HORIBA Scientific

Application Note

Analysis of Biopolymers with Laser Diffraction

AN196

Considerable research has been conducted exploring the use of nanoparticles composed of biopolymers as a drug delivery vehicle. Since the design goal for nanoparticles is in the range of 100 nm, much of the particle size analysis in this field has been done using dynamic light scattering (DLS). However, certain materials will feature larger particles that are outside the upper size range of DLS, but within the capability of laser diffraction. Therein is the unique value of a single analyzer that can accurately measure particles both less than 100 nm and greater than several microns. This application note describes two experiments where laser diffraction proved capable of determining both the base biopolymer nanoparticles and also larger particles outside of the range of DLS.

Introduction

Biodegradable polymers are frequently studied as potential carriers for controlled release formulations of active pharmaceutical ingredients (APIs). One common biopolymer used for drug delivery is polylactide. Poly(lactic acid) or polylactide (PLA) is a thermoplastic aliphatic polyester (see Figure 1) derived from renewable resources, such as corn starch. PLA used for drug delivery has been studied for several decades. PLA or PLA surface modified with polyethylene glycose (PEG) can be manufactured as nanoparticles in the range of 50 – 500 nm using several techniques (1).

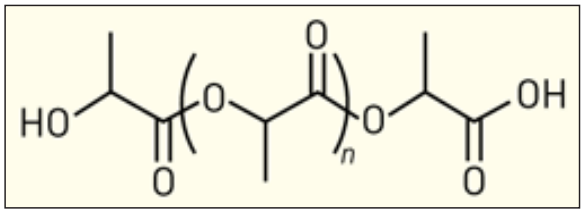


Figure 1: Polylactic acid (PLA)

Proving ability to detect larger population

The first experiment was designed to prove the ability of laser diffraction to measure both PLA-PEG nanoparticles in the 100 nm range and spiked 1 micron latex spheres meant to model the presence of agglomerates. The particles used for this study were supplied by a potential customer who made the test particles using a double emulsion (W/O/W) procedure in a sonicator. Sample number 1 contains only the PLA-PEG nanoparticles. Sample number 2 contains the nanoparticles plus a second population of several percent 1 micron (nominal) polystyrene latex particles. The particle size distribution analysis was performed using a competitive DLS system and on the HORIBA LA-960 laser diffraction analyzer, see Figure 2.

Figure 2: HORIBA LA-960

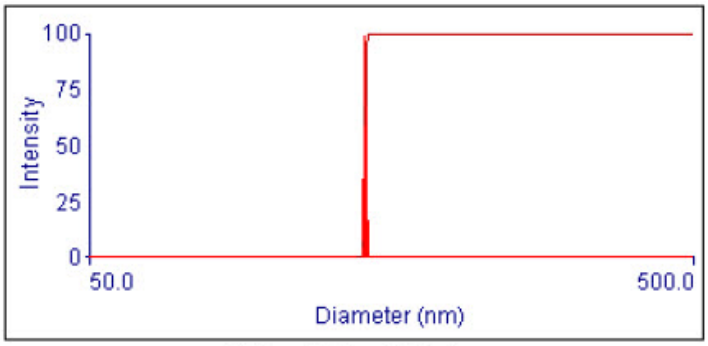
Experimental

Two LPA nanoparticle samples (1 and 2) were analyzed using both DLS and laser diffraction. The DLS measurements were made on a competitive system, so specific analysis procedures were not available to report. The samples were analyzed by laser diffraction using the HORIBA LA-960 system using the Fraction Cell accessory in order to minimize the amount of sample required for the measurement. The Fraction Cell is a unique LA-960 accessory that reduces the required sample volume to less than 1 mg. The samples were analyzed using the quick and simple procedure described below:

1. Fill the Fraction Cell with DI water
2. Activate magnetic stirrer
3. Align the system (automatic)
4. Take a background reading
5. Pipette sample directly into fraction cell
6. Measure at desired concentration (%T)
7. Measure three times, calculate COV

Results and Discussion

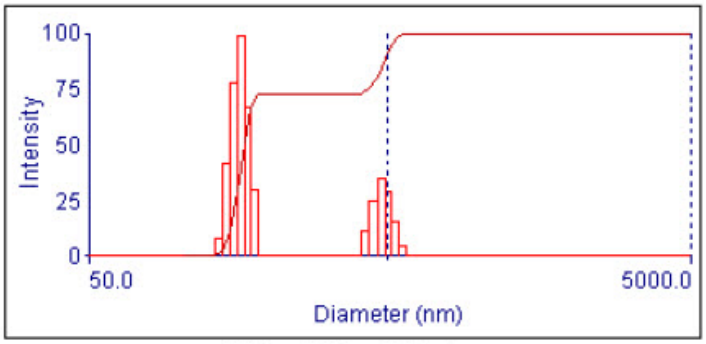
The results from analyzing samples 1 and 2 using DLS reported as intensity distributions are shown in Figures 3 and 4.



Multimodal Size Distribution

d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)
138.4	0	0	142.1	15	4	145.2	0	100
138.7	0	0	142.4	35	11	145.7	0	100
139.0	0	0	142.7	63	23	146.1	0	100
139.3	0	0	143.0	89	40	146.4	0	100
139.6	0	0	143.2	100	60	146.7	0	100
139.9	0	0	143.5	89	77	147.0	0	100
140.7	0	0	143.8	63	89	147.4	0	100
141.0	0	0	144.1	35	96	147.7	0	100
141.3	0	0	144.4	15	99	148.0	0	100
141.6	0	0	144.7	5	100	148.4	0	100
141.8	5	1	145.0	0	100	148.7	0	100

Figure 3: DLS results for sample 1



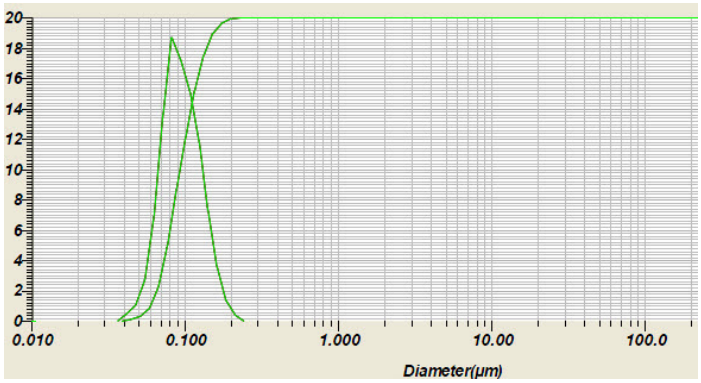
Multimodal Size Distribution

d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)
107.5	0	0	199.9	0	74	371.7	0	74
113.7	0	0	211.5	0	74	393.2	0	74
120.3	0	0	223.7	0	74	416.0	11	76
127.3	0	0	236.7	0	74	440.2	24	81
134.7	8	2	250.5	0	74	465.7	35	89
142.5	41	11	265.0	0	74	492.7	28	96
150.8	78	29	280.4	0	74	521.3	15	99
159.5	100	52	296.6	0	74	551.5	3	100
168.8	67	67	313.8	0	74	583.5	0	100
178.6	30	74	332.0	0	74	617.4	0	100
188.9	0	74	351.3	0	74	653.2	0	100

Figure 4: DLS results for sample 2

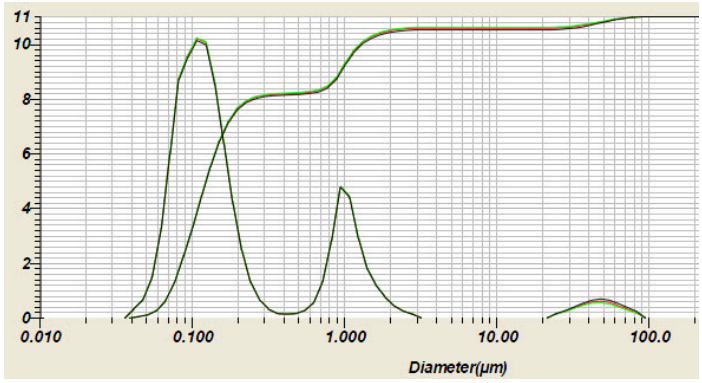
The peak of the nanoparticles alone as shown in Figure 3 is centered at 143 nm. When the 1 micron PSL particles are added the reported bi-modal distribution seen in Figure 4 shows two peaks centered at 160 nm and 465 nm. Both of these peaks are in the wrong position. Several attempts by the customer and vendor to optimize the algorithm to properly split the distributions proved unsuccessful.

The results for two similar samples analyzed by laser diffraction are shown in Figures 5 and 6.



Sample Name	D(v,0.1)	D(v,0.5)	D(v,0.9)
50928-6-1	0.06541(µm)	0.09222(µm)	0.13789(µm)
50928-6-1	0.06541(µm)	0.09222(µm)	0.13788(µm)
50928-6-1	0.06540(µm)	0.09221(µm)	0.13787(µm)

Figure 5: LA-960 Results for sample 1



Sample Name	D(v,0.1)	D(v,0.5)	D(v,0.9)
50928-6-2	0.07348(µm)	0.13085(µm)	1.21951(µm)
50928-6-2	0.07345(µm)	0.13065(µm)	1.20702(µm)
50928-6-2	0.07360(µm)	0.13155(µm)	1.25225(µm)

Figure 6: LA-960 Results for sample 2

The results for sample 1 in Figure 5 report the main population as being centered at 92 nm based on the volume distribution, smaller than the reported size based on intensity distribution by DLS as expected. The results for sample 2 shows the first peak shifted slightly to the larger size, but very accurately reports the 1 micron particles at 1.02 micron. In addition, a third peak of larger agglomerates at 46 micron is detected – well beyond the range of any DLS system.

Test for repeatability

Sample 2 was measured three times to test for repeatability, which proved excellent as shown in Figure 7 where the coefficient of variation (CV) is 0.44 % for the D(v,0.5).

SAMPLE NAME	D(v,0.1)	D(v,0.5)	D(v,0.9)
50928-6-2A.NGB	0.073	0.131	1.220
50928-6-2B.NGB	0.073	0.131	1.207
50928-6-2C.NGB	0.074	0.132	1.252
Average	0.073	0.131	1.226
Std. Dev.	0.001	0.001	0.023
CV (%)	0.787	0.440	1.888

Figure 7: Repeatability of sample 2

Laser diffraction for process control

A different PLA-based engineered nanoparticle used for drug delivery was analyzed by the LA-960 on a regular basis at a customer site as a process control and QA tool. This customer encapsulates API's into a matrix of biodegradable and biocompatible polymers engineered to provide the desired drug release profile. Optimal batches of the product contained only a single population centered near 80 nm as shown in Figure 8.

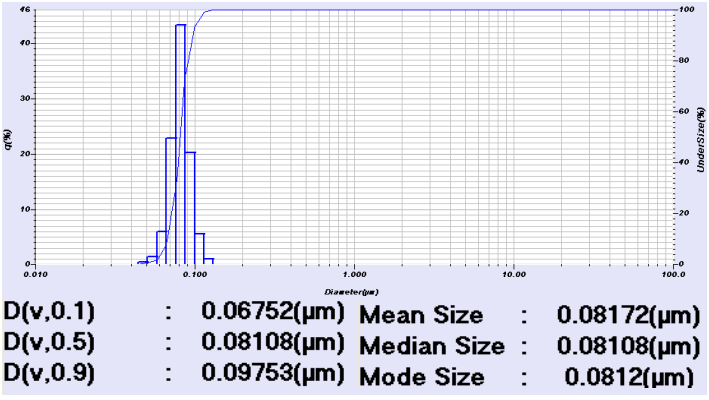


Figure 8: LA-960 results of PLA nanoparticles, good batch

On rare occasions the same formulation generated both the same 80 nm particles but also a small population of agglomerates in the range of anywhere between 10 and 50 micron. It was important for the customer to detect batches containing these agglomerates so all batches were routinely tested on the LA-960 looking for their presence. The results from a bad batch of the PLA nanoparticles are shown in Figure 9.

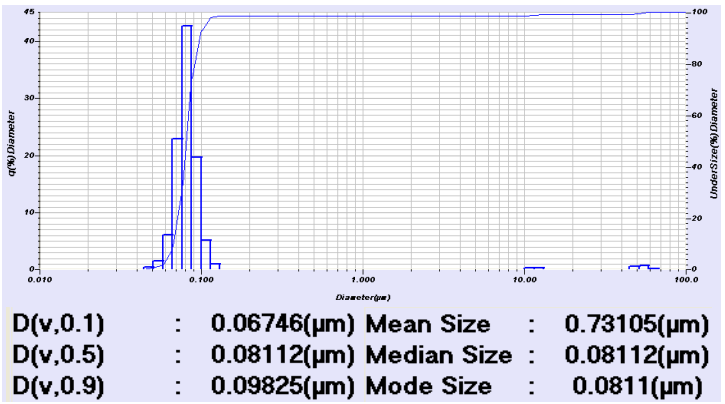


Figure 9: LA-960 results of PLA nanoparticles, bad batch

Data interpretation is critical in order to set specifications to flag the bad batch since the D(v,0.1), D(v,0.5), and D(v,0.9) for both batches are essentially the same. But the volume mean D(4,3) reports a nine fold increase from 0.082 to 0.731 micron. Therefore, the volume mean is the best result value to use to detect the presence of the agglomerates.

Conclusions

The HORIBA LA-960 proved capable of detecting both nano-scaled particles smaller than 100 nm in addition to larger model or agglomerated particles. This highlights several unique capabilities including the low end dynamic range to measure down to 30 nm via laser diffraction, the ability to accurately split multiple populations, and the sensitivity to detect a small percentage of agglomerates in the presence of a main peak in the nanoparticle range. The LA-960 is both sensitive enough for the most demanding R&D requirements and easy enough to use that it can be a day to day process control monitor.

References

1. Avgoustakis K., Pegylated poly(lactide) and poly(lactide-co-glycolide) nanoparticles: preparation, properties and possible applications in drug delivery, Curr Drug Deliv, 2004 Oct:1(4):321-33