

DEVELOPMENT AND ACTIVITY TESTING OF SPRAY DRIED PHOSPHORYLATED HEXAACYL DISACCHARIDE FOR NASAL ADMINISTRATION

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Key Message:

Spray dried PHAD formulation has been developed for administration as a micellar nasal spray to treat viral respiratory infections and allergies. Investigations found that PHAD concentration within spray drying feed solutions influenced the reconstitution properties of the resultant powder formulations, with reconstitution time, solution turbidity and micelle uniformity affected.

1. Introduction

Phosphorylated Hexaacyl Disaccharide (PHAD) is a synthetic structural analogue of Monophosphoryl Lipid A (MPLA), a phospholipid with immunostimulatory activity [1]. When in micellar form, it activates Toll-like receptor 4 (TLR4), leading to the production of protective cytokines and chemokines, and activation of CD4+ and CD8+ T-cells [1,2,3].

It is believed that PHAD must be in particle form, like a micelle, for best recognition and binding to TLR4, likely as the micellar shape mimics a globular protein [4].

Micelles can be prepared by wetting PHAD with a solvent and adding water. However, liquid solutions have shown poor solubility and high irritation on administration due to the presence of the non-aqueous solvent.

2. Aim

To spray dry PHAD in its micellar form to produce a powder formulation that would reconstitute in water back to its original micellar form and produce an immune response.

3. Experimental Methods

Micellar Feed Solution Preparation Preparation of Micellar Feed Solution for the Spray Drying of 2:49:49 (%w/w/w) PHAD:HP-β-CD:Trehalose Formulation

- Prepared at PHAD concentrations varying from 0.3-1.0 mg/mL. Due to poor solubility of PHAD in water, higher ethanol contents were required for increased PHAD concentrations (Table 1).
- PHAD was sonicated at 40°C in 95% ethanol to produce a turbid nanosuspension.
- Excipient solution of Hydroxypropyl-β-Cyclodextrin (HP-β-CD) and Trehalose Dihydrate (Trehalose) in Water for Injection (WFI) was heated to 40°C and added to the ethanolic PHAD at 10 g/min while sonicating at 40°C.
- Following addition, the micellar feed solution was further sonicated at 40°C for 15 minutes and then equilibrated to room temperature.

Table 1: Composition of spray drying feed solutions at varying PHAD concentrations

Feed Solution Batch Codes	PHAD Concentration (mg/mL)	HP-β-CD Concentration (mg/mL)	Trehalose Concentration (mg/mL)	WFI in PHAD Solution (%)	95% Ethanol in PHAD Solution
A-Feed	1.0	24.5	24.5	50	50
B-Feed	0.8	19.6	19.6	60	40
C-Feed	0.6	14.7	14.7	70	30
D-Feed	0.5	12.3	12.3	75	25
E-Feed	0.4	9.8	9.8	80	20
F-Feed	0.3	7.4	7.4	85	15

Spray Drying

Spray Drying of Micellar Feed Solutions

- Micellar feed solutions were spray dried using the ProCepT 4M8-Trix Spray Dryer with a two-fluid nozzle (0.8 mm tip), using target spray drying parameters outlined in Table 2.
- Formulations were produced as white cohesive powders in 70-80% yield.

Table 2: Target Spray Drying Parameters for PHAD Feed Solutions

Inlet Temperature (°C)	Outlet Temperature (°C)	Nozzle Air (bar)	Cooling Air (bar)	Drying Airflow (m ³ /min)	Liquid Feed Rate (g/min)
113-120	70	1.0	0.5	0.7	7.5-8.0

Reconstitution

Reconstitution of Spray Dried PHAD Formulations and Analysis of Micelles by Dynamic Light Scattering (DLS)

- Spray dried PHAD formulations were reconstituted in triplicate at 0.6 mg/mL PHAD in WFI with light vortexing.
- Dissolution time and visual observations of the resultant dispersion were recorded.
- Dispersions were analysed via DLS with backscatter at 20°C.

In Vitro Analysis

In Vitro Comparison of Liquid and Spray Dried PHAD Formulations

- Murine J774A.1 macrophages were cultured in DMEM medium containing 10% foetal bovine serum.
- 100K cells were plated per well of a 24-well plate and treated with 50 ng/mL PHAD in a liquid solution (ethanol in water) or spray dried and reconstituted in water.
- Cells were harvested 24 hours after seeding and pelleted by centrifugation at 250 x g.
- Supernatant was collected and centrifugation at 250 x g was performed to remove any residual cells. Supernatant then analysed per manufacturer's protocols for the presence of IP-10 or TNFα using Invitrogen and R&D Systems ELISA kits.

5. Conclusion

- Decreasing the initial PHAD concentration resulted in:
 - Reduced reconstitution times
 - Decrease in turbidity of the micellar solutions.
 - Uniform micelles – monomodal in size, with lower Pdl values.
- 0.5 mg/mL PHAD showed to be the most effective in maintaining the balance of spray drying processing times without compromising the integrity of the reconstituted micelles.
- Reconstituted solutions maintained *in vitro* activity, being able to produce a cytokine response comparable to the original PHAD in ethanolic solution.
- The powder will be further investigated as a stable form of PHAD that can be administered intranasally, either as a reconstituted solution or as a powder

4. Results and Discussion

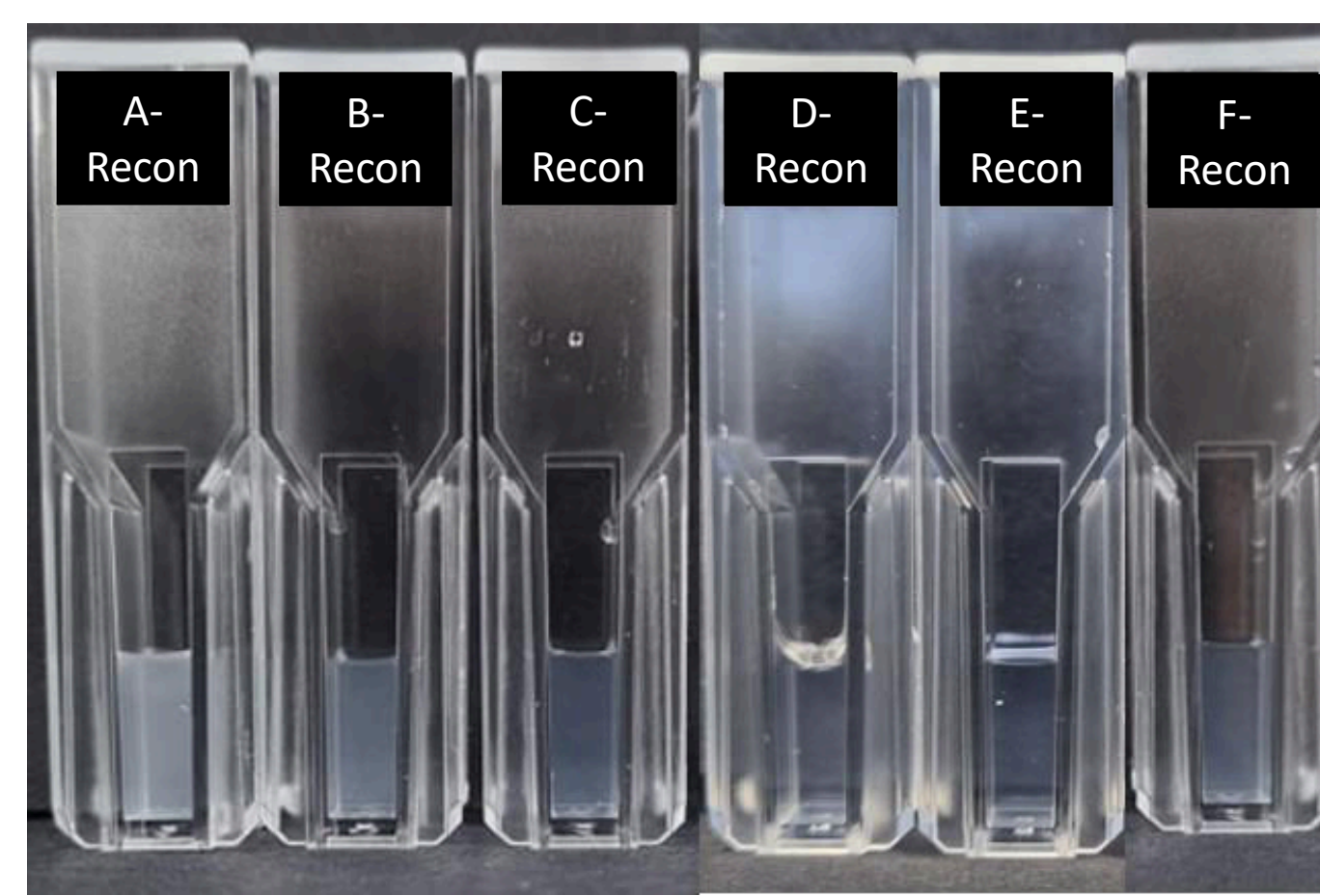


Figure 1: Spray dried PHAD formulations reconstituted at 0.6 mg/mL PHAD in WFI. Reconstituted batch codes correspond to feed solution batch codes outlined in Table 1

Turbidity of the reconstituted spray dried PHAD decreased with decreasing PHAD concentration within the feed solution (Figure 1). Decreasing initial PHAD concentration also increased ease of dissolution when reconstituting samples (Table 3).

Table 3: Summary of feed solution batches, their reconstitution times and micelle size distributions

Batch	PHAD Concentration in Feed Solution (mg/mL)	DLS – Size Distribution by Intensity		Reconstitution Time (Seconds)
		Z-Avg (d.nm)	Pdl	
A	1.0	854.2	1.000	122
B	0.8	213.6	0.769	35
C	0.6	150.8	0.350	29
D	0.5	130.4	0.287	25
E	0.4	133.4	0.239	31
F	0.3	124.1	0.221	34

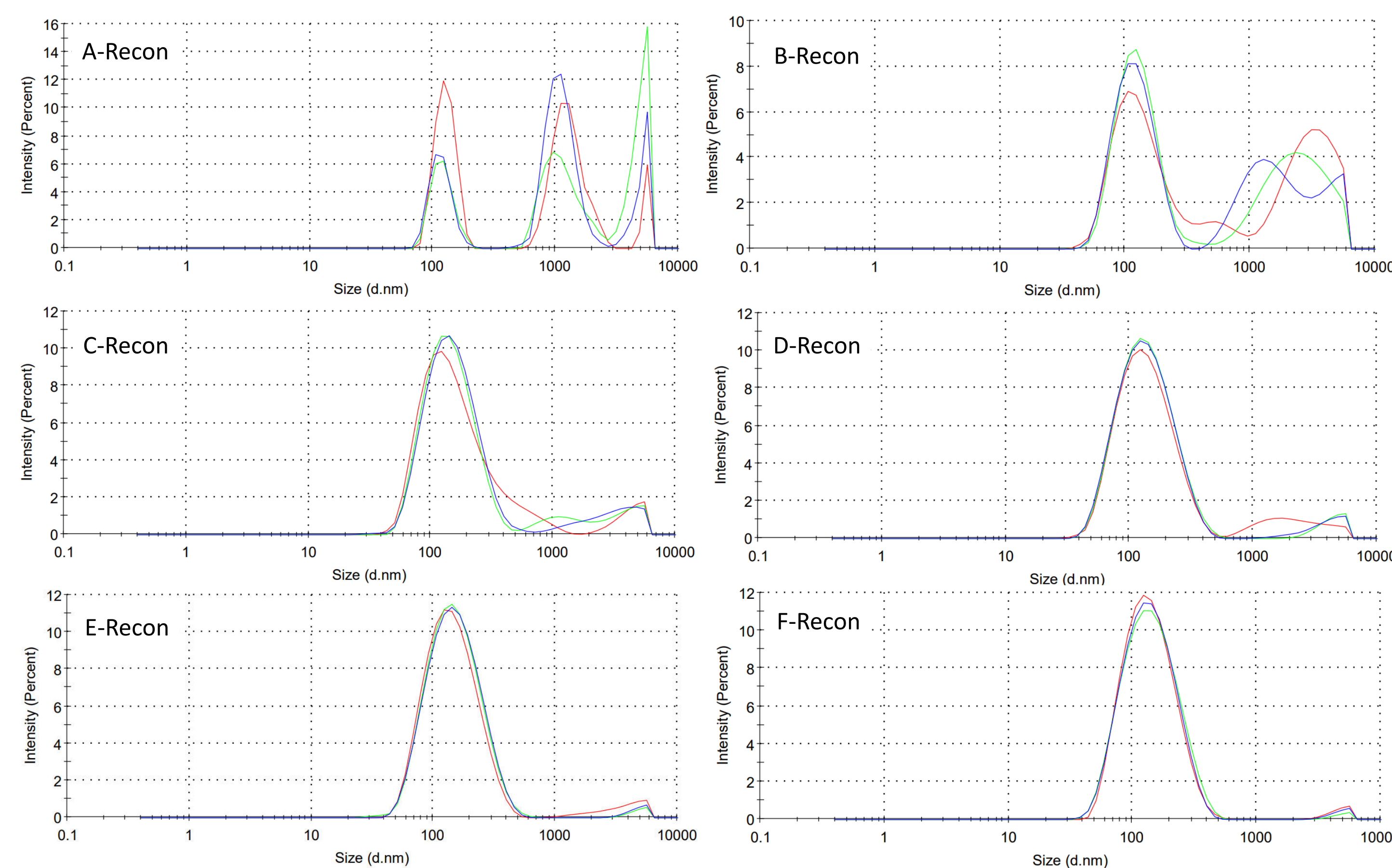


Figure 2: Size distribution traces of micelles formed from spray dried PHAD formulations reconstituted at 0.6 mg/mL PHAD in WFI (3 average intensity measurements per batch)

Micelles within formulations spray dried from lower concentration feed solutions appeared more monodisperse, with lower Pdl values observed (Table 3 and Figure 2).

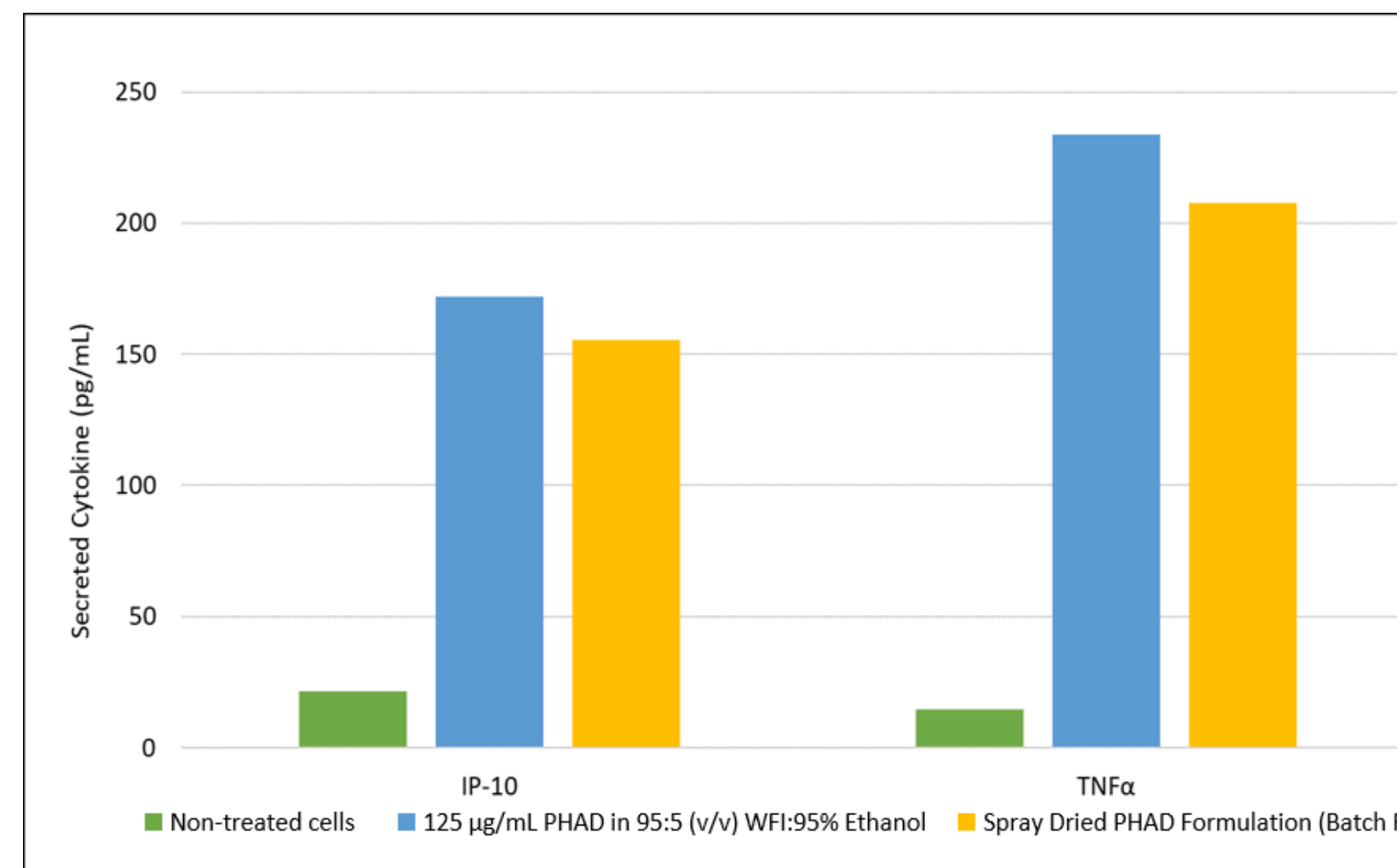


Figure 3: Levels of IP-10 and TNFα following 24-hour treatment of J774A.1 macrophages with 50 ng/mL of a 125 µg/mL PHAD in 95:5 WFI:95% Ethanol (v/v) and spray dried PHAD formulation (Batch F) (n=1)

In vitro activity was maintained after spray drying and mediated similar levels cytokine of production following treatment as PHAD in an ethanol solution (Figure 3).

References
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 [3] Kamphuis, T., Meijerhof, T., Stegmann, T., Lederhofer, J., Wilschut, J., and de Haan, A. (2012, May). Immunogenicity and Protective Capacity of a Viroosomal Respiratory Syncytial Virus Vaccine Adjuvanted with Monophosphoryl Lipid A in Mice. *PLOS ONE*, 7(5), e36812. DOI: 10.1371/journal.pone.0036812
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