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TECHNICAL NOTE

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Analysis of Glass Fragments by Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry and Principal Component Analysis*

ABSTRACT: Laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) is used to differentiate glass samples with similar optical and physical properties based on trace elemental composition. Laser ablation increases the number of elements that can be used for differentiation by eliminating problems commonly associated with dissolution and contamination. In this study, standard residential window and tempered glass samples that could not be differentiated by refractive index or density were successfully differentiated by LA-ICP-MS. The primary analysis approach used is Principal Component Analysis (PCA) of the complete mass spectrum. PCA, a multivariate analysis technique, provides rapid analysis of samples without time-consuming pair-wise comparison of calibrated analyses or prior knowledge of the elements present in the samples. Probabilities for positive association of the individual samples are derived from PCA. Utilization of the Q-statistic with PCA allowed us to distinguish all samples within the set to a certainty greater than the 99% confidence interval.

KEYWORDS: forensic science, glass comparisons, elemental analysis, laser ablation-inductively coupled plasma-mass spectrometry, principal component analysis

Glass fragments are often recovered as trace evidence during criminal investigations. Characterization is normally accomplished by measuring the physical and optical properties of thickness, density, and refractive index. Advances in the quality of glass manufacturing technology have made further discrimination, such as identification of a suspected source, more difficult as the range of densities and refractive indices have narrowed within glass subtypes. Glasses from the same subtype may have the same gross elemental composition, but can have different trace and ultra-trace elemental signatures. An obvious significant source of this variation lies in the trace and ultra-trace elemental compositions of raw materials used in the manufacturing process, which is dependent on the source of the raw materials (e.g., mined, recycled). The presence, absence, and relative abundance of elements in specific association patterns provide a unique means of the trace elemental signatures for distinguishing samples and is easily understandable.

Consequently, scientists have been investigating the use of elemental analysis techniques, particularly inductively coupled plasma-atomic emission spectrometry (1) and -mass spectrometry (2–7), for discrimination or differentiation between glasses within a particular class (e.g., window glass) based on their trace elemental contents. The implementation of laser ablation as a sampling technique has also been investigated to simplify and extend elemental analyses to much smaller sizes, typical of trace samples encountered (8–11). Laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) is rapid, eliminates the need for extensive sample preparation, and is virtually a nondestructive technique due to the extremely small amount of material consumed (several hundred nanograms) (12), allowing for the possibility of further analysis of questioned samples by corroborative techniques. Furthermore, LA increases the number of analytically useful elements, compared to ICP-MS with sample dissolution and nebulization, by eliminating problems with some elements due to poor dissolution and contamination that may occur in a standard solution nebulization ICP-MS analysis.

In order to exploit the large amount of information collected using this technique, the criteria and protocols for the comparison and differentiation of glass fragments from different sources based on LA-ICP-MS and Principal Component Analysis (PCA) techniques are presented. PCA is a statistical data reduction technique that examines the variance patterns in complex multidimensional datasets, providing clear ways for visual comparisons by allowing interpretation to be done graphically. A significant advantage of this type of analysis is that spectral comparison by PCA results in a quantitative statistical comparison (yielding probabilities) without the need for elemental calibration, matrix-matched standards, or prior knowledge of the elements present in the sample.

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TABLE 1—*Glass samples undifferentiated by refractive index.*

Group A					
Sample 1010	Window	$N_D = 1.5148$	$N_F = 1.5212$	$N_C = 1.5121$	···
Sample 1030	Tempered	$N_D = 1.5149$	$N_F = 1.5211$	$N_C = 1.5123$	···
Group B					
Sample 1024	Window	$N_D = 1.5155$	$N_F = 1.5216$	$N_C = 1.5130$	···
Sample 1032	Window	$N_D = 1.5157$	$N_F = 1.5221$	$N_C = 1.5130$	···
Group C					
Sample 10	Window	$N_D = 1.5169$	$N_F = 1.5232$	$N_C = 1.5143$	$D = 2.4871$
Sample 8	Window	$N_D = 1.5171$	$N_F = 1.5234$	$N_C = 1.5145$	$D = 2.4936$
Sample 1002	Laminated	$N_D = 1.5171$	$N_F = 1.5235$	$N_C = 1.5144$	$D = 2.4909$
Sample 11	Tempered	$N_D = 1.5172$	$N_F = 1.5237$	$N_C = 1.5145$	$D = 2.4868$
Group D					
Sample 1035	Plate	$N_D = 1.5175$	$N_F = 1.5238$	$N_C = 1.5148$	···
Sample 1033	Window	$N_D = 1.5176$	$N_F = 1.5240$	$N_C = 1.5149$	$D = 2.4946$
Sample 1009	Window	$N_D = 1.5177$	$N_F = 1.5239$	$N_C = 1.5151$	$D = 2.5016$
Group E					
Sample 1003	Tempered	$N_D = 1.5183$	$N_F = 1.5246$	$N_C = 1.5156$	$D = 2.4911$
Sample 4	Wire Reinforced	$N_D = 1.5185$	$N_F = 1.5247$	$N_C = 1.5159$	$D = 2.4940$
Sample 1029	Tempered	$N_D = 1.5185$	$N_F = 1.5250$	$N_C = 1.5158$	···
Sample 1013	Window	$N_D = 1.5186$	$N_F = 1.5249$	$N_C = 1.5159$	$D = 2.4939$
Sample 1036	Tempered	$N_D = 1.5187$	$N_F = 1.5251$	$N_C = 1.5159$	···
Sample 1001	Tempered	$N_D = 1.5189$	$N_F = 1.5255$	$N_C = 1.5161$	$D = 2.4942$
Group F					
Sample 1	Gray Window	$N_D = 1.5196$	$N_F = 1.5259$	$N_C = 1.5170$	$D = 2.4975$
Sample 1017	Tempered	$N_D = 1.5197$	$N_F = 1.5263$	$N_C = 1.5169$	$D = 2.4964$
Group G					
Sample 14	Wire Reinforced	$N_D = 1.5127$	$N_F = 1.5292$	$N_C = 1.5199$	···
Sample 16	Window	$N_D = 1.5129$	$N_F = 1.5294$	$N_C = 1.5201$	···
Group H					
Sample 1006	Window	$N_D = 1.5138$	$N_F = 1.5302$	$N_C = 1.5210$	···
Sample 1034	Dark Gray	$N_D = 1.5138$	$N_F = 1.5302$	$N_C = 1.5210$	···
Group I					
Sample 18	Patterned	$N_D = 1.5147$	$N_F = 1.5313$	$N_C = 1.5219$	$D = 2.5250$
Sample 17	Window	$N_D = 1.5148$	$N_F = 1.5311$	$N_C = 1.5221$	$D = 2.5139$
Sample 19	Plate	$N_D = 1.5149$	$N_F = 1.5314$	$N_C = 1.5221$	$D = 2.5210$
Differentiation	Criteria	$N_D \pm 0.0002$	$N_F \pm 0.0004$	$N_C \pm 0.0004$	

Methods

The Illinois State Police Science Command supplied 26 glass samples for analysis along with their measured refractive indices and density values, if available. Glass samples that could not be differentiated by refractive index were separated into groups. These groups are listed in Table 1.

ICP-MS

The mass spectrometer used in this study was a Thermo Finnigan Element 1 ICP-MS. This device employs magnetic and electrostatic analyzers configured in a reverse Nier-Johnson geometry to provide both mass and kinetic energy selection. The mass spectrometer has excellent detection limits (80 ppq aqueous ^{115}In), linear dynamic range (>109), and sensitivity (5 × 109 cps ppm-1 aqueous ^{115}In at low resolution) for ultra-trace analysis of solid fragments by laser ablation. For this study, the ICP-MS was operated in low resolution ($R = 300$).

The ICP load coil was "shielded" (CD-1 torch, Thermo Finnigan) to improve the ion transmission compared to a standard quartz ICP torch. With the shield grounded, the ICP is sustained only by inductive coupling, and the secondary discharge between the ICP and sampling cone is attenuated. Compared to analyses performed with a standard ICP torch, the shielded torch improves sensitivity by a factor of 5 to 20 (depending upon mass) while maintaining the extremely low background and high precision of the double-focusing instrument (13).

Laser Ablation

An in-house constructed argon fluoride (ArF) laser ablation system was used in this study. This system consists of an MPB Technologies Inc., PSX-100 excimer laser. The ArF laser emits at 193 nm, with an average power of 4.0 mJ pulse^{-1} at a repetition rate of 10 Hz. The ablation cell was mounted on a computer controlled xy-translation stage (Oriel, Inc.).

An argon flow rate of 1.3 L min^{-1} was used to transport the ablated particles to the plasma through a Tygon tube approximately 1.5-m long × 3-mm internal diameter. For the sampling position and power used to operate the ICP, this gas flow rate maximized atomic ion signals for all the elements measured during ablation of a glass standard (NIST 612).

Data Analysis for Comparison of Samples

A low-resolution full mass spectrum in the 4 to 240 mass range was acquired for each sample. Five spectra were acquired for each sample while ablating a raster pattern on the surface of the sample. The side of the glass fragment selected for analysis was the non-"float" side, determined by using a handheld UV-lamp. The float side fluoresced when exposed to UV light. The full mass spectrum was used for analysis (i.e., no pre- or post-selection of isotopes was done).

Data preprocessing consisted of peak-area integration, background subtraction, and arrangement of the data into a form suitable for multivariate analysis. The data were saved as ASCII text and

imported into Matlab v 6.1 (the MathWorks, Inc., Natick, MA) for PCA using PLS Toolbox 2.01f (Eigenvector Research, Manson, WA).

Principal component analysis for chemical and spectral analysis has been reviewed elsewhere (14). Basically, it is a multivariate data reduction method that examines the variance patterns within a multidimensional dataset. The dimensionality of the dataset is reduced while retaining a major portion of the original information. This is accomplished by decomposing the correlation matrix of the variables (i.e., measured elemental MS signals) of the data into a new set of axes, principal components, which define the directions of the major variances in the data set. The principal components are linear combinations of the variables (elements) and comprise three matrices that define each of the principal components: scores, loadings, and residuals. These matrices facilitate visualization of the relationships of the samples in the dataset and interpretation of the data. Scores describe the variance or relationship among the samples in the dataset and represent the contribution of the principal components in each sample. Loadings represent the contribution of the variables to the principal components and describe which variables (or masses (i.e., m/z ratio)) are responsible for the variance in the data. The residuals represent random variations within the dataset and are generally attributed to noise.

Samples within a particular group were compared by generating a PCA model for the data from one sample. Spectra for other samples in the group were then compared to the model. The difference or variance of the sample spectra from the developed model was determined by the Q-statistic, which indicates how well each sample conforms to the model. The Q-statistic is simply the measure of the difference, or residual, between the mass spectrum from one sample and its projection into the PCA model created from the data for another sample. Probabilities for each of the samples within a particular group were calculated from the Q-statistic. More in-depth discussion of the Q-statistic can be found in reference (15).

Results and Discussion

The refractive indices and densities, if available, of glass samples used in this study are listed in Table 1. Two pieces of glass are considered indistinguishable if their refractive indices overlap within the following ranges: $N_D \pm 0.0002$, $N_F \pm 0.0004$, and $N_C \pm 0.0004$. Based on these refractive index criteria, the glass samples within groups A, B, D, F, G, H, and I are indistinguishable. Within Groups C and E, some differentiation for some of the samples is possible. However, there are five possible pairs of glass samples in Group C that cannot be differentiated: 8 and 10, 8 and 1002, 10 and 1002, 8 and 11, and 11 and 1002. If the density of these samples were known, then all five pairs might possibly be differentiable. However, in typical casework, sample fragments are often not large enough to determine density. In group E, there are nine possible pairs of glass samples that cannot be differentiated by refractive index alone: 4 and 1003, 4 and 1029, 1003 and 1029, 4 and 1013, 4 and 1036, 1013 and 1029, 1013 and 1036, 1029 and 1036, 1001 and 1036.

Principal component analysis was performed on the acquired LA-ICP-MS mass spectra of each of the datasets and groups (26 glass samples separated into 9 groups). In all cases more than 99% of the variance within a group was accounted for in the first two principal components. To illustrate how PCA is used for pair-wise comparisons of samples, the score plot of the six samples (five repetitions for each sample) in Group E is shown in Fig. 1. Three of the glass samples (4, 1013, and 1029) cluster into well defined, separate groups in the score plot. They are largely differentiated

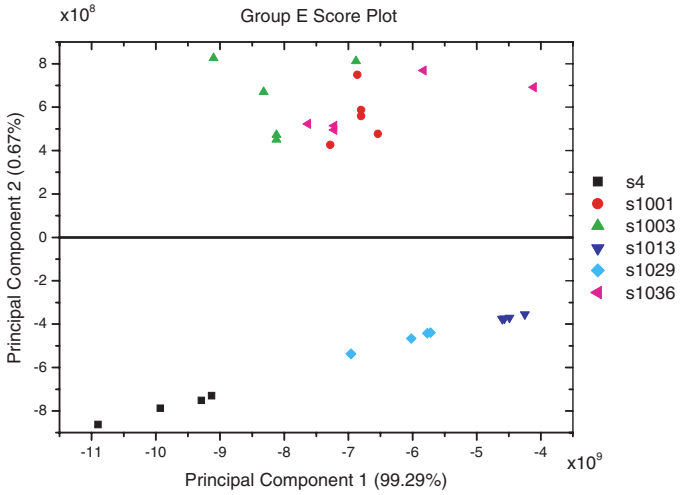

FIG. 1—*Score plot for glass samples in Group E. The PCA was performed on all six samples of the group. The numbers in parentheses indicate the amount of variance captured by the principal components.*

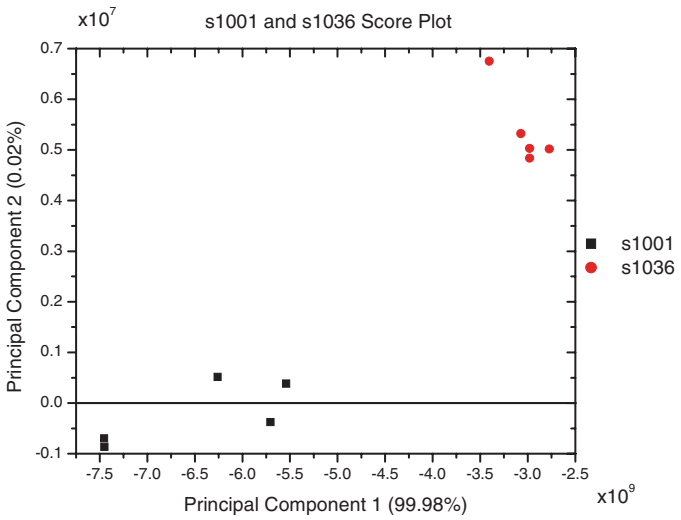

FIG. 2—*Score plot for glass samples 1001 and 1036. The PCA was performed on these two samples. The numbers in parentheses indicate the amount of variance captured by the principal components.*

by differences in the first PC. This separation indicates that the samples within those groups are different from the other members of Group E, based on the acquired mass spectra (i.e., elemental composition). The other three samples (1001, 1003, and 1036) from Group E are distinguished from the former samples by PC 2 and appear to occupy the same area on the score plot, indicating that they are not distinguishable from one another using PCA when all six of the samples are used in the model. In order to determine if the overlapping samples are distinguishable from each other, additional analysis of the data from these three samples is required.

Since samples 1001 and 1036 overlap and are not distinguishable in the score plot in Fig. 1, a new PCA is performed using the existing data from just these two samples. The resulting score plot (Fig. 2) shows the new model based on just the two samples (five repetitions each). The two samples clearly group separately from one another in the score plot, indicating that they have distinguishable elemental compositions. Performing additional PCA comparisons with sample-pair combinations incorporating sample 1003 yields analogous score plots, indicating that the three samples have different elemental compositions and are therefore distinguishable.

TABLE 2—*Probability of sample being indistinguishable from model.*

Group A						
	s1010	s1030				
Model 1010	...	$<10^{-14}$				
Model 1030	$<10^{-14}$	...				
Group B						
	s1024	s1032				
Model 1024	...	$<10^{-14}$				
Model 1032	$<10^{-14}$	...				
Group C						
	S8	s10	s11	s1002		
Model 8	...	$<10^{-14}$	$<10^{-14}$	$<10^{-14}$		
Model 10	7×10^{-11}	...	$<10^{-14}$	$<10^{-14}$		
Model 11	$<10^{-14}$	$<10^{-14}$	...	$<10^{-14}$		
Model 1002	1×10^{-07}	3×10^{-04}	$<10^{-14}$	...		
Group D						
	s1009	s1033	s1035			
Model 1009	...	7×10^{-11}	$<10^{-14}$			
Model 1033	$<10^{-14}$	-	$<10^{-14}$			
Model 1035	8×10^{-07}	3×10^{-08}	...			
Group E						
	s4	s1001	s1003	s1013	s1029	s1036
Model 4	...	$<10^{-14}$	$<10^{-14}$	$<10^{-14}$	5×10^{-04}	$<10^{-14}$
Model 1001	$<10^{-14}$	...	5×10^{-07}	5×10^{-12}	5×10^{-13}	2×10^{-06}
Model 1003	$<10^{-14}$	3×10^{-05}	...	1×10^{-12}	2×10^{-11}	6×10^{-04}
Model 1013	$<10^{-14}$	$<10^{-14}$	$<10^{-14}$	...	$<10^{-14}$	$<10^{-14}$
Model 1029	8×10^{-07}	$<10^{-14}$	$<10^{-14}$	$<10^{-14}$	...	$<10^{-14}$
Model 1036	$<10^{-14}$	9×10^{-12}	2×10^{-07}	$<10^{-14}$	1×10^{-11}	...
Group F						
	s1	s1017				
Model 1	...	5×10^{-09}				
Model 1017	2×10^{-12}	...				
Group G						
	s14	s16				
Model 14	...	$<10^{-14}$				
Model 16	3×10^{-07}	...				
Group H						
	s1006	s1034				
Model 1006	...	7×10^{-07}				
Model 1034	$<10^{-14}$	...				
Group I						
	s17	s18	s19			
Model 17	...	$<10^{-14}$	$<10^{-14}$			
Model 18	$<10^{-14}$	...	$<10^{-14}$			
Model 19	9×10^{-07}	$<10^{-14}$	...			

The elemental differences between samples can also be qualitatively ascertained from the principal component analysis. This information is contained in the loadings and represents the presence or relative difference of elements in the sample-to-sample comparisons. Figure 3 shows the Q-residual elemental contribution of the second repetition of sample 1001 compared to the PCA model of sample 1036. This plot shows that the major elemental differences between samples 1001 and 1036 is in the amount of Mg, Ca, Ti, Fe, Cd, In, Sb and Sn present. From the plot, sample 1001 is characterized as having more Sn and less Mg, Ca, Ti, Fe, Cd, In, and Sb than sample 1036.

The assignment of a quality value or probability for a pair-wise comparison utilizing this multivariate analysis approach requires that a sample dataset be compared to a PCA-generated model of another sample (or group of samples) in order to determine how well the sample data fit the model. The difference in the fit between the sample and the model is known as the Q-statistic, or Q-residual. From the Q-statistic one can calculate the probability of a sample being indistinguishable from the model.

Table 2 lists the calculated probabilities for the sample-to-sample comparisons for all of the groups. The probabilities are calculated from the average Q-statistic for all of the repetitions in each sample. For example, consider the overlapping samples in the Group E score plot (Fig. 1); when sample 1001 is compared to the sample 1003 PCA model, 1001 has no better than a 1 in 10^5 chance of being the same as sample 1003. Similarly, sample 1003 has no better than a 1 in 10^7 chance of being the same as sample 1001 when sample 1003 is compared to the sample 1001 PCA model. The difference in the probabilities, although only two samples are being compared, arises from the scatter within several repetitions of the mass spectra. PCA automatically assesses precision.

Conclusion

This work demonstrates LA-ICP-MS as a rapid and reliable technique the forensic scientist can use to uniquely identify, characterize, and perform pair-wise comparisons of glass trace evidence found at a crime scene, with a high level of certainty. Furthermore,

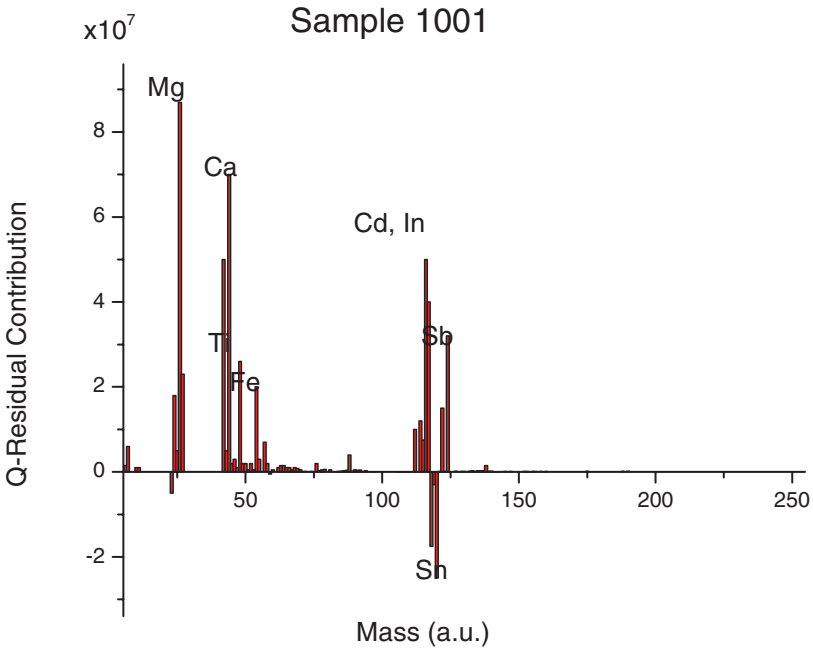

FIG. 3—*Plot showing the Q-residual contribution of the elements from the second repetition of sample 1001 compared to the PCA model of sample 1036.*

analyzing mass spectrometry data by PCA allows the forensic scientist to perform a quantitative statistical comparison (yielding confidence intervals) for positive association of a questioned glass sample based on its trace elemental composition.

While quantitative elemental analysis is possible with LA-ICP-MS, a significant advantage of using PCA to compare samples is the elimination of the need for elemental calibration and matrix-matched standards. PCA is essentially a pattern-matching analysis and does not rely on calibration of the spectra to known concentrations. This reduces the need for expensive, hazardous, and costly materials and procedures for analysis. A second significant advantage is the elimination of bias due to pre-selection of elements. Since the analyst does not need to decide in advance which elements to calibrate and determine, the full mass spectrum may be used, and the statistical comparison will determine which elements are significant for elimination or inclusion in the analysis.

Acknowledgments

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