Molecular Luminescence Spectroscopy

Methods and Applications: Part 2

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PREFACE

If there was a pervasive aspect of the expositions of analytical luminescence spectroscopy in Part 1 of *Molecular Luminescence Spectroscopy*, it was that of the currency of the methodologies covered. For example, the luminescence spectroscopies of pharmaceuticals, natural products, and inorganics are practiced daily in thousands of laboratories around the world. Fluorescence detection in chromatography and luminescence immunoassay have emerged at the forefront of the analysis of real samples, especially those of biological origin.

Where, then, can analytical luminescence spectroscopy be expected to go from here? In Part 2, we attempt to answer or at least give some insight into possible answers to this question.

In recent years, analytical luminescence spectroscopists have turned their attention from the simple spectral analysis of homogeneous solutions to more elegant methodologies and more complex media. Herein we consider various aspects of the analysis of solids and solid solutions. In addition, we describe analysis based on the temporal and phase characteristics of luminescence spectra and the application of these forms of analysis to the evaluation of very fast chemical reactions. Finally, we consider the theory and applications of fluorescence optical sensors.

Some of these subjects have already found their way into analytical practice; others have yet to do so. In any event, it is the editor's opinion that these will constitute the "hot" areas of analytical luminescence spectroscopy, at least until the turn of the century. As in Part 1, the various chapters in Part 2 have been written by scientists who are at the "cutting edges" of their respective areas.

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CHAPTER

1

LUMINESCENCE FROM SOLID SURFACES

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1.1. INTRODUCTION

The analytical measurement of the fluorescence or phosphorescence of components adsorbed on solid materials constitutes the general area of solid-surface luminescence analysis. A considerable number of solid materials have been used in chemical analysis. These include filter paper, silica gel, aluminum oxide, silicone rubber, sodium acetate, potassium bromide, and cellulose. Several of the arguments presented for sensitivity and selectivity in solution luminescence analysis can be applied to solid-surface luminescence analysis (1–4).

One important difference between solid-surface luminescence and solution luminescence is that in solid-surface luminescence the luminescent molecules are usually adsorbed on small particles or a solid-surface like filter paper. However, in solution luminescence, the molecules are dissolved in a solvent. The adsorbed molecules and the solid matrix will cause both the source and luminescent radiation to be scattered. The scattered source radiation and scattered luminescent radiation are reflected from the surface of the solid matrix and can also be transmitted through the solid material. Of course, for transmitted radiation, the experimental conditions have to allow for the transmission of the radiation. Wendlandt and Hecht (5) discussed the difference between specular reflection and diffuse reflection. These phenomena are important in solid-surface luminescence analysis. Specular reflection or mirror reflection is defined by Fresnel's equations and occurs from a very smooth surface. Diffuse reflection of exciting radiation results from penetration of the incident radiation into the interior of the solid substrate, and multiple scattering occurs at the boundaries of individual particles. Ideal diffuse reflection takes place when the angular distribution of the reflected radiation is independent of the angle of incidence of source radiation (6). Körtum (6) has pointed out that specular reflection and diffuse reflection are two important limiting cases, and all possible variations are found, in practice, between these two extremes. Normally, with solid-surface luminescence analysis, diffuse luminescence is measured. A fraction of the sample of interest penetrates into the solid matrix, and the sample luminescence is excited at the surface and within the solid matrix at a given depth depending on the characteristics of the solid matrix. Usually, the excited luminescence is scattered diffusely. In this chapter, reflected luminescence refers to diffusely reflected luminescence, and it appears at the same side as the excitation radiation. Generally, any specular reflection is considered insignificant. Transmitted luminescence refers to diffusely transmitted luminescence, and the luminescence appears at the unexcited surface.

Several commercial and laboratory-constructed instruments are used to measure solid-surface luminescence. Commercial instruments for solid-

surface fluorescence measurements became available about 1968. Laboratory-constructed instruments and modifications to commercial instruments have appeared in the last 10 years for measuring both fluorescence and phosphorescence from solid surfaces. Hundreds of applications have appeared in the literature in areas such as environmental research, forensic science, pesticide analysis, food analysis, pharmaceutical analysis, biochemistry, medicine, and clinical chemistry. Most of the applications have been with solid-surface fluorescence. However, more and more applications with room-temperature phosphorescence (RTP) are appearing in the literature. In this chapter, emphasis will be given to recent developments in the theoretical and practical aspects of solid-surface RTP. A recent monograph details the theory, instrumentation, and applications in solid-surface fluorescence and phosphorescence (7). In addition, another recent monograph provides a basic introduction to the analytical principles and practice of RTP (8).

1.2. PRACTICAL CONSIDERATIONS

In this section, the application of the sample to the solid surface, choice of substrate, and other experimental conditions will be discussed. Several procedural details for thin-layer chromatography (TLC) with subsequent quantitation of separated components have been considered elsewhere (9,10). Many of these procedural details are applicable to solid-surface luminescence analysis. Hurtubise (7) has considered several procedural aspects in solid-surface luminescence analysis, and Vo-Dinh (8) has discussed in detail the practical aspects of RTP.

1.2.1. Application of the Sample to the Solid Surface

Two general methods have been used to deposit luminescent components on solid surfaces. Syringes or micropipets have been employed which can deliver microliter amounts of solution to a solid material such as filter paper. Also, luminescent components have been adsorbed onto powders by evaporating the solution in which the components are dissolved. For solutions deposited on flat surfaces, the size of the initial spot should be as uniform as possible. If a syringe is used, it is possible that some of the solution can "creep back" on the outside of the stem. Part of the drop can curl back around the tip of the syringe and remain after the sample is placed on the surface. This source of error can be minimized with a very fine tip or by coating the outside of the stem with silicone (11). However, generally, adequate precision can be obtained without taking special precautions. Folded nichrome wire loops

were used by Samuels and Fisher (12) to apply nanoliter volumes to flat solid surfaces. Normally, 1–5 μL volumes are employed to deposit the sample on the surface in solid-surface luminescence analysis.

The technique used by von Wandruszka and Hurtubise (13) for the deposition of various samples on sodium acetate for RTP studies is typical of the approaches for dealing with powdered substrates. A 25-μL volume of ethanol was introduced into a 4 × 0.4 cm test tube from a micropipet, and then 1–6 μL volumes of standard or sample solution were added from a 10-μL Hamilton syringe. A constant amount of sodium acetate was added to each tube with a measuring spoon which had the same volume as the depressions in a special sample plate (13). The tube was placed in an oven at 80°C until the ethanol was evaporated. The dry solid was transferred quantitatively to a small mortar and pestle, which was used to gently break up conglomerate particles. The powder was then transferred to a special sample plate.

Spotting a sample on a flat surface with a microsyringe or a micropipet is more rapid than the solvent evaporation technique used for powders. However, particularly in RTP work, a powdered substrate may offer greater selectivity or have a lower luminescence background than flat surface material.

1.2.2. Choice of Substrate

If solid-surface fluorescence analysis is used in conjunction with thin-layer or paper chromatography, one is normally limited by the choice of adsorbent because the chromatographic separation is usually the overriding factor. However, if two chromatographic adsorbents give identical or similar separation of components, but one adsorbent yields greater fluorescence from the components than the other adsorbent, then the former should be chosen for the experiment. Sawicki (14) and Sawicki and Sawicki (15) have discussed several aspects of the use of thin-layer chromatography adsorbents with room-temperature fluorescence (RTF) and low-temperature phosphorescence in air pollution research. Also, Hurtubise (7) has summarized numerous examples of the use of fluorescence with thin-layer chromatography and some examples for paper chromatography. Solid-surface RTP has not yet been used extensively in thin-layer and paper chromatography. This is due to the special conditions needed to induce RTP from a solid surface. As one application, Ford and Hurtubise (16) have shown how the phthalic acid isomers could be separated by thin-layer chromatography and then detected by their RTP signals from the chromatoplate.

Only certain substrates are useful for inducing RTP from adsorbed organic compounds. Filter paper is the most widely used solid surface to date

Table 1.1. Several Solid Surfaces for Inducing RTP

Solid Surface	References
Several brands of filter paper	8, 17–19
Polyacrylic acid–treated filter paper	20
Ion-exchange filter papers	21,22
Sodium acetate	
Powder	8, 23
Peliets	8, 24
Impregnated paper	8, 24, 25
Silica gel chromatoplates with a salt of polyacrylic acid as a binder	8, 26
Polyacrylic acid–sodium chloride or sodium bromide mixtures	8, 27–30
Chalk, H_3BO_3/T-7 clay/NaOH, $CaHPO_4$/T-7 clay/cornstarch/NaOH	8, 31

for inducing RTP. Because several aspects of solid surface RTP are not understood, solid surfaces for inducing RTP are still chosen somewhat empirically. However, several solid substrates are available for RTP, which allows for the analysis of numerous organic compounds.

Vo-Dinh (8) has considered several of the solid substrates available for inducing RTP and various criteria for the selection of solid substrates. No detailed guidelines have been developed despite the importance of selecting the proper solid surface for good sensitivity and selectivity. This points out the need for more research in this particular area. It is important to consult the literature to determine if compounds of a similar nature have been investigated by RTP. Table 1.1 lists several of the solid surfaces that have been used in RTP work. Generally, a good starting surface is filter paper.

1.2.3. Other Experimental Considerations

The control of experimental conditions for inducing RTF from organic compounds adsorbed on solid surfaces is not as important as controlling the experimental conditions for solid surface RTP. This is partially due to the short lifetime of fluorescence and the relatively long lifetime for phosphorescence, although several other factors are involved. Sawicki (14) has considered several examples of earlier work for enhancing and quenching of solid-surface RTF. Hurtubise (7) has discussed a variety of the more recent experimental aspects of solid-surface RTF. It is beyond the scope of this

chapter to consider in detail the experimental conditions for solid-surface RTF, and refs. 7 and 14 can be consulted by the reader. Because of the rapid growth of solid-surface RTP, a general outline of important experimental conditions for RTP will be given.

Vo-Dinh (8) has discussed in detail the variety of experimental needs for solid-surface RTP. Solvents, drying time, moisture, background luminescence, and variation of the properties of similar solid substrates are some of the more important experimental aspects. Solvents can have an important effect on RTP. For example, von Wandruszka and Hurtubise (23) found that common alcohol solvents were useful solvents for the RTP of *p*-aminobenzoic acid adsorbed on sodium acetate. However, aprotic solvents such as ether, acetone, dimethylformamide, and cyclohexane permitted no RTP from *p*-aminobenzoic acid adsorbed on sodium acetate. Ramasamy and Hurtubise (28) showed that a 0.1 *M* HBr–methanol solution of benzo[*f*]quinoline adsorbed onto 0.5% polyacrylic acid–sodium chloride yielded an RTP signal 3.5 times greater than a comparable sample adsorbed from a 0.1 *M* HBr ethanol solution. Dalterio and Hurtubise (29) used ethanol–water solutions of 4-phenylphenol to enhance the RTP of the hydroxyl aromatic adsorbed on 0.8% polyacrylic acid–sodium bromide. Some general considerations for solvents are the solubility of the luminescent component, the chemical compatibility between the solvent and the solid surface, and the purity of the solvent. Ethanol has been the most widely used solvent for RTP work (8).

Water can have a deleterious effect on the RTP of many compounds; thus it is important to dry the sample and substrate prior to the RTP measurement step. Various techniques can be used to dry the samples. For example, blowing hot air onto the sample, keeping the sample inside a desiccator, heating the sample in an oven, and drying the sample under an infrared heating lamp can be employed. Vo-Dinh (8) has given a detailed discussion of drying samples for RTP measurements. McAleese et al. (32) used sodium citrate–treated filter paper to minimize moisture an oxygen quenching of the RTP of several polar compounds.

Background luminescence from solid surfaces can cause problems in both solid-surface fluorescence and phosphorescence. Filter paper and chromatoplates can be developed in ethanol several times to move impurities to one end before spotting the sample. However, this approach is not always satisfactory. In RTP work, Lue-Yen Bower et al. (18) reported several pretreatment methods for substrates. These included baking for various periods at different oven temperatures, eluting with polar and nonpolar solvents, and irradiation with several light sources. They found no combination of substrates and pretreatments that would enhance the signal-to-noise ratio. Bateh and Winefordner (33) conducted further studies on the treatment of cellulose materials as substrates in RTP. The treatments involved

soaking techniques using dioxane–water, diethylenetriamine–pentaacetic acid: water, ether, boiling water, sodium hydroxide, and periodic acid. These authors concluded that pretreatment would improve the adsorption characteristics of filter paper but that phosphorescence background probably would not be reduced substantially. Vo-Dinh (8) has discussed general background correction techniques for use in quantitative RTP, and Su et al. (34) have described a computer approach for background correction in RTP measurements. Recently, McAleese and Dunlap (35) showed that the phosphorescence background of filter paper could be reduced considerably by exposing the filter paper to 285-nm radiation or white light from a xenon lamp for several hours. Of all the approaches investigated so far to reduce background phosphorescence from filter paper, the irradiation technique by McAleese and Dunlap seems to be the most promising.

Variation among different lots of paper and chromatoplates of the same type can contribute to errors in the final results. Bateh and Winefordner (33) compared various lots of one type of filter paper. They found that the various lots were consistent in quality for use in RTP work. The relative standard deviation for the blank signal varied from 3.5 to 4.7%, and the relative standard deviation for the sample (*p*-aminobenzoic acid) changed from 2.2% to 3.5%. Ford and Hurtubise (36) showed that with 100 ng of benzo[*f*]quinoline adsorbed on three different chromatoplates, the relative RTP intensities of 62.0, 54.8, and 57.6 were obtained. To minimize the problem of variations from the same chromatoplate, reference standards and the unknown sample can be applied to the same chromatoplate. In comparing results from chromatoplate to chromatoplate, the same reference standard can be applied to the different chromatoplates and the RTP intensities normalized to the RTP intensity of a given chromatoplate. This approach assumes that instrumental conditions are the same in all measurements. As Vo-Dinh (8) has pointed out, lot-to-lot variation with filter paper is not a major problem because one RTP assay requires little material, and it takes a large number of measurements to consume one lot.

Other factors that the analyst should consider are optimal sample volume, choice of drying gas, optimal excitation and emission wavelengths, sample decomposition, and contamination related to sample holders (8).

1.3. INSTRUMENTATION

1.3.1. Commercial Instruments

Commercial instruments for quantitative thin-layer chromatography have been available since about 1968. These instruments can be used for quantitative and qualitative analysis of a variety of luminescent components

adsorbed on surfaces, such as silica gel, alumina, filter paper, gels, potassium bromide, silicone rubber, and sodium acetate. Several reviews have appeared which describe self-contained units and motorized thin-film scanners as attachments to spectrofluorometers (37–40). Commercial instruments have been modified for use in solid-surface luminescence analysis, and some of these modifications will be discussed in the next section. Some of the companies that supply spectrodensitometers or scanning attachments are Carl Zeiss, Inc.; Kontes Glass Co.; Camag; Perkin-Elmer; and Shimadzu Scientific Instruments, Inc. The yearly Labguide issue of *Analytical Chemistry* and yearly Buyer's Guide edition of *American Laboratory* list several companies that manufacture luminescence instrumentation.

1.3.2. Research Instruments, Modified Instruments, and Accessories

Researchers have designed and built their own instruments and accessories. For the measurement of luminescence from solid surfaces, Hurtubise (7) has discussed instruments for both fluorescence and phosphorescence measurements, while recently Vo-Dinh (8) has considered instruments for phosphorescence measurements. This section will summarize some of the instruments designed, accessories, and innovative experimental techniques. First, equipment for fluorescence measurements will be considered.

1.3.2.1. Fluorescence Equipment

Goldman and Goodall (41) modified a Chromoscan densitometer (manufactured by Joyce Loebl & Co. Ltd., Gateshead, England) to use for absorption measurements for components separated on thin-layer chromatoplates. Even though this instrument was not designed for fluorescence measurements, it is of historical interest and the design principles could be used in the construction of an instrument for fluorescence measurements. The most important modification involved a scanning apparatus of their design that fit into the sample compartment of the Chromoscan densitometer. The scanning apparatus resulted in a sawtooth motion to the chromatoplate. This approach is called the flying-spot technique, and the sawtooth motion of the scanning device allowed for more accurate absorbance measurements. Later Goldman and Goodall (42) built a very sophisticated instrument that permitted the measurement of transmittance of components on silica gel chromatoplates in the ultraviolet region down to 240 nm. The ultraviolet transmittance at 0.5 × 0.5 mm intervals in the two dimensions of the chromatoplate was calculated and recorded on computer tape. Silica gel absorbs radiation in the region 200–280 nm, which is an important consideration in absorption analysis and luminescence analysis.

Goldman and Goodall (42) showed that the absorption by silica gel decreased the transmittance of a silica gel chromatoplate to 0.001 of that normally observed above 280 nm. The computer system employed with the Goldman and Goodall instrument allowed for two iterative procedures at each data point, aligned the data, determined the length of each spot zone, and interpolated the background transmittance in the zone. Goodall (43) modified the instrument just discussed so that transmission could be presented continuously as a negative logarithm on a flatbed recorder in the ultraviolet or visible regions. With the first version of the instrument, the signals were digitized and recorded on paper tape, and sometimes there were delays in processing the tapes. The modified instrument permitted both encoded digital tape and instantaneous graphical recordings. This feature allowed rapid review of results to decide if more accurate computer processing was required.

Instrument theory and design for quantitative absorption analysis of components on solid surfaces such as thin-layer chromatoplates have been considered extensively by Pollak and Boulton (44–51), Boulton and Pollak (52,53), and Pollak (54–59). Pollak and Boulton (50) and Pollak (57,60–62) discussed theoretically the performance of photometric methods for the quantitative evaluation of components on thin-layer chromatoplates by fluorescence. An instrument designed by Pollak and Boulton (63) and Boulton et al. (64) that can be used in the reflectance and transmittance absorption modes and the fluorescence mode will be discussed briefly. This illustrates the similarity of instrument design for absorption and luminescence measurements. The main thrust of Pollak and Boulton's research was in designing an instrument that could be used in all types of photometric determinations which are used in thin-layer chromatography and related techniques. They discussed several aspects for the optimum performance of the instrument. One aspect was a double-beam design that avoided any spatial or time separation of the beams before the beams interacted with the chromatoplate (Fig. 1.1). They adopted the principle of flying-spot scanning to compensate for nonhomogeneous distribution of the sample. The two beam signals were linearized in terms of concentration by logarithm forming for transmittance and simple inversion for reflectance measurements. The reasons for logarithm forming were twofold. First, optical noise was decreased considerably, and the signal was almost independent of the output of the light source. Second, a signal was obtained which was almost a linear function of the concentration. The previous aspect is very important for a flying-spot system to function efficiently. For solid-surface luminescence analysis, linearization is not required (50,57). Pollak (60) mentioned that the double-beam method is advantageous for fluorescence measurements, but the combination of the two beams to minimize optical noise is less

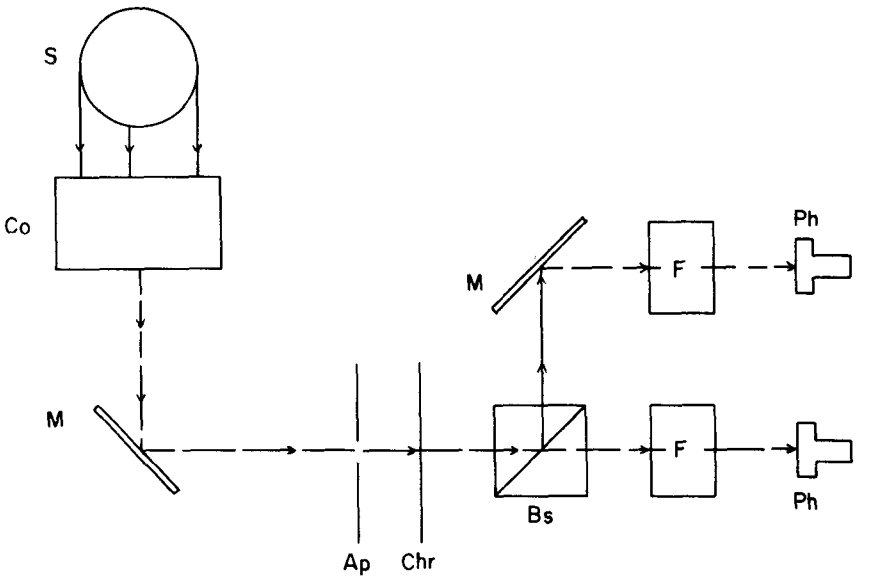

Fig. 1.1. Schematic diagram of the optical path of a double-beam scanning device. S, light source; Co, collimator; M, mirror; Ap, aperture; Chr, chromatogram; Bs, beam splitter; F, optical filter or monochromator; Ph, photodetector. (From ref. 63.)

straightforward compared to transmission or reflection absorption measurements. In addition, the theoretical principles for luminescence measurements from solid surfaces need to be further clarified for instrument design. Fluorescence was measured in the single-beam mode with Pollak and Boulton's instrument. Boulton et al. (64) reported data on the reproducibility and on the stability of their new instrument. The fluorophor, dansyl ethylamine, was separated by paper chromatography and used to evaluate the performance of the instrument. In addition, the instrument was used only in the fluorescence transmission mode. A straight-line relationship was obtained for fluorescence when log area versus log sample (nanograms) was plotted. With 500 ng the deviation from the mean was $\pm 0.07\%$, while with 10 ng the deviation from the mean was $\pm 12\%$. The instrument was capable of handling thin-layer absorbents on supports such as glass, polyethylene, and aluminum, provided that their width was 5 cm or less.

Guilbault and co-worker (65–69) designed equipment and developed methods for the assays of enzymes, substrates, activators, and inhibitors by solid-surface fluorescence analysis. The approach developed by these workers was to place an Aminco filter instrument on its side, and a cell holder was adapted to accept a metal slide on which a silicone rubber pad was

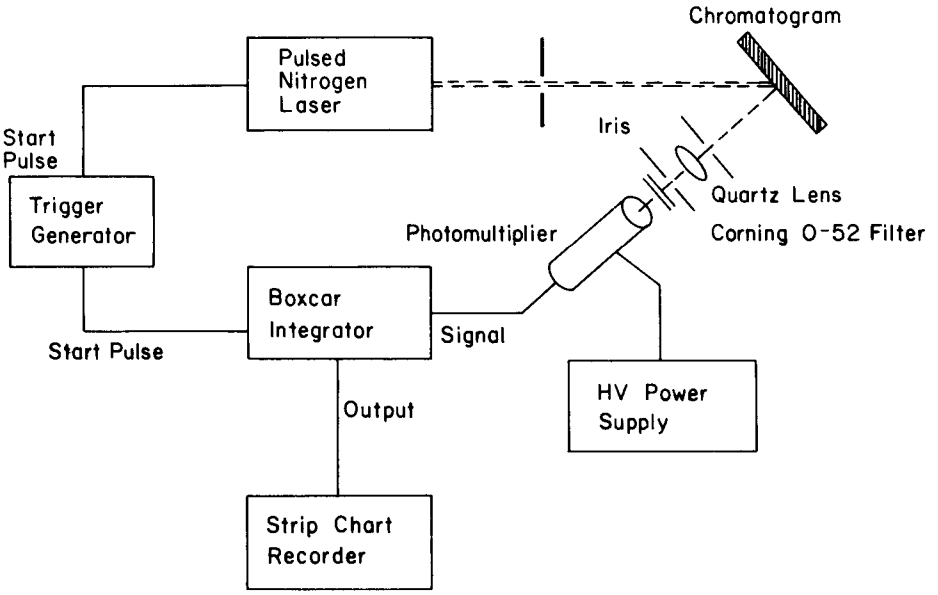

Fig. 1.2. Experimental system for laser-induced fluorescence of aflatoxins on thin-layer chromatoplates. Reprinted with permission from M. R. Berman and R. N. Zare, "Laser fluorescence analysis of chromatograms: sub-nanogram detection of aflatoxins," *Analytical Chemistry*, **47**, 1201 (1975). Copyright 1975 American Chemical Society.

placed that contained the reagents for an assay. The position of the filter fluorometer prevented any reagents from falling off the surface of the pad. The concentration of the substance to be determined was related to the change in fluorescence with respect to time. The silicone rubber pads were made by pressing uncured rubber between a glass plate and a stainless steel mold. Several steps were used in the final preparation of the pads. However, the reagent pads were simple to prepare and hundreds could be easily manufactured at one time (69).

Lasers have been used sparingly in solid-surface fluorescence analysis and certainly much potential exists for the application of lasers in this area. Berman and Zare (70) used time-resolved and wavelength-resolved laser-induced fluorescence for the analysis of aflatoxins on thin-layer chromatoplates. The experimental system is shown in Fig. 1.2. A pulsed nitrogen laser (337.1 nm) was used as the source and an RCA 7265 photomultiplier with appropriate aperatures and wavelength filters was employed for the detection of fluorescence. Berman and Zare stated that the technique of laser fluorescence analysis would have several advantages over fluorescence