Cannabis Emulsion Droplet Size Analysis

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INTRODUCTION

Cannabis was first domesticated over 10,000 years ago and has been used for various purposes for over the course of human history. Uses include both medical and recreational. Cannabidiol (CBD) is a phytocannabinoid from the cannabis plant whose medical uses include the treatment of nausea due to chemotherapy, spasticity, and possibly neuropathic pain.[1] Epidiolex® CBD oral solution, a CBD oil preparation, was approved by the U.S. Food and Drug Administration (FDA) for the treatment of seizures associated with Dravet and Lennox-Gastaut syndromes. Three synthetic cannabinoid/THC drugs have been approved by the FDA: Cesamet™ (nabilone), Marinol® (dronabinol), and Syndros™ (dronabinol).

With the legalization of cannabis products in much of the USA and many other countries worldwide, the legal cannabis industry is growing rapidly. Cannabis products available for general consumption include a wide range of delivery paths including inhalation, edible, and beverage products. Edible and beverage products have the advantages of avoiding smoke inhalation and discreet consumption of agreeable food products. This application note will focus on cannabis infused beverages, one of the fastest growing cannabis delivery routes.

Cannabis Beverage Processing

The path from cannabis flower to infused beverage requires multiple processing steps, Figure 1. The flower is grown, de-stemmed, dried, and size reduced to create a feedstock. The feedstock then undergoes an extraction process to create an oil concentrate. The oil concentrate may then be further refined to create a distillate or other delivery form. The extracted/refined oil can then be used to create a cannabis oil in water emulsion that can be used as is or diluted to a specified dosage level. Emulsion products can also be freeze dried to create a powder that can later be redispersed into a beverage. Formulating a cannabis oil emulsion requires surfactant optimization (see Appendix I for details) and selection of an energy source such as homogenizer, ultrasonicator, or microfluidizer.

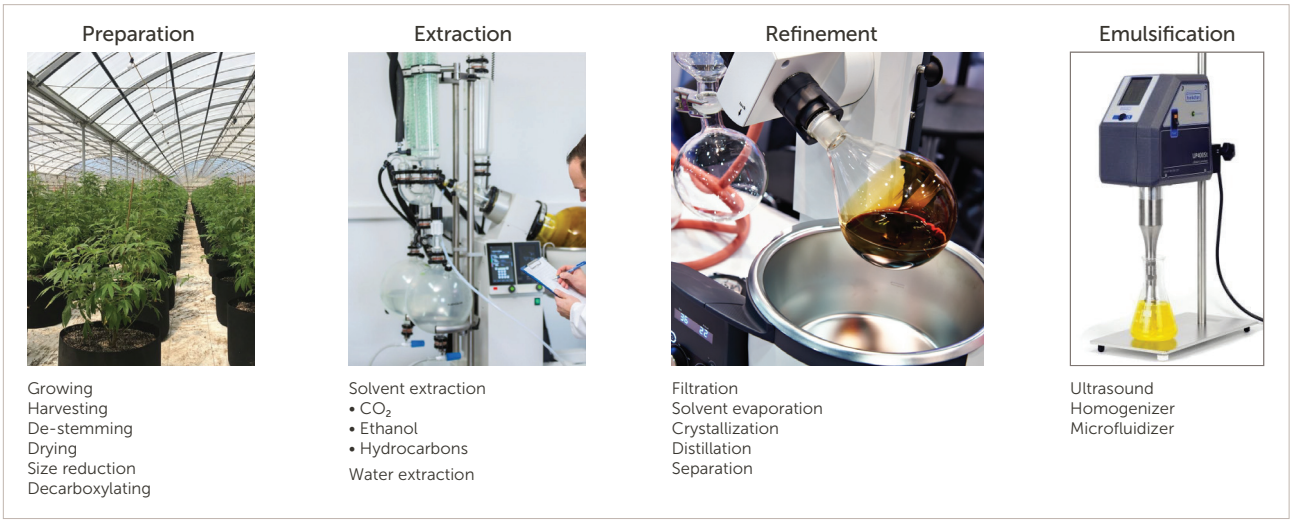

Figure 1. Cannabis processing.

EXPERIMENTAL

A range of cannabis oil in water emulsions were created using commercially available CBD oil, several surfactant formulations, and a high energy ultrasonicator. Emulsion droplet size was analyzed by dynamic light scattering (DLS) to determine mean size and zeta potential and single particle optical sizing (SPOS) to quantify the volume of large droplets – the tail of the distribution.

Materials

Cheef Botanicals[2] CBD oil was purchased locally in California. The sample components from the certificate of analysis for the product as analyzed by CannaSafe[3] is shown in Figure 2.

Analyte	Results
CBD	37.0096 (mg/mL)
CDC	1.3279 (mg/mL)
Δ9-THC	0.8284 (mg/mL)
CBDV	0.7144 (mg/mL)
CBG	0.6405 (mg/mL)
Δ8-THC	0.1125 (mg/mL)
Total	40.6335 (mg/mL)

Figure 2. Sample CBD oil composition.

Surfactants used in this study included:

- Tween™ 80, Sigma-Aldrich P1754 HLB = 15
- Span™ 80, Sigma-Aldrich S6760 HLB = 4.3
- StuphCorp™ Part B, no published HLB value

Instrumentation used in the study included:

- Entegris Nicomp® ZLS3000 DLS system for submicron size + zeta potential, see Appendix II for details
- Entegris AccuSizer® APS SPOS instrument for emulsion stability analysis, see Appendix III for details

Ultrasonicator used:

- Hielscher UP400St[4]

Formulation 1 and 2 used in this study were mixtures of Tween 80 and Span 80 to create different HLB numbers and Stuff[5] component B was used as described by the manufacturer.

Formulation 1	4 parts Span 80 + 1 part Tween 80, combined HLB = 6.97
Formulation 2	1 part Span 80 + 1 part Tween 80, combined HLB = 9.65
Formulation 3	Part B

Mixing Procedure

Part B procedure:

1. Pour 25 grams of component B into beaker 1
2. Heat beaker 1 to a temperature range of 55°C (131°F)
3. When step 2 is at temperature, add 3 grams of oil to beaker 1
4. Fill beaker 2 with 70 grams of water, heat cup to 55°C (131°F)
5. Place beaker 2 under the ultrasonic sonotrode
6. Position the bottom of the sonotrode just below the surface of the water
7. Start sonicating beaker 2
8. Pour beaker 1 slowly and steadily into beaker 2
9. Move beaker 2 in a circle motion while sonicating
10. Remove samples from beaker 2 at defined time interval for analysis
11. Stop process when all liquid is homogenized and clear

Formulation 1 and 2 procedures:

1. Heat Span 80 and Tween 80 to 55°C (131°F)
2. Mix Span 80 and Tween 80 in desired proportions for a total of 2 mL
3. Vortex combination of surfactants until blended
4. Add 200 µL oil into 2 mL surfactant
5. Heat 50 mL water to 55°C (131°F), start to sonicate
6. Slowly add oil/surfactant to water
7. Move beaker in circular motion while sonicating

8. Remove samples from beaker at defined time interval for analysis

9. Stop after 10 minutes

Measurement Protocol, DLS Measurements

- Laser = 35 mW, 632.8 nm
- Detector = APD for size, PMT for zeta potential
- Measurement angle = 90 deg for size, -14.1 deg for zeta potential
- Measurement duration = 5 minutes
- Number of measurements = 2
- Channel width and sensitivity = Automatic
- Temp = 23°C (73°F)
- Viscosity = 0.933 cP
- Zeta potential mode = phase analysis light scattering (PALS)
- Applied electric field = 4 V/cm

A 2 mL sample was removed from the beaker during ultrasonic processing. The sample was diluted 200 µL emulsion into 20 deionized (DI) water for a 1000:1 dilution ratio. Then 300 µL of diluted emulsion was pipetted into a disposable glass cell for measurement for particle size and 2.5 mL was pipetted into a square plastic disposable cell for zeta potential analysis.

Prior to routine analysis of multiple samples quick studies were performed to check the effects of sample concentration and measurement duration. A dilution study was performed to assure the concentration was below the level where coincidence or hindered diffusion affected results.[5] The first sample was diluted 0.5 mL emulsion into 10 mL of DI water, for a dilution factor of 20 (D20). The second sample was diluted 100 µL in 10 mL of DI water for a dilution factor of 100 (D100). The intensity mean result for D20 = 26.0 nm and for D100 = 25.9 nm, proving that the concentration did not influence the results. The next two five-minute measurements were made and the time history plot was reviewed to determine if five minutes is sufficient to achieve stable results. The raw data for the results were also checked for number of decays in the raw data correlation plot (all were greater than 2.3), channel error plots, and automatic baseline adjustment.

Measurement Protocol, SPOS Measurements

A diluted sample used for the DLS measurements was tested on the AccuSizer APS system to determine an acceptable measurement protocol.

Sensor = LE400

Measurement mode = Single stage exponential dilution, manual injection

Injection volume = 100 µl to 1.5 mL, depending on sample

Sensor mode = Extinction for formulations 1 and 2

Sensor mode = Summation for formulation 3

Sample run time = 60 sec

Oil density = 0.927 gm/mL

Oil concentration varied with formulation preparation and predilution

PFAT5 calculation = On

The AccuSizer APS system was flushed to a background level below 100 particles/mL prior to measurements. The sample was directly pipetted into the 11 mL dilution vessel and measurements were analyzed automatically using the settings protocol as described above.

Results

DLS Results

The DLS size results shown in Figure 3 plot the intensity mean size in nm vs. minutes of ultrasonication for the three CBD emulsion formulations.

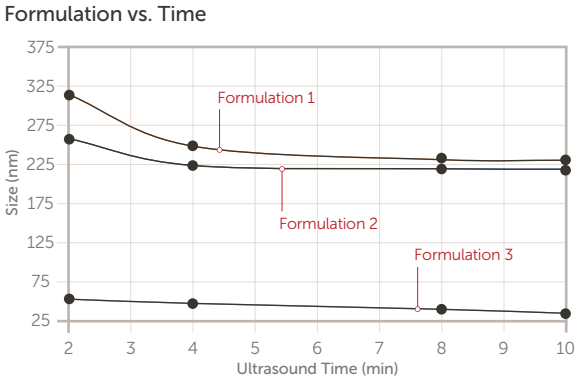

Figure 3. Size vs. ultrasound time exposure.

Figure 4 shows the change in particle size distribution for Formulation 1 when converting from an intensity to volume distribution. Note that the volume distribution is always smaller than the intensity distribution (219.4 vs. 152.4 nm).

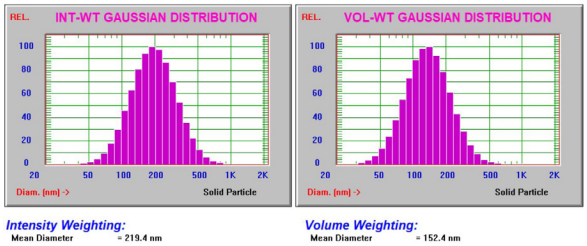

Figure 4. Intensity vs. volume distribution results.

The ISO standard for DLS[6] states that DLS test reports should include the intensity mean and polydispersity index. The intensity distribution can be converted to volume for comparison to laser diffraction or to a number basis for comparison to SEM, but the primary calculated results when using the DLS method are based on intensity. Emulsion droplet size by DLS results in the cannabis industry are currently reported on an intensity, volume, and sometimes unspecified basis making it difficult to assess the actual state of emulsification. Results claiming to be nanoemulsions without supporting data are common and without merit.

The zeta potential results for the three formulations are shown in Figure 5.

Formulation 1 ZP	-28.59 mV
Formulation 2 ZP	-34.41 mV
Formulation 3 ZP	-24.32 mV

Figure 5. Zeta potential results.

SPOS Results

The SPOS size results shown in Figure 6 plots counts/mL vs. size in microns for the three CBD emulsions. Note that these are only the largest droplets at the far end of the size distribution. In this plot the size scale on the x axis is 2.5 to 10 micron and the y axis shows particle counts/mL (calculated back to the actual concentration in the original sample).

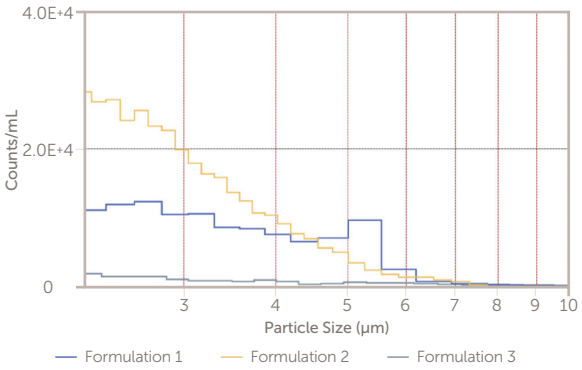

Figure 6. SPOS size vs. counts/mL.

The same results shown in Figure 6 are converted to a volume basis and shown in Figure 7.

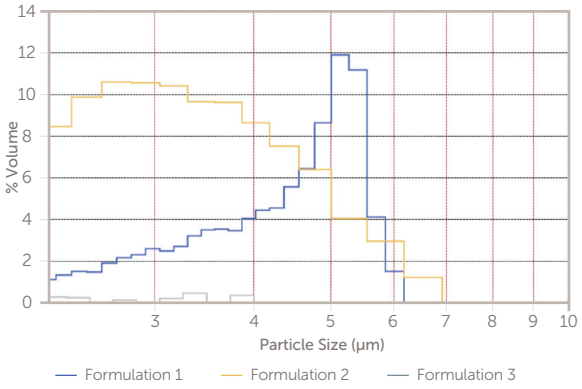

Figure 7. SPOS size vs. volume %.

In the pharmaceutical industry USP test <729>[7,8] was created in the 1990s to help assure the safety of patients receiving injectable lipid emulsions. USP <729> test requires two analytical techniques: Method I, dynamic light scattering, or laser diffraction to measure the mean and standard deviation of the distribution, and Method II, light obscuration (or SPOS) to measure the large tails >5 µm. The mean size from Method I must be less than 500 nanometers (or 0.5 microns) and the volume percent greater than 5 microns (also called PFAT5) from Method II must be <0.05%. Taking this same criterion, the CBD emulsions from this study were analyzed by SPOS and the PFAT5 calculation was reported to quantify the large particle tail – an indicator of emulsion stability. The PFAT results using an oil density = 0.927 g/mL for CBD oil are shown in Figure 8.

Formulation	PFAT5	Result
1	0.036	Pass
2	0.09915	Fail
3	0.0084	Pass

Figure 8. PFAT5 results.

CONCLUSIONS

Both the DLS and SPOS results indicate the same rating of the state of emulsification for the three CBD formulations; formulation 3 > formulation 1 > formulation 2. A combination of both DLS and SPOS results provides a more complete picture of both formulation quality and predicted stability. Products claiming to be nanoemulsions should include droplet size data to support the claim of the state of emulsification. DLS size results should be presented as the intensity mean and polydispersity index as suggested in the ISO standard and the accepted general practice in other industries. The data presented from this study provides an excellent approach to determining optimum formulation and emulsification processing.

References

[1] Allen, G.M. et al., *Systematic Review of Systematic Reviews of Medical Cannabinoids: Pain, Nausea and Vomiting, Spasticity, and Harms*, Canadian Family Physician, Feb 2018, 64(2), e78-94

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[5] Stuff Corp, Las Vegas, NV, https://www.stuphcorp.com/

[6] *ISO Standard 22412:2017 Particle Size Analysis – Dynamic light scattering*, https://www.iso.org/standard/65410.html

[7] *USP <729>, Globule Size Distribution in Lipid Injectable Emulsions*, https://www.uspnf.com/

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[9] Mikulcová, V. et al, *Formulation, Characterization and Properties of Hemp Seed Oil and Its Emulsions*, Molecules 2017, 22, 700

[10] Entegris Technical Note, *DLS Data Interpretation*

Acknowledgements

The author wishes to thank David Schaible of Pharmacann for devoting a day of time to helping him understand the various steps in cannabis processing and Michael Hnatow from Stuff Corp. for providing formulation component B and the Hielscher ultrasonicator.

APPENDIX I: EMULSIONS AND HLB THEORY

An emulsion is a mixture of two or more liquids that are not typically miscible. Most are a two-phase system with a dispersed phase (smaller volume) and a continuous phase (greater volume). Types of emulsions include oil in water (o/w), water in oil (w/o), and double emulsions such as a water in oil in water (w/o/w) emulsion. Cannabis emulsions are typically an o/w emulsion where the dispersed phase is the oil, and the continuous phase is the water.

Creating an emulsion usually requires an energy source such as shaking, stirring, ultrasound, homogenizer, or microfluidizer. Most emulsions destabilize over time, sometimes immediately after the energy input has ceased. Chemicals known as emulsifiers are added to extend the stable period and delay phase separation.

Emulsifiers are typically surfactants containing a hydrophilic head and a hydrophobic R-C chain. The hydrophobic tail orients towards the organic phase and the hydrophilic head orients towards the water. By positioning itself in this orientation at the interface the emulsifier reduces the surface tension and increases the charge (the zeta potential) on the droplet surface, resulting in a stabilizing influence on the emulsion. Types of emulsifiers include food products such as lecithin, sodium phosphates, and surfactants (both ionic and nonionic). Viscosity modifiers such as PEG can also be added to increase emulsion stability. An image of an oil in water emulsion droplet stabilized by a surfactant is shown in Figure 9.

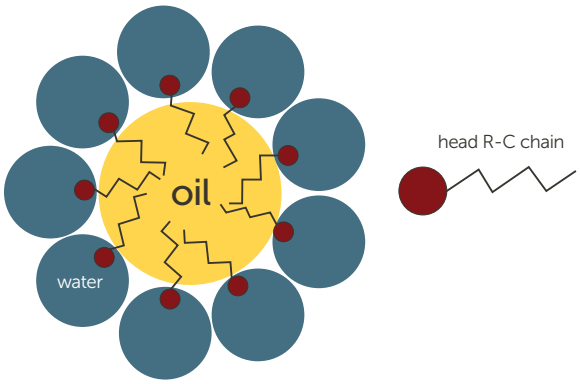


Figure 9. Oil in water emulsion droplet.

The choice of surfactant(s) to formulate a stable emulsion is a critical step in creating a new product. The choice can be guided by theory, common practice, and sometimes trial and error. Surfactant choice for cannabis emulsions is typically limited to food grade or FDA acceptable emulsions. The additional concern of taste effects should also be considered.

One theoretic approach helpful for beginning the choice of surfactants is HLB theory. HLB stands for "Hydrophile-Lipophile Balance" where molecules that are attracted to and dissolve in water are hydrophilic and molecules that are attracted to and dissolve in oils and nonpolar solvents are lipophilic. An emulsifier that is lipophilic in character is assigned a low HLB number (below 9.0), and one that is hydrophilic is assigned a high HLB number (above 11.0), as shown in Figure 10. Those in the range of 9 – 11 are intermediate.

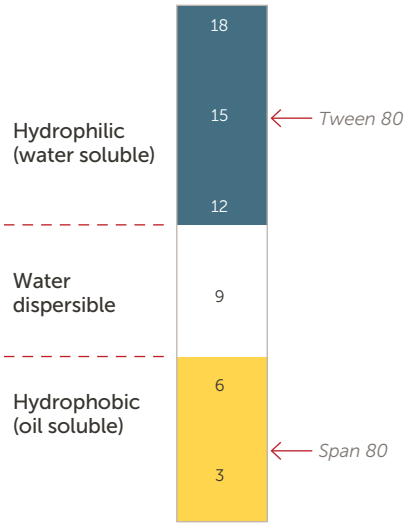


Figure 10. HLB scale.

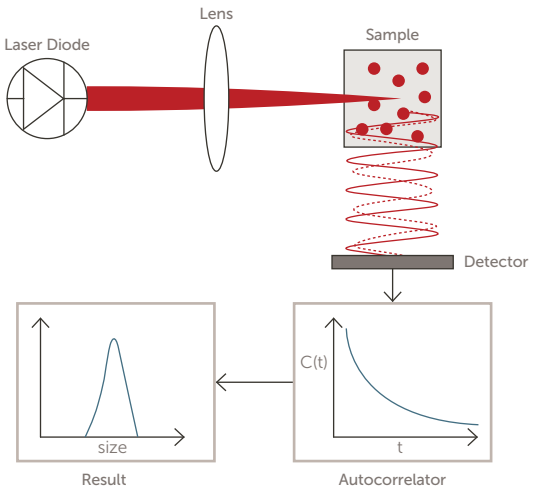
Several publications[9] suggest an HLB number near 8 – 9 is appropriate for cannabis oil in water emulsions. In this study a mixture of Tween 80 and Span 80 surfactants was used for creating formulations 1 and 2. Basic arithmetic means were used to calculate the composite HLB values for the surfactant mixtures:

Formulation 1	25% Tween 80, 75% Span 80: (0.25×15)+(0.75×4.3)=3.75+3.22=6.97
Formulation 2	50% Tween 80, 50% Span 80: (0.5×15)+(0.5×4.3)=7.5+2.15=9.65

Formulation 3 was created using a proprietary surfactant mixture provided by Stuff Corp. The HLB number for this surfactant mixture was not disclosed.

APPENDIX II: DYNAMIC LIGHT SCATTERING (DLS) AND ZETA POTENTIAL

Dynamic light scattering (DLS) is the preferred method for particle size analysis of nanoparticles. To make a measurement the sample is placed in a cuvette where the particles experience Brownian motion. Smaller particles move faster than larger particles. The cuvette is placed in the instrument where it is illuminated by a laser, Figure 11. The scattered light due to the Brownian motion is captured on a detector at a specific angle. The time signature of the scattered light is used to create an autocorrelation function that decays more rapidly for smaller particles and more slowly for larger particles. The translational diffusion coefficient (D) is determined from the autocorrelation function. The Stokes-Einstein equation is then used to calculate the particle radius R.



$D = kT/6\pi\eta R$

Where:
D = Diffusion coefficient
R = Particle radius
k = Boltzmann's constant
T = Temperature Kelvin
η= Shear viscosity of the solvent

Figure 11. Basic principles of DLS.

The basic results from the Nicomp system measurement include the intensity mean size, the width of the distribution (polydispersity index, PI)[10], and the Chi Square calculation. If the Chi Square value is greater than around 3, then the multimodal Nicomp system algorithm should be considered rather than the single mode Gaussian result. Note that DLS provides the size distribution of the sample, not the concentration. The primary size result from DLS is the intensity distribution. This result can be converted to a volume or number distribution, but this is only suggested for the purpose of comparing results to other techniques such as laser diffraction (volume) or image analysis (number).

Zeta potential is a measure of the charge on the surface of particles or emulsion droplets. Figure 12 illustrates that the zeta potential is a potential measured in mV at a small distance from the surface of the droplet known as the slipping plane. This charge is an indication of dispersion stability. Dispersions with a zeta potential near zero are typically unstable and prone to aggregation or phase separation. A higher zeta potential indicates expected greater stability.

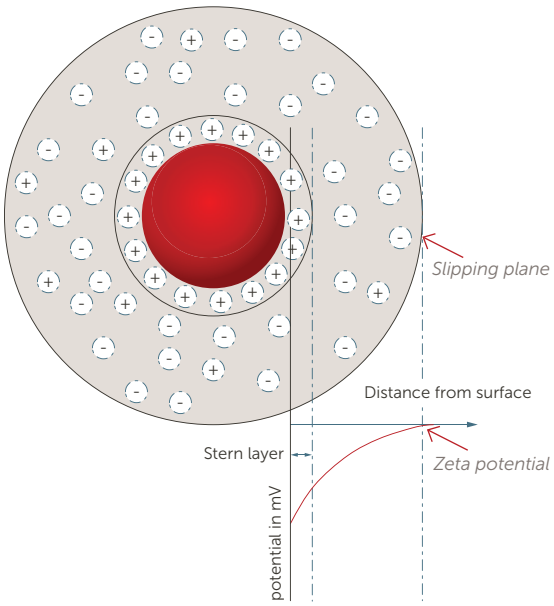


Figure 12. Zeta potential.

To make a measurement, a sample is pipetted into a standard square cuvette and a dip cell containing two palladium electrodes is inserted into the cuvette. The electrodes apply an electric field, causing the particles to migrate to the pole of opposite charge. The direction of the particle motion indicates if the particles are positively or negatively charged. The speed of the particle motion is used to calculate the magnitude of the charge.

The Nicomp system measures the particle motion using a patented DSP-based phase analysis light scattering (PALS) technique that is extremely sensitive and robust. Measurements can be made at low electric field strength, which is much gentler on fragile samples such as proteins or other biomolecules.

APPENDIX III: SINGLE PARTICLE OPTICAL SIZING (SPOS)

The SPOS technique is both a liquid particle counter and a sophisticated particle size analyzer that provides both size and concentration results for the sample analyzed.

All AccuSizer systems consist of a sensor, pulse height analyzer (counter), and fluidics to transport the sample through the sensor. Particles flowing through the sensor scatter and obscure the incident laser beam. This light interaction creates pulses that are proportion to the size of the particle. The counter converts these pulses to particle size.

The AccuSizer APS system used in this study incorporated the LE400 sensor, and single stage exponential dilution fluidics to control sample concentration. The LE400 sensor includes both extinction and scattering detectors to measure particles in liquid from 0.5 – 400 µm, Figure 13.

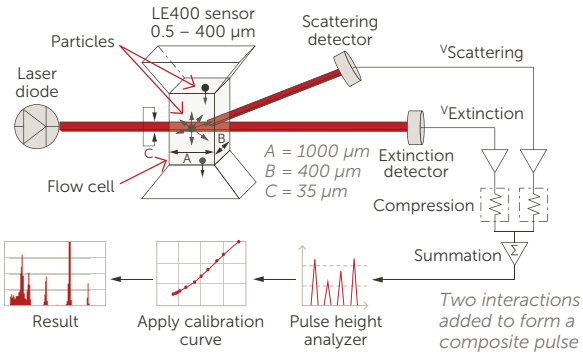


Figure 13. Basic principles of SPOS.

The results from the AccuSizer SPOS system include size and concentration in particles/mL. The system tracks sample dilution to accurately calculate the concentration in the original sample independent of dilution level. Results can also be converted to a volume-based distribution, which is useful for emulsion stability testing such as the USP <729> for lipid injectable emulsions.[7]

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