

Low Volume Particle Size and Count Analysis

AccuSizer® SIS Syringe Injection Sampler

OVERVIEW

For certain applications, only a small volume of sample is available for analysis using multiple techniques. It isn't unusual, in pharmaceutical research, to have 1 mL or less of a protein-based sample and require many assays to be performed. The AccuSizer® is capable of measuring particle size and concentration at extremely low sample volumes, as proven in the data shown in this technical note.

INTRODUCTION

Single particle optical sizing (SPOS) is a particle size and count technique that provides results at high accuracy and resolution. Particles in liquid suspensions flow through a sensing zone illuminated by a laser beam. Each particle obscures and scatters the incident light source. With the AccuSizer LE400 sensor, both the light obscuration and scattering are used to relate particle/light interactions with particle size. Each particle is sized and counted one at a time, providing the concentration in particles/mL. The sample volume must also be accurate in order to generate accurate concentration data. This technical note presents data generated at customer request to prove the size, count, and volume accuracy of the AccuSizer SIS/syringe injection sampler system.

MATERIALS AND METHODS

The sample used to perform all tests described in this technical note was a 15 µm particle count standard from micro measurement labs, Inc., lot #NK20C. The reference count value for this standard is 3,118 – 4,218 particles/mL. All measurements were made on the PSS AccuSizer SIS system equipped with the LE400 sensor, calibrated and used at a flow rate of 15 mL/min. A 1 mL syringe was installed onto the SIS sampler. The measurement procedure used is described below:

- Flushes of 0.5 mL were performed before and after sampling (an air gap took place after the 'before' flush, but before the sampling).
- Fresh 900 µL aliquots were used for each sample, regardless of sampling required.
- An air gap of 0.05 mL was used in each run prior to sampling.
- A tare volume of 0.15 mL was used for each measurement.

Measurements were performed at the following sample volumes: 650, 550, 450, 350, 250, 150, and 50 µL. All measurements were performed in triplicate.

RESULTS

The results from the experiments are shown in Table 1 below and in Figure 1 on the next page.

Volume	Counts	Counts/mL	Counts average	Average/mL	Std dev	Std. Dev./mL	%RSD
650 µL	2418	3720					
650 µL	2326	3578					
650 µL	2328	3582	2357	3627	53	81	2.2%
550 µL	2016	3665					
550 µL	1947	3540					
550 µL	2050	3727	2004	3644	52	95	2.6%
450 µL	1720	3822					
450 µL	1657	3682					
450 µL	1678	3729	1685	3744	32	71	1.9%
350 µL	1283	3666					
350 µL	1256	3589					
350 µL	1256	3589	1265	3614	16	45	1.2%
250 µL	907	3628					
250 µL	928	3712					
250 µL	961	3844	932	3728	27	109	2.9%
150 µL	577	3847					
150 µL	580	3867					
150 µL	592	3947	583	3887	8	53	1.4%
50 µL	179	3580					
50 µL	180	3600					
50 µL	187	3740	182	3640	4	87	2.4%

Table 1.

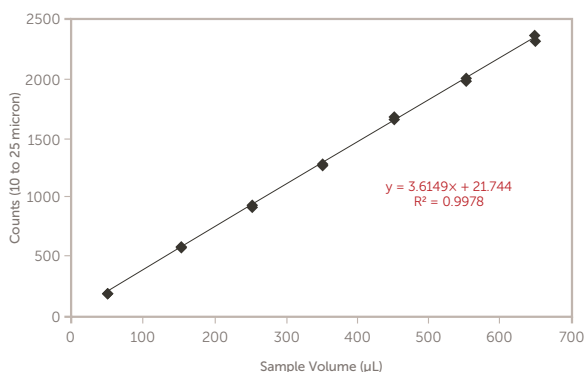


Figure 1.

All concentration experiments generated results within the pass/fail criteria for the count standard – all the way down to 50 µL of sample volume. The results shown in Figure 1 exhibit the expected, well-correlated linear relationship between sample volume and counts.

CONCLUSIONS

This study proved that the AccuSizer SIS system can generate accurate size and count data even at very low sample volumes. Although the data was accurate down to 50 µL, we suggest using slightly larger sample volumes whenever possible, to make the measurement easier on the operator.

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