

# Center for Microencapsulation and Drug Delivery

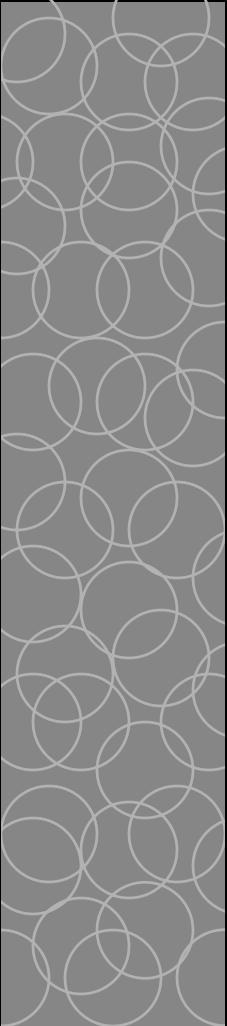

*Allison C. Rice-Ficht, Director*

- ◆ Texas A&M University System
- ◆ New materials and delivery systems
- ◆ Range of technologies - Protein composites, PEGs, PLGA, PVA, alginates, dendrimers
- ◆ Chemistry, scale-up, testing



# Novel Biopolymers for Controlled Release

---

- 
- ◆ Protein composites
  - ◆ Microspheres as carriers for vaccines
  - ◆ Subcutaneous and oral delivery
- 

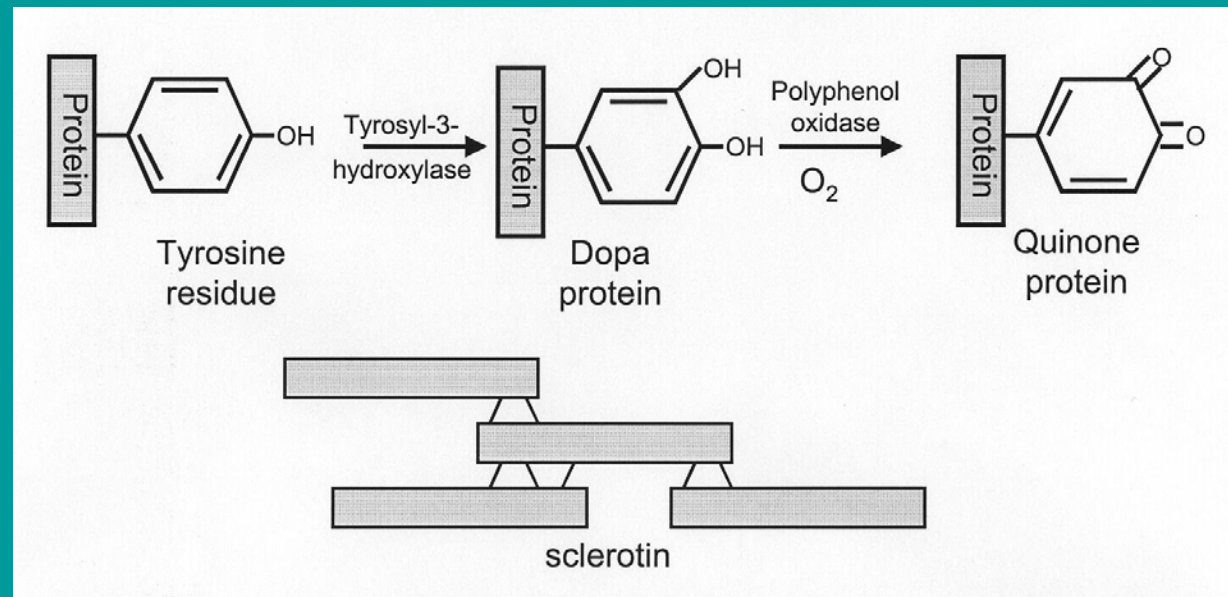
# A Unique Biopolymer Produced by *Fasciola hepatica*

The liver fluke *Fasciola hepatica* encapsulates its eggs with a mechanically tough and chemically resistant scleroprotein (VpB). It has adhesive properties similar to the marine mussel proteins. **This protein can be purified as a recombinant in gram quantities. High content of glycine, lysine, DOPA and positively charged amino acids.**

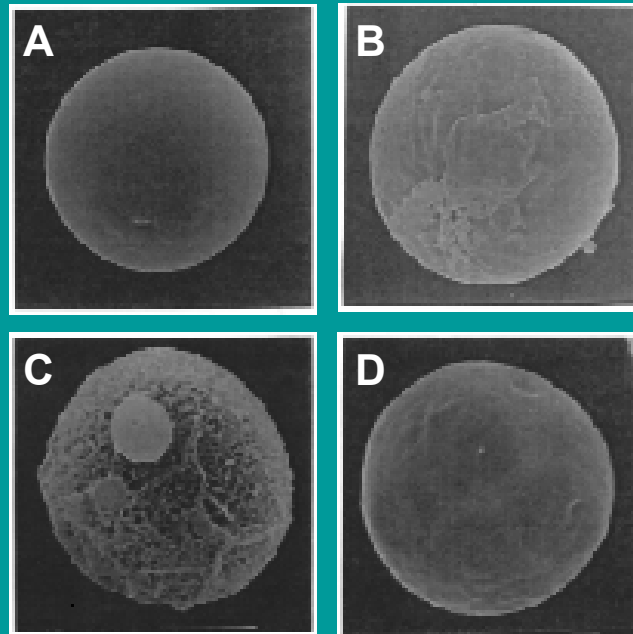
***Fasciola hepatica*** (liver fluke) stained with molybdate-nitrate reagent to detect the presence of DOPA containing protein, the precursor to eggshell.



# Production of Quinone in *Fasciola hepatica* from eggshell precursor protein



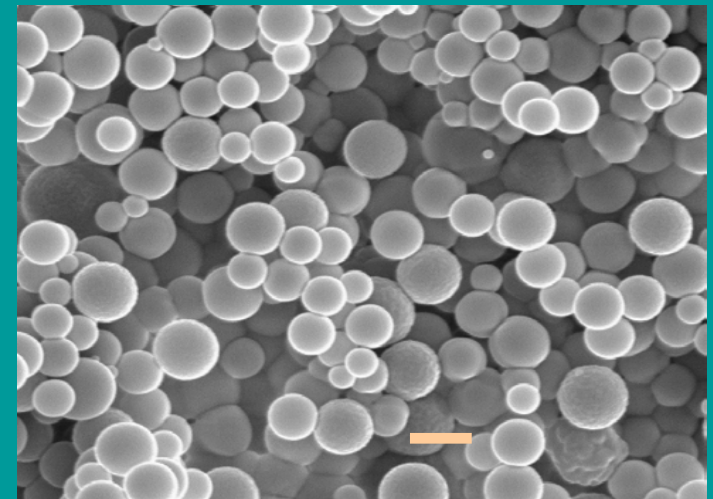
# Capsule Surface Characteristics



- Scanning electron micrographs of microcapsules formed with
- A) 100% BSA cross-linked with 25% glutaraldehyde,
  - B) 100% BSA cross-linked with 1% glutaraldehyde,
  - C) 96% BSA, 4% biopolymer protein, 1% glutaraldehyde,
  - D) 98% poly-DL-lactide co-glycolide, 2% staphylococcal enterotoxin A.

Microcapsule diameters are 17.5, 11.4, 12.8 and 5.1 microns, respectively.

# Emulsion Technology for capsule production



# Focus on monolithic capsule

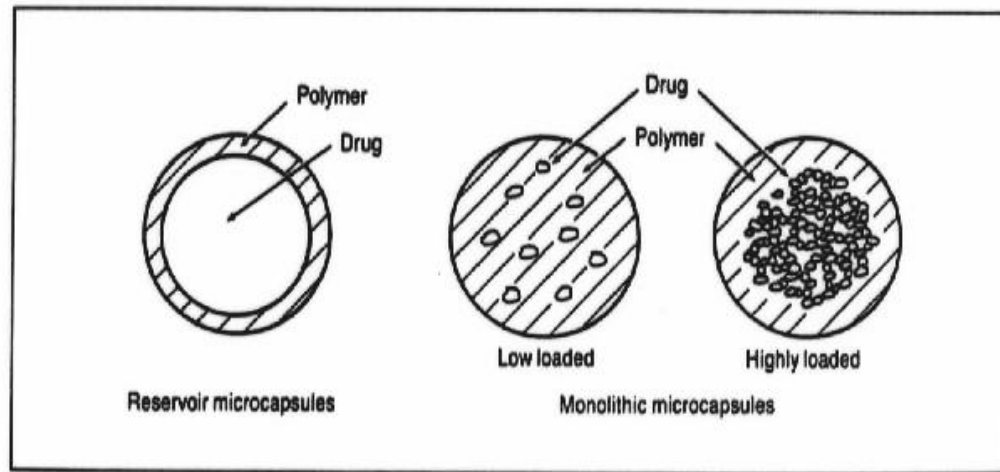
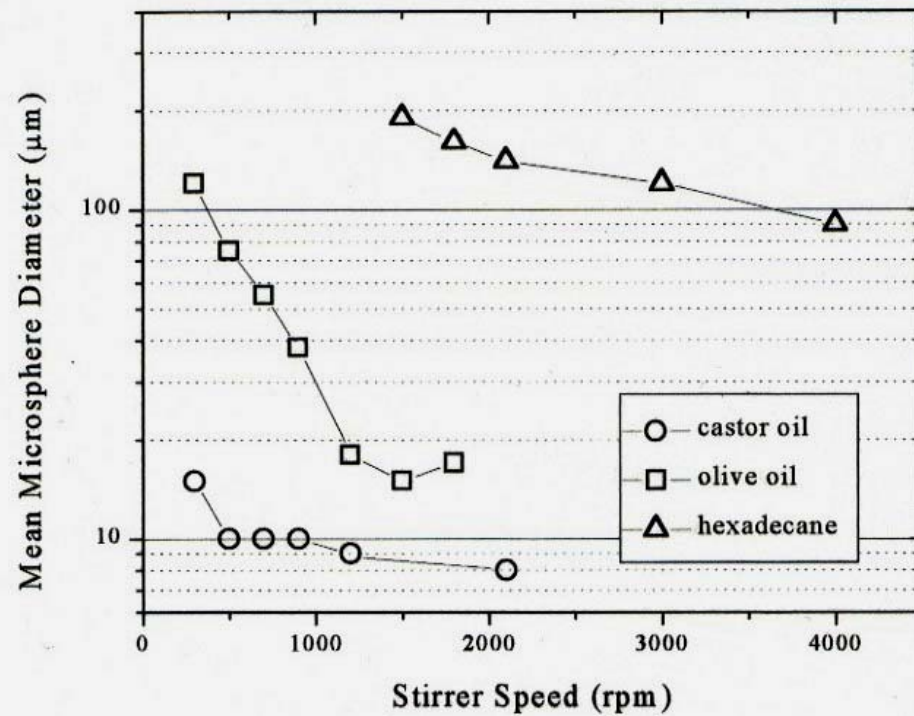


Figure 1: Internal structure of controlled-release microcapsules.

# Capsule Manufacturing

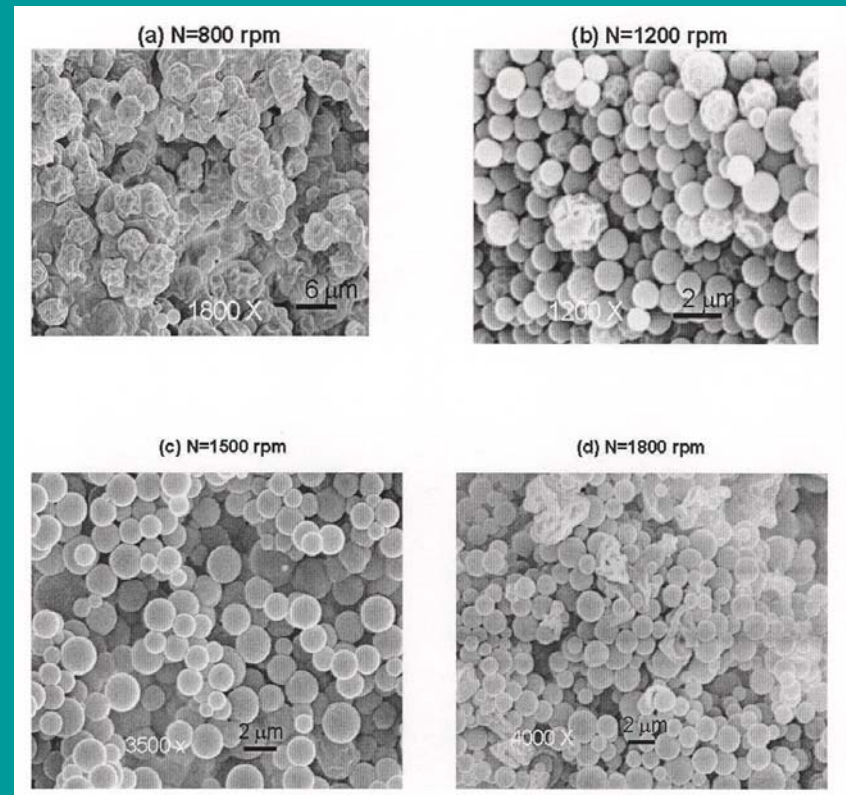




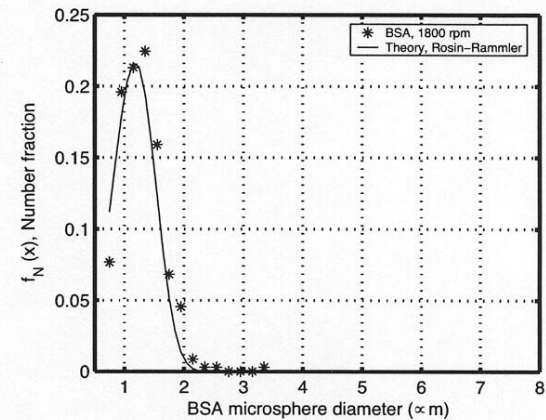
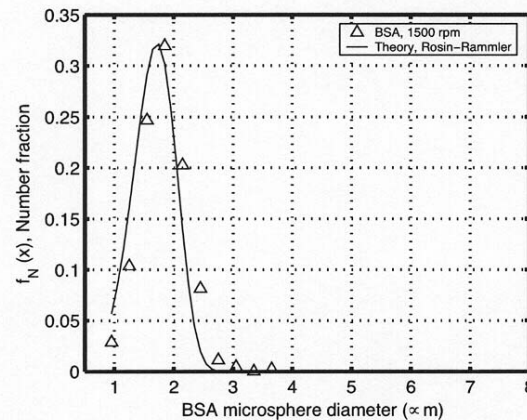
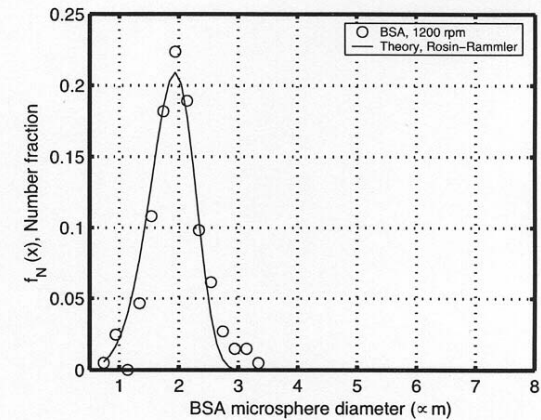
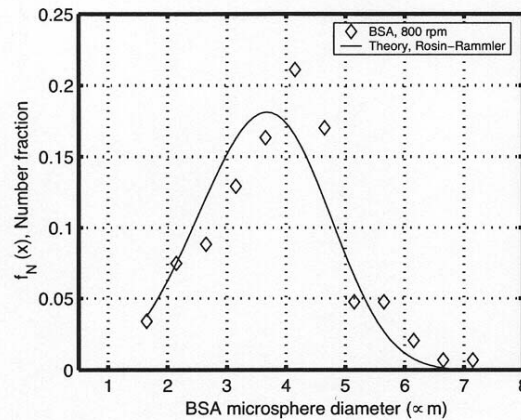
# Stirring speed as a factor

## Integrity:

SEM micrographs of BSA microspheres prepared using the water-in-oil emulsion technique in olive oil at (a)  $N = 800$  rpm, (b)  $N = 1200$  rpm, (c)  $N = 1500$  rpm, and (d)  $N = 1800$  rpm.



# Stirring speed: size distributions of BSA microspheres



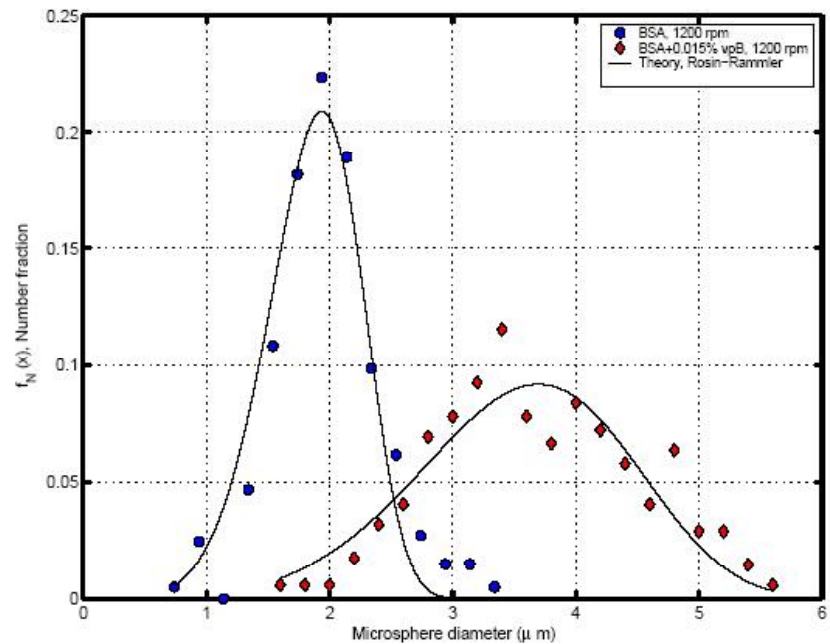
# Microsphere diameter as a function of protein composition

Normalized number fraction distributions of

(a) BSA microspheres prepared in olive oil at  $N = 1200$  rpm, and

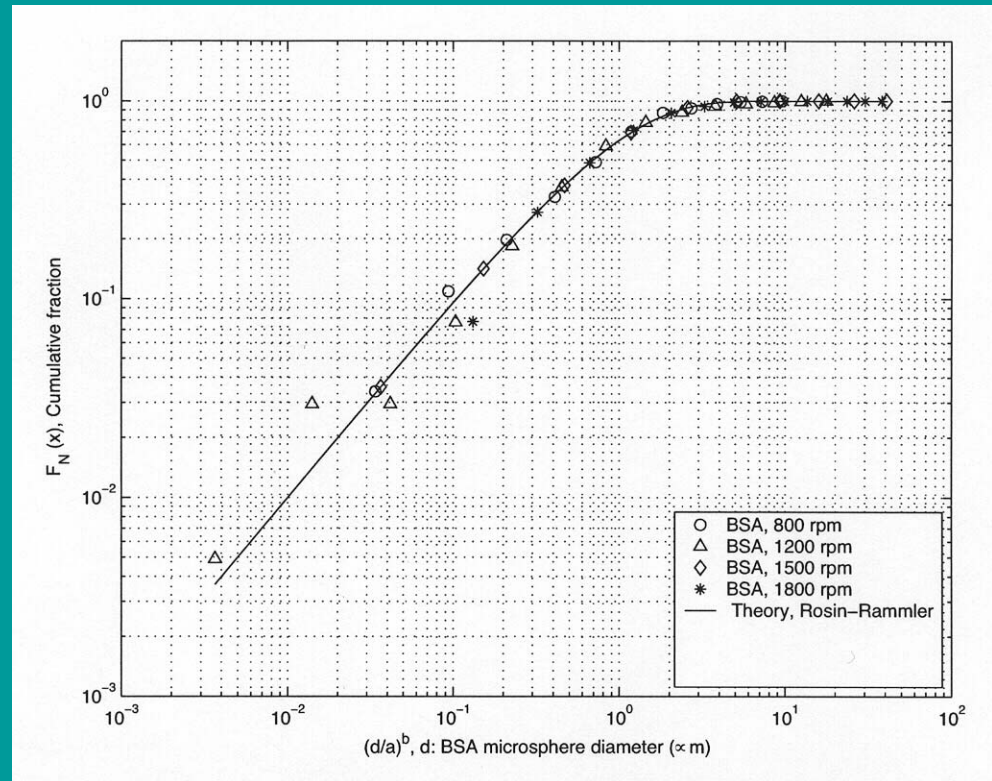
(b) BSA + 0.015% vpB microspheres prepared in olive oil at  $N = 1200$  rpm.

A Rosin-Rammler distribution function is used to fit the experimental data.



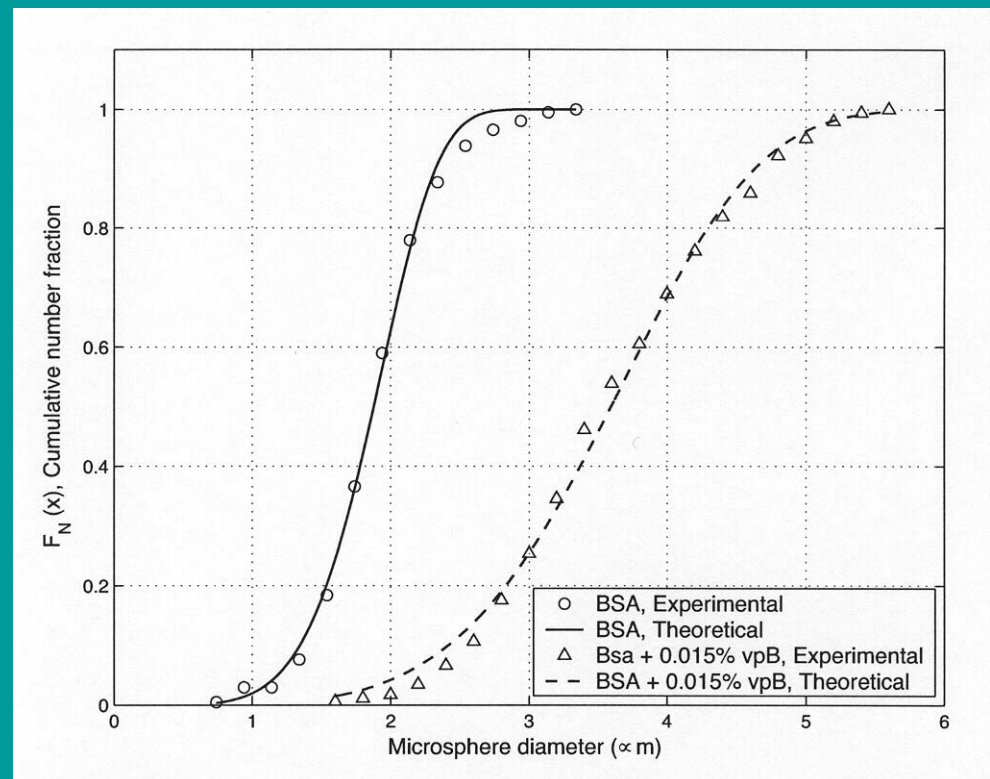
# Predictive value of Rosin-Rammler Distribution for BSA Microspheres

Universal cumulative number fraction distribution of BSA microspheres prepared in olive oil. The cumulative size distributions of BSA microspheres prepared at  $N = 800$ , 1200, 1500, and 1800 rpm are collapsed onto a single distribution curve.



# Predictive Value of Rosin-Rammler Distribution for Composite Microspheres

Cumulative number fraction distributions of (a) BSA microspheres prepared in olive oil at  $N = 1200$  rpm, and (b) BSA + 0.015% vpB microspheres prepared in olive oil at  $N = 1200$  rpm. A Rosin-Rammler distribution function is used to fit the experimental data.



# Properties of Microspheres: Release kinetics

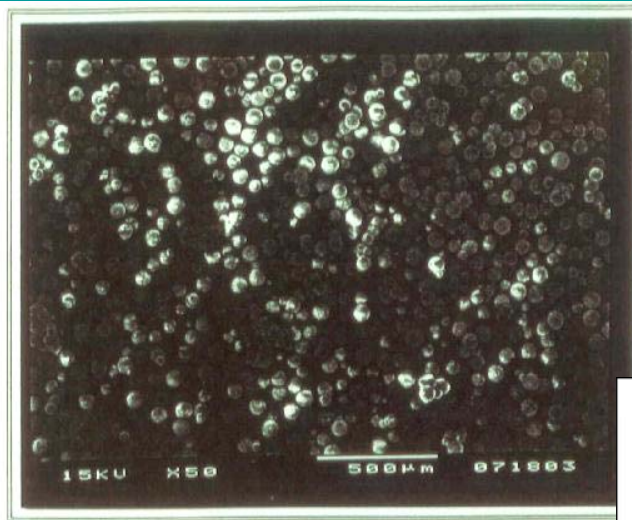
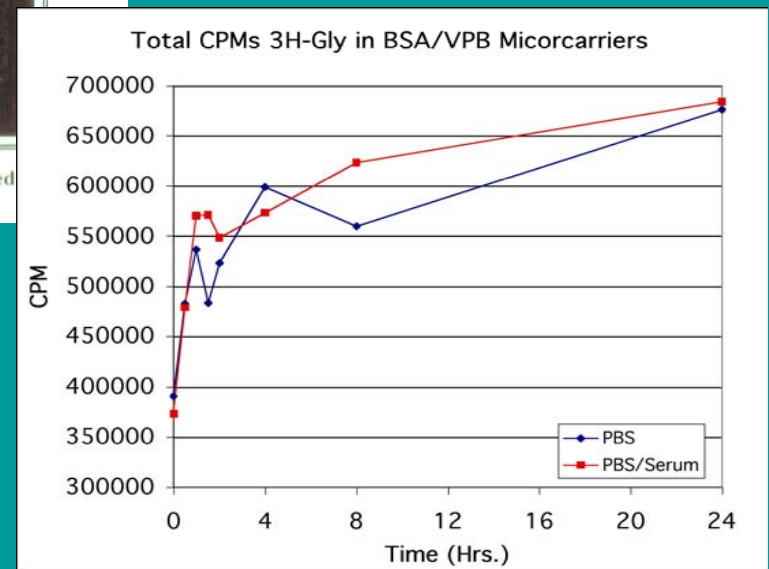


Figure 1. SEM micrograph (50X) of BSA microspheres prepared oil at 600 r.p.m. Approximate size of spheres = 50 µm

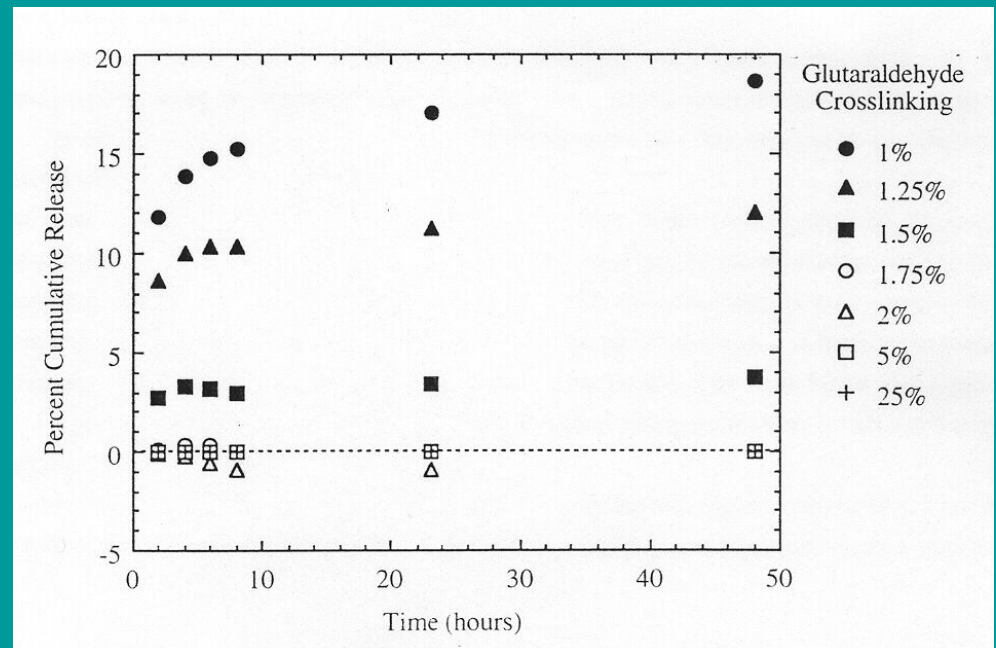
Uniform size  
distribution

Release kinetics for  
small molecules



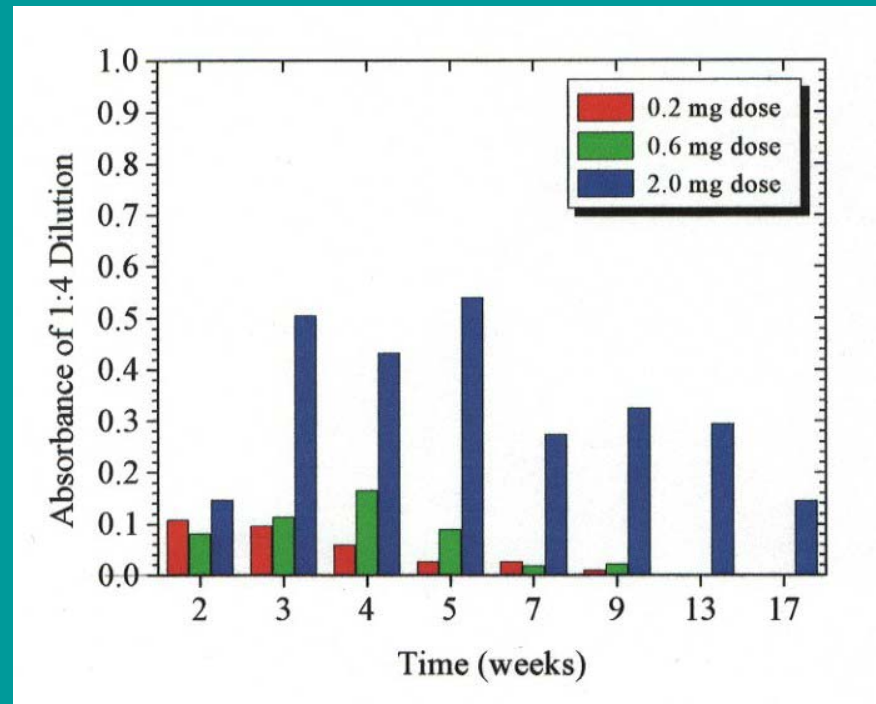
# Protein release kinetics

## Protein release kinetics from BSA microspheres



# Immune Response: Depot delivery and Capsule loading

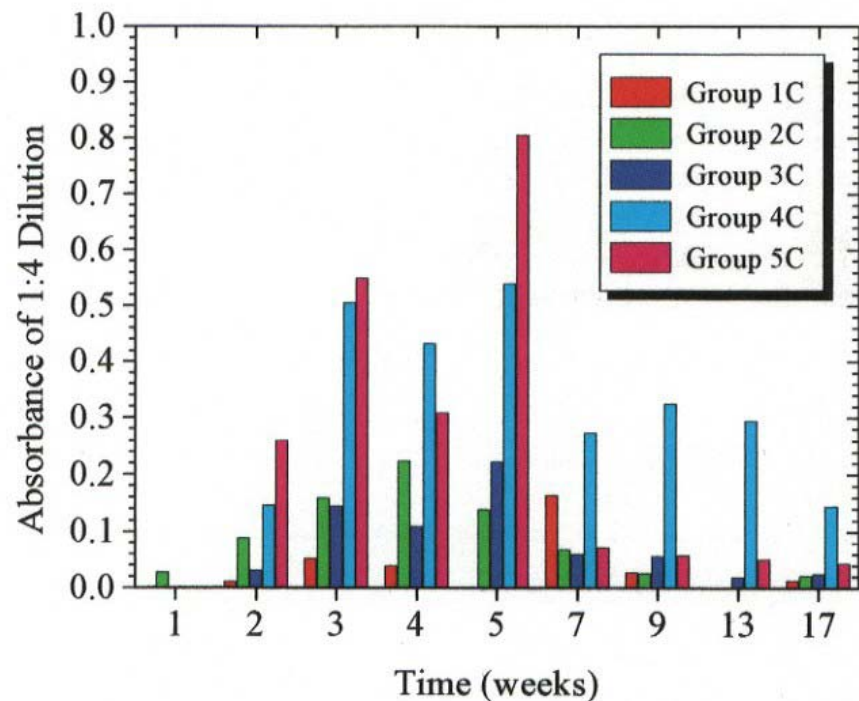
Encapsulated  
botulinum toxin  
delivered as a  
depot





# Immune Response: Depot delivery and sustained release kinetics

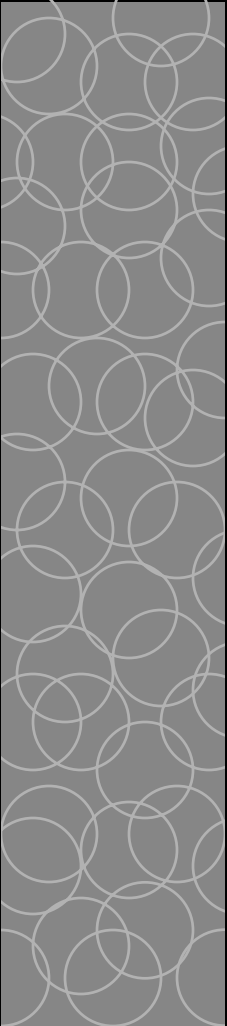

Botulinum toxin fragment delivered in a free form (5C) and encapsulated in protein (1C - 4C)





# Oral delivery

---

- 
- ◆ Direct to Peyer's patches
  - ◆ 1-5 micron capsules are taken up by Peyer's patches and delivered to local lymph nodes and spleen.
  - ◆ 5-10 micron capsules are retained in M cells of Peyer's patch.
- 

# Subcutaneous vs. oral delivery

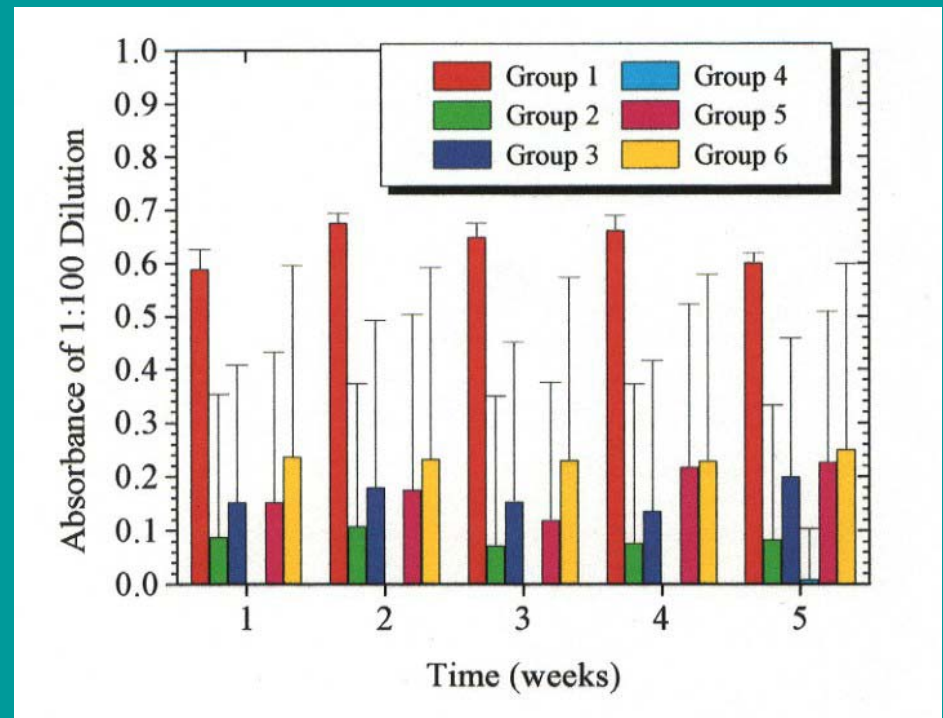
Groups (single dose):

1- subcutaneous

2,3 - protein based capsules

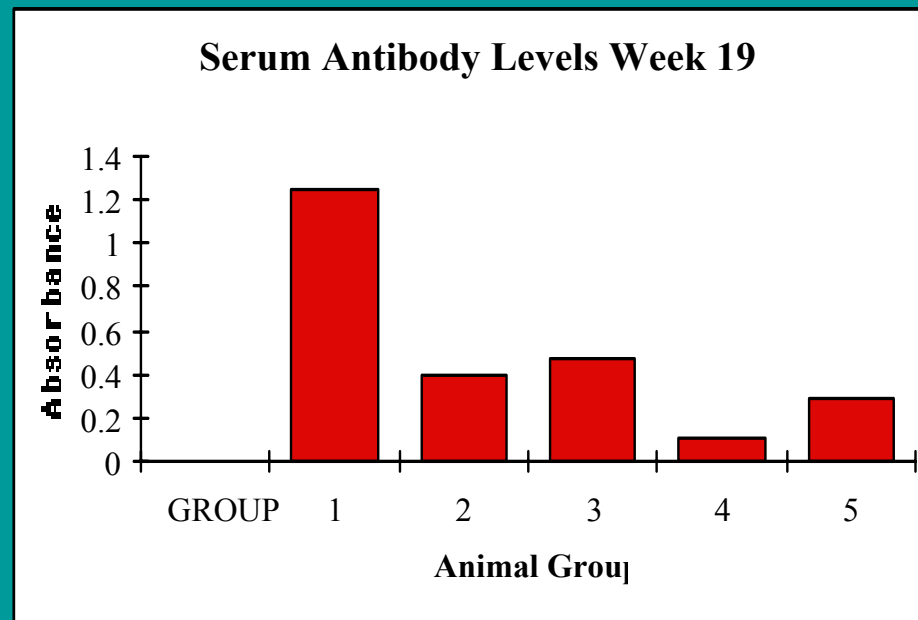
4,5 - PLG

6 - PLG with corn oil



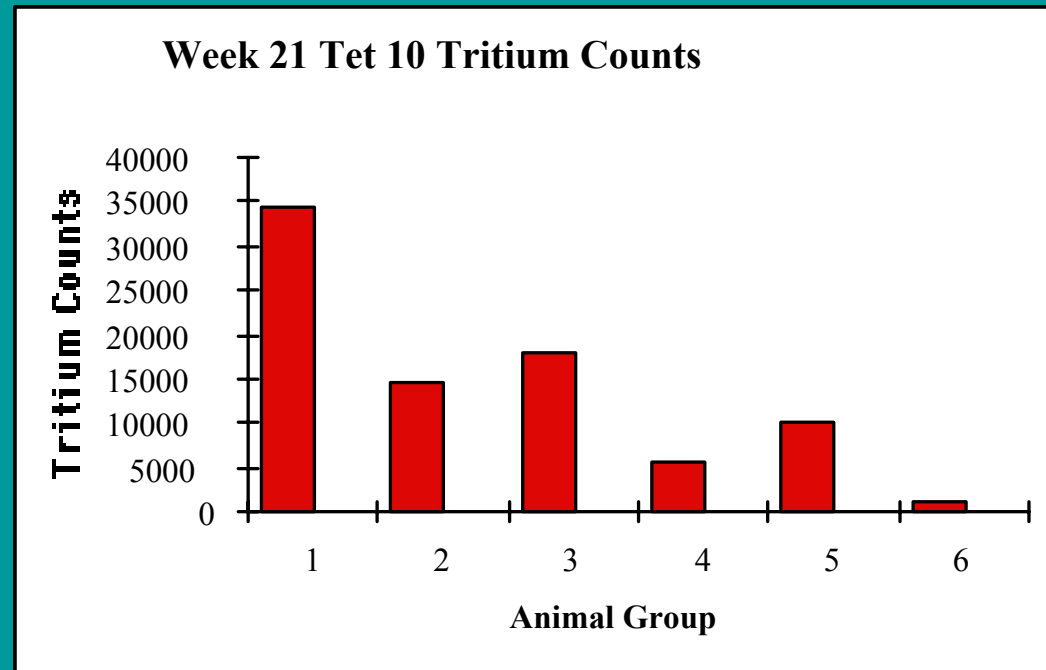
# Encapsulated tetanus toxoid

Antibody titers following a single subcutaneous injection - pooled sera



# Encapsulated tetanus toxoid

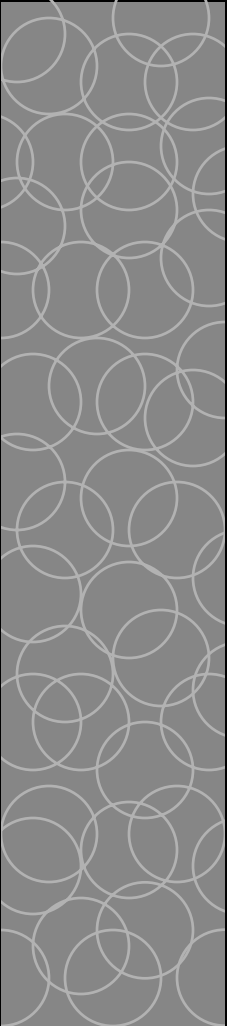

## Lymphocyte stimulation





# Conclusions

---

- 
- ◆ VpB is a promising component of a composite delivery system
  - ◆ Affords extended release, potentiates the immune response
  - ◆ Subcutaneous routes are effective
  - ◆ Oral routes require further development
- 



# Collaborators

---



## **Texas A&M University**

Dr. Malcolm Andrews

Dr. Ali Beskok

Gopinath Subramanian,

Pradipkumar Bahukudumbi

Jesus Mendoza

## **Texas A&M University System HSC**

Ken Carson

Jane Miller

## **University of California Santa Barbara**

Dr. Herbert Waite

## **Lynntech, Inc.**

Duncan Hitchens

Toni Giletto