Multicomponent Analysis by Synchronous Luminescence Spectrometry

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A methodology for the synchronous excitation technique is developed to improve the selectivity of luminescence spectrometry. This approach offers several advantages, including narrowing of spectral bands, an enhancement in selectivity by spectral simplification, and a decrease of measurement time in multicomponent analysis.

Luminescence spectrometry with its excellent sensitivity has provided a useful analytical tool for monitoring trace organic compounds. Nevertheless, despite the ability to select both the excitation and the emission wavelengths, the conventional luminescence methods have limited applicability since most spectra of complex mixtures often cannot be resolved satisfactorily. The present awareness of the large variety of pollutants in our environment has increased the necessity to have instruments and methods for monitoring complex samples. Our present approach to this need for multicomponent analysis is to develop and extend the applicability of simple methods which can be used on a routine basis without resorting to techniques that are expensive or excessively time consuming.

In this work, an attempt will be made to evaluate the applicability of simultaneous analysis of multicomponent mixtures by the so-called synchronous excitation technique. The idea of synchronous excitation luminescence was first suggested by Lloyd (*1*). Although forensic researchers and oil-spill analysts have often employed this technique in an empirical manner (*2*, *3*), the effective use of this technique in the general field of analytical luminescence spectrometry has been limited somewhat. This might be partly due to a lack of specific information and methodology which makes it difficult for analysts who are not familiar with spectroscopic techniques to exploit fully the possibilities offered by this simple approach. Practical applications have been limited so far only to providing fingerprints of complex samples such as crude oils of various origins (*3*). The intention of this paper is to develop and present a simple method for organic trace analysis and to investigate how the technique of synchronous excitation can be applied to obtain not only spectral signatures from complex samples but also specific information of analytical interest. A methodology for multicomponent analysis is suggested. Specific examples of the fluorometric characterization of various representatives of the important class of polynuclear aromatic hydrocarbons (PAH) are presented. The results illustrate that this technique has a great potential to offer an effective, rapid, and simple "screening type" method of analysis.

BASIC PRINCIPLE

In conventional luminescence spectrometry, an emission spectrum can be monitored by scanning the emission wavelength λ_{em} while the luminescent compound is excited at fixed excitation wavelength λ_{exc}. On the other hand, an excitation spectrum can be obtained by scanning λ_{exc} while the emission is monitored at a given λ_{em}. It was suggested that a third possibility consists of varying simultaneously (or "synchronously") both λ_{exc} and λ_{em} while keeping a constant wavelength interval $\Delta\lambda$ between them (*1*, *2*). At first glance, the use of a constantly changing excitation energy may seem undesirable for spectrometric applications. This continuous variation of the excitation would not allow it to be used as a light source to record emission spectra in the usual manner (constant excitation energy) that spectrometrists employ. This feature however can be a distinct advantage. In order to assess the figures of merit of the synchronous technique, it is necessary to discuss the corresponding luminescence expressions.

Consider a luminescent substance excited at a given wavelength λ'. $E_M(\lambda)$ is defined as the intensity distribution pattern of the emission (also referred to as an emission spectrum). The recorded luminescence signal at a given emission wavelength λ, I_λ, which depends on the value of E_M at λ, is also proportional to the spectral radiance of luminescence $R_{\lambda'}$ emitted by the compound excited at λ':

$$I(\lambda) = kR_{\lambda'}E_M(\lambda) \tag{1}$$

where k = a constant factor.

In the above expression and in further development, $\lambda(\lambda')$ denotes the wavelength variable that corresponds to the actual wavelength position of the emission (excitation, respectively) monochromator. The effect of instrumental factors (spectrometer profile function, detection system response, transmission factor of all optics, etc.) not essential to our discussion is neglected. Assuming the validity of the well-known Lambert law for dilute solutions, $R_{\lambda'}$ can also be expressed as:

$$R_{\lambda'} = k'Y_L(\lambda')I_0(\lambda')\epsilon(\lambda')cd \tag{2}$$

where Y_L = the luminescence quantum yield, I_0 = the incident exciting light intensity, ϵ = the molar extinction coefficient, c = the concentration of the analyte, d = the thickness of the sample, k' = an experimental constant factor.

The product, $Y_L I_0 \epsilon$, which depends exclusively on λ' can be related to the excitation function. It is proportional to the excitation spectrum $E_X(\lambda')$ which is experimentally measured when the excitation wavelength is scanned:

$$E_X(\lambda') = k''Y_L(\lambda')I_0(\lambda')\epsilon(\lambda') \tag{3}$$

where k'' = constant factor.

By combining Equations 1, 2, and 3, we can obtain the synchronous luminescence intensity (I_s) expression as a function of λ and λ':

$$I_s(\lambda',\lambda) = KcdE_X(\lambda')E_M(\lambda) \tag{4}$$

with $K = k\,k'\,k''^{-1}$. One specific condition of the synchronous technique is

$$\lambda - \lambda' = \Delta\lambda \ (= \text{constant})$$

or

$$\lambda = \lambda' + \Delta\lambda \tag{5}$$

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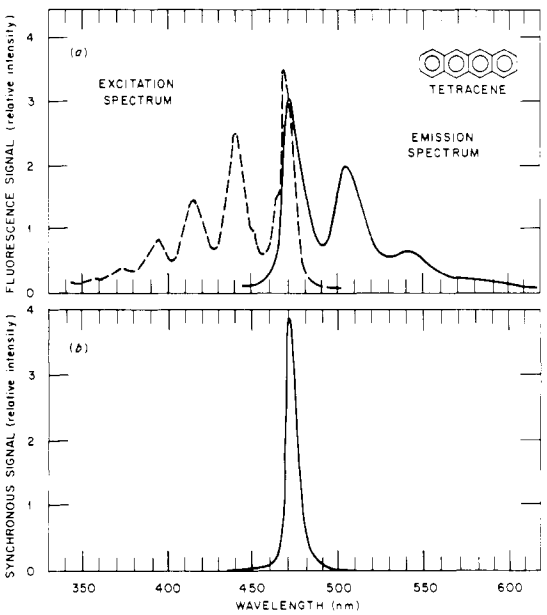

Figure 1. (a) Fluorescence emission and excitation spectra of tetracene. (b) Synchronous fluorescence signal of tetracene

In order to introduce explicitly the parameter $\Delta\lambda$, Equation 4 can be expressed as

$$I_s = KcdE_X(\lambda - \Delta\lambda)E_M(\lambda) \qquad (6)$$

This relation represents the basic equation of synchronous spectrometry. In conventional luminescence, the intensity of the emission spectrum depends upon the excitation wavelength. This dependence, however, is restricted solely to the intensity factor of the spectrum but the spectral features remain generally unchanged. In other words, the excitation wavelength acts only as a multiplicative parameter in the emission expressions of conventional luminescence spectrometry. On the other hand, in the synchronous technique, as shown in Equation 4, the luminescence intensity expression is an explicit function of λ as well as λ'. The improvement in sensitivity of this technique is indeed reflected in Equation 6 which involves two functions instead of only one in the conventional luminescence method. In addition a new degree of selectivity is introduced by the parameter $\Delta\lambda$ which can be selected by the experimenter.

EXPERIMENTAL

Apparatus. In this work, a Perkin-Elmer spectrofluorimeter (Model 43A, Perkin Elmer, Norwalk, Conn.) was used for spectrometric measurements. Both excitation and emission wavelengths can be locked together and scanned synchronously: this possibility was primarily designed in the commercial instrument for absorption measurements. A 150-W xenon arc lamp was used as an excitation light source. The detection device was a R508 photomultiplier (Hamamatsu Co., Middlesex, N.J.) that has a spectral response from 200 to 750 nm. Spectral resolution less than 2 nm was used. No correction for instrumental response was applied. All spectra were recorded on a strip-chart recorder (Perkin-Elmer, Model 023).

Reagents. All compounds investigated were commercially available and used without further purification.

RESULTS AND DISCUSSION

The Synchronous Signal. The basic performance of the synchronous technique is illustrated in Figure 1. The conventional excitation and emission spectra of tetracene in a solution of ethanol is shown in Figure 1a. The fluorescence spectrum (with λ_{exc} = 442 nm), showing three distinct emission bands at 473 nm, 507 nm, and 546 nm, covers the spectral range from 460 nm up to 600 nm under our experimental conditions. The excitation spectrum (with λ_{em} = 507 nm) ranges from 480 nm to 350 nm, revealing absorption bands at 470 nm, 442 nm, 416 nm, and 397 nm. There is a small wavelength difference of 3 nm, often called the "Stokes shift," between the peaks of the 0–0 band in the emission and in the excitation spectra. With a wavelength interval $\Delta\lambda$ = 3 nm, matching the Stokes shift, the synchronous spectrum of tetracene is shown in Figure 1b. Instead of a spectrum covering several hundreds of nanometers, the synchronous signal consists simply of one single peak located at 473 nm. This unique feature is the consequence of the restrictive character of the product of the two nearly mirror-symmetric functions $E_M(\lambda)$ and $E_X(\lambda')$. Since $E_M(\lambda)$ is a function which is limited on the short wavelength range and $E_X(\lambda')$ is a function limited on the long wavelength range, the corresponding synchronous signal, resulting from their product, must necessarily have a limited spectral band width.

Note that what is usually called a "synchronously excited emission spectrum" is referred to here as simply a "synchronous signal" or "synchronous spectrum" since it can be considered either as an emission or as an excitation spectrum. Effectively, it can be noticed that Equation 4 does not give priority to the emission wavelength λ or to the excitation wavelength λ'. Expressed explicitly in function of λ', Equation 6 could also be written as:

$$I_s(\lambda,\lambda') = KcdE_X(\lambda')E_M(\lambda' + \Delta\lambda) \qquad (7)$$

Relation 7 shows that the synchronous signal could also be considered as an excitation spectrum with a synchronously scanned emission wavelength.

Narrowing of Spectral Bands. There are various causes for diffuseness in the spectrum of complex mixtures. One trivial reason is that the emission bands of each individual spectrum are intrinsically broad. Severe overlapping of various spectra are often another cause for diffuseness. It will be shown that the synchronous technique can decrease the adverse effect of these two sources of diffuseness.

In conventional luminescence spectrometry, the spectrum can show a resolved structure only when the monitored luminescence function consists of narrow bands. But with the synchronous technique, it is sufficient, in order to observe a narrow peak, that either one of the two functions $E_M(\lambda)$ or $E_X(\lambda')$ has resolved structure in a given spectral range. This increases the chance of obtaining spectra having resolved structure. The synchronous signal fails to show a resolved peak only when both functions $E_X(\lambda')$ and $E_M(\lambda)$ are featureless. We consider the example shown in Figure 2, where $E_M(\lambda)$ and $E_X(\lambda')$ represent two bands belonging to an emission and an excitation spectrum. In this case, the optimal condition is achieved when $\Delta\lambda$ is chosen to match the wavelength interval between the maxima of these two peaks. This situation provides the most intense synchronous signal with narrowest half-width. In Figure 2a, the hypothetical emission $E_M(\lambda)$ and excitation $E_X(\lambda')$ bands were represented for the sake of simplicity, with Gaussian shapes and identical intensities. If the sample is excited monochromatically at λ_1' (λ_2' or λ_3', respectively), the observed emission spectrum I_L would show an intensity represented by I_{L_1} (I_{L_2}, I_{L_3}, respectively). As shown in Figure 2b, the intensity is increased proportionally with the excitation (absorption) intensity but the band width of the observed emission remains unchanged. However, as depicted in Figure 2c, if the emission is monitored while the exciting radiation is varied (synchronous method) the signal would show a more narrow peak having the intensity that corresponds to the maximum value I_{L_1} obtained with fixed λ_{exc}. This band-narrowing effect is essentially a consequence of the multiplication of two functions increasing and/or decreasing simultaneously. This process is illustrated in Figure 3. The dashed curve shows a portion of the fluorescence

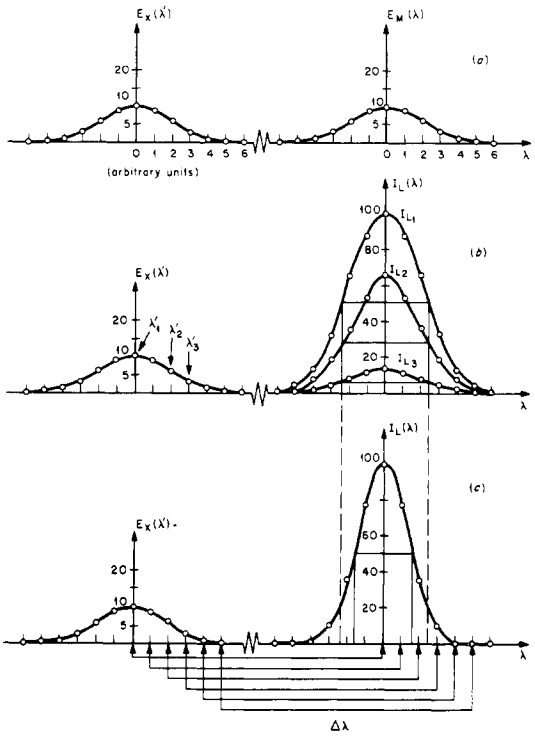

Figure 2. Schematic representation of the band narrowing effect

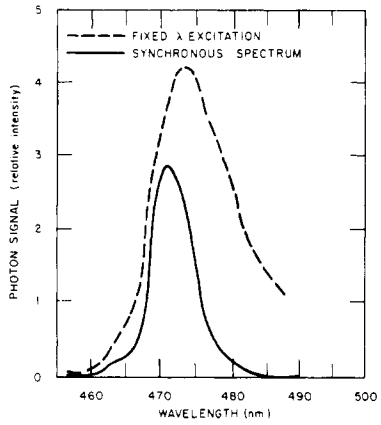

Figure 3. Band width of conventional and synchronous fluorescence signal of tetracene

spectrum of tetracene using fixed excitation at 440 nm. The solid line curve shows the significantly narrower synchronous signal (with $\Delta\lambda$ = 3 nm) of the same sample.

Simplification of Emission Spectra. In some cases, the synchronous technique can greatly simplify complex quasi-linear spectra. In order to bring out the situation more clearly, two graphical examples are given in Figure 4. A signal is observed only when $\Delta\lambda$ matches the interval between one absorption band and one emission band. If it is possible to select and use one particular $\Delta\lambda$ which matches one unique pair of absorption and emission bands, the synchronous spectrum will show only one single peak. This situation is shown in Figure 4a, where $\Delta\lambda$ ($= \lambda_1 - \lambda_0'$) is assumed to be the wavelength interval which matches only the absorption band at λ_0' and the emission peak at λ_1. As can be observed, instead of a complex emission spectrum, only one peak at λ_1 should be observed. In multicomponent mixtures, the spectra of various compounds will be consequently simplified and interferences resulting from spectral superposition will be greatly reduced. An interesting feature is the possibility to analyze a specific compound in a mixture by selecting an

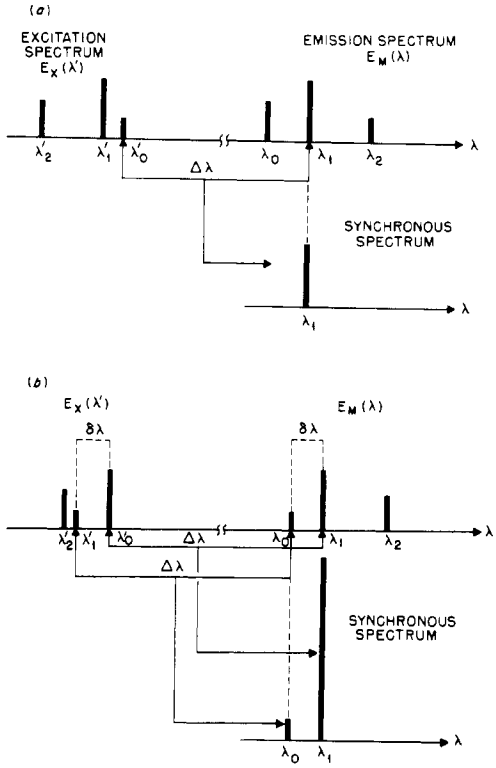

Figure 4. Spectral simplification effect. $E_M(\lambda)$ = emission spectrum; $E_X(\lambda')$ = excitation spectrum

appropriate $\Delta\lambda$ value among the numerous combinations of wavelength intervals. This would offer another opportunity to study selectively a certain component in a mixture in the case where the traditional approach with fixed excitation would be unsuccessful.

In order to have a situation where an interval $\Delta\lambda$ can be found to match solely one pair of excitation and emission bands, it is necessary that the emission and the excitation spectra consist of bands which are not separated by similar wavelength intervals. The situation where, by accidental coincidence, two (or several) pairs of bands in the emission and excitation spectra show identical intervals ($= \delta\lambda$) is illustrated in Figure 4b. Even in this case, if $\Delta\lambda$ is chosen to match the two strong bands (in Figure 4b, at λ_0' and λ_1), the intense peak at λ_1 is enhanced more strongly than the weak peak at λ_0. It is important to emphasize here again that, whereas in conventional spectrometry with fixed λ_{exc} one can only increase the intensity of all the emission bands at the same time, the synchronous technique can increase selectively the stronger peaks when a proper $\Delta\lambda$ is used. The situations discussed above show how the synchronous technique, if applied properly, can enhance the selectivity: characteristic intense peaks are increased strongly while, on the other hand, the interfering effect of weak bands can be reduced.

Reduction of the Spectral Range. For the spectrometrist involved in fundamental research, the detailed structure of the entire emission spectrum is of crucial importance since it reflects directly the physical properties in which he is interested. For the analytical chemist, however, the details of the whole spectrum might not be of vital importance: usually he selects only one or several spectral bands useful for his analysis, provided these spectrometric data are suitable for his needs (for example, direct correlation with the amount of analytes). Most of the other spectrometric details are generally not considered and their presence serves only to confuse the total spectrum by interfering with the emission of other components in the mixture. The previous discussions show the various processes by which the synchronous tech-

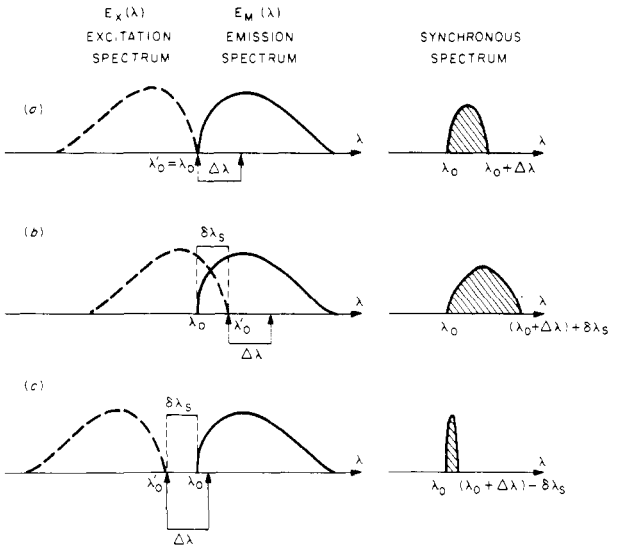

Figure 5. Influence of the Stokes shift and the wavelength interval on the synchronous signal

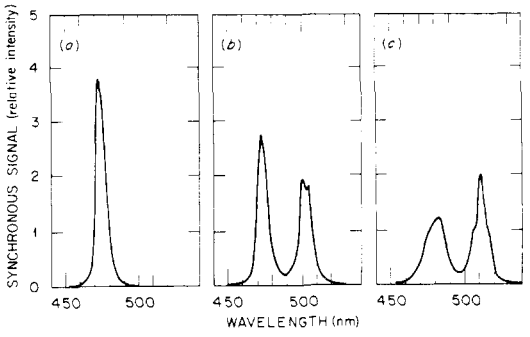

Figure 6. Effect of the wavelength interval on the synchronous fluorescence signal of tetracene: (a) $\Delta\lambda$ = 3 nm, (b) $\Delta\lambda$ = 30 nm, (c) $\Delta\lambda$ = 45 nm

nique can simplify the emission signal and reduce spectral interference; this section will describe how the choice of $\Delta\lambda$ can affect the spectral range of the synchronous signal. The influence of the Stokes shift is also discussed.

The emission and excitation spectra with various spectral overlaps are schematically shown in Figure 5. The shortest wavelength of the emission spectrum is depicted as λ_0 and the longest wavelength of the excitation spectrum is λ_0'. In Figure 5a, the spectral overlap is assumed to have zero value ($\lambda_0' = \lambda_0$). If a given wavelength interval $\Delta\lambda$ is used, the synchronous signal would have a band width of $\Delta\lambda$, covering from λ_0 (where the compound starts its emission) to $\lambda_0' + \Delta\lambda$ (where the compound no longer absorbs the excitation radiation). Figure 5b illustrates the situation where the emission and the excitation spectra overlap each other ($\lambda_0 < \lambda_0'$); in this case the synchronous spectrum covers from λ_0 to $\lambda_0 + \Delta\lambda + \delta\lambda_s$, having therefore a band width of $\Delta\lambda + \delta\lambda_s$, $\delta\lambda_s$ being the spectral overlap. In Figure 5c, the situation involving a Stokes shift of $\delta\lambda_s$ is illustrated: in this case ($\lambda_0' < \lambda_0$), the spectral band width of the synchronous signal is equal to $\Delta\lambda - \delta\lambda_s$ (from λ_0 to $\lambda_0 + \Delta\lambda - \delta\lambda_s$). It is therefore experimentally possible to modify the spectral band width of the synchronous signal by varying $\delta\lambda_s$ and $\Delta\lambda$. The Stokes shift can be varied by changing the solvent environment. But of most interest, the width of the synchronous spectrum can be directly compressed or expanded just by decreasing or increasing the experimental parameter $\Delta\lambda$. Whenever it is experimentally possible, the decrease of the band width of the synchronous signal would be advantageous since spectral overlap could be greatly reduced. This possibility to modify the spectral band width of

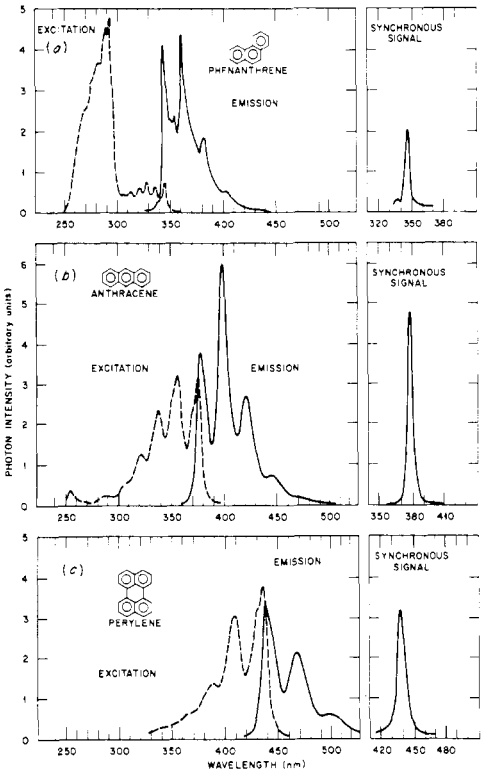

Figure 7. (a) Fluorescence excitation, emission, and synchronous spectra of phenanthrene. (b) Fluorescence excitation, emission, and synchronous spectra of anthracene. (c) Fluorescence excitation, emission, and synchronous spectra of perylene

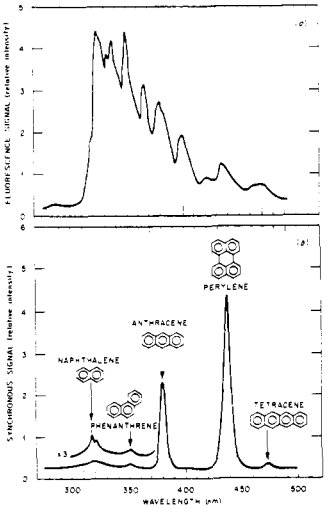

Figure 8. (a) Conventional fluorescence spectrum of a mixture of naphthalene, phenanthrene, anthracene, perylene, and tetracene. (b) Synchronous spectrum of the mixture

the emission signal of each individual component in a mixture is the most outstanding feature offered by the synchronous technique. Figure 6 shows the effect of various $\Delta\lambda$ values upon the synchronous fluorescence spectra of tetracene: the $\Delta\lambda$ values were 3 nm (Figure 6a), 30 nm (Figure 6b) and 45 nm (Figure 6c).

Multicomponent Analysis. One illustration of the methodology developed in this paper is given in Figures 7 and 8. Figure 7 shows the fluorescence excitation and emission spectra as well as the synchronous luminescence (SL) signal of several compounds such as phenanthrene, anthracene, and perylene. The conventional fluorescence spectrum shows several typical vibronic bands of the compound that covers a large spectral range of several hundreds of nanometers. In

the synchronous spectrum (using $\Delta\lambda$ = 3 nm, which is close to most Stokes shifts), only one single band, having approximately 10 to 15 nm in halfwidth, appears at the 0–0 band positions: 347 nm for phenanthrene, 381 nm for anthracene, and 440 nm for perylene.

The conventional fluorescence spectrum of a mixture of five PAHs of various sizes and configurations in a solution of ethanol is shown in Figure 8a: naphthalene, phenanthrene, anthracene, perylene, and tetracene. The excitation wavelength was at 258 nm. Although the total spectrum reveals several peaks, the analysis of such a mixture is not simple and straightforward. On the other hand, if the synchronous technique is used, the resulting spectrum, illustrated in Figure 8b, consists of a series of exceptionally well-resolved peaks. Each band (or group of bands for naphthalene) corresponds unequivocally to one component is the mixture and can be correlated perfectly with its synchronous signal in each individual spectrum (compare with Figure 7).

Correlation of the Synchronous Signal with the Structure of the PAH Compound. The first correlation between the structure of a PAH compound and its fluorescence spectrum is reflected by the dependence of the energy of the 0–0 band with the ring size of the compound. The information provided by the vibronic structure is less typical because, for most PAHs, the fluorescence spectra usually consist of a principal series of vibronic bands of diminshing intensity which are evenly spaced at intervals of equal frequency, 1400 cm^{-1}, due to the dominant C–C vibrational modes (*4*). On the other hand, the spectrum of a higher ring number linear cyclic compound occurs generally at a longer wavelength than a lower ring number compound. Nonlinear PAHs also follow, to a certain extent, this basic rule. With conventional spectrometry, because of severe spectral overlap, this simple rule cannot be efficiently applied, especially when a large number of components in a mixture have to be analyzed. With the synchronous technique, however, the effect of limiting each individual spectrum to a definite spectral band provides the most useful feature to locate the presence of specific compounds in a mixture. The method is most suitable to give information about the presence of a given compound or group of compounds. Its simplicity makes it very suitable as a rapid "screening method". As shown in Figure 8a and 8b, the synchronous technique can achieve some sort of "spectral confinement" or "spectral separation" into individual components without requiring any actual physical separation process.

Data Chart for Synchronous Spectra. Because of the simplicity of the signal which shows generally one or a limited number of emission bands within a definite spectral range, one can construct some type of graphical classification. The spectral location of the synchronous signal can be determined from spectrometric data already available in the literature. An example of such a chart for a variety of polyaromatic hydrocarbons is given in Figure 9; most of the spectral information used to construct this chart was deduced from data in Berlman's Handbook (*5*). The synchronous signal of each compound is limited by λ_0, the shortest wavelength of the emission, and by $\lambda_0' + \Delta\lambda$ (λ_0' being the longest wavelength of the absorption and $\Delta\lambda$ the assumed wavelength interval); $\Delta\lambda$ used for the chart in Figure 9 was taken to be 3 nm. It is clear from Figure 9 that such a chart is useful in the analysis of mixtures. Note the shaded areas in Figure 9 which should theoretically represent the synchronous signals of naphthalene, phenathrene, anthracene, perylene, and tetracene. These bands are in excellent agreement with the experimental data in Figure 8.

Analytical Considerations. As expressed in Equation 4, the linear relationship between the measured synchronous signal I_s and the concentration of the luminescent compound is preserved in the same way as with conventional spectrometry. Quantitative analysis is therefore straightforward. Synchronous spectrometry should offer the same possibilities and have the same limitations as those that are encountered in conventional spectrometry. The figures of merit of luminescence spectrometry such as linear and dynamic range of analytical curves, accuracy of experimental data, etc., are well documented and, so, are not discussed here. The general applicability of luminescence analysis has been amply presented in a large number of works (*6*, *7*). The possibility of quantitative analysis by synchronous spectrometry is demonstrated in a study of shale oil processed water (*8*). Conversely, synchronous spectrometry has also the limitations inherent to the luminescence technique, such as spectral distortions caused by intermolecular interactions and by static, as well as dynamic, quenching processes. Especially at high concentrations, energy transfer processes can become quite effective; in these cases, it is expected that the intensities of the emission from compounds with high excited state energies should decrease whereas those from compounds with lower energies should increase. These observations have been reported by John and Soutar in their studies of crude oil (*3*).

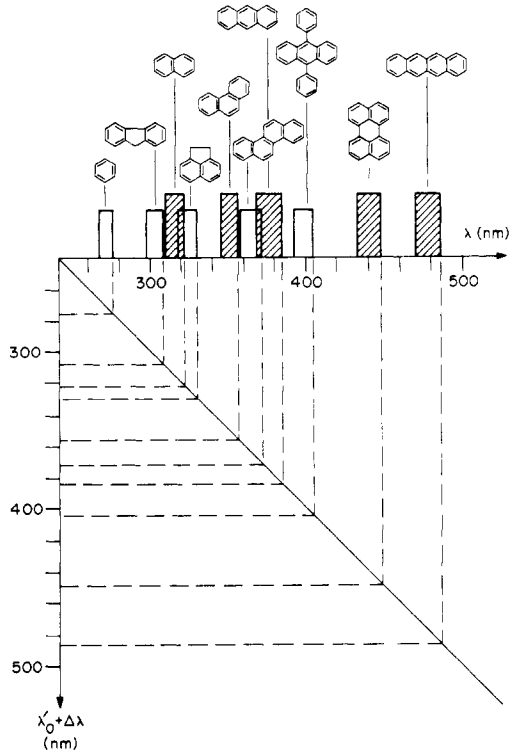

Figure 9. Data chart for synchronous spectra

Sensitivity. One important factor in any analytical technique is the sensitivity of the method. The synchronous spectra shown in the previous examples were measured with relatively narrow slit widths (1–2 nm). Because of the small value of the wavelength interval $\Delta\lambda$ (3 nm) employed, the use of larger slits would create undesired scattering and stray light interferences. One might think, therefore, that the synchronous method would be limited by the use of such narrow slits and consequently by low sensitivity. Nevertheless, the tradeoff between spectral resolution and sensitivity must be considered. In Figures 7 and 8, the vibronic band widths are of the order of 4 nm. In low temperature studies which use special solvents such as the Shpolskii matrices (*9*), the quasilinear structure can even have emission band widths of the order of 0.1 nm. The use of broad slit widths would increase the radiance throughput but at the same time would

alter the spectral structure. The same tradeoff consideration applies also for excitation: an excitation covering a large spectral band would increase the limit of detection but also remove the selectivity of the excitation since several compounds would also be excited simultaneously. There is another factor the analyst should keep in mind: for a mixture, the method with the lowest detection limit is not necessarily the one which provides the strongest signal because only the signal-to-noise ratio determines the detection limit. This feature favors the synchronous technique since a synchronous signal, as previously discussed, shows less contribution from emission of other components in the mixture. The photon noise associated with the emission from other compounds would decrease the signal-to-noise ratio. Finally, the simplicity of this technique makes it particularly attractive as a monitoring method for organic pollutants on a routine basis. It can easily be applied to fluorimetry as well as phosphorimetry, which are two complementary luminescence tools. No additional equipment is required and synchronous measurement can be performed directly using any commercial spectrometer in which excitation and emission monochromators can be interlocked.

Selectivity and Multicomponent Analysis Approach. It is noteworthy to emphasize again the multicomponent excitation approach of the synchronous technique in contrast with the fixed wavelength excitation method. Even if in those situations where it is possible to excite selectively each component present in a mixture, several measurements have to be performed, each using a different excitation most suitable for one specific component. In contrast, it was shown in Figure 8 that for compounds of a given group (such as the PAHs), it is possible with the synchronous method to obtain in the same measurement all the information specific to each compound. This would result in a shorter measurement time. It could be argued that the information obtained by this "multicomponent excitation approach" has to be traded against the loss of spectral information that would have been contained in the complete spectrum obtained with the conventional fixed excitation method. Fortunately this loss of spectral information that is contained in the other part of the spectrum does not have any adverse effect on the analysis but, on the contrary, can reduce interfering spectral overlap. It is interesting to note that the multicomponent feature offered by the synchronous approach is provided by the simultaneous scanning of both excitation and emission wavelengths, which allows each component to be excited and measured at a specific spectral range most suitable to them.

This simple method of analysis using synchronous excitation and detection opens up a host of possibilities for monitoring organic pollutants by luminescence spectrometry. Some recent practical applications include the characterization of polynuclear aromatic compounds (PNA) in by-product water from the Synthane gasifier and the multicomponent analysis by room temperature phosphorimetry of organic compounds absorbed on filter paper (*8*).

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Experimental and Theoretical Considerations of Flow Cell Design in Analytical Chemiluminescence

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A modular flow cell has been designed for chemiluminescence (CL) analysis. The cell features inert construction, detection of light not front emitted, and ability to vary cell geometry to optimize for a particular chemical system. A mathematical model was developed to predict observed CL intensity as a function of solution absorbance (ϵc), cell depth (l), and degree of reflectance at the back wall of the cell (r). The cell and theory were tested with several geometries including cell depths between 0.2 cm and 1.0 cm (volumes between 0.25 mL and 4.50 mL). The chemical systems employed were luminol, which has no significant absorption of the CL emission, and gallic acid, which has significant absorption. Theory and experimental observation show excellent agreement. A practical limit on cell depths is such that ϵcl is less than about 0.65.

For our studies of the analytical applications of chemiluminescent (CL) systems (*1*), we have found it necessary to develop a flow cell suited particularly for CL measurements rather than the more common measurements of solution absorbance or fluorescence. This cell was designed with four goals in mind. The geometry of the cell should be easily variable in order to see the effect of changes in cell dimensions on the observed CL. The cell should collect light which is not emitted in the direction of the detector. (This approach has been suggested, but not systematically investigated, in discussions of other CL measurement systems (*2–4*), and is presently employed in certain commerical fluorescence cells (*5*).) Flow characteristics must ensure an even, well-rinsing flow through the cell. Finally, the cell must be chemically inert, since many of the CL reactions studied are sensitive to trace concentrations of metal ions and are performed in strongly alkaline H_2O_2. Because the first goals are related to the absorbance of the resident emitting solution, we have chosen two CL reactions, luminol and gallic acid, to evaluate the flow cell. In the gallic acid system, the products are strongly absorbing (*1*, *6*); however, in the luminol system, neither the reactants nor products absorb significantly over

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