USP <729> Globule Size Distribution in Lipid Injectable Emulsions

Measure the means with the Nicomp® DLS system and the tails with the AccuSizer® SPOS system

The size of lipid droplets in injectable emulsions is critical because larger size fat globules (>5 µm) can be trapped in the lungs and are also an indicator that the emulsion is destabilizing. The USP <729>1 test requires two analytical techniques: dynamic light scattering (DLS), or laser diffraction to measure the mean and standard deviation of the distribution, and light obscuration to measure the large tails >5 µm. The Nicomp® DLS system can be used to measure the mean size, and the AccuSizer® single particle optical sizing (SPOS) system is the preferred method to measure the tails >5 µm

INTRODUCTION

Injectable lipid emulsions, Figure 1, have been clinically used for decades as an energy source for hospitalized patients by providing essential fatty acids and vitamins. Intralipid, and other balanced lipid emulsions, provide essential fatty acids, linoleic acid (LA), an omega-6 fatty acid, alpha-linolenic acid (ALA), an omega-3 fatty acid. The critical size characteristics of lipid injectable emulsions include the mean droplet size and the large diameter tail >5 µm. No single technique, or test, can adequately measure both parameters so two methods exist in USP <729>; Method I to determine the mean size and Method II to quantify the percent >5 µm.

Figure 1. Injectable lipid emulsions provide essential fatty acids and vitamins.

Method I – Measurement of Mean Droplet Diameter by Dynamic Light Scattering or Classical Light Scattering

Either DLS or laser diffraction (referred to as classic light scattering in the method), is used to measure the mean size that must be less than 500 nm. The Nicomp DLS system, Figure 2, is the ideal system to use for Method I testing to determine the mean droplet size.

Figure 2. The Nicomp DLS system.

Follow these steps to comply with the Method I requirements set in USP <729>:

- Verify system performance in triplicate with polystyrene latex (PSL) standards at 100, 250, and 400 nm. The reported mean value must be "within acceptable errors",[1] and the coefficient of variation (COV) must be <15% of the reference values.

Note: Entegris does not suggest using 400 nm PSL for verifying the Nicomp DLS system.[2]

- Dilute the sample to an appropriate concentration and measure the size at 90°. Check that the chi square error calculation is "acceptable low",[1] and record the intensity weighted mean diameter and standard deviation. The intensity mean value must be <0.5 µm (500 nm) irrespective of dilution.

Results from a new and old intralipid sample are shown in Figure 3.

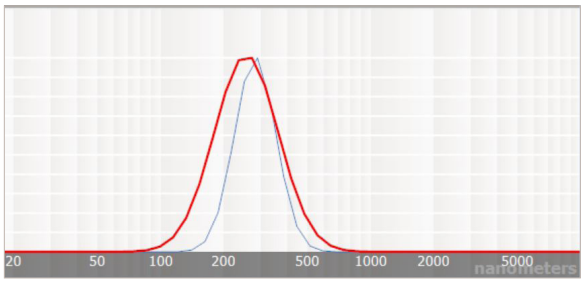


Figure 3. Old (red) and new (blue) intralipid size results.

Method II – Measurement of Large Globule Content by Light Obscuration or Extinction Method

The large diameter droplet tail is measured using a light obscuration/extinction liquid particle counter that employs the SPOS technique, such as the AccuSizer system. Additional guidance on using this technique can be found in USP<788>[3] and USP <1788>.[4]

Follow these steps to comply with the requirements set in USP <729>:

- Check the sizing and counting accuracy of the light obscuration instrument using two different size standards of ~5 and 10 µm (triplicate analyses per size). The average mean diameter on a number weighted basis should be within 10% of the expected size and concentration values. Results of a bimodal mix of 5 and 10 µm PSL standard[5] are shown in Figure 4.

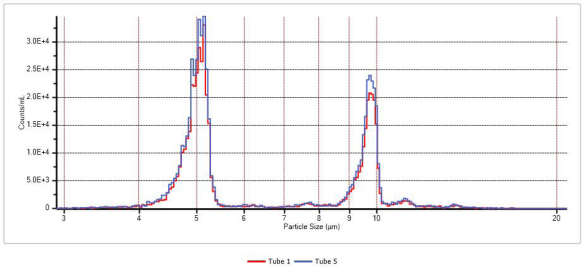
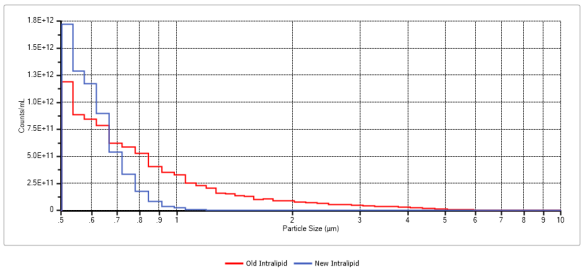


Figure 4. 5 and 10 µm PSL standard results.

- Set the lower size limit at 1.8 µm, and upper limit at 50 µm. Vary the concentration and/or measurement time so there is a factor of two difference >5 µm between two runs. The volume-weighted result >5 µm (PFAT5) must be <0.05%.

Results from a new and old intralipid sample are shown in Figure 5.



Fat concentration	Oil density	$PFAT_5$
10.0%	0.9213 g/mL	0.01711%

Test criteria	Result
$PFAT_5$<0.05%(PASS)	PASS

Fat concentration	Oil density	$PFAT_5$
10.0%	0.9213 g/mL	0.14833%

Test criteria	Result
$PFAT_5$<0.05%(PASS)	FAIL

Figure 5. Old (red) and new (blue) intralipid results.

Automation

Both the Nicomp system and AccuSizer system can be interfaced with an autosampler for high sample throughput requirements. Entegris' AccuSizer APS and Autosampler[6] systems are shown in Figure 6.

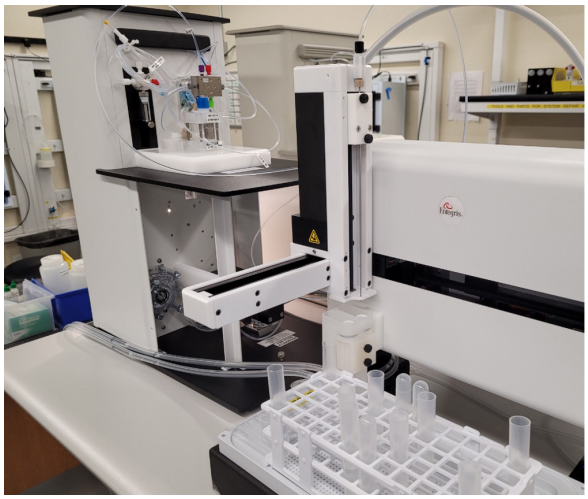


Figure 6. AccuSizer APS system (left) and Autosampler (right).

Automating the USP <729> test facilitates larger studies performed by both pharmaceutical manufacturers and compounding pharmacies. Different concentrations, run times, and flow rates can be analyzed in the same sample tray by using different measurement protocols for each sample. Automated reporting and exporting results to Excel keeps data management easy. Full 21 CFR part 11 features[7] allow for result review, approval, and audit trails.

CONCLUSIONS

Entegris provides ideal solutions for both Method I, with the Nicomp DLS system, and Method II, with the AccuSizer SPOS system. The AccuSizer system has become the industry standard for Method II tests for the PFAT 5 due to it having the highest resolution and best autodilution features available. Integrating the AccuSizer APS and Autosampler systems is the ideal configuration for large projects and high sample throughput.

References

[1] USP <729> Globule Size Distribution in Lipid Injectable Emulsions

[2] Entegris technical note, *Selection of Particle Size Standards for Verifying and Validating Dynamic Light Scattering Instruments Used in Life Sciences*

[3] USP <788> Particulate Matter in Injections

[4] USP<1788> Light Obscuration Method for the Determination of Subvisible Particulate Matter

[5] Available through Micro Measurement Labs, http://www.mmlabs.com/

[6] Entegris data sheet, *AccuSizer® Autosampler*

[7] Entegris manual, *Assessment for Software Systems*, 21 CFR Part 11 Compliance

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