Rapid Analytical Methods for Quality Control of Hemp Emulsions

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Definition of Food

- **"Articles used for food or drink for man or other animals, chewing gum, and articles used for components of any such article"**
- **Consumed for taste, aroma, nutrition**
- **Safe by definition**
- **Regulations**
  - FSMA (Food Safety Modernization Act)
  - CODEX (Deals mainly with labeling)
  - GMPs (Manufacturing Regulations)

Definition of Dietary Supplement

- **Classified as food**
- "...any product (other than tobacco) that contains a vitamin, mineral, herb, or other botanical or amino acid and is ***intended as a supplement to the diet***."
  - A concentrate, metabolite, constituent, or combination of the above
  - Don't need to prove safety before selling
  - Don't need to prove product is effective
- **Regulations**
  - cGMPs

Definition of Drug

- **Articles intended to:**
  - "...diagnose, cure, mitigate, treat or prevent disease in man or animals"
  - "…affect structure or function of the body"
  - "…listed in an official compendium"
- **Unsafe by definition**
- **Must be Generally Recognized as Safe and Effective (GRASE) e.g., premarket approval via the IND/NDA route**
- **Regulations**
  - GMPs

Cannabis Regulatory Status

- **Cannabis**
  - Same species as Hemp but contains more then 0.3% THC
  - Although in some states is considered legal as a recreational drug, Cannabis is still considered a Schedule 1 drug Federally
  - Medical use has also not been approved on the Federal level
  - Canada is the exception
    - Has legalized Cannabis use throughout the country

Hemp Regulations

- **Hemp**
  - Hemp Seed and fiber are considered GRAS
    - Sold as food - hemp seeds, hemp hearts, hemp seed oil etc.
    - Fiber used for making clothes, rope, shoes, paper etc.
  - Extract of flower not approved as a DS but regulators have used discretion in policing it on the market
    - Should not exceed 0.3% THC according to Farm Bill
- **CBD**
  - Isolate has been approved as a pharmaceutical drug therefore products claiming pure CBD are technically illegal and unapproved drugs

Recent Hemp Regulations

- **USDA Published final rule for the Domestic Production of Hemp (January 15th, 2021)**
- **Some examples of changes in the final rule**
  - Timing of sample collection-agents have 30-day window to collect samples for THC compliance
  - Raises negligent violation from 0.5% THC to 1% THC.
    - Plants that test above 0.3% but below 1% not considered to be in violation
  - Testing at DEA-Registered labs and other
    - Requirement for testing hemp at FDA Registered Labs suspended until December 31st, 2022

Recent Hemp Regulations

- **California and AB-45**
  - **October 6th, 2021 signed in to law**
    - Allows for hemp and cannabinoids (e.g. CBD,), extracts or derivatives of hemp in food and beverages, dietary supplements, cosmetics and processed pet food
    - Must contain less than 0.3% THC
  - **Defines THC as:**
    - Tetrahydrocannabinolic acid
    - Well known isomers Delta-8 THC and Delta-10-THC
    - Any other cannabinoid besides CBD that the Department of Health determines causes intoxication

Chromatography (HPLC, GC, HPTLC)

- **Chromatography Advantages**
  - Has been around a long time and is used in most labs worldwide
    - Pharmaceutical and Dietary Supplement Companies
    - Contract Manufacturers and Testing Laboratories
    - Research Labs and Organizations
    - Environmental Labs
    - Biopharma
    - Food and Beverage etc.
  - Used for most applications
    - Q/C and Q/A of incoming materials and release of finished products
    - Research
    - Regulatory Compliance etc.

A-TEEM: Aqualog and FAST-01 Autosampler

The Future of Fluorescence

A-TEEM™ MOLECULAR FINGERPRINTING

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Aqualog/ATEEM Advantages

- **Speed of Analysis**
  - Takes seconds (35 - 40 sec for cannabinoids) versus ≥ 6mins for GC and HPLC
- **Initial cost of equipment much less compared to most others**
  - Does not require column heater, mobile phase degasser etc.
  - Annual cost of running of Aqualog almost non-existent when compared to other equipment (e.g., HPLC/UV can cost $14,000+ to run a year which does not include maintenance costs - LC/MS maintenance can range from $25K-$50K/year)
- **Does not require expensive consumables**
  - Reference standards extremely expensive and unreliable for many botanicals
  - Cost of columns @ $500+ and ideally should be dedicated to a single method.
- **Leaves a small carbon footprint**

Who Are the Potential End-Users

- **Food and Beverage**
- **Dietary Supplements/Natural Health Products**
- **Pharmaceutical**
- **Government/Regulatory Agencies**
  - FDA, USDA, EPA etc.
- **State Labs**
  - Colorado, Oregon etc. Dept. of Agriculture Industrial Hemp Programs
- **Research Institutes and Universities**
- **Contract Manufacturers and Testing Labs**
- **Cannabis and Hemp**
  - Growers
  - Finished Product Manufacturers

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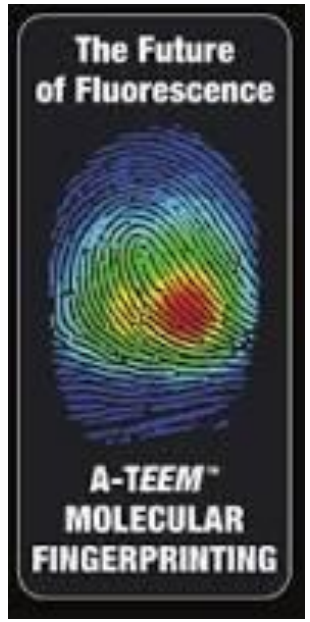
Who Are the Potential End-Users

- **Contract Testing Laboratories**
  - Testing of incoming materials for identity for companies without in house labs
  - Testing of finished goods/product release and label claim for companies without In house labs or for 3rd party verification
  - Examples
    - SC Labs - (CA) - Specialize in Cannabis testing. Annual revenue @ $75 million
    - Eurofins (Worldwide-WI, CA etc.)-annual revenue @ $62.19 million just in US
    - Alkemist Labs (CA) - Single lab with annual revenue @ $5 million
    - ElSohly Labs (MS) - Single lab with annual revenue @ $4 million

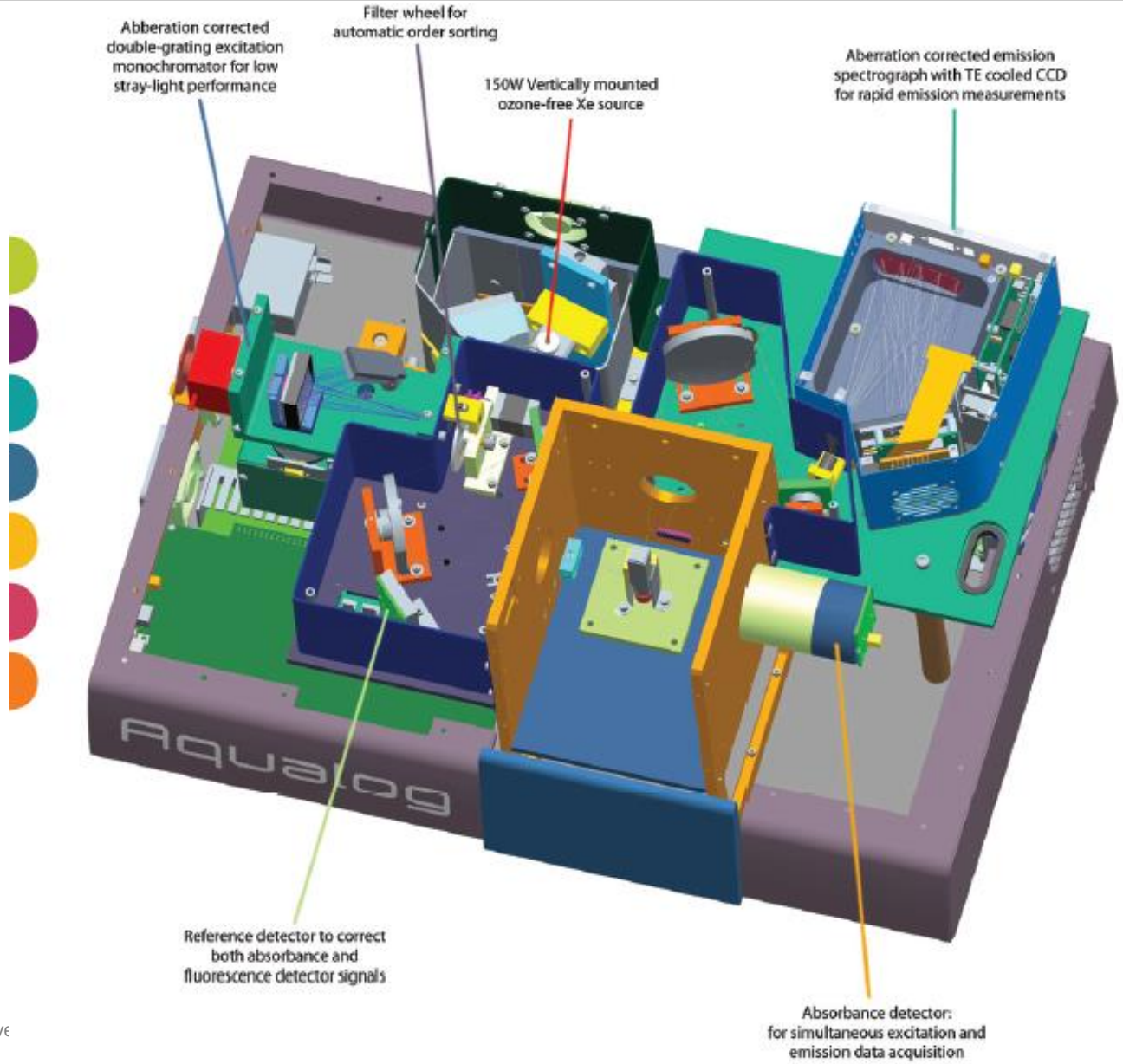
Introduction: The A-TEEM Solution

- QA/QC Analyses of Beverages, Food and Dietary supplements are complicated by the general requirement for expensive, time-consuming chromatography
  - Sample extraction, concentration and cleanup
  - Column and pump maintenance and expensive mobile phase solvents and or gases
  - Expensive, fragile equipment requiring maintenance
  - Requirement for expert operator training

- Introducing the **A-TEEM**, short for **A**bsorbance-**T**ransmittance fluorescence **E**xcitation-**EM**ission spectroscopy.
  - Simple sample preparation
  - Rapid acquisition (s-min)
  - Robust analysis toolbox (easy to apply advanced machine learning principles)
  - Excellent Intra- and Inter-laboratory Performance (completely traceable to NIST references)
  - Effectively column-free chromatography!



A-TEEM: Aqualog Optical Layout



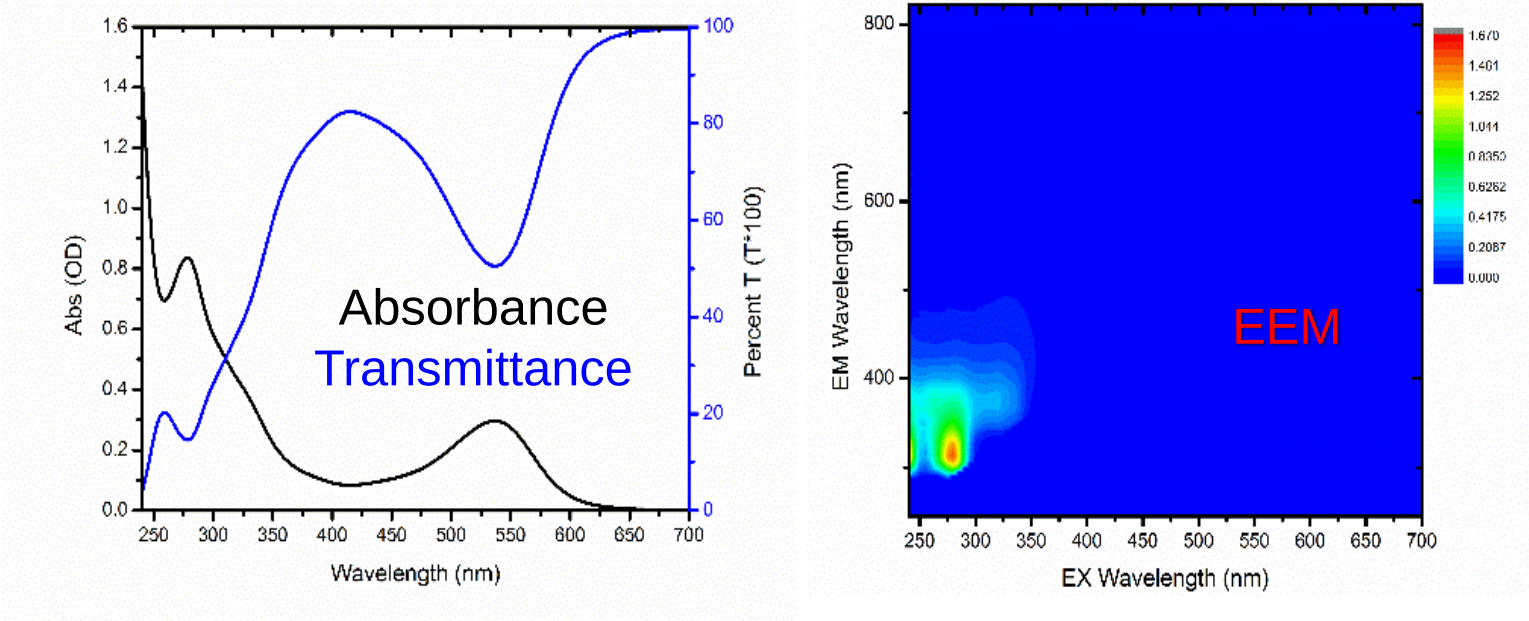
A-TEEM: Synergy of Unique Abs and Fluor Variables

***Patented* Instrument Method:**

Absorbance: measures all colored species independent of fluorescence

Transmittance: measures color and chromaticity

EEM: measures excitation and emission of all fluorescent components



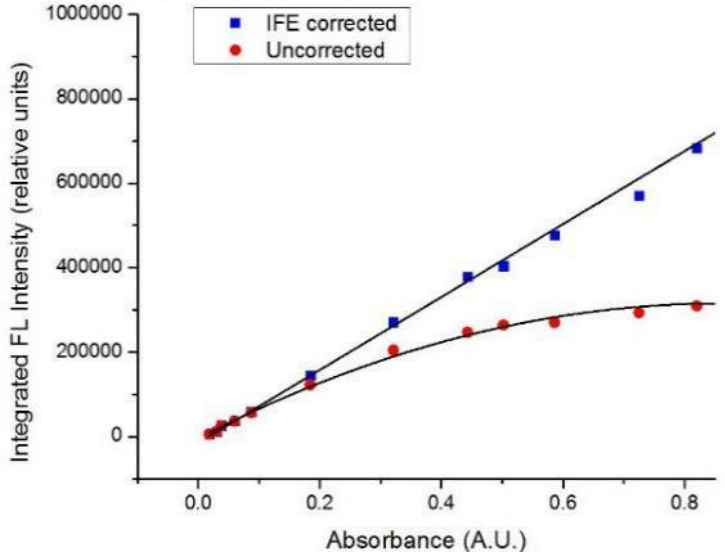
Inner-Filter Effect Correction:

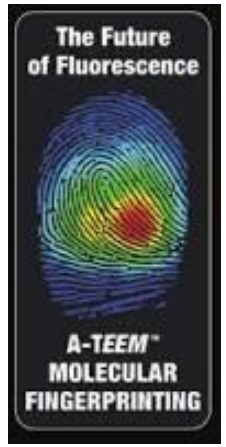
Expands linear concentration range for fluorescence

High sensitivity

Can resolve compounds in ppt to ppb range from high background (ppm) matrices.

Compound libraries and model databases independent of concentration





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A-TEEM: 5-Dimensional Optical Chromatography

Any Chromophoric compound in a given solvent and temperature condition will have a unique:

1. Extinction coefficient (ε)
2. Absorbance Spectrum

And If Fluorescent, also:

3. Fluorescence Quantum efficiency (ΦF)
4. Fluorescence Excitation Spectrum
5. Fluorescence Emission Spectrum

The A-TEEM uniquely provides access to all of these variables

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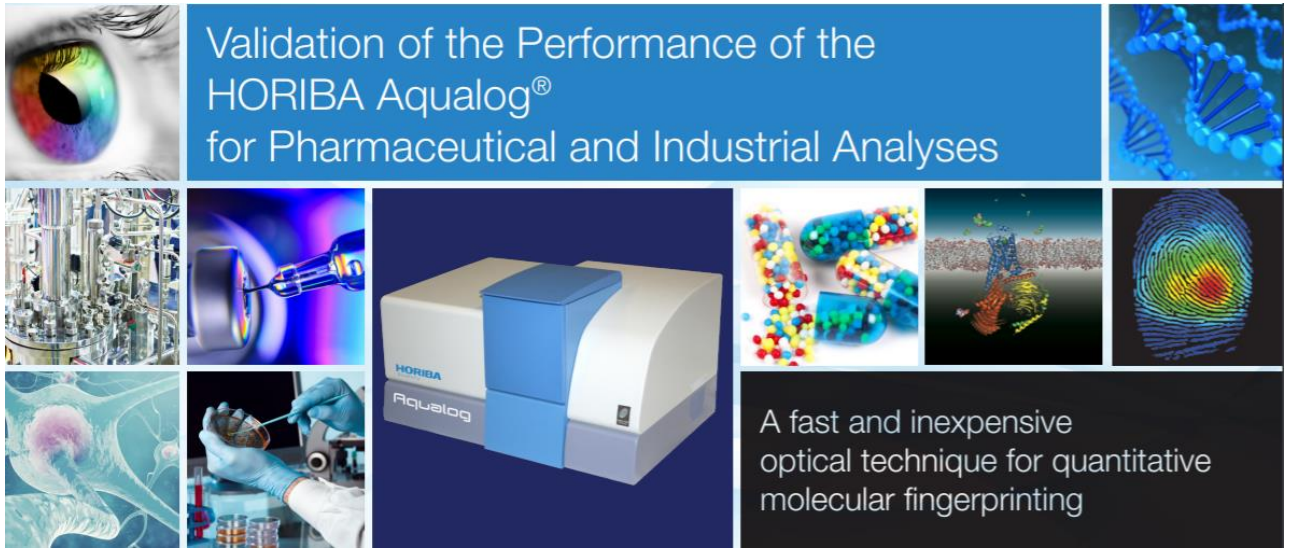
A-TEEM: USP Calibration/Validation Toolbox

The Aqualog software is fully equipped to facilitate both calibration and validation of all instrumental absorbance and fluorescence performance functions, including the following tests:

- Photometric accuracy
- Absorbance and Excitation Wavelength Accuracy
- Stray Light
- Emission Wavelength Accuracy
- Emission Spectral Shape Accuracy
- Water Raman Band Accuracy
- Water Raman Band Signal to Noise (SNR)
- Water Raman Band Area Scattering Units (RSU)
- Quinine Sulfate Units (QSU)



The Aqualog toolbox is modular and intuitive, and along with HORIBA IQ/OQ documentation, guides users through the validation process. Individual Pass/Fail Tests can easily be selected individually to minimize test redundancy and facilitate more routine testing requirements.

A-TEEM: Advantages over Conventional Technologies

Technology	Pros	Cons	Notes
GC, GC/MS, LC/MS	• High Sensitivity/Selectivity	• **Capital,OpEx** • Slow/Complex operation	Not generally compatible for real-time
HPLC/UPLC	• High Sensitivity • High Selectivity	• Capital,OpEx • Low Dynamic Range • Slow	Not generally compatible for real-time
Raman	• Chemical specificity • Noninvasive • Adaptable Probes	• **Capital,OpEx** • Complex operation • Low Sensitivity	Real-time capabilities
Near-infrared (FTIR)	• Noninvasive • Various Materials • Speed/Low Cost	• Low Sensitivity • Low Selectivity	Real-time capabilities
UV-VIS	• Noninvasive • Adaptable Probes • Speed/Low Cost	• Low Sensitivity • Low Selectivity	Real-time capabilities
A-TEEM	• Noninvasive • Adaptable Probes • Speed/Low Cost • High Sensitivity Selectivity	- Too sensitive?	Real-time capabilities On line At line Effective Variable Selection

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A-TEEM: Method Publication

“Cannabis and Cannabinoid Research”

Accepted: 2022/02/19

A-TEEM Method for Quantification of Major Cannabinoids and Corresponding Acids: A Rapid Alternative to Chromatography for Rapid Chemotype Discrimination of Cannabis sativa Varieties

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A-TEEM: Introduction to Cannabis

- A dioecious, annual herb.
- Family: **Cannabaceae**
- One species, many varieties:

***Cannabis sativa;* Var. sativa** –
A tall conical plant found in warmer lowland climates (Mexico, S. Africa, Thailand).

***Cannabis sativa;* Var. indica** –
A squat, bushy highland plant (Northern India).

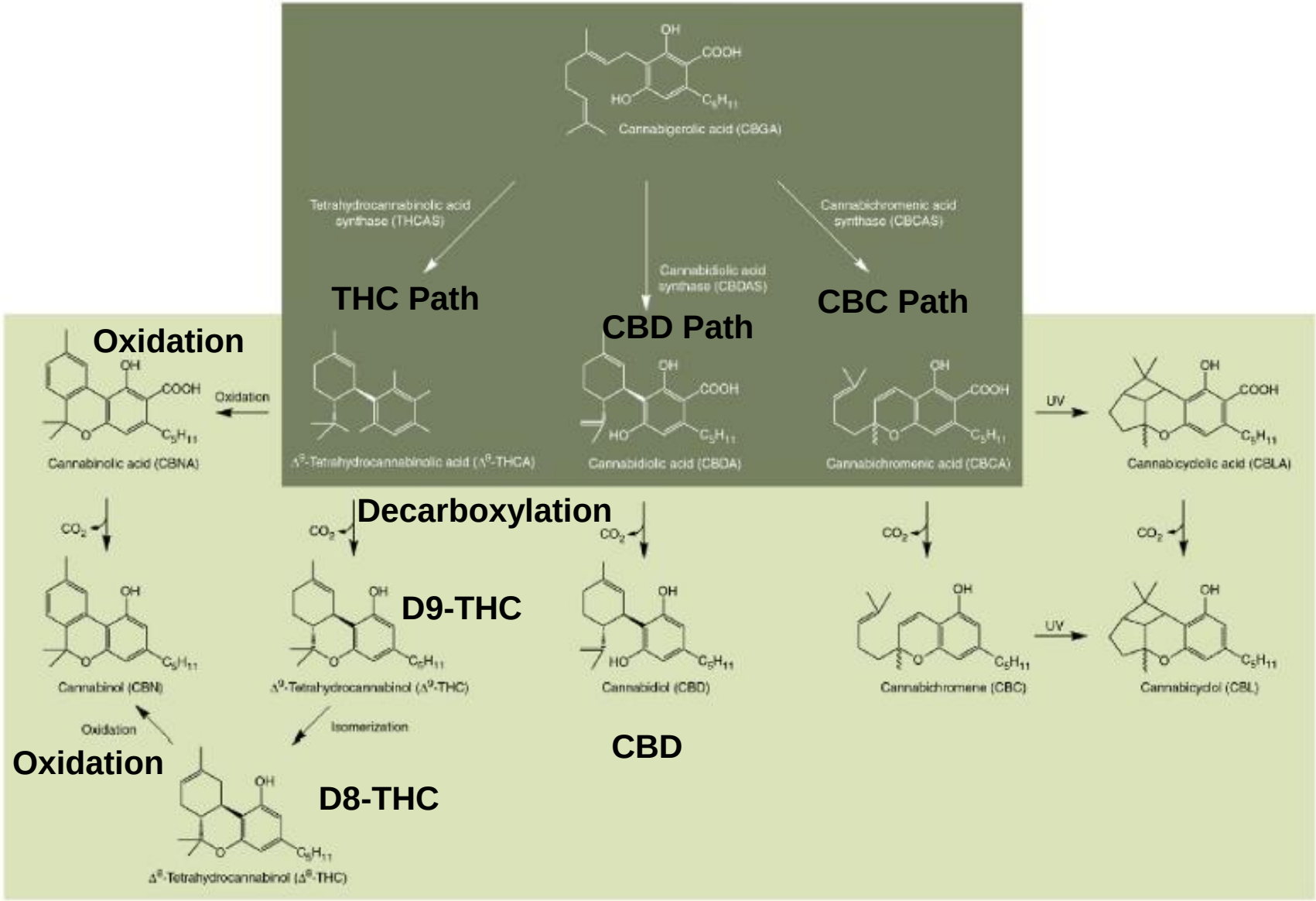
***Cannabis sativa;* Var. ruderalis** –
A small plant less than 1.0 meter at full maturity (Central Asia).

https://en.wikipedia.org/wiki/Cannabis

A-TEEM: Cannabinoid Biosynthetic Pathway

Cannabinoid Chemistry:

- US Farm Bill Legality: Total THC+THCA <0.3% wt %
- Living plants enriched in Acid Forms.
- Heat generally required in harvested plant material for decarboxylation to neutral forms.
- CBN(A) is an oxidation product of THC(A)



A-TEEM: Purified Major THC(A) and CBD(A)

Purified Standards:

1 mg/L in MeOH

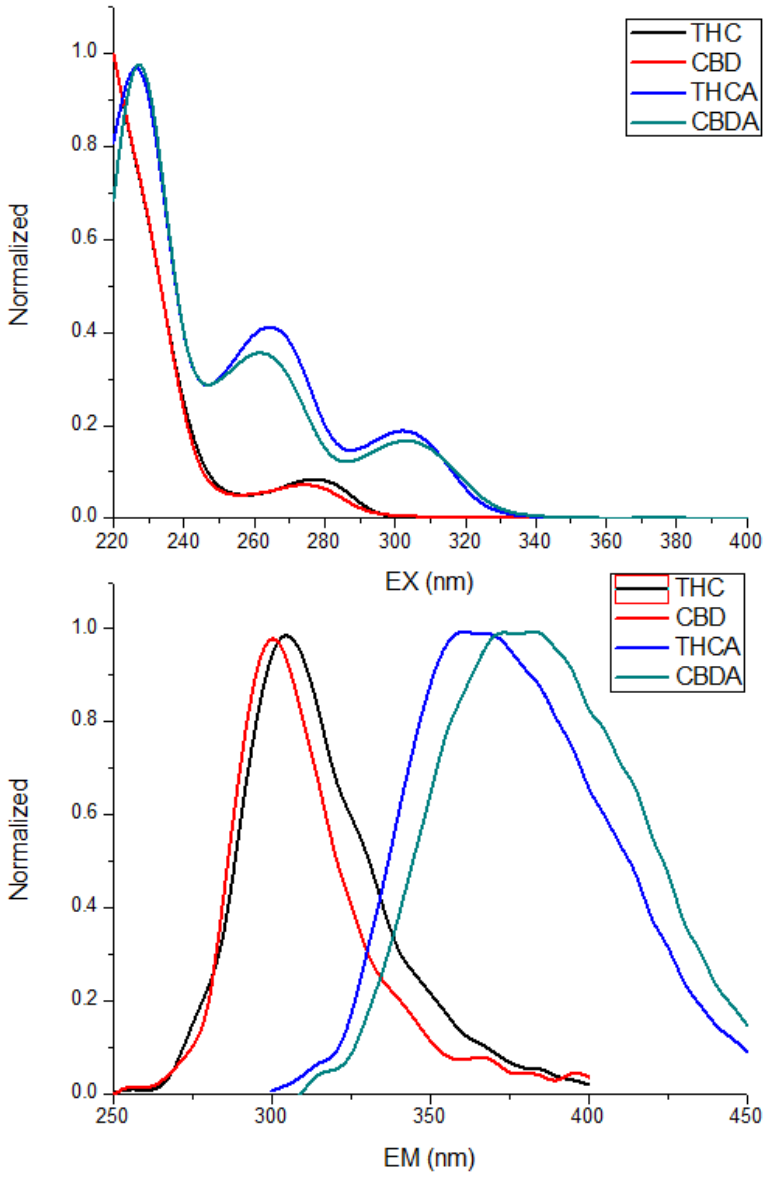
Acid Forms:

Three absorbance/excitation peaks

Broader more red shifted emission

Neutral Forms:

One major and one minor peak



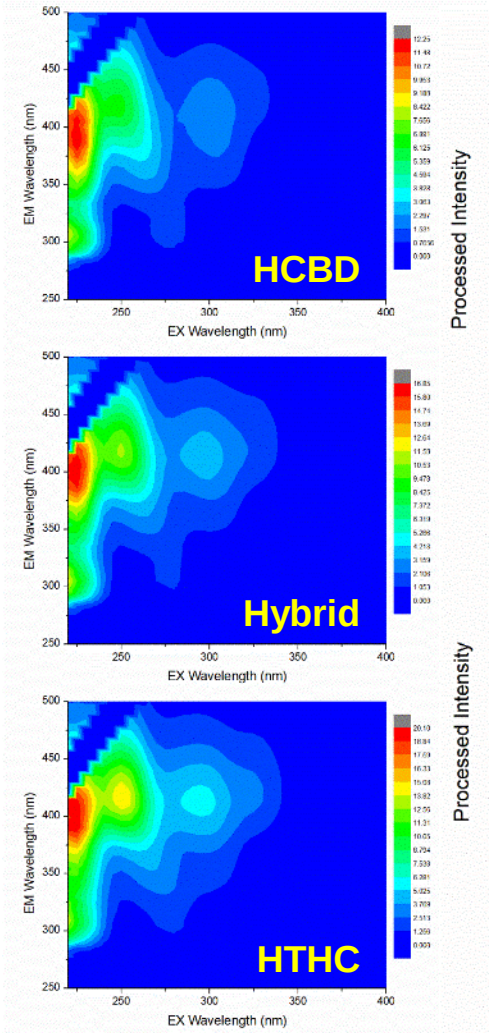
A-TEEM: CBD and THC Cannabis Strains

HEMP
<0.3% total THC

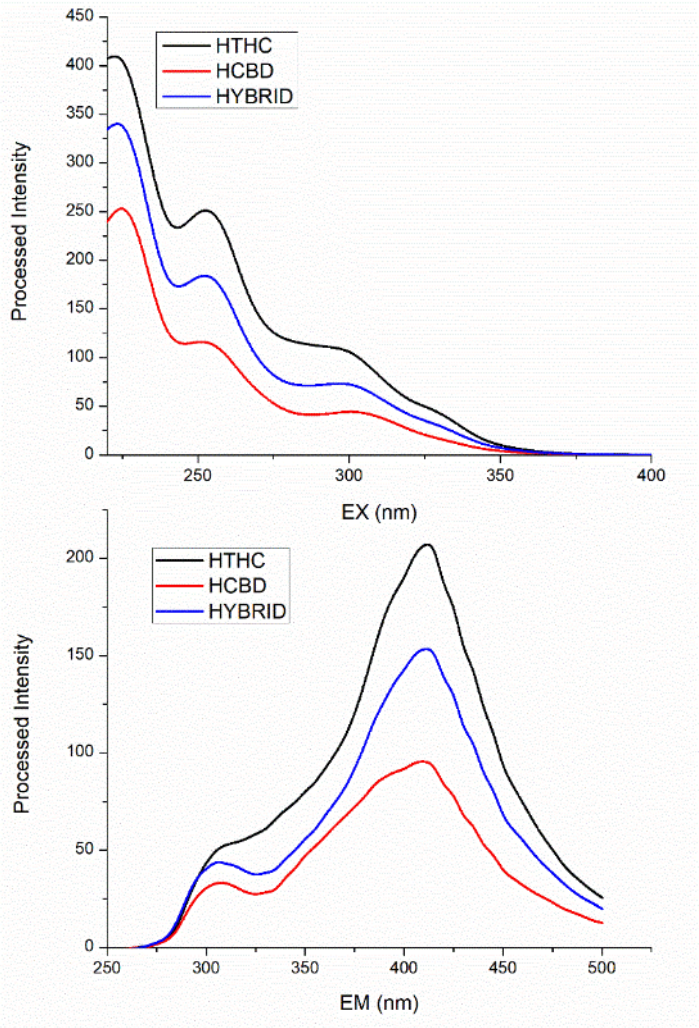
Marijuana/Hemp
≥0.3% total THC

Marijuana
≥0.3% total THC

A-TEEM Contours Scaled to Maximum for Each Strain



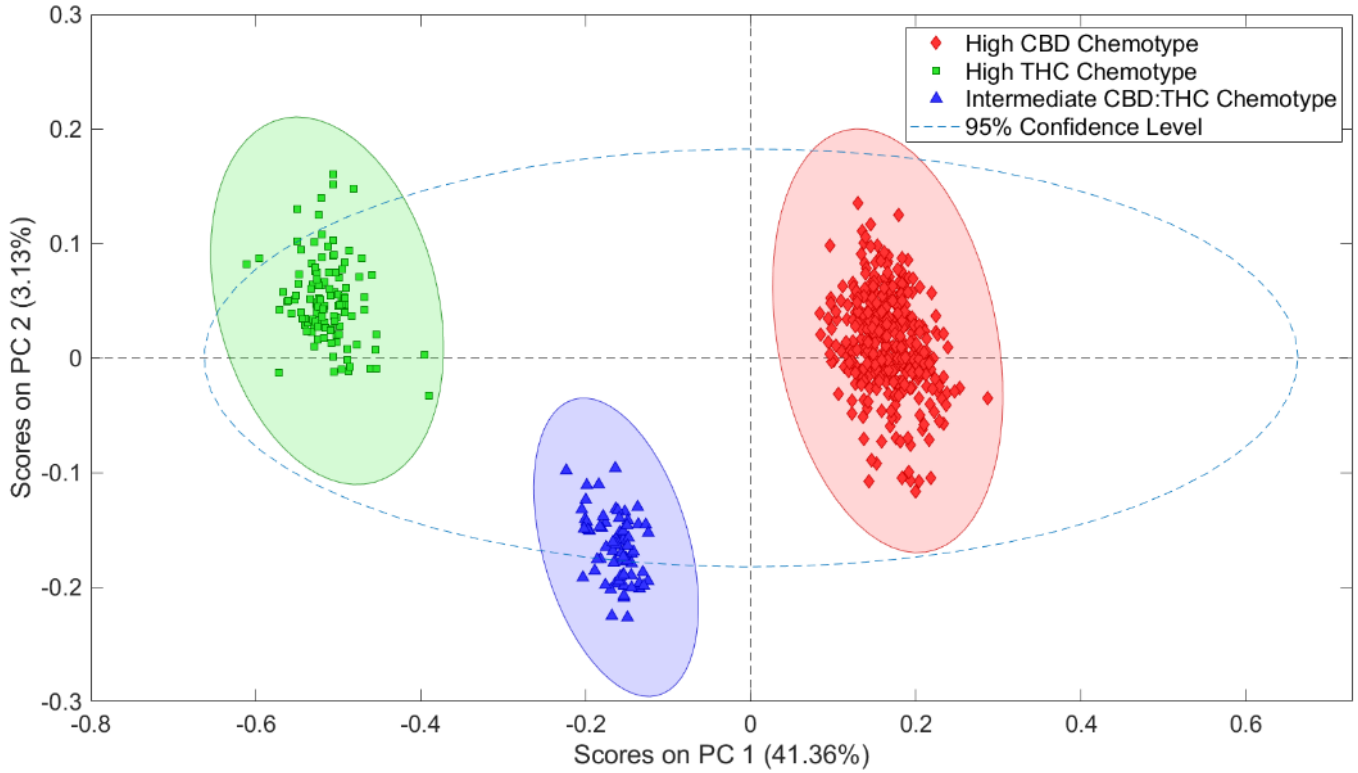
Excitation (TOP) and Emission (Bottom) Integrals



A-TEEM contours and integral profiles exhibit spectral peak center, width, shape and intensities consistent with the varying levels of CBD(A) and THC(A).

A-TEEM: Cannabis Varietal PCA Calibration-Validation

Calibration and Validation Classes Evaluated Simultaneously
(99.99% Confidence Ellipses)



PCA Spontaneously Reveals Classes of High-THC, Hybrid and High-CBD strains!

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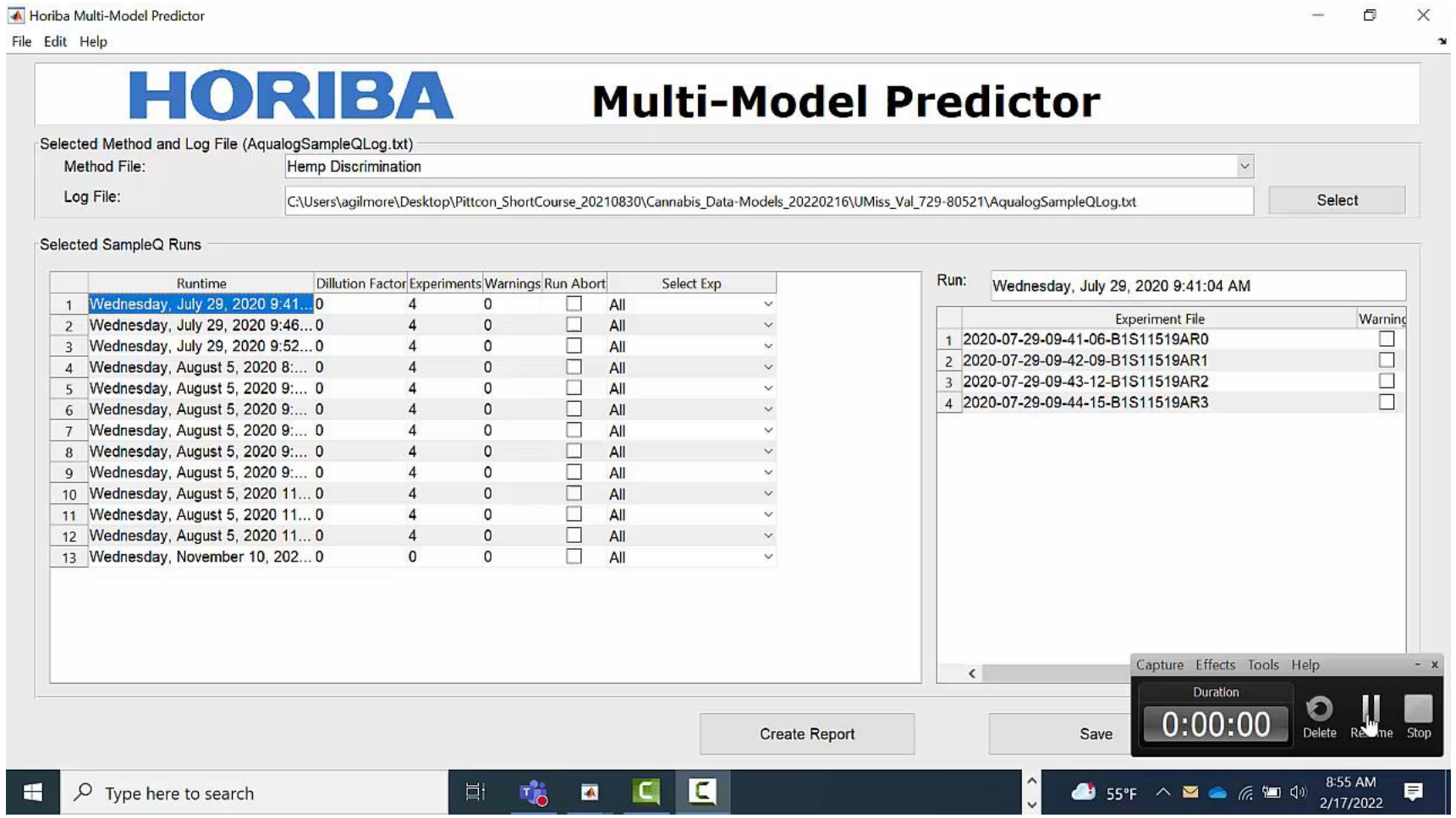
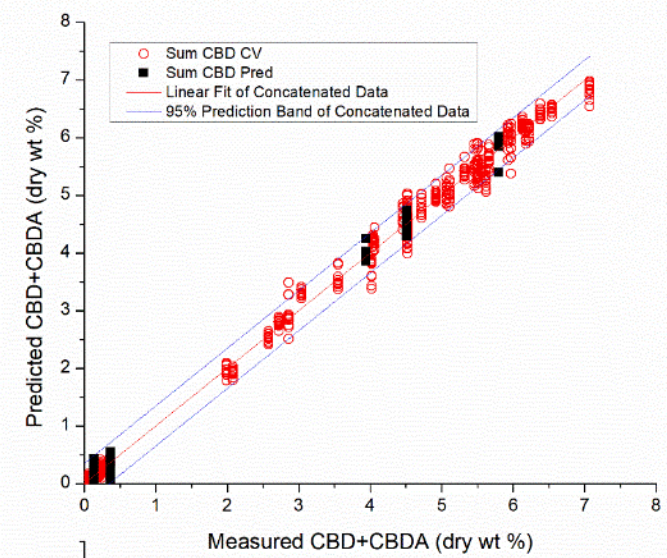
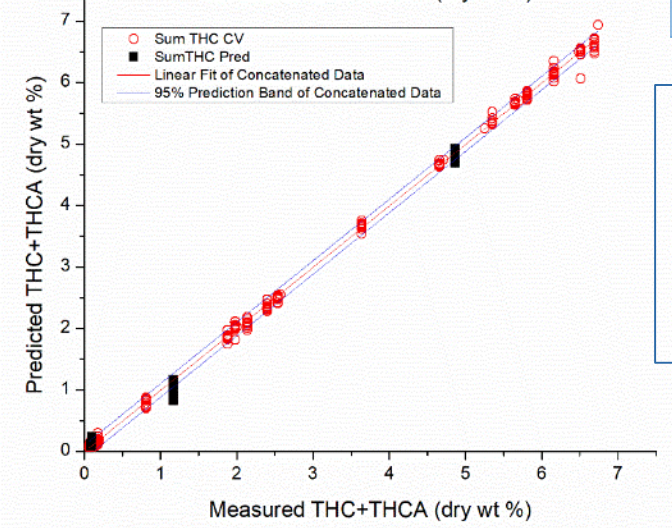
A-TEEM: HMMP Discrimination Report



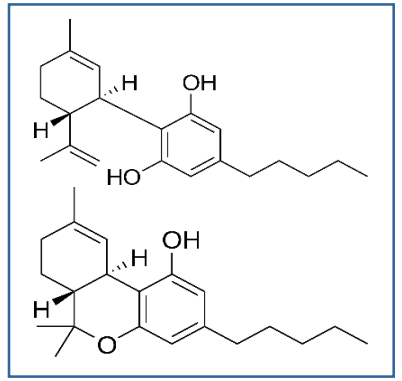
A-TEEM: XGB Major Cannabinoid Regression (HPLC)





XGB Regression Statistics (R^2)

XGB Fit	Calibration	Cross-Validation	Prediction
CBD	0.992538	0.993634	0.981454
CBDA	0.990559	0.99237	0.996454
THC	0.998983	0.989893	0.993457
THCA	0.998296	0.999509	0.995188



Concatenated CV-Prediction for HPLC

ATEEM HPLC Parameter	Intercept	SE	Slope	Adjusted R2	LOD	LOQ	CV n	Pred n
CBD+CBDA	0.001	0.007	1	0.9936	0.024	0.080	517	59
THC+THCA	-0.006	0.002	1	0.9993	0.008	0.026	516	47
HPLC: CBD+CBDA					0.015	0.047		
HPLC: THC+THCA					0.007	0.021		

A-TEEM XGB Regression can report CBD+CBDA and THC+THCA concentrations with high linearity. Importantly, as with GC, the LOD/LOQ for THC+THCA (0.008/0.026 dry wt %) for HPLC detection is well below the 0.3 % threshold for distinguishing illegal hemp varieties.

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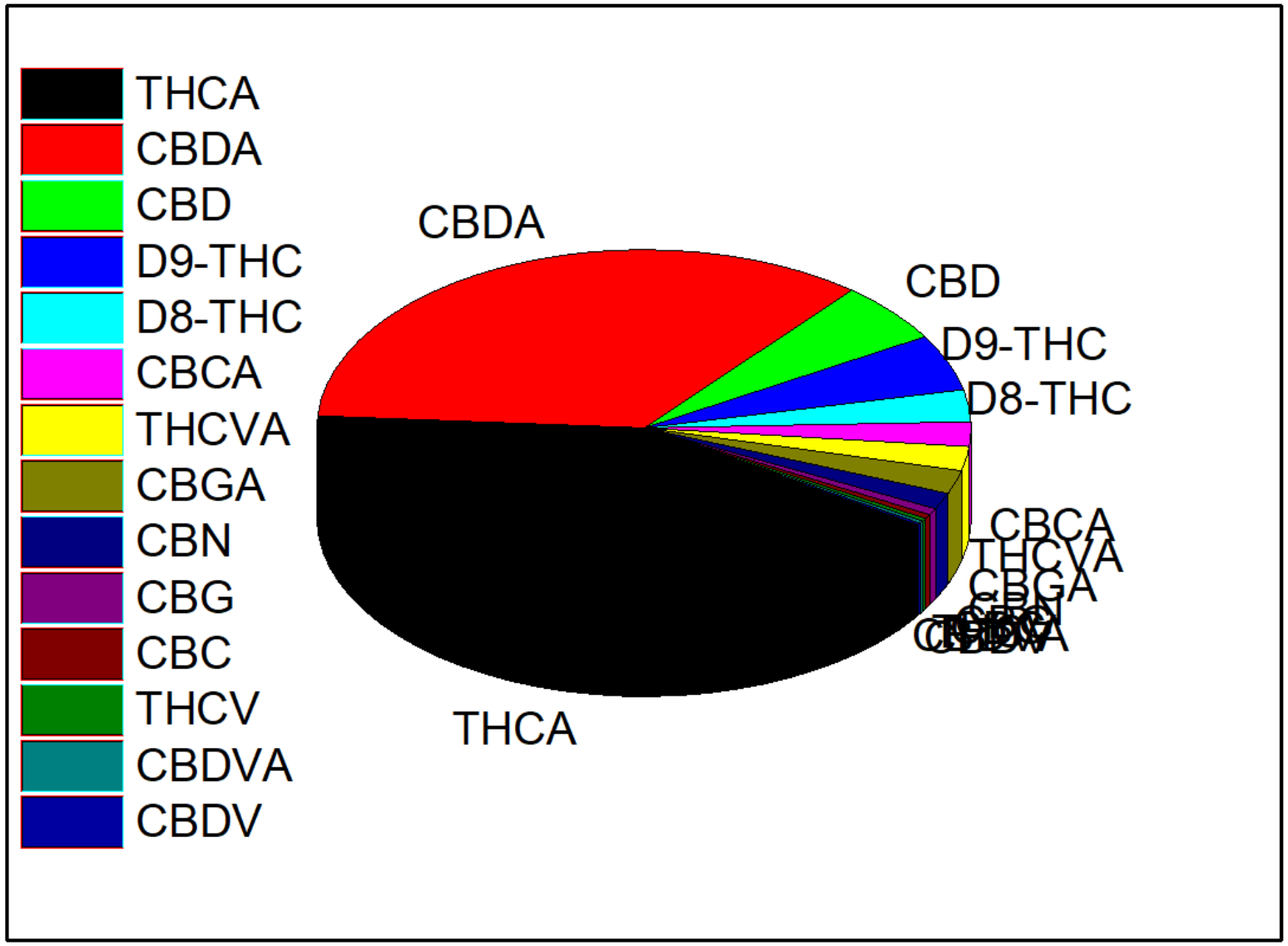
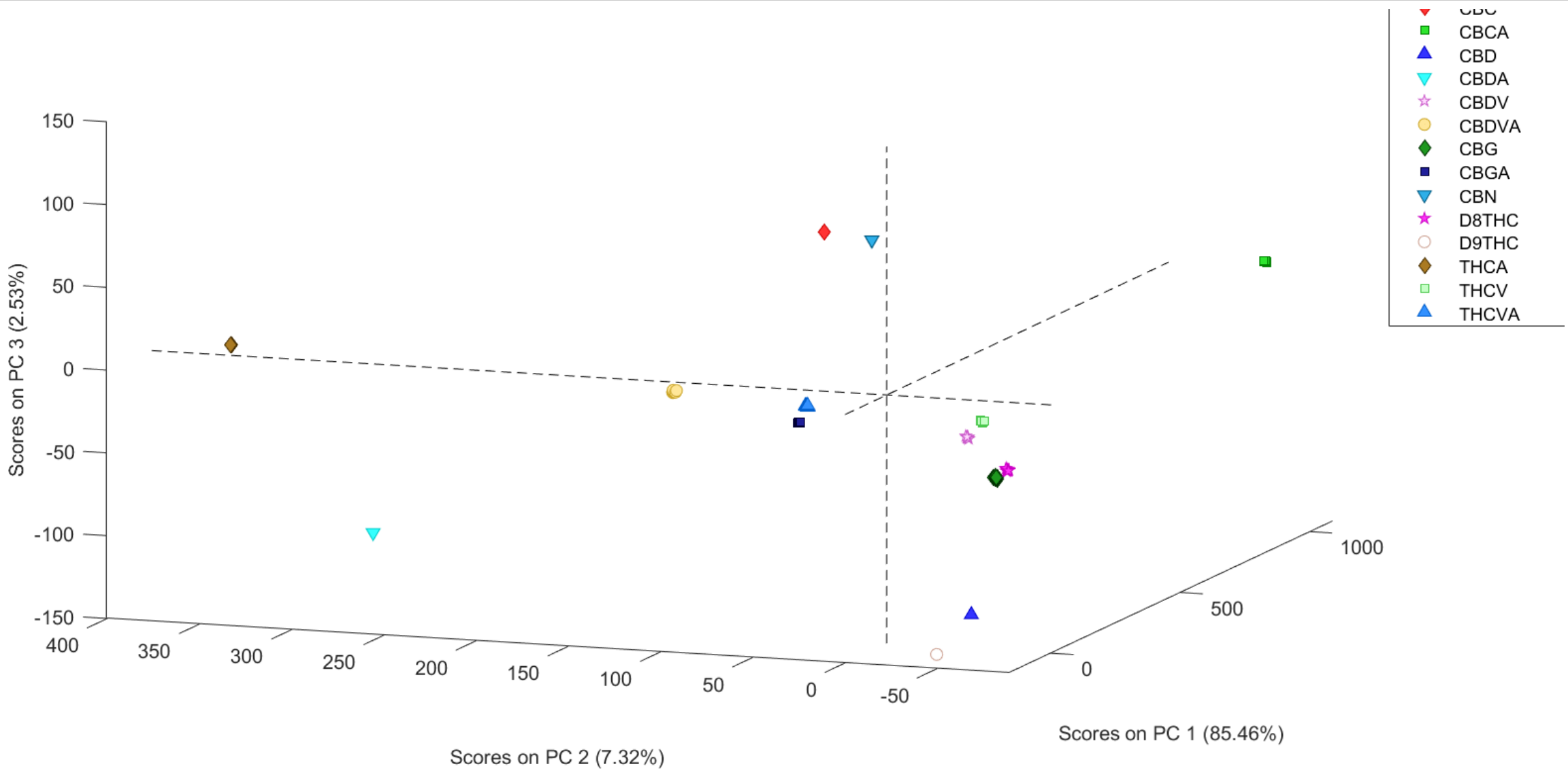
A-TEEM: Cannabinoid Distribution (wt% Max)



A-TEEM: PCA Resolution of 14 Cannabinoid Stds



A-TEEM: Full Cannabinoid Potency Profile

Full Cannabinoid Data Set:

- All Flower Extracts
- All Sourced from Ole Miss
- All HPLC Integrated Values
- Calibration: n= 509
- Validation: n= 97

Concatenated CV and P Set Values

#	Cannabinoid	Adj. R-Square	LOD	LOQ	Mean Res	SD	[Max]
1	THCA	0.9983	0.0129	0.0427	0.0000	0.0807	6.545
2	CBDA	0.9870	0.0512	0.1691	0.0000	0.2048	5.450
3	CBD	0.9892	0.0049	0.0162	0.0000	0.0213	0.860
4	D9-THC	0.9958	0.0029	0.0096	0.0000	0.0176	0.799
5	D8-THC	0.9984	0.0010	0.0032	0.0000	0.0059	0.453
6	CBCA	0.9754	0.0039	0.0130	0.0000	0.0102	0.345
7	THCVA	0.9775	0.0016	0.0052	0.0000	0.0101	0.342
8	CBGA	0.9537	0.0042	0.0139	0.0000	0.0167	0.335
9	CBN	0.9875	0.0014	0.0046	0.0000	0.0078	0.235
10	CBG	0.9746	0.0005	0.0018	0.0000	0.0026	0.094
11	CBC	0.9654	0.0005	0.0017	0.0000	0.0021	0.059
12	THCV	0.9729	0.0005	0.0015	0.0000	0.0023	0.045
13	CBDVA	0.9695	0.0004	0.0012	0.0000	0.0016	0.034
14	CBDV	0.9424	0.0002	0.0007	0.0000	0.0012	0.026
	Mean	0.9937	0.0146	0.0481	0.0000	0.0661	
	SD	0.0053	0.0210	0.0693	0.0000		
	Mean	0.9735	0.0028	0.0092	0.0000	0.0112	
	SD	0.0142	0.0015	0.0049	0.0000		
	Mean	0.9649	0.0004	0.0014	0.0000	0.0019	
	SD	0.0131	0.0001	0.0004	0.0000		

A-TEEM: CBD Emulsions for Food/Topical Apps

Why Emulsify Cannabinoids?

- Cannabinoids are insoluble in water so preparing them for consumption in water based products presents a problem.
- Emulsifying them facilitates dispersion in beverages, edibles, topical creams etc..
- Typical cannabinoid emulsification involves dissolving in oil followed by ultrasonication to render nanparticulate dispersal (*nano-emulsification*).

Types of CBD Emulsions*:

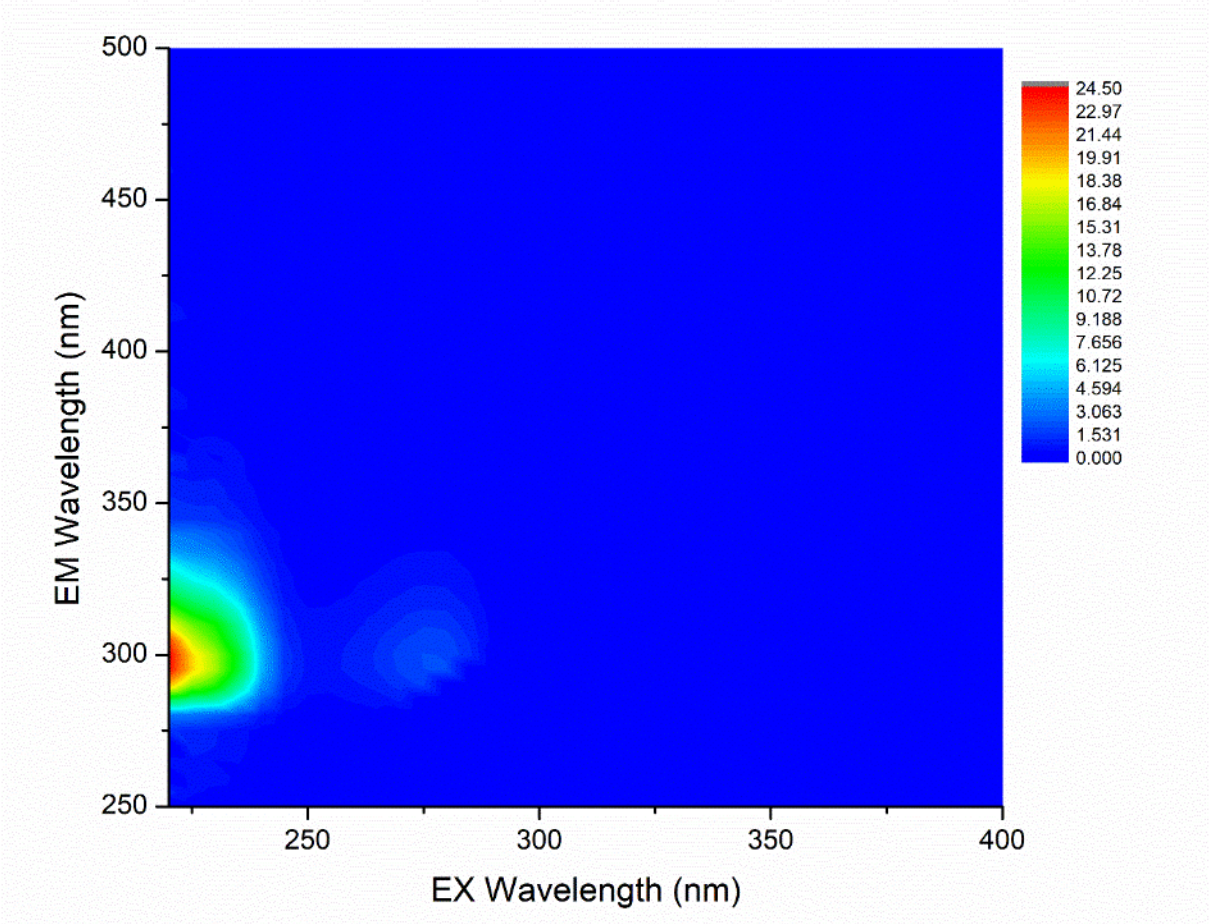
1. Full Spectrum: All plant components with minor amounts of THC
2. Broad spectrum: All Plant components but no THC
3. CBD Isolate: Purified CBD

*Typically evaluated using LC or GC

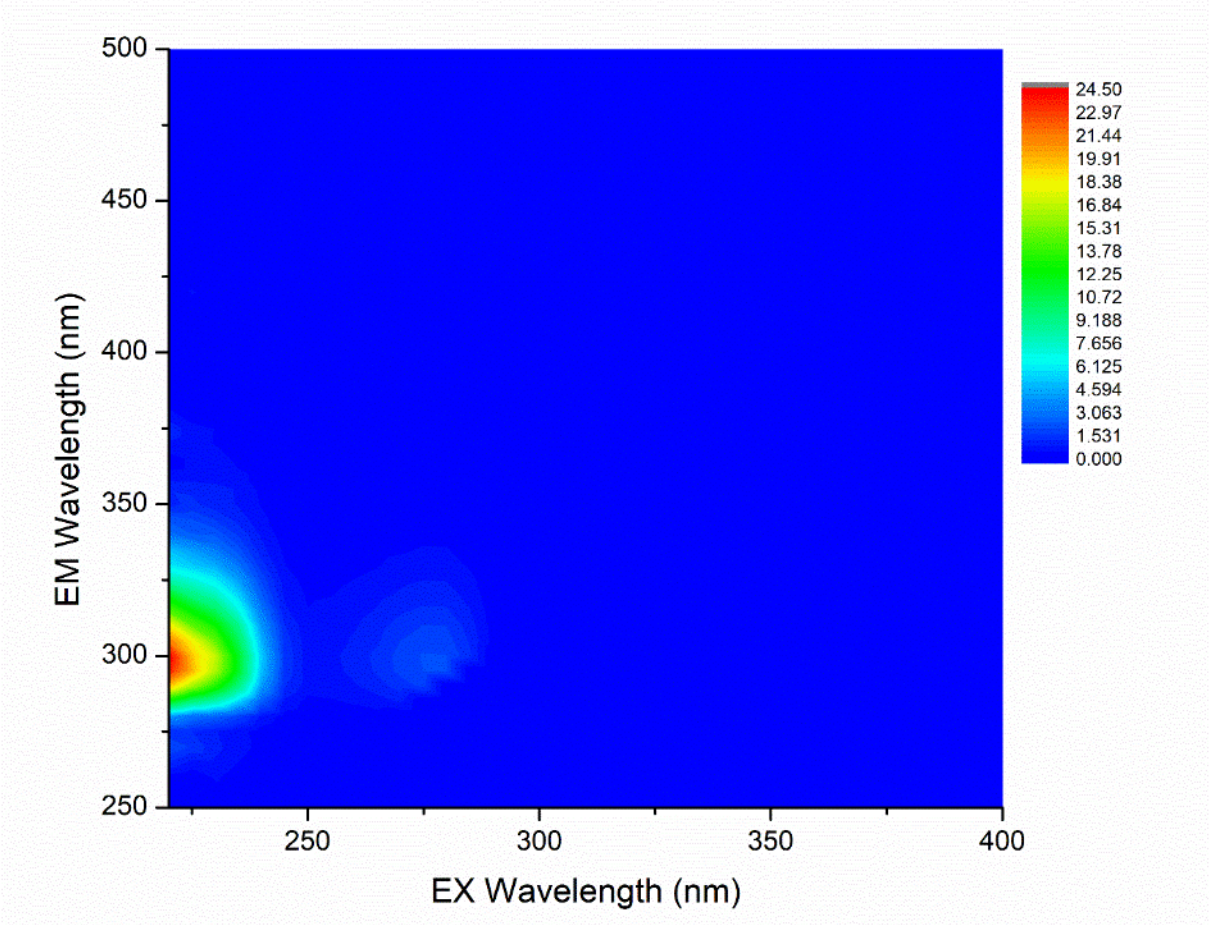
CBD vs CBDA

1. Neutral forms more stable.
2. CBDA and CBD interact with the body differently.
3. CBDA not believed to bind to cannabinoid receptors.

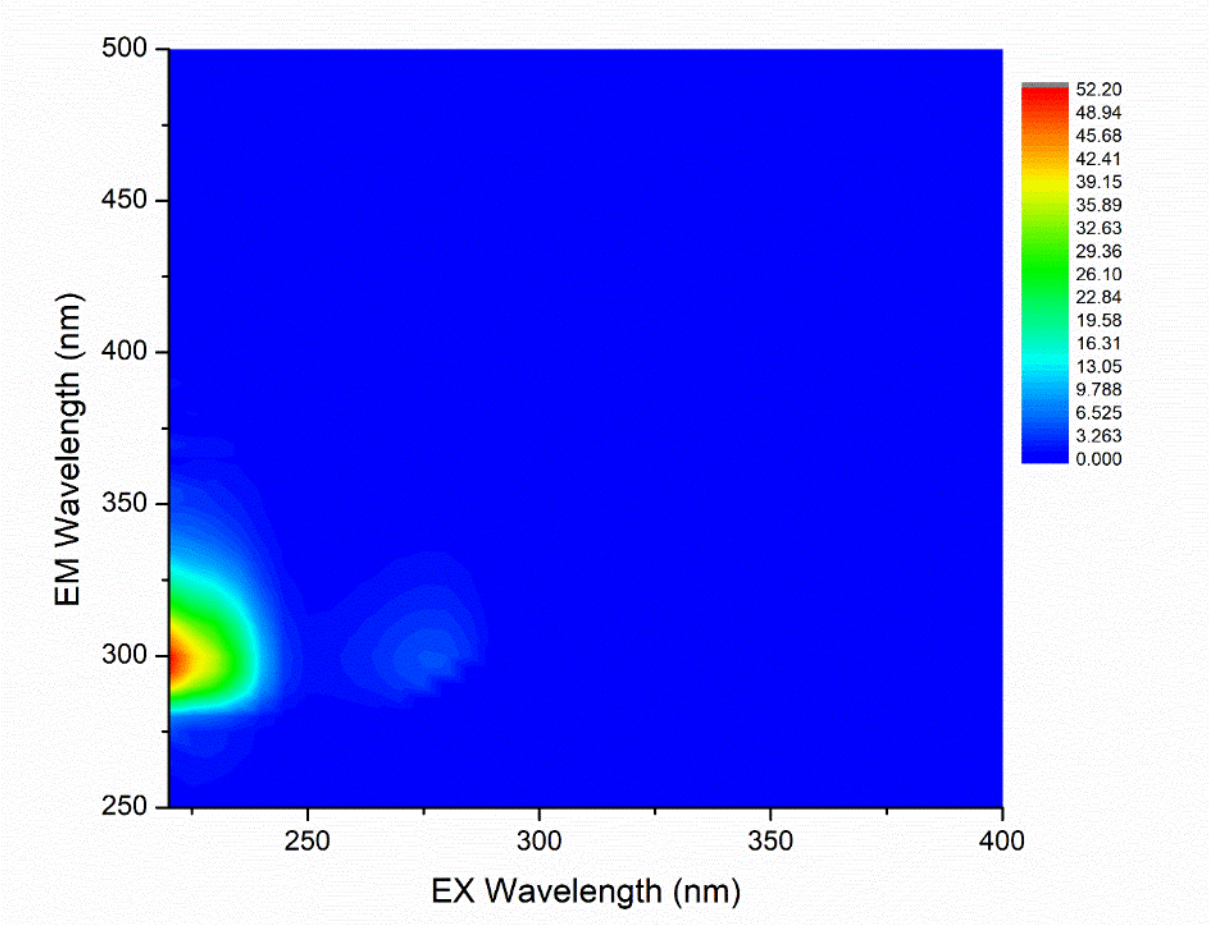
ISA 3% Emulsion: Hsquared Ind. LLC



BSA 3% Emulsion: Hsquared Ind. LLC



Topical 7.5% Emulsion: Hsquared Ind. LLC

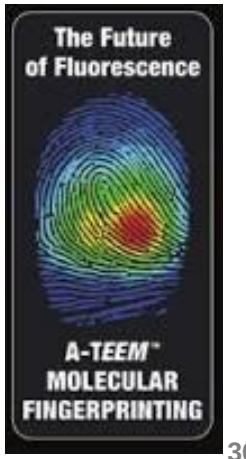


Emulsion CBD Content: Hsquared Ind. LLC

	A	B	C	D
1	Sample	mean (n=4), CBD µg/mL	sd	HPLC Values (% wt)
2	10% (No Certificate)	13.99	0.12	NA
3	ISA 3% (PC: LA-035-ISA-X-UNF)	3.10	0.02	3.08E+00
4	BSA 3% (PC:LA-035-BSA-X-UNF)	3.32	0.07	2.84E+00
5	Topical 7.5% (PC: LA-070-ISA-X-UNF)	7.55	0.10	8.10E+00

The A-TEEM can:

- Discriminate Cannabis varieties based on cannabinoid content *and* quantify total THC at <0.1% dry wt.
- Provide comprehensive potency profile with 14 Cannabinoids
- Measure cannabinoid (CBD) purity and content in Emulsified samples.
- Other potential Apps:
  - Monitor purifications (distillation)
  - Monitor decarboxylation and isomerizaiton processes
  - Monitor stability
- **Most importantly** facilitate autosampling and operator-level analysis and reporting for regression and discrimination.



Emulsion basics

A fine dispersion of minute droplets of one liquid in another in which it is not soluble or miscible.

- Two components that normally do not mix + emulsifier
- Two types: Oil in water, and inverse water in oil
- Sodas, beverages, medications, condiments, cosmetics, creams
- Salad dressing (temporary emulsion) oil + vinegar
- Mixing requires components to be shaken together, quickly separates again
- CBD emulsions requires something more consistent

Image Source: https://bbsr2chemistry.wordpress.com/2013/01/24/adam-smith-emulsifiers-in-food/

Emulsion basics

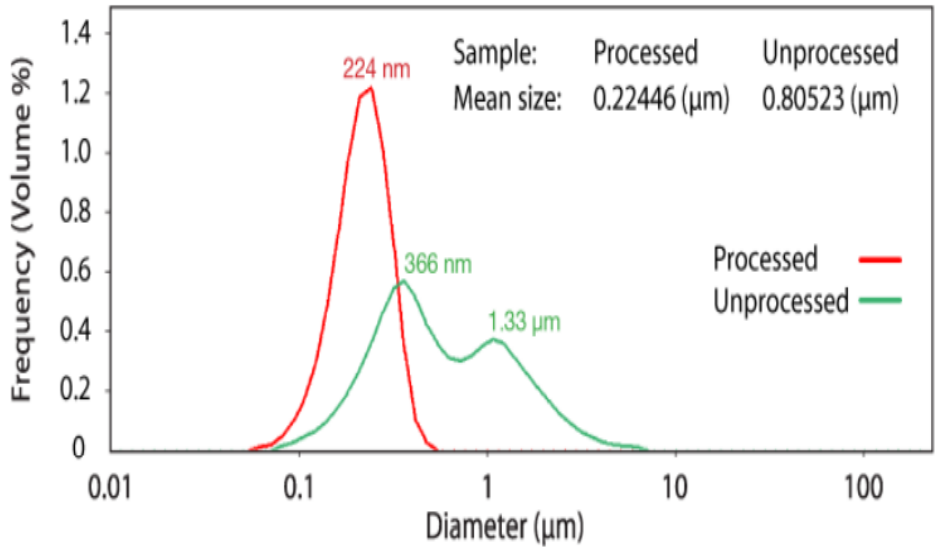
Beverages and creams (permanent emulsion)
– oil + water + surfactant/emulsifier

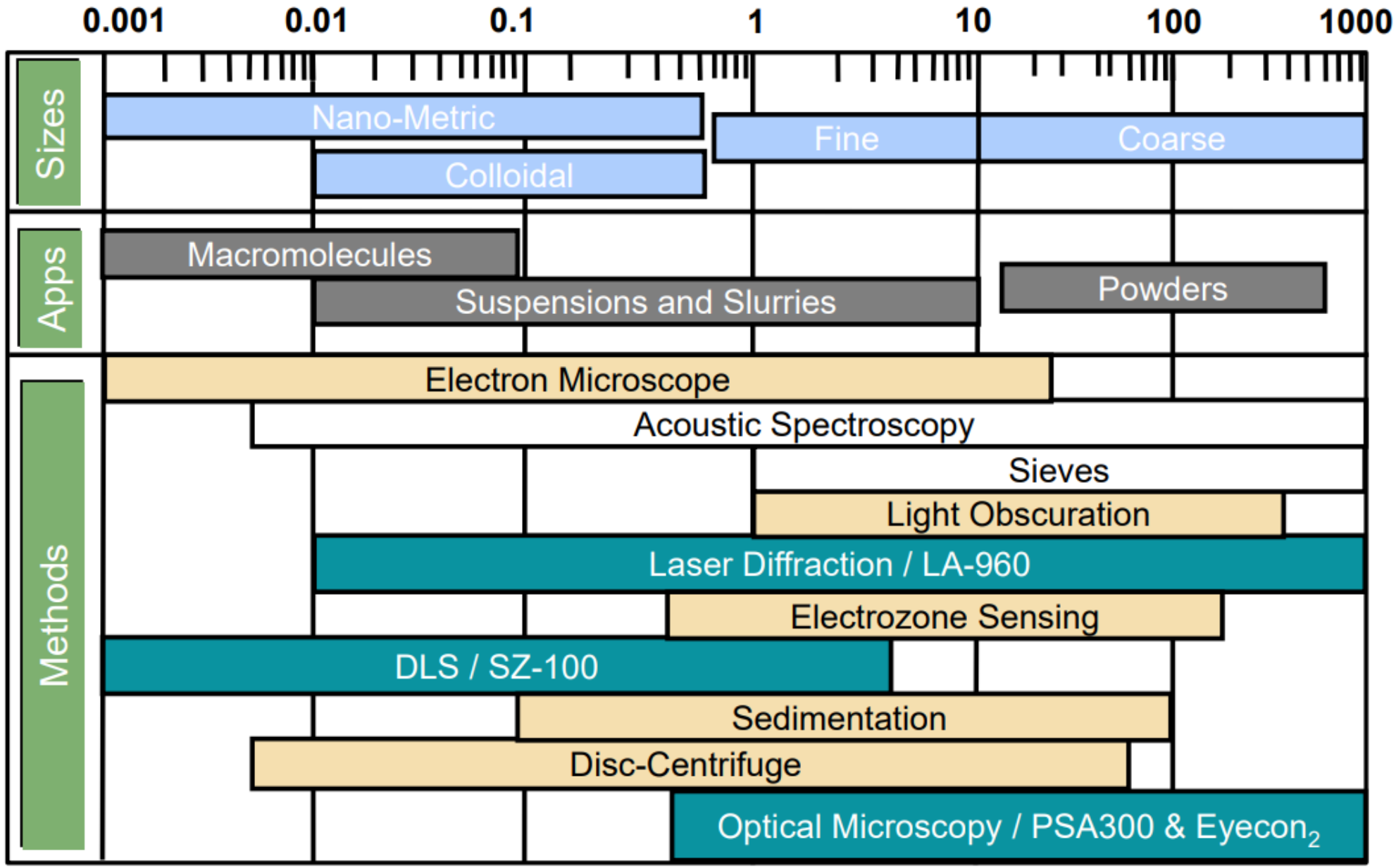
- Natural emulsifiers found in gum from trees: Gum Arabic
- Emulsions destabilize over time, as particles collide with each other and grow
- Smaller the particles are in an emulsion the more time it will take for emulsions to break
- Smaller particles and lack of large particles = long-term stability

Image Source: https://bbsr2chemistry.wordpress.com/2013/01/24/adam-smith-emulsifiers-in-food/

CBD Emulsions

- **Interested in CBD oil being dispersed in water and its stability over time (Oil in Water)**
  - Quantity of oil in mixture also has an effect (Aqualog)
  - **Strongly influenced by particle size (DLS & LA960)**
  - **Particle size is not a simple measurement, displayed as a distribution**
  - **Charge on particles has a smaller effect on stability than particle size, still significant (Zeta Potential)**
  - **Best emulsion is typically measured in nano meters and is monodisperse (single peak)**

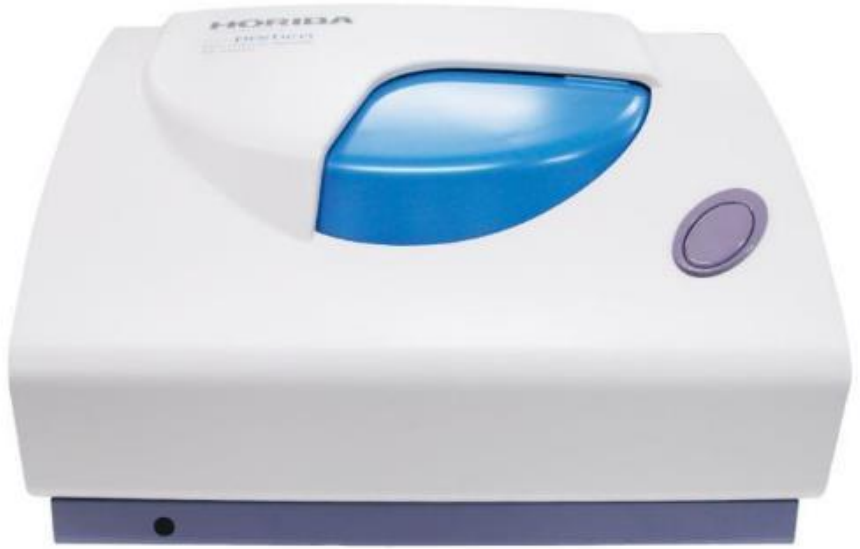




What is Dynamic Light Scattering (DLS) ?

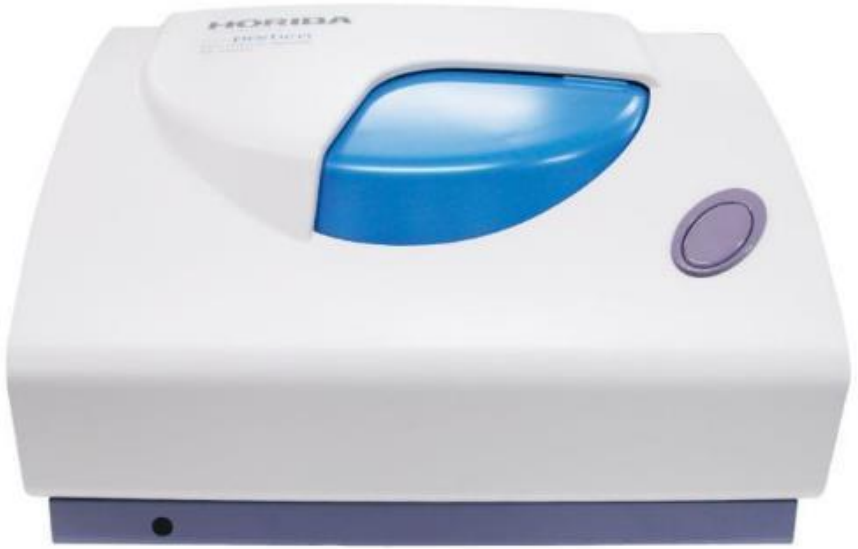
- **Dynamic light scattering refers to the measurement and interpretation of light scattering data on a microsecond time scale**

- **DLS can be used to measure**
  - Particle/molecular size
  - Size distribution
  - Reported as hydrodynamic size
  - Zeta potential

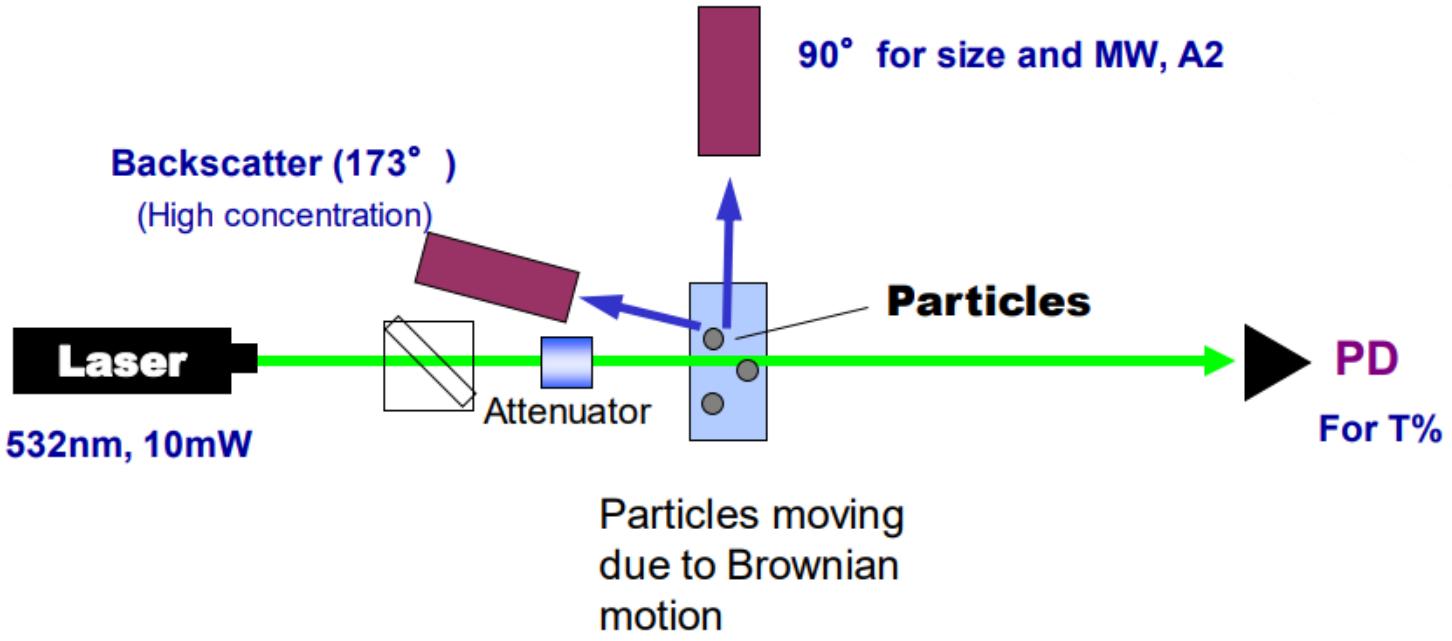


DLS for Emulsion Analysis

- **Results given as an average size**
- **Can use a very small amount of sample ~ 10uL**
- **Upper limit of DLS is 8 microns!!**
  - Can be a problem, as larger aggregations can easily exceed this value and be missed
  - Especially problematic in studying stability over time
  - If you expect particles larger than 1 micron, at any point in your stability study, use LA960 (Laser Diffraction) instead



SZ-100 Size Measurement



Volume: (mL)	**1 – 5 mL**
Range: (µm)	**0.001 – 10**

What is Laser Diffraction (LA-960)?

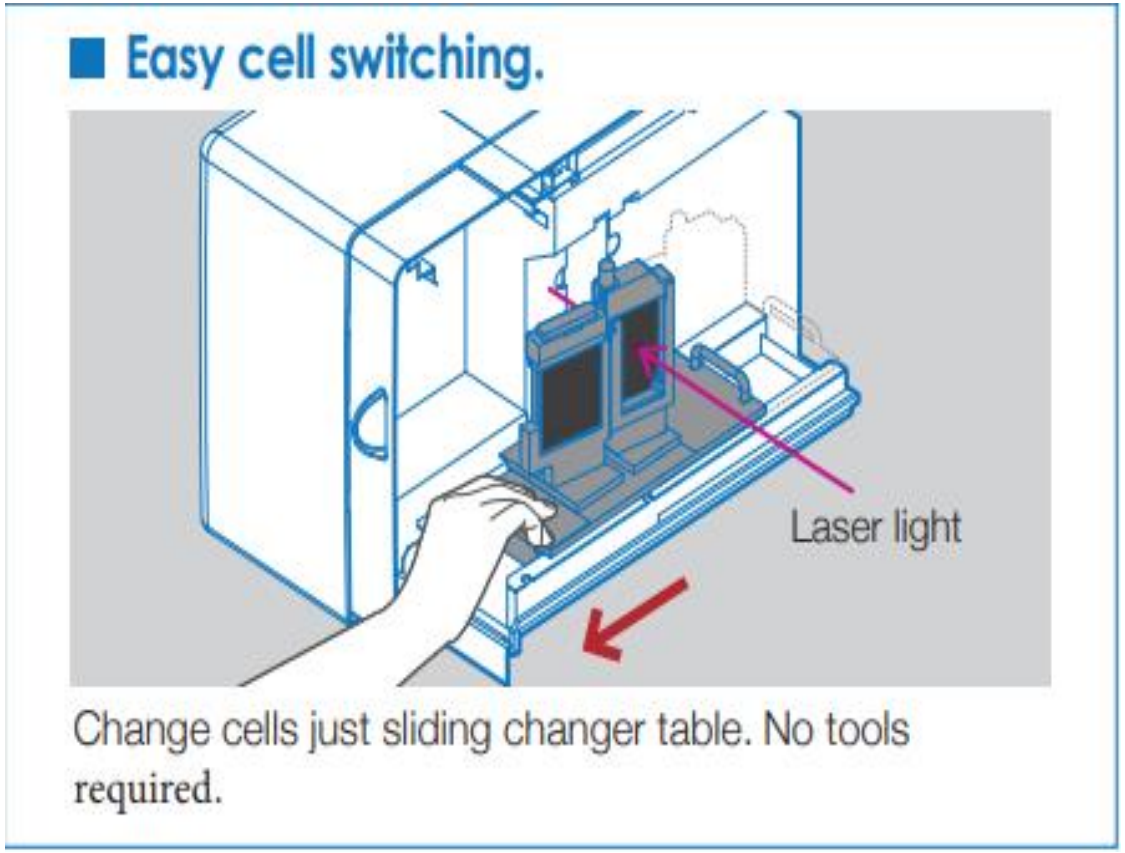
- **Utilizes diffraction patterns of a laser passed through any object ranging from nanometers to millimeters in size, to quickly measure a particle**
- **Incredibly fast and precise**
- **Very easy to use, large install base, many instructional videos**
- **LA-960 can measure**

  Solids

  Liquids

  Emulsion/Pastes
- **Displays data across a wide range enabling user to see presence of large particles**

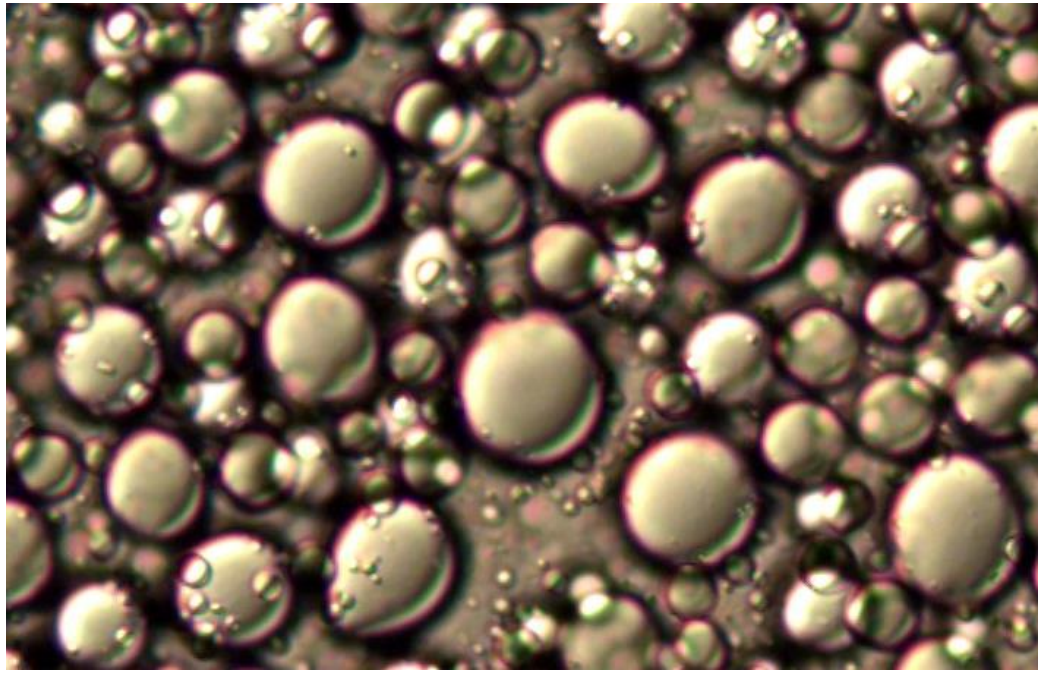
LA-960 Emulsion Measurement used Fraction Cell

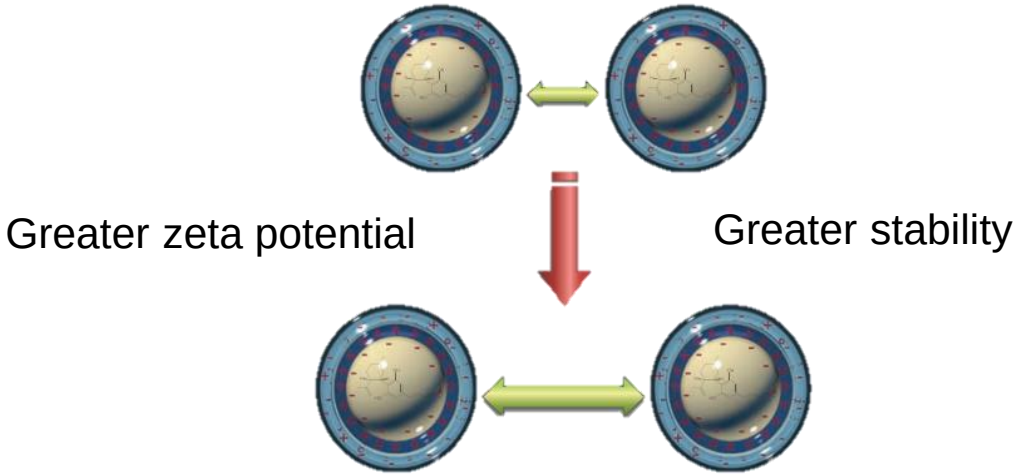


Volume: (mL)	5 – 15
Range: (µm)	0.01 – 3000

Effect of Particle Size and Zeta Potential on Emulsions

- Particle size correlates directly to stability of emulsions
- Small particles as a monodispersed emulsion gives great stability
- Presence of large particles = less stability
- Collision of particles allows them to grow, break emulsions
- Higher Zeta potential = more stability
  - Regardless of +/-
  - Magnitude of the charge is key factor, larger charge increased repulsion between particles

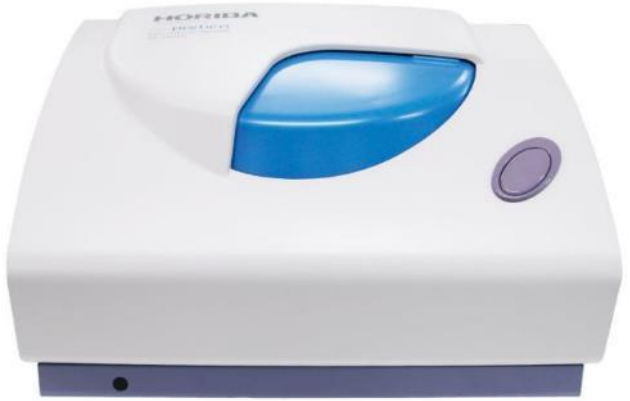




Available Techniques for Sizing

Laser Diffraction

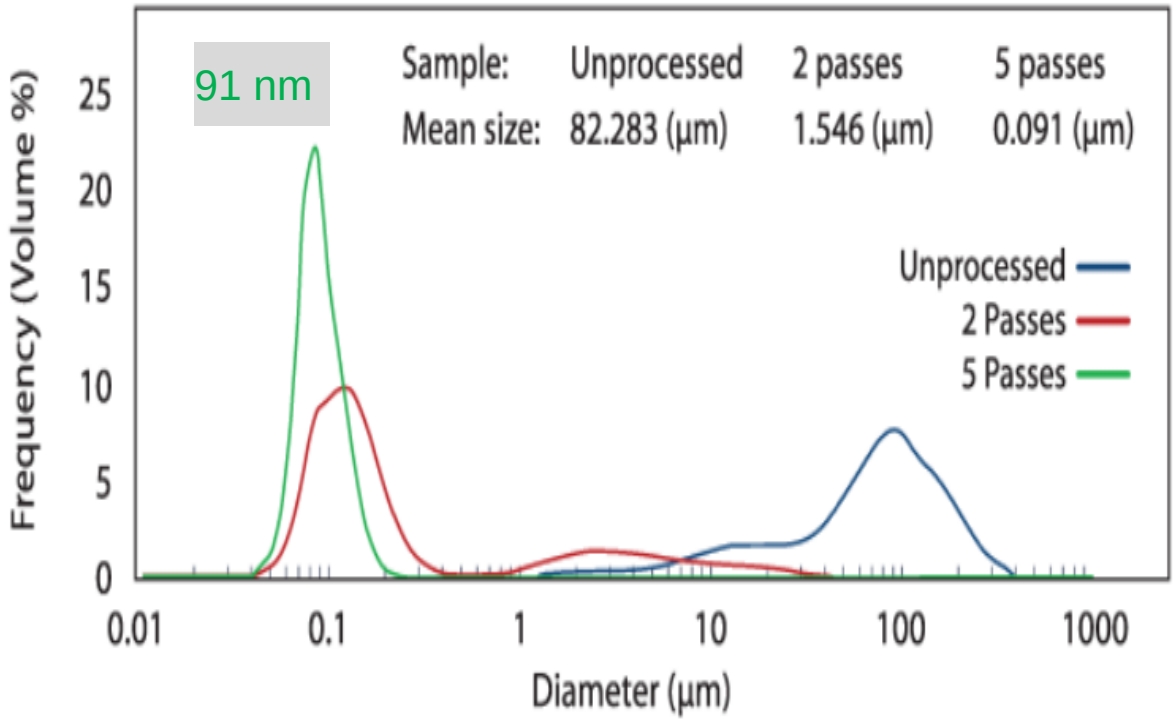
Dynamic Light Scattering

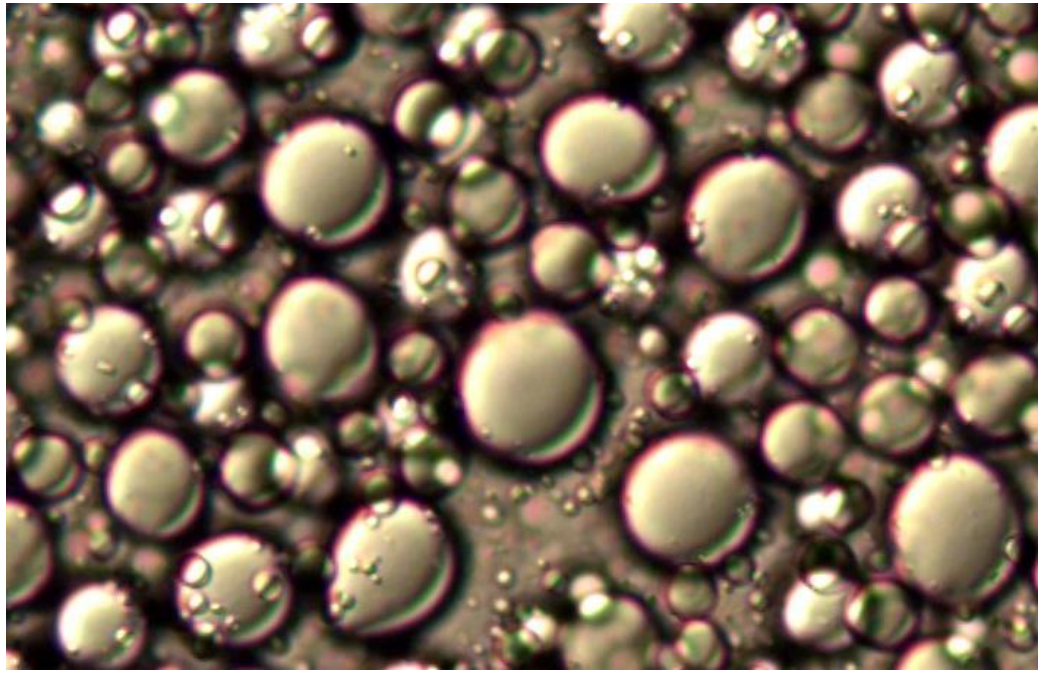


	LA-960V2	SZ-100V2
Measurement Principle	Mie scattering & Fraunhofer diffraction	Dynamic Light Scattering
Measurement Range	10 nm – 5000 µm	0.3 nm – 10 µm

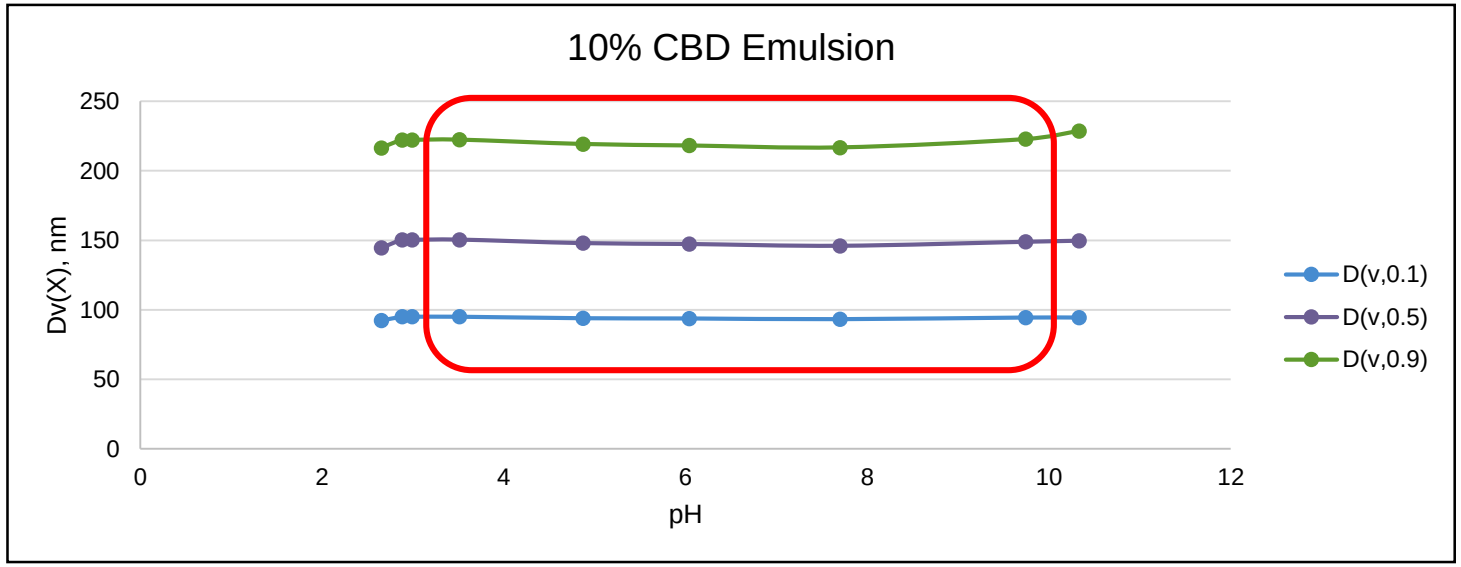
Techniques used to enhance emulsion stability

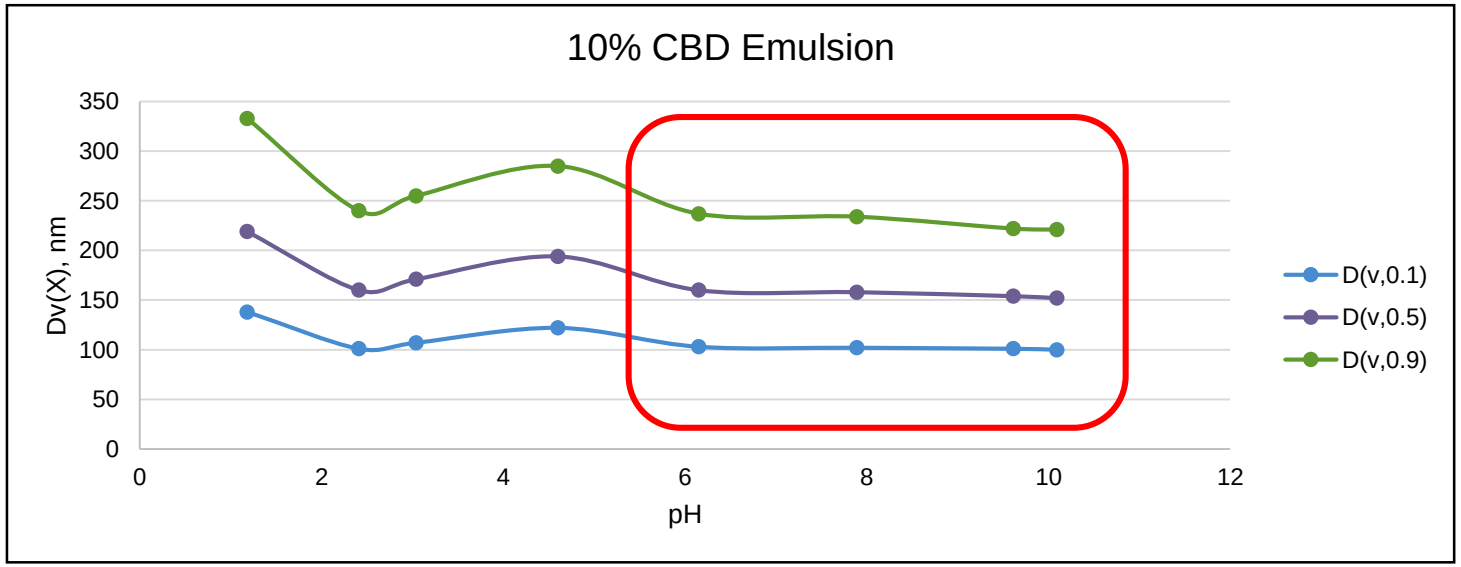
Microfluidizer High-Shear Homogenization



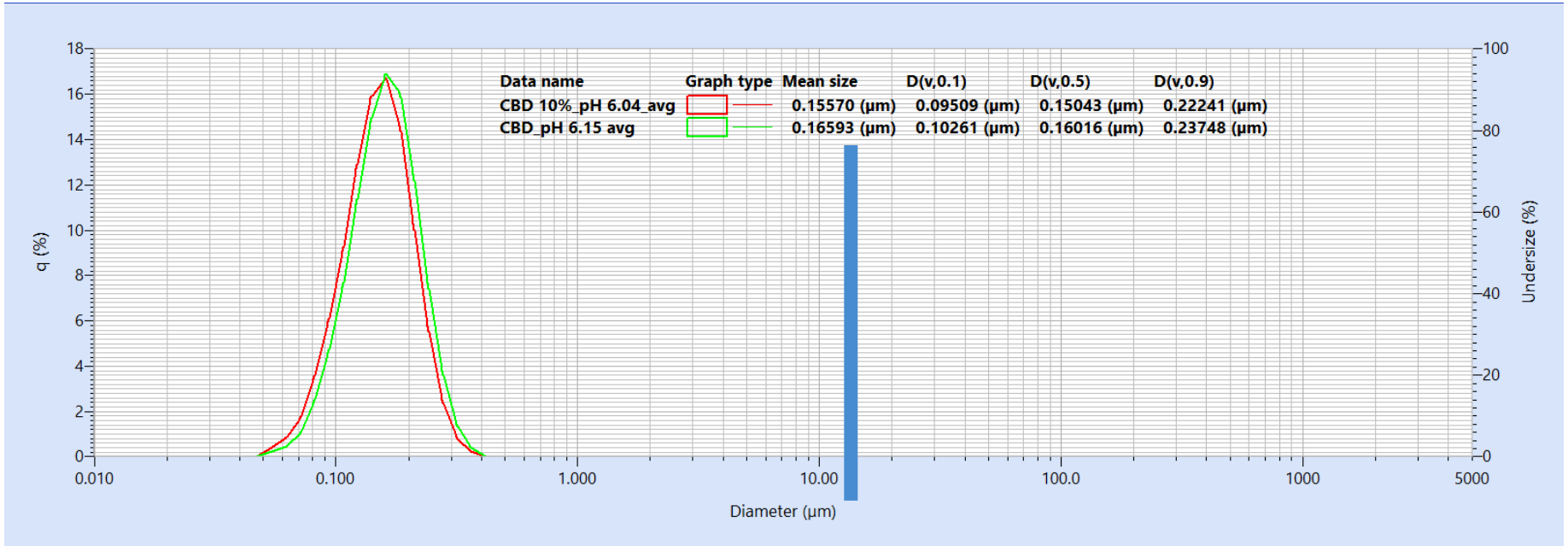


Effect of pH on CBD Droplet Size (H Squared)

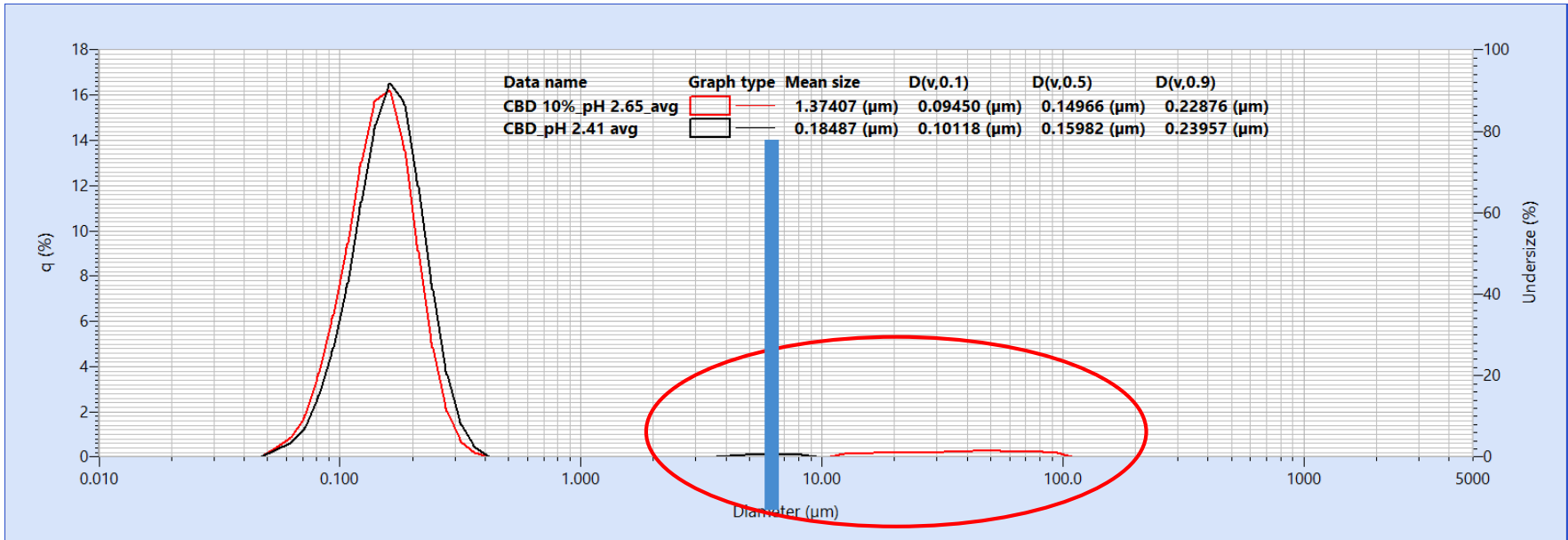




Stable vs. Unstable (H Squared)



~Size cutoff between SZ-100 and LA-960



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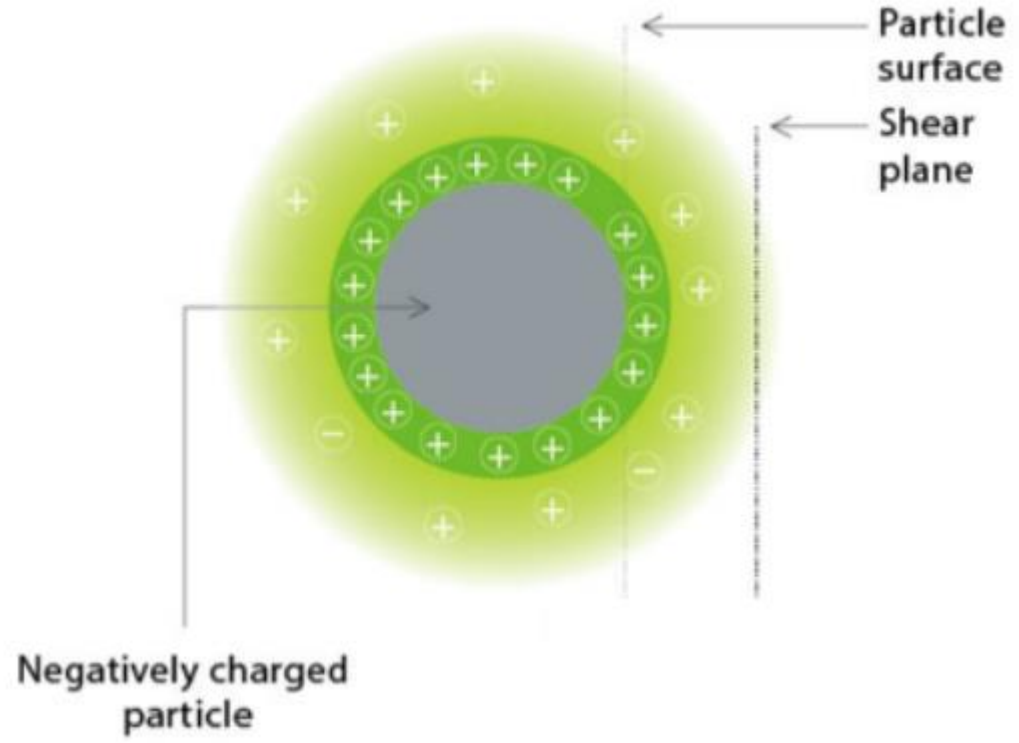
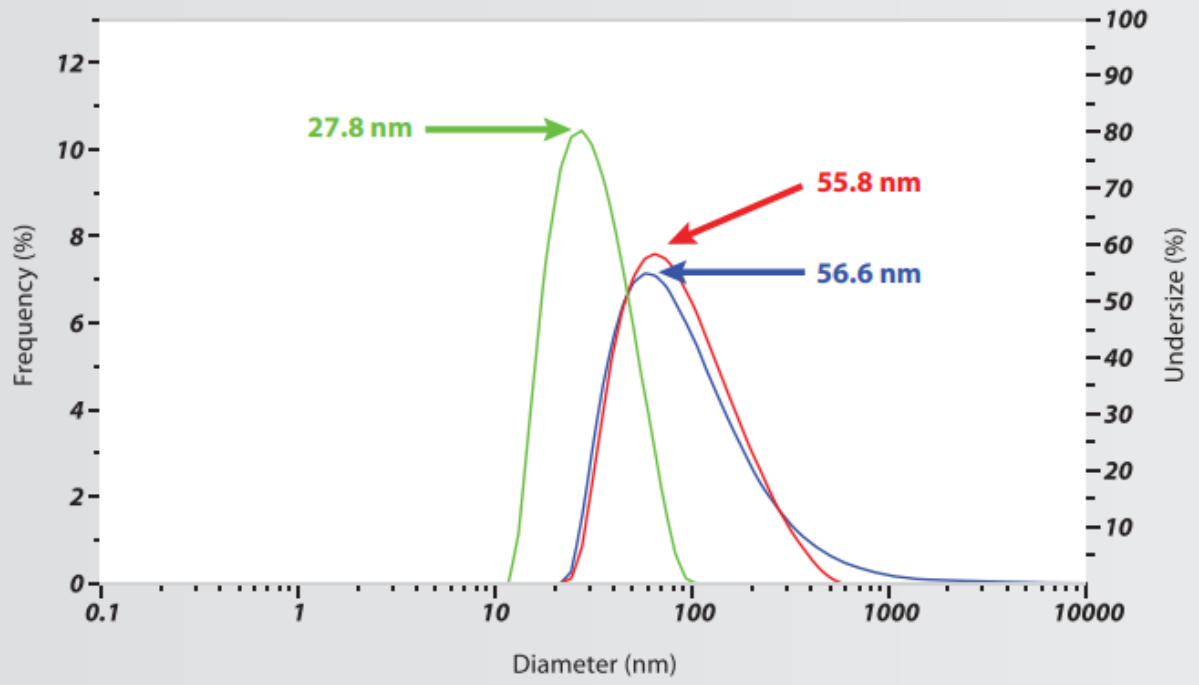
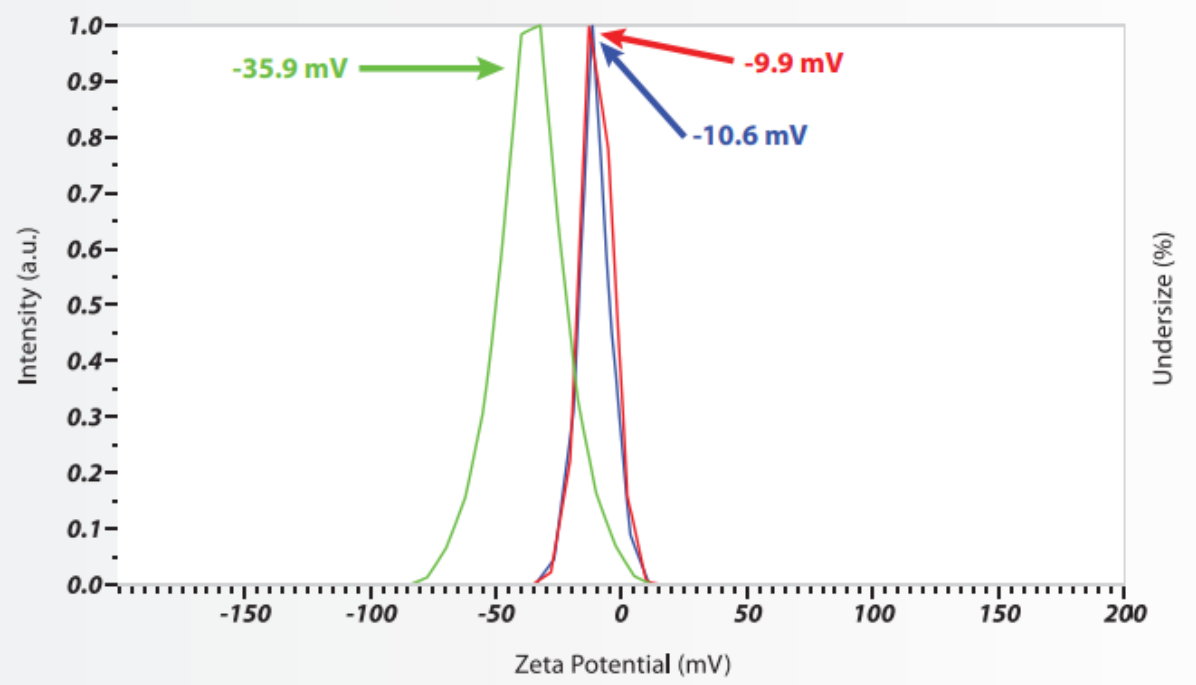
Zeta Potential

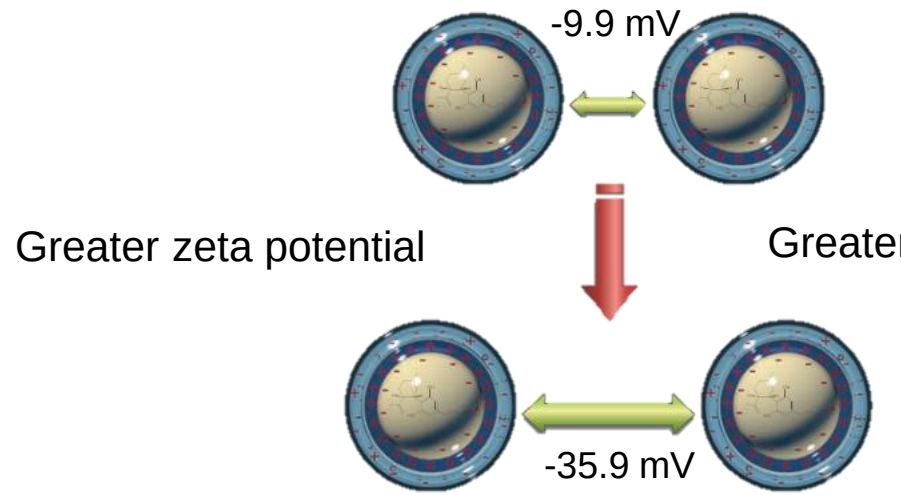
- Zeta potential is measured via SZ-100, same cuvette
- Positive or negative charge is irrelevant
- Magnitude of the charge is relevant to emulsion stability
- Particles grow when they collide
- Larger zeta charge makes them more likely to repel than collide and form a larger particle



Size by DLS and Zeta Potential







Concluding Comments

Careful monitoring of emulsions is crucial to the burgeoning CBD market

Quantification of Cannabinoid components and particle size analysis are crucial to quality control of emulsions

HORIBA offers a suite of products to help with these analyses

Aqualog with A-Teem fluorescence offers the ability to quantify oil components, monitoring both CBD content AND THC components at the same time in a single cuvette measurement.

SZ-100 and LA-960 are excellent tools for characterizing particle size

- SZ-100 measures from low nanometer range – ~5 microns, small sample size 10uL, AND Zeta potential
- LA-960 allows versatile sample types (solid, liquid, paste) along a wide range from nanometer to millimeter range

Taken together HORIBA tools present an excellent suite of products to monitor Quality Control of emulsions and quantify critical components of interest in natural products market

Collaborators, Data and Sample Sources

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Thank you