit width and scanning speed effects but also by the dwell time per channel in the MCA. Most MCAs have an internal clock with which the channel advance can be controlled. If not, or if dwell times unattainable with the MCA clock are desired, clocks designed specifically for control of multichannel scaling are available as NIM standard modules (see Section 5.2.10). If channel advance is controlled by an external clock this must normally be started at the same time as the data collection. Automatic operation of the data collection is of course possible by means of a simple electronic circuit. Since MCA channels now correspond to increments in wavelength the accumulated histogram of counts is a wavelength-resolved spectrum of the photons emitted during the interval δt. It should be remembered that these time-resolved spectra are not corrected for the wavelength response of the detecting system.

For instrument response functions of finite FWHM the delay time Δt must be defined arbitrarily since a unique time of excitation cannot be established, as reference to Fig. 7.6 demonstrates. It is therefore usual to specify Δt as the time elapsed between the time the instrument response function reaches its maximum and the midpoint of the time interval δt. In order to determine this time the MCA is returned to pulse height analysis mode and counts collected (and stored) without alteration of the upper and lower level discriminators. Then these levels are adjusted to the normal settings for decay curve measurements and an instrument response function collected. There is now a record of the position of Δt with respect to the instrument response function as well as of the gate width δt.

Constant intensity in the pump pulse is of crucial importance when TRES are measured in this simple way. However, since this stability is necessary only during one wavelength scan a simple stratagem can be adopted to correct for steady drifts in intensity. The same spectrum is scanned through fairly rapidly a number of times and alternate scans are driven in a different wavelength direction. Since the channel can usually be advanced only in the forward direction the alternate scans must be added to different memory groups of the MCA. Finally the spectrum in the second memory group is altered to run in the same wavelength direction as the first spectrum and added to it (in the computer processing). A more satisfactory procedure is to advance the wavelength and channel number in accordance with a predetermined photon flux from the exciting light, which could be monitored with an additional photomultiplier or photodiode (Ware *et al.*, 1971). A circuit designed for this purpose has been described by Rughooputh *et al.* (1983). Background counts could also be averaged out by means of a rotating shutter.

As we have already stated, optical resolution in the spectrum is dependent on the slit width and scanning speed of the monochromator. In addition, many MCAs have a maximum of 1024 channels, so that, if it is desired to scan a wide wavelength range, the number of available channels may also impose a limitation on the optical resolution. This limitation could obviously be overcome by a rapid transfer of data from the MCA into a secondary store (e.g., a computer) and an automatic control over the wavelength drive.

Time resolution in this type of TRES can be quite high since counts accumulating in a single MCA channel could be selected for spectral analysis. Unfortunately, as was stated previously an absolute zero-time is sometimes impossible to define; the width of the instrument response function relative to the delay time, Δt, is crucial in the determination of time resolution. For example, a spectrum of photons emitted at late times, when the excitation has long ceased, will not be badly distorted since convolution at very late times is negligible. It is the spectra of photons emitted in a time window occurring shortly after the maximum in the instrument response function that have low, convolution affected, time resolution.

In the attainment of good time resolution intensities may have to be drastically curtailed, especially if the delay time is set at very short or very long times. A flash lamp monochromator excitation source will probably require very careful optical alignment if the intensities necessary for the recording of accurate (the accuracy depending on pulse stability and therefore short collection times) and well resolved spectra are to be achieved. Some recent semi-quantitative studies into flash lamp generated TRES demonstrate the power of the technique (Hara *et al.*, 1980; Hoyle and Guillet, 1979). The disadvantages, related to intensity and time resolution, associated with

TRES measurement are greatly reduced if a pulsed laser serves as the excitation source. High photon fluxes in a narrow energy band, and sometimes in a pulse of a few picoseconds duration, render measurement of TRES from photons even in a very narrow gate width relatively facile.

TRES generated by three techniques are illustrated in Fig. 7.7. The phenomenon under investigation was exciplex formation, in cyclohexane solution, between 1,4-dicyanonaphthalene in its S_1 state and ground state 2,5-dimethyl-2,4-hexadiene. The total fluorescence spectrum, in which the structureless red-shifted exciplex band is clearly visible, is illustrated in Fig. 7.8. A CW dye laser (pumped by an Ar^+ ion laser) with cavity-dumped and frequency doubled output provided excitation pulses of 7 ns duration at 5 MHz. In Fig. 7.7(a) are shown the separated monomer and exciplex spectra, the latter obtained by subtracting the fluorescence spectrum of the sample to which oxygen was added (see Section 7.3) from the spectrum of the degassed sample (Johnson, 1975). The spectra in Fig. 7.7(b) are TRES measured as outlined in this section. Although the late gated spectrum was measured at a delay time of 58 ns, well removed from the maximum in the instrument response function, it is obviously contaminated with some monomer emission. This spectrum would tend to confirm the view of Easter *et al.* (1976) that TRES distorted by convolution are unsuitable for quantitative kinetic analysis, for which it is preferable to have spectra of the type illustrated in Fig. 7.7(c). Generation of these spectra is described in the following section.

7.5 Construction of TRES from Deconvolved Decay Functions

TRES measured directly are composed not of the true decay functions as given by Equation 7.5, but of convolved decay functions,

$$S(\lambda,t_k) \sim I(\lambda_1,t_k), I(\lambda_2,t_k), \ldots, I(\lambda_j,t_k). \tag{7.6}$$

In order to construct TRES free of convolution effects it is necessary to measure decay curves at a number of wavelengths, extract true decay functions from them by deconvolution and normalize and rearrange these $G(\lambda,t)$s (Easter *et al.*, 1976; Rayner and Szabo, 1978). In Appendix 7.A1 is listed a computer routine with which these steps may be carried out. A detailed description of the procedure will now be given.

First, decay curves are collected at various wavelengths spanning the emission spectrum of the sample. Since spectral resolution is determined by the number of decays that go to make up the TRES (see Equation 7.5), a large number of measurements will be necessary for even moderate resolution. Time profiles of the pump pulse (instrument response functions) are collected

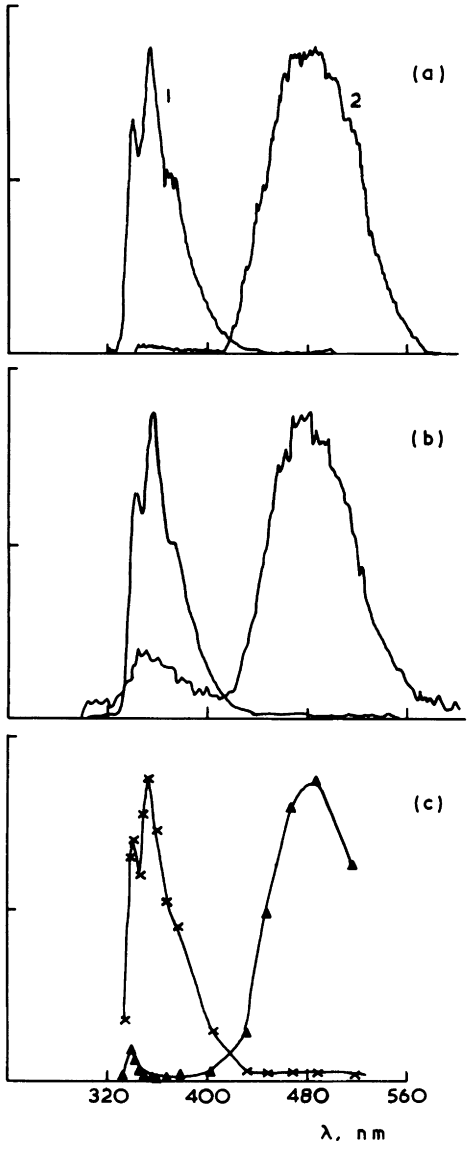

Figure 7.7 Spectra for the 1,4-dicyanonaphthalene (DCNN) 2,5-dimethyl-2,4-hexadiene (HD) exciplex system in degassed cyclohexane. (a) Spectra separated using the differential quenching technique: (1) monomer; (2) exciplex. (b) Directly measured TRES: (1) EGS $\Delta t = 2.56$ ns, $\delta t = 8.32$ ns; (2) LGS $\Delta t = 57.6$ ns, $\delta t = 19.84$ ns. (c) TRES constructed from deconvolved decays: (1) EGS $\Delta t = 1.28$ ns, $\delta t = 0.64$ ns; (2) LGS $\Delta t = 54.4$ ns, $\delta t = 0.64$ ns. Spectra are normalized to maximum intensity (cf. Fig. 7.8).

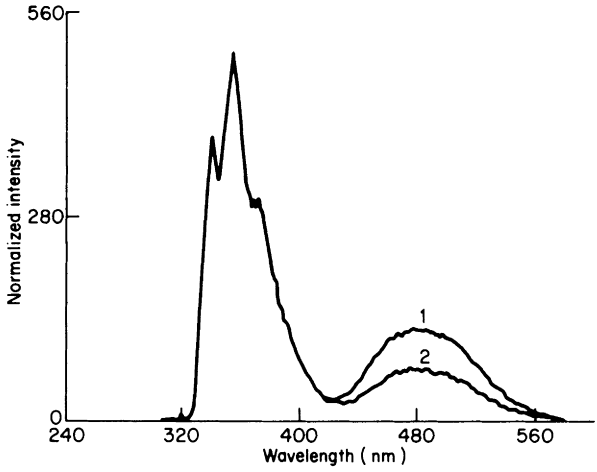

Figure 7.8 Fluorescence spectra of DCNN–HD in (1) degassed cyclohexane; (2) aerated cyclohexane.

at regular intervals, although with a stable excitation source it should not be necessary to measure an instrument response function for each decay.

These decays are now deconvolved. If the system under study is one for which the form of the decay functions, $G(t)$, cannot be specified, deconvolution is perhaps best achieved with the exponential series method (Section 6.5.8), although Fourier transform techniques (Section 6.5.6) could also be used. In the deconvolution routine CEDRIC listed in Appendix 7.A1 $G(t)$ is represented by a sum of ten exponentials and, in fitting, only the pre-exponential factors are allowed to vary (Ghiggino *et al.*, 1981). Given that TRES are usually desired to isolate short time processes from those occurring at longer times, least-squares fitting should be carried out over the whole curve rather than merely over the decay. It must be emphasized that when fitting encompasses the rising edge of the decay curve there is a heightened need to eliminate experimental distortions affecting short time data, such as scattered light and photomultiplier wavelength effects. (In the listed routines the deconvolved decay functions, $G(\lambda,t)$ are transferred to an output file and can be plotted in another routine (not listed).)

It is now necessary to normalize the decay functions to the intensity in the total fluorescence spectrum, $F(\lambda)$ or $F(\nu)$, as consideration of Fig. 7.1 and Equation 7.4 indicates. It would be equally valid to normalize to constant excitation light flux intensity. Normalization to the spectrum is usually chosen since, if this spectrum has been corrected for the wavelength dependence of the detector*, corrected TRES are generated at this stage. In

*This correction must obviously be distinguished from the wavelength correction in deconvolution.

addition, if the total spectrum is known as a function of energy, $F(\bar{\nu})$, the TRES will also be known as a function of energy, $S(\bar{\nu},t)$, a form that is most suitable for spectral calculations. As Easter *et al.* (1976) point out, the total spectrum and the decays should be measured under the same conditions of spectral bandwidth. Normalized decays, $G^0(\bar{\nu},t)$ are calculated with the equation (written in terms of wavenumber),

$$G^0(\bar{\nu}_j,t) = \frac{G(\bar{\nu}_j,t)F(\bar{\nu}_j)}{\int_0^\infty G(\bar{\nu}_j,t)\,\mathrm{d}t}, \tag{7.7}$$

Where $F(\bar{\nu}_j)$ is the intensity in the total spectrum at wavenumber, $\bar{\nu}_j$ (normalization is carried out in the routine CLAY).

There are now available from the normalized decay functions, derived for, say, m wavenumbers, the array of intensity data, $G^0(\bar{\nu}_j,t_k)$, j running from 0 to m and k from 0 to n, say. Time-resolved spectra, $S(\bar{\nu},t_k)$ may therefore be assembled for any time, t_k, merely by taking the t_k point from the decay functions ordered according to the wavenumber at which they were measured (see Equation 7.5). (This array manipulation is carried out in the routine TUMBLE. TRES may then be listed and plotted (Routine BUMBLE).)

Most of the crucial aspects of this procedure for construction of TRES have been covered in the preceding chapter on data analysis. Some further points, however, are worthy of mention. As we have already stated, spectral resolution is determined both by the number of wavelengths at which decay curves were measured and by the more usual slit width factor. Collection of the requisite number of decay curves and instrument response functions, and their ensuing deconvolution, may be both a time consuming and a tedious operation, which might be rendered less so by computer control of data collection. Laser excitation sources can reduce collection times by perhaps an order of magnitude. Time resolution in the spectrum depends on the accuracy with which deconvolution has been achieved and is probably greater than that determined by the MCA channel width.

Choice of a ten exponential function as a representation for $G(t)$ may give rise to the difficulties with spurious oscillations referred to in Section 6.5.8, so that fitting to a sum of three or four exponentials might be preferred. However, the latter may not lead to as acceptable a fit (Meech *et al.*, 1981). If a ten or fifteen exponential series is chosen we would emphasize the need to inspect the recovered decay functions graphically for oscillations. If present, they usually affect only the tails of the decay functions, at which times TRES may not be required. Normalization to the intensity in the total fluorescence spectrum will be seriously affected by these oscillations, since the area under the decay function (see Equation 7.7), if calculated analytically, will be grossly in error. The area under the function specified previously in Equation 6.57 as:

$$G(t) = \sum_{j=1}^{l} a_j \exp(-t/\gamma_j)$$

is given by:

$$\int_0^\infty G(t)\,\mathrm{d}t = \sum_{j=1}^{l} a_j \gamma_j. \tag{7.8}$$

If oscillations occur in the function after the time, say t_n, integration to infinity is obviously unacceptable. The area under the actual curve could be calculated either numerically (Equation 7.9) or analytically (Equation 7.10):

$$\int_0^{t_n} G(t)\,\mathrm{d}t = \sum_{i=1}^{n} G(t_i) \tag{7.9}$$

$$\int_0^{t_n} G(t)\,\mathrm{d}t = \sum_{j=1}^{l} a_j \gamma_j (1 - \mathrm{e}^{-t_n/\gamma_j}) \tag{7.10}$$

A "cut-off" correction would have to be devised for functions that had not decayed almost to zero by the time t_n. For data that give rise to spurious oscillations it would be preferable to reduce the number of exponentials in the fitting function to three or four and allow the γ_j also to vary. (The routine CREWS listed in Appendix 6.A2 could easily be adapted to this purpose.)

To conclude this section we refer to the spectra presented in Fig. 7.7(c), which are TRES assembled from 15 deconvolved decay functions derived using a sum of four exponentials in the fitting routine. The deconvolved functions were normalized to the intensity in the total spectrum (Fig. 7.8). The system under study was again the exciplex between 1,4-dicyanonaphthalene and 2,5-dimethyl-2,4-hexadiene in cyclohexane solution. In Fig. 7.9 are shown the deconvolved decay functions at 354 nm and 486 nm as well as the fits to the observed data. The unquenched lifetime of 1,4-dicyanonaphthalene is 1.7 ns while the lifetime of the exciplex (as derived from a measurement at very high diene concentrations) is 35 ns. These decay times are widely enough spaced so that, if convolution effects were not present, a direct measurement of TRES at early and late times would separate the monomer from the exciplex spectrum. Convolution effects however lead to the distorted exciplex spectrum shown in Fig. 7.7(b). Given that the resolution in the TRES constructed from decay curves is clearly not satisfactory, these spectra (Fig. 7.7(c)) and the true separated spectra (Fig. 7.7(a)) are quite similar, the former being obviously superior to the directly measured TRES with respect to time resolution and convolution effects.

7.6 Decay-associated Spectra

It will be clear that in a mixture of two fluorophores, time-resolved emission spectra can reproduce the emission of the separate components only if the

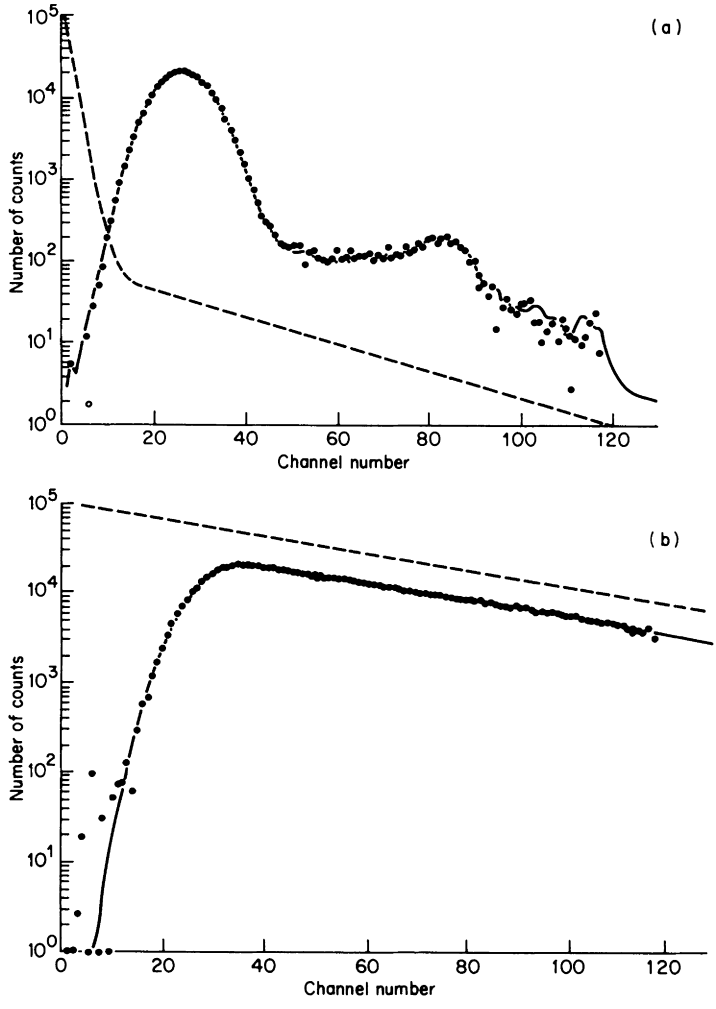

Figure 7.9 Decay data for DCNN–HD in degassed cyclohexane. ●●●, observed decays, 0.64 ns per channel; ——, reconvolved curves; - - - - -, decay functions, $G(t)$. (a) 354 nm; (b) 486 nm.

associated lifetimes are quite different. If the fluorophore lifetimes are similar a further decomposition of the TRES is required in order to obtain spectra characteristic of the individual emitting species. Such spectra have been termed decay-associated spectra and may be derived in the following way (Knutson *et al.*, 1982): suppose the observed emission results from two components, having decay times, τ_1 and τ_2, and spectral distributions $f_1(\lambda)$

and $f_2(\lambda)$. The directly measured TRES will then consist of two contributions, namely

$$S(\lambda,\Delta t_j)=f_1(\lambda)i_1(\Delta t_j)+f_2(\lambda)i_2(\Delta t_j), \tag{7.11}$$

where

$$i_i(\Delta t_j)=\int_{\Delta t_j}^{\Delta t_j+\delta t} I_i(t)\,\mathrm{d}t \tag{7.12}$$

with

$$I_i(t)=a_1\int_0^t P(t')\mathrm{e}^{-(t-t')/\tau_i}\,\mathrm{d}t' \tag{7.13}$$

Suppose now we measure TRES at two delay times Δt_1 and Δt_2. We have the equations

$$\left.\begin{aligned} S(\lambda,\Delta t_1)&=f_1(\lambda)i_1(\Delta t_1)+f_2(\lambda)i_2(\Delta t_1)\\ S(\lambda,\Delta t_2)&=f_1(\lambda)i_1(\Delta t_2)+f_2(\lambda)i_2(\Delta t_2)\end{aligned}\right\} \tag{7.14}$$

and we wish to determine $f_1(\lambda)$ and $f_2(\lambda)$. First, the decay at any wavelength is deconvolved and τ_1 and τ_2 determined. Then $i_1(\Delta t_1)$, $i_1(\Delta t_2)$, $i_2(\Delta t_1)$ and $i_2(\Delta t_2)$ are reconstructed using Equations 7.13 and 7.12, and Equation 7.14 is solved for $f_1(\lambda)$ and $f_2(\lambda)$. If deconvolved TRES are available Equation 7.11 becomes

$$S(\lambda,t_j)=f_1(\lambda)\mathrm{e}^{-t_j/\tau_1}+f_2(\lambda)\mathrm{e}^{-t_j/\tau_2}, \tag{7.15}$$

and the solution when τ_1 and τ_2 are known is even more straightforward. The extension to cases with three or more fluorophores and solution of a system of equations like Equation 7.14 by matrix inversion is equally simple. Figure 7.10 is presented as an example of two-component separation. In the upper part are shown early gated and late gated TRES, measured directly as outlined in Section 7.4, for a mixture of anthracene and 9-cyanoanthracene in ethanol. The decomposition of these spectra into decay-associated spectra is shown in the lower part where it can be seen that the individual spectra of anthracene and 9-cyanoanthracene are reproduced almost exactly. This method is simple but offers a means of elucidating vital information about many complex phenomena, notably heterogeneity in biological systems (Knuston *et al.*, 1982).

7.7 Conclusion

As the discussion in Sections 7.4 and 7.5 indicate, convolution effects are difficult to eliminate from directly measured time-resolved spectra. The result

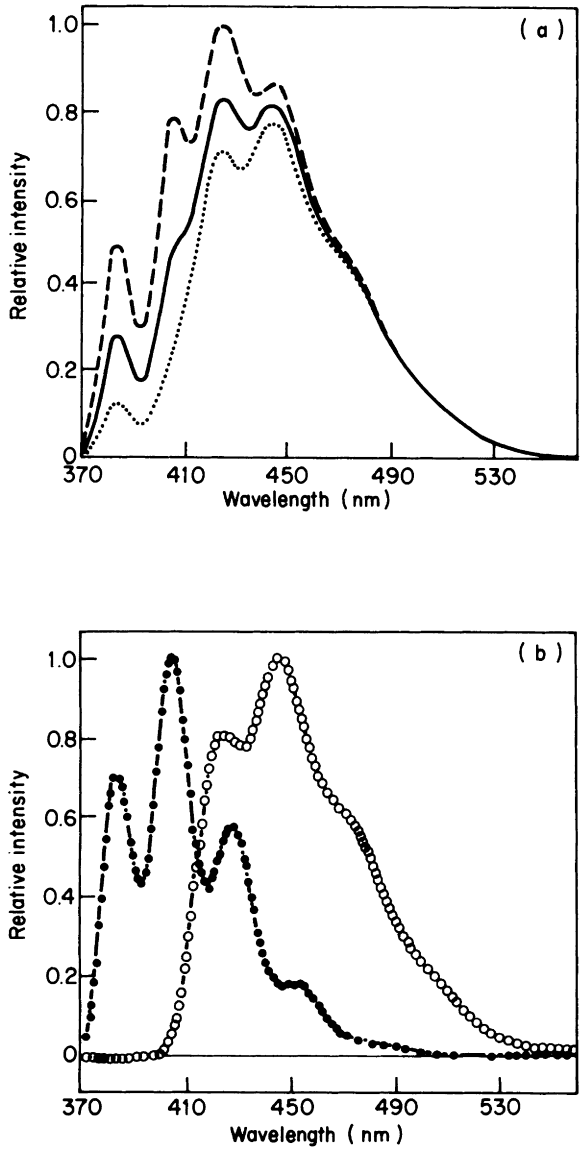

Figure 7.10 Fluorescence spectra from a mixture of anthracene and 9-cyanoanthracene in ethanol. (a) Solid curve, steady state spectrum; dashed curve, EGS, $\Delta t = 1$ channel, $\delta t = 121$ channels; dotted curve, LGS, $\Delta t = 122$ channels, $\delta t = 387$ channels. (b) Solid curves, spectra of pure anthracene and pure 9-cyanoanthracene; solid circles, normalized decay-associated spectrum of component 1; open circles, normalized decay-associated spectrum of component 2 (after Knutson *et al.*, 1982).

is that these types of TRES are, on the whole, unsuitable for quantitative analysis (Gafni *et al.*, 1977). On the other hand, measurement and analysis (in terms of six or more variable parameters) of numerous decay curves is certainly time consuming and could perhaps be quite costly as regards computer time. In addition, TRES constructed from deconvolved decays will invariably have poorer optical resolution than directly measured TRES. Furthermore, while distortions other than those arising from convolution have not been discussed in this chapter, their presence, unless corrected for, could seriously interfere with the accurate construction of spectra. If kinetic analysis related, say, to study of the phenomena referred to in Section 7.2 were to be attempted through the construction of TRES, great care would have to be taken in eliminating experimental distortions and in manipulating and analysing decay curve data. For an initial qualitative investigation of a system under study, directly measured TRES are obviously to be preferred. In short, the type of TRES required depends on the information sought.

Every TRES is merely a time slice from the total spectrum and may therefore include contributions from individual components. We have illustrated a simple means by which the latter can be extracted from the TRES. In many complex systems, for instance where the exact form of the decay law is unknown, determination of decay-associated spectra may be difficult or impossible. Nevertheless, by combining time resolution with spectral resolution in the generation of TRES it should be possible to achieve a qualitative picture and to construct a model of the physics involved upon which further analysis can be based.

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Appendix 7.A1

In this appendix are listed Fortran computer routines designed for the construction of time-resolved emission spectra from decay data. Since the routines were written for use in a fairly small Perkin-Elmer Interdata 7/32 minicomputer with a Tektronix Model 43 plotter hard-wired, the code may have features puzzling to the reader about which some brief comments might be in order. It is assumed that data on disc files are stored in unformatted files with 64 data-words to a record. In order to minimize the size of routines at run time we have divided the TRES construction operations into four main sections. Dimensioned arrays are kept to a minimum so that data are usually written to holding disc files when not being manipulated. It is also assumed that raw decay data were collected with fast repetition rate laser excitation and consequently with the TAC in "inverted" mode (Section 2.2.6). Raw decay data on disc must therefore be inverted with respect to time in the software. Data for the deconvolution routine are

assumed to be stored in a single file in the order $P_1(t)$, $I_1(t)$, $P_2(t)$, $I_2(t)$, etc., even if an instrument response was not measured for each decay curve. Finally, while we have not listed the two main programs that are designed primarily for plotting purposes we would emphasize that the deconvolved decay functions should always be inspected graphically for irregular behaviour. It should also be mentioned that in the main routine (not listed) for plotting the total fluorescence spectrum, this spectrum is corrected for the wavelength dependence of the detecting optics and converted from a wavelength to an energy spectrum if so desired.

The deconvolution routine CEDRIC, which carries out least-squares fitting to a 10 exponential function (Section 6.5.8) was tested in the following way. A 3-exponential decay curve was synthesized in the routine CRACK (Appendix 6.A1) from a real instrument response function $P_{or}(t)$ and the decay function

$$G(t) = 0.1e^{-t/1.0} + 1.0e^{-t/5.0} + 0.5e^{-t/10.0}.$$

Poisson noise was added (Section 6.8) and the data deconvolved in the routine CEDRIC with the set of (fixed) γ_j (see Equation 6.57) chosen as

$$\gamma_1 = 1.0, \qquad \gamma_2 = 2.0, \ldots, \qquad \gamma_{10} = 10.0.$$

After fitting ($\chi_v^2 = 0.97$) the values of the variable pre-exponential coefficients were, 0.17; −0.32; 0.56; −0.19; 0.45; 0.47; 0.11; −0.15; 0.005; 0.50. Next, this 10 exponential function was convolved with $P_{or}(t)$ in CRACK, noise was added and the decay curve so synthesized was deconvolved to a sum of 3 exponentials ($\chi_v^2 = 1.04$). The recovered function was

$$G(t) = 0.12e^{-t/0.99} + 0.97e^{-t/4.99} + 0.53e^{-t/9.75}$$

in good agreement with the original function. This testing procedure was carried out principally to check the mathematical operations (and possible typing errors) of the code. Some such procedure should be followed initially by the user adapting the listing to his or her own computer.

Except for the plotting sections, most of which are perhaps specific to the computer–plotter interface with which the routines were first run, COMMENT statements have been inserted to explain the operations performed.

```
LIST FX:CEDRIC.FTN

 1:   $BATCH
 2:   C  PROGRAM CEDRIC.FTN
 3:   C  DECONVOLVES SPC DECAY CURVES USING EXPONENTIAL SERIES METHOD
 4:   C  DIMENSIONED FOR 10 EXPONENTIALS AND 512 DATA POINTS
 5:         INTEGER*2 IFP(9),IFR(9)
```

```
      DIMENSION A(10),T(10)
      COMMON/AREA2/Y(512),C(512)
C  IFP : FILENAME FOR INPUT DATA
C  IFR : FILENAME FOR OUTPUT DECAY FNS AND RECONVOLVED DECAYS
C  A : PRE-EXPONENTIAL FACTORS IN EXP SERIES
C  T : TAU VALUES IN EXP SERIES(FIXED)
C  C(512) : FINAL RECONVOLVED CURVE
C  Y(512) : RAW DECAY CURVE DATA AND CORRECTED DATA
C  NCU : NUMBER OF DECAY CURVES(WAVELENGTHS)
C  NC : NUMBER OF CHANNELS
C  NPAR : NUMBER OF FITTING PARAMETERS(10)
C  FACT : NANOSECONDS/CHANNEL
C
C  IN REPLY TO QUESTIONS ASKING FOR YES OR NO,
C  GIVE 1 FOR YES, 0 FOR NO
C
      WRITE(4,2)
2     FORMAT(1X,' HOW MANY CURVES:(I3) ?')
      READ(5,3) NCU
3     FORMAT(I3)
      WRITE(4,4)
4     FORMAT(1X,' HOW MANY CHANNELS:(I3) ?')
      READ(5,3) NC
      WRITE(4,5)
5     FORMAT(1X,' HOW MANY NANOSECONDS/CHANNEL:(F7.3) ?')
      READ(5,6) FACT
6     FORMAT(F7.3)
71    WRITE(4,7)
7     FORMAT(1X,' WHAT IS THE FILENAME FOR THE DECAY CURVES ?')
      READ(5,8) IFP
8     FORMAT(9A2)
C  INPUT FILE IS OPENED ON LOGICAL DEVICE 1
      CALL OPENW(1,IFP,7,0,0,I)
      IF(I.NE.0) GO TO 71
14    WRITE(4,13)
13    FORMAT(1X,' WHAT IS THE FILENAME FOR THE RESULTS ?')
      READ(5,8) IFR
C  OUTPUT FILE IS MADE AND THEN OPENED ON LOGICAL DEVICE 2
      CALL CFILW(IFR,2,256,1,1,0,0,I)
      IF(I.NE.0) GO TO 14
      CALL OPENW(2,IFR,7,0,0,I)
      IF(I.NE.0) GO TO 14
C  FILE RECORDS MUST BE WRITTEN SEQUENTIALLY. HENCE
C  SINCE ALL THE INFORMATION TO BE WRITTEN ON RECORD 1
C  IS NOT YET KNOWN A DUMMY IS WRITTEN. IT WILL BE
C  OVERWRITTEN LATER
      WRITE(2,REC=1) FACT
      NP=NC/2
      NPAR=10
      IK=NC/64
      IK1=IK+1
      KJI=0
C  THE DECAYS ARE ANALYSED ONE BY ONE
```

Appendix 7.A1 (*continued*)

```
 59:          DO 1001 JI=1,NCU
-60:          CALL RSET(JI,IK,NC,NCU,FACT,NP,N1,N2,SB)
-61:          NI=0
-62:    C     IF THE FLAG NI IS SET=1 IN THE ROUTINE CALC10 THE FIT
-63:    C     HAS FAILED AND THE RESULTS ARE REJECTED
-64:    C
-65:    C     FOR THE DATA AFTER THE FIRST SET THE STARTING VALUES FOR
-66:    C     THE PARAMETERS ARE TAKEN AS THE RECOVERED VALUES FROM THE
-67:    C     PREVIOUS SET
-68:          IF(JI.GT.1) GO TO 10
-69:    111   NI=0
-70:    C     A SET OF 10 TAU VALUES IS CHOSEN.THEY ARE NOT VARIED IN
-71:    C     THE FIT OR FROM DATA SET TO ANOTHER.
-72:    C     CHOOSE TAU VALUES SUCH THAT FACT/TAU < 80
-73:          WRITE(4,9)
 74:    9     FORMAT(1X,' CHOOSE THE 10 TAU VALUES:(10F6.2)')
-75:          READ(5,12)(T(J),J=1,NPAR)
-76:    12    FORMAT(10F6.2)
-77:    C     FOR THE FIRST DECAY STARTING VALUES FOR THE VARIABLE A FACTORS
-78:    C     ARE CHOSEN. IT IS RECOMMENDED TO SET THEM ALL AT THE SAME
-79:    C     VALUE(E.G. 0.1) BUT ALTERNATELY +VE AND -VE
-80:          WRITE(4,11)
-81:    11    FORMAT(1X,' GIVE STARTING VALUES FOR THE A FACTORS:(10F6.2)')
-82:          READ(5,12)(A(J),J=1,NPAR)
-83:    C
 84:    C     CURVE FITTING
-85:    10    CALL CALC10(FACT,NC,N1,N2,NI,NPAR,A,T,SB)
-86:    C     IF THE FITTING HAS FAILED TRY NEW STARTING VALUES
-87:          IF(NI.EQ.1) GO TO 111
-88:    C     THE QUALITY OF THE FIT IS JUDGED BY THE VALUE OF THE
-89:    C     REDUCED CHI-SQUARE AND THE DATA ACCEPTED,REJECTED OR
-90:    C     RE-ANALYSED
-91:          WRITE(4,15)
-92:    15    FORMAT(1X,' DO YOU WANT TO INCLUDE THIS RESULT ?')
-93:          READ(5,16) INC
-94:    16    FORMAT(I1)
-95:          IF(INC.EQ.1) GO TO 20
-96:          WRITE(4,17)
-97:    17    FORMAT(1X,' DO YOU WANT TO RE-ANALYSE THIS CURVE ?')
-98:          READ(5,16) IRE
-99:          IF(IRE.EQ.1) GO TO 111
-100:         GO TO 1001
-101:   C     THE ACCEPTED DECAY FNS AND RECONVOLVED CURVES ARE WRITTEN
-102:   C     TO THE OUTPUT FILE TOGETHER WITH SOME INFORMATION TO BE
-103:   C     USED BY SUBSEQUENT ROUTINES
-104:   20    WRITE(4,21)
-105:   21    FORMAT(1X,' DO YOU WANT A PLOT ?')
-106:         READ(5,16) IP
-107:         WRITE(4,22)
-108:   22    FORMAT(1X,' WHAT IS THE WAVELENGTH:(F7.3) ?')
-109:         READ(5,6) Z
-110:         M=(KJI*IK1)+2
```

```
-111:        KJI=KJI+1
-112:        WRITE(2,REC=M)SB,JI,IP,Z,N1,N2,(A(J),J=1,NPAR),(T(J),J=1,NPAR)
-113:        DO 18 J=1,IK
-114:        KJ3=M+J
-115: 18     WRITE(2,REC=KJ3)(C(I),I=(J-1)*64+1,J*64)
-116: 1001   CONTINUE
-117: C   THE OUTPUT FILE IS NOW REWOUND AND DATA CHARACTERISING
-118: C   THE COMPLETE DATA SET IS WRITTEN ON RECORD 1
-119:        REWIND 2
-120:        WRITE(2,REC=1)KJI,NC,FACT,NPAR
-121: C   KJI IS A COUNT OF THE NUMBER OF CURVES ACCEPTED
-122: C
-123: C   INPUT AND OUTPUT FILES ARE CLOSED
-124:        CALL CLOSE(1,I)
-125:        CALL CLOSE(2,I)
-126:        STOP
-127:        END
-128: C   SUBROUTINE RSET
-129: C   READS IN THE RAW DATA,INVERTS THE CURVES,CALCULATES AND
-130: C   SUBTRACTS BACKGROUNDS AND DETERMINES CHANNEL FITTING RANGE
-131: C   JI : DECAY CURVE NUMBER
-132: C   IK : NUMBER OF RECORDS ON THE FILE OCCUPIED BY ONE CURVE
-133: C   NP : NC/2 : NEEDED FOR INVERTING CURVES
-134: C   N1,N2 : LIMITS OF CHANNEL FITTING RANGE
-135: C   SB : BACKGROUND NOISE(COUNTS/CHANNEL) IN DECAY CURVE
-136: C   P(512) : PUMP PULSE PROFILE
-137: C
-138:        SUBROUTINE RSET(JI,IK,NC,NCU,FACT,NP,N1,N2,SB)
-139:        COMMON/AREA1/P(512)
-140:        COMMON/AREA2/Y(512),C(512)
-141: C   ON THE INPUT FILE THE DATA ARE ARRANGED IN RECORDS OF
-142: C   LENGTH 64(UNFORMATTED DATA). THE CURVES FOLLOW EACH OTHER
-143: C   IN SEQUENCE P(1,NC),Y(1,NC),P(2,NC),Y(2,NC)..P(NCU,NC),Y(NCU,NC)
-144:        KI=1+(JI-1)*IK*2
-145:        KJ=KI+IK-1
-146:        MI=KJ+1
-147:        MJ=MI+IK-1
-148:        IM=IK*2
-149:        DO 911 J=KI,KJ
-150:        JM=MOD(J,IM)
-151:        READ(1,REC=J)(P(I),I=(JM-1)*64+1,JM*64)
-152: 911    CONTINUE
-153:        DO 912 J=MI,MJ
-154:        IK2=J-IK
-155:        JM=MOD(IK2,IM)
-156:        READ(1,REC=J)(Y(I),I=(JM-1)*64+1,JM*64)
-157: 912    CONTINUE
-158: C   THE DATA ARE ASSUMED TO BE STORED IN THE MCA WITH
-159: C   TIME INCREASING FROM RIGHT TO LEFT,AS THEY WOULD BE
-160: C   E.G. FOR MHZ EXCITATION RATE
-161:        DO 3 I=1,NP
-162:        K=NC+1-I
-163:        R=P(K)
```

Appendix 7.A1 (*continued*)

```
      S=Y(K)
      P(K)=P(I)
      Y(K)=Y(I)
      P(I)=R
      Y(I)=S
3     CONTINUE
C  THE BACKGROUNDS ARE CALCULATED ON THE ASSUMPTION THAT
C  THE FIRST 21 CHANNELS IN EACH CURVE CONTAIN ONLY
C  DARK COUNTS
      RB=0.0
      SB=0.0
      DO 91 I=2,21
      RB=RB+P(I)
      SB=SB+Y(I)
91    CONTINUE
      RB=RB/20.0
      SB=SB/20.0
C  THE CURVES ARE CORRECTED FOR THE DARK BACKGROUND
      P(1)=RB
      Y(1)=SB
      DO 5 I=1,NC
      P(I)=P(I)-RB
      IF(P(I).LT.0.0) P(I)=0.0
      Y(I)=Y(I)-SB
      IF(Y(I).LT.0.0) Y(I)=0.0
5     CONTINUE
C  THE CHANNEL FITTING RANGE IS CHOSEN FROM Y(N1)=1000
C  TO Y(N2)=75(OR Y(NC-12) IF Y(NC)>75)
      DO 6 I=1,NC
      IF(Y(I).LT.1000.) GO TO 6
      N1=I
      GO TO 7
6     CONTINUE
7     DO 8 I=N1,NC
      IF(Y(I).GT.75.0) GO TO 8
      N2=I
      GO TO 9
8     CONTINUE
      N2=NC-12
9     RETURN
      END
C
C  SUBROUTINE CALC10
C  CURVE FITTING TO A 10 EXPONENTIAL SERIES WITH VARIABLE
C  A FACTORS
C  FOLLOWS CLOSELY BEVINGTON P.237
      SUBROUTINE CALC10(FACT,NC,N1,N2,NI,NPAR,A,T,SB)
      DIMENSION ALPHA(10,10),BETA(10),B(10),ARRAY(10,10),A(10),T(10)
      COMMON/AREA1/P(512)
      COMMON/AREA2/Y(512),C(512)
      COMMON/AREA6/D(10,512)
C  ALPHA(10,10) : MATRIX OF PRODUCTS OF PARTIAL DERIVATIVES
```

```
-216: C                  ALSO CALLED THE ERROR MATRIX
-217: C  ARRAY(10,10) : ERROR MATRIX ADJUSTED FOR EASE OF INVERSION
-218: C  ALPHA AND ARRAY ALSO ARE USED FOR THE INVERTED MATRIX
-219: C  BETA(10) : ARRAY OF THE PRODUCTS (OBS-CALC)*PART.DERIV.
-220: C  D(10,512) : ARRAY OF PARTIAL DERIVATIVES
-221: C  B(10) : STORE FOR A VALUES
-222: C
-223: C  INITIAL CHI-SQUARE AND PARTIAL DERIVATIVES ARE CALCULATED
-224:       CALL FUNC10(N1,N2,FACT,CHISQR,NC,A,T,SB,1,NPAR)
-225:       WRITE(4,671) CHISQR
-226: 671   FORMAT(1X,' CHISQR = ',E14.6)
-227:       JCOUNT=0
 228: C  JCOUNT IS THE NUMBER OF RECONVOLUTIONS THAT PRODUCE A
-229: C  LOWER VALUE OF CHISQR. IT IS NOT ALLOWED TO EXCEED 30
-230:       FLAMDA=0.05
 231: C  FOR THE MEANING OF FLAMDA SEE TEXT (SECTION 6.5.7)
-232: 80    JCOUNT=JCOUNT+1
-233: C  CALCULATE THE ALPHA AND BETA ARRAYS
-234:       DO 91 J=1,NPAR
-235:       BETA(J)=0.0
-236:       DO 91 K=1,J
-237: 91    ALPHA(J,K)=0.0
 238:       DO 110 I=N1,N2
-239: C  THE FIT IS POISSON WEIGHTED WITH THE RECIPROCALS OF
 240: C  THE RAW DATA COUNTS
-241:       W=Y(I)+SB
-242:       IF(W.LT.1.0) W=1.0
-243:       W=1.0/W
-244:       DO 100 J=1,NPAR
-245:       BETA(J)=BETA(J)+W*(Y(I)-C(I))*D(J,I)
-246:       DO 100 K=1,J
-247: 100   ALPHA(J,K)=ALPHA(J,K)+W*D(J,I)*D(K,I)
-248: 110   CONTINUE
-249: C  THE ALPHA MATRIX IS SYMMETRICAL
-250:       DO 150 J=1,NPAR
-251:       DO 150 K=1,J
-252: 150   ALPHA(K,J)=ALPHA(J,K)
-253:       CHISQ1=CHISQR
-254:       ICOUNT=0
 255: C  ICOUNT IS THE NUMBER OF TIMES THE ERROR MATRIX(WITH
 256: C  DIFFERENT VALUES OF FLAMDA EACH TIME) IS INVERTED
 257: C  IT NOT ALLOWED TO EXCEED 6
 258: C
-259: C  THE ERROR MATRIX IS ADJUSTED FOR EASE OF INVERSION
 260: 160   DO 174 J=1,NPAR
 261:       DO 173 K=1,NPAR
 262:       SROOT=SQRT(ALPHA(J,J)*ALPHA(K,K))
-263:       IF(SROOT.EQ.0.0) GO TO 561
 264: 173   ARRAY(J,K)=ALPHA(J,K)/SROOT
-265: C  APPLICATION OF THE MARQUARDT METHOD
 266: 174   ARRAY(J,J)=1.0+FLAMDA
 267:       GO TO 562
 268: C  HERE THE FITTING HAS FAILED. SET NI=1 AND TRY AGAIN
```

Appendix 7.A1 (*continued*)

```
 269: 561   WRITE(4,563)
 270: 563   FORMAT(1X,' DIVISION BY 0')
 271:       NI=1
 272:       GO TO 901
 273: C  THE ADJUSTED ERROR MATRIX IS INVERTED
 274: 562   CALL MATINV(ARRAY,NPAR,DET)
 275:       ICOUNT=ICOUNT+1
 276: C  INCREMENTS IN THE FITTING PARAMETERS ARE CALCULATED AND
 277: C  ADDED TO THE CURRENT ESTIMATES OF THE PARAMETERS
 278: C  THE CALCULATION TAKES ACCOUNT OF THE ADJUSTMENT PERFORMED
 279: C  IN THE DO LOOP 173
 280:       DO 201 J=1,NPAR
 281:       B(J)=A(J)
 282:       DO 201 K=1,NPAR
 283:       B(J)=B(J)+BETA(K)*ARRAY(J,K)/SQRT(ALPHA(J,J)*ALPHA(K,K))
 284: 201   CONTINUE
 285: C  WITH THE RECALCULATED PARAMETERS A NEW VALUE OF
 286: C  CHI-SQUARE AND PARTIAL DERIVATIVES ARE CALCULATED
 287:       CALL FUNC10(N1,N2,FACT,CHISQR,NC,B,T,SB,1,NPAR)
 288: C  IF NEW CHI-SQUARE IS < OLD KEEP CURRENT PARAMETER VALUES
 289: C  IF OLD < NEW, RE-INVERT ERROR MATRIX ACCORDING TO
 290: C  MARQUARDT METHOD
 291:       IF(CHISQ1-CHISQR) 302,303,303
 292: 303   DO 304 J=1,NPAR
 293: 304   A(J)=B(J)
 294:       IF(JCOUNT.EQ.30) GO TO 560
 295: C  CHECK FOR CONVERGENCE
 296:       IF((CHISQ1-CHISQR).LT.0.000001) GO TO 560
 297: C  THE SEARCH HAS NOW REACHED A POINT ON THE HYPERSURFACE
 298: C  CLOSER TO THE MINIMUM. SO REDUCE FLAMDA AND RE-CALCULATE
 299: C  PARAMETER INCREMENTS
 300:       FLAMDA=FLAMDA/10.0
 301:       GO TO 80
 302: C
 303: 302   FLAMDA=FLAMDA*10.0
 304:       IF(ICOUNT.EQ.5) GO TO 305
 305:       IF(ICOUNT.EQ.6.AND.JCOUNT.LE.2) GO TO 361
 306:       IF(ICOUNT.EQ.6.AND.JCOUNT.GT.2) GO TO 330
 307:       GO TO 160
 308: C  IF NO MORE THAN 2 RECONVOLUTIONS HAVE BEEN PERFORMED
 309: C  THE FIT IS CONSIDERED UNSUCCESSFUL
 310: 361   NI=1
 311:       GO TO 901
 312: 305   FLAMDA=FLAMDA/10**6
 313:       GO TO 160
 314: 330   CHISQR=CHISQ1
 315: 560   WRITE(4,622)JCOUNT,N1,N2,CHISQR
 316: 622   FORMAT(1X,I3,' CONVOLUTIONS',/,1X,' FITTING BETWEEN',
 317:      1 ' CHANNELS ',I3,' AND ',I3,2X,' CHISQR = ',E14.5,/,
 318:      1 7X,' A FACTORS',11X,'TAU')
 319:       DO 623 J=1,NPAR
 320:       WRITE(4,624) A(J),T(J)
```

```
 321: 623   CONTINUE
 322: 624   FORMAT(4X,E14.6,5X,F10.4)
 323: C  RECALCULATE FITTED CURVE FOR BEST PARAMETERS
 324:       CALL FUNC10(N1,N2,FACT,CHISQR,NC,A,T,SB,0,NPAR)
 325: 901   RETURN
 326:       END
 327: C
 328: C  SUBROUTINE FUNC10
 329: C  CALCULATES THE CONVOLUTION PRODUCT BETWEEN P(NC) AND
 330: C  THE 10 EXPONENTIAL SERIES. ALSO CALCULATES THE PARTIAL
 331: C  DERIVATIVES.
 332:       SUBROUTINE FUNC10(N1,N2,FACT,CHISQR,NC,A,T,SB,ID,NP)
 333:       DIMENSION A(10),T(10),CC(10)
 334:       COMMON/AREA1/P(512)
 335:       COMMON/AREA2/Y(512),C(512)
 336:       COMMON/AREA6/D(10,512)
 337: C
 338: C  ID IS A FLAG:=1 CALCULATE DERIVS,=0 DO NOT
 339:       IF(ID.EQ.0) GO TO 303
 340:       DO 1 J=1,NP
 341:       DO 1 I=N1,N2
 342:       D(J,I)=0.0
 343: 1     CONTINUE
 344: C  THE INTEGRATION IS PERFORMED USING THE TRAPEZOIDAL RULE
 345: 303   CMULT=FACT*0.5
 346:       C(1)=0.0
 347:       DO 304 J=I,NP
 348:       CC(J)=CMULT*A(J)*P(1)
 349: 304   C(1)=C(1)+CC(J)
 350:       DO 2 I=2,N2
 351:       C(I)=0.0
 352:       DO 21 J=1,NP
 353:       CC(J)=EXP(-FACT/T(J))*CC(J)+A(J)*CMULT*(P(I-1)
 354:      1 *EXP(-FACT/T(J))+P(I))
 355:       IF(ID.EQ.0) GO TO 21
 356:       D(J,I)=CC(J)/A(J)
 357: 21    C(I)=C(I)+CC(J)
 358: 2     CONTINUE
 359: C  THE REDUCED CHI-SQUARE IS CALCULATED
 360:       NPTS=N2-N1+1
 361:       NFREE=NPTS-10
 362:       FREE=FLOAT(NFREE)
 363:       CHISQ=0.0
 364:       DO 3 I=N1,N2
 365: C  THE FITTING IS POISSON WEIGHTED WITH THE RECIPROCALS OF
 366: C  THE RAW DATA POINTS
 367:       W=Y(I)+SB
 368:       IF(W.LT.1.0) W=1.0
 369:       W=1.0/W
 370:       CHISQ=CHISQ+W*(Y(I)-C(I))*(Y(I)-C(I))
 371: 3     CONTINUE
 372:       CHISQR=CHISQ/FREE
 373:       RETURN
```

Appendix 7.A1 (*continued*)

```
374:       END
375: C
376: C  SUBROUTINE MATINV
377: C  MATRIX INVERSION ROUTINE TAKEN DIRECT FROM BEVINGTON
378: C  FOR COMMENTS SEE BEVINGTON P.301
379:       SUBROUTINE MATINV(ARRAY,NORDER,DET)
380:       DIMENSION ARRAY(NORDER,NORDER)
381:       DIMENSION IK(10),JK(10)
382:       DET=1.0
383:       DO 100 K=1,NORDER
384:       AMAX=0.0
385: 21    DO 30 I=K,NORDER
386:       DO 30 J=K,NORDER
387:       IF(ABS(AMAX)-ABS(ARRAY(I,J))) 24,24,30
388: 24    AMAX=ARRAY(I,J)
389:       IK(K)=I
390:       JK(K)=J
391: 30    CONTINUE
392:       IF(AMAX) 41,32,41
393: 32    DET=0.0
394:       GO TO 140
395: 41    I=IK(K)
396:       IF(I-K) 21,51,43
397: 43    DO 50 J=1,NORDER
398:       SAVE=ARRAY(K,J)
399:       ARRAY(K,J)=ARRAY(I,J)
400: 50    ARRAY(I,J)=-SAVE
401: 51    J=JK(K)
402:       IF(J-K) 21,61,53
403: 53    DO 60 I=1,NORDER
404:       SAVE=ARRAY(I,K)
405:       ARRAY(I,K)=ARRAY(I,J)
406: 60    ARRAY(I,J)=-SAVE
407: 61    DO 70 I=1,NORDER
408:       IF(I-K) 63,70,63
409: 63    ARRAY(I,K)=-ARRAY(I,K)/AMAX
410: 70    CONTINUE
411:       DO 80 I=1,NORDER
412:       DO 80 J=1,NORDER
413:       IF(I-K) 74,80,74
414: 74    IF(J-K) 75,80,75
415: 75    ARRAY(I,J)=ARRAY(I,J)+ARRAY(I,K)*ARRAY(K,J)
416: 80    CONTINUE
417:       DO 90 J=1,NORDER
418:       IF(J-K) 83,90,83
419: 83    ARRAY(K,J)=ARRAY(K,J)/AMAX
420: 90    CONTINUE
421:       ARRAY(K,K)=1.0/AMAX
422: 100   DET=DET*AMAX
423:       DO 130 L=1,NORDER
424:       K=NORDER-L+1
425:       J=IK(K)
```

```
-426:        IF(J-K) 111,111,105
-427: 105    DO 110 I=1,NORDER
-428:        SAVE=ARRAY(I,K)
-429:        ARRAY(I,K)=-ARRAY(I,J)
-430: 110    ARRAY(I,J)=SAVE
-431: 111    I=JK(K)
-432:        IF(I-K) 130,130,113
-433: 113    DO 120 J=1,NORDER
-434:        SAVE=ARRAY(K,J)
-435:        ARRAY(K,J)=-ARRAY(I,J)
-436: 120    ARRAY(I,J)=SAVE
-437: 130    CONTINUE
-438: 140    RETURN
-439:        END
-440: $BEND


 LIST FX:CLAY.FTN

-1:    $BATCH
-2:    C  PROGRAM CLAY.FTN
-3:    C  CONSTRUCTS TIME-RESOLVED SPECTRA FROM DECONVOLVED
-4:    C  DECAY FNS AND A TOTAL FLUORESCENCE SPECTRUM
-5:          INTEGER*2 IFR(9),IFC(9),IGRAF(3)
-6:          DIMENSION MIXER(64),S(512)
-7:          COMMON/AREA7/A(20)
-8:          COMMON/AREA8/G(512)
-9:          COMMON/AREA1/IFC
-10:         COMMON/AREA2/TRES(64),TLAM(64)
-11:         DATA IGRAF/'GRAF: '/
-12:   C  IFR : NAME OF FILE CONTAINING THE DECAY FNS
-13:   C         I.E. OUTPUT FILE FROM CEDRIC
-14:   C  IFC : NAME OF FILE CONTAINING (CORRECTED) SPECTRUM
-15:   C         ALSO USED FOR HOLDING FILES
-16:   C  IGRAF : NAME USED TO IDENTIFY THE PLOTTER
-17:   C  MIXER(64) : ARRAY CONTAINING CHANNEL NOS. OF TRES TO BE
-18:   C                 PLOTTED
-19:   C  S(512) : (CORRECTED) SPECTRUM DATA POINTS
-20:   C  A(10),T(10) : A FACTORS AND TAU VALUES OF DECAY FNS
-21:   C  G(512) : ARRAY CONTAINING DECONVOLVED DECAY CURVE
-22:   C  TRES(64) : ARRAY CONTAINING THE TIME-RESOLVED SPECTRM
-23:   C  TLAM(64) : WAVELENGTHS OF THE POINTS IN TRES
-24:   C
-25:   C  TOTAL SPECTRUM READ IN FROM FILE ON LOGICAL DEVICE 2
-26:   7     WRITE(4,6)
-27:   6     FORMAT(1X,' TYPE CORRECTED SPECTRUM FILENAME')
-28:         READ(5,3) IFC
-29:         CALL OPENW(2,IFC,7,0,0,I)
-30:         IF(I.NE.0) GO TO 7
-31:   C  ON RECORD 1 IN SPECTRUM FILE IS THE INFORMATION:
-32:   C  NS : NUMBER OF POINTS
-33:   C  FSLAM : WAVELENGTH OF FIRST POINT
-34:   C  CHIPPY : INCREASE IN WAVELENGTH PER DATA POINT
```

Appendix 7.A1 (*continued*)

```
-35:  C  LUD : FLAG. IF =1 TRES IN WAVELENGTH. =2 IN ENERGY
-36:        READ(2,REC=1) NS,FSLAM,CHIPPY,LUD
-37:        IM=NS/64+1
-38:        DO 8 J=2,IM
-39:        READ(2,REC=J)(S(I),I=((J-2)*64+1),(J-1)*64)
-40:  8     CONTINUE
-41:        CALL CLOSE(2,I)
-42:  C  OPEN DECAY FNS FILE ON LOGICAL DEVICE 1
-43:  1     WRITE(4,2)
-44:  2     FORMAT(1X,' TYPE DECONVOLUTION RESULTS FILENAME')
-45:        READ(5,3) IFR
-46:  3     FORMAT(9A2)
-47:        CALL OPENW(1,IFR,7,0,0,I)
-48:        IF(I.NE.0) GO TO 1
-49:        READ(1,REC=1) KJI,NC,FACT,NPAR
-50:        NP2=NPAR*2
-51:  C  IK : NUMBER OF RECORDS ON THE OUTPUT FILE THAT WILL BE
-52:  C       OCCUPIED BY A DECAY CURVE
-53:        IK=NC/64
-54:        IK1=IK+1
-55:  C  TO REDUCE THE NUMBER OF ARRAYS,THE NORMALISED DECAY CURVES
-56:  C  ARE STORED IN A HOLDING FILE. IT IS NOW MADE AND OPENED ON
-57:  C  LOGICAL DEVICE 3
-58:  9     WRITE(4,10)
-59:  10    FORMAT(1X,' TYPE FILENAME FOR DECAYS NORMALISED TO THE TFS')
-60:        READ(5,3) IFC
-61:        CALL CFILW(IFC,2,256,1,1,0,0,I)
-62:        IF(I.NE.0) GO TO 9
-63:        CALL OPENW(3,IFC,7,0,0,I)
-64:        IF(I.NE.0) GO TO 9
-65:  C  THE DECAY FNS ARE READ IN ONE AT A TIME
-66:        DO 1002 JN=1,KJI
-67:        KJ2=(JN-1)*(IK+1)+2
-68:  C  FOR THE INFORMATION IN THIS FILE SEE CEDRIC
-69:        READ(1,REC=KJ2)SB,JI,IPL,FLAM,NQ,NW,(A(J),J=1,NP2)
-70:        TLAM(JN)=FLAM
-71:        IF(LUD.EQ.1) GO TO 501
-72:  C  IF LUD=2 CONVERT WAVELENGTHS TO ENERGY
-73:        TLAM(JN)=100000./FLAM
-74:  C  CALCULATE THE DECAY CURVES
-75:  501   CALL DECFUN(NC,FACT,NPAR)
-76:  C  THE THEORETICAL AREA UNDER THE DECAY CURVE IS CALCULATED
-77:  C  IF G(T) SHOWS OSCILLATIONS AND PROVIDED IT HAS DECAYED
-78:  C  TO 0 AT LAST CHANNEL REPLACE THIS CALCULATION BY A
-79:  C  CALCULATION OF THE ACTUAL AREA(SUM OF POINTS)
-80:        ARG=0.0
-81:        DO 4 LA=1,NPAR
-82:        LA2=LA+NPAR
-83:  4     ARG=ARG+A(LA)*A(LA2)
-84:  C  NORMALISE THE DECAY TO THE INTENSITY IN THE SPECTRUM
-85:        DO 5 I=1,NC
-86:  5     G(I)=G(I)/ARG
```

```
 87:  C  INTERPOLATE THE SPECTRUM TO FIND THE INTENSITY AT THE
 88:  C  WAVELENGTH AT WHICH THE DECAY CURVE WAS MEASURED
 89:        DO 68 J=1,NS
 90:        FJ=FLOAT(J)
 91:        SLAM=FSLAM+(FJ-1.)*CHIPPY
 92:        R=FLAM-SLAM
 93:        IF(R.LT.0.0) GO TO 69
 94:  68    CONTINUE
 95:  69    P1F=(S(J)-S(J-1))/CHIPPY
 96:        PF=(P1F*(FLAM-SLAM+CHIPPY))+S(J-1)
 97:        DO 71 L=1,NC
 98:  71    G(L)=G(L)*PF
 99:  C  WRITE THE NORMALISED DECAY CURVES TO THE HOLDING FILE
100:        DO 11 J=1,IK
101:        KJ3=(JN-1)*IK+J
102:  11    WRITE(3,REC=KJ3)(G(I),I=(J-1)*64+1,J*64)
103: 1002  CONTINUE
104:  C  WHEN ALL THE NORMALISED DECAY CURVES HAVE BEEN WRITTEN
105:  C  TO THE FILE CLOSE THE HOLDING FILE AND THE DECAY FNS FILE
106:        CALL CLOSE(1,I)
107:        CALL CLOSE(3,I)
108:  C  THE TRES ARE CALCULATED
109:  C  KIK : FLAG. IF =0 NORMALISED DECAY CURVES FILE COULD NOT
110:  C        BE OPENED IN SUBROUTINE TUMBLE
111:        CALL TUMBLE(KIK,IK,KJI,LUD)
112:        IF(KIK.NE.0) GO TO 1003
113:  C  TRES CAN BE LISTED
114:        WRITE(4,12)
115:  12    FORMAT(1X,' DO YOU WANT TO LIST SOME TRES ?')
116:        READ(5,13) IL
117:  13    FORMAT(I1)
118:        IF(IL.EQ.0) GO TO 101
119:        WRITE(4,14)
120:  14    FORMAT(1X,' HOW MANY:(I2) ?')
121:        READ(5,15) NTR
122:  15    FORMAT(I2)
123:  C  WAVELENGTHS OR WAVENUMBERS ARRANGED IN GROUPS OF 8
124:        CALL RANCH(KJI,8,L1,L2)
125:        IF(LUD.EQ.1) GO TO 502
126:  C  LUD INDICATES WAVELENGTHS OR WAVENUMBERS
127:        WRITE(4,161)
128: 161   FORMAT(20X,' WAVENUMBERS * 0.01')
129:        GO TO 503
130: 502   WRITE(4,16)
131:  16    FORMAT(30X,' WAVELENGTHS')
132: 503   IF(L1.EQ.0) GO TO 20
133:        DO 17 J=1,L1
134:        J1=8*J
135:        J2=(J-1)*8+1
136:        WRITE(4,18)(TLAM(K),K=J2,J1)
137:  17    CONTINUE
138:  20    L3=L1*8
139:        DO 19 J=1,L2
```

Appendix 7.A1 (*continued*)

```
      L4=L3+J
      WRITE(4,24) TLAM(L4)
19    CONTINUE
18    FORMAT(1X,8(F6.2,2X))
24    FORMAT(1X,F6.2)
35    FORMAT(I3)
C  SPECTRUM POINTS ARRANGED IN GROUPS OF 5
      CALL RANCH(KJI,5,L1,L2)
      L3=5*L1
      DO 25 JL=1,NTR
      WRITE(4,34) JL
34    FORMAT(1X,' TYPE CHANNEL NUMBER OF TRES',I2,1X,':(I3)')
      READ(5,35)LJ
C  TRES ARE READ IN FROM HOLDING FILE
      READ(2,REC=LJ)(TRES(K),K=1,KJI)
      IF(L1.EQ.0) GO TO 27
      DO 28 JK=1,L1
      J1=5*JK
      J2=(JK-1)*5+1
      WRITE(4,29)(TRES(K1),K1=J2,J1)
28    CONTINUE
27    DO 30 J=1,L2
      L4=L3+J
      WRITE(4,31) TRES(L4)
30    CONTINUE
29    FORMAT(1X,5(E12.5,1X))
31    FORMAT(1X,E12.5)
25    CONTINUE
C  TRES CAN BE PLOTTED
101   WRITE(4,32)
32    FORMAT(1X,' DO YOU WANT TO PLOT SOME TRES ?')
      READ(5,13) IL
      IF(IL.EQ.0) GO TO 1003
      WRITE(4,14)
      READ(5,15) NUM
      WRITE(4,26)
26    FORMAT(1X,' TYPE CHANNEL NO.S OF TRES TO BE PLOTTED:(I3)')
      DO 33 JL=1,NUM
33    READ(5,35) MIXER(JL)
C  THE PLOTTER IS OPENED FOR PLOTTING
36    CALL OPENW(3,IGRAF,7,0,0,I)
      IF(I.EQ.0) GO TO 37
      WRITE(4,38)
38    FORMAT(1X,' THE PLOTTER IS NOT READY.WHEN IT IS TYPE 1')
      READ(5,13) IME
      GO TO 36
C  PLOTTING
37    CALL BUMBLE(NUM,MIXER,KJI,NS,FSLAM,CHIPPY,LUD)
C  CLOSE PLOTTER AND HOLDING FILE
      CALL CLOSE(3,I)
      GO TO 101
1003  CALL CLOSE(2,I)
```

```
      STOP
      END
C
C  SUBROUTINE DECFUN
C  CALCULATES A SUM OF EXPONENTIALS
C  DIMENSIONED FOR UP TO TEN EXPONENTIALS
      SUBROUTINE DECFUN(N,F,NPAR)
      COMMON/AREA7/A(20)
      COMMON/AREA8/G(512)
      DO 1 I=1,N
      G(I)=0.0
      FI=FLOAT(I)
      DO 2 K=1,NPAR
      K2=K+NPAR
      R=FI*F/A(K2)
      IF(R.GT.85.0) R=85.0
      P1=A(K)*EXP(-R)
      G(I)=G(I)+P1
2     CONTINUE
1     CONTINUE
      DO 3 I=1,N
      IF(G(I).LT.0.0) G(I)=0.0
3     CONTINUE
C  NORMALISE THE CURVE TO 100000 COUNTS IN THE
C  CHANNEL OF MAXIMUM COUNTS
      GMAX=G(1)
      DO 4 I=2,N
4     GMAX=AMAX1(G(I),GMAX)
      DO 5 I=1,N
5     G(I)=G(I)*100000./GMAX
      RETURN
      END
C
C  SUBROUTINE RANCH
C  CALCULATES HOW MANY TIMES(L1) L GOES INTO KJ1 AND
C  THE REMAINDER(L2)
      SUBROUTINE RANCH(KJ1,L,L1,L2)
      RL=FLOAT(KJ1)/FLOAT(L)
      L2=MOD(KJ1,L)
      IF(L2)1,1,2
1     L1=KJ1/L
      GO TO 3
2     L1=INT(RL)
3     RETURN
      END
C
C  SUBROUTINE TUMBLE
C  REARRANGES A FILE CONTAINING NORMALISED DECAY CURVES
C  TO GIVE TIME-RESOLVED SPECTRA.THE SPECTRA ARE ASSUMED
C  TO HAVE <64 POINTS AND ARE WRITTEN TO AN OUTPUT
C  FILE, ONE TRES PER RECORD
      SUBROUTINE TUMBLE(KIK,IK,NLAM,LUD)
      INTEGER*2 IFC(9),IFT(9)
```

Appendix 7.A1 (*continued*)

```
      DIMENSION XFQ(512)
      COMMON/AREA1/IFC
      COMMON/AREA8/G(512)
      COMMON/AREA2/TRES(64),TLAM(64)
C  NLAM : NUMBER OF CURVES ON THE INPUT FILE
C         = NO. OF POINTS IN THE TRES
C  IFC : INPUT FILE CONTAINING NORMALISED DECAY CURVES
C  IFT : OUTPUT FILE CONTAINING TRES
C  XFQ(512) : ARRAY OF MEAN FREQUENCIES OF EACH TRES
      KIK=0
      CALL OPENW(1,IFC,7,0,0,I)
      IF(I.NE.0) GO TO 1000
1     WRITE(4,2)
2     FORMAT(1X,' TYPE FILENAME FOR TRES')
      READ(5,3) IFT
3     FORMAT(9A2)
      CALL CFILW(IFT,2,256,1,1,0,0,I)
      IF(I.NE.0) GO TO 1
      CALL OPENW(2,IFT,7,0,0,I)
      IF(I.NE.0) GO TO 1
C  READ IN THE DECAY CURVES AND CONSTRUCT THE TRES
      DO 23 K=1,IK
      K1=64*(K-1)+1
      K2=64*K
      DO 22 I=K1,K2
      DO 21 J=1,NLAM
      IK4=IK*(J-1)+K
      READ(1,REC=IK4)(G(L),L=K1,K2)
      TRES(J)=G(I)
21    CONTINUE
C  CORRECT THE TRES IF INTENSITY VS. ENERGY IS REQUIRED
C  AND CALCULATE THE MEAN FREQUENCY OF EACH TRES
      IF(LUD.EQ.1) GO TO 11
      XF=0.0
      XM=0.0
      DO 12 L=1,NLAM
      TRES(L)=TRES(L)*TLAM(L)*TLAM(L)*0.0001
      XF=XF+TRES(L)*TLAM(L)
      XM=XM+TRES(L)
12    CONTINUE
C  WRITE TRES TO OUTPUT FILE
11    WRITE(2)(TRES(J),J=1,NLAM)
C  LIST THE MEAN FREQUENCIES
      IF(LUD.EQ.1) GO TO 22
      XFQ(I)=XF/XM
22    CONTINUE
      IF(LUD.EQ.1) GO TO 23
      WRITE(4,2105)K1,K2
2105  FORMAT(1X,'MEAN FREQ. TRES  ',I3,' TO ',I3)
      DO 2106 I=K1,K2,8
      M=I+7
      WRITE(4,2107)(XFQ(J),J=I,M)
```

```
-297: 2106  CONTINUE
-298: 2107  FORMAT(1X,8(F7.1,1X))
-299:       WRITE(4,2108)
-300: 2108  FORMAT(1X,'TYPE 1 TO CONTINUE')
-301:       READ(5,2109)MILL
-302: 2109  FORMAT(I1)
-303: 23    CONTINUE
-304: C  LEAVE OUTPUT FILE OPEN. IS READ FROM IN MAIN PROGRAM
-305:       GO TO 1001
-306: 1000  KIK=1
-307: 1001  RETURN
-308:       END
-309: C
-310: C  SUBROUTINE BUMBLE
-311: C  NO COMMENTS ARE GIVEN FOR THIS ROUTINE AS IT
-312: C  IS FOR THE MOST PART RELEVANT TO A SPECIFIC PLOTTER
-313:       SUBROUTINE BUMBLE(NUM,M,KJ1,NS,FSLAM,CHIPPY,LUD)
-314:       INTEGER*2 IUS
-315:       COMMON/AREA2/TRES(64),TLAM(64)
-316:       DIMENSION M(64)
-317:       DATA IUS/31/
-318:       RANGY=2390.0/10000.0
-319:       FNS=FLOAT(NS)
-320:       SLAMF=FSLAM+(FNS-1.0)*CHIPPY
-321:       FS=FSLAM
-322:       SL=SLAMF
-323:       IF(LUD.EQ.1) GO TO 501
-324:       FS=100000./SLAMF
-325:       SL=100000./FSLAM
-326: 501   ICE=0
-327: 101   WRITE(4,1)
-328: 1     FORMAT(1X,' TYPE 0 TO FINISH PLOTTING')
-329:       READ(5,2) IPLF
-330: 2     FORMAT(I1)
-331:       IF(IPLF.EQ.0) GO TO 401
-332:       WRITE(4,3)
-333: 3     FORMAT(1X,' TYPE 1 FOR UNNORMALISED,SAME PLOT',/,
-334:      1 1X,'       2 FOR UNNORMALISED,DIFFERENT PLOTS',/,
-335:      2 1X,'       3 FOR NORMALISED,SAME PLOT',/,
-336:      3 1X,'       4 FOR NORMALISED,DIFFERENT PLOTS')
-337:       READ(5,2) ICAT
-338:       IF(ICAT.GE.3) GO TO 6
-339:       IF(ICE.EQ.1) GO TO 6
-340:       TMAX=0.0
-341:       ICE=1
-342:       A=0.
-343:       DO 1000 MI=1,NUM
-344:       READ(2,REC=M(MI))(TRES(K),K=1,KJ1)
-345:       TMAX=TRES(1)
-346:       DO 4 J=2,KJ1
-347: 4     TMAX=AMAX1(TRES(J),TMAX)
-348:       IF(TMAX-A)1000,1000,5
-349: 5     A=TMAX
```

Appendix 7.A1 (*continued*)

```
-350: 1000  CONTINUE
-351:       REWIND 2
-352: 6     IF(ICAT.EQ.2.OR.ICAT.EQ.4) GO TO 61
-353:       WRITE(4,10)
-354:       READ(5,2) MAB
-355:       CALL SIDLE
-356:       CALL APLOT(56.0,2944.0,-1)
-357:       IF(ICAT.EQ.3) GO TO 63
-358:       WRITE(3,64)IUS
-359: 64    FORMAT(A2,'UNNORMALISED TIME RESOLVED SPECTRA')
-360:       GO TO 611
-361: 63    WRITE(3,65)IUS
-362: 65    FORMAT(A2,'NORMALISED TIME RESOLVED SPECTRA')
-363: 611   CALL PAXIS(FS,SL,FIX1,FIX2,LUD)
-364: 61    DO 1001 MI=1,NUM
-365:       READ(2,REC=M(MI))(TRES(K),K=1,KJ1)
-366:       IF(ICAT.EQ.2.OR.ICAT.EQ.4) GO TO 7
-367:       IF(MI.EQ.1) GO TO 8
-368:       WRITE(4,66)
-369: 66    FORMAT(1X,' CHOOSE A PEN. READY ?')
-370:       READ(5,2)MAB
-371:       GO TO 8
-372: 7     WRITE(4,10)
-373: 10    FORMAT(1X,' SET UP PLOTTER.   READY ?')
-374:       READ(5,2) MAB
-375:       CALL SIDLE
-376:       CALL APLOT(56.0,3030.0,-1)
-377:       WRITE(3,67)IUS,M(MI)
-378: 67    FORMAT(A2,'FOR CHANNEL ',I3)
-379:       CALL APLOT(56.0,2944.0,-1)
-380:       IF(ICAT.EQ.2) GO TO 68
-381:       WRITE(3,69)IUS
-382: 69    FORMAT(A2,'NORMALISED TIME RESOLVED SPECTRUM')
-383:       GO TO 70
-384: 68    WRITE(3,71)IUS
-385: 71    FORMAT(A2,'UNNORMALISED TIME RESOLVED SPECTRUM')
-386: 70    CALL PAXIS(FS,SL,FIX1,FIX2,LUD)
-387: 8     IF(ICAT.LT.3) GO TO 12
-388:       TRENT=TRES(1)
-389:       DO 9 MK=2,KJ1
-390: 9     TRENT=AMAX1(TRES(MK),TRENT)
-391: 12    DO 11 MK=1,KJ1
-392:       IF(ICAT.GE.3) GO TO 14
-393:       TRES(MK)=TRES(MK)*10000./A
-394:       GO TO 11
-395: 14    TRES(MK)=TRES(MK)*10000./TRENT
-396: 11    CONTINUE
-397:       DO 13 MK=1,KJ1
-398:       Y=TRES(MK)
-399:       PW=TLAM(MK)-FIX1
-400:       X=460.0+PW*3480.0/(FIX2-FIX1)
-401:       Y=410.0+RANGY*Y
```

```
 402:        KLM=0
 403:        IF(MK.EQ.1) KLM=-1
 404:        CALL APLOT(X,Y,KLM)
 405: 13     CONTINUE
 406: 1001   CONTINUE
 407:        REWIND 2
 408:        GO TO 101
 409: 401    RETURN
 410:        END
 411:        SUBROUTINE APLOT(X,Y,IG)
 412:        DIMENSION ICOORD(5)
 413:        DATA IGS/486539264/,ISYN/369098752/,ISP/'    '/
 414:        ICOORD(1)=16777216*INT(AMOD(Y/128.0,32.0)+32.0)
 415:        ICOORD(2)=16777216*INT(AMOD(Y,4.0)*4.0+AMOD(X,4.0)+96.0)
 416:        ICOORD(3)=16777216*INT(AMOD(Y/4.0,32.0)+96.0)
 417:        ICOORD(4)=16777216*INT(AMOD(X/128.0,32.0)+32.0)
 418:        ICOORD(5)=16777216*INT(AMOD(X/4.0,32.0)+64.0)
 419:        IF(IG)199,200,201
 420: 199    WRITE(3,12) IGS,(ICOORD(I),I=1,5)
 421:        RETURN
 422: 200    WRITE(3,14) ISYN,ISP,(ICOORD(I),I=1,5)
 423:        RETURN
 424: 201    WRITE(3,16) IGS,(ICOORD(I),I=1,5),(ICOORD(I),I=1,5)
 425:        RETURN
 426: 12     FORMAT(1H ,A1,5A1)
 427: 14     FORMAT(1H ,2A1,5A1)
 428: 16     FORMAT(1H ,A1,5A1,5A1)
 429:        END
 430:        SUBROUTINE SIDLE
 431:        INTEGER*2 IESC,IA,IE,IN
 432:        DATA IESC/27/,IA/65/,IE/69/,IN/78/
 433:        WRITE(3,1) IESC,IA,IE,IESC,IA,IN
 434: 1      FORMAT(6A2)
 435:        CALL APLOT(0.0,3124.0,-1)
 436:        CALL APLOT(0.0,0.0,0)
 437:        CALL APLOT(4095.0,0.0,0)
 438:        CALL APLOT(4095.0,3124.0,0)
 439:        CALL APLOT(0.0,3124.0,0)
 440:        RETURN
 441:        END
 442:        SUBROUTINE PAXIS(XMIN,XMAX,FIX1,FIX2,L)
 443:        INTEGER*2 IESC,IA,IE,IN,IJ,II,ICR,IUS,ICOM,ITF,ISEV
 444:        DATA IESC/27/,IA/65/,IE/69/,IN/78/,IJ/74/,ISEV/70/
 445:        DATA ICR/13/,IUS/31/,ICOM/44/,II/73/,ITF/35/
 446:        CALL APLOT(460.0,2800.0,-1)
 447:        CALL APLOT(460.0,410.0,0)
 448:        CALL APLOT(3940.0,410.0,0)
 449:        CALL APLOT(100.0,1213.0,-1)
 450:        WRITE(3,1) IESC,IA,IJ,ICR
 451: 1      FORMAT(3A2,'90',A2)
 452:        WRITE(3,2) IUS,IESC,IA,IN
 453: 2      FORMAT(A2,'INTENSITY',3A2)
 454:        IXMIN=INT(XMIN)
```

Appendix 7.A1 (*continued*)

```
      IXM=MOD(IXMIN,10)
      IXMIN=IXMIN-IXM
      FIX1=FLOAT(IXMIN)
      IXMAX=INT(XMAX)
      IXM=MOD(IXMAX,10)
      IXMAX=IXMAX-IXM+10
      FIX2=FLOAT(IXMAX)
      JOB=IXMAX-IXMIN
      DO 3 I=0,10
      FI=FLOAT(I)
      X=460.0+FI*348.0
      Y=410.0
      IF(I.EQ.0) GO TO 11
      CALL APLOT(X,Y,-1)
11    Y=Y+17.0
      IF(I.EQ.0) GO TO 12
      CALL APLOT(X,Y,0)
12    X=X-56.0
      Y=Y-95.0
      CALL APLOT(X,Y,-1)
      IQ=IXMIN+(JOB*I/10)
      WRITE(3,4)IUS,IQ
4     FORMAT(A2,I3)
3     CONTINUE
      IF(L.EQ.1) GO TO 51
      Y=120.0
      X=1584.0
      CALL APLOT(X,Y,-1)
      WRITE(3,52)IUS
52    FORMAT(A2,'WAVENUMBER X 10')
      X=2424.0
      Y=208.0
      CALL APLOT(X,Y,-1)
      WRITE(3,53)IUS
53    FORMAT(A2,'-2')
      X=2536.0
      Y=120.0
      CALL APLOT(X,Y,-1)
      WRITE(3,54)IUS
54    FORMAT(A2,',CM')
      X=2704.0
      Y=208.0
      CALL APLOT(X,Y,-1)
      WRITE(3,55)IUS
55    FORMAT(A2,'-1')
      GO TO 56
51    Y=146.0
      X=1808.0
      CALL APLOT(X,Y,-1)
      WRITE(3,5)IUS
5     FORMAT(A2,'WAVELENGTH,NM')
56    CALL WAIT(30,2,I)
```

```
-507:        DO 7 I=0,10
-508:        FI=FLOAT(I)
-509:        X=460.0
-510:        Y=410.0+239.0*FI
-511:        IF(I.EQ.0) GO TO 14
-512:        CALL APLOT(X,Y,-1)
-513: 14     X=485.0
-514:        IF(I.EQ.0) GO TO 13
-515:        CALL APLOT(X,Y,0)
-516:        X=150.0
-517:        GO TO 15
-518: 13     X=110.0
-519: 15     Z=Y-20
-520:        IQ=I*1000
-521:        CALL APLOT(X,Z,-1)
-522:        WRITE(3,8)IUS,IQ
-523: 8      FORMAT(A2,I5)
-524: 7      CONTINUE
-525:        CALL WAIT(15,2,I)
-526:        WRITE(3,9)IESC,IA,IN
-527: 9      FORMAT(3A2)
-528:        RETURN
-529:        END
-530: $BEND
-
```