FOOD stars — Innovative Food Product Development Cycle: Frame for Stepping Up Research Excellence of FINS

Application of NADES to Natural Products Research

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Young Hae Choi

Natural Products Laboratory, Institute of Biology

Leiden University

Before start…

- Not chemical engineer but pharmacist or biologist Working for metabolomics
- 'I' means 'We' in this presentation
- Collaboration between Leiden University and TU-Delft for 4 years
- Not for results but for your 'HELP'

If water is unique media in organisms,

There are many questions…

1. SOLUBILITY OF METABOLITES
2. PLANT DESSICATION TOLERANCE
3. GERMINATION OF SEEDS
4. HIGH-ENERGY ELECTORON BIOPROCESSING

1. Water solubility of metabolites?

1. Over 30,000 Metabolites in single organisms
2. Wide range polarity
3. Secondary metabolites non-water soluble
4. Biosynthesis, storage, transportation

2. Life in sever condition?

1. Desert without water?
2. (Anta)-archtic with frozen water?
3. Bioprocessing stopped?
4. But they still survive, How?

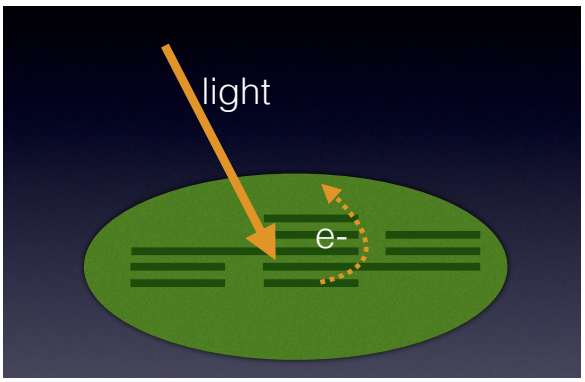
in dry season

with rains

3. Germination of seeds, saps?

1. Seeds keep life for long time
2. How do they keep life?
3. Plants excrete sap for interaction
4. If water, they will easily dry

4. Water loss challenge?



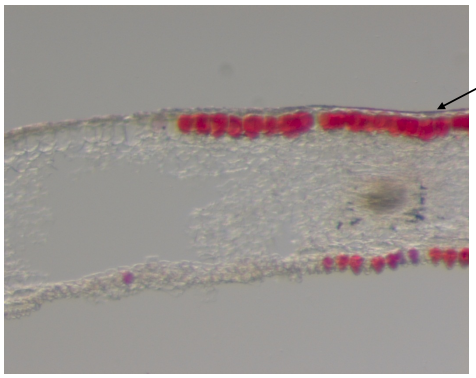
- **Normal biochemistry no longer processes high-energy electrons in photosynthesis or respiration.**
- **ROS accumulate**

Some solutions in the past

Mechanical	Elastic Walls "Accordion" Microanatomy Water Replacement / Vacuolation
Structural	LEA Proteins HSPs Compatible Solutes, Vitrification, Water Replacement?
Oxidative / Chemical	Antioxidants Catabolise chlorophyll (poikilychlorophylly) Or hide it (homoiochlorophylly)

- **But they are not fundamental but very fractional!**

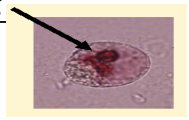
Other media in plants, together with water

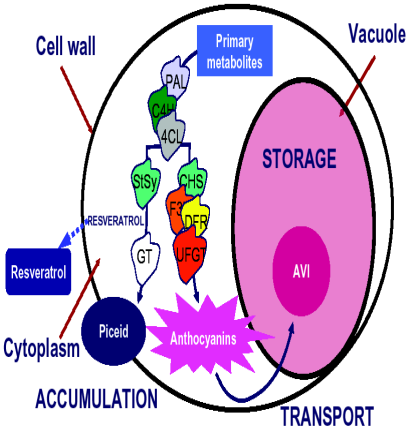


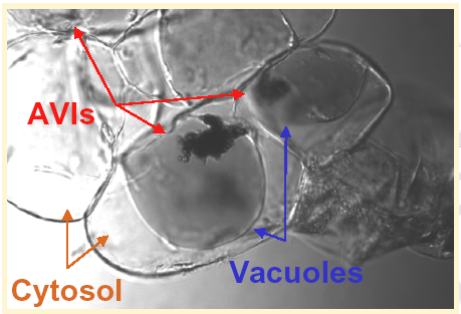
- Very localized
- In other solvents?

Photo of Red Rose Flower

Anthocyanic Vacuolar Inclusion (AVI)?



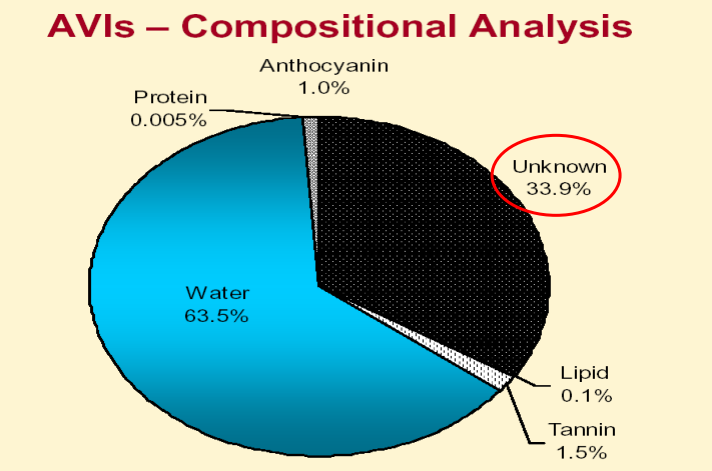




Transmission Image

Dr. Wei Zhang, *13th International Biotechnology Symposium & Exhibition Dalian, China, 12-17 October 2008*

It can be, But?

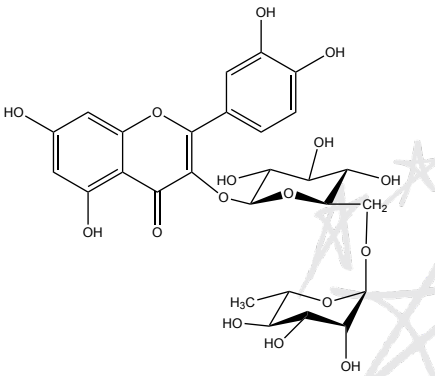


Dr. Wei Zhang, *13th International Biotechnology Symposium & Exhibition Dalian, China, 12-17 October 2008*

In some cases…

- ***Sophora japonica*** **flowers**

  30-40% Rutin (Dry weight)
- **But not soluble in water at all**



Rutin

How do plants synthesize and store?

A hypothesis for solution

New alternative media in organisms?

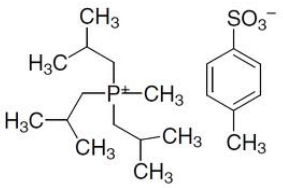
- **No other solvents in organisms**
- **Lipids have limited function as media**
- **New theory required e.g. liquids from solids?**

Evidences of Natural Ionic Liquids and Natural Deep Eutectic Solvents

Liquids from solids?

Ionic liquids and deep eutectic solvents

1. In 1920 chemistry
2. Ionic liquids (ILs)
3. Deep eutectic solvents (DES)
4. Why not in nature?



Natural Deep Eutectic Solvents (NADES)

Screening of Natural Ionic Liquids and Natural Deep Eutectic Solvents

Any single metabolite must have the reason for the presence in organisms but…

- For the most of them, the roles are not clear
- Why malic acid and citric acid in TCA-cycle related metabolites?
- Choline, Proline?
- Sucrose, Trehalose, Glucose, Fructose?

Other Example: In Water Deficiency

- Plants can still survive in shortage of water: Water deficiency
- Some specific metabolites accumulate more by water deficiency
- Sugars, Proline, Proline betaine (Methyl proline), Choline deriv.

Alternative Solvents in Plants?

Screening Natural Ionic or Deep Eutectic Sovents

- We have done 'Metabolomics (Metabolic Profiling)' of Plants for long time.
- Based on the data, Common Metabolites Selected
- Malic, Citric, Fumaric, Maleic acids
- Choline, Proline
- Sucrose, Trehalose, Glucose, Fructose

Chemical or *In Vitro* Proof of Natural Ionic Liquids and Natural Deep Eutectic Solvents

Most Combination are Liquids

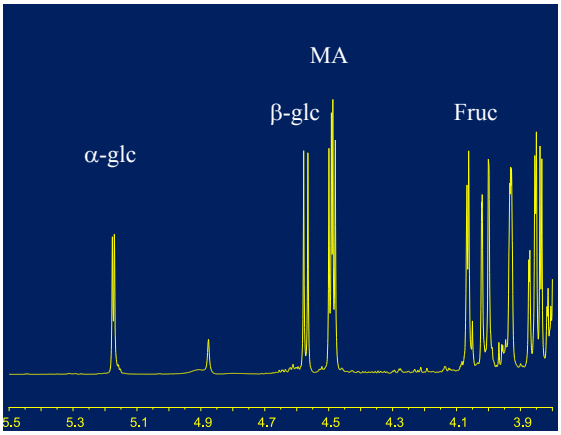
- Add a certain mole to de ionized water (ca. 40g/100 mL)
- Keep for 1 hour
- Evaporation with Rotary Evaporator at 50 ^{o}C
- Measure mole ratio by ^{1}H-NMR spectroscopy
- ca. 100 combinations are liquids

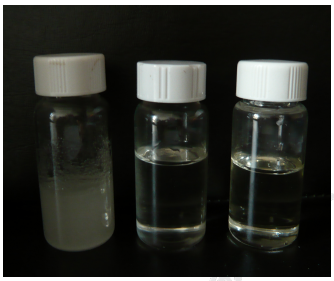
Example

1 2 3 4 5 6

1: Frucrose
2: Glucose
3: Malic acid
4: Choline Cl
5: Malic acid-Choline Cl (1:1)
6: Fructose-Glucose-Malic acid (1:1:1)

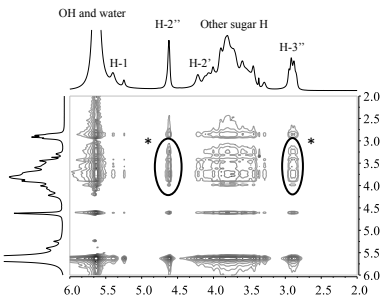
Mole Ratio by ^{1}H-NMR

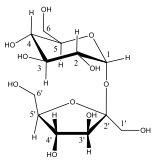




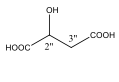
Glucose-Fructose-Malic acid = 1:1:1

Correlation between molecules by^{1}H-^{1}H-NOESY

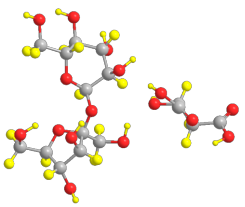




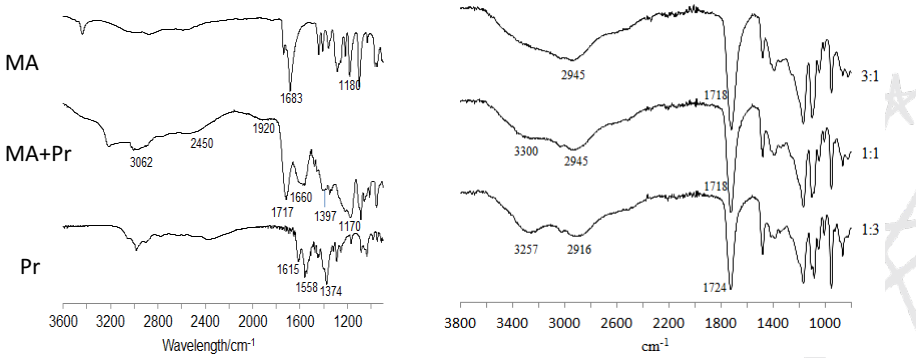
Sucrose



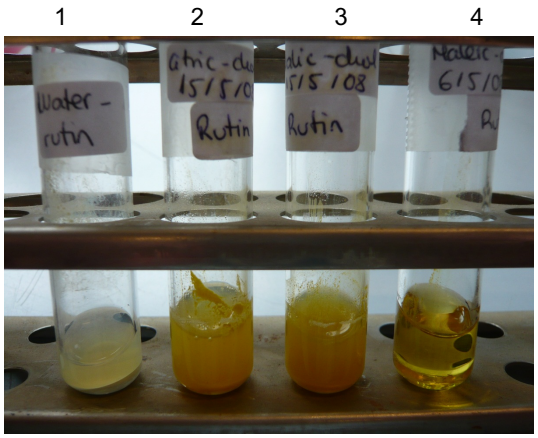
Malic acid

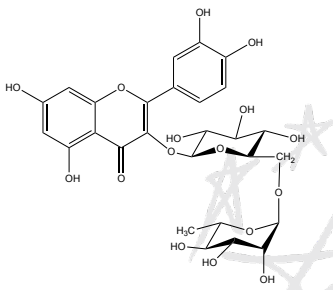


IR application to NADES assessment



Solubility of Pigments (Rutin)

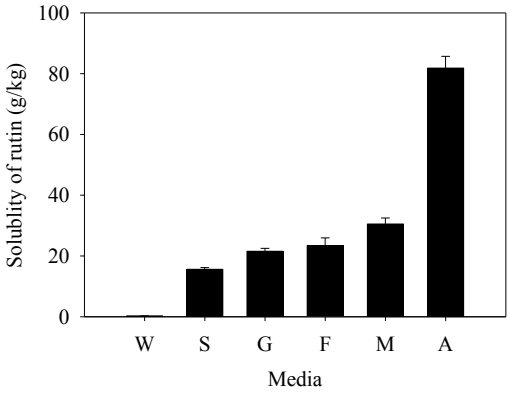




Rutin

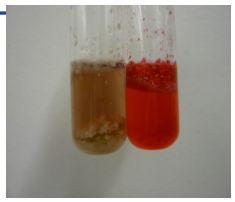
1; in Water (Not soluble)
2; in Citric acid-Choline Cl (1:2) (partially soluble)
3; in Malic acid-Choline Cl (1:1) (Partially soluble)
4; in Maleic acid-Choline (1:1) (Completely soluble)

Solubility of Rutin in diverse NADES



Water (W), sucrose-choline chloride (S), glucose-choline Cl (G), fructose-choline Cl (F), maleic acid-choline Cl (M), and aconitic acid-choline Cl (A).

Extractability of Pigments (Tulip)



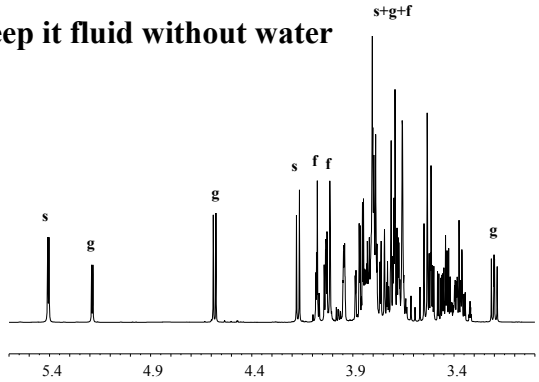
1 2 3

1 2 3

1: Malic acid-Choline Cl (1:1)
2: Fructose-Glucose-Malic acid (1:1:1)
3: Water

A Plant sap excreted: Sugar Mixtures but Liquid

- Plants excrete saps to interact with other organism
- or for catching insects
- How to keep it fluid without water



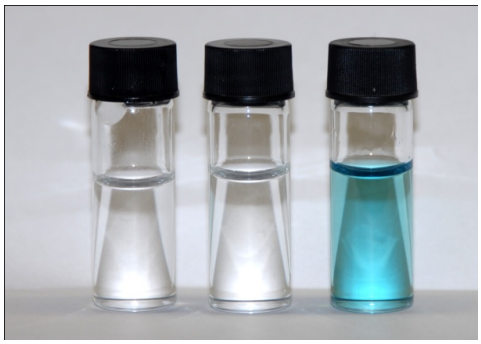
^{1}H NMR spectrum of *Cleome hasselorana*. s: sucrose, g: glucose, f: fructose.

A Plant sap excreted: Sugar Mixtures but Liquid

1 2 3 4 5 6

Typical natural eutectic solvents. 1: sucrose, 2: fructose, 3: glucose, 4: malic acid, 5: sucrose-fructose-glucose (1:1:1, mole/mole), 6: sucrose-malic acid (1:1, mole/mole).

If NADES is present in plants, enzyme may be activated therein.



1 2 3

Laccase activity in malic acid-choline Cl (1:1) with addition of water.
1: 0 % water, 2: 25% water, 3: 50% water.

Where do we stand now?

- *It is still Hypothesis-level*
- *Some Chemical or In Vitro Proof*
- *Some possible applications*

Possible Applications

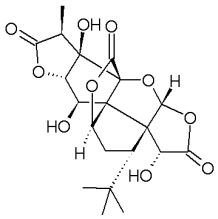
- Solubilizing non-water soluble medicines

  like Taxol and Ginkgolides combined with liposome

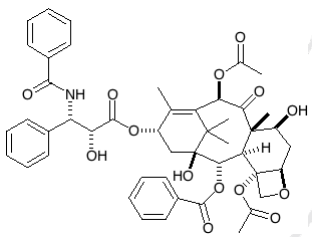
  (Drug Delivery System for Pharmaceutics and Cosmetics)
- Dissolving macro molecules (Proteins, DNA, or Polysaccharides)
- Chromatography resins

Solubility of water-insoluble Taxol and Ginkgolides

- In Glucose-Choline Cl
- 0.81 mg/mL (Ginkgolide B)
- 5.85 mg/mL (Taxol)



Ginkgolide

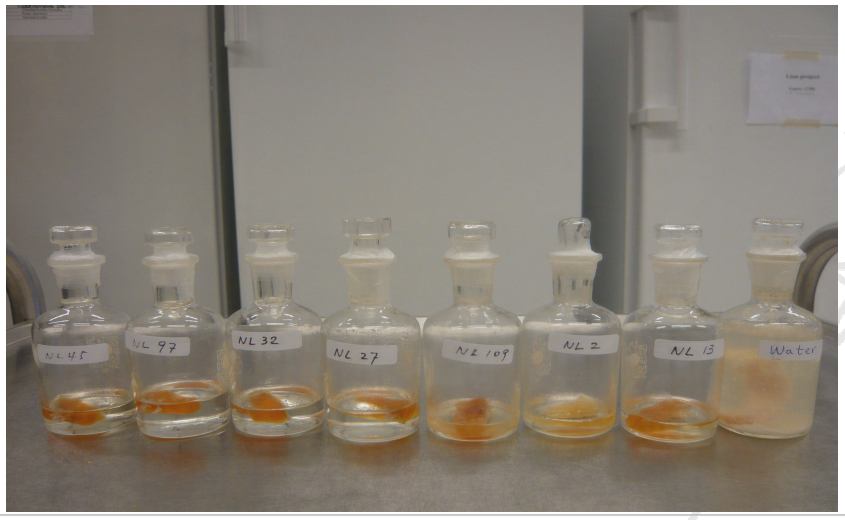


Taxol

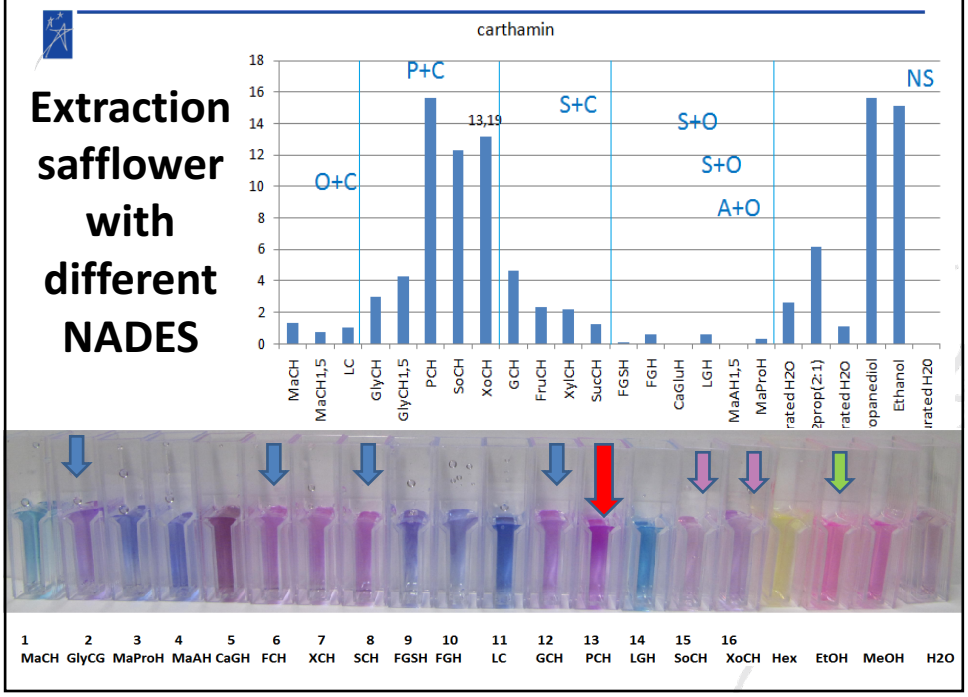
On-going works

- Collecting physical Property
  (b.p. water activity, vapor pressure…)
- Enzyme activity
- Application to Natural Fine Chemicals
- Dissolving Proteins
- Study Plant Saps with Naturalis

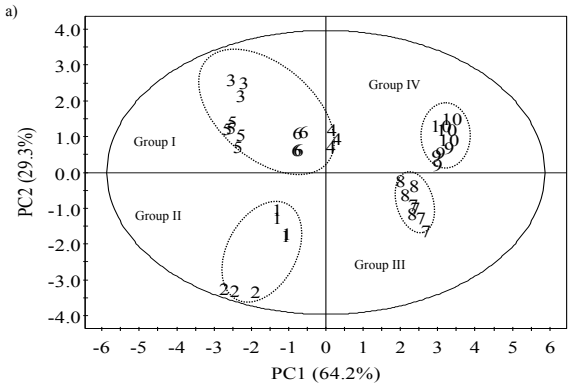
Preservation with NADES



Extraction safflower with different NADES

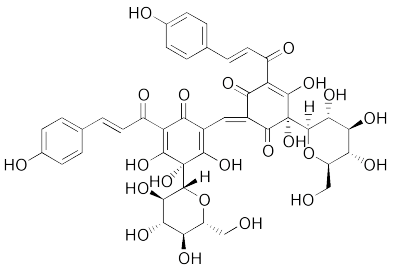


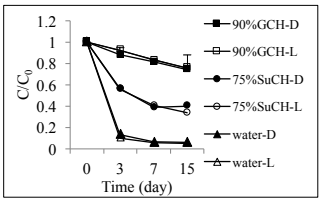
NADES extraction of safflower

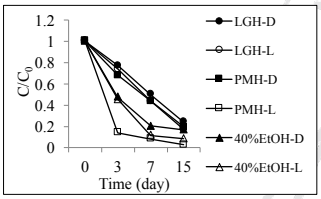


Score plot (a) and loading plot (b) of principal component analysis of the extracts from safflower with different solvents. (In the score plot (a): 1, 1,2-propanediol–choline chloride; 2, ethanol; 3, 40% ethanol; 4, sucrose–choline chloride; 5, lactic acid–glucose; 6, proline–malic acid; 7, glucose–choline chloride; 8, sorbitol–choline chloride; 9, fructose–glucose–sucrose; 10, water.

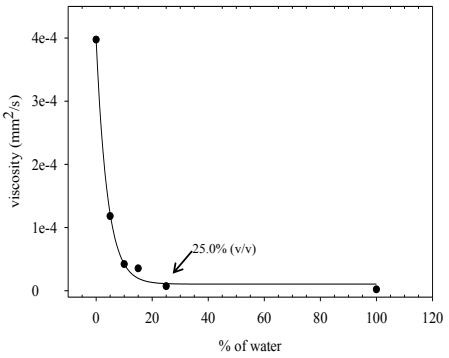
Prevent degradation: Carthamine in NADES

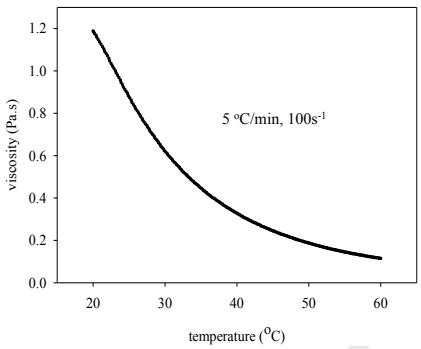




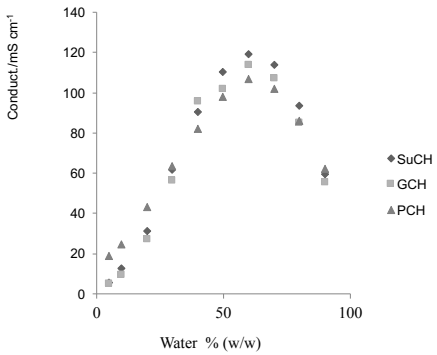


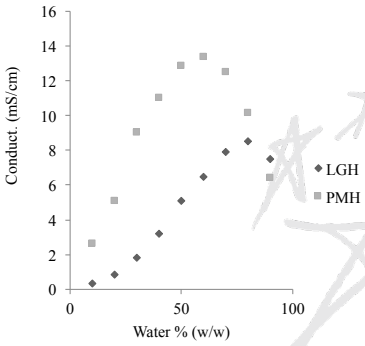
Viscosity – Temp, Water Content (Glc-Choline)



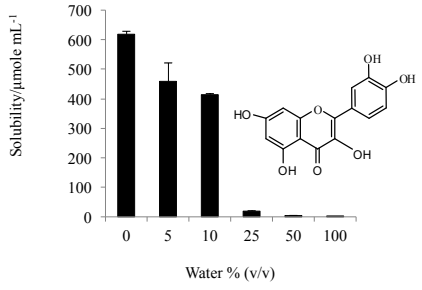


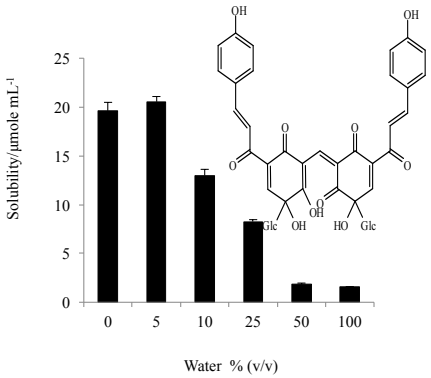
Conductivity-Water Content





Solubility-Water Content





NADES for Today

1. Betaine-Citric acid (1:1, 39-64 g) added by 10.3 mL (10%)
2. Betaine-Citric acid (1:1, 39-64 g) added by 20.6 mL (20%)
3. β-Alanine-Citric acid (1:1, 29.6-64 g) added by 9.3 mL (10%)
4. β-Alanine-Citric acid (1:1, 29.6-64 g) added by 18.6 mL (20%)
5. Xylitol-Citric acid (1:1, 50.6-64 g) added by 11.8 mL (10%)
6. Xylitol-Citric acid (1:1, 50.6-64 g) added by 23.6 mL (20%)

Extraction

1. 200 mg of *Sophora japonica* flowers
2. 4 mL of NADES for 2 min Vortexing
3. 30 min in water bath (40 ^{o}C)
4. Ultrasonication for 30 min
5. Centrifuge for 15 min
6. Transfer 0.5 mL Solution and mix with 0.5 mL of HPLC solvents
7. Filtering for HPLC