STUDY 1A RESULTS: EFFECT OF TREATMENT ON LA FIBER INTEGRITY
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STUDY OBJECTIVE

The objective of Study 1A was to investigate whether the treatments utilized in EPA Analytical Method 100.1 (sonication and ozone/ultraviolet [UV] treatment), alone or in combination, resulted in any significant increases in fiber concentrations or skewed fiber size distributions (smaller) when applied to suspensions of LA in water.

STUDY DESIGN

The LA test material utilized in this study was collected from the Libby mine by USGS in 2007. Samples of LA from the mine were mixed, crushed, and ground to a "raw" sample by USGS in the laboratory at the Federal Center in Denver, Colorado. This material is referred to as "Libby 2007 Raw".

The laboratory prepared the stock suspension of LA in accord with the procedures in Study 1A, Modification #1. In brief, on day 1 of the study, 10 liters of moderately –hard reconstituted laboratory water (supplemented with natural organic material) were treated with ozonation and UV light . On day 2 of the study, 100 mg of test material was added to 1 liter of the ozone-treated water and the solution was mixed thoroughly for 20 minutes using a magnetic stirrer. After mixing, the suspension was allowed to settle for approximately 30 minutes. A 100 mL aliquot of the stock suspension was removed from the upper portion of the cylinder and diluted to 5 liters using the ozone-treated water.

A 100 mL aliquot of the stock suspension was placed in each of twenty wide-mouth glass bottles and randomized into four treatment groups of 5 bottles each. Each bottle was treated in basic accord with Method 100.1 (Section 6.2) as follows:

Group	Bottles	Treatment
1	1-5	None
2	6-10	Sonication alone
3	11-15	Ozonation/UV alone
4	16-20	Ozonation/UV + Sonication

Following the treatment of each bottle, an aliquot was promptly removed and filtered through a 25 mm diameter polycarbonate (PC) filter with a pore size of 0.1 µm. The total time between initial preparation of the stock suspension and filtration of all aliquots on day 2 was approximately 6 hours. Three transmission electron microscopy (TEM) grids were prepared and analyzed from each filter. The five replicates from Group 1 and Group 4 were analyzed by TEM

in basic accord with ISO (1995), as modified by the most recent versions of Libby-specific laboratory modifications LB-000016A, LB-000019, and LB-000030.

DATA EVALUATION

Effect of Treatment on Fiber Concentration

Table 1 presents the results for each sample from Treatment Group 1 (Bottles #1-5) and Treatment Group 4 (Bottles #16-20). As shown, the mean concentration of LA in Treatment Group 1 and Treatment Group 4 was 46 million fibers per liter (MFL) and 57 MFL, respectively. These mean concentrations were compared using the t-test. Concentrations in Treatment Group 4 were not statistically different from concentrations in Treatment Group 1 (p-value = 0.49). These results support the conclusion that Treatment 4 (ozonation/UV with sonication) does not significantly increase fiber concentrations of LA in water.

Effect of Treatment on Fiber Integrity

Figure 1 presents the cumulative distribution function (cdf) of the length and width distributions for all LA structures observed in samples from Treatment Group 1 and Group 4. The summary table at the bottom of this figure presents the frequency of each type[1] of LA structure for each treatment group. As shown, the cdfs for length and width are generally similar across treatment groups. While a few LA structures were associated with disperse clusters and matrices in Group 1, all LA structures in Group 4 were either free fibers or bundles. These results support the conclusion that Treatment 4 (ozonation/UV with sonication) does not skew the LA structure size distribution (i.e., breakage of long fibers is not expected) and successfully disaggregates structures in disperse clusters and matrices.

Filter Loading

This study also demonstrated that the structure loading on the filter tended to be fairly uneven, with two filters from Treatment Group 1 and one filter from Treatment Group 4 failing the Chi-square test for uneven loading (see **Table 1**). Even for those filters that passed the Chi-square test, the resulting p-values show that structure loading on the filter was rather uneven for both treatment groups. These results suggest that the stock suspension is fairly heterogeneous and that sonication (as part of Treatment 4) did not appear to improve the evenness of the structure loading on the filter.

As stated in ISO (1995), the precision of the result from a filter that fails the Chi-square test will be uncertain and results may be rejected on this basis. **Table 2** presents the mean concentration of LA in Treatment Group 1 and Treatment Group 4 excluding the three results

[1] F = free fiber, B = free bundle, M = fiber contained within a disperse matrix, CF = fiber contained within a disperse cluster, CB = bundle contained within a disperse cluster

that failed the Chi-square test. As shown, while the mean and standard deviation decreased for both treatment groups after excluding these three results, concentrations in Treatment Group 4 were not statistically different from concentrations in Treatment Group 1 (p-value = 0.26).

Structure Yield from Test Material

The estimated number of LA structures per gram of test material (spg) is calculated as follows:

$$spg = k \bullet Conc \text{ (as MFL)}$$

where:

$$k = V / M \bullet d / a \bullet CF_1 \bullet CF_2 = 5E{+}08$$

V = suspension volume (1 L)
M = mass of test material (100 mg)
d = dilution volume (5 L)
a = Aliquot volume (0.1 L)
CF_1 = conversion factor of 1E+06 (MFL to f/L)
CF_2 = conversion factor of 1E+03 (mg to g)

As shown in **Table 1**, the number of LA structures obtained in suspension per gram of test material in this study was about 2.6E+10. This structure yield is about 10 times lower than originally anticipated. However, it may be possible to increase the structure yield from the solid test material by using sonication during the preparation of the stock solution. This will be investigated in Study 3A. The results of this study demonstrate that sonication will not significantly impact the particle size distribution of the resulting water suspensions of LA.

QC RESULTS

A repreparation is an analysis of TEM grids that have been prepared from a new portion of the same filter as was used to prepare the original grids. Repreparation analyses are compared to the original analysis results using the ratio method for statistical comparison of two Poisson rates recommended by Nelson (1982). For the purposes of this study, two replicates within Group 4 were selected for repreparation (Bottle #17 and Bottle #18). Bottle #18 was specifically chosen because the Chi-square test indicated that there was uneven loading on the portion of the filter that was analyzed.

Table 3 presents a comparison of the original analysis results with the repreparation results. As shown, neither repreparation was statistically different from the original analysis at the 90% confidence interval. These results show that, despite the fact that the filter loading continues to

be uneven (as evidenced by the Chi-square test p-values), the reported water concentrations of LA are reproducible.

REFERENCES

Nelson, W. 1982. *Applied Life Data Analysis.* John Wiley & Sons, New York. pp 438-446.

Libby OU3, Water Pilot Study 1A

Table 1 - TEM Results Summary

Group	Index ID	Rep #	EFA (mm^2)	GO Size (mm^2)	GO Count	Vol Applied to Filter (mL)	ChiSq p value	Sensitivity (1/L)	Total N Struc LA	Total LA Conc (MFL)	LA (s/g)
1	WPS-1A-01	1	360	0.013	6	20	6.0E-07	2.3E+05	398	92	4.6E+10
	WPS-1A-02	2	360	0.013	13	10	2.4E-01	2.1E+05	160	34	1.7E+10
	WPS-1A-03	3	360	0.013	13	10	1.0E-02	2.1E+05	150	32	1.6E+10
	WPS-1A-04	4	360	0.013	11	10	8.6E-01	2.5E+05	158	40	2.0E+10
	WPS-1A-05	5	360	0.013	7	20	8.9E-09	2.0E+05	152	30	1.5E+10
4	WPS-1A-16	1	360	0.013	19	10	1.3E-01	1.5E+05	155	23	1.1E+10
	WPS-1A-17	2	360	0.013	10	10	2.1E-02	2.8E+05	230	64	3.2E+10
	WPS-1A-18	3	360	0.013	9	10	1.6E-05	3.1E+05	299	92	4.6E+10
	WPS-1A-19	4	360	0.013	7	10	6.5E-02	4.0E+05	159	63	3.1E+10
	WPS-1A-20	5	360	0.013	9	10	6.5E-01	3.1E+05	150	46	2.3E+10

Chi square test indicates filter loading is uneven

Total LA Conc (MFL)		
Group	Mean	Stdev
1	45.5	26.1
4	57.5	25.5

t-test p value: 0.4863

LA s/g	
Mean	Stdev
2.6E+10	1.3E+10

Libby OU3, Water Pilot Study 1A

Table 2 - TEM Results Summary (Chi-square Failures are Excluded)

Group	Index ID	Rep #	EFA (mm^2)	GO Size (mm^2)	GO Count	Vol Applied to Filter (mL)	ChiSq p value	Sensitivity (1/L)	Total N Struc LA	Total LA Conc (MFL)	LA (s/g)
1	WPS-1A-01	1	360	0.013	6	20	6.0E-07		*rejected*		
	WPS-1A-02	2	360	0.013	13	10	2.4E-01	2.1E+05	160	34	1.7E+10
	WPS-1A-03	3	360	0.013	13	10	1.0E-02	2.1E+05	150	32	1.6E+10
	WPS-1A-04	4	360	0.013	11	10	8.6E-01	2.5E+05	158	40	2.0E+10
	WPS-1A-05	5	360	0.013	7	20	8.9E-09		*rejected*		
4	WPS-1A-16	1	360	0.013	19	10	1.3E-01	1.5E+05	155	23	1.1E+10
	WPS-1A-17	2	360	0.013	10	10	2.1E-02	2.8E+05	230	64	3.2E+10
	WPS-1A-18	3	360	0.013	9	10	1.6E-05		*rejected*		
	WPS-1A-19	4	360	0.013	7	10	6.5E-02	4.0E+05	159	63	3.1E+10
	WPS-1A-20	5	360	0.013	9	10	6.5E-01	3.1E+05	150	46	2.3E+10

Chi-square test indicates filter loading is uneven

Total LA Conc (MFL)		
Group	**Mean**	**Stdev**
1	35.3	4.0
4	48.8	19.3

t-test p value: *0.2560*

LA s/g	
Mean	**Stdev**
2.2E+10	7.8E+09

Libby OU3, Water Pilot Study 1A

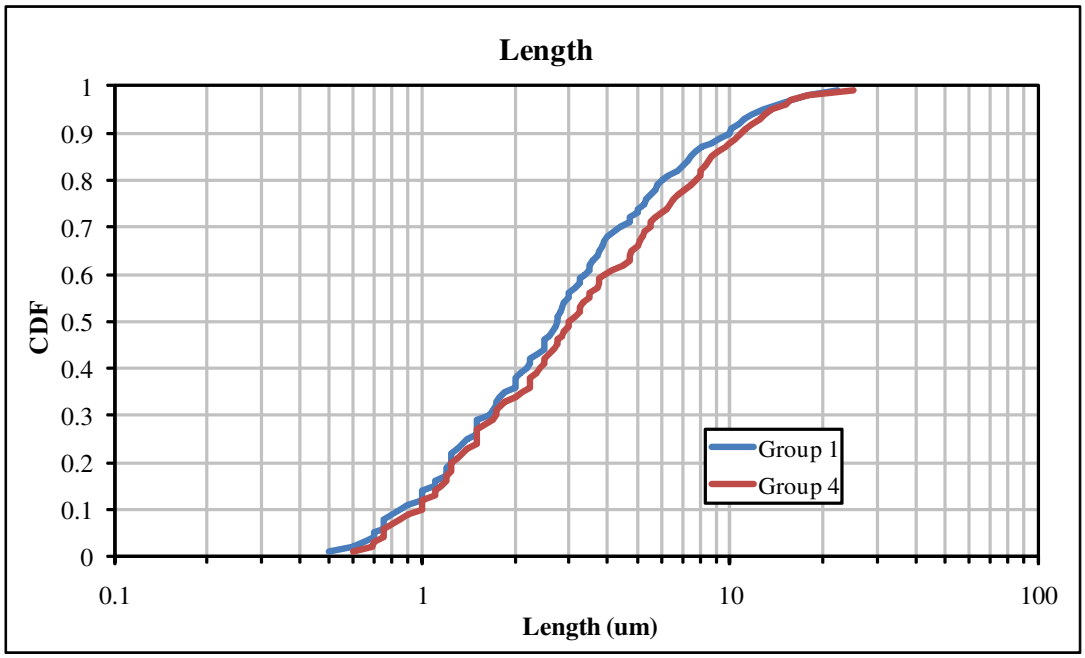
Table 3 - Comparison of Repreparation Analyses with Original Results

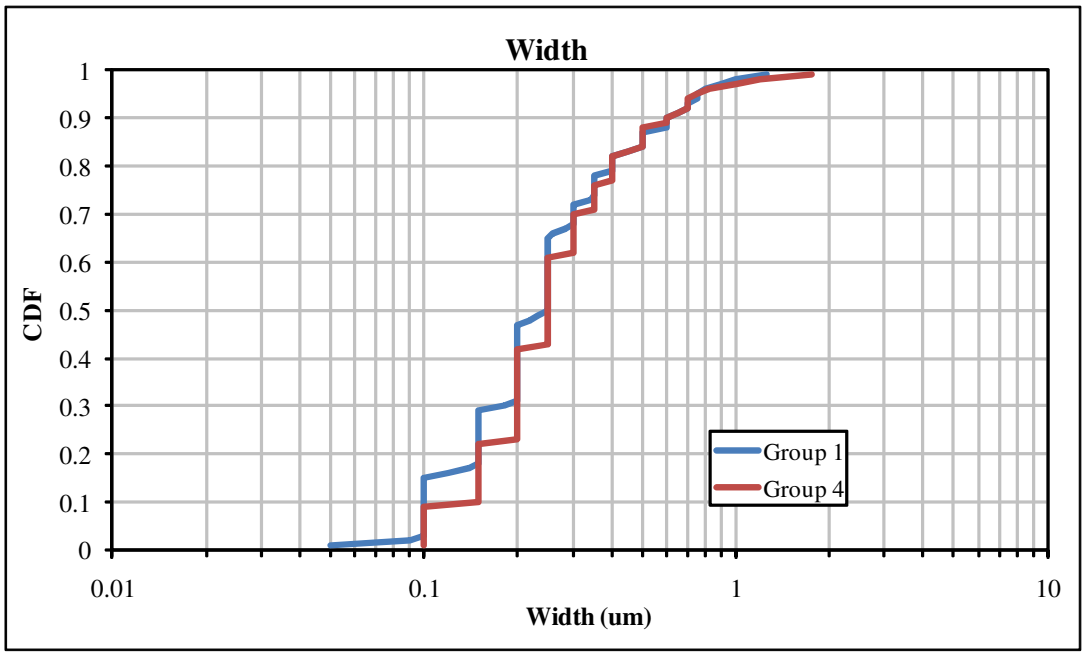
Index ID	Analysis Type	GOs Counted	ChiSq p value	Sensitivity (1/L)	Total N Struc LA	Total LA Conc (MFL)	LA (s/g)	Poisson Ratio test (90% CI)
WPS-1A-17	original	10	2.1E-02	2.8E+05	230	64	3.2E+10	[0.68-1.03] The rates are not different
	reprep	6	2.2E-01	4.6E+05	165	76	3.8E+10	
WPS-1A-18	original	9	1.6E-05	3.1E+05	299	92	4.6E+10	[0.93-1.33] The rates are not different
	reprep	7	1.8E-06	4.0E+05	210	83	4.2E+10	

Chi-square test indicates filter loading is uneven

Libby OU3, Water Pilot Study 1A

Figure 1 - LA Particle Size Distributions





Group 1

Number of LA Structures		
Total	1,018	
F	854	83.9%
B	123	12.1%
M	2	0.2%
CF	32	3.1%
CB	7	0.7%

Group 4

Number of LA Structures		
Total	993	
F	898	90.4%
B	95	9.6%
M	0	0.0%
CF	0	0.0%
CB	0	0.0%