A Multi-Source Portable Light Emitting Diode Spectrofluorometer

SAFWAN OBEIDAT, BAOLONG BAI, GARY D. RAYSON,* DEAN M. ANDERSON, ADAM D. PUSCHECK, SERGE Y. LANDAU, and TZACH GLASSER

Box 30001 MSC 3C, Department of Chemistry and Biochemistry, New Mexico State University, Las Cruces, New Mexico 88003-0001 (S.O., B.B., G.D.R., A.D.P.); Box 30001 MSC JER, United States Department of Agriculture, Agriculture Research Service, Jornada Experimental Range, Las Cruces, New Mexico 88003-0003 (D.M.A.); and Department of Natural Resources and Agronomy, Institute of Plant Science, Agriculture Research Organization, The Volcani Centre, Bet Dagan, Israel 50250 (S.Y.L., T.G.)

A portable luminescence spectrofluorometer weighing only 1.5 kg that uses multiple light emitting diodes (LEDs) as excitation sources was developed and evaluated. Excitation using a sequence of seven individual broad-band LED emission sources enabled the generation of excitation-emission spectra using a light weight (<1.5 kg) spectrometer. Limits of detection for rhodamine 6G, rhodamine B, and fluorescein were 2.9, 3.2, and 11.0 nM, respectively. Generation of excitation-emission matrices (EEMs) enabled the analysis of samples containing mixtures of rhodamine B and fluorescein. Buffered saline plant and animal feed extracts were also analyzed using this instrument. These samples included the woody plants *Pistacia lentiscus* (Evergreen pistache or Mastic) and *Philyria latifolia*, and the herbaceous species *Medicago sativa* (alfalfa), *Trifolium spp.* (clover), and a feed concentrate. Application of multi-way principal component analysis (MPCA) to the resulting three-dimensional data sets enabled discernment among these various diet constituents.

Index Headings: **Portable spectrometer; Fluorescence; Light emitting diodes; LEDs; Animal forage; Excitation-emission matrices; EEM.**

INTRODUCTION

Movement of chemical analysis from the laboratory to the field requires the continual development of both portable and transportable instrumentation.[1] These developments have included optical spectroscopic techniques.[2] Because of its inherent sensitivity, photoluminescence instrumentation has also been subjected to minimization efforts.[3] The majority of these efforts have involved the measured emission resulting from excitation at a single wavelength.[3,4] Although this has been successfully applied to the analysis of samples containing a single luminescent species,[4] in-field samples are often considerably more complex, e.g., the determination of pesticides[5] or polycyclic aromatic hydrocarbons in the environment.[6,7] Such complex samples have been successfully analyzed using the corresponding excitation-emission spectral response surfaces. Examples of such applications include the detection of dissolved organic material (DOM),[8] carbamate pesticides, polycyclic aromatic hydrocarbons,[9] and animal diet composition.[10,11]

A portable spectrofluorometer capable of in-field collection of excitation-emission spectra was described by Alarie et al. in 1993.[5] Although this instrument exhibited favorable figures of merit, it was described as having a total mass of 22.5 kilograms. It consisted of two separate units including two scanning monochromators (for selection of excitation and emission wavelengths, respectively), with photomultiplier detection and Xe-arc lamp excitation (11 kg) and a battery power supply (11 kg). The design and construction of an instrument with improved portability and similar analytical capabilities was undertaken.

Light emitting diodes (LEDs) have been increasingly applied as excitation sources for fluorescence measurements. Examples include the determination of inhibitory effects on the monoamine oxidase B (MAO-B) activity of probe compounds using blue LED excitation ($\lambda_{max} = 412$ nm).[12] The fluorescence decay of a protein excited using an ultraviolet LED ($\lambda_{max} = 280$ nm) was also described by McGuinness.[13] Single LEDs have also been used as fluorescence excitation sources in both environmental and industrial applications.[14]

Use of LEDs as excitation sources has been driven by their small size, low power consumption demands, high stability, low cost, and nanosecond pulsing capabilities.[15] These characteristics make them ideal for use in portable instrumentation for fluorescence measurements.

A portable spectrofluorometer developed in our laboratory uses seven different colored LEDs as excitation sources.[15] Use of the battery power supply of a laptop computer to drive the LEDs eliminated the need for an external bulky power supply.

Hart and Jiji[16] have described an LED-based spectrometer that is capable of recording excitation-emission matrices (EEMs) using a two-dimensional charge-coupled device (CCD) camera imaging detector. Although they reported many favorable figures of merit, the instrument was limited to benchtop operation. We believe the present work is the first application of multiple LED devices as separate excitation sources for the generation of EEMs using a portable spectrometer. Similarly to Hart and Jiji, eight different colored LEDs (blue through red, and white) were available to sequentially provide excitation radiation covering most of the visible spectrum (i.e., 380–700 nm). The use of these intense LED devices in conjunction with a miniature spectrograph equipped with an array detector enabled the rapid collection of the EEM for various samples.

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* Author to whom correspondence should be sent. E-mail: garayson@nmsu.edu.

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TABLE I. Colors and central wavelengths for each LED.

LED	Color	Central wavelength (nm)	Part number[a]
1	Red	640	MV8015
2	Orange	620	MV8716
3	Yellow	590	MV8317
4	White	570	TLWY8600
5	Green	525	MV8412
6	Light green	505	MV8G03
7	Blue	435	MV8B11
8	Violet	405	[b]

[a] All indicated parts obtained from Newark Electronics.
[b] LED was obtained from a "UV" illuminator.

The performance of this portable LED-based spectrofluorometer was compared to that of a commercial bench-top fluorometer (Varian, model Cary Eclipse). Solutions containing rhodamine 6G, rhodamine B, fluorescein, riboflavin, and a mixture of rhodamine 6G and fluorescein were investigated using both instruments. Additionally, buffered saline extracts of components found in animal diets used in a parallel study[17] were investigated using the LED instrument.

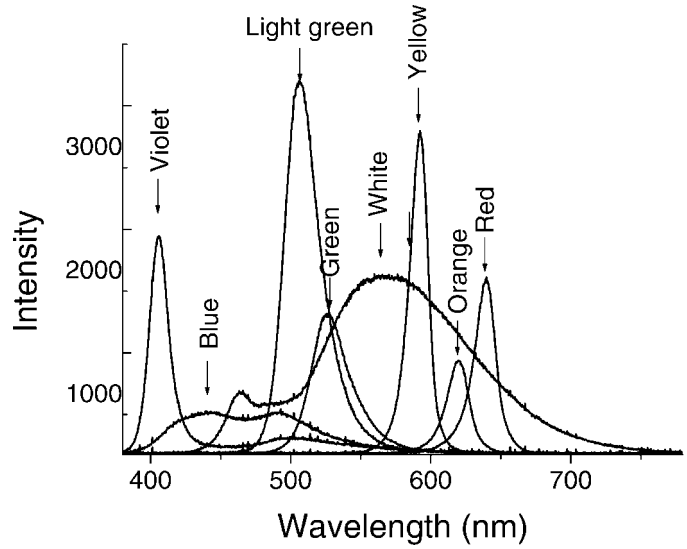

FIG. 1. Emission wavelength of each of the eight LEDs representing the excitation source for the instrument.

EXPERIMENTAL

Instrumentation. Dimensions of the spectrometer were 24.0 × 15.0 × 5.0 cm, with a total mass of 1.5 kg. The dimensions and mass make this instrument portable and applicable for on-site analyses. The instrument includes multiple LED excitation sources, a fiber optic to collect and transfer emitted radiation to a miniature spectrograph configured with a CCD detector (Model USB2000 FL, Ocean Optics, FL), and associated control electronics. A laptop computer operating under Windows XP (Microsoft) provided instrument control for data acquisition, storage, and processing. The computer's normal battery power supply supported both the LED excitation sources and the detector electronics of the spectrometer in addition to the normal operation of the computer's hardware.

Excitation Source. The excitation sources consisted of eight separate, high intensity LEDs (Newark. IL). The color designation and wavelength of maximum emission for each LED is listed in Table I with the available part numbers. The LEDs were symmetrically arranged around a 4.5 mm diameter cylindrical sample chamber. This geometry enabled the illumination of the sample solution by each LED at 90° to the collection optics located equidistant from each source at the base of the cylindrical cuvette. The additional incorporation of a light-tight cap prevented stray radiation from entering the sample chamber during signal acquisition. Although this prohibited the acquisition of true simultaneous excitation-emission spectra,[16] it allowed for the rapid effective scanning of the wavelength ranges provided by each LED without requiring homogeneity of a static sample throughout the cuvette.

The emission spectrum for each LED is shown in Fig. 1. Again, it is readily apparent from these spectra that wavelengths throughout the visible spectrum (i.e., 380–700 nm) are available from this combination of light sources. Although significant spectral overlap was present among the LED sources (Fig. 1),[16] each LED exhibits a unique wavelength of maximum emission (Table I).

Instrumental Control. Each light source was software selected using the digital output of the universal serial bus (i.e., USB) interface unit (DATAQ model DI-148U, Akron, OH). Again the current required for lighting the LEDs for the duration of emission signal integration (typically < 200 ms each) was provided by the computer's battery power supply.

Each LED was activated for a specified period of time (~200 ms). The resulting fluorescence signal was then collected using the CCD array detector of the spectrograph. Power provided through the USB interface was inverted using a potential inverter (7660) to yield 8 volts. Using a voltage adjustment unit (LM 317L), an appropriate voltage and current was applied to drive each selected LED with a transistor (model MPSA 13).

For performance comparison, a fluorometer (Varian, model Cary Eclipse, Palo Alto, CA) was used. It was equipped with a Xenon pulse lamp source pulsed at 80 Hz. The pulse width at half peak height was ~ 2 μs, with a peak power equivalent to 75 W. The wavelength accuracy was (nm) ±1.5 nm. The detector was a photomultiplier tube (Hamamatsu, model R928).

Sample Preparation. Rhodamine 6G and riboflavin (Sigma, St. Louis, MO) and rhodamine B and fluorescein (Aldrich Co., Milwaukee, WI) were each dissolved in absolute ethanol without further purification. Plants and a commercial feed concentrate analyzed in a previous study[17] were again analyzed using this portable device. These samples consisted of filtered extract solutions of each material using a pH 12.5 phosphate buffered saline (PBS) solution. The plant samples consisted of dried samples of *Pistacia. lentiscus* (*P. lentiscus*), *Phillyrea latifolia* (*P. latifolia*), *Medicago sativa* (alfalfa), and *Trifolium spp.* (clover).

Data Analysis. Multi-way principal component analysis (MPCA) is a powerful tool for the processing of three-dimensional data sets.[18] It involves the generation of a representation of the eigenvectors for the covariance or correlation matrix of the original measured variable data matrix.

Such a matrix consists of the measurement of intensity at each *j*th ($j = 1$ to J) emission wavelength for every *k*th excitation wavelength ($k = 1, \ldots, K$) corresponding to the *i*th

TABLE II. Emission and excitation shown for maximum luminescence intensity using both the commercial (Cary Eclipse) and the LED spectrometers.

Sample	Spectrometer	Excitation source/ wavelength (nm)	Emission wavelength (nm)
Rhodamine 6G	LED	Green	560
	Cary Eclipse	525	555
Rhodamine B	LED	Green	568
	Cary Eclipse	525	570
Riboflavin	LED	Violet	500
	Cary Eclipse	435	527
Fluorescein	LED	Light green	522
	Cary Eclipse	505	518

sample ($i = 1, \ldots, I$). In MPCA, the resulting unfolded three-dimensional matrix **Y** (i.e., $I \times J \times K$), is subsequently decomposed into a large two-dimensional matrix, **X**, followed by conventional PCA. Simply stated, MPCA is the summation of the product of score vectors (t_r) and loading vectors (P_r), plus a residual or error array (E), which is minimized in a least squares sense (Eq. 1):

$$X = \sum_{r=1}^{R} t_r \otimes p_r + E \quad (1)$$

Each element of score vectors (t_r) corresponds to a particular data set. The loading vectors (p_r) are then directions of a maximum variability and define the reduced dimension space (R). In most cases, only a few principal components are required to express the maximum variability. This is especially true for data with a high degree of correlation ($R << \min(I, JK)$). The choice of R is made such that most of the systematic variability of the data can be described by these few principal components.[19] MPCA was therefore applied to data sets generated using the present LED-based, multi-dimension luminescence spectrometer.

RESULTS AND DISCUSSION

Instrument Analytical Performance Characterization. Portable instrument designs should exhibit several important characteristics. Any portable instrument should use a power supply that is suitable for on-site analysis in terms of weight and availability. The instrument itself should also have a small size. It should be fast and easy to operate. It should be reliable and robust. Instrument affordability and low cost of sample analysis are other desirable features. The portable spectrofluorometer developed in our laboratories meets many of these criteria. Similar analytical figures of merit were determined for both this instrument and a commercial spectrofluorometer (Cary Eclipse) in parallel for comparison purposes. (It should be noted that the commercial spectrometer was not operated under optimal conditions but was set to closely emulate those settings of the portable LED spectrometer.) Because the maximum band pass of the commercial instrument (2.5 nm)

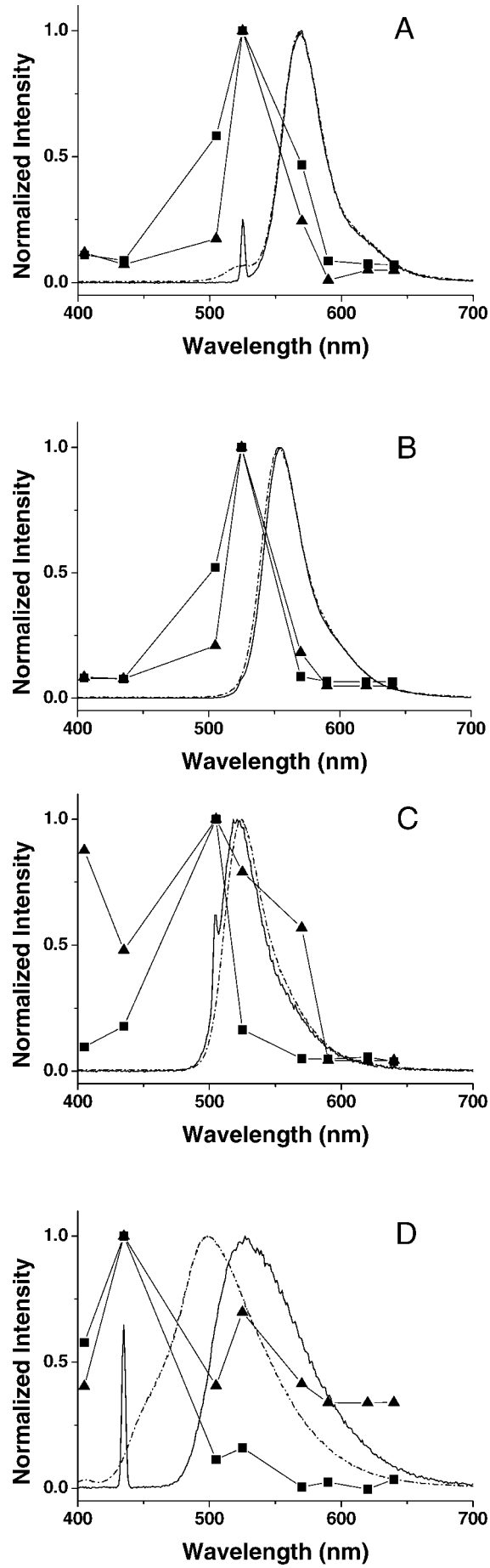

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FIG. 2. Excitation by the portable (▲) and Eclipse (■) spectrofluorometers and emission recorded by the portable (– - –) and Eclipse (——) profiles obtained for 1.0 μM of (**A**) rhodamine 6G, (**B**) rhodamine B, (**C**) fluorescein, and (**D**) riboflavin.

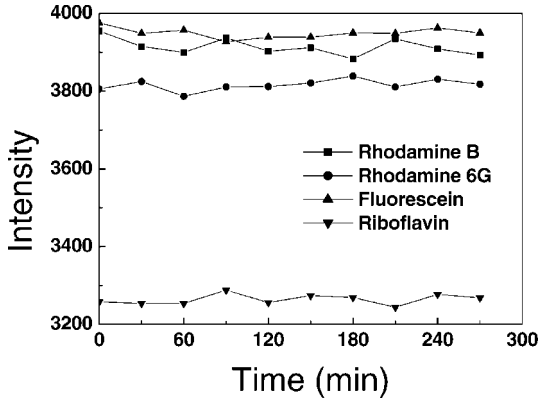

FIG. 3. The intensity of the emission signal against the time for 1.0 μM solutions of rhodamine 6G, rhodamine B excited by the green LED, fluorescein excited by the light green LED, and riboflavin excited by the violet LED.

was considerably less than the spectral bandwidth of each LED (10–80 nm), selected wavelengths of excitation were used corresponding to λ_{max} of each LED source (Table I).

The validity of this operating parameter for comparison of these two different instruments was supported by the similarities in observed maximum luminescence wavelengths observed for 1.0 μM solutions of rhodamine 6G, rhodamine B, fluorescein, and riboflavin using each spectrometer (Table II).

The corresponding excitation and emission spectra are shown in Figs. 2A–2D. The portable device yielded data exhibiting spectral shifts and broader peaks compared to those from the commercial spectrometer. These differences can be attributed to the broader range of wavelengths present with each LED source compared to the narrow wavelength range selected by the excitation monochromator within the commercial instrument. These similar spectra were each collected in less than 200 ms using the LED spectrometer.

Reproducibility and the Limits of Detection. Both short-term and long-term stabilities of the measured emission signals using this portable fluorometer were investigated. This was accomplished by recording the fluorescence spectrum of solutions of each of these four fluorophores ten times once every 30 minutes. Figure 3 shows the intensity as a function of time for each 1.0 μM solution. A long-term coefficient of variation (CV) of less than 15% was calculated for the measured maximum emission wavelength for both rhodamine 6G and fluorescein. Table III lists the signal-to-noise ratios and percent relative standard deviation for each 1.0 μM sample solution, the standard deviation of the corresponding blank signals, and the resulting limits of detection (S/N = 3) for each analyte using this instrument. These values compare favorably with those reported by Aleri et al. using their (22 kg) portable spectrometer.[5]

TABLE III. Signal-to-noise ratio (S/N), percentage relative standard deviation (% RSD), and limit of detection (LOD) for each 1.0 μM solution of analytes in ethanol using the portable spectrofluorometer.

Analyte	LED	% RSD	S/N	LOD (nM)
Rhodamine 6G	Green	0.096	1043	2.9
Fluorescein	Light green	0.37	274	11
Rhodamine B	Green	0.089	1125	3.2
Riboflavin	Violet	0.098	1023	18

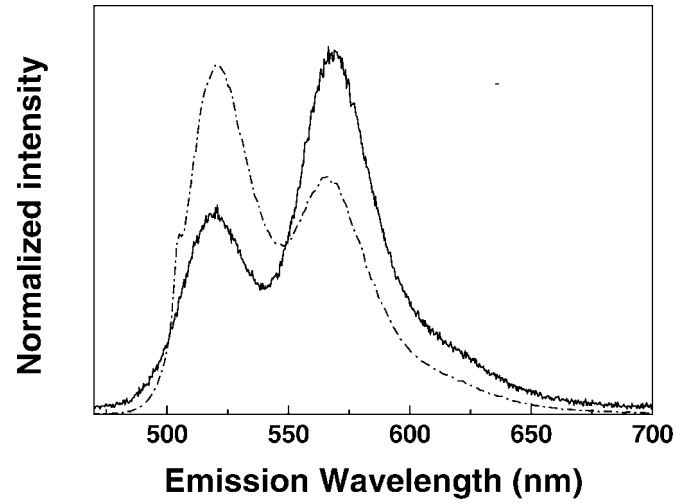

FIG. 4. Emission scans for a mixture of rhodamine B (1×10^{-7} M) and fluorescein (1 μM) excited at 505 nm using the commercial instrument (– – –) and using the light green LED (——, central 505 nm) for the portable spectrofluorometer.

Pure Chemical Mixtures. The analytical utility of any instrument capable of collecting complete EEMs lies in the ability to analyze samples containing a mixture of fluorophores. Therefore, a mixture of these same fluorophores was analyzed using the LED spectrometer. Because of similarities in the measured spectra (Table II and Fig. 2), only a solution containing 0.10 μM rhodamine B and 1.0 μM fluorescein was investigated. For comparison purposes, samples containing the same mixture were also analyzed using the commercial spectrofluorometer. Emission spectra (Figs. 4 and 5) were collected from each instrument using these two different sources of incident radiation. Specifically, excitation wavelengths of 505 and 525 nm and the light green and green LEDs (Table I) were used. Both spectra easily resolved the spectral features (Fig. 4) associated with each analyte when excited at either 505.0 nm or using the light green LED. An inversion of relative intensities of these fluorophores using these two

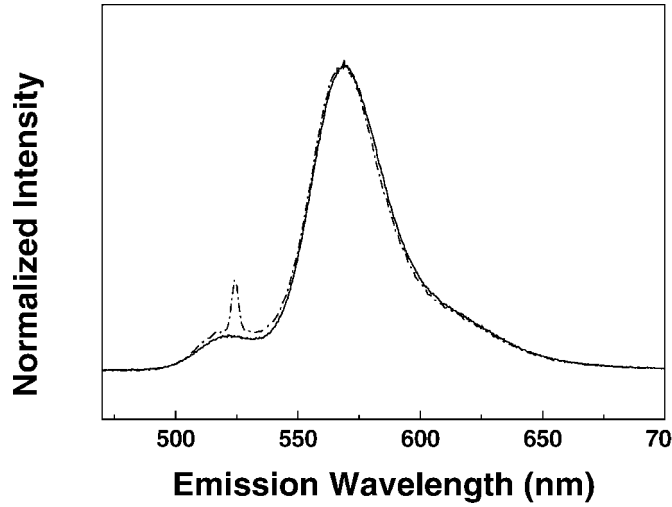

FIG. 5. Emission scans for the mixture with excitation at 525 nm using the commercial instrument (– – –) and using the green LED (——, central 525 nm) for the portable spectrofluorometer.

TABLE IV. Sample codes and relative standard deviations for the clusters appearing in the MPCA model (Fig. 7).

Sample	Sample identification
Alfalfa hay	1–5
Clover hay	6–10
Feed concentrate	11–15
P. lentiscus	16–20
P. latifolia	21–25
Blank	26–30

instruments may be explained by the comparatively wide excitation bandwidth exhibited by the LED (10–80 nm) relative to the excitation bandpass of the commercial instrument (2.5 nm).

Emission spectra for the same rhodamine B and fluorescein mixture at either 525.0 nm or using the green LED as the source of incident radiation (Fig. 5) were similar, with the fluorescein feature considerably less intense than the feature for the rhodamine B. The rhodamine B emission signal at 568 nm was more intense than the signal obtained for the fluorescein at 521 nm. This is consistent with the data collected during the excitation-emission scan using both instruments. At an excitation wavelength of 525 nm, rhodamine B tends to result in the most intense emission signal.

Analysis of two-, three-, and four-component mixtures of these fluorophores was attempted using MPCA with a model derived from the spectral signatures of each component. Although the model was able to account for 100% of the variance using only four principal components, these initial efforts were unsuccessful in identifying the composition of these mixtures. This is the focus of ongoing work pertaining to the further development of this spectrometer.

Animal Diet Components. Work in our laboratory has been directed toward the utility of luminescence spectroscopy to differentiate various plant species within animal diets.[12,17] The availability of a portable spectrometer for in-field analysis of plant and fecal samples would greatly facilitate those studies. As a result, this portable spectrometer was evaluated using a series of animal diet samples included in another study.[17] Specifically, these samples consisted of phosphate buffered saline extracts (pH 12) of alfalfa, clover, *P. lentiscus*, *P. latifolia*, and a feed concentrate.

Five replicates of each sample and its corresponding blank were coded and analyzed (Table IV). Excitation-emission spectra for each sample were collected by sequentially illuminating it with each LED and recording the resulting emission spectrum. These spectra were concatenated using MATLAB 7.0.1 into a three-dimensional data matrix ($2048 \times 30 \times 8$). Following mean centering of these data, MPCA was applied.

In an effort to ascertain whether samples with intensities below the detection level could be identified, sample emission spectra were not blank corrected. Instead, spectra corresponding to the blanks were treated as separate samples. Figure 6 shows the scores plot resulting from the application of MPCA to this data set. The first two principal components accounted for more than 80% of the total variance in the data. Readily visible is the ability of this model to segregate each of the five diet components and the blank signal.

Separation of these clusters reflects the variation in fluorescence signature among these samples. The greater the distance between samples, the greater the difference in their spectral signature. Also, the same MPCA model (Fig. 6) indicates sample-to-sample variations. These depend on the sample nature itself and vary from one species to another, as can be seen in Fig. 6. For further sample classification analysis, soft independent modeling of class analogy (SIMCA) was used. Generally, SIMCA is a combination of PCA models for each class in the data set.[20] Each group of samples belonging to the same diet (class) was investigated using separate PCA models. Figure 7 indicates that one or two principal components were responsible for the total variation in each. Sample distances within each model (T^2) were evaluated relative to the residuals (Q) for all of the samples. Each of the six classes exhibited clustering separate from the others using a 95% confidence limit. This indicates that samples belonging to the same diet fall in a separate class.

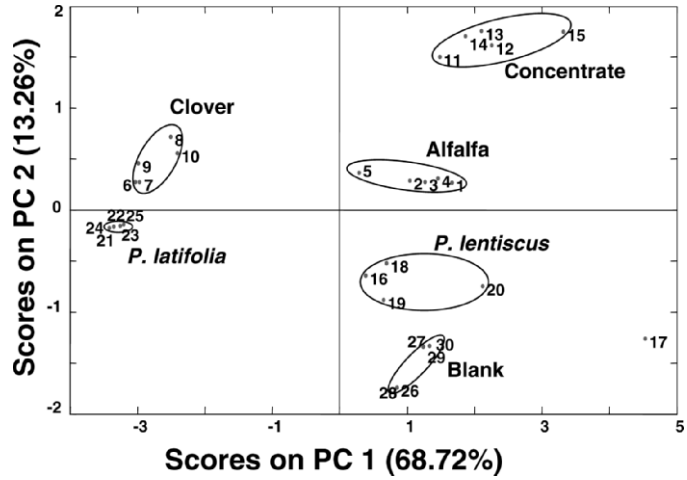

FIG. 6. Two-dimensional MPCA model showing five resolved clusters. Each cluster represents different plant species and an additional cluster represents the blank.

CONCLUSION

A light-weight prototype portable spectrofluorometer capable of being powered by a typical laptop computer that uses multiple LEDs as excitation sources exhibited promising performance when four chemical compounds, four different

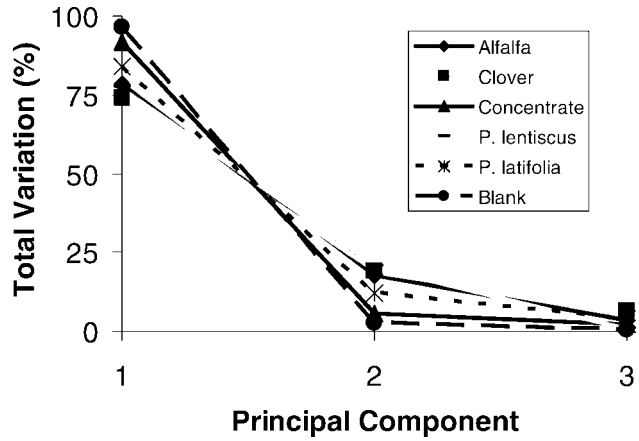

FIG. 7. The percentage of the total variation associated with the first three principal components in each class for plant identification.

plant extracts, and a commercial feed supplement were evaluated using fluorescence measurements. This new prototype portable spectrofluorometer yielded comparable spectra to those obtained from a commercial spectrofluorometer under similar operational constraints. Limits of detection (LOD) for these analytes varied from 2.67 to 11.0 nM. The spectrofluorometer was also capable of analyzing a mixture (i.e., rhodamine B and fluorescein). Extracts from different animal diet components (i.e., *P. lentiscus*, *P. latifolia*, alfalfa, clover, and a feed concentrate) were successfully discerned using MPCA. Class identification was also achieved using sub PCA models (SIMCA). All samples belonging to the same diet were identified as belonging to separate classes within a 95% confidence limit. Although this device has yet to be applied to in-field studies, the portability features and the analytical performance of this portable spectrofluorometer offer a promising technique for future applications to on-site analysis of free-ranging animal diets.

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