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APPLICATION OF MOLECULAR FLUORESCENCE SPECTROSCOPY FOR THE ELUCIDATION OF DIET COMPOSITION FOR FREE-RANGING HERBIVORES

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The determination of the dietary intake of domestic livestock is necessary for their effective management. This becomes more difficult when the animals are allowed to feed on native flora in open rangeland. Efforts in the past to provide information regarding a particular animal's dietary habits have used either highly invasive techniques or methods that are time and labor intensive and very subjective. The development of instrumentation to enable the investigation of samples for the elucidation of the animals' dietary intake is a challenging analytical problem.

Diets are currently determined using various techniques. Probably the oldest and least automated technique is that of direct observation of foraging animals (Bjugstad et al. 1970). This bite count procedure (Doherty et al. 1999) is often inappropriate for some animal species and landscapes, and the effect of observer influence may influence those data. All other animal-based techniques require sampling digesta from 1 or more locations along the digestive tract (Holechek et al. 1984). The microhistological technique is the predominant method currently used for identifying dietary botanical composition of free-ranging animals (Bennett et al. 1999, Bontti et al. 1999), with feces being the most commonly used samples since they represent a broad spatial and temporal range of dietary intakes (Norbury and Sanson 1992). Plant fragments too small to identify taxonomically, the overestimation of indigestible materials and the underestimation of readily digested materials such as leaf material, are just some of the

problems that beset this technique. The amount of time required for sample preparation and analysis prevents its usefulness for real-time decision-making. Two recent automated techniques for determining botanical composition of animal diets having shorter analytical time requirements are near infrared reflectance spectroscopy (NIRS; Garcia-Criado et al. 1991, Walker et al. 1998) and laser induced fluorescence (LIF; Anderson et al. 1996, Anderson et al. 1998). Although NIRS (Foley et al. 1998) and fluorometry (Lakowitz 1983, Guilbault 1990) both rely on molecular properties, they differ in several important ways.

Our solution to this daunting analytical problem entails the use of multi-dimensional fluorescence measurements for the rapid, objective identification of those plants eaten by specific animals. Although the application of molecular fluorescence greatly simplifies the number of molecular species detected, as compared to the broader technique of NIRS, it enables the collection of multidimensional response surfaces to define the species-specific signatures. The present work has been restricted to the acquisition and analysis of two-dimensional fluorescence data sets for the initial assessment of this technique.

Initial work has concentrated on the generation of a spectral database for each of six plants. These plants were selected because of their consumption by free-ranging sheep and cattle in the northern Chihuahuan desert of the southwestern United States. The plants were collected from with tie USDA Jornada Experimental Range in southern New Mexico. These plants include species of grasses, (tobosa hay and mesa dropseed, *Pleuraphis mutica* and *Sporobolus flexuosus*, respectively), forbs (spectacle pod and pale globemallow, *Dimorphocarpa wislizenii* and *Sphaeralcea incana*, respectively), and shrubs (tarbush and four-wing salt bush, *Flourensia cernua* and *Atriplex canescens*, respectively). Normalized average spectra of chloroform extracts from each of these species are shown in Figures 1 and 2. Each of these spectra is representative of spectra from triplicate samples of at least five different plants (Table 1). An excitation wavelength of 369 nm from a 500 W Hg/Xe-arc lamp was used for each determination. The emission spectra were collected using a 1.0 m monochromator with a band pass of 1.2 nm.

Table 1. Sampling criteria used.

Plant form	Plant species	Plants	Locations	Spectra Averaged
Grass	Mesa Dropseed	5	1	15
Grass	Tobosa	5	3	45
Forb	Spectaclepod	5	1	15
Forb	Pale Globemallow	5	1	15
Shrub	Tarbush	5	2	30
Shrub	Fourwing Saltbush	5	3	45

The application of post-processing algorithms to these fluorescence emission spectra has also been pursued. Excitations of extract solutions from five plants of each species at 369 nm as yielded significant spectral signatures in the wavelength region of 400-600 nm (Figures 1-2). The spectra shown in Figure 1 suggest that the distinguishing of different species using their respective spectral fluorescence signatures would be relatively simple. However, as shown in Figure 2, the spectral response curves with single-wavelength excitation may not be readily assigned to a specific plant species. One approach to the generation of identifying criteria has been the deconvolution of the measured spectral envelope into separate components. The application of this to spectra from each of several species is illustrated in Figure 3. It should be noted that the purpose of this deconvolution is not to elucidate the fluorescence spectra of each component of the plant extracts but to provide spectroscopically relevant parameters for the application of pattern recognition and regression analysis algorithms for the identification of plant species in complex mixtures (i.e., extracts of post-digestive samples). Samples of this spectral database will be presented. The ability of each of several algorithms to determine the composition of pre- and post-digestive samples will be discussed.

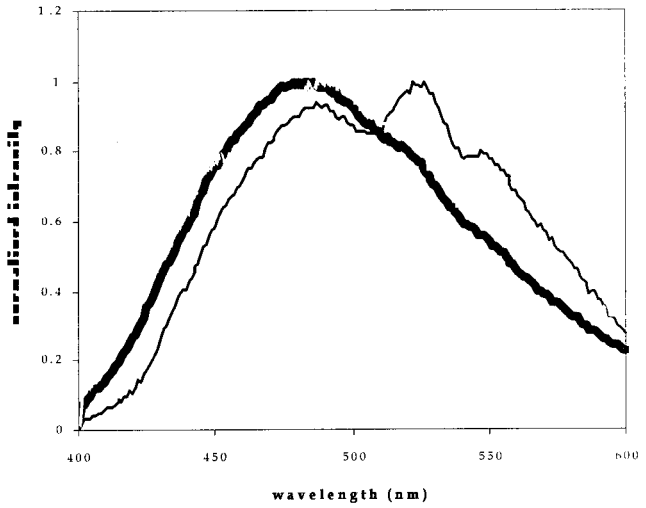


Figure 1. Fluorescence spectra of extracts of plants in chloroform for spectaclepod (__), tarbush (__), and tobosa hay (....).

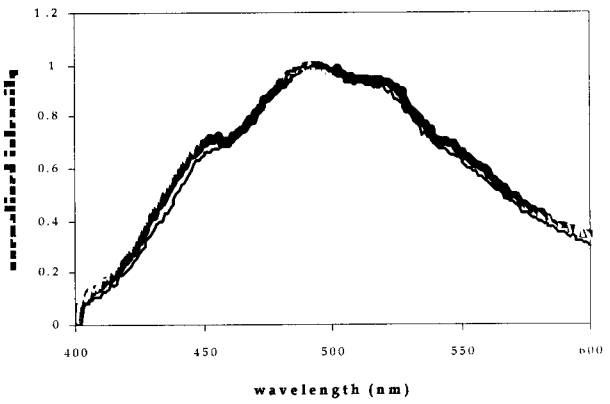
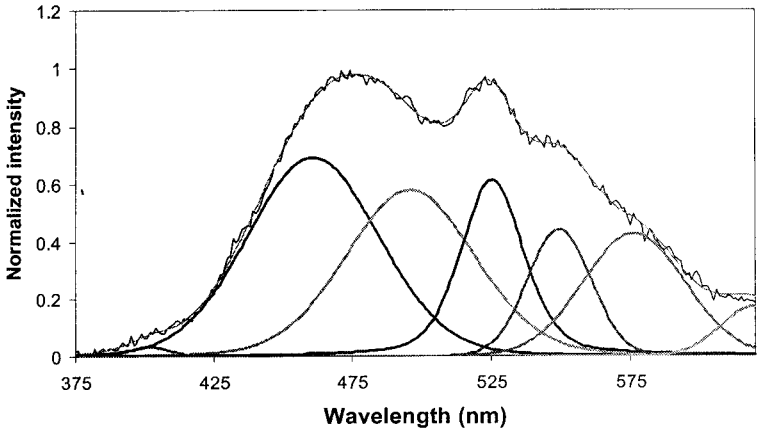
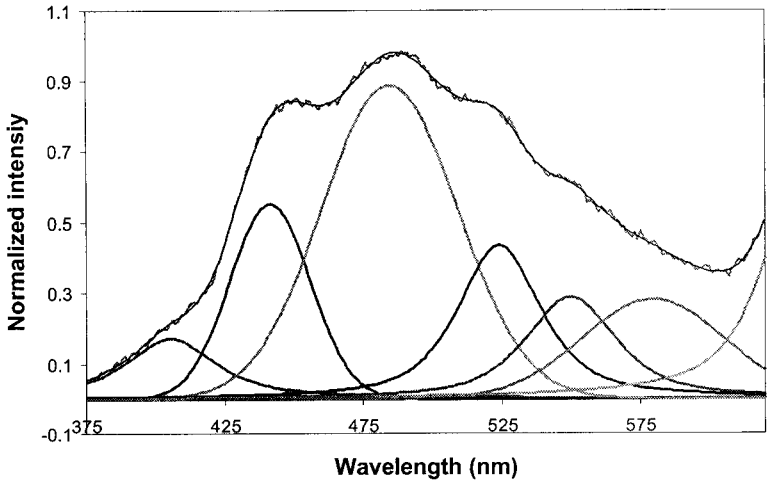


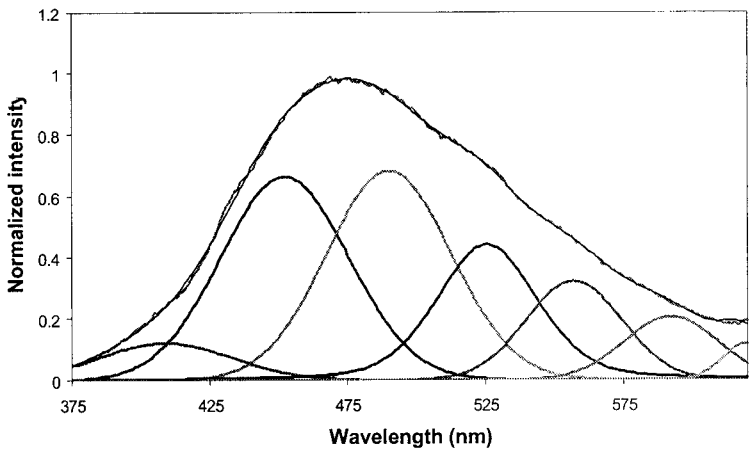
Figure 2. Fluorescence spectra of extracts of plants in chloroform for fourwing saltbush (__), pale globemallow (__), and mesa dropseed (....).



(a)



(b)



(c)

Figure 3. Deconvolution of fluorescence spectra for tarbush (a), spectacle pod (b), and pale globemallow.

In conclusion, it has been shown that each the three plant forms, grasses, forbs, and shrubs may be distinguished using their fluorescence signatures. The use of UV radiation for the excitation of the plant material seems to be applicable. Identifying plant forms by fluorescence is the first step. This technique can lead toward the use of fluorescence in the identification of plant species. If one can characterize a spectral signature for an individual plant species, an extremely large amount of information can be gained. This technique can lead the way to a rapid, accurate and precise method in investigating the dietary habits of rangeland animals.

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