DATING SILK AND OTHER INNOVATIONS IN MASS SPECTROMETRY

MARY W. BALLARD, CHRISTINE A.M. FRANCE, MEHDI MOINI, AND CAROLINE SOLAZZO

ABSTRACT – This paper introduces three recent mass spectrometric methods that can aid the field of conservation. Amino acid racemization and protein deamidation using CE-MS in conjunction with proteomics are new techniques for dating and authenticating old or new protein fibers that cannot otherwise be matched to a specific date by more common analytical techniques such as C-14 dating or dye analysis. These techniques are useful when extant archaeological fibers, often protein, have degraded or damaged scale patterns that prevent conclusive identification by polarized light microscopy. The second method identifies species by peptide mass finger printing using matrix assisted laser desorption ionization in conjunction with time-of-flight mass spectrometry. This technique allows for rapid identification of the animal species whose fibers were used in the construction of textile and ethnographic objects. The final method, stable isotope mass spectrometry, can identify ecological characteristics about the animal from which a natural fiber originated, thus providing clues about the interaction of humans with their environment. These three methods are especially viable for textile and costume analysis because they require only milligrams (0.001 grams) or sub-milligrams (<0.001 grams) samples to provide precise and accurate information about the proteinaceous specimens.

RESUMEN – Este documento presenta tres métodos recientes de espectometría de masas que pueden ayudar en el campo de la conservación. La racemización de aminoácidos y la desaminación de proteínas con CE-MS junto con proteomicos son técnicas nuevas para datar y autenticar fibras proteicas viejas o nuevas que no puedan ser asociadas con una fecha específica mediante técnicas analíticas más comunes como la datación C-14 o el análisis de tintura. Estas técnicas son útiles cuando las fibras arqueológicas sobrevivientes, generalmente proteínas, tienen patrones de escamas degradados o dañados que impiden la identificación concluyente mediante el microscopio de luz polarizada. El segundo método identifica a las especies mediante la impresión digital de la masa péptica usando ionización de desabsorción de láser asistida por una matriz junto con espectometría de masas por tiempo de vuelo. Esta técnica permite identificar rápidamente a las especies animales cuyas fibras fueron utilizadas en la construcción de telas y objetos etnográficos. El último método, la espectometría de masas por isotopos estables, puede identificar las características ecológicas del animal en el que se originó una fibra natural, brindando así pistas sobre la interacción de los humanos con su medioambiente. Estos tres métodos son especialmente viables para el análisis de telas y vestimentas porque solo se necesitan muestras de miligramos (0,001 gramos) o sub-miligramos ((<0,001 gramos) para obtener información precisa y exacta sobre los especímenes proteináceos.

1. INTRODUCTION

Mass spectrometry (MS) was developed in the early 20th century as a means of identifying atoms and their isotopes. By the mid 20th century, MS became a tool for the identification of molecules in complex mixtures; by the late 20th century, it became one of the fastest-growing analytical techniques with many diverse applications. MS is essentially an expensive balance that measures the weights of atoms and molecules. It does so by converting the analyte atoms and molecules into ions in the gas phase using a variety of ionization techniques. The masses of these ions are then measured according to their behavior inside either electric or magnetic fields (the MS analyzer). Using high resolution, high mass accuracy mass analyzers, the mass of the ions can be measured to such a high degree of accuracy that the

chemical compositions of the parent molecules can be identified. Molecular ions or protonated molecules can be further fragmented by a variety of fragmentation techniques to provide more information about the chemical structure. All of this means that mass spectrometers have several important characteristics for conservation science applications: they provide molecular weights and chemical structure; they provide this information using minute quantities of specimens; they can provide this information even if the specimens are in complex mixtures; they can analyze gas, liquid, and solids; and they can also provide both qualitative and quantitative information.

There are four fundamental parts to all mass spectrometers (fig.1): an *inlet port* introduces the sample into the mass spectrometer's ion source; an *ion source* generates ions in the gas phase; the *mass analyzer* separates the molecular ions and their fragments based on their mass to charge ratios (m/z); and the *detector* which detects the ions. To control and minimize the interaction of ions with neutral molecules, the ion transfer lenses, analyzer, and MS detector are kept in a *vacuum system* which provides an extremely low-pressure environment, free of atmospheric gases.

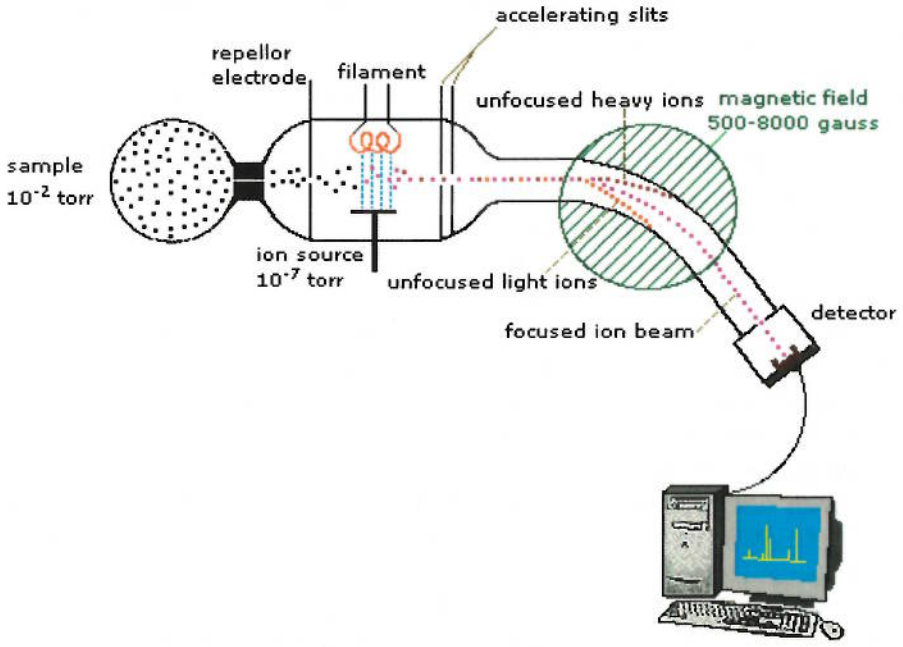

Figure1: Diagram of the Principle Parts of a Mass Spectrometer ("Mass Spectrometry" website, no date). The sample enters, in this example at low vacuum; it proceeds to the ion generating area; its components are separated by the mass analyzer; and the results are enumerated by the detector.

Mass spectrometers are divided into two general categories, atmospheric pressure and conventional, based on whether ions are generated at atmospheric pressure or in a vacuum. Each category has a variety of ionization techniques associated with it. Electrospray ionization and atmospheric pressure chemical ionization are examples of atmospheric pressure ionization techniques, while electron ionization and matrix assisted laser desorption ionization (MALDI) are examples of ionization techniques used under a vacuum in conventional systems. Both positive and negative ions can be generated and analyzed, as one

mode of ionization may provide more information at higher sensitivity than the other for a given sample. Each method of mass spectrometry has its own sample preparation and protocol, which may include weighing, digestion, and dilution, before the sample is injected through the inlet port. Protein fibers are insoluble in water and must be manipulated, broken up, or digested before reaching the ion source. For amino acid racemization or peptide sequencing analyses, a homogeneous solution must be prepared and the sample divided for multiple experimental trials. For stable isotope analyses, proteins are introduced in their solid form, oxidatively combusted, and reacted at high temperature across solid reagants to convert it into gaseous components. The low pressure environment encourages the molecules to disperse and to enter the ion source at a smooth, consistent rate.

For the analysis of very complex mixtures, a separation technique is usually used in conjunction with mass spectrometry. These separation techniques divide the components of the complex mixture into less complex mixtures, (ideally into individual components), and introduce them into MS in a serial fashion. The most common separation techniques include gas chromatography, which separates volatile and semi-volatile compounds based on their boiling points, liquid chromatography, which separates compounds based on their hydrophobicity, and capillary electrophoresis, which separates compounds based on their charge and three-dimensional structures.

2. KINDS OF MASS SPECTROMETERS USED FOR TEXTILE ANALYSES

Capillary electrophoresis mass spectrometry (CE-MS) is a powerful analytical technique that can separate and analyze a wide range of chemicals from amino acids to protein complexes, while only consuming minimal amounts of samples (approximately a nano-liter, 10^{-6} of a milliliter). CE uses a very narrow capillary for compound separation, which allows for high separation efficiency and high sensitivity at fast speeds. Co-author Dr. Mehdi Moini has developed a method to use CE-MS to date silk by detecting amino acid racemization (Moini et al., 2011). Most living things contain proteins that are formed from L-amino acids (optically, L-amino acids will rotate a beam of light to the left, so they are characterized as left handed amino acids --"L" for *levo-rotary*). However, once proteins are synthesized, the amino acids begin to equilibrate to D-amino acids (right handedness --"D" for *dextro-rotary*), a process known as racemization. When this happens, the combination of L and D amino acids is known as a racemic mixture. With silk, the aspartic amino acid has a ratio of D to L that builds up consistently over 2,500 years (fig.2). CE-MS can be used to determine the aspartic acid D:L ratio for a given sample, and thus the extent of racemization that has occurred over time. Since the ratio changes at a constant rate, it can be used to determine the age of the silk. This makes CE-MS a very useful tool for textile conservators and historians. In fact, a similar system is being developed by Moini for sheep wool and other proteins. Currently, the principal aim of Moini's research is to obtain more silk samples from museum textiles with an absolute known date of origin to add to his curve of standards (fig.3)."

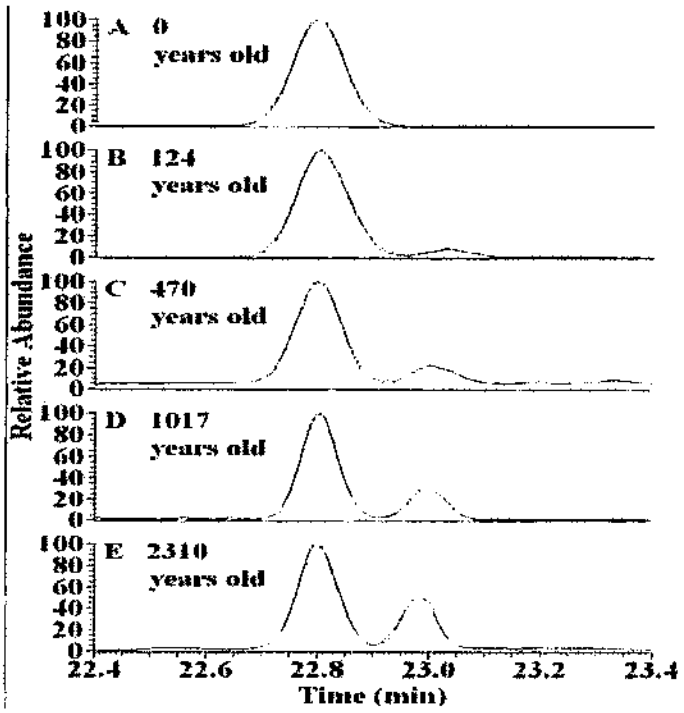

Figure2: Electropherogram of the D- and L-aspartic acid peaks at 574 m/z for fresh silk (2010), a silk flag from 1883-8, silk from the 1540's, silk from a Tiraz fragment (933 AD), and silk from 200-400 B.C.

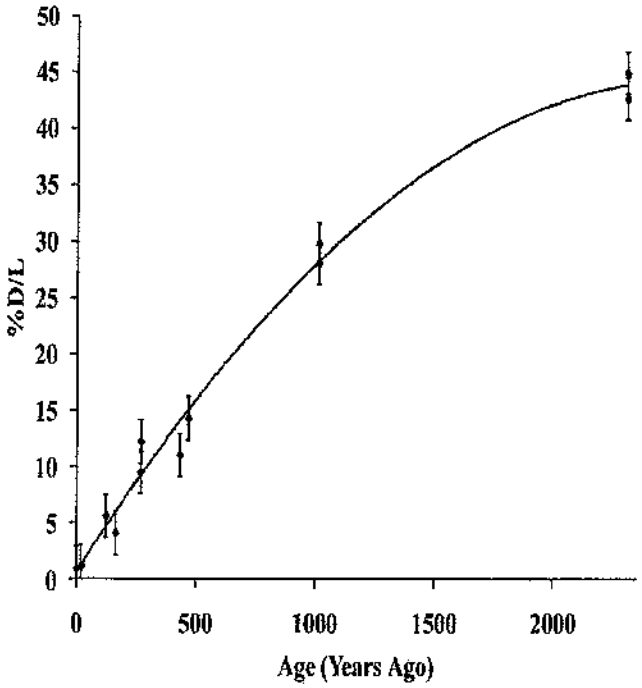

Figure3: The slope of the D/L ratio is plotted as a percentage against Time. Note that the D/L percentage is graphed on the basis on the age of the protein from when it was synthesized ("years ago") rather than on the *anno domini* age of the western calendar, like "1350 AD.

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Matrix assisted laser desorption time of flight mass spectrometry (MALDI-TOF-MS) is a type of mass spectrometry that uses the energy of a laser to ionize molecules deposited on a plate. Entire proteins can be analyzed by mass using this technique. However, the bigger the molecule, the less accurate the m/z is, making precise identification of the protein difficult. Protein samples are therefore broken down using enzymes and purified to obtain smaller fragments called peptides before deposition on the plate. The set of peptides produced by the enzymatic digestion is not only unique to a type of proteins, but also to the species from which the sample originated. The more related two species are, the more peptides they will have in common. The profile of peptides extracted from the mass spectrometer after MALDI-TOF-MS is called the peptide mass fingerprint (PMF), and can be matched to one or more parent proteins. The peptide sequences have been mapped out for certain species and unknown protein samples can be identified by comparison. . When the sequence of a peptide has not been mapped and is unknown, it can be fragmented to obtain structural information and reconstitute its amino acid sequence. This process is called MS/MS as it happens in two steps: first the characterization by m/z of the peptides in the mixture; then the fragmentation of each peptide into an amino acid profile where amino acids are recognized by their m/z position. This allows pinpointing differences between species, resulting from substitution of amino acids in peptidic sequences. In addition, databases (for example, that of the National Center for Biotechnology Information (NCBI), found at http://www.ncbi.nlm.nih.gov/protein/, accessed 10/17/12) are available and regularly updated with new sequences.

Using this method, Dr. Caroline Solazzo recently resolved a major question about the Coast Salish and the Mountain Salish Indian blankets in the Smithsonian collections using this type of proteomic analysis. Her research focused on the blended fibers of these ceremonial blankets and determining whether they included protein fibers from a wooly coastal island dog, a mountain goat, and/or sheep. Historically, for the Salish culture, fibers from the wild mountain goats were difficult to obtain and had high status; the wooly dogs were raised by the coastal Salish as an alternative fiber source (Barsh et al., 2002; Murray, et al., 2005; Schulting, 1994). First, documented specimens of the wooly dog and the mountain goat, held by the vertebrate zoology collection of the National Museum of Natural History (NMNH), were sampled, tested, and evaluated to find dissimilar peptide markers. Comparison of profiles between dog and goat resulted in many different peptides as these species are genetically distant, but goat was differentiated from sheep by only one peptide, whose sequence was determined using MS/MS analysis, and it was found to have one amino acid of difference (Solazzo, et al., 2011). Samples from a ceremonial blanket and pelt robe in the NMNH collection, as well as samples from blankets in the National Museum of the American Indian (NMAI) were then analyzed and compared to the references from the documented specimens. NMNH's ceremonial blanket, collected in 1919, proved to be pure mountain goat; their pelt-robe, possibly from 1838, was found to be dog; two other blankets and a sash from the 1830's were a mixture of goat and dog. No dog hair fibers were found in the NMAI blankets, but sheep fibers were found in some of their 19th century blankets (Solazzo, et al., 2011). This work confirms a spectrum of weaving fibers, and, by confirming the presence of dog, substantiates the oral history passed down by generations of Coast Salish Indians (Murray et al., 2005).

Another way to identify fiber sources is based on the ratio of stable isotopes that make up the fiber. A stable isotope is an atom that has obtained an extra neutron or two, making it slightly heavier than average atoms of its type, and creating differences in bonding properties between the heavy and light species (Table 1). At MCI, Dr. Christine France looks at stable isotopes of carbon and nitrogen and establishes the relative ratios of heavy to light species with an elemental analyzer (EA) coupled to a continuous flow isotope ratio mass spectrometer (CF-IRMS). This is useful, because animals accumulate stable isotopes into their tissue throughout their lifetimes based on their diets. Measuring the ratio of

stable isotopes present in samples can provide information about the type of animal from which the sample originated, which can aid in identification. For example,the animals in the Salish blanket project had different food sources (fish for the Coastal Salish dogs, mountain shrubs for the terrestrial browsing mountain goat, and grasses for the herbivore sheep), all of which incorporate heavy and light carbon and nitrogen into their tissues in different ratios (fig. 4). Fish tends to incorporate a relatively high percentage of heavy nitrogen atoms, mountain shrubs incorporate a relatively high percentage of light carbon atoms, and grasses incorporate a relatively high percentage of heavy carbon atoms. This results in the dog, goat, and sheep reflecting these different carbon and nitrogen isotopic ratios in their fur fibers after consuming these variable foods. The stable isotope ratios of unidentified fur fibers from blankets can therefore serve as a marker indicating the species.

Element	Predominant Isotope	% Relative Abundance	Secondary Stable Isotope	% Relative Abundance	Secondary Stable Isotope	% Relative Abundance
Carbon	^{12}C	98.90	^{13}C	1.10		
Hydrogen	^{1}H	99.985	^{2}H	0.015		
Nitrogen	^{14}N	99.63	^{15}N	0.37		
Oxygen	^{16}O	99.762	^{17}O	0.038	^{18}O	0.200
Sulfur	^{32}S	95.02	^{33}S	0.75	^{34}S	4.21
Chlorine	^{35}Cl	75.77			^{37}Cl	24.23

Table 1: The predominant isotope and secondary stable isotope measured by mass spectrometry and their relative abundance in the atmosphere (CRC Handbook, 1985/6)

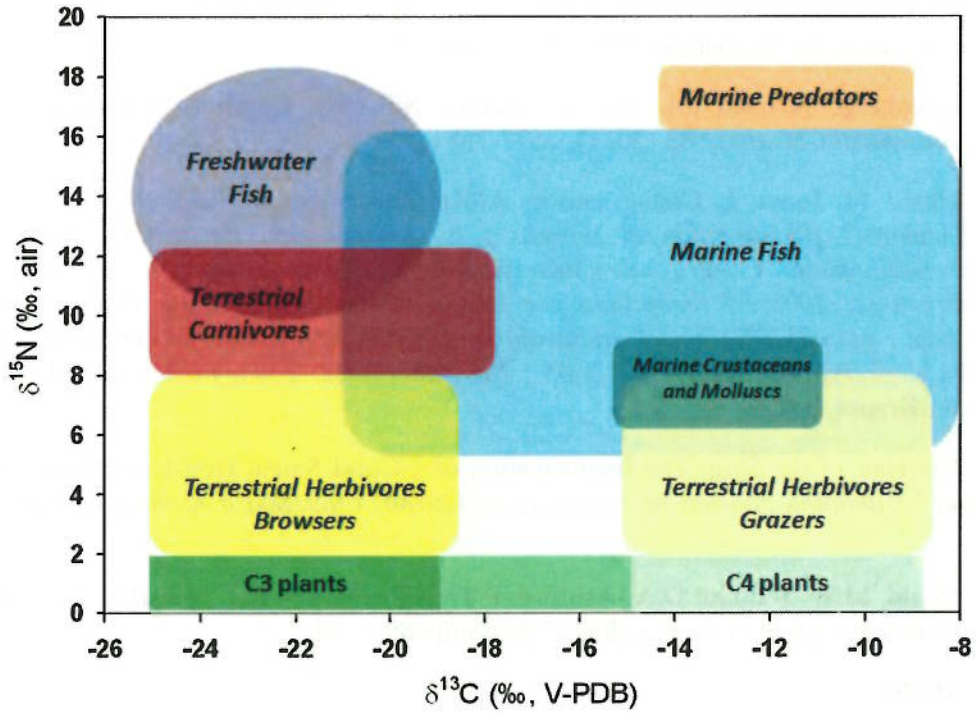

Figure 4: The effect of food source on the ratios stable isotope ratio of various animals.

3. CONCLUSION

Analytical methods using mass spectrometry are providing new and valuable answers to perplexing textile history problems that were previously unanswerable as a result of sample condition. With the small size of sample now required by each of these methods, 1 thousandth of a gram to 5 thousandths of a gram, textile conservators can advocate for the use of these methods to resolve issues of dating or authenticity.

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