Example Protocol for (1,000ml)
Nanosized Oil in Water Emulsion: CBD

(Using customer developed surfactant mix)

Model #Q700CA Sonicator

Materials and Equipment:

- CBD isolate
- Purified water
- Mixed surfactant: average HLB of ~11-14 (see Workflow document for details)
- Beakers of various volumes: 100-2000ml
- Calibrated balance
- Weigh boats
- Spatulas
- Stir bar
- Heated stir plate
- Stir bar retriever
- Overhead homogenizer
- Calibrated thermometer / thermometer gun
- #Q700CA Sonicator system
- Compressed air with inlet/outlet
- Ice bath: composition can vary based on user preference
- .45-micron filter
- .22-micron filter
- Pipettes
- Flask weights (optional)
- Parafilm or equivalent (optional)
- Laser pointer (optional)
- Small glass bottles (optional)
- Paper with black print text (optional)

Formula:

Purified Water	**92.5%**
Surfactant	**5.0%**
CBD Isolate	**2.5%**

This protocol creates a 25mg/ml concentration.

*Common compounding practice allows ≤5% error

General Preparatory Work:

- Gather appropriate materials and calibrate any balances to be used
- Place 925g of purified water into a 2,000ml beaker
- Place 50g surfactant into a weigh boat or beaker (pre-melt if solid at room temperature)
- Place 25g CBD isolate into a weigh boat or beaker
- Begin heating hot plate to ≥60℃
- Place stir bar into the beaker containing 925g purified water
- Prepare ice bath and store in cool place until needed

Note: Ice bath should be composed of ≥70% water. It is optional to use alcohols and sodium chloride in conjunction with ice water for a greater cooling effect. Operator should account for volume displacement of ice as well as potential vapor pressures from alcohol evaporation.

925g Purified Water

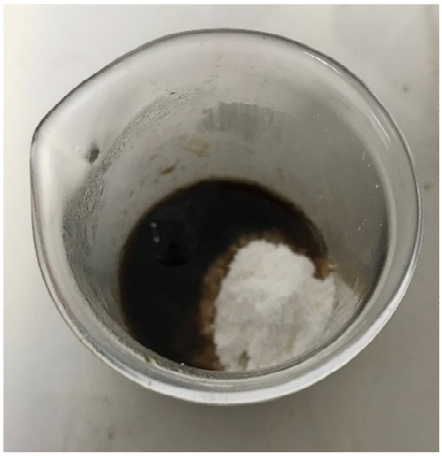
25g CBD Isolate

50g Surfactant Blend

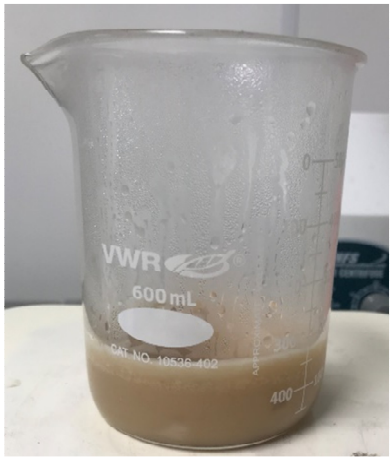
Coarse Emulsion:

- Place purified water onto heated stir plate and activate the magnet
- Heat water to ≥60℃
- Place pre-weighed surfactant into the heated water solution
- Place CBD isolate into the water: surfactant solution
  (Optional step - pre-mix CBD isolate with surfactant then add to heated water)
- Stir on heat for ≥ 30 minutes
- Cool to room temperature
- Remove stir bar

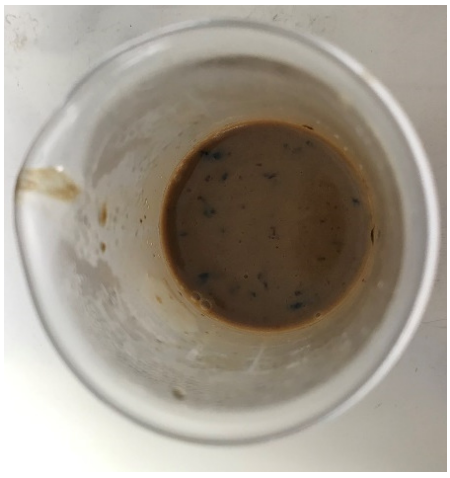
Note: do not exceed 100℃ as water will begin to vaporize



CBD added to surfactant before mixing



Completed coarse emulsion



CBD added to surfactant after mixing

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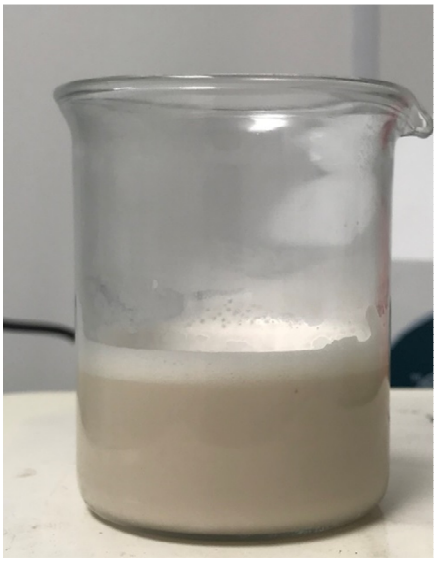
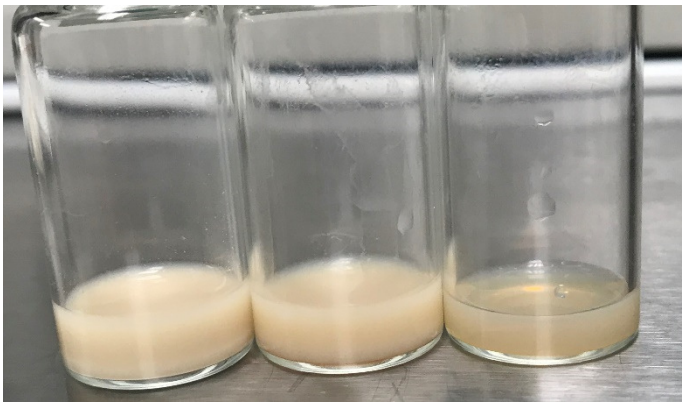
Microemulsion:

- Place homogenizer/mixer into the coarse emulsion
- Homogenize at maximum speed permitted for ≥5 minutes
- Pipette aliquot into small glass bottle for comparison (optional)

Overhead homogenization to form microemulsion



Completed microemulsion



Sample aliquots:

unfiltered (left), .45 micron (middle), and .22 micron

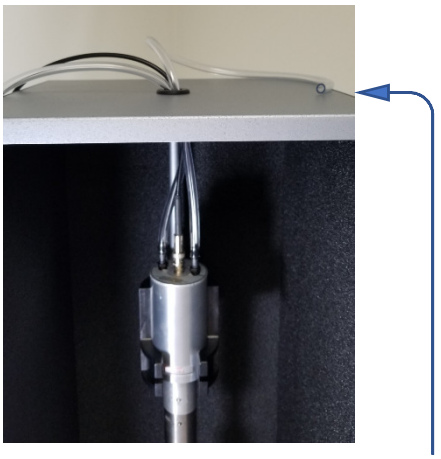
Nanoemulsion Preparatory Work:

- When using 1” probe (#4205), operator must use compressed air to cool the Converter
- See Converter cooling instructions to prevent voiding warranty
- Place ice bath vessel into Sound Enclosure
- Secure beaker in the ice bath with flask weights or clamp (red weights used in photo)
- Close Sound Enclosure door

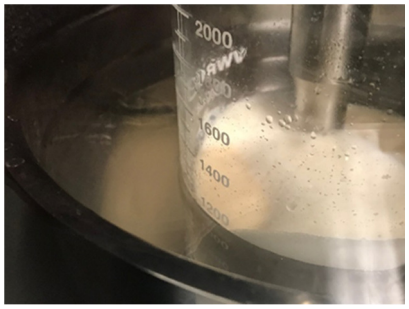
Note: For best results, ensure ice water volume corresponds to the microemulsion

- Only use a 1,500mL or 2,000mL beaker for a 1,000mL sample volume
- If the probe is too shallow or deep it will not deliver consistent results
- Place cover onto top of beaker to prevent splashing or aerosol (optional)
- Determine appropriate processing parameters

Note: If solution is overheating or foaming excessively, decrease ice bath temperature or increase pulse OFF time. Check probe depth and confirm beaker size.



Note air cooling hoses - one enters the Converter and the other exits the enclosure to exhaust air to the room



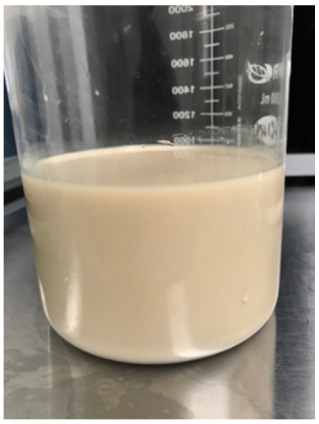
Ensure ice bath volume corresponds to sample volume

Program screen for current protocol

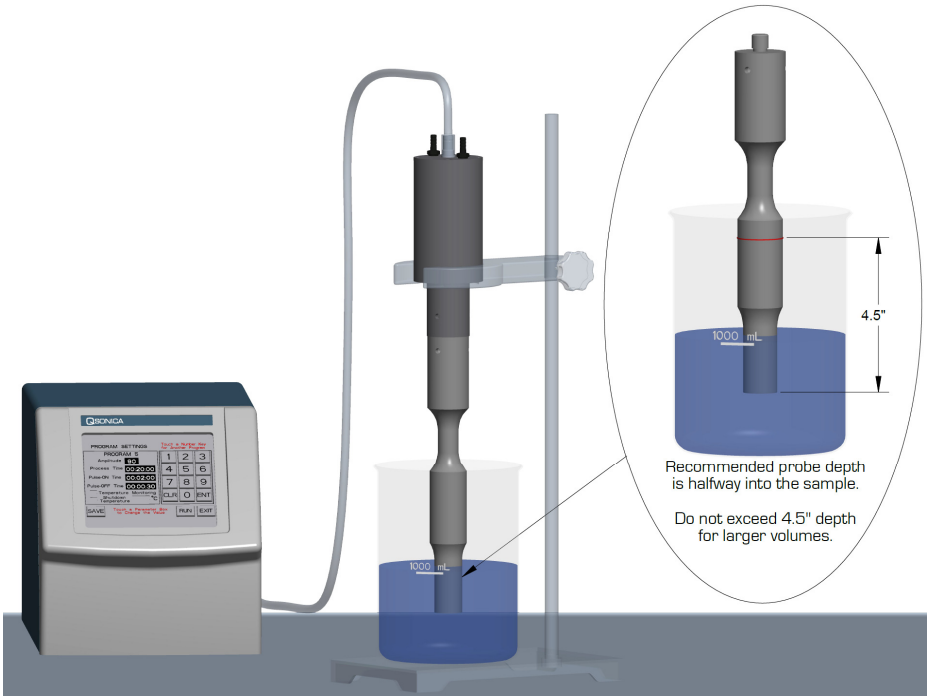
Nanoemulsion:

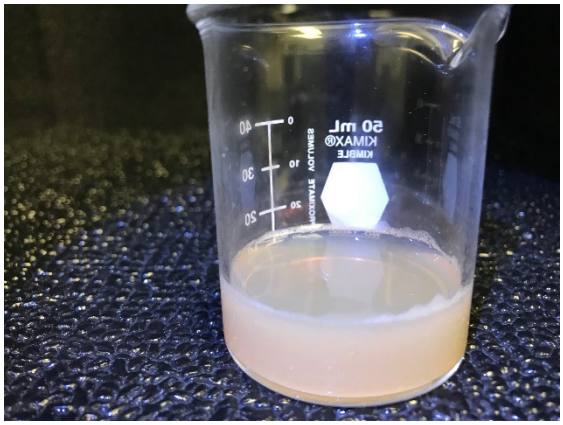
- Power on the generator using the switch on the back of the unit
- Indicate you are not using a microtip (“Are you using a microtip?” - select NO)
- Select: “To Select or Modify a Program or Sequence, Press HERE”
- Set amplitude to 90
- Set process time to 1 hour (Note: lower concentrations will require less processing time)
- Set pulse ON time to 4 minutes
- Set pulse OFF time to 1 minute
- Save program
- Select RUN
- Closely monitor solution temperature and refresh ice bath as needed
- Once run is completed, open door and allow solution to cool to room temperature

Sample secured in ice bath



Sample appearance at T=30min





Sample aliquot appearance after .22 micron filter (volume = ~10mL)

Custom Hardware Processing Parameters:

While each sample matrix is individual, and most relationships in colloidal chemistry are not linear, below is a table with general guidelines for customizing processing time in relation to oil load assuming 90% amplitude/intensity setting.

Oil Amount	Run Time
1%	~30 min
1.5%	~36 min
2%	~48 min
2.5%	~60 min
3%	~72 min

Filtering:

Most nanoemulsions must be filtered at least once in order to achieve translucency. Additionally, filtering prevents the presence of probe titanium in the solution.

- Ensure filter matrix is compatible with solution
- Filter sample through .45-micron filter until appearance does not change-it is not uncommon to filter multiple times.
- Filter sample through .22-micron filter until appearance does not change -it is not uncommon to filter multiple times.

When selecting proper filtering matrix, most vendors include a table which lists solvent compatibility.

Syringe Filters Solvent Compatibility Chart

Group of Substance & Chemical Reagents	Cellulose Acetate	Nylon	PES	PTFE	PVDF
ACIDS					
Acetic, 5%	L	R	R	R	R
Acetic, 10%	L	R	R	R	R
Acetic, 25%	N	L	R	R	R
Acetic, Glacial	N	N	R	R	R
Boric	-	L	-	R	-
Formic 25%	L	N	-	R	-
Hydrochloric 15%	L	L	R	R	L
Hydrochloric 25%	N	N	R	R	-
Hydrochloric concentrated	N	N	L	R	N
Hydrofluoric 10%	N	N	-	-	-
Hydrofluoric 35%	N	N	-	R	-
Nitric 25%	N	N	R	R	-
Nitric 6N, 38%	N	N	L	R	R
Nitric concentrated	N	N	N	R	N
Phosphoric 25%	L	N	R	R	-
Sulfuric 25%	N	N	N	R	-
Sulfuric 6N, 29%	N	N	N	R	-
Sulfuric concentrated	N	N	N	R	N
Trichloroacetic 10%	N	N	-	R	R
ALKALINES					
Ammonium Hydroxide 25%	N	R	R	R	L
Formalin 30%	L	L	R	-	-
Sodium Hydroxide 3N, 12%	N	R	R	R	R

Top 10 Reasons to Use Restek Syringe Filters

1. Protect any analytical system.
2. Extend LC column lifetime.
3. Achieve more reproducible analyses.
4. Variety of membranes, porosities, and diameters available.
5. Luer lock inlet provides strong, leak-tight syringe connection to withstand filtration pressure.
6. Rugged polypropylene construction— autoclavable to 121 °C for 15 minutes (75 psi).

Sample Filter Chart from Restek

Full Chart:
https://www.restek.com/pdfs/GNTS2122-UNV.pdf

Particle Size Estimation (Optional):

While the most credible way to determine particle size is dynamic light scattering (DLS), there are a few economic methods to check your work.

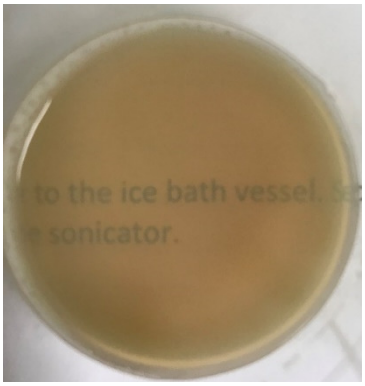
Option 1: Printed text test

- Place up to 5mLs of filtered aliquot into a small glass bottle/beaker-beakers often yield best results
- Place beaker/bottle over printed text
- If the particles are nanosized, the text should be extremely easy to read

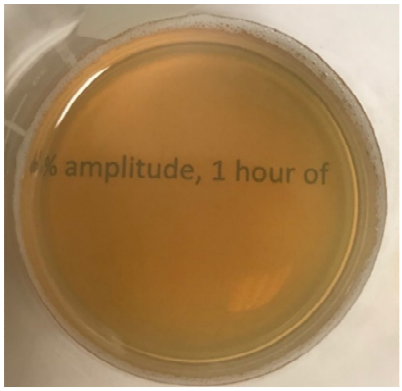
Option 2: laser-pointer method

- Place up to 5mLs of filtered aliquot into a small glass bottle/beaker
- Place beaker/bottle in front of a plain colored background such as a lab notebook cover. Use plain white or plain black for best results
- Turn off lights (optional)
- Shine laser beam through solution and check for penetration onto the background.
- This may be done from the anterior or posterior aspect of the solution
- Nanoemulsions will allow light to pass through them

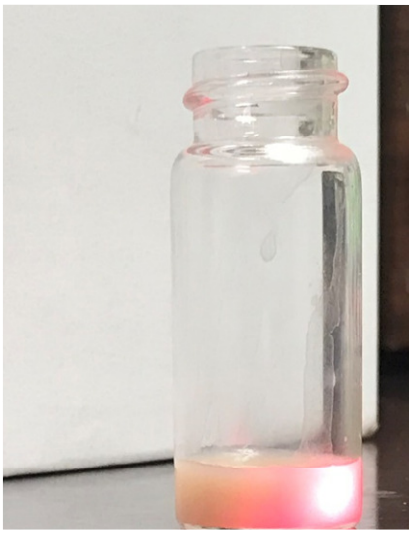
Unfiltered printed text test



.45 micron printed text test

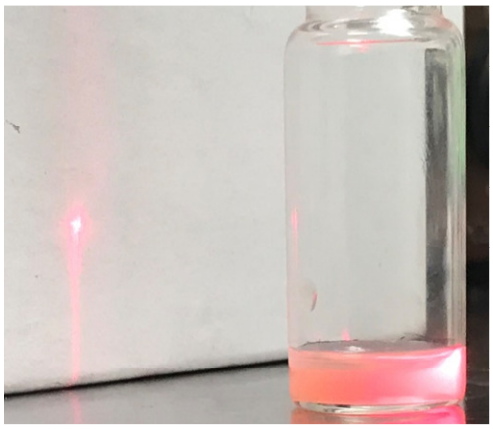


.22 micron printed text test



Unfiltered laser pointer test

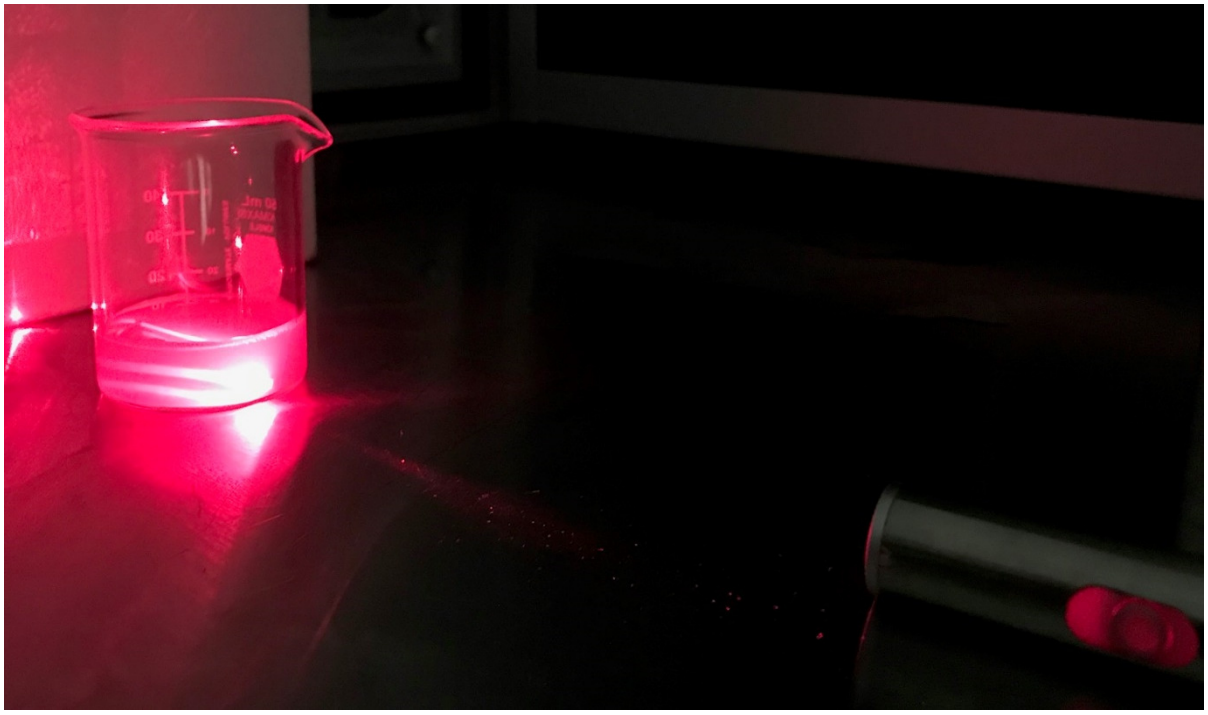
.45 micron laser pointer



.22 micron laser pointer test

Determine API recovery (Optional):

The conditions of sonicating in order to form a nanoemulsion can cause side reactions or degradation for a multitude of reasons. The most common being poor condition monitoring (such as inappropriately high temperatures). If you are nanosizing a product for commercial retail, all label claims must be verified with a potency assay via instrumentation. Common methods are liquid and gas chromatography although liquid chromatography is the most used.



.22 micron laser pointer test in the dark

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