ABSTRACT

Multivariate Analysis of Luminescence Spectra as a Means of Determining Postmortem Interval

Patricia A. Diamond, M.S.

Co-Charipersons: Kenneth W. Busch Ph.D.
Marianna A. Busch Ph.D.

Post-mortem interval (PMI) is the time elapsed since a person died. Currently there is no accurate method for determining PMI of skeletal remains. Existing methods are best suited for deciding whether a bone is of forensic interest, meaning less than fifty years old. This is a problem for areas that have extreme climates, specifically those areas that experience high heat and high humidity, which accelerate decomposition.

The objective of this study was to develop a method to accurately predict PMI of skeletal remains through luminescence studies of the change in the intensity of the luminol reaction with skeletal remains over time. Previous research in the area demonstrated that a correlation can be found between the PMI and the change in intensity over long periods of time. This research aims to demonstrate a similar correlation with PMI and to correctly predict the PMI of skeletal remains over much shorter age ranges.

Multivariate Analysis of Luminescence Spectra as a Means
of Determining Postmortem Interval

by

Patricia A. Diamond, B.S.

A Thesis

Approved by the Department of Chemistry and Biochemistry

_____
Patrick J. Farmer, Ph.D., Chairperson

Submitted to the Graduate Faculty of
Baylor University in Partial Fulfillment of the
Requirements for the Degree
of
Master of Science

Approved by the Thesis Committee

_____
Kenneth W. Busch, Ph.D., Co-Chairperson

_____
Marianna A. Busch, Ph.D., Co-Chairperson

_____
Patrick J. Farmer, Ph.D.

_____
Stephen L. Gipson, Ph.D.

_____
Carlos E. Manzanares, Ph.D.

_____
Lori E. Baker, Ph.D.

Accepted by the Graduate School
August 2011

_____
J. Larry Lyon, Ph.D., Dean

Page bearing signatures is kept on file in the Graduate School.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGMENTS

I'd like to thank God. I believe I have felt His presence more during this experience than any other time in my life. Without Him I would not be here.

I also have to thank my wonderful husband Chris. I can't thank you enough for the love and support that you've shown over the past few years. I know it was hard for you to push me, and even though I resisted I appreciated your effort.

To my family: I feel as though I've been neglecting you all throughout the research and writing process. Mom and Dad thank you for understanding and always giving your love. To my siblings: Thank you for listening to me even when you didn't understand what I was talking about. Special thanks too for Sarah and Aunt Mary for all the encouragement and the late night phone calls.

Thank you to all my friends, Beth, Robin, Amanda, Richard, Jose, Chris, Jason and Nelson who have offered their support each in their own way. Thank you to my girlfriends, Becky Vanessa, Yany, Tiffany, Elky and Melinda. You have all been so helpful to me, and there are not enough words for me to express how much I appreciated the hugs and all the calm words you've given me. I look forward to the day when we can all get together to just have fun without thinking about chemistry! Special thanks to Jody. I've learned so much thanks to you, and I was a better student because of your help. It was you that made working in the lab fun, and I missed you so much after you had moved on to bigger and better things.

I also want to thank some of the professors here at Baylor. Dr. Rizalia Klausmeyer, as an undergrad you were my favorite teacher, but in the past few years you've become a good friend. Thank you so much for all the advice and all the help you've given me. Dr. Karen Humphrey, I want to thank you for your friendship, your support, and your willingness to loan me books and supplies. Also, Dr. Baldridge, thank you for listening and brightening my days.

Thank you to my advisors Dr. Kenneth Busch and Dr. Marianna Busch. I appreciated that you gave me a chance and always believed in me. You were both very encouraging and always seemed to know when I was in need of a good pep talk.

I also want to thank the other members of my committee. Dr. Patrick Farmer and Dr. Stephen Gipson, thank you for reading my thesis and for being here today. Dr. Carlos Manzanares, thank you for the advice and support you've given me. I've enjoyed getting to know you during our lunches this past year. Also, I want to thank Dr. Lori Baker who offered her expertise from the beginning and working with me throughout the research process.

Dr. James Karban, thank you so much for supporting me as I continued my education. Finally, I want to thank the Chemistry department for funding all of my research, and I want to thank Baylor University for providing me the opportunity to return to school as a full-time employee. As I move on in my career I hope to represent Baylor well in all that I do.

CHAPTER ONE

Introduction

Postmortem Interval

Postmortem interval (PMI) is an important part of any death investigation. It helps to set the scene and can help point to possible suspects in a criminal investigation. However, in many cases PMI is hard to predict with accuracy due to the number of factors affecting the rate of decomposition. [1] Some of the factors may include the body type and clothing of the victim[1]. Also, the location of the body, the weather, and presence of insects or scavengers could also affect the rate of decay.[1] Due to the variety of factors involved, the investigator's ability to obtain accurate estimates of PMI increases the earlier a body is discovered.[1]

During the first few hours after death, PMI may be determined by estimating algor mortis (body cooling), rigor mortis (the stiffening of limbs), and livor mortis (blood settling in the body).[1] Once the decay reaches a point where these medical diagnostics are no longer useful, the ability to accurately determine PMI decreases. Investigators then turn to entomological (insect interaction with corpse) evidence.[2] While insect activity may be greatly influenced by environmental factors, it can be relied upon for approximately seven weeks after death.[2] However, as the soft tissue continues to decay and eventually becomes skeletonized, none of the entomological methods can be relied upon for an accurate estimation of PMI.[3] With no established

methods for determining PMI of skeletal remains, a forensic pathologist must rely on previous experience to make a prediction.[4, 5]

The majority of research on PMI has focused on the earlier stages of decomposition with very little emphasis on skeletal remains. Most of the accepted techniques for dating skeletal remains are more appropriately used with ancient remains (thousands of years), rather than those that are of forensic interest or less than fifty years old.[4, 6-16] For example, radiocarbon dating has been routinely used by archeologists.[9, 13, 14] The half-life of ^{14}C is 5730 years which is not practical for determining PMI.[9, 13, 14] Some efforts have been made to utilize radioactive nuclei such as ^{3}H, ^{210}Pb, ^{210}Po, ^{90}Sr, and ^{137}Cs, which have shorter half-lives and could be slightly more useful in a forensic context.[8, 12, 15-17] A positive correlation between the radionucleotide concentrations and PMI is promising, and there are some who believe these methods have the most potential use in forensic investigations.[18] However, much more research is required for these methods to be considered in a legal investigation.

A majority of techniques that have shown the most promise for measuring PMI focus on the degradation of organic material in the bone, specifically proteins. Six methods have had the most consistent results. The first involved measuring nitrogen content and would determine the PMI based on the percentage of nitrogen in the bone[4]. Two methods focused on the amino acid content of the bone[4]. The first studied total amino acid content, while the other focused on the presence of prolines to determine PMI.[4] Two other methods, the benzidene reaction and the UV fluorescence, were limited in their use, but were successful in distinguishing modern

from ancient remains.[4] The benzidene reaction depended on the amount of blood remnants left in the bone, since an enzyme in the blood catalyzed the reaction much like in the luminol reaction described later.[4, 5] Benzidine's use decreased after it was discovered to be carcinogenic.[19] The UV fluorescence depended on the general organic content of the bone.[4, 45] The final test considered functional by Knight and Lauder was an immunological test.[45] For the majority of the cases, immunological activity could only be seen for up to five years.[4] However a few positive results were seen as late as 150 years after death, which led to reliability issues with that test.[4, 5]

In a multivariate study, Castellano *et al.* tested nine factors of bone composition including total lipids, triglycerides, cholesterol, free fatty acids, total proteins, zinc, iron , manganese and phosphorous.[20] It was determined that out of the nine factors tested, the decomposition of protein had the best correlation to PMI.[20] Results were improved upon by adding triglycerides and cholesterol.[20] However, only 11.29% of the variance was explained by the addition of triglycerides and cholesterol, thus leaving 74.31% of the variance to be explained by protein composition.[20]

Previous research at Baylor addressed the problem of determining PMI through a multivariate approach.[21] Using Near-Infrared (NIR) spectroscopy, Dogra attempted to track water loss and protein decomposition of bone.[21] Figure 1-1 shows the general change in spectra over time due to the breakdown of protein and water lost from the samples.[21] This was the first study to attempt to trace the decomposition of bone over a small period of time versus decades.[21] All samples were tested over a period of three months.[21] In a series of control studies and

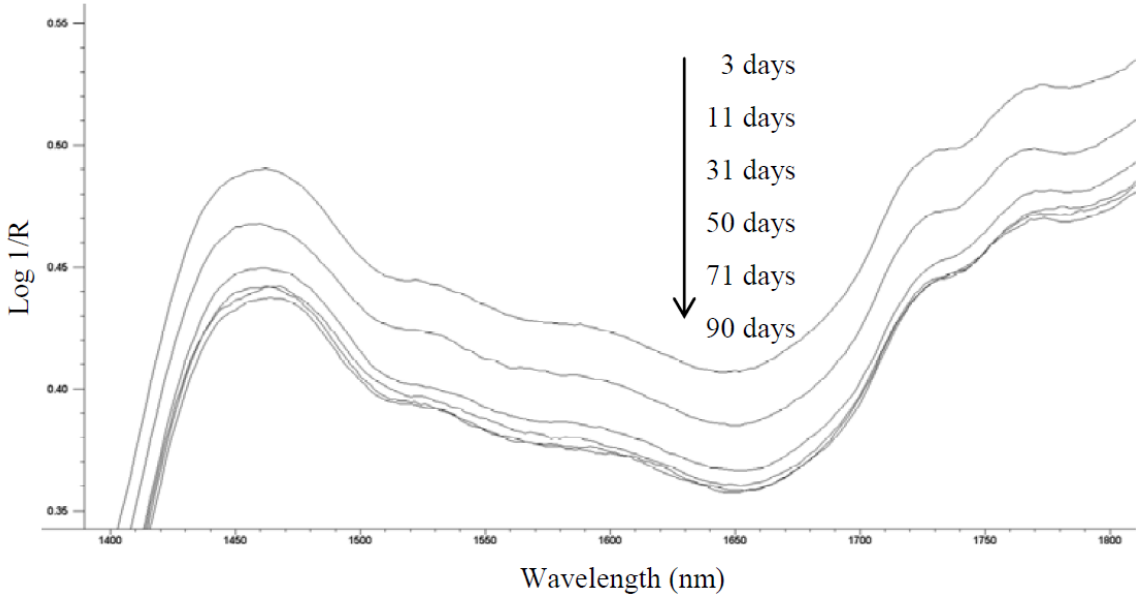


Figure 1-1. General change in NIR spectra with time.[21]

a brief field study, spectra were collected over a three-month period and used to develop PLS-1 models.[21] Predictions from the various models gave average absolute errors ranging from ± 2 days to ±14 days.[21] While the study showed promise, there was some uncertainty as to its effectiveness since water loss would vary greatly depending on the environment.

A different approach by Introna, Di Vella and Campobasso shifted from analyzing the decomposition of total protein composition to studying the decomposition of hemoglobin from blood remnants in the bone.[11] This was accomplished by exposing the bone powder to a luminol solution, and the chemiluminescence was recorded through a camera and analyzed visually.[11] The results showed a decrease in intensity of the light as the age of the samples increased.[11] Their samples ranged in age from less than 3 years up to 80 years in age.[11]

More recently, Creamer and Buck attempted to improve upon the original study by Introna, but much of the premise of the study was similar to the original. A smaller number of samples was used with ages ranging from six months to 375 years.[22] However instead of using a camera to record the reaction, Creamer and Buck used a photomultipier to measure the intensity of the light emitted for each sample.[22] The research yielded similar results to those found in the original study.[22] Figure 1-2 shows the intensity of the reaction of hemoglobin with the luminol decreased over time.[22] Still both studies were only successful in separating recent bones from historic bones.

Bone Composition

All bones are a blend of two phases, an organic phase and an inorganic phase.[23]A diagram of bone can be seen in Figure 1-3. The rigid, inorganic phase is made of calcium salts with hydroxyapatite, $Ca_5(PO_4)_3(OH)$, being the most abundant.[23] The elastic, flexible, organic phase is made of mostly collagen.[23] There are three types of bone tissue: the cortical, the cancellous, and the subchondral.[23] Each type of bone tissue has the same basic structure, but the difference between the three comes from their interaction with the blood supply of the body.[23] The cortical bone, which makes up the exterior of the skeleton as well as the bone shafts, consists of blood vessels surrounded by bone tissue.[23] Cancellous, or spongy bone, is the opposite of the cortical bone where the blood vessels surround the bone tissue.[23] The vascularization of subchondral bone is a balance between the cancellous and cortical bone.[23]

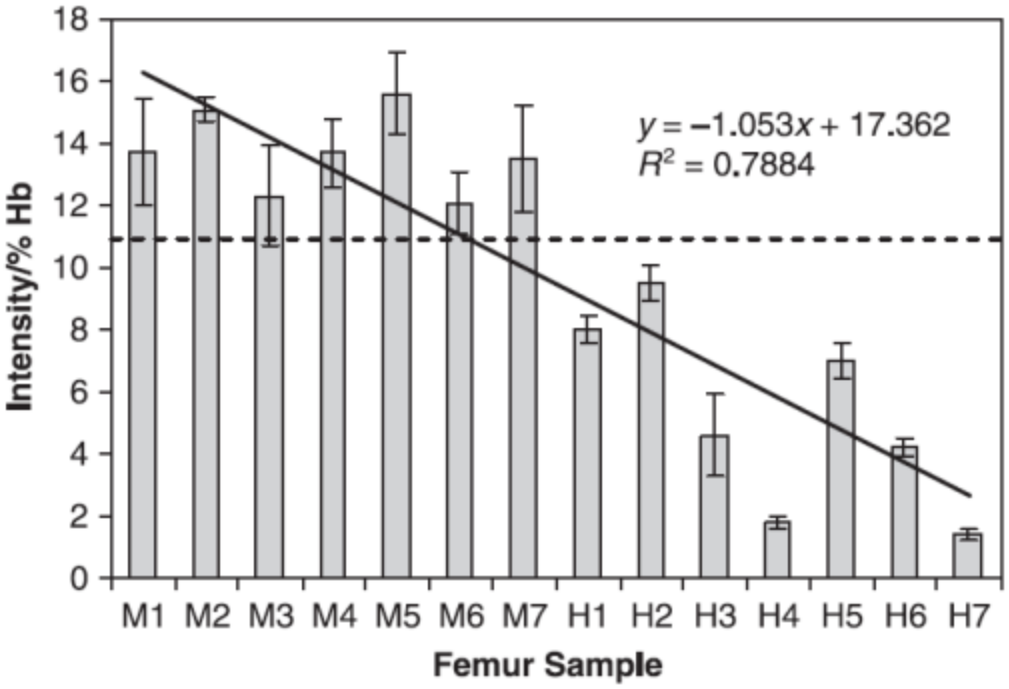


Figure 1.2 A plot demonstrating the decreasing intensity of the luminol reaction as the skeletal remains increase in age, with M samples representing modern samples and H samples representing historical samples.[22]

Cancellous bone tissue contains the bone marrow.[24] There are two types of bone marrow: the yellow and the red.[24] Yellow marrow, which is found mostly in long bones of adults, is responsible for production of fat cells.[24] On the other hand, red marrow is filled with hemopoietic tissue, which is responsible for producing red blood cells.[24] The red marrow also produces heme, which is an iron containing portion of hemoglobin.[24] In adults, the red marrow can be found in the ends of the humerus and the femur as well as in the ribs, sternum, vertebrae and pelvis.[24]

Hemoglobin is a protein seen in Figure 1.4, which is found in red blood cells and is responsible for the transportation of oxygen throughout the bodies of vertebrates.[25] Specifically in mammals, 97% of the content of dry red blood cells or approximately 35% of the total content is composed of hemoglobin.[26]

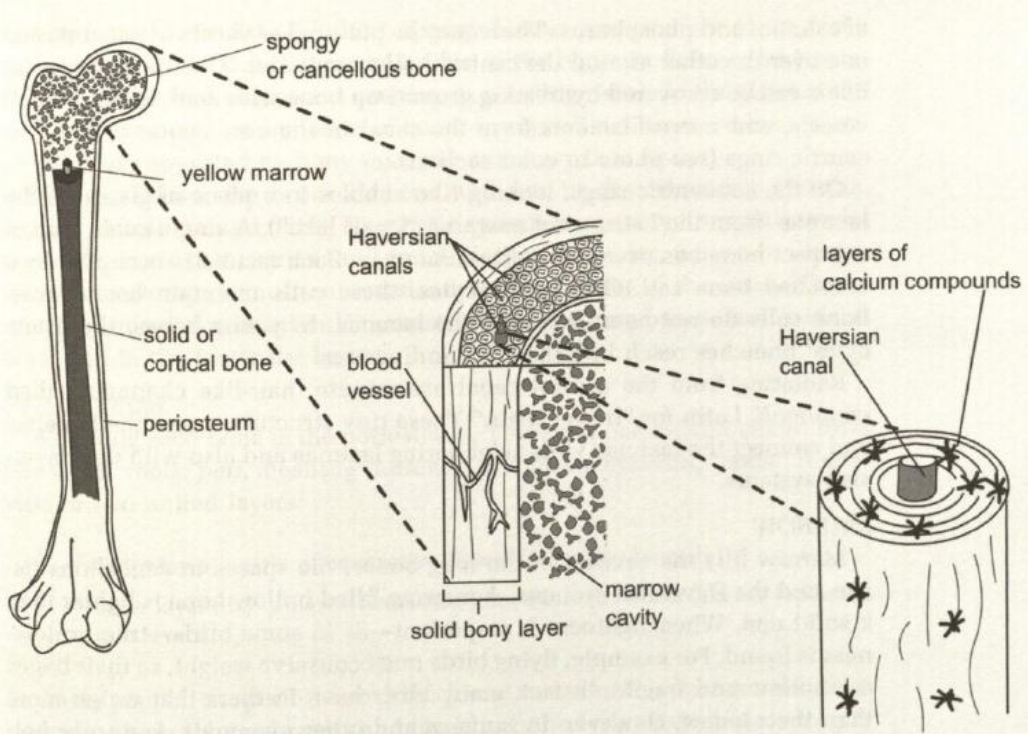


Figure 1-3. Diagram of bone.[24]

In humans, the protein is composed of subunits, which are closely associated with a heme group.[28, 29] The heme is a nonprotein group composed of an iron ion within a porphyrin ring, which can be seen in Figure 1-4.[27] In the body, the iron ion has an oxidation state of +2,[27,30,31]but upon degradation it rapidly oxidizes to Fe^{+3}.[31-36] The heme molecules and the red blood cells are produced by the red marrow within bones such as the ribs, sternum, vertebrae and in some of the long bones as well.[24]

Luminol

The chemiluminescent properties of 5-amino-2,3-dihydro-1,4-phthalazienedione, more commonly known as luminol, were first reported in 1928 by Albrecht.[37] Since that time, the reaction mechanism has been thoroughly studied. The potential pathways the reaction can take have been summarized by Mereyani

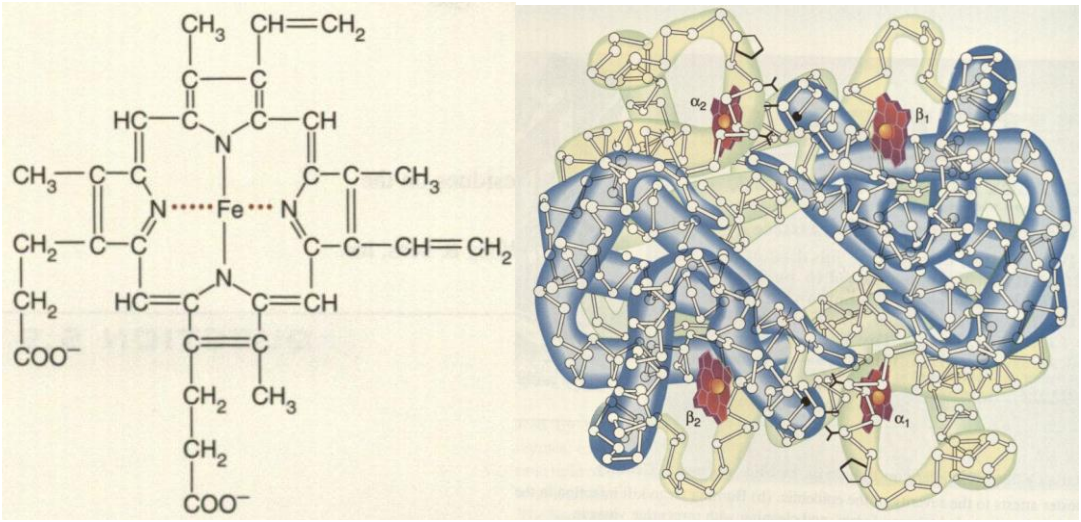


Figure 1-4. a)The heme group[27]. b) Hemoglobin[27]

and can be seen in Figure 1.5[38]. As demonstrated in the figure, all the thick arrows represent the paths leading to the formation of 3-aminophthalate and the emission of light.[38] The remaining pathways are referred to as the dark reactions because ultimately no light is produced.[38, 39]

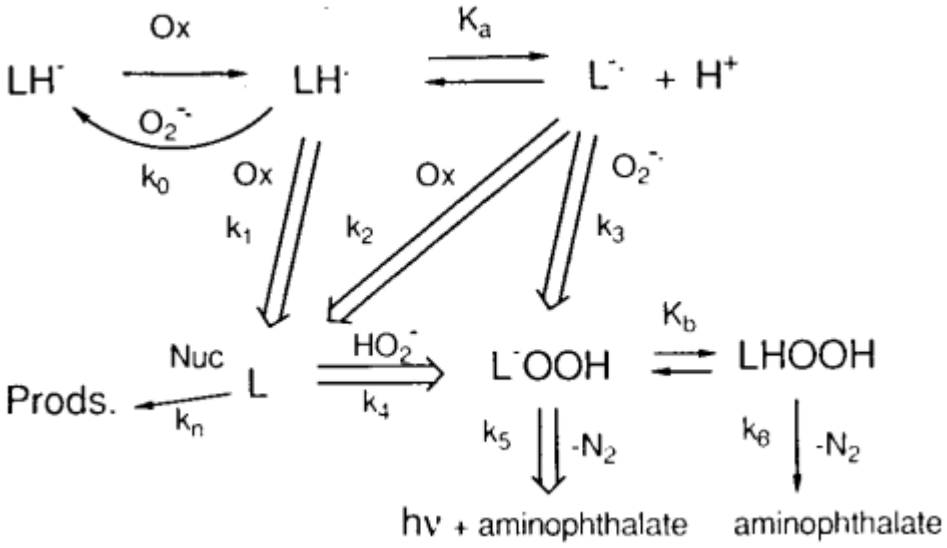


Figure 1-5. The chemical pathways of the luminol reaction.[38]

A proposed mechanism for the chemiluminescent reaction of luminol is shown in Figure 1-6.[38, 40-42] A wide variety of catalysts can be used in the reaction. Heme containing proteins such as hemoglobin and horseradish peroxidase are commonly used and require basic conditions, typically with a pH of 10. In the mechanism presented, the iron of the hemoglobin has an oxidation state of Fe^{3+} [27]which is oxidized by hydrogen peroxide to Fe^{4+} as seen in the reaction. The hydrogen peroxide breaks down into a hydroxide ion and a hydroxide radical. Luminol (1) loses hydrogen to form an anion in (2). A hydroxide radical takes an electron from (2) to create (3), producing an hydroxide ion in the process. A hydroxide takes the hydrogen from the remaining NH group to create (4). Oxygen then removes the electron from the radical creating (5), which rearranges forming a double bond between the two nitrogens shown in (6). An $^{-}$OOH anion then attacks one of the carbonyls, forming (7). A lone pair from an oxygen in the $^{-}$OOH group attacks the other carbonyl, creating a bridge seen in the dianion (8). Another hydroxide ion takes the hydrogen from the other oxygen in the bridge, leading to (9). Further rearrangement of electrons reforms two carbonyls, nitrogen is lost, and the light emitting species 3-aminophthalate dianion (10) is produced.

In a multivariate study performed by Bastos *et al*. researchers alternately varied the concentrations of the luminol, the hemin and the hydrogen peroxide to observe the changes in the response curves for initial chemiluminescence intensity (a), the area under the emission curve (b) and the observed rate constants shown(c) in Figure 1-7.[43] They found that the best conditions to achieve high chemiluminescence intensity emissions required high hemin concentration and medium concentrations

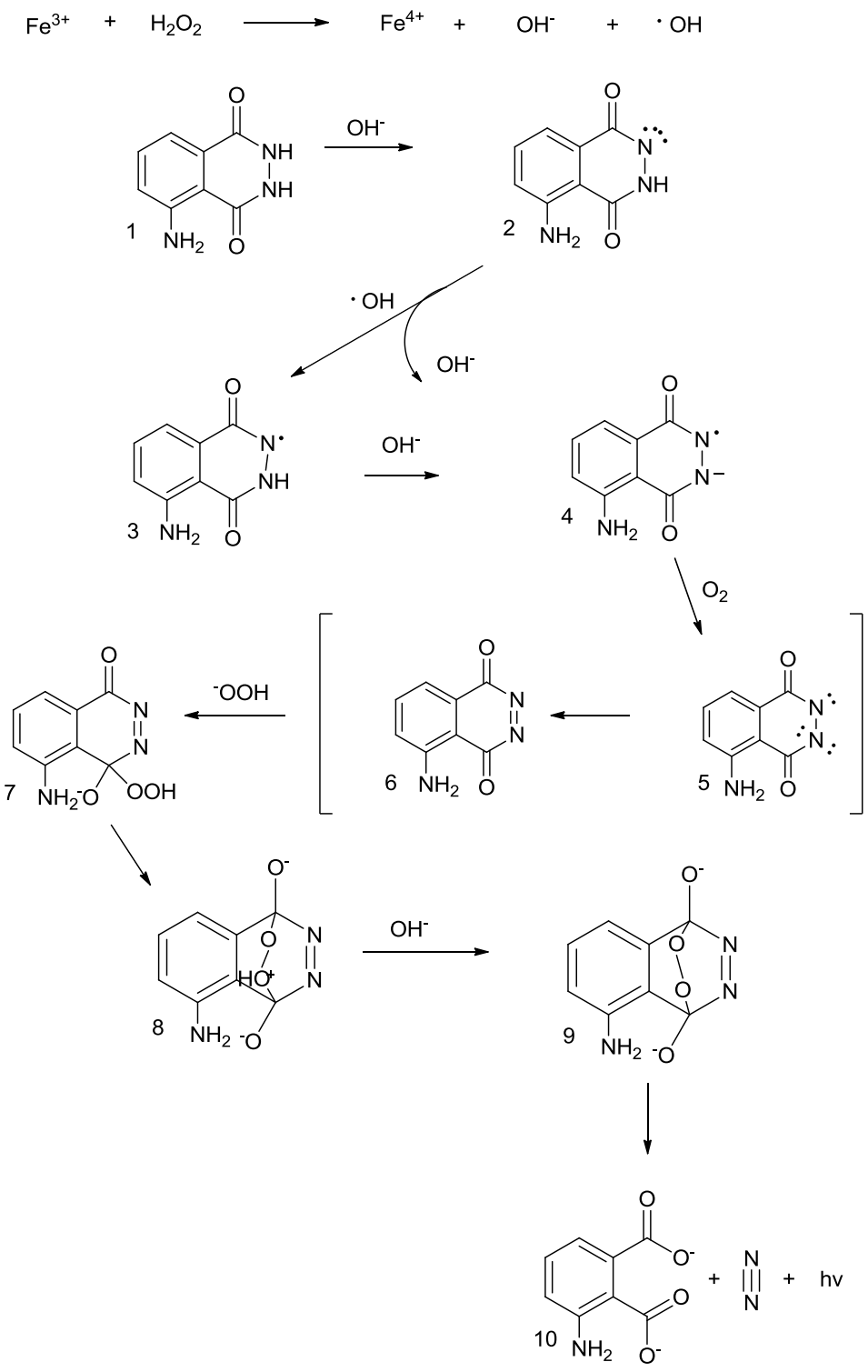
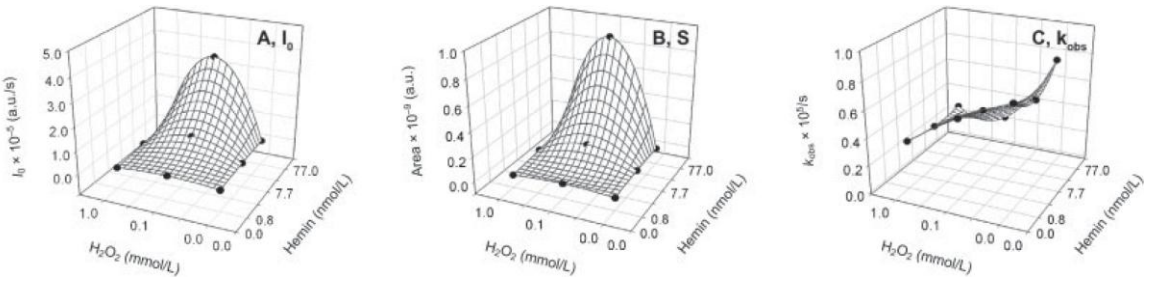


Figure 1-6. Proposed mechanism of the luminol reaction.[38, 40-42]

for both the luminol and the hydrogen peroxide.[43] High levels of peroxide actually led to the destruction of hemin, and high levels of luminol led to very fast reaction rates that recycled the hemin[43]. The researchers also determined, in agreement with previous research, that the likely rate determining steps for the luminol reaction were first the oxidation of the luminol anion, which is dependent on the concentrations of all compounds involved[41, 43], and secondly the addition of the peroxide to diazaquinone, represented in steps 6-7 in the proposed reaction shown in Figure 1-6, which would affect the reaction at low peroxide concentrations.[43]

Luminol's chemiluminescent reaction is used quite often in analytical chemistry. Often the reaction is used to detect the presence of hydrogen peroxide in a system or as a way to observe a reaction with a peroxide-labeled compound.[45] The use of this reaction is also well established in the forensic field where it has been used



(a) (b) (c)

Figure 1-7. The response curves from the multivariate factorial anyalsis of the (a) initial intensity, I_0, (b) area under emission curve, S, and (c) observed rate constant, K_{obs} and their dependence on the concentrations of hemin and peroxide with a concentration of 1.0 mmol/L of luminol. [44]

since the 1930's to detect the presence of blood in criminal investigations.[46] The luminol reaction is highly sensitive and can detect hematin in a dilution of $1:10^8$.[47] Specht determined that luminol reacts better with dried, decomposed blood than it

does with fresh blood.[48] In general, blood is stable enough to last over extended periods of time and able to withstand exposure to heat as well.[46]

Luminol's use has gained popularity in the forensic field for many reasons besides its sensitivity. The luminol solution is not corrosive, it does not stain, it won't react with other biological fluids, and most importantly it will not destroy the blood in a sample.[46] Also, one of its biggest benefits is that the reaction is accepted as a valid test by both the legal and scientific communities.[46]

However, the luminol test does have some drawbacks. The main disadvantage affecting its use for investigating PMI is that luminol does not react specifically with blood or iron.[46] Many plant peroxidases, metals and other chemicals can react with luminol to give false positives.[46] Two of the most common substances responsible for producing false positives are copper compounds and bleach.[46] Another drawback of conventional chemiluminescence is the transient nature of the signal, which requires careful timing of data acquisition.

Purpose

The purpose of this research was to examine the feasibility of using fluorescence spectroscopy to measure the products of luminol's reaction with blood remnants of skeletal remains while using chemometric modeling of spectral data to accurately determine PMI. While luminol has been used as a means of determining PMI, it has not been studied with young samples with a relatively small age range.

CHAPTER TWO

Chemistry Background Information

Luminescence

Molecular luminescence spectrometry consists of three optical methods: fluorescence, phosphorescence and chemiluminescence .[49] The first two are similar in that the absorption of photons leads to excitation, while with chemiluminescence the excitation results from a chemical reaction.[49]

In fluorescence and phosphorescence, an electron must be moved from the ground state to an excited state.[49] In the ground state, the Pauli Exclusion principle is followed, which means no two electrons may have exactly the same four quantum numbers.[49] This means that for two electrons to share an orbital they must have opposing spins.[49] In the excited singlet state the electrons still have opposing spins.[49] However, in the triplet excited state, the spin state of electrons in the higher energy level becomes parallel (both electrons have the same spin).[49]

Once a photon is absorbed, there are a few deactivation processes the electron can follow on its way back down to the ground state.[49] Figure 2-1 shows the energy diagram of a photoluminescent system.[49] Of all the processes shown, the only two which lead to the emission of a photon are fluorescence and phosphorescence.[49] Vibrational relaxation, intersystem crossing, as well as internal and external conversion do not lead to the emission of a photon and are referred to as

radiationless processes.[49] The process that allows for the shortest lifetime in the excited state will be the preferred process.[49]

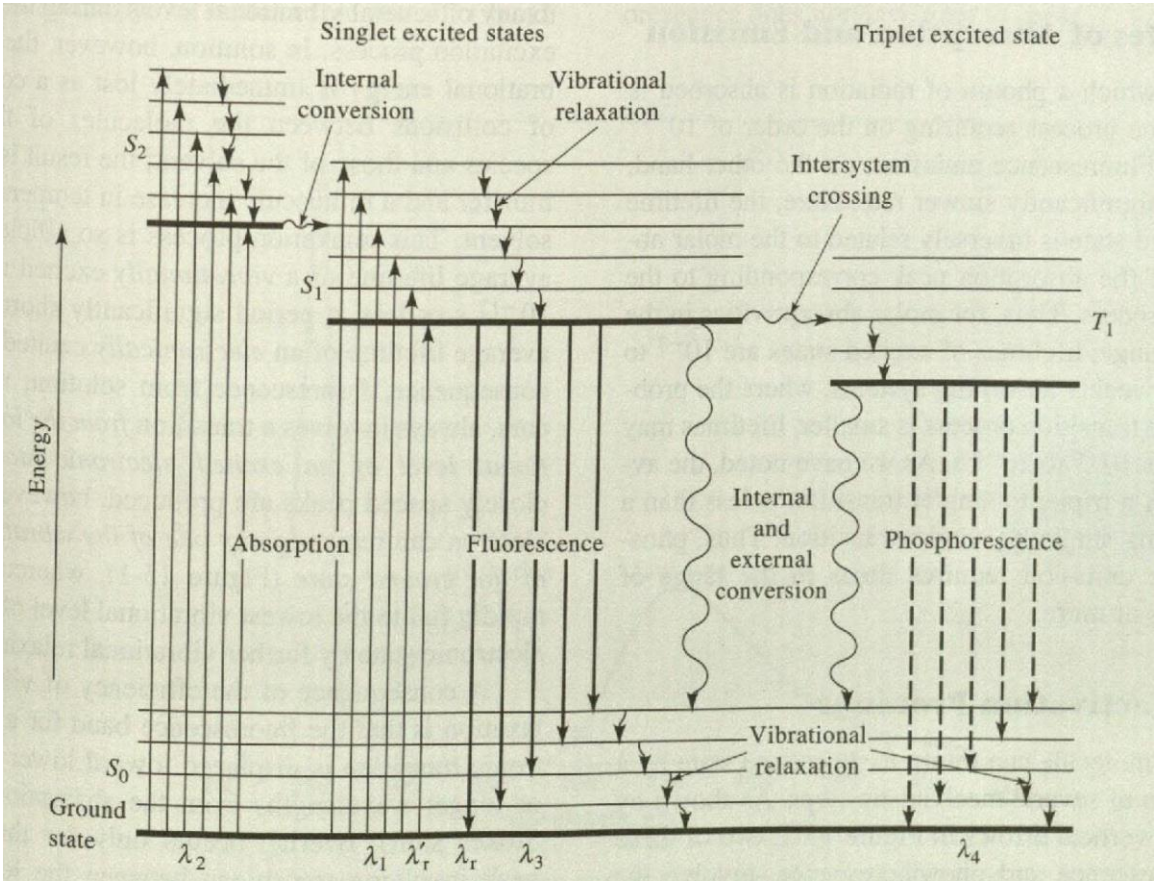


Figure 2-1. Energy diagram for a photoluminescent system[49]

In chemiluminescence (CL), the process is very similar to photoluminescence except that it is a chemical reaction that provides the energy necessary to generate an excited state.[42,49] This may happen in one of three ways. First a high energy molecule can decompose into lower energy molecules represented by[42]

$$A \rightarrow {}^*B \quad \text{or} \quad {}^*B + C \tag{1}$$

Secondly, CL can be caused by an exothermic reaction represented by[42]

$$A + B \longrightarrow {}^*C + D \tag{2}$$

or *C

Finally, chemiluminescence can occur through electron transfer reactions as represented by[42]

$$A^+ + B^- \longrightarrow \begin{matrix} *A + B \\ A + *B \end{matrix} \quad (3)$$

All three processes require that the reaction produce enough energy to reach the excited state.[42] It is possible for energy shortfalls to be made up with thermal energy.[42] Also required is an efficient pathway back to the ground state.[42]

The luminol reaction, discussed at length in the introduction, is an example of a chemiluminescent reaction caused by an exothermic reaction as was seen in Equation 2.[42]

Fluorescence Spectroscopy

Fluorescence measurements are made using either a fluorometer or a spectrofluorometer.[49, 50] The two types of instruments share many of the same basic components, as seen in Figure 2-2.[49] A source emits a beam that first is sent through a monochromator or excitation filter.[49, 50] The light which passes through will allow for excitation to occur, but will exclude the wavelength of fluorescence emission.[49, 50]

Fluorescence spreads in all directions, but it is best to observe the fluorescence at right angles where there is less scattering, and interference from the excitation source is at a minimum.[49, 50] The emitted radiation must go through another filter or monochromator before reaching the photransducer, typically a photomultiplier tube, which is responsible for generating an electrical signal from the fluorescence to be measured.[49, 50] A reference beam is also necessary.[49, 50] This beam is sent through an

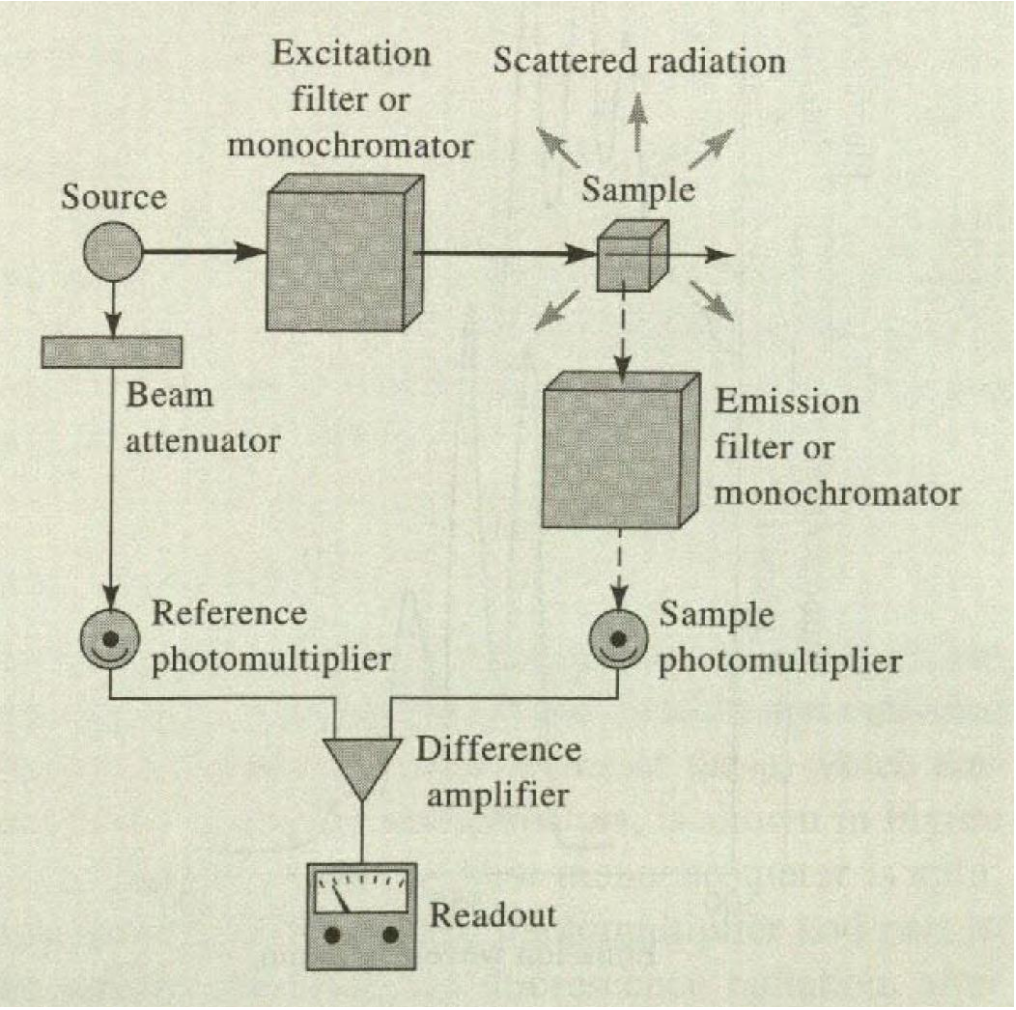


Figure 2-2 Basic components of a fluorometer or spectrofluorometer.[49]

attenuator which lowers the power to match that of the fluorescence radiation. [49, 50] Both the signals are then amplified by a difference amplifier which sends the information to a recorder. [49, 50]

One of the biggest differences in the two types of instruments is their lamps.[49] Fluorometers use a low-pressure mercury vapor lamp. [49] Spectrofluorometers tend to use a high-pressure xenon arc lamp. [49] Spectrofluorometers also differ from fluorometers in that they are able to produce both excitation and emission spectra. [49]

Chemometrics

In 1971, a Swedish chemist, named Svante Wold, first introduced the term chemometrics.[55, 56] The word can be split into two main parts, chemo and metrics. Chemo means chemical, and metrics or metron meaning to measure.[51] The literal translation of the word is to measure chemistry.[51] However, the literal translation may seem much more simplistic than Wold meant for it to be; a fairly accepted definition would be that chemometrics is concerned with using mathematical approaches to analyze chemical information.[57, 58]

Multivariate Data in Chemistry

Chemical systems are, by nature, complicated or multivariate, which necessitates a form of data reduction to make interpretation possible.[51] Chemometrics provides a way to take advantage of multivariate data based on models, multidimentional spaces, and by making projections onto these multidimensional spaces.[51] However, Wold found that the main concepts used in the field of statistics, such as concepts concerning populations and multivariate normal distributions, do not work as well for chemical applications.[51] Chemists tend to be interested in the properties of individual chemical systems rather than those properties of a population of chemical systems.[51] Similarly, multivariate normal distributions do not adequately explain surface tensions, enzymatic rate reactions or other chemical properties.[51]

The discipline of chemometrics has proven to be quite useful. In the academic setting, chemometrics may be used in any area where large amounts of data are acquired.[51] Also, it has been found to be useful in the industrial setting,

particularly in quality assurance and process monitoring.[51] Chemometric methods can be divided into two types: regression methods and pattern recognition methods.[51,52] Regression methods are used to determine if there is a mathematical relationship between two sets of information, also known as the predictor and the response variables.[52] Once a relationship is established, it can allow the prediction of response values of future samples based on the predictor variables.[52] Pattern recognition methods may be used to find the similarities and differences between samples based on their predictor variables.[52] In the research presented in this thesis, the predictor variables are measured spectral data.

Multivariate Regression

There are many methods used to perform multivariate regression, such as multiple linear regression (MLR), principal components regression (PCR) and partial least-squares regression (PLSR).[51-53] Of these methods, the best suited for spectral data are PCR and PLSR.[51-54] These two methods work particularly well because they can be used (1) when the there is a large number of predictor variables, and (2) they work even when correlation exists between the predictor variables within the original data set.[51-53] MLR has two requirements that only allow for limited use with spectral data.[51-52] The first requirement for MLR is that there must be more samples than predictor variables.[51-52] This requirement is difficult to satisfy because there could be hundreds of data points in a spectrum, and in most cases there are not hundreds of samples.[51-52] The second requirement is that the variables must be linearly independent of each other.[51-52] This requirement is often not met because spectral data are naturally collinear.[51-52]

All regression methods involve two processes: calibration and prediction.[51-53] During the calibration phase, a mathematical model is constructed to relate the spectral data and the response variables.[51-53] The data set consists of samples with known response variables that have been measured independently.[51-53] In the calibration phase, a data array or matrix composed of two smaller matrices, **X** and **Y**, is produced. The **X** matrix contains the spectral data, while the **Y** matrix contains the response variables.[51-53] The relationship between the two matrices is then mathematically estimated.[51-53] Matrix notation of this relationship may be expressed as $\mathbf{y} = \mathbf{Xb}$, where **y** is a column vector of a single response variable, and **b** is the regression vector containing the coefficients of the model.[51-53] In solving the previous equation, **b** equals $(\mathbf{X}^T\mathbf{X})^{-1}\mathbf{X}^T\mathbf{y}$ where the superscript T denotes the transpose of the matrix and superscript -1 denotes the inverse of the matrix.[51-53] Once the regression vector is established, it can be used in the prediction process by applying the model equation to calculate the response given the measured spectal data.[51-53] In this equation

$$y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + \ldots + b_nX_n \quad (4)$$

where y is the response value, b_i are regression coefficients, and X_i are measured intensities at different wavelengths.[51-53] MLR operates in the same manner.[51-53] A problem that arises in applying MLR to spectral data results from its dependence upon the stability and existence of $(\mathbf{X}^T\mathbf{X})^{-1}$.[51-53] **X** must have as many samples as wavelengths for the inverse to exist.[51-53] Additionally, collinearity problems will arise from the inversion of the matrix if the **X** variables are not linearly independent.[51-53] The regression will be unreliable if **X**-data are collinear or nearly collinear.[51-53]

The development of PCR and PLSR took these matrix inversion problems into account.[51-53] The concept of projections solved these problems leading to the success of several chemometric methods.[51-53] PCR and PLSR are both referred to as projection methods.[51-53] These methods decompose the original **X**-matrix into a new matrix, **T**, comprised of orthogonal principal components (PCs).[51-53] It is the PCs that are used instead of the **X**-matrix in both PCR and PLSR.[51-53] The PCs are estimated iteratively, with the first PC explaining the greatest amount of variance in the spectral data.[51-53] The next PC is then found by using the remaining variance.[51-53] As each PC is determined, it is used as a column vector in the new data matrix.[51-53] This process will continue until all variance is accounted for or until the maximum number of PCs is reached (the matrix inversion allows for n-1 PCs).[51-53] This approach reduces the dimensionality of the original data by concentrating the information into fewer variables, as established in the successive PCs.[51-53] The data reduction overcomes the problem of a limited number of predictor variables.[51-53] Additionally, the series of orthogonal PCs are used to define a new coordinate system, on which the samples and variables can be projected.[51-53] The creation of the new coordinate system eliminates the collinearity problem.[51-53]

Individual PCs are linear functions of the original **X**-variables; each contributes to the explanation of the variance along that component.[51-53] The regression vector contains these contributions (coefficients), **b**. Here, **b** is estimated as $(\mathbf{T}^T\mathbf{T})^{-1}\mathbf{T}^T\mathbf{y}$.[51-53] This is similar to what is seen in MLR except for the replacement of **X** (original data matrix) with **T** (new matrix containing PCs).[51-53] The transformation of the original matrix into a new matrix of PCs occurs in both PCR

and PLSR, but the methods differ in how the transformation is performed.[51-53] PCR considers only the **X**-data in its transformation.[51-53] However, PLSR actively involves both the **X**-data and the **Y**-data in the performance of the transformation.[51-53] This leads to increased data reduction as only the spectral variations correlating with the desired response variable are used in calculating the PCs.[51-53] However, if all possible PCs are used in both predictions then the two methods are equivalent.[51-53] PCR has a disadvantage in that it risks the inclusion of irrelevant spectral variations in calculating the PCs.[51-54] This can lead to poor predictive performance of the model. As a result, PLSR is the most commonly used regression method for spectroscopic data.[51-54]

Multivariate Classification

Classification is a method that uses pattern recognition with the goal of placing new samples into previously defined classes in a population.[51-52] The two most common classification methods in chemometrics are soft independent modeling of class analogy (SIMCA) and PLS-discriminant analysis (PLS-DA).[51-52] Every classification scheme has two steps. First, a model is constructed using a training set of samples, which is made up of samples whose class memberships are known.[51-52] Because the class memberships of the samples are known they can be used to define the classes mathematically.[51-52] Then in the second step, new samples are introduced, and the model is used to predict the membership classes of the new samples.[51-52] SIMCA and PLS-DA methods are quite different in a way comparable to the differences in the PCR and PLSR regression methods.[51-52] SIMCA and PCR are concerned only with the **X**-data, while PLS-DA and PLSR consider both the **X**-

data and **Y**-data . SIMCA will be discussed in further detail since it was the method chosen for this research. [51-52]

The focus of the SIMCA method is on the similarities of samples belonging to the same class. [51-52] Every class used must have its own model, which is created through principal components analysis (PCA). [51-52] The beginning step of principal components regression is PCA. [51-52] In this process the **X**-data are broken down to create a new matrix. [51-52] This new matrix is made from PCs that use only **X**-data to estimate those PCs. [51-52] A new coordinate systems is then created to represent the samples. [51-52] PCA uses zones to define each class where the dimensions are equal to the number of PCs retained for the class. [51-52] Individual PCA models are not required to have the same dimensions, and in an ideal case they would be positioned differently in the overall measurement space. [51-52]

All PCA models are used to make a prediction in the SIMCA approach. [51-52] Using the determined mathematical relationship between the spectral data and PCs, new samples are projected onto the "map" of PCs. [51-52] A sample may be identified as a member of a class if it occupies the defined space for the class. [51-52] Similarly, a sample can be a member of two or more classes if it falls within the boundaries of those classes. [51-52] Likewise, if a sample falls outside of all class borders then it cannot belong to any of the classes. [51-52] SIMCA models lack the discriminative power in cases of multiple class membership due to overlap that can exist in measurement space. [51-52] However, the inclusion of additional training samples or variables can improve discriminatory power. [51-52] Samples that do not fall into any class are either outliers or they belong to a class not represented by the data set. [51-52] Changing the

significance level, describing relationship of sample to the established class, determines the outcome of a SIMCA classification.[51-52] Altering the significance level changes the class boundaries.[51-52] Raising the significance level narrows the class limits, rejecting more samples.[51-52] Lowering the significance level extends the class limits, rejecting fewer samples.[51-52]

Model Optimization

In both regression and classification methods, the data generated are expressed in four main plots: scores, loadings, influence and total residual variance plots.[51-52] The total residual variance plot is important in determining the number of PCs that should be used in a model.[51-52] For each PC, the plot depicts the amount of variance in the data that the PC explains.[51-52] Figure 2-3 shows the characteristics of what an ideal total residual variance plot should look like.[21] The first characteristic is that the residual variance should be small, which means that most of the variation in **X** can be explained.[51-52] Secondly, in an ideal case most of the variation can be explained by the first few PCs.[51-52] Finally, the end of the curve should increase slightly, which would mean that additional PCs would lead to overfitting.[51-52] If an increase at the tail is not seen then it makes it harder to determine if the correct number of PCs were used in the model.[51-52] Either overfitting or underfitting of a model will lead to inferior performance.[51-52] In the case of overfitting, the model actually tries to explain uninformative information in the data or noise.[51-52] With underfitting the issue is that there aren't enough PCs to explain all of the relevant information.[51-52] Any erratic behavior seen in this plot could indicate the existence of outliers in the data set.[51-52]

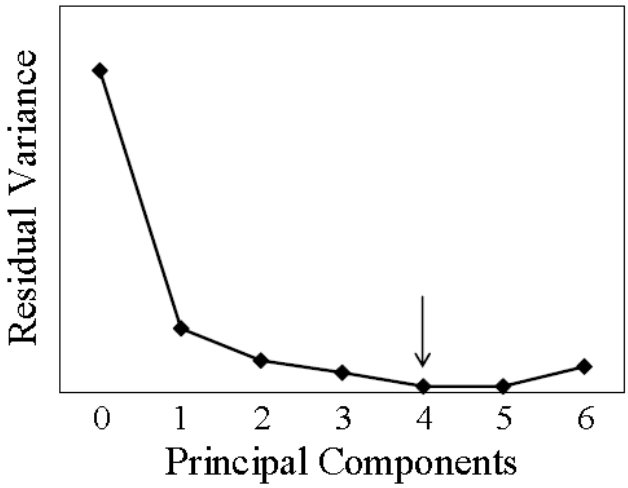


Figure 2-3. An example of an ideal residual variance plot showing the appropriate amount of PCs.[21]

The second type of plot used in all projection methods is the scores plot.[51-52] Projection methods represent the data on a new coordinate system, and the scores plot is produced by projecting samples onto this new coordinate system.[51-52] Information regarding patterns in the data can be visualized through the model's scores plot.[51-52] If samples are close to each other in the plot it means they are alike with respect to those particular PCs.[51-52] If they are further apart, it means those samples are different from each other.[51-52] Outliers can be identified when a sample in the scores plot is separated from the rest of the points.[51-52] Since most of the information is explained by the first few PCs, plots comparing those PCs will be the most useful.[51-52] Figure 2-4 shows a two-dimensional scores plot that contains three clusters as well as one outlier.[21]

Next is the loadings plot, which is the projections of the variables onto the new coordinate system.[51-52] It is necessary to look at the loadings for every PC to find which **X**-variables are the most critical to the model.[51-52] Just like with the scores, it's

the first few PCs that are the most important.[51-52] The greatest amount of variation in the data is accounted for by those PCs.[51-52] An example of a loadings plot can be seen in Figure 2.5. If the loading value of a variable is high, then it means that the contribution of that variable to the model is high.[51-52] The contribution to the model can be either positive or negative.[51-52] As demonstrated in Figure2-5, variables 4 and 8 have the highest values in either direction and thus contribute the most to the model for that PC.[21] However, variables 2 and 6 do not make a contribution for this PC since their loading values are zero.[21]

The loadings plot can also assist in determining the appropriate number of PCs for a model.[51-52] The model should only include PCs which explain chemical information and leave out those that try to explain noise.[51-52] One can determine whether a PC is explaining noise simply by looking at the line plot.[51-52] If it appears fuzzy and does not resemble spectra then it is explaining noise and should not be included.[51-52] Only the last loading vector is given to show that the model was not overfit.[51-52]

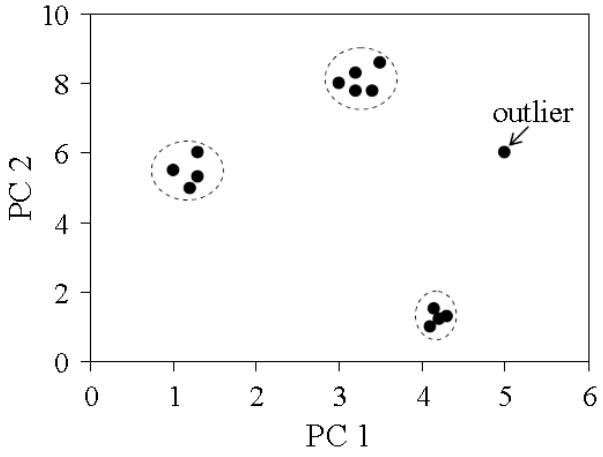


Figure 2-4. An example of an ideal scores plot showing three distinct clusters.[21]

Another useful plot that helps to identify outliers in the data set is the influence plot.[51-52] Samples are plotted as a function of leverage versus residual variances.[51-52] As the name implies, the plot shows which samples are the most influential.[51-52] Samples that have high leverage values pull the model in a certain

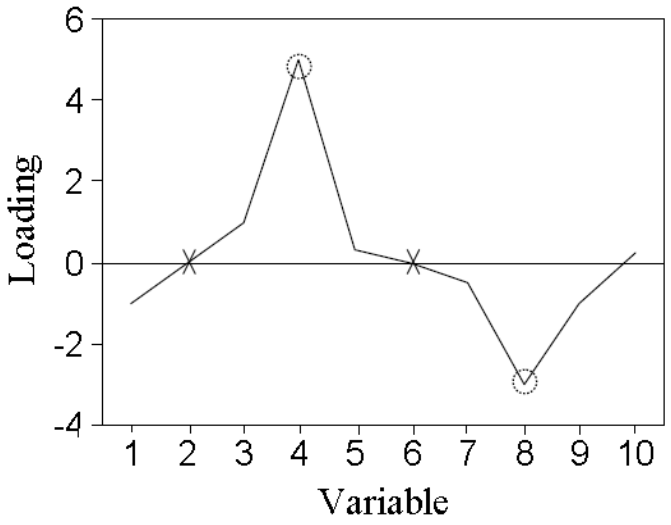


Figure 2-5. A hypothetical example of a loadings plot showing two important variables (4 and 8) as well as two variables that are not important to this PC. (2 and 6).

direction ultimately giving a better description of the model.[51-52] Figure 2-6 gives an example of an influence plot showing both outliers and influential samples.[21] Samples with a low leverage value and high residual variance are likely to be outliers, and samples that have high residual variance and high leverage are considered to be dangerous outliers.[51-52] A dangerous outlier is one that is not well described by the model, distorts the model, and makes the model less able to describe the average samples.[51-52]

Any samples that fit these three situations should be examined and removed if necessary.[51-52]

The final plot that must be discussed is the predicted-versus-measured-values plot that gives information used to determine the quality of the regression model. [51-52] In an ideal plot, the points will all fall onto a straight line that runs through the origin and has a slope equal to one. [51-52] A poor quality model will have points whose average distances are far from the fit line. [51-52]

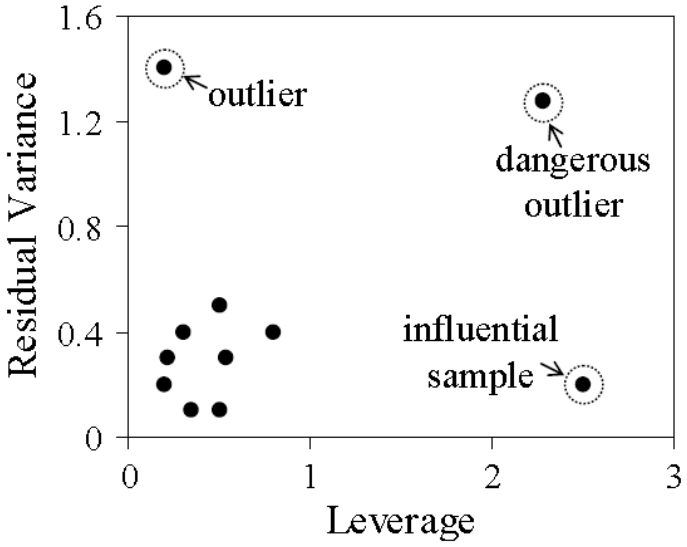


Figure 2-6. A hypothetical influence plot depicting 3 potential problem cases.[21]

An ideal version of a predicted versus measured plot is shown in Figure 2-7 The R-squared value is given in this plot and is also used to determine the quality of the model. [51-52] This value helps to show how strong a relationship exists between the **Y**-data and the model's predicted **Y**-values, and gives the predictive ability of the model. [51-52] The R-squared value is calculated by taking the square of the correlation coefficient between the predicted and measured values. [51-52] The value will always fall between 0 and 1 with values closer to 1 indicating better fit. [51-52]

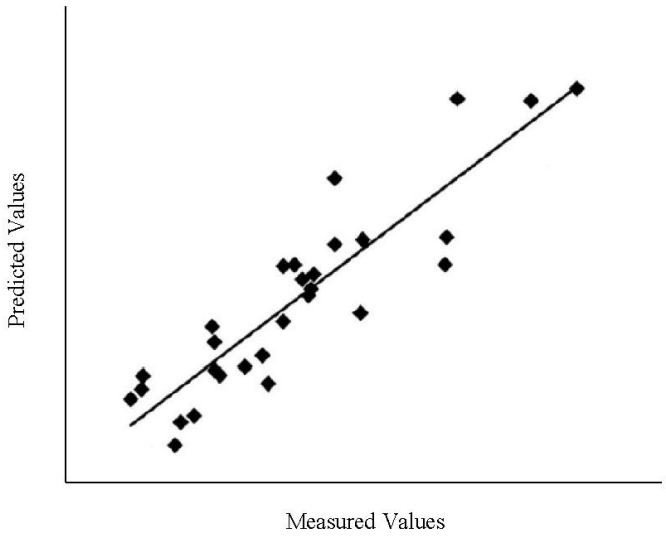


Figure 2-7. A hypothetical predicted versus measured plot.

CHAPTER THREE

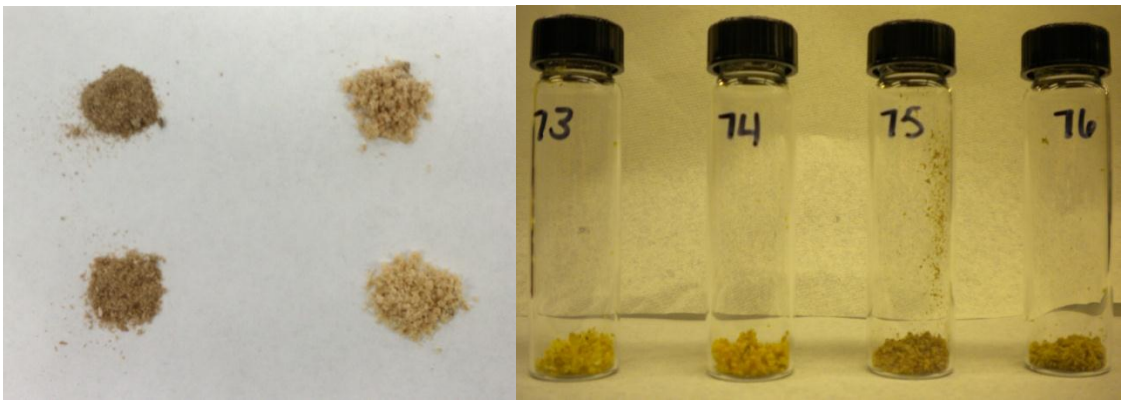
Experimental

Preparation of Bone Samples

While human remains would have been ideal for this research, such remains could not be obtained easily. In that instance, it is common to rely upon pigs to act as human analogs. However, due to space and time constraints, it was not possible to use whole pigs. As a result, during the course of the research pork ribs purchased from a local meat market were used as samples. For the indoor control study the soft tissue was removed from the bone by hand. The bones were then cut into one inch pieces using a hand saw before being placed in a single layer in a plastic tub to decompose in a laboratory hood. For the field studies, the whole racks of ribs were placed in a cage on the ground in an open grassy area. Holes in the cage allowed the samples to be in contact with soil and grass. The soft tissue was allowed to fully decompose before the bones were analyzed.

Previous research at Baylor focused on relatively non-destructive techniques.[21] In this study bone powder was used in the hope of getting a more complete representation of the bone when compared with a cross section. The one-inch bone pieces were allowed to freeze in liquid nitrogen for approximately five minutes. The frozen bone was placed between paper towels and crushed with a hammer. The crushed bone was then transferred to a dish, where the fine powder used in analysis

was separated from any larger bone fragments. Examples of the bone powder can be seen in Figure 3-1.



a. b.

Figure 3-1. (a) Bone powder where samples on the left are from the field study and samples to the right are from the control study. (b) Bone powder taken from one bone in control study.

Reagents

A luminol solution was used in both the control and the field study. Since luminol solutions have a short shelf life, a series of three standard solutions were prepared as outlined by Weber[11]. Luminol, sodium hydroxide and hydrogen peroxide were purchased from TCI America, EMD, and Alfa Aesar. Deionized water was used as a solvent. Solution A consisted of 0.354 g of luminol, 62.5 mL of 0.4 N sodium hydroxide, and 437.5 mL of deionized water. Solution B contained 8 g sodium hydroxide and 500 mL deionized water. Solution C consisted of 10 mL of 30% hydrogen peroxide and 490 mL of deionized water. The final solution used in the testing of samples was made fresh each day by taking 10 mL of Solutions A, B and C and mixing with 70 mL of deionized water.

Collection of Spectral Data

Spectra were taken using a Hitachi F7000 fluorescence spectrophotometer. Scans were performed over a wavelength range from 200 nm to 900 nm with an excitation wavelength of 360 nm. To run the samples, a drop of luminol solution was placed on the window of the sample holder. Bone powder was then poured onto the luminol, and the lid was secured to keep the powder from shifting. In the control study, three to five of the one inch bone pieces were examined at a time and tests were performed periodically over a 116-day period. For the field study, two different sets of bones were used. The first set was allowed to decompose for a period of 240 days before fluorescence analysis began, while the second set was allowed to decompose for a period of 360 days prior to testing. Bones from each set were assessed periodically over the course of 21 days.

Finally, Lin *et al.* showed the differences in the absorption spectra of luminol, heme, the combination of the two in a reaction and finally the heme mixed with hydrogen peroxide which can be seen in Figure 3-2.[40] The excitation wavelength was chosen based on this figure because there is absorption of heme combined with luminol at 360 nm. Similar steps were taken for this research in showing the fluorescence spectra of the bone, pure hemoglobin, the luminol solution, as well as spectra from the reaction of the luminol with the bone sample and the luminol with the hemoglobin as seen in Figure 3-5. No fluorescence was observed for the bone sample or the pure hemoglobin. The intensity of luminol's fluorescence was much higher compared to either the chemiluminescence produced by the reaction of the

bone with luminol or that produced by the reaction of hemoglobin with luminol. The chemiluminescence intensity from the reaction of bone and luminol was quite similar to that from the reaction of hemoglobin and luminol, but reaction with the bone produced more chemiluminescence than that with pure hemoglobin.

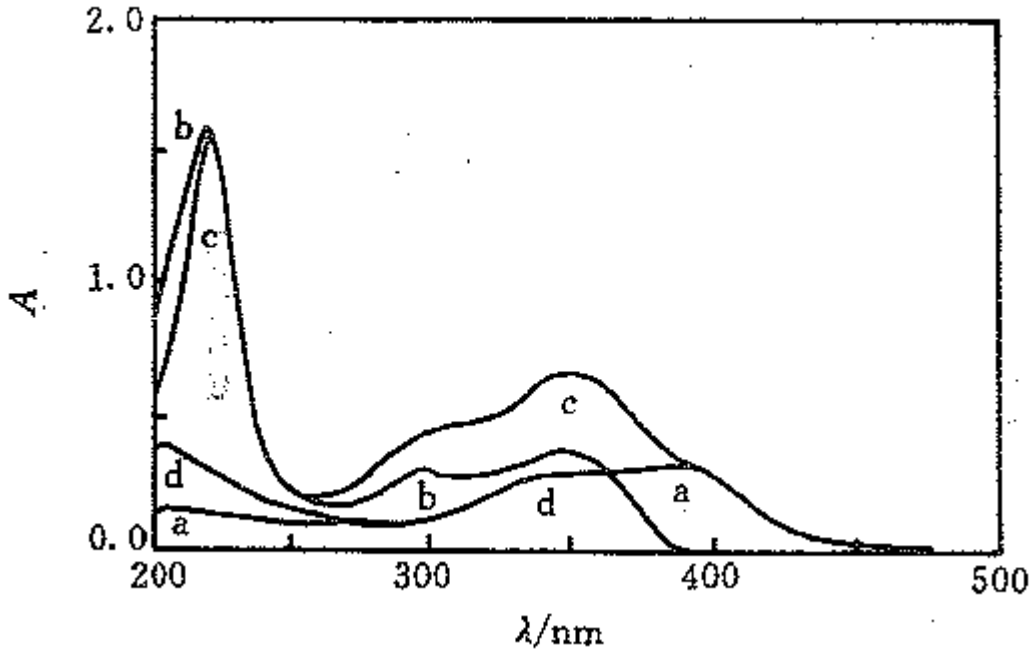


Figure 3-2. UV absorption spectra for: (a)8.358 x 10^{-6} mol/L of heme; (b) 5.015 x 10^{-5} mol/L of luminol; (c) mixture of a & b; (d) heme & 1.543 x 10^{-3} mol/L H_2O_2[40]

Data Treatment

The spectral data were compiled into Excel files and analyzed using Unscramber 9.8 chemometrics software from CAMO Inc. Figures 3-3 and 3-4 show the unprocessed spectra for the control study and the field study, respectively. The useful wavelength range for the control was from 378 nm to 515 nm. A wavelength range of 385.6 nm to 524.6 nm was used for the field study. Spectra were smoothed using 5-point Sovitsky-Golay smoothing[59]. Finally, a column containing the known

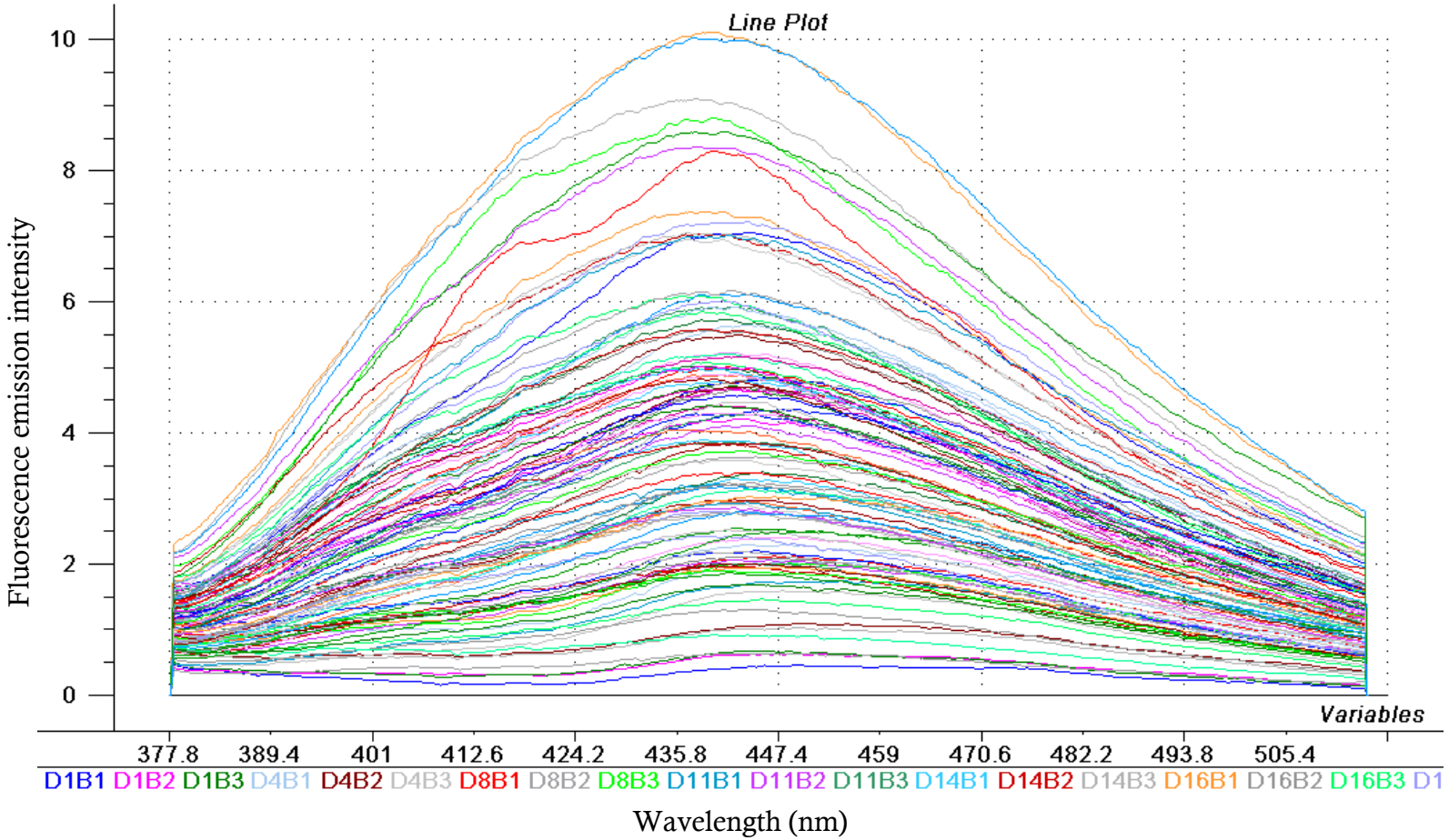


Figure 3-3. Fluorescence spectra for each individual bone sample from the control study. In general, the emission intensity increases as the samples age.

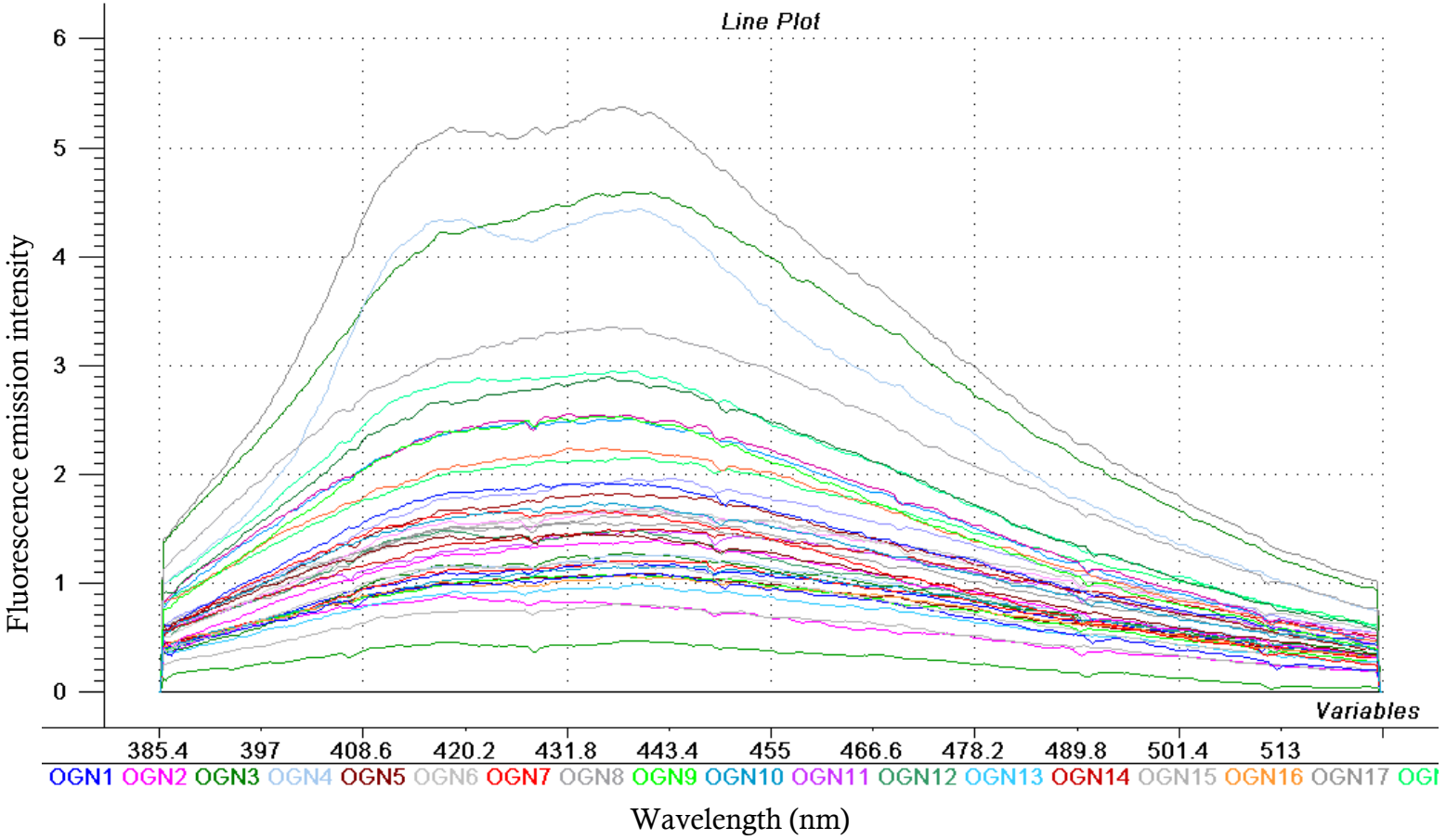


Figure 3-4. Fluorescence spectra for each individual bone sample from the field study. In general, the emission intensity increases with time.

PMI for all samples was added to the data matrices before data analysis could be performed.

One disadvantage to using ribs for samples is that each bone varies in diameter from one end of the bone to the other as well as from the first rib through the last rib. As a result, the color and texture of the powder in each piece could vary as well. Color and texture differences could also be seen between the samples of the

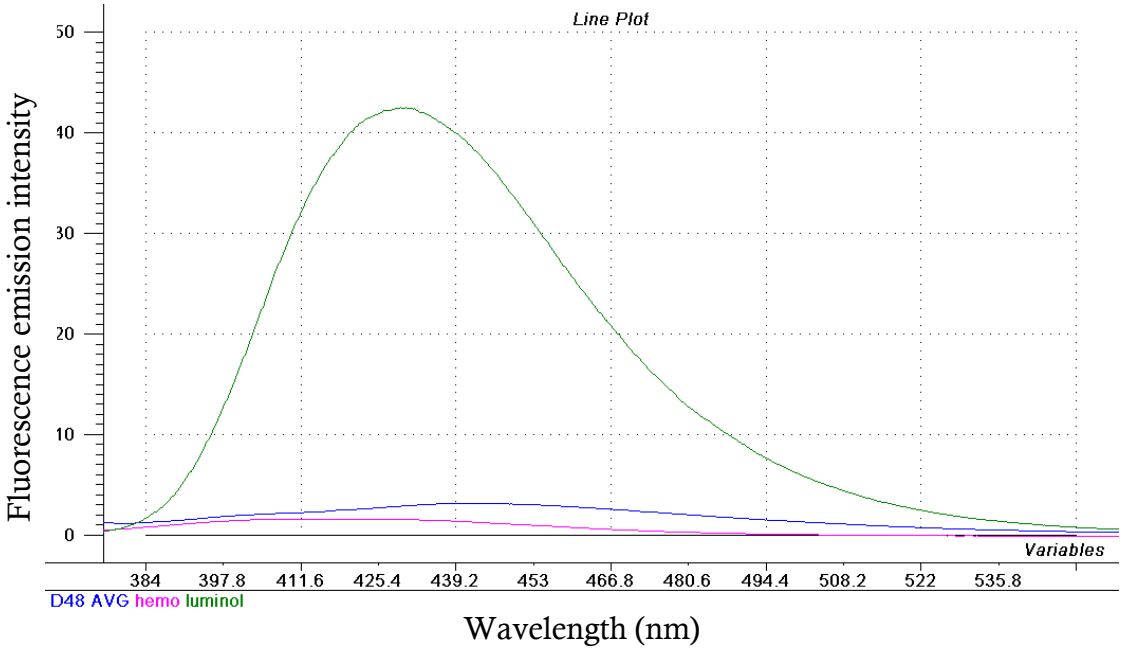


Figure 3-5. Comparison of the fluorescence spectra of luminol solution (green) and its reaction with the bone sample (blue) and with pure hemoglobin (pink).

control study versus the samples in the field study. To ensure that this variation did not affect the model's spectral data, spectral data for samples of the same age were averaged. Analysis was then performed on the averaged spectra for comparison. Unprocessed averaged spectra for the control study and the field study are shown in figures 3-6 and 3-7, respectively.

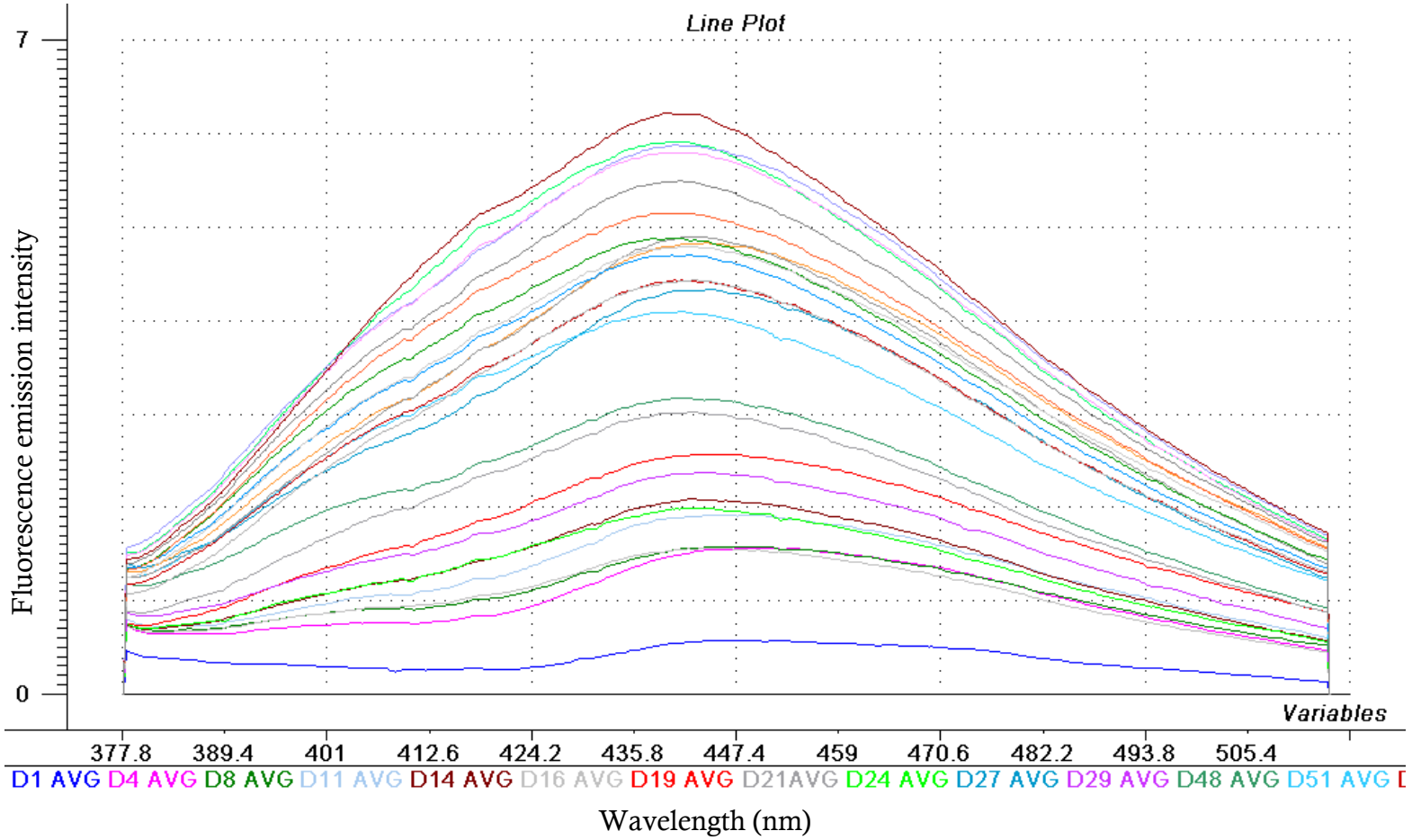


Figure 3-6. Fluorescence spectra for the control study where spectra from bone samples of the same age were averaged. In general, the emission intensity increases as the bone ages.

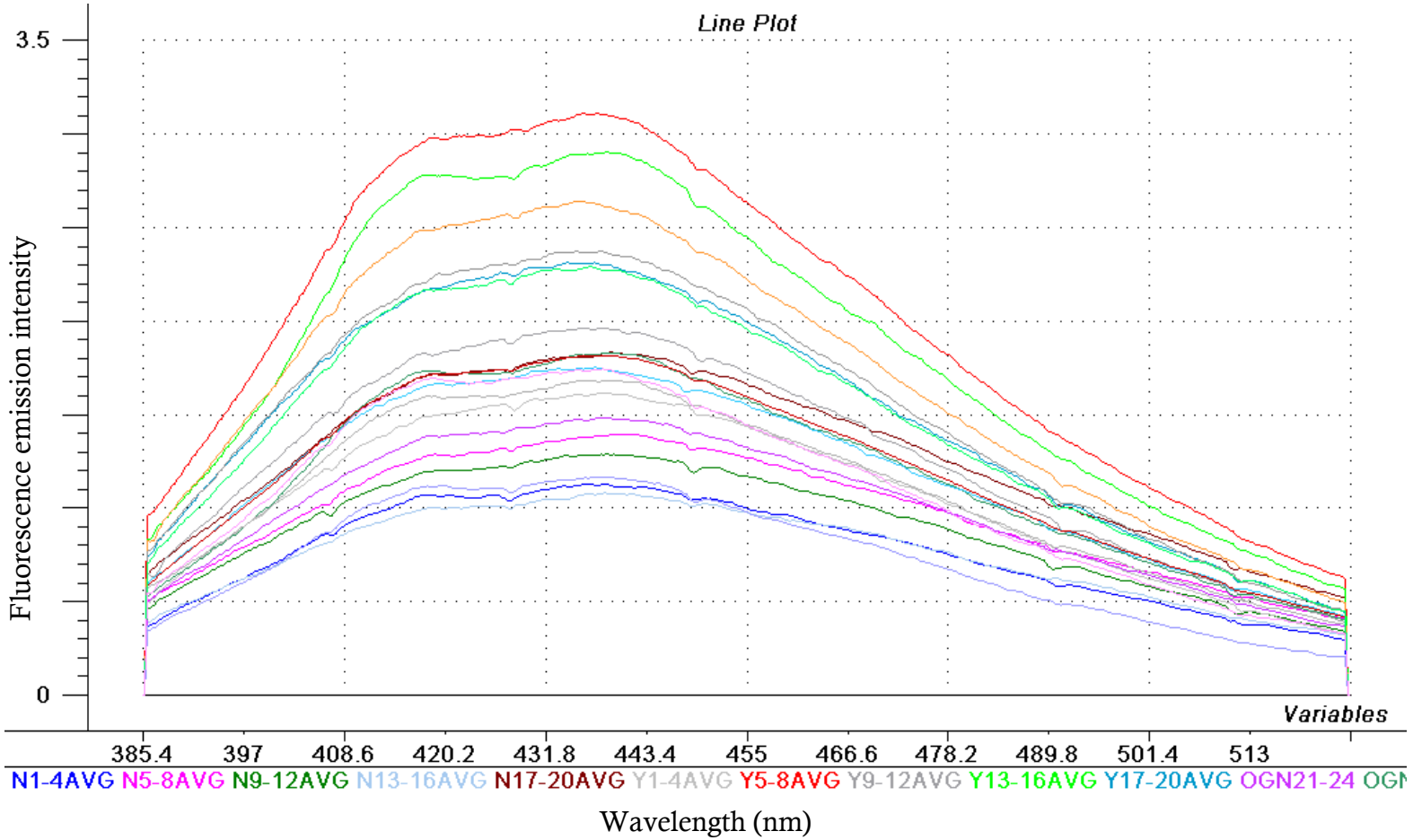


Figure 3-7. Fluorescence spectra for field study where spectra from bone samples of the same age were averaged. In general, there is an increase in emission intensity over time.

Spectra for all data sets were analyzed using PLS-1 regression analysis. Eight PCs were used, although most of the data sets did not require all eight PCs. To check for overfitting, regression analysis was performed using fewer PC's and the results remained the same. The resulting models were then used to predict the age of unknown samples

In addition, SIMCA classification studies were performed for both the control and field studies. Instead of predicting an age of unknown samples, the goal was to correctly classify the unknown samples into one of two groups. In the control study, the groups were divided approximately at the half way point giving a group of bones aged 1 to 50 days and the other with ages 51 to 116 days. In the field study, the two groups were the nine-month samples and the year-old samples.

CHAPTER FOUR

Results and Discussion

Control Study

The purpose of the control phase of the study was to determine if fluorescence intensity could be correlated with PMI. A general trend can be observed in Figure 4-1. The spectra show a general increase in intensity over time.

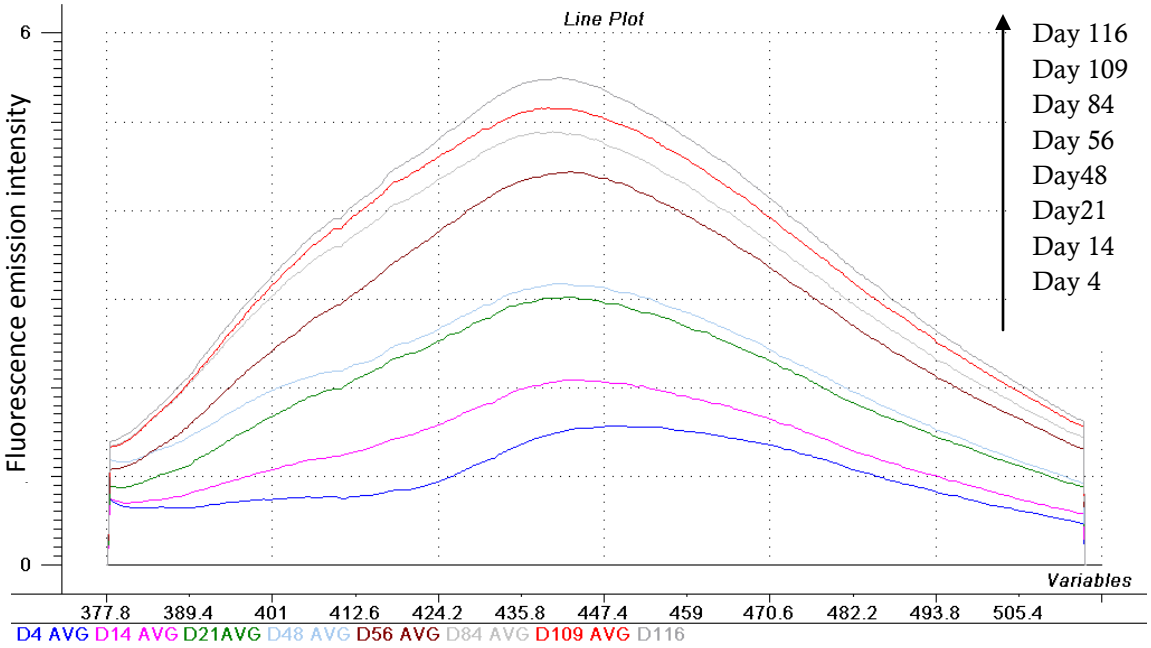


Figure 4-1. Fluorescence spectra demonstrating the increase of the fluorescence intensity with bone material over time.

Initially these results were perplexing in view of the long term studies of Introna et al.[11] and Creamer et al.[22] that showed a decrease in intensity with time.

However, the results make sense when keeping in mind the research of Specht.[48] This work demonstrated that dried blood reacts better with luminol than wet blood. This conclusion was supported by Proesher and Moody who proposed that chemiluminescence emission intensity could increase for up to three years after death.[31, 33] The control samples in this study contained fresh blood in the beginning and were still "wet" throughout. Whereas the samples used by Introna and Buck were much older and had already reached the point in their decomposition where they were completely dry. While not observed in this study, the results might suggest that the intensity of the reaction of the luminol would continue to increase up to the point where the blood remnants had completely dried, after which the intensity should begin to fall as the remains continue to age.

Regression models were constructed and examined for the set of individual spectra and the set of averaged spectra. The first model representing the set of individual spectra, shown in Figure 4-2, explains 99% of the **X** variance, but only 44% of the **Y** variance and has an R^2 value of 0.57. Six PC's were required to explain the variance for this data set. However, the second model, which represents the averaged spectra, seen in Figure 4-3, explains 100% of the **X** variance and 84% of the **Y** variance with an R^2 value of 0.89. The averaged data also required six PC's to account for the variation. The results show that the varying widths of the bone fragments had a negative effect on the models. Once the fragments of the same age were averaged together, the model improved significantly.

Next, new regression models were made by removing one sample at a time to be used as an unknown. These new models were then used to make predictions for

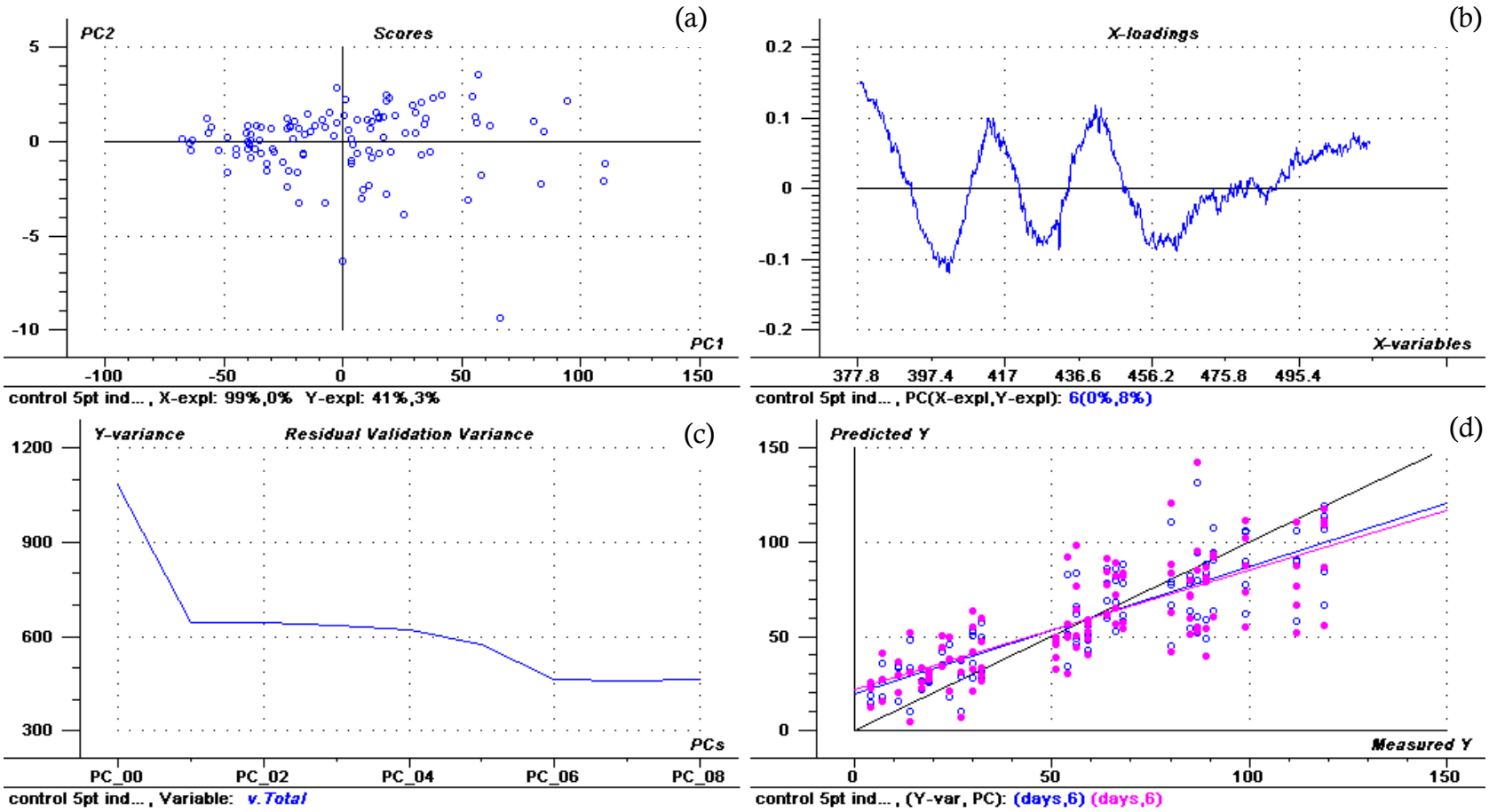


Figure 4-2. PLS-1 Regression overview for control study: (a) scores plotted along the first two PCs; (b) loadings for the sixth PC as a function of wavelength; (c) Y validation variance curve plotted as a function of PCs; and (d) plot of predicted versus measured values, R-squared value is 0.57.

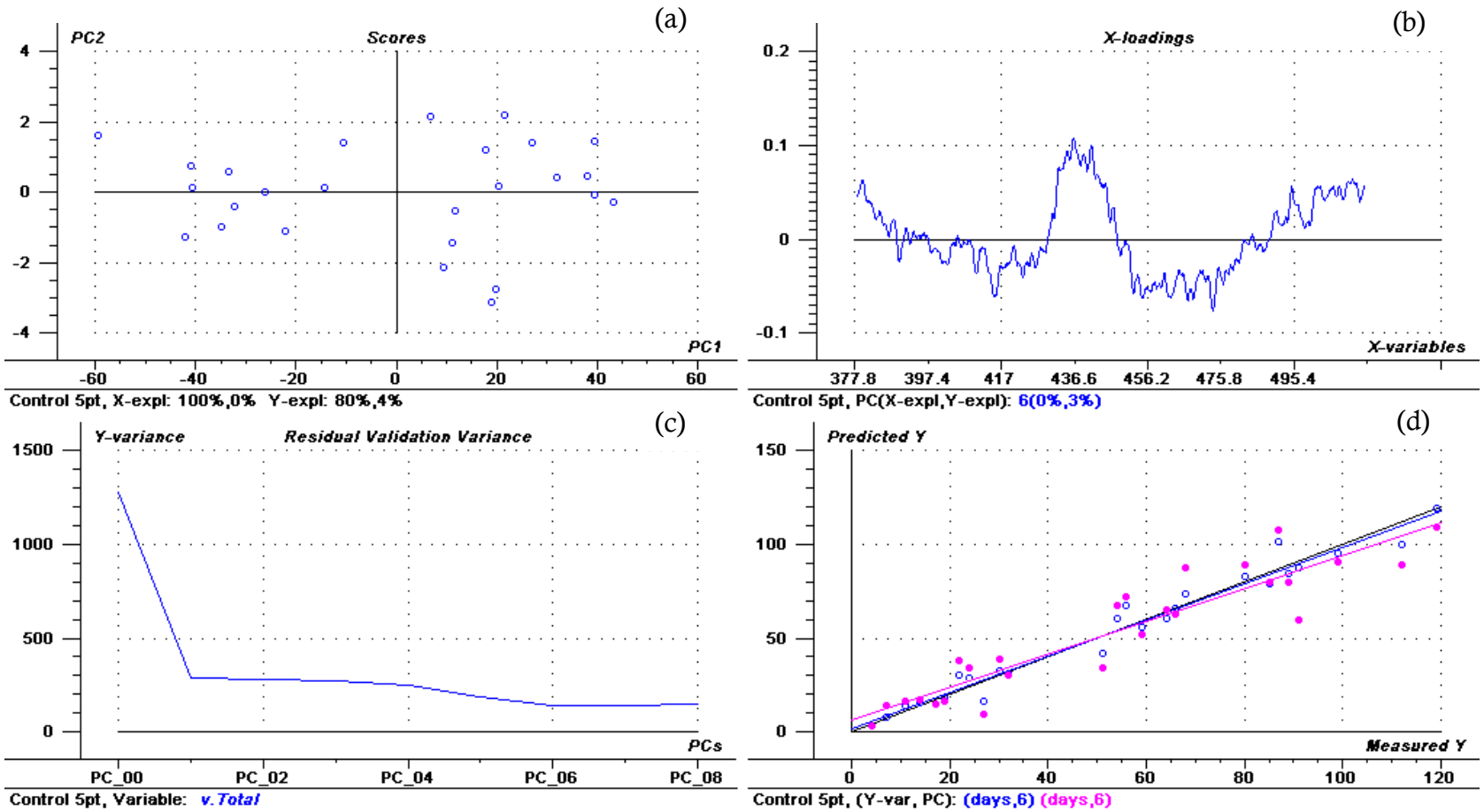


Figure 4-3. PLS-1 overview for control study with data averaged: (a) scores plotted along the first two PC's; (b) loadings for the sixth PC as a function of wavelength; (c) Y validation variance curve plotted as a function of PCs; and (d) plot of predicted versus measured values, R-squared value is 0.89.

each unknown. The results for the control study predictions can be seen in Table 4-1. For the individual samples the average absolute error ranges from zero days to twenty-three days, but the average absolute error for all the unknown samples was thirteen days.

Table 4-1 Prediction results for the control study.

Unknown Sample	Predicted (days)	Reference (days)	Average absolute error (days)
Day 1	4	4	0
Day 8	17	11	6
Day 19	40	22	18
Day 24	15	27	12
Day 51	68	54	14
Day 63	43	66	23
Day 77	90	80	10
Day 88	79	91	12
Day 96	88	99	11
Day 109	90	112	22
Total average absolute error			13

SIMCA classification was also used to try to assign unknown samples to one of two groups. The first group contained samples less than fifty days old, while the second contains all the samples more than fifty days old. The classification results can be seen in Table 4-2. Overall the classification correctly assigned samples 96% of

the time with the sample for Day 27 as the only sample classified incorrectly. This particular sample must have had an influence on the model because when it was removed the model changed, thus explaining the incorrect prediction. The influence plots both with and without Day 27 can be seen in Figure 4-4. Without Sample 27, Sample 1 moves from being somewhat influential to possibly being a dangerous outlier. Sample 48 also moves from being a possible outlier to a dangerous outlier.

There was nothing noted during sample prep or while running the sample to indicate anything unusual had happened to Sample 27. Sample 51 was predicted as being a member of both groups. Since the cut off was at fifty days, it is understandable that a sample close to the cutoff could be placed in both groups. The results from the control study seem to indicate there is a correlation between PMI and the fluorescence intensity of the sample. It was surprising that for this time period, the change in intensity was opposite of what was expected from previous work.

Field Study

In order for the method to be useful to forensic investigators, it had to achieve similar results in the field. The averaged spectra for the field study do not show quite as clear of a trend as was seen in the control study. Even though the field samples were much older than those used in the control study, there still appears to be an increase in the fluorescence intensity over time as was seen in the control study. Rather than naming each sample by its age in days, a separate naming convention was used in the field study. For samples in the nine-month set were labeled first with the letter N followed by a number representing the order in which the pieces were

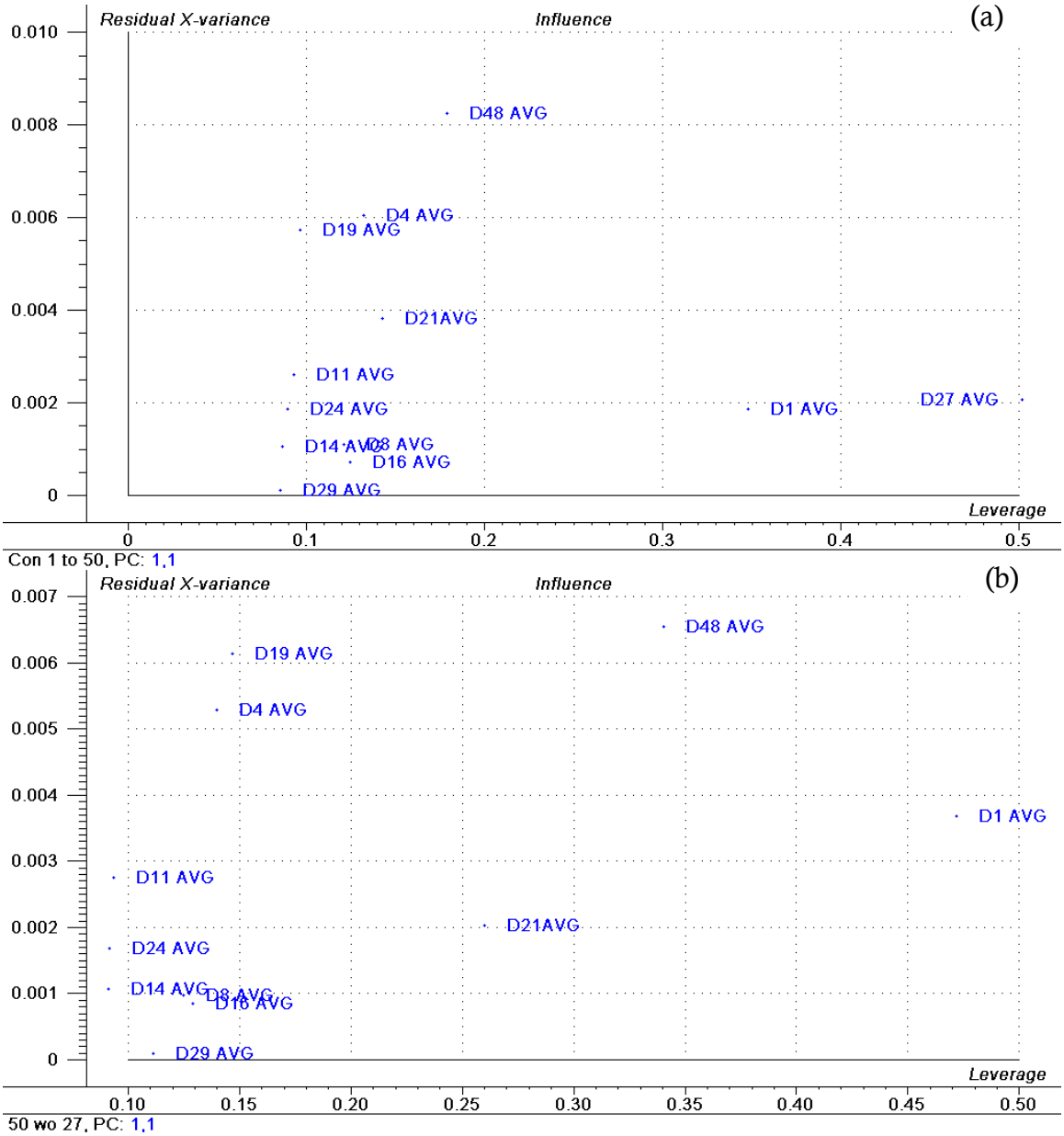


Figure 4-4. Influence plots (a) with Day 27 and (b) without Day 27

used. After averaging the spectra, the name was changed to incorporate all the samples averaged together, so N1-4 indicates the averaged spectra of bone pieces N1, N2, N3, and N4. The same convention was used for the year-old set of samples except they were labeled with the letter Y.

Table 4-2. SIMCA predictions for the control study with the * representing inclusion in the designated group.

Unknown Sample	Group 1	Group 2
Day 1	*	
Day 4	*	
Day 8	*	
Day 11	*	
Day 14	*	
Day 16	*	
Day 19	*	
Day 21	*	
Day 24	*	
Day 27		*
Day 29	*	
Day 48	*	
Day 51	*	*
Day 53		*
Day 56		*
Day 61		*
Day 63		*
Day 65		*
Day 77		*
Day 82		*
Day 84		*
Day 86		*
Day 88		*
Day 96		*
Day 109		*
Day 116		*

Regression models were created for the averaged spectra since the averaged spectra produced better results in the control study. The model for the field study,

seen in Figure 4-5, predicted 100% of the X variance and 83% of the Y variance with an R^2 value of 0.75. The predicted X and Y were very similar to the results of the control, but the R^2 value dropped slightly. Predictions for the control set were performed in a manner similar to the control study. In this case there were so few samples that each sample was alternately removed from the model, and used as an unknown. Results from the prediction can be seen in Table 4-3. The best result came for sample N21-24 with an error of 10 days. The average absolute error was 36 days.

Table 4-3 Prediction results for field study.

Unknown Sample	Predicted (days)	Reference (days)	Average absolute error (days)
N1-4	289	241	48
N5-8	229	245	16
N17-20	176	262	86
N21-24	280	270	10
N37-40	323	282	41
Y1-4	313	361	48
Y5-8	337	372	35
Y17-20	370	382	12
Y21-24	411	390	21
Y31-34	353	398	45
Total average absolute error			36

Considering the large age difference in the two sets of samples, new models were created for each set separately. The results for the first model, N set, can be seen in Figure 4-6, and the results for the second set, the Y set, can be found in Figure 4-7. For the N set, the model explained 100% of the X variance and 81% of the Y variance with an R^2 value of 0.81. The N set required seven PCs to explain the variation. For the Y set, the model explained 100% of the X variance and 81% of the Y variance with an R^2 value of 0.76. The Y set required only 4 PCs to explain the variance. The model for the N set more closely resembles the control, while the Y set still closely resembles the full set of samples for the field study.

Predictions were performed again separating the two sets of samples. The results for the N set can be found in Table 4-4, and the results for the Y set can be found in Table 4-5. The lowest prediction for the N set had an error of one day, while the largest error was nineteen days. The average error for the N set was eleven days. For the Y set, the lowest error was four days and the highest error was fifteen days. The average error for the Y set was nine days.

One major difference between the control study and the field study can be observed simply by comparing the spectra for each. The spectra for the field study have a bump suggesting that there might actually be two peaks compared to only one in the control study. Since the luminol reaction is not selective, a possible explanation is the luminol is reacting to some contaminant that was not present in the control. Several factors could contribute. Luminol will react with plant peroxidases, and while the bones were placed on mud in the beginning, by the time samples were taken the grass had grown over most of the bones. The few that were

still exposed were covered in a green film. In an attempt to determine if plant material caused the change in the spectra, samples of grass were taken, chopped up and exposed to the luminol solution. No chemiluminescence was produced upon

Table 4-4. Prediction results of field study using only nine-month old samples.

Unknown Sample	Predicted (days)	Reference (days)	Average absolute error (days)
N1-4	256	241	15
N5-8	260	245	15
N9-12	256	249	7
N13-16	235	252	17
N17-20	281	262	19
N21-24	261	270	9
N25-28	284	272	12
N29-32	278	275	3
N33-36	277	278	1
N37-40	273	282	9
Average error			11

exposure to the plant material. Another possibility could be a contaminant in the soil or even rust from the cages where the bones were kept. Whatever the reason, predictions were still possible, and the results were slightly better than the control study.

Classification of the unknowns was also attempted using SIMCA. The samples were classified as belonging to the N set, the Y set, part of both, or part of

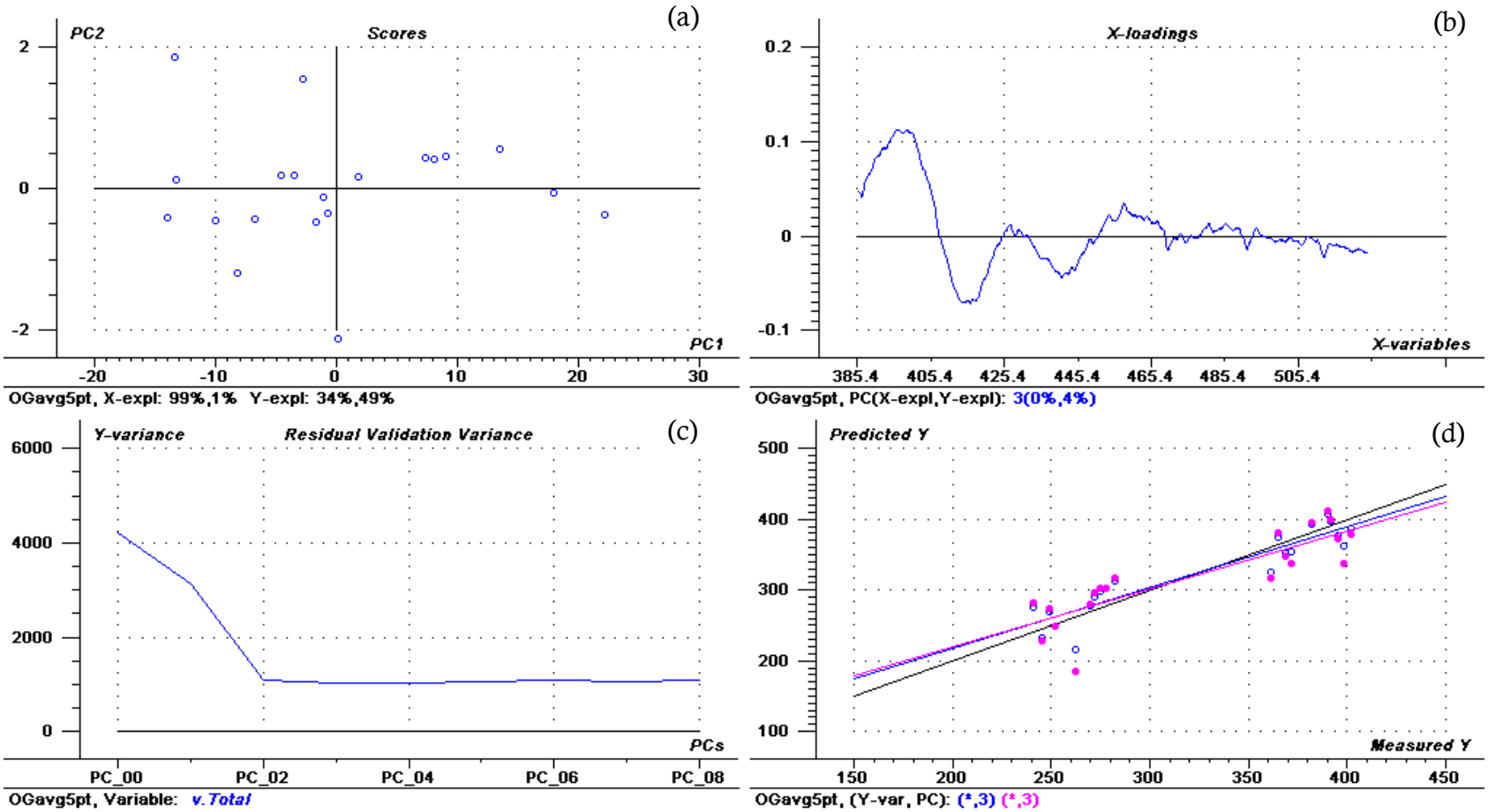


Figure 4-5. Regression overview for the field study: (a) scores plotted along the first two PCs; (b) loadings for the third PC as a function of wavelength; (c) Y validation variance curve plotted as a function of PCs; and (d) plot of predicted versus measured values, R-squared value is 0.75.

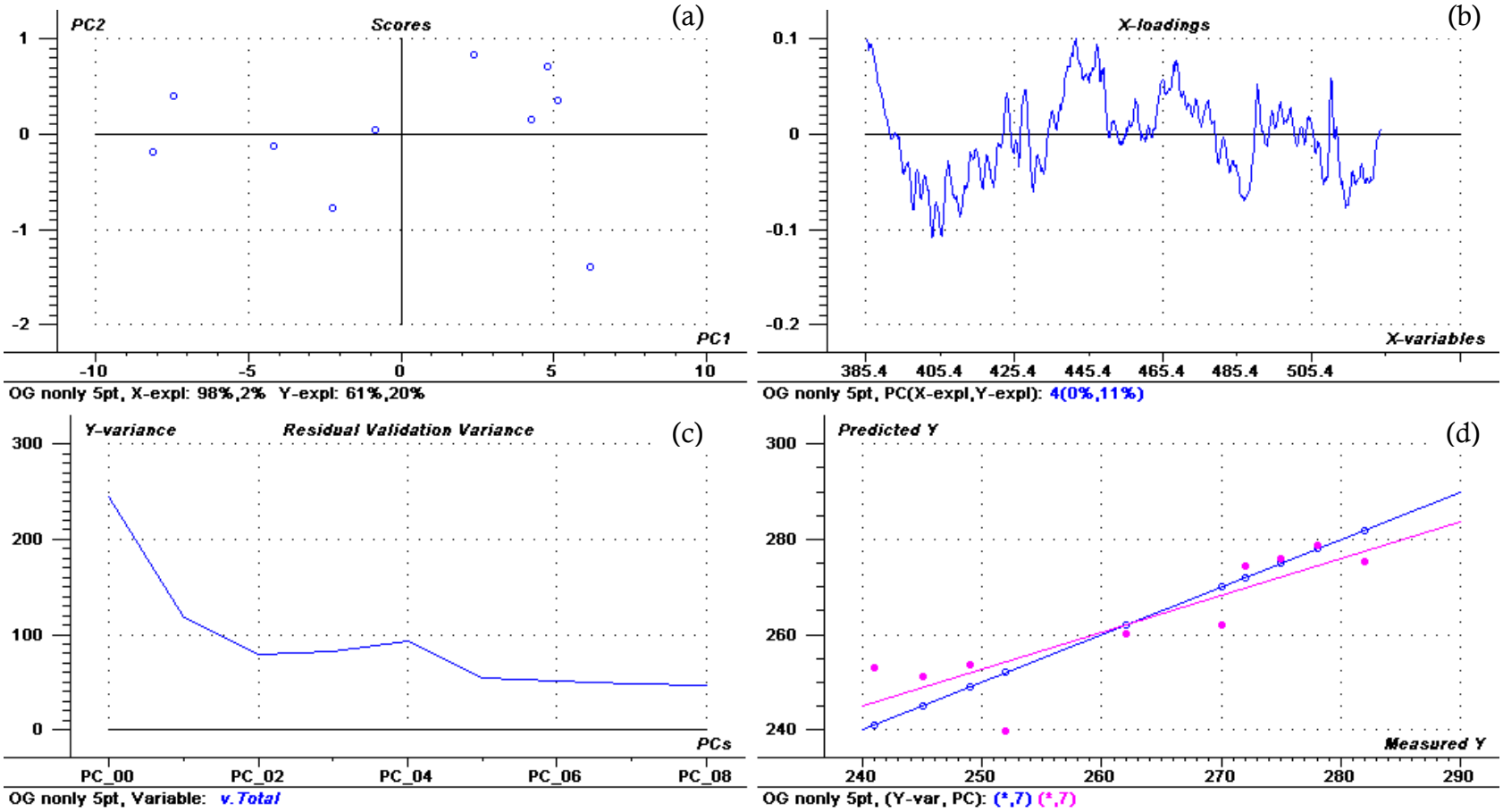


Figure 4-6. Regression overview for N set of field study: (a) scores plotted along the first two PCs; (b) loadings for the forth PC as a function of wavelength; (c) Y validation variance curve plotted as a function of PCs; and (d) plot of predicted versus measured values, R-squared value is 0.80.

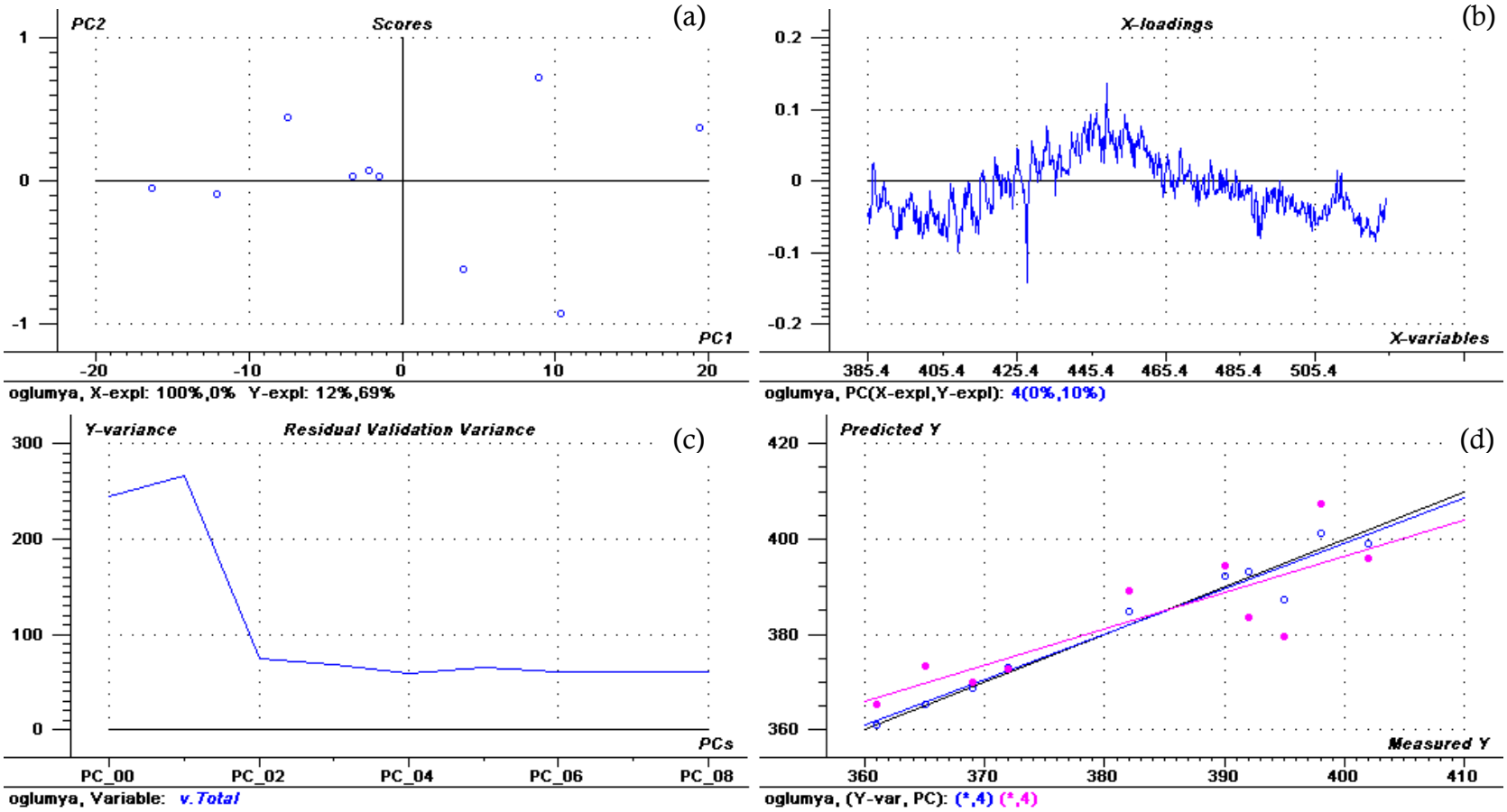


Figure 4-7. Regression overview for Y set of field study: (a) scores plotted along the first two PCs; (b) loadings for the forth PC as a function of wavelength; (c) Y validation variance curve plotted as a function of PCs; and (d) plot of predicted versus measured values, R-squared value is 0.75

neither. Results for the predictions can be found in Table 4.6. Two samples were classified as being part of both groups, while another sample was not placed in either of the two groups. None of the samples was classified as only being in the

Table 4-5. Prediction results for field study using only year-old samples.

Unknown Sample	Predicted (days)	Reference (days)	Average absolute error (days)
Y1-4	368	361	7
Y5-8	379	365	14
Y9-12	370	369	1
Y13-16	361	372	11
Y17-20	391	382	9
Y21-24	394	390	4
Y25-27	381	392	11
Y28-30	380	395	15
Y31-34	407	398	9
Y35-38	406	402	4
Total average absolute error			9

incorrect group. There is no clear explanation as to why two samples were classified as being part of both groups. One possibility could be a difference in the diameters of the bones used for those samples. If thinner bones were used, it is possible they could have decomposed at a faster rate than those with a larger diameter causing, them to be mistaken for older samples.

Table 4-6. SIMCA results for field study.

Unknown sample	N Group	Y Group
N 1-4	*	
N5-8	*	*
N9-12	*	*
N13-16	*	
N17-20		
Y1-4		*
Y5-8		*
Y9-12		*
Y13-16		*
Y17-20		*

CHAPTER FIVE

Conclusion

Once skeletonization occurs, there are no established scientific methods for use in the estimation of post-mortem interval. Previous works suffer from lack of reliability and a lack of samples that have a similar age range.

The idea of combining spectroscopic methods with chemometrics had not been attempted before the work by Dogra using NIR spectroscopy and chemometric analysis. The current work took that same idea, but attempted to narrow the focus from measuring protein decomposition and water loss to measuring only the decomposition of hemoglobin in the bone. This led to the use of fluorescence spectroscopy instead of NIR. Using spectroscopic methods allowed for the measurements of minute changes in the decomposition of bone, and allowed for the accumulation of large amounts of data across a range of wavelengths. The incorporation of chemometrics was necessary to analyze the data and to create the models used for predictions.

In this work, as well as in the work by Dogra, both control and field studies were performed. Both studies shared similar goals, though different in their approach. Similarities can be seen in comparing the control versus field studies in the two sets of work. Control studies were able to provide an environment free of contaminants, but the error for the controlled experiments was higher. The field studies were advantageous in that they provide a more realistic view of what is

expected in a forensic setting. The results in the field research produced lower error than the control studies. While one would predict that more error should occur with the possible presence of contaminants, this proved not to be the case. Instead results may have improved as a result of the exposure to the elements, which could result in faster rates of decomposition and by providing a way for the decomposition products to be lost to the environment.

In view of this, it seems the method developed in this work could hold promise. Results indicate that the method could possibly be used in the future as a way of estimating PMI of skeletal remains. Fluorescence spectroscopy coupled with chemometrics proved to be capable of detecting minute changes in the spectra from luminol's reaction with the bone samples. The regression models constructed were able to predict PMI with errors ranging from ±9 days to ± 36 days, a considerable accomplishment considering that most methods can only predict whether a sample is of forensic interest or not. SIMCA methods were also capable of predicting the classes of samples with high efficiency. With additional research and development, the method might be considered at least as a preliminary test in forensic investigations. Future research should focus on accumulating data and trying to understand the variables that exist in a real world setting. Further work to attempt to optimize the luminol solution to enhance the intensity of the reaction may also be beneficial.

APPENDIX

Permissions for Copyrighted Materials

From: Dogra, Jody
To: Diamond, Tricia
Subject: Re: permissions request
Date: Thursday, March 24, 2011 10:53:32 AM

Tricia,

You are welcome to use these figures as you please.

--Jody

On 3/24/11 11:21 AM, "Diamond, Tricia" <Patricia_Diamond@baylor.edu> wrote:

> Dr. Dogra,
>
> I'd like to ask your permission to use several figures from your dissertation listed below which would help in explaining the hypothesis of my graduate thesis which is tentatively entitled *Multivariate Analysis of Luminescence Spectra as a Means of Determining Postmortem Interval*.
>
> Figure 1.4
> Figure 1.5
> Figure 1.6
> Figure 1.7
> Figure 1.8
> Figure 2.6
>
> Looking forward to hearing from you.
>
> Tricia Diamond
>
> Stockroom Manager
>
> Baylor University
>
> Sciences Stockroom F103
> 254-710-2078

Rightslink Printable License
https://s100.copyright.com/CustomerAdmin/PLF.jsp?lID=2011030_1299257975746[3/24/2011 11:16:38 AM]

JOHN WILEY AND SONS LICENSE
TERMS AND CONDITIONS

Mar 24, 2011

This is a License Agreement between Patricia A Diamond ("You") and John Wiley and Sons ("John Wiley and Sons") provided by Copyright Clearance Center ("CCC"). The license consists of your order details, the terms and conditions provided by John Wiley and Sons, and the payment terms and conditions.

All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.

License Number 2621991487746
License date Mar 04, 2011
Licensed content publisher John Wiley and Sons

Licensed content publication Luminescence
Licensed content title The assaying of haemoglobin using luminol chemiluminescence and its application to the dating of human skeletal remains
Licensed content author J. I. Creamer,A. M. Buck
Licensed content date Sep 1, 2009
Start page 311
End page 316
Type of use Dissertation/Thesis
Requestor type University/Academic
Format Electronic
Portion Figure/table
Number of figures/tables 1
Number of extracts
Original Wiley figure/table number(s)
Figure 2.
Will you be translating? No
Order reference number
Total 0.00 USD
Terms and Conditions

TERMS AND CONDITIONS

This copyrighted material is owned by or exclusively licensed to John Wiley & Sons, Inc. or one of its group companies (each a "Wiley Company") or a society for whom a Wiley Company has exclusive publishing rights in relation to a particular journal (collectively "WILEY"). By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the billing and payment terms and conditions established by the Copyright Clearance Center Inc., ("CCC's Billing and Payment terms and conditions"), at the time that you opened your Rightslink account (these are available at any time at http://myaccount.copyright.com)

Terms and Conditions

1. The materials you have requested permission to reproduce (the "Materials") are protected by copyright.

2. You are hereby granted a personal, non-exclusive, non-sublicensable, non-transferable, worldwide, limited license to reproduce the Materials for the purpose specified in the licensing process. This license is for a one-time use only with a maximum distribution equal to the number that you identified in the licensing process. Any form of republication granted by this licence must be completed within two years of the date of the grant of this licence (although copies prepared before may be distributed thereafter). The Materials shall not be used in any other manner or for any other purpose. Permission is granted subject to an appropriate acknowledgement given to the author, title of the material/book/journal and the publisher and on the understanding that nowhere in the text is a previously published source acknowledged for all or part of this Material.

Any third party material is expressly excluded from this permission.

3. With respect to the Materials, all rights are reserved. Except as expressly granted by the terms of the license, no part of the Materials may be copied, modified, adapted (except for minor

reformatting required by the new Publication), translated, reproduced, transferred or distributed,
in any form or by any means, and no derivative works may be made based on the Materials without the prior permission of the respective copyright owner. You may not alter, remove or suppress in any manner any copyright, trademark or other notices displayed by the Materials. You may not license, rent, sell, loan, lease, pledge, offer as security, transfer or assign the Materials, or any of the rights granted to you hereunder to any other person.
4. The Materials and all of the intellectual property rights therein shall at all times remain the exclusive property of John Wiley & Sons Inc or one of its related companies (WILEY) or their respective licensors, and your interest therein is only that of having possession of and the right
to reproduce the Materials pursuant to Section 2 herein during the continuance of this Agreement. You agree that you own no right, title or interest in or to the Materials or any of the
intellectual property rights therein. You shall have no rights hereunder other than the license as
provided for above in Section 2. No right, license or interest to any trademark, trade name, service mark or other branding ("Marks") of WILEY or its licensors is granted hereunder, and you
agree that you shall not assert any such right, license or interest with respect thereto.
5. NEITHER WILEY NOR ITS LICENSORS MAKES ANY WARRANTY OR REPRESENTATION OF ANY
KIND TO YOU OR ANY THIRD PARTY, EXPRESS, IMPLIED OR STATUTORY, WITH RESPECT TO THE
MATERIALS OR THE ACCURACY OF ANY INFORMATION CONTAINED IN THE MATERIALS, INCLUDING, WITHOUT LIMITATION, ANY IMPLIED WARRANTY OF MERCHANTABILITY, ACCURACY, SATISFACTORY QUALITY, FITNESS FOR A PARTICULAR PURPOSE, USABILITY, INTEGRATION OR NON-INFRINGEMENT AND ALL SUCH WARRANTIES ARE HEREBY EXCLUDED BY
WILEY AND ITS LICENSORS AND WAIVED BY YOU.
6. WILEY shall have the right to terminate this Agreement immediately upon breach of this Agreement by you.
7. You shall indemnify, defend and hold harmless WILEY, its Licensors and their respective directors, officers, agents and employees, from and against any actual or threatened claims, demands, causes of action or proceedings arising from any breach of this Agreement by you.
8. IN NO EVENT SHALL WILEY OR ITS LICENSORS BE LIABLE TO YOU OR ANY OTHER PARTY OR
ANY OTHER PERSON OR ENTITY FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL, INDIRECT,
EXEMPLARY OR PUNITIVE DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION
WITH THE DOWNLOADING, PROVISIONING, VIEWING OR USE OF THE MATERIALS REGARDLESS
OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.
9. Should any provision of this Agreement be held by a court of competent jurisdiction to be illegal, invalid, or unenforceable, that provision shall be deemed amended to achieve as nearly as
possible the same economic effect as the original provision, and the legality, validity and enforceability of the remaining provisions of this Agreement shall not be affected or impaired thereby.
10. The failure of either party to enforce any term or condition of this Agreement shall not constitute a waiver of either party's right to enforce each and every term and condition of this

Agreement. No breach under this agreement shall be deemed waived or excused by either party
unless such waiver or consent is in writing signed by the party granting such waiver or consent.
The waiver by or consent of a party to a breach of any provision of this Agreement shall not operate or be construed as a waiver of or consent to any other or subsequent breach by such

other party.
11. This Agreement may not be assigned (including by operation of law or otherwise) by you without WILEY's prior written consent.
12. Any fee required for this permission shall be non-refundable after thirty (30) days from receipt.
13. These terms and conditions together with CCC's Billing and Payment terms and conditions (which are incorporated herein) form the entire agreement between you and WILEY concerning
this licensing transaction and (in the absence of fraud) supersedes all prior agreements and representations of the parties, oral or written. This Agreement may not be amended except in writing signed by both parties. This Agreement shall be binding upon and inure to the benefit of
the parties' successors, legal representatives, and authorized assigns.
14. In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms
and conditions shall prevail.
15. WILEY expressly reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these
terms and conditions and (iii) CCC's Billing and Payment terms and conditions.
16. This Agreement will be void if the Type of Use, Format, Circulation, or Requestor Type was misrepresented during the licensing process.
17. This Agreement shall be governed by and construed in accordance with the laws of the State
of New York, USA, without regards to such state's conflict of law rules. Any legal action, suit or proceeding arising out of or relating to these Terms and Conditions or the breach thereof shall be
instituted in a court of competent jurisdiction in New York County in the State of New York in the
United States of America and each party hereby consents and submits to the personal jurisdiction of such court, waives any objection to venue in such court and consents to service of
process by registred or certified mail, return receipt requested, at the last known address of such
party. . BY CLICKING ON THE "I ACCEPT" BUTTON, YOU ACKNOWLEDGE THAT YOU HAVE READ
AND FULLY UNDERSTAND EACH OF THE SECTIONS OF AND PROVISIONS SET FORTH IN THIS AGREEMENT AND THAT YOU ARE IN AGREEMENT WITH AND ARE WILLING TO ACCEPT ALL OF YOUR OBLIGATIONS AS SET FORTH IN THIS AGREEMENT.
v1.4
Gratis licenses (referencing $0 in the Total field) are free. Please retain this printable
license for your reference. No payment is required.
If you would like to pay for this license now, please remit this license along with your
payment made payable to "COPYRIGHT CLEARANCE CENTER" otherwise you will be

invoiced within 48 hours of the license date. Payment should be in the form of a check
or money order referencing your account number and this invoice number RLNK10943224.
Once you receive your invoice for this order, you may pay your invoice by credit card.
Please follow instructions provided at that time.
Make Payment To:
Copyright Clearance Center
Dept 001
P.O. Box 843006
Boston, MA 02284-3006
For suggestions or comments regarding this order, contact Rightslink Customer Support: customercare@copyright.com or +1-877-622-5543 (toll free in the US) or +1-978-646-2777.

JOHN WILEY AND SONS LICENSE TERMS AND CONDITIONS

Mar 24, 2011

This is a License Agreement between Patricia A Diamond ("You") and John Wiley and Sons ("John Wiley and Sons") provided by Copyright Clearance Center ("CCC"). The license consists of your order details, the terms and conditions provided by John Wiley and Sons, and the payment terms and conditions.

All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.

License Number 2622000176806
License date Mar 04, 2011
Licensed content publisher John Wiley and Sons
Licensed content publication Luminescence
Licensed content title Luminol chemiluminescence: Chemistry, excitation, emitter
Licensed content author Gábor Merényi,Johan Lind,Trygve E. Eriksen
Licensed content date Jan 1, 1990
Start page 53
End page 56
Type of use Dissertation/Thesis
Requestor type University/Academic
Format Electronic
Portion Figure/table
Number of figures/tables 1
Number of extracts
Original Wiley figure/table number(s)
Scheme 1.
Will you be translating? No
Order reference number
Total 0.00 USD
Terms and Conditions

TERMS AND CONDITIONS

This copyrighted material is owned by or exclusively licensed to John Wiley & Sons, Inc. or one of
its group companies (each a "Wiley Company") or a society for whom a Wiley Company has exclusive publishing rights in relation to a particular journal (collectively "WILEY"). By clicking

"accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the billing and payment terms and conditions established by the Copyright Clearance Center Inc., ("CCC's Billing and Payment terms
and conditions"), at the time that you opened your Rightslink account (these are available at any
time at http://myaccount.copyright.com)
Terms and Conditions
1. The materials you have requested permission to reproduce (the "Materials") are protected by
copyright.

2. You are hereby granted a personal, non-exclusive, non-sublicensable, non-transferable, worldwide, limited license to reproduce the Materials for the purpose specified in the licensing process. This license is for a one-time use only with a maximum distribution equal to the number
that you identified in the licensing process. Any form of republication granted by this licence must
be completed within two years of the date of the grant of this licence (although copies prepared
before may be distributed thereafter). The Materials shall not be used in any other manner or for
any other purpose. Permission is granted subject to an appropriate acknowledgement given to the author, title of the material/book/journal and the publisher and on the understanding that nowhere in the text is a previously published source acknowledged for all or part of this Material.
Any third party material is expressly excluded from this permission.
3. With respect to the Materials, all rights are reserved. Except as expressly granted by the terms
of the license, no part of the Materials may be copied, modified, adapted (except for minor reformatting required by the new Publication), translated, reproduced, transferred or distributed,
in any form or by any means, and no derivative works may be made based on the Materials without the prior permission of the respective copyright owner. You may not alter, remove or suppress in any manner any copyright, trademark or other notices displayed by the Materials. You may not license, rent, sell, loan, lease, pledge, offer as security, transfer or assign the Materials, or any of the rights granted to you hereunder to any other person.
4. The Materials and all of the intellectual property rights therein shall at all times remain the exclusive property of John Wiley & Sons Inc or one of its related companies (WILEY) or their respective licensors, and your interest therein is only that of having possession of and the right
to reproduce the Materials pursuant to Section 2 herein during the continuance of this Agreement. You agree that you own no right, title or interest in or to the Materials or any of the
intellectual property rights therein. You shall have no rights hereunder other than the license as
provided for above in Section 2. No right, license or interest to any trademark, trade name, service mark or other branding ("Marks") of WILEY or its licensors is granted hereunder, and you
agree that you shall not assert any such right, license or interest with respect thereto.
5. NEITHER WILEY NOR ITS LICENSORS MAKES ANY WARRANTY OR REPRESENTATION OF ANY
KIND TO YOU OR ANY THIRD PARTY, EXPRESS, IMPLIED OR STATUTORY, WITH RESPECT TO THE
MATERIALS OR THE ACCURACY OF ANY INFORMATION CONTAINED IN THE MATERIALS,

INCLUDING, WITHOUT LIMITATION, ANY IMPLIED WARRANTY OF MERCHANTABILITY, ACCURACY, SATISFACTORY QUALITY, FITNESS FOR A PARTICULAR PURPOSE, USABILITY, INTEGRATION OR NON-INFRINGEMENT AND ALL SUCH WARRANTIES ARE HEREBY EXCLUDED BY
WILEY AND ITS LICENSORS AND WAIVED BY YOU.
6. WILEY shall have the right to terminate this Agreement immediately upon breach of this Agreement by you.
7. You shall indemnify, defend and hold harmless WILEY, its Licensors and their respective directors, officers, agents and employees, from and against any actual or threatened claims, demands, causes of action or proceedings arising from any breach of this Agreement by you.
8. IN NO EVENT SHALL WILEY OR ITS LICENSORS BE LIABLE TO YOU OR ANY OTHER PARTY OR
ANY OTHER PERSON OR ENTITY FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL, INDIRECT,
EXEMPLARY OR PUNITIVE DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION
WITH THE DOWNLOADING, PROVISIONING, VIEWING OR USE OF THE MATERIALS REGARDLESS
OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.
9. Should any provision of this Agreement be held by a court of competent jurisdiction to be illegal, invalid, or unenforceable, that provision shall be deemed amended to achieve as nearly as
possible the same economic effect as the original provision, and the legality, validity and enforceability of the remaining provisions of this Agreement shall not be affected or impaired thereby.
10. The failure of either party to enforce any term or condition of this Agreement shall not constitute a waiver of either party's right to enforce each and every term and condition of this Agreement. No breach under this agreement shall be deemed waived or excused by either party
unless such waiver or consent is in writing signed by the party granting such waiver or consent.
The waiver by or consent of a party to a breach of any provision of this Agreement shall not operate or be construed as a waiver of or consent to any other or subsequent breach by such other party.

11. This Agreement may not be assigned (including by operation of law or otherwise) by you without WILEY's prior written consent.
12. Any fee required for this permission shall be non-refundable after thirty (30) days from receipt.
13. These terms and conditions together with CCC’s Billing and Payment terms and conditions (which are incorporated herein) form the entire agreement between you and WILEY concerning
this licensing transaction and (in the absence of fraud) supersedes all prior agreements and representations of the parties, oral or written. This Agreement may not be amended except in writing signed by both parties. This Agreement shall be binding upon and inure to the benefit of
the parties' successors, legal representatives, and authorized assigns.
14. In the event of any conflict between your obligations established by these terms and conditions and those established by CCC’s Billing and Payment terms and conditions, these terms

and conditions shall prevail.
15. WILEY expressly reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these
terms and conditions and (iii) CCC's Billing and Payment terms and conditions.
16. This Agreement will be void if the Type of Use, Format, Circulation, or Requestor Type was misrepresented during the licensing process.
17. This Agreement shall be governed by and construed in accordance with the laws of the State
of New York, USA, without regards to such state's conflict of law rules. Any legal action, suit or proceeding arising out of or relating to these Terms and Conditions or the breach thereof shall be
instituted in a court of competent jurisdiction in New York County in the State of New York in the
United States of America and each party hereby consents and submits to the personal jurisdiction of such court, waives any objection to venue in such court and consents to service of
process by registred or certified mail, return receipt requested, at the last known address of such
party. . BY CLICKING ON THE "I ACCEPT" BUTTON, YOU ACKNOWLEDGE THAT YOU HAVE READ
AND FULLY UNDERSTAND EACH OF THE SECTIONS OF AND PROVISIONS SET FORTH IN THIS AGREEMENT AND THAT YOU ARE IN AGREEMENT WITH AND ARE WILLING TO ACCEPT ALL OF YOUR OBLIGATIONS AS SET FORTH IN THIS AGREEMENT.
v1.4
**Gratis licenses (referencing $0 in the Total field) are free. Please retain this printable
license for your reference. No payment is required.
If you would like to pay for this license now, please remit this license along with your
payment made payable to "COPYRIGHT CLEARANCE CENTER" otherwise you will be invoiced within 48 hours of the license date. Payment should be in the form of a check
or money order referencing your account number and this invoice number RLNK10943230.
Once you receive your invoice for this order, you may pay your invoice by credit card.
Please follow instructions provided at that time.
Make Payment To:
Copyright Clearance Center
Dept 001
P.O. Box 843006
Boston, MA 02284-3006
For suggestions or comments regarding this order, contact Rightslink Customer Support: customercare@copyright.com or +1-877-622-5543 (toll free in the US) or +1-
978-646-2777.**

Rightslink Printable License
https://s100.copyright.com/CustomerAdmin/PLF.jsp?lID=2011030_1299257654048[3/24/2011 11:17:44 AM]

JOHN WILEY AND SONS LICENSE TERMS AND CONDITIONS

Mar 24, 2011

This is a License Agreement between Patricia A Diamond ("You") and John Wiley and Sons ("John Wiley and Sons") provided by Copyright Clearance Center

("CCC"). The license consists of your order details, the terms and conditions provided by John Wiley and Sons, and the payment terms and conditions.
All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.
License Number 2621991166048
License date Mar 04, 2011
Licensed content publisher John Wiley and Sons
Licensed content publication Luminescence
Licensed content title Studies on PVP hydrogel supported luminol chemiluminescence: 2. Luminometer calibration and potential analytical applications
Licensed content author Erick Leite Bastos,Luiz Francisco Monteiro Leite Ciscato,Fernando Heering Bartoloni,Luiz Henrique Catalani,Paulete Romoff,Wilhelm Josef Baader
Licensed content date Mar 1, 2007
Start page 126
End page 133
Type of use Dissertation/Thesis
Requestor type University/Academic
Format Electronic
Portion Figure/table
Number of figures/tables 2
Number of extracts
Original Wiley figure/table number(s)
Figure 2
Will you be translating? No
Order reference number
Total 0.00 USD
Terms and Conditions
TERMS AND CONDITIONS
This copyrighted material is owned by or exclusively licensed to John Wiley & Sons, Inc. or one of
its group companies (each a "Wiley Company") or a society for whom a Wiley Company has exclusive publishing rights in relation to a particular journal (collectively "WILEY"). By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the billing and payment terms and conditions established by the Copyright Clearance Center Inc., ("CCC's Billing and Payment terms
and conditions"), at the time that you opened your Rightslink account (these are available at any
time at http://myaccount.copyright.com)
Terms and Conditions
Rightslink Printable License
https://s100.copyright.com/CustomerAdmin/PLF.jsp?lID=2011030_1299257654048[3/24/2011 11:17:44 AM]
1. The materials you have requested permission to reproduce (the "Materials") are protected by
copyright.
2. You are hereby granted a personal, non-exclusive, non-sublicensable, non-transferable, worldwide, limited license to reproduce the Materials for the purpose specified in the licensing process. This license is for a one-time use only with a maximum distribution equal to the number
that you identified in the licensing process. Any form of republication granted by this licence must
be completed within two years of the date of the grant of this licence (although copies prepared

POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.
9. Should any provision of this Agreement be held by a court of competent jurisdiction to be illegal, invalid, or unenforceable, that provision shall be deemed amended to achieve as nearly as
possible the same economic effect as the original provision, and the legality, validity and enforceability of the remaining provisions of this Agreement shall not be affected or impaired thereby.
10. The failure of either party to enforce any term or condition of this Agreement shall not constitute a waiver of either party's right to enforce each and every term and condition of this Agreement. No breach under this agreement shall be deemed waived or excused by either party
unless such waiver or consent is in writing signed by the party granting such waiver or consent.

The waiver by or consent of a party to a breach of any provision of this Agreement shall not operate or be construed as a waiver of or consent to any other or subsequent breach by such other party.
11. This Agreement may not be assigned (including by operation of law or otherwise) by you without WILEY's prior written consent.
12. Any fee required for this permission shall be non-refundable after thirty (30) days from receipt.
13. These terms and conditions together with CCC’s Billing and Payment terms and conditions (which are incorporated herein) form the entire agreement between you and WILEY concerning
this licensing transaction and (in the absence of fraud) supersedes all prior agreements and representations of the parties, oral or written. This Agreement may not be amended except in writing signed by both parties. This Agreement shall be binding upon and inure to the benefit of
the parties' successors, legal representatives, and authorized assigns.
14. In the event of any conflict between your obligations established by these terms and conditions and those established by CCC’s Billing and Payment terms and conditions, these terms
and conditions shall prevail.
15. WILEY expressly reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these
terms and conditions and (iii) CCC’s Billing and Payment terms and conditions.
16. This Agreement will be void if the Type of Use, Format, Circulation, or Requestor Type was misrepresented during the licensing process.
17. This Agreement shall be governed by and construed in accordance with the laws of the State
of New York, USA, without regards to such state’s conflict of law rules. Any legal action, suit or proceeding arising out of or relating to these Terms and Conditions or the breach thereof shall be
instituted in a court of competent jurisdiction in New York County in the State of New York in the
United States of America and each party hereby consents and submits to the personal jurisdiction of such court, waives any objection to venue in such court and consents to service of
process by registred or certified mail, return receipt requested, at the last known address of such
party. . BY CLICKING ON THE "I ACCEPT" BUTTON, YOU ACKNOWLEDGE THAT YOU HAVE READ
AND FULLY UNDERSTAND EACH OF THE SECTIONS OF AND PROVISIONS SET FORTH IN THIS

AGREEMENT AND THAT YOU ARE IN AGREEMENT WITH AND ARE WILLING TO ACCEPT ALL OF YOUR OBLIGATIONS AS SET FORTH IN THIS AGREEMENT.
v1.4
**Gratis licenses (referencing $0 in the Total field) are free. Please retain this printable
license for your reference. No payment is required.
If you would like to pay for this license now, please remit this license along with your
payment made payable to "COPYRIGHT CLEARANCE CENTER" otherwise you will be invoiced within 48 hours of the license date. Payment should be in the form of a check
or money order referencing your account number and this invoice number RLNK10943214.
Once you receive your invoice for this order, you may pay your invoice by credit card.
Please follow instructions provided at that time.
Make Payment To:
Copyright Clearance Center
Dept 001
P.O. Box 843006
Boston, MA 02284-3006
For suggestions or comments regarding this order, contact Rightslink Customer Support: customercare@copyright.com or +1-877-622-5543 (toll free in the US) or +1-
978-646-2777.**

CENGAGE Learning

NELSON EDUCATION

Global Rights and Permissions Administration

20 Davis Drive, Belmont, California 94002 USA
Phone: 800-730-2214 or 650-413-7456 Fax: 800-730-2215 or 650-595-4609
Email: permissionrequests@cengage.com

Submit all requests online at www.cengage.com/permissions.

Response # 250870

03/14/2011

Patricia A Diamond
Baylor University
Chemistry and Biochemistry
OBP #97348
Waco, TX 76798

Thank you for your interest in the following Cengage Learning/Nelson Education, or one of their respective subsidiaries, divisions or affiliates (collectively, "Cengage/Nelson") material.

Title:	Principles of Instrumental Analysis 5E		
Author(s):	SKOOG	ISBN:	9780030020780 (0030020786)
Publisher:	Brooks/Cole	Year:	1998

Specific material: pages 357; pages 365; Figure 15-1 and Figure 15-4
Total pages: 2

For use by:
Name: Diamond
School/University/Company:
Course title/number: Multivariate Analysis of Luminescence Spectra as a Means of Determining Postmortem Interval
Term of use: 2011

Intended use:
For inclusion in a research project, master's thesis, or doctoral dissertation. May also be stored electronically for on-demand delivery through a dissertation storage system such as UMI system or as listed above. This permission is for non-exclusive rights for the US and Canada in English. Permission extends only to the work specified in this agreement, not to any future editions, versions, or publications. Applicant will not attempt to assign rights given herein to others, and the publication of this material in the work herein approved does not permit quotation therefrom in any other work. If, at a later date, a publishing contract is achieved, additional permission will be required.

The non-exclusive permission granted in this letter extends only to material that is original to the aforementioned text. As the requestor, you will need to check all on-page credit references (as well as any other credit / acknowledgement section(s) in the front and/or back of the book) to identify all materials reprinted therein by permission of another source. Please give special consideration to all photos, figures, quotations, and any other material with a credit line attached. You are responsible for obtaining separate permission from the copyright holder for use of all such material. For your convenience, we may also identify here below some material for which you will need to obtain separate permission. For your reference, we identify the following material for which you will need to obtain separate permission.

all third party material

This credit line must appear on the first page of text selection and with each individual figure or photo:

From SKOOG. *Principles of Instrumental Analysis*, 5E. © 1998 Brooks/Cole, a part of Cengage Learning, Inc. Reproduced by permission. www.cengage.com/permissions

Sincerely,

Karen Lee
Senior Grant Coordinator

From: Suzanne Paris
To: Diamond, Tricia
Subject: ABC-CLIO Web Permission Request
Date: Tuesday, April 26, 2011 2:19:40 PM

April 26, 2011

Dear Ms. Diamond,

Thank you for your recent request (copy below) for use of material from an ABC-CLIO, LLC title or (one of its imprints). *Page 53, 1 Figure from Kelly, Evelyn, The Skeletal System, Greenwood, 2004.*

ABC-CLIO, LLC is pleased to grant you a complimentary permission for one-time use of the material as described in your request. Non-transferable, non-exclusive one time only permission is extended to you for one for distribution as described below. Non-exclusive permission is extended to special non-profit editions for the handicapped. No changes, adaptations, or deletions are to be made except as approved.

Thesis - Electronic Copy. English Language One Time Only Use. Display granted for a term of 1 Academic Year, 2011-2012.

This permission does not include any material (including photos, illustrations, tables, and figures) reprinted by ABC-CLIO, LLC from another source. Permission for such use must be separately requested from the original copyright holder, as specified in our credit notice. Credit notice can be found on the same page as the material, in chapter notes or in front matter for the citation.

We require that the following notice appear on our copyrighted material: BOOK title, Author/Editor(s) of BOOK (exactly as on title page). Copyright (c) (date) by _____ (exact copyright notice from reverse of title page). All rights reserved. Reproduced with permission of ABC-CLIO, LLC, Santa Barbara, CA.

Regards,

Suzanne Paris
Rights & Permissions Coordinator
ABC-CLIO LLC
130 Cremona Drive, Suite C
Santa Barbara, CA 93117-5505
Tel: 805-880-6808
Fax: 805- 880-5834

REFERENCES

(1) Reichs, K. J., Ed.; In *Forensic Osteology: Advances in the Identification of Human Remains;* Charles C. Thomas Publisher, Ltd.: Springfield IL, 1998.

(2) Byrd, J. H.; Castner, J. L., Eds.; In *Forensic Entomology: The Utility of Anthropods in Legal Investigations;* CRC Press: Boca Raton, 2001.

(3) Buchan, M. J.; Anderson, G. S. *J. Can. Soc. Forensic Sci.* **2001***, 34*, 1-22.

(4) Knight, B.; Lauder, I. Methods of Dating Skeletal Remains. *Hum. Bio.* **1969***, 41(3)*, 322-341.

(5) Berg, S. The Determination of Bone Age. *Methods of forensic science* **1963***, 2*, 231-252.

(6) Facchini, F.; Pettener, D. Chemical and physical methods in dating human skeletal remains. *Am. J. Phy. Anthropology* **1977***, 47(1)*, 65-70.

(7) Prieto-Castello, M. J.; Hernandez del Rincon, J. P.; Perez-Sirvent, C.; Alvarez-Jimenez, P.; Perez-Carceles, M. D.; Osuna, E.; Luna, A. *Forensic Sci. Int.* **2007***, 172*, 112-118.

(8) Swift, B. Dating human skeletal remains: Investigation the viability of measuring the equilibrium between 210Po and 210Pb as a means of estimating the post-mortem interval. *Forensic Sci. Int.* **1998***, 98*, 119-126.

(9) Ubelaker, D. H.; Buchholz, B. A.; Stewart, J. E. B. Analysis of Artificial Radiocarbon in Different Skeletal and Dental Tissue Types to Evaluate Date of Death. *J. Forensic Sci.* **2006***, 51*, 484-488.

(10) Villanueva, P. E.; Girela, F.; Castellanos, M. J. *J. Forensic Sci.* **1976***, 21*, 822-830.

(11) Introna, F.; Di Vella, G.; Campobasso, C. P. Determination of Postmortem Interval from Old Skeletal Remains by Image Analysis of Luminol Test Results. *J Forensic Sci* **1999***, 44(3)*, 535-538.

(12) Swift, B.; Lauder, I.; Black, S.; Norris, J. An estimation of the post-mortem interval in human skeletal remains: a radionuclide and trace element approach. *Forensic Sci. Int.* **2001***, 117*, 73-87.

(13) Taylor, R. E.; Suchey, J. M.; Payen, L. A.; Slota, P. J. J. The Use of Radiocarbon (14C) to Identify Human Skeletal Materials of Forensic Science Interest. *J. Forensic Sci.* **1989***, 34*, 1196-1205.

(14) Wild, E. M.; Arlamovsky, K. A.; Golser, R.; Kutschera, W.; Priller, A.; Puchegger, S.; Rom, W.; Steier, P.; Vycidilik, W. 14C dating with the bomb peak: An application to forensic medicine. *Nucl. Instr. and Meth. in Phys. Res. B* **2000***, 172*, 944-950.

(15) Neis, P.; Hille, R.; Paschke, M.; Pilwat, G.; Schnabel, A.; Niess, C.; Bratzke, H. *Forensic Sci. Int.* **1999***, 99*, 47-51.

(16) Maclaughlin-Black, S. M.; Herd, R. J. M.; Willson, K.; Myers, M.; West, I. E. Strontium-90 as an indicator of time since death: A pilot investigation. *Forensic Sci. Int.* **1992***, 57(1)*, 51-56.

(17) Jimenez, S. B. In *In In TIme Since Deathe in Forensic Anthropology: A Discussion of Tritium Method;* Annual Meeting of the American Academy of Forensic Sciences; Boston, MA, 1993; .

(18) Ramsthaler, F.; Kreutz, K.; Verhoff, M. A. Dating skeletal remains with luminol-chemiluminescence. Validity, in interobserver error. *Forensic Sci. Int.* **2009***, 187*, 47-50.

(19) AnonymousKnown and Probable Human Carcinogens. http://www.cancer.org/Cancer/CancerCauses/OtherCarcinogens/GeneralInformationaboutCarcinogens/known-and-probable-human-carcinogens?sitearea=PED (accessed 03/23, 2011).

(20) Castellano, M. A.; Villanueva, E. C.; von Frenckel, R. *J Forensic Sci* **1984***, 29(2*), 527-534.

(21) Dogra, J.; Busch, K. W.; Busch, M. A. Multivariate analysis of near-infrared and UV spectral data, Baylor University, Waco, TX, 2009.

(22) Creamer, J. I.; Buck, A. M. The assaying of haemoglobin using luminol chemiluminescence and its application to the dating of human skeletal remains. *Luminescence* **2009**, 24, 311-316.

(23) Shipman, P.; Walker, A.; Bichell, D. In *The Human Skeleton;* Harvard University Press: Cambridge, MA, 1985, pp 343.

(24) Kelly, E., Ed.; In *The Skeletal System;* Windelspecht, M., Ed.; Human Body Systems; Greenwood Press: Westport, CT, 2004; Vol. 1, pp 231.

(25) Maton, A.; Hopkins, J.; McLaughlin, C. M.; Johnson, S.; Warner, M. Q.; LaHart, D.; Wright, J. D., Eds.; In *Human Biology and Health;* Prentice Hall: Englewood Cliffs, NJ, 1993.

(26) Dominguez de Villota, E. D.; Ruiz Carmona, M. T.; Rubio, J. J.; de Andres, S. *Br. J. Anaesth.* **1981**, *5*3, 1325-1328.

(27) McKee, T.; McKee, J. R., Eds.; In *Biochemistry;* McGraw Hill: Boston, 2003; pp 771.

(28) Steinberg, M. H., Ed.; In *Disorders of Hemoglobin: Genetics, Pathophysiology, and Clinical Management;* Cambridge University Press: New York, 2001.

(29) Hardison, R. C. "A brief history of hemoglobins: plant, animal, protist, and bacteria". http://www.pebmedcentral.gov/articlerender.fcgi?tool=pubmed& pubmedid=8650150 (accessed June, 11, 1996).

(30) Bunn, H. F.; Forget, B. G. In *Hemoglobin: Molecular, Genetic, and Clinical Aspects;* Saunders WB Co.: Philadelphia, 1986.

(31) Barni, F.; Lewis, S. W.; Berti, A.; Miskelly, G. M.; Lago, G. *Talanta* **2007**, *7*2, 896-913.

(32) Gaensslen, R. E. In *Sourcebook in Forensic Serology, Immunology, and Biochemistry;* U. S. Department of Justice: Washington DC, 1983.

(33) Proescher, F.; Moody, A. M. Detection of blood by means of chemiluminescence. *J. Lab. Clin. Med.* **1939**, 24, 1183-1189.

(34) Yeshion, T. E. In Stanley, P. E., Kricka, L. J., Eds.; Proceedings of the 6th International Symposium on Bioluminescence and Chemiluminescence; John Wiley & Sons Ltd.: Chichester, 1991; pp 379-384.

(35) Grispino, R. R. J. *Prosecutor* **1991**, 25, 28-32.

(36) Kadish, K.; Smith, K. M.; Guilard, R. In *The Porphyrin Handbook;* Academic Press: London, 1999; Vol. 4.

(37) Albrecht, H. O. *Z. Physiol. Chem.* **1928**, 136, 321-330.

(38) Merenyi, G.; Lind, J.; Eriksen, T. E. Luminol Chemiluminescence: Chemistry, Excitation, Emitter. *J. Biolumin. Chemilumin.* **1990**, 5, 53-56.

(39) Lee, J.; Seigler, H. Quantum yields of the luminol chemiluminescence reaction in aqueous and aprotic solvents. *Photochem. Photobiol.* **1972**, *15;*2, 227-237.

(40) Lin, D.; Zhang, H.; Yang, X.; Huang, Y. *Acta Scientiarum Naturalium Universitatis Sunyatseni* **1997**, 36, 59-63.

(41) Merenyi, G.; Lind, J. Role of a Peroxide Intermediate in the Chemiluminescence of Luminol. A Mechanistic Study. *J. Am. Chem. Soc.* **1980**, 102, 5830-5835.

(42) Kirk, R. E.; Othmer, D. F., Eds.; In *Encyclopedia of Chemical Technology;* Academic Press: New York, 1992; Vol. 8, pp 159-180.

(43) Bastos, E. L.; Ciscato, L.; Bartoloni, F. H.; Catalani, L. H.; Baader, W. J. Studies on PVP hydrogel-supported luminol chemiluminescence: 1. Kinetic and mechanistic aspects using multivariate factorial analysis. *Luminescence* **2007**, 22, 113-125.

(44) Bastos, E. L.; Ciscato, L.; Bartoloni, F. H.; Catalani, L. H.; Romoff, P.; Baader, W. J. Studies on PVP hydrogel-supported luminol chemiluminescence: 2. Luminol calibration and potential analytical applications. *Luminescence* **2007**, 22, 126-133.

(45) Marquette, C. A.; Blum, L. J. Applications of the luminol chemiluminescent reaction in analytical chemistry. *Anal Bioannal Chem.* **2006**, 385, 546-554.

(46) Yeshion, T. E. The Forensic Application of Luminol as a Presumptive Blood Test. *Bioluminescence and Chemiluminescence* **1991**, 1990(379), 379-384.

(47) Gibson, E. P. *J. Forensic Sci.* **1977**, 22, 680-696.

(48) Specht, W. *Angew. Chemie.* **1937**, 50, 155-157.

(49) Skoog, D. A.; Holler, F. J.; Nieman, T. A. In *Principles of Instrumental Analysis;* Brooks/Cole: United States of America, 1998; pp 849.

(50) Sharma, A.; Schulman, S. G. In *Introduction to Fluorescence Spectroscopy;* Techniques in Analytical Chemistry; John Wiley and Sons Inc.: New York, 1999; pp 173.

(51) Bu, D.; Hopkins, D. W., Eds.; In *Multivariate Data Analysis with Chemometrics and Spectroscopic Investigations;* Camo, Inc.: Piscataway, NJ, 2007.

(52) Beebe, K. R.; Pell, R. J.; Seaholtz, M. B. In *Chemometrics: A Practical Guide;* John Wiley and Sons: New York, 1998.

(53) Kramer, R., Ed.; In *Chemometric Techniques for Quantitative Analysis;* Marcel Dekker Inc.: New York, 1998.

(54) Barker, M.; Rayens, W. *J. Chemom.* **2003**, 17, 166-173.

(55) Umea University 40 Aspects of Umea University. http://www.umu.se/info enheten/jubileum05/images/pdf/jubskrift_eng.pdf (accessed October 25, 2008).

(56) Editorial *Chemom. Intell. Lab. Syst.* **1995**, 29, 155-156.

(57) Wold, S. *Chemom. Intell. Lab. Syst.* **1995**, 30, 109-115.

(58) Wold, S.; Sjostrom, M. *Chemom. Intell. Lab. Syst.* **1998**, 44, 3-14.

(59) Savitzky, A.; Golay, J. E. *Anal. Chem*. **1964**, 36, 1627-1.