Apr 17th, 11:00 AM - 12:00 PM

Focused Ultrasonication-Assisted Preparation of Aqueous Nanodispersions for Selected Novel C-type Lectin Receptor Ligands

Alexander Riffey
University of Montana, Missoula, alexander.riffey@umconnect.umt.edu

Walid Abdelwahab
University of Montana, Missoula, walid.abdelwahab@mso.umt.edu

Cassandra Buhl
University of Montana, Missoula, cassandra.buhl@mso.umt.edu

Craig Johnson
University of Montana, Missoula, craig.johnson@mso.umt.edu

George Ettenger
University of Montana, Missoula, george.ettenger@mso.umt.edu

See next page for additional authors

Presenter Information
Alexander Riffey, Walid Abdelwahab, Cassandra Buhl, Craig Johnson, George Ettenger, Kendal Ryter, Jay Evans, and David Burkhart

Focused Ultrasonication-Assisted Preparation of Aqueous Nanodispersions for Selected Novel C-type Lectin Receptor Ligands

Alexander Riffey*§, Walid M. Abdelwahab*§, Cassandra Buhl*†, Craig Johnson*‡, George Ettenger*‡, Kendal Ryter*‡, Jay T. Evans*†, David J. Burkhart*§

*Center for Translational Medicine, †Department of Biological Sciences, ‡Department of Chemistry, § Department of Biomedical and Pharmaceutical Sciences, University of Montana, 32 Campus Drive, Missoula, MT 59812

Developing a Next-Generation TB Vaccine

Mycobacterium tuberculosis (Mtb), the causative pathogen of tuberculosis (TB), is a global threat. Tuberculosis remains one of the top ten causes of death, with 10 million people affected by infections in 2017, resulting in 1.6 million deaths.

Adjuvants are substances used in the creation of vaccines which boost immune response to an antigen, enhancing immune protection.

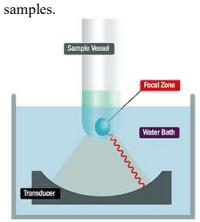
- Recent evidence suggests a T-helper cell 17 (Th17) response, part of the body's adaptive immune system, may protect against TB, but no Th17 adjuvants are clinically approved at this time.
- C-type lectin receptors (CLRs), such as Mincle, are a class of cellular immunostimulatory receptor which have shown promise in driving a Th17 response when stimulated by another molecule.
- Trehalose 6,6'-dimycolate (TDM) is the main immunostimulatory component of the Mtb cell wall and a potent Mincle stimulator.
- TDM is too toxic to be used in a vaccine, but may lead to discovery of a drug with low toxicity which can create a strong enough Th17 response necessary to protect against TB.

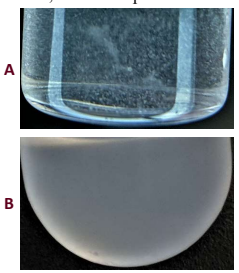
In this study, immune responses were tested for an array of CLR-binding adjuvant formulations in order to identify lead CLR adjuvant candidates for use in next-generation TB vaccines.

Methods

Molecules similar to TDM are amphiphilic in nature, meaning they are attracted to both water and fats, and can self-assemble in water to form different sized particles. A technique utilizing focused ultrasonic energy was used to prepare water-based dispersions of the studied compounds. These aqueous formulations were characterized via a variety of analytical methods including: dynamic light scattering (DLS), transmission electron cryomicroscopy, high-performance liquid chromatography (HPLC), and osmometry before *in vitro*, human cellular culture, tests were performed.

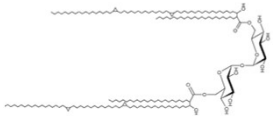
(Below) A diagram showing the method used to focus sonic energy in order to produce the studied samples.



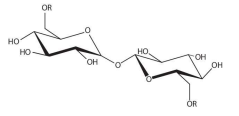


(Above, Right: A, B) An example of the dispersions prepared via traditional bath sonication shows settling and aggregation of particles, while **(Above, Right: B)** the same dispersion prepared via focused ultra-sonication resulted in a uniform suspension.

Compound Structures



(Left) A representation of the structure of trehalose 6,6'-dimycolate (TDM.)



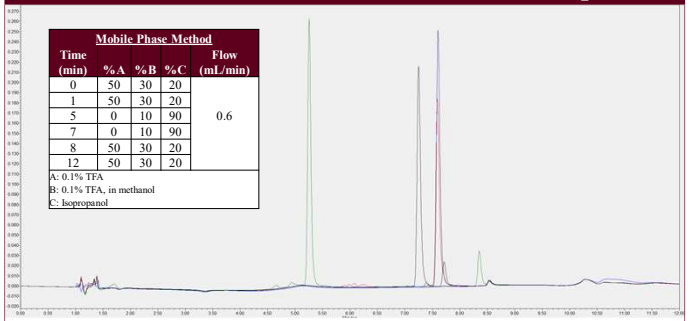
(Right) Compounds used in these experiments are trehalose diesters based upon the structure of TDM.

Concentrations and a Tool For Future Development

Mobile Phase Method

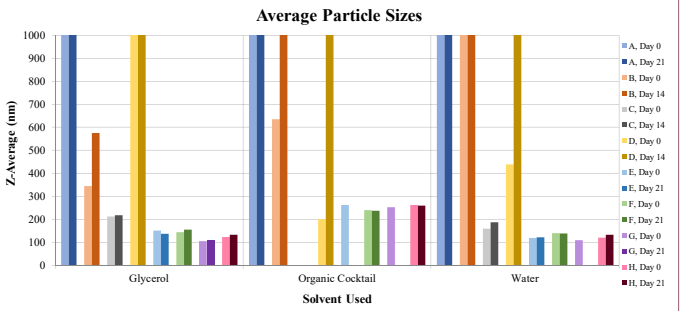
Time (min)	%A	%B	%C	Flow (mL/min)
0	50	30	20	0.6
1	50	30	20	
5	0	10	90	
7	0	10	90	
8	50	30	20	
12	50	30	20	

A: 0.1% TFA
B: 0.1% TFA, in methanol
C: Isopropanol



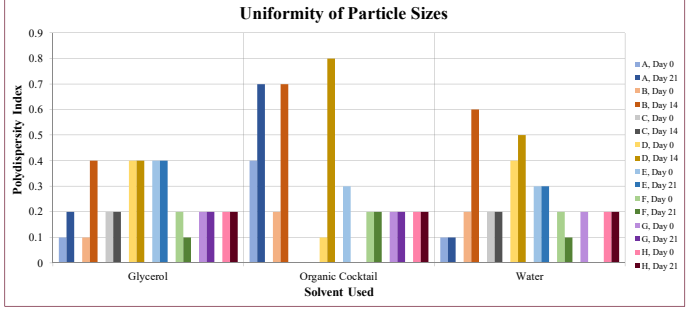
(Above) A HPLC method developed to determine concentration of the compounds used in this study. Results showed 100% recovery with most compounds, the lowest observed value being 87%. The above method worked universally for the studied compounds and allows for determination of LogP for future studies. Determination of LogP allows for greater accuracy in determining the best solvent to use for a formulation.

Formulations Remain Relatively Stable Over Time



(Above) Average particle sizes for suspensions of the studied compounds in a variety of solvents. Samples were tested at day 0 and day 14 or 21 after being kept between 2˚C-8˚C to determine changes in particle size over time. Sizes under 200 nm are desirable as this allows for sterile filtration; the majority of compounds resulted in a formulation with initial particle sizes below 200 nm.

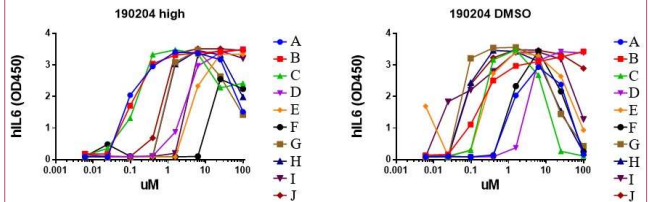
(Below) Uniformity of the above particle sizes based upon polydispersity index (PDL) PDI can be used to determine the uniformity of particle sizes as well as the accuracy of the above particle sizes. A PDI below 0.2 suggests good uniformity of particles in the sample and reliability of the measured particle size.



Target Characteristics vs. Experimental Results

☐ Stable or easy to resuspend.	☑ Size was fairly stable in most samples, however some samples grew from nano to micron size.
☐ Size less than 200 nm to allow sterile-filtration.	☑ Initial particle size of the majority of formulations were below 200 nm.
☐ Increased immune response compared to compounds in dimethyl sulfoxide (DMSO.)	☑ Immunogenicity was increased in the majority of nanodispersions when compared to the same compound in DMSO.
☐ High adsorption of antigen to particles.	☒ A method for determining antigen adsorption remains in development.
☐ Isotonicity close to that of human blood.	☑ Isotonicity of samples was close to that of blood in glycerol solution, but lower when in water.
☐ Explore effects of different diluents.	☑ Water and glycerol samples showed increased immune response over the organic cocktail with water generally resulting in easier formulatability.

Improved Response Over Compounds in DMSO



(Above) *In vitro* results showing human interleukin-6 (hIL6) response from human peripheral blood mononuclear cells (hPBMCs.) The above results show that the nanodispersions of the adjuvants resulted in a higher immune response than the same compounds in DMSO.

Conclusions

- Use of focused ultrasonication can produce smaller and more uniform particles than traditional bath sonication.
- Using aqueous glycerol solution as the solvent results in favorable characteristics for dispersions of the studied compounds when paired with focused ultrasonication.
- *In vitro* data suggests these dispersions can stimulate a strong immune response in human cells, which is dependent upon both size and molecular structure.

Future Directions

- Determine antigen adsorption to the particles in the dispersions.
- Add stabilizers to these formulations to increase size stability over time.
- Explore the effects of formulation macromolecular structure on immune response.

Acknowledgments

This project was supported by the National Institutes of Health (NIH) Adjuvant Discovery Contract # HHSN272201400050C. The contents are solely the responsibility of the author and do not necessarily represent the official views of the NIH. Special thanks go to the University of Montana College of Translational Medicine staff for their continuing guidance and support. Special thanks go to GlaxoSmithKline for their contribution with development of the proprietary TDEs used in this project.