

FORENSIC APPLICATIONS OF LA-ICP-MS: ELEMENTAL PROFILING AND EVALUATION OF HOMOGENEITY IN SODA-LIME CONTAINER GLASS

K. J. Harrington, B.S.^{a,b}, D.P. Land Ph.D.^a, E.M. Pollock, B.S.^b, F.A. Springer, B.S.^{a,b}, D.G. Howitt, Ph.D.^a

^aUniversity of California at Davis

^bSacramento County Office of the District Attorney, Laboratory of Forensic Services

LA-ICP-MS has applications in a variety of fields, including forensic science for examining glass fragments found in relation to a crime. The natural elemental variation of soda-lime container glass, as a result of the manufacturing process has continued to present difficulties in establishing the extent of discrimination possible between bottles manufactured from the same plant. Therefore, it is difficult to determine the value this evidence might carry; this study aimed toward evaluating to what degree soda-lime container samples can be identified based on manufacturing production runs. A sample of glass bottles from a local glass company was evaluated using LA-ICP-MS. Results show that samples originating from the same furnace were distinguishable outside of an approximate 8-11 day manufacturing run. Samples originating from different furnaces were found to be distinguishable. This demonstrates that LA-ICP-MS can reasonably be applied to soda-lime container glass for forensic application as long as these constraints are considered.

Introduction

Trace evidence, however minute in volume, plays a sizeable role in the field of forensics by potentially providing an association between a suspect and a crime scene or victim. Glass is one important type of trace evidence found in crime scene investigations. Glass can be present in a wide array of colors, sizes and shapes. However, due to the small quantity and physical form often found at a scene, it is not always clear what type of glass is present or where it originated.

In the past, refractive index measurements served as the prominent method for glass evidence comparison. However, the discrimination capability of refractive index measurements has been affected considerably as a result of the improved quality control measures implemented by glass manufacturers (13). Elemental analysis is a method of adding discrimination to glass evidence examinations. Studies comparing refractive index and elemental analysis have demonstrated that elemental analysis has discrimination where refractive index does not (15, 16, 17). The introduction of various methods of inductively coupled plasma mass spectrometry (ICP-MS) has been described as a discriminating technique for forensic analysis (1-3, 6, 14).

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) makes use of a high power laser to create an aerosol of super-fine glass fragments by irradiating the surface of a glass fragment; the resulting aerosol can then be analyzed using ICP-MS. The advantage of LA-ICP-MS is primarily the elimination of dangerous, costly and time-consuming acid digestion of samples. Additionally, it requires less sample preparation and consumption, allows for safer techniques to handle and prepare samples, and provides less risk of contamination and interference related to aqueous solutions while still maintaining much of the sensitivity and precision seen with other methods of ICP-MS (4).

Previous studies suggest that the natural elemental variation of glass is statistically insignificant when discriminating between samples originating from different categorical sources (for example, windshield vs. container) (6-8, 10, 11,13). It has also been demonstrated that statistically indistinguishable quantitative elemental analysis profiles have a high probability of originating from the same manufacturing source (5). Additionally, some studies suggest that glass maintains enough internal homogeneity that glass fragments originating from different parent sources are relatively easy to discriminate (5,9). While the manufacturing process has been shown to produce a homogenous material overall, LA-ICP-MS has revealed small differences along and across panes of glass (9). The manufacturing process of soda-lime container glass (specifically the use of molding parts) has been noted to result in a similar natural elemental variation within a single bottle which falls outside normal instrumental variation; both wine bottles from the same case and beer bottles from the same case were determined to be indistinguishable, while 45 random bottles originating from different sources were discernible (14). Based on the results of previous research, it is proposed here that a parent source might be defined as the specific furnace that manufactured a bottle within a single glass plant.

The purpose of this research is to expand on the phenomenon of homogeneity in glass bottles to determine the degree to which elemental profiles are consistent (per bottle, furnace or manufacturer) in samples of soda-lime glass originating from four furnaces within one manufacturer (Gallo Glass Company, Modesto, California). This research consisted of two stages. Stage one verified a valid set of match-criteria, which allowed for within-bottle variations while still allowing for forensic application. Stage two examined normal manufacturer variability within furnaces and the implications for forensic glass evidence analyzed with LA-ICP-MS. The ultimate goal of this project was to establish a set of match-criteria that remains strict enough to minimize false positives but allows for enough variability to reduce false negatives (treat a bottle as a uniform unit) for future forensic applications of container glass. These match-criteria will ideally provide valuable insight into specific origins of glass fragments on a case by case basis:

originating from the same bottle, originating from the same furnace, originating from the same manufacturer but different furnaces (chance profile similarities), similar glass classifications originating from different manufacturers and sources (chance profile similarities) or differing glass classifications originating from different manufacturers and sources (chance profile similarities) (9).

Materials and Methods

Sample Preparation and Selection

All bottles originated from four furnaces at the Gallo Glass Company in Modesto, California; they were made using typical soda-lime glass composition ratios (~70% sand, ~10% lime, ~12% soda ash, provided locally, in addition to cullet from flawed or recycled bottles). Bottles were smashed in a 12 ½" x 19" sealed air-cushioned mailer using a Carver laboratory hydraulic press (Carver Inc., Wabash, IN, USA). Samples were then selected from the broken glass and etched with location on the inside surface prior to being washed according to standard laboratory procedure. First they were soaked in methanol (VWR, West Chester, PA, USA) for 10 minutes. Then they were washed with 10% Nitric Acid (HNO₃, Omnitrace Ultra; EM Sciences, Merck KgaA, Darmstadt, Germany) for ~30 minutes and finally washed with 18 MO-cm deionized water (Barnstead, Dubuque, IA, USA) and dried. For stage one of this research, ten bottles were selected to represent the population of bottles sampled from two of the furnaces used for sample collection. Homogeneity within a bottle was analyzed by selecting three samples from the neck, three from the sidewall and three from the base of the bottles. For stage two (comparing normal manufacturer variability), one sample was selected from the sidewall of bottles.

Analyte Selection

Analyte selection was determined using an acid digestion and solution analysis according to laboratory procedure (12). A total of five samples were selected for digestion: four bottles and 1 NIST SRM 612. The four bottles were selected to represent each of the four furnaces and variety of colors found in the sample population: sample #1 (furnace 1 - clear), sample #47 (furnace 2 - amber), sample #156 (furnace 3 - clear) and sample # 143 (furnace 4 – green). A NIST/SRM 621 sample was additionally analyzed as an external standard. From these five samples, a list of general analytes found in glass bottles was determined. Based on several literature sources of ICP-MS container analytes, SRM reference guides and literature on colorants, a preliminary list of analytes was compiled for the sample analysis (6, 7, 2, 8, 9, 11).

Following analysis of the digested samples, the revised list of elements was used to select ratios for comparison. Elemental ratios were selected based on a comparison of variations (relative standard deviations) and mass-to-charge values to accommodate for the inherent instrumental drift seen with this type of ICP-MS analysis (2, 19): Sr/Zr, Na/Al, Ca/Mn, Zn/Rb, Fe/Cr, Ba/Pb, Ni/V, Ti/Li, Ce/Nd, Sn/La, Mg/Cu, As/Y, Hf/Th, Sb/Pr, U/Ta.

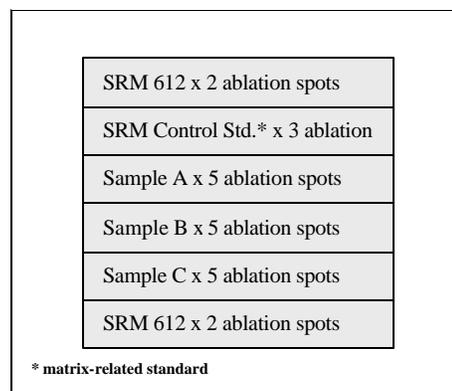
Instrumental Conditions

The inductively coupled plasma mass spectrometer used for this project was an ELAN® DRC II (PerkinElmer Life and Analytical Sciences, Boston, MA, USA). An RF power of 1500 W was used with a 10 ms dwell time. The laser ablation system utilized was a New Wave UP-213 (New Wave Research, Fremont, CA, USA). See Table 1 for laser operating parameters.

Table 1 – Laser Operating Parameters

Spot Size	60µm
Power	100%
Fluence	~27.25 J/cm²
Repetition Rate	10 Hz
Transport Gas Flow - Helium	1.04 L/Min
Argon make-up gas post-cell	~.95 L/Min
Time of Ablation	90 s

Figure 1 – Analytical Sequence



Experimental Set-up

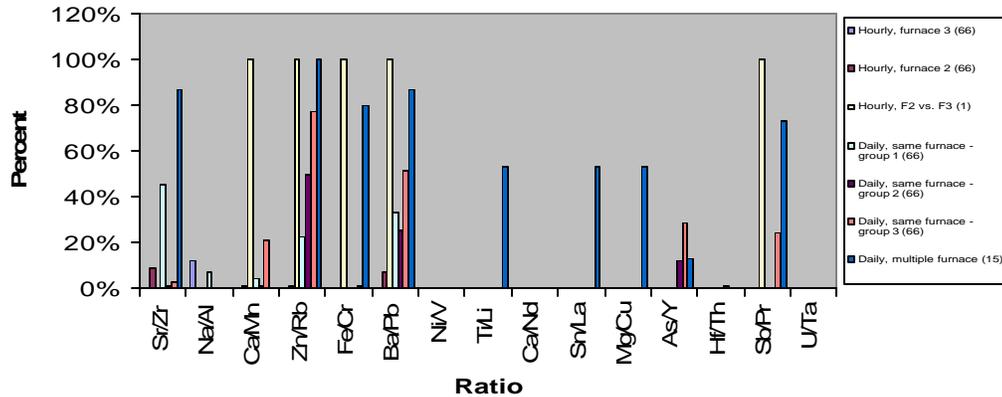
Samples were analyzed in the scheme shown in Figure 1 (7). For stage 1, runs were divided into bottle regions. Each bottle consisted of 6 runs (inside neck, inside sidewall, inside base, outside neck, outside sidewall and outside base). Stage 2 runs incorporated only sidewall fragments (inside surfaces of three bottles for one run, then outside surfaces of the same three bottles for the next run, for a total of ten ablation measurements per bottle).

Data Reduction

Data acquired from the ELAN® was processed using GLITTER software (GEMOC, Macquarie University, Australia) and then exported to an Excel worksheet to provide descriptive statistics for comparison analysis.

Results

Figure 2 – Percent distinguishable pair-wise comparisons with $\pm 2SD$ ($\pm 3SD$ for Sr/Zr and Fe/Cr). Demonstrates overall trends of bottle discrimination as a comparison across all ratios. (number of pair-wise comparisons in parenthesis).



Homogeneity within a single bottle (Stage 1)

The purpose of this comparison was to determine match-criteria that would allow for the variability resulting from natural elemental variations of a glass bottle; this consideration is essential for forensic casework application. Ten bottles from the two main sampling furnaces were analyzed for within bottle homogeneity. Comparisons made included: inside neck vs. inside sidewall, inside sidewall vs. inside base, inside base vs. inside neck, outside neck vs. outside sidewall, outside sidewall vs. outside base, outside base vs. outside neck, compiled neck vs. compiled sidewall, compiled sidewall vs. compiled base, compiled base vs. compiled neck and inside vs. outside. As a result of the natural elemental variation of bottles detected by the instrument utilized for this particular project, initial T-tests and ANOVA tests eliminated all same-bottle samples from each other (all samples were distinguishable from each other). However, application of $\pm 2SD$ range overlap comparison criteria of ratios (with the exception of some of the more consistently problematic elements Fe, Zr, Cr of which $\pm 3SD$ was applied to the ratios Sr/Zr and Fe/Cr) provided complete same-bottle grouping for this project (18). Comparisons revealed that K was too variable to provide useful information and was eliminated from the given ratios.

Preliminary results of homogeneity - hourly time variable, single furnace (Stage 2)

This comparison was performed on two groups of twelve bottles originating from the same furnace, pulled from the final production line every hour. The purpose was to determine if bottles from the same parent furnace were distinguishable when produced in close time

proximity. Sixty-six pair-wise comparisons were made using a compilation of inside and outside sidewall surface ablations for elemental ratios (Table 3). The range overlap comparison as described in the previous section was used for two separate groupings of bottles. The first group of bottles originated from furnace three within a 24-hour period. The first 6 bottles were from 12 am to 5 am, the second 6 bottles were from 6 pm to 11 pm. The second group of bottles originated from furnace two with the same time collection as the first group. The two groups were also compared.

Table 3 – Relative Discrimination Capabilities ± 2 SD (± 3 SD for Sr/Zr and Fe/Cr)
Numbers in parenthesis = ± 3 SD (± 4 SD for Sr/Zr and Fe/Cr)

Elemental Ratios	Hourly Time Variable		
	Group 1	Group 2	Group 1 vs. Group 2
Number of Samples: 12/group (66 comparisons)	No. of distinguishable pairs		
Sr/Zr	0 (0)	6 (0)	0 (0)
Na/Al	8 (4)	0 (0)	0 (0)
Ca/Mn	0 (0)	1 (0)	1 (1)
Zn/Rb	0 (0)	1 (0)	1 (1)
Fe/Cr	0 (0)	0 (0)	1 (1)
Ba/Pb	0 (0)	5 (2)	1 (1)
Ni/V	0 (0)	0 (0)	0 (0)
Ti/Li	0 (0)	0 (0)	0 (0)
Ca/Nd	0 (0)	0 (0)	0 (0)
Sn/La	0 (0)	0 (0)	0 (0)
Mg/Cu	0 (0)	0 (0)	0 (0)
As/Y	0 (0)	0 (0)	0 (0)
Hf/Th	0 (0)	0 (0)	0 (0)
Sb/Pr	0 (0)	0 (0)	1 (0)
U/Ta	0 (0)	0 (0)	0 (0)

Preliminary results of homogeneity - daily time variable, single furnace (Stage 2)

This comparison was performed on 3 groups of bottles from furnace one, collected from production lines at the same time for a series of consecutive days. A possible 66 pair-wise comparisons were made for each ratio (Table 4). Group 3 was collected 7/19/05 – 7/30/05 at 8 am. Group 4 was collected from 7/31/-5 – 8/15/05 at 8 am. Group 5 was collected from 8/9/05 – 8/21/05.

Table 4 – Relative Discrimination Capabilities ± 2 SD (± 3 SD for Sr/Zr and Fe/Cr)**Numbers in parenthesis = ± 3 SD (± 4 SD for Sr/Zr and Fe/Cr)****Daily Time Variable**

Elemental Ratios	No. of distinguishable pairs		
	Group 3	Group 4	Group 5
Sr/Zr	0 (0)	30 (21)	1 (0)
Na/Al	0 (0)	5 (1)	0 (0)
Ca/Mn	1 (1)	3 (0)	1 (0)
Zn/Rb	1 (1)	15 (1)	33 (21)
Fe/Cr	1 (1)	0 (0)	0 (0)
Ba/Pb	1 (1)	22 (14)	17 (10)
Ni/V	0 (0)	0 (0)	0 (0)
Ti/Li	0 (0)	0 (0)	0 (0)
Ca/Nd	0 (0)	0 (0)	0 (0)
Sn/La	0 (0)	0 (0)	0 (0)
Mg/Cu	0 (0)	0 (0)	0 (0)
As/Y	0 (0)	0 (0)	8 (0)
Hf/Th	0 (0)	0 (0)	1 (0)
Sb/Pr	1 (0)	0 (0)	0 (0)
U/Ta	0 (0)	0 (0)	0 (0)

Preliminary results of homogeneity –daily, simultaneous three furnace (Stage 2)

This comparison was conducted on a set of six bottles originating from three different furnaces at the same time (8 am) on two consecutive days (15 possible pair-wise comparisons per ratio).

Table 5 – Relative Discrimination Capabilities ± 2 SD (± 3 SD for Sr/Zr and Fe/Cr)**Numbers in parenthesis = ± 3 SD (± 4 SD for Sr/Zr and Fe/Cr)****Daily Time Variable, simultaneous multiple furnace**

Elemental Ratios	No. of distinguishable pairs	
	Group 6	
Sr/Zr	0 (0)	
Na/Al	0 (0)	
Ca/Mn	13 (12)	
Zn/Rb	15 (12)	
Fe/Cr	12 (12)	
Ba/Pb	13 (12)	
Ni/V	0 (0)	
Ti/Li	8 (4)	
Ca/Nd	0 (0)	
Sn/La	8 (6)	
Mg/Cu	8 (8)	
As/Y	2 (0)	
Hf/Th	0 (0)	
Sb/Pr	11 (8)	
U/Ta	0 (0)	

Conclusion/Discussion

The purpose of this research was to determine to what degree soda-lime container bottles could be distinguished. The presence of natural elemental variations within a single bottle presents interpretational difficulties in determining appropriate match-criteria to eliminate within-bottle variation as a factor in comparing forensic samples. This research demonstrates that the use of ± 2 SD is a reasonable match-criteria when comparing soda-lime container samples. The first stage of this research compared inside and outside surfaces as well as neck, sidewall and base regions within a bottle; the ± 2 SD (± 3 SD for consistently problematic ratios) match-criteria proved to be appropriate for eliminating within-bottle elemental variations as a factor for sample comparisons. The most informative ratios for all comparisons were Sr/Zr, Ca/Mn, Zn/Rb and Ba/Pb.

Comparisons for group 1 suggest that within a 24-hour period, bottles from the same furnace shared matching elemental profiles. The pair-wise comparisons that provided distinguishable results were all related to a single bottle, suggesting that the sample might contain some contaminant not present in the other samples. The hourly comparisons for group 2 demonstrated a similar pattern, except that two bottles seemed to consistently result in distinguishable comparisons. This might suggest that for soda-lime container samples manufactured from the same furnace within a 24 hour time period, 80 – 90% of bottles within a one day production lot show indistinguishable elemental profiles.

Daily comparisons from the same furnace did not suggest that one bottle was consistently distinguishable from other bottles; rather, bottles seemed to be distinguishable based on time groups. For group 3, bottles were almost all distinguishable if manufactured at least 5 days apart with the exception of 1 bottle (11 days). For group 4, bottles were all distinguishable if manufactured at least 8 days apart. For group 5, bottles were all distinguishable when manufactured at least 4 days apart. This pattern would imply that for known and question samples resulting in indistinguishable elemental profiles, fragments had a high likelihood of originating from the same furnace within, at a maximum of 8 - 11 days.

Simultaneous daily comparisons from three furnaces on two consecutive days demonstrated that every bottle was distinguishable from every other bottle, except from the bottles originating from the same furnace. Furthermore, for the two bottles originating from furnace one, they were distinguishable from each other on two consecutive days. These results reiterate that bottles originating from different furnaces are distinguishable; additionally, bottles originating from the same furnace may be distinguishable as only 80-90% of those within a daily manufacturing run have indistinguishable elemental profiles.

Previous research suggests that bottles originating from the same case or six-pack were indistinguishable (14) and samples with indistinguishable elemental profiles originate from the same manufacturing source (5). The data from this research supports both of these previous conclusions and provides more specific information as to the limitations of parent source similarities. This research suggests that bottles originating from different furnaces within a single manufacturing plant are distinguishable, and that those originating from the same furnace are most likely distinguishable if produced at least 8 - 11 days apart. The particular manufacturer from which these bottles were sampled produces around 800 cases per hour and 1 billion bottles per year. Identifying whether bottles likely originated from the same furnace or different furnaces within a single manufacturer provides reasonable statistical strength for forensic application of fragments to casework. However, due to the natural elemental variation of glass bottles seen in stage 1 of this research, it remains important that reference samples are collected as extensively as possible for forensic examinations (14). Although the research presented here is only preliminary data from a much larger survey of bottle production, it provides an excellent stage to set future data against. Future considerations might include further assessments of manufacturing homogeneity across multiple manufacturers, how bottles are distributed into cases (mix and match or same furnace distribution), how same-furnace bottles are distributed to vendors (locally, nationally or world-wise) and surveys of better statistical techniques to provide benchmarks for forensic applicability (Principal Component Analysis, Mean Square Error and standard deviation match-criteria).

Acknowledgements

The authors would like to particularly thank the Sacramento County Office of the District Attorney Laboratory of Forensic Services for providing facility workspace, technical support, financial support and instrument resources. Additionally, the Gallo Glass Company kindly provided all bottles. The University of California, Davis Forensic Science Masters Program is also recognized for funding research and providing resources. Finally, the authors acknowledge the California Criminalistics Institute for providing workspace and instrument resources.

References

1. S. Umpierrez, T. Trejos, K. Neubauer and J. Almirall. Determination of Iron in Glass by Solution and Laser Ablation DRC-ICP-MS. *At. Spectrosc.* 27(3), 76-79 (2006).
2. T. Trejos, S. Montero and J.R. Almirall. Analysis and comparison of glass fragments by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) and ICP-MS. *Analytical and Bioanalytical Chemistry.* 376(8), 1255 – 1264 (2003).
3. ASTM Method E 2330, Standard Test Method for Determination of Trace Elements in Glass Using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Volume 14 (2004).
4. K. Smith, T. Trejos, R.J. Watling, J. Almirall. A Guide for the Quantitative Elemental Analysis of Glass Using Laser Ablation Inductively Coupled Plasma Mass Spectrometry. *At. Spectrosc.* 27(3), 69-75 (2006).

5. J.R. Almirall. Glass as evidence of association. In *Mute Witness; When Trace Evidence Makes the Case*, M. Houck (Ed.), Academic Press, San Diego, CA, USA, 139-155 (2001).
6. C. Latkoczy, S. Becker, M. Dücking, D. Günther, J. Hoogewerff, J.R. Almirall, J. Buscaglia, A. Dobney, R. Koons, S. Montero, G.J.Q. van der Peijl, W.R.S. Stoecklein, T. Trejos, J.R. Watling and V. Zdanowicz. Development and Evaluation of a Standard Method for the Quantitative Determination of Elements in Float Glass Samples by LA-ICP-MS. *J Forensic Sci.* 50(6), 1327-1341 (Nov. 2005).
7. T. Parouchais, I. Warner, L. Palmer and H. Kobus. The Analysis of Small Glass Fragments Using Inductively Coupled Plasma Mass Spectrometry. *J Forensic Sci.* 41, 351-360 (1996).
8. S. Becker. Guidelines for the Forensic Analysis of Float Glass with LA-ICP-MS. Nite Crime work package 3.2 glass. Nite Crime Network.
9. S. Montero. Forensic float-glass analysis using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). *Method Validation – NFI Scientific Report.* 8 (2005).
10. J.R. Almirall. Elemental analysis of glass fragments. In: *Forensic examination of glass and paint: Analysis and interpretation*. London, New York: Taylor & Francis, 65-83 (2001).
11. D.B. Aeschliman, S.J. Bajic, D.P. Baldwin, R.S. Houk. Multivariate pattern matching of trace elements in solids by laser ablation inductively coupled plasma mass spectrometry: Source attribution and preliminary diagnosis of fractionation. *Anal Chem.* 76, 3119-3125 (2004).
12. Sacramento County District Attorney, Laboratory of Forensic Services. *Trace Evidence Procedures Manual Ch. 5: 22-32* (2006).
13. J.R. Almirall. "Glass examination and comparison with a focus on refractive index measurements, elemental analysis and interpretation of data: a workshop for practicing forensic scientists." Colorado Bureau of Investigation Forensic Laboratory. Denver, Colorado. December 2003.
14. T. Trejos and J.R. Almirall. Sampling Strategies for the analysis of glass fragments by LA-ICP-MS Part I. Micro-homogeneity study of glass and its application to the interpretation of forensic evidence. *Talanta.* 67(2) 388 – 395 (August 2005).
15. R. Koons, C. Fiedler and R. Rawalt. Classification and discrimination of sheet and container glasses by inductively coupled plasma-atomic emission spectrometry and pattern recognition. *J. of For. Sci.* 33, 49 – 67 (1988).
16. R. Koons, C. Peters and P. Rebbert. Comparison of refractive index, energy dispersive x-ray fluorescence and inductively coupled plasma atomic emission spectrometry for forensic characterization of sheet glass fragments. *J. of Analy. At. Spect.* 6, 451-456 (1991).
17. R. Coleman and G. Goode. Comparison of glass fragments by neutron activation analysis. *J. of Radioanalytical Chemistry* 15, 367 – 388 (1973).
18. Almirall, J.R. *Manslaughter Caused by a Hit and Run; Glass as Evidence of Association*. International Forensic Research Institute. 1 - 250.
19. A.J. Dodds. *Forensic Glass Analysis by ICP-MS, Volume I: Sample introduction Techniques [Thesis]*. Davis (CA); Univ. of California, 2005.