# Macro research: fabrication of nanoparticles for drug delivery

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**Biological and Agricultural Engineering**

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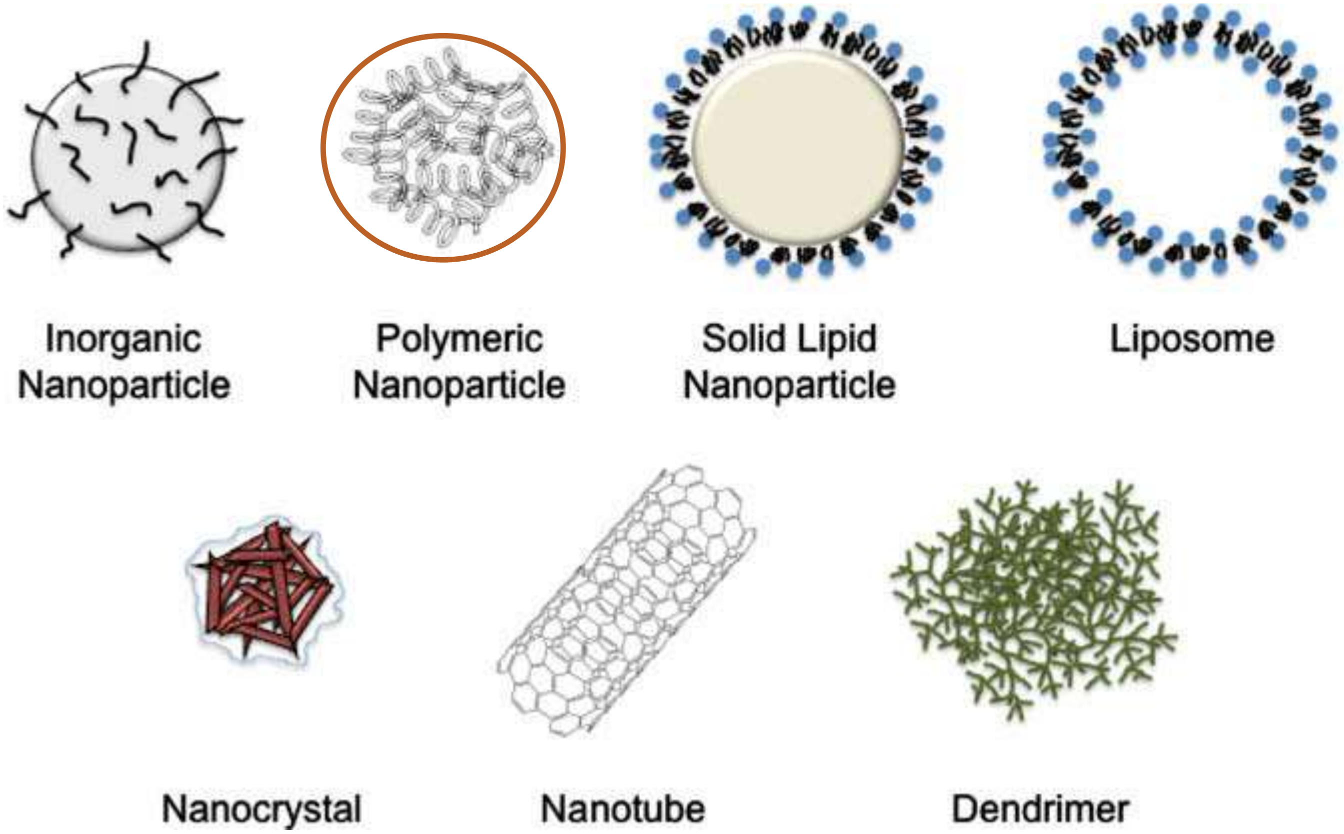
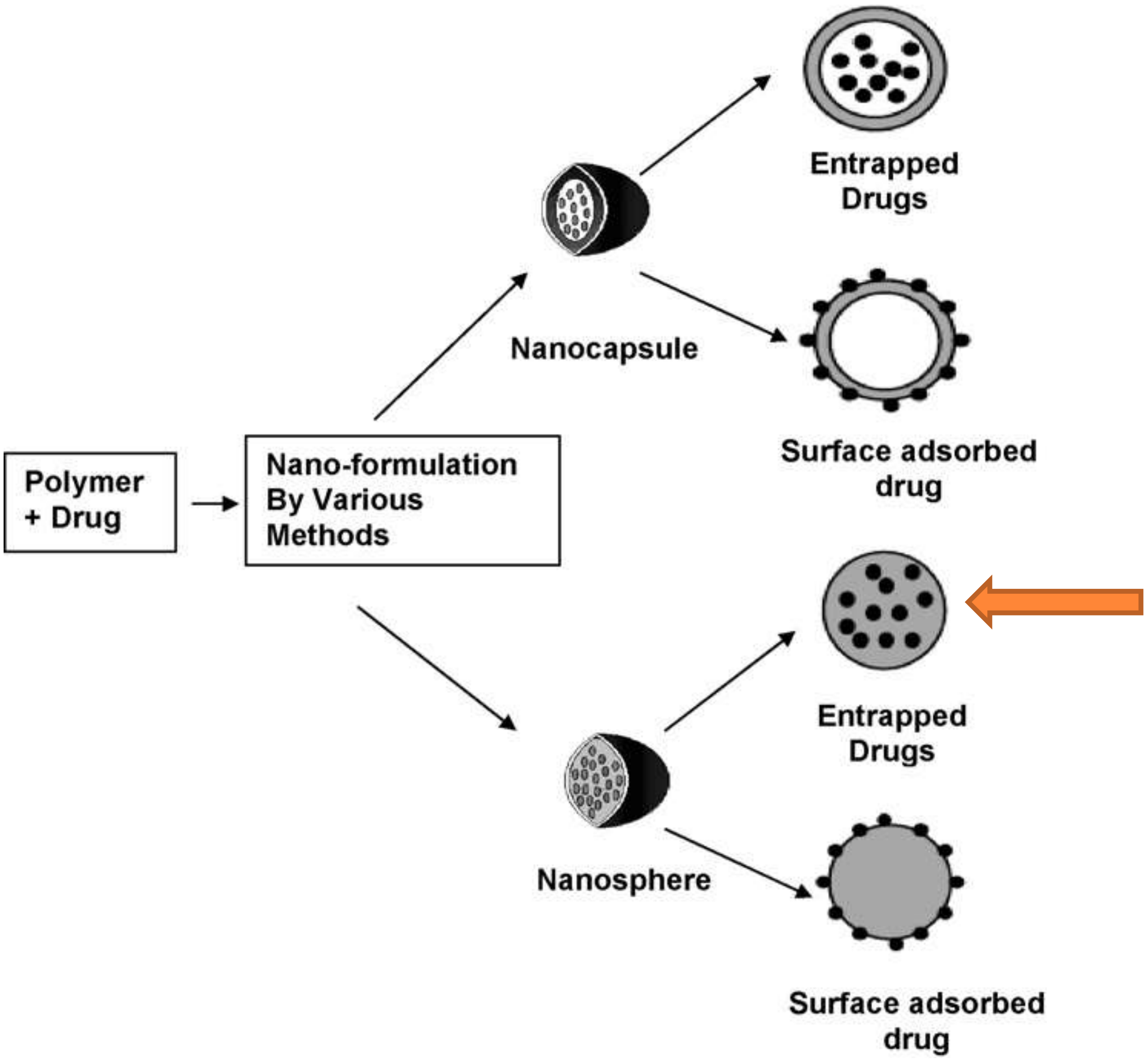
# NANOPARTICLES

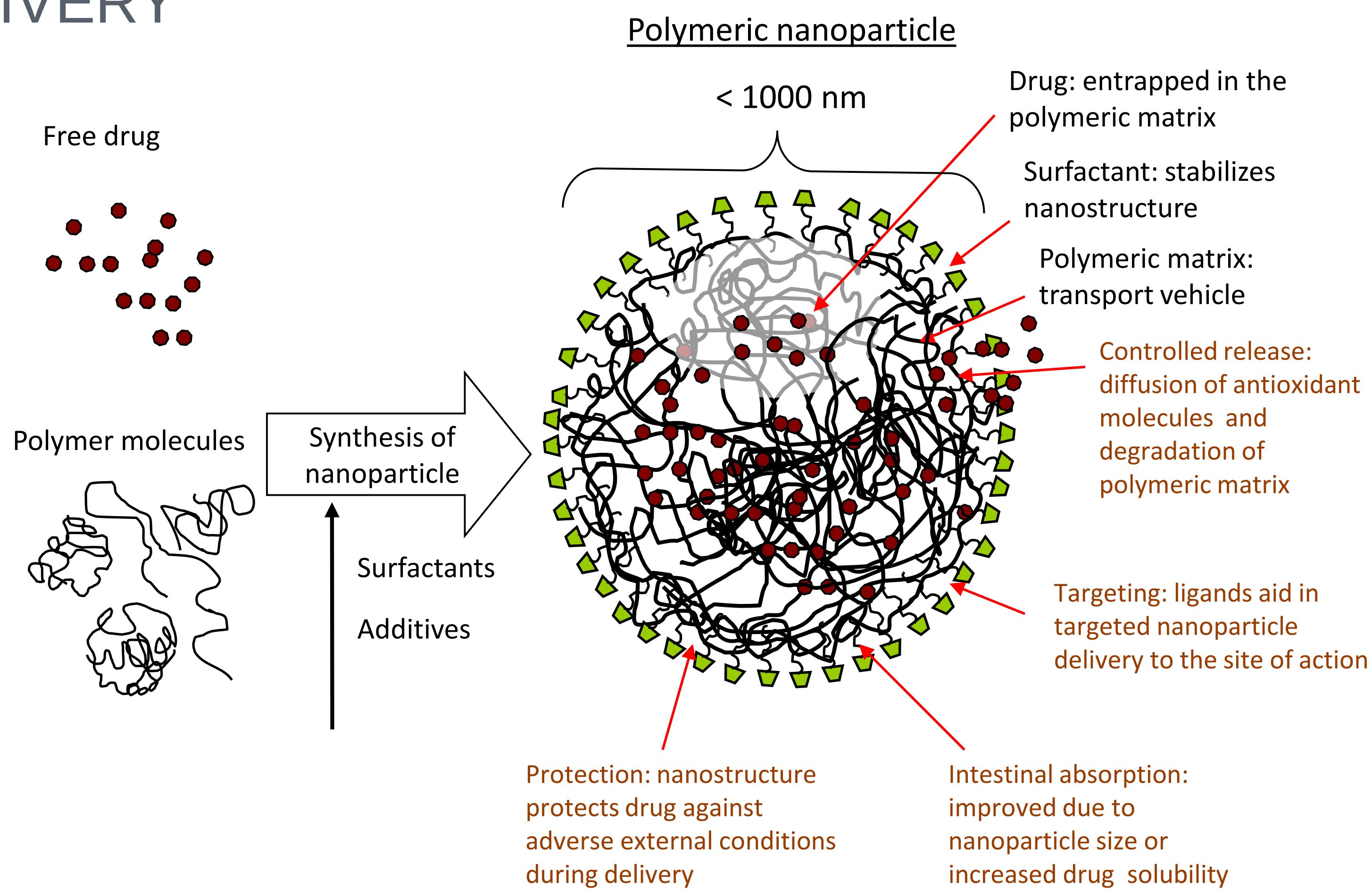



A. H. Faraji, P. Wipf. 2009. Nanoparticles in cellular drug delivery. Bioorganic and Medicinal Chemistry. 17: 2950-2962.




Kumari, A., S. K. Yadav, and S. C. Yadav. 2009. Biodegradable polymeric nanoparticles based drug delivery systems-Review. Colloids and Surfaces B: Biointerfaces. Doi: 10.1016/j.colsurfb.2009.09.001.

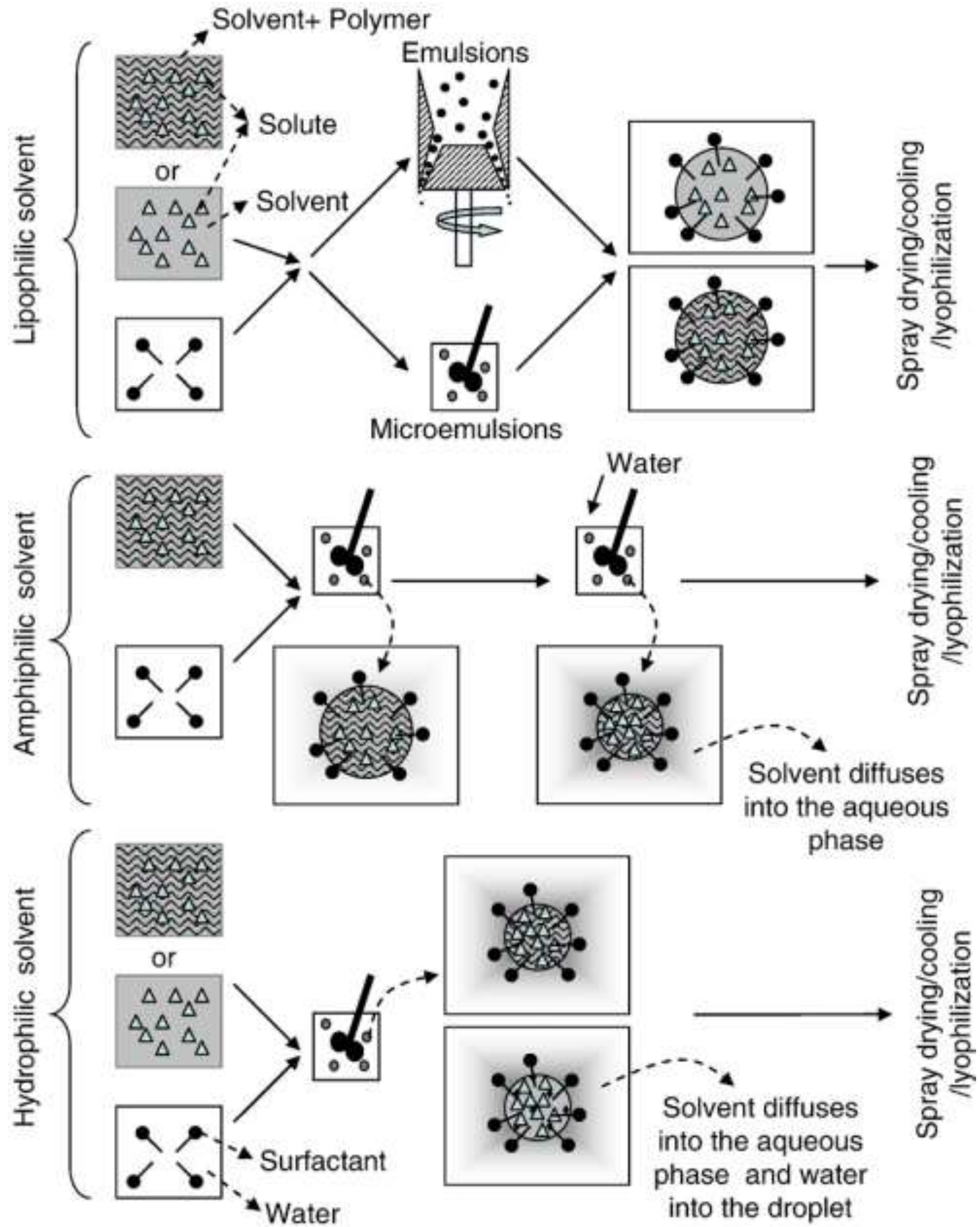
# Polymeric nanoparticles in drug delivery





Sabliov, C. M. and C. E. Astete. 2008. Chapter 17: Encapsulation and controlled release of antioxidants and vitamins via polymeric nanoparticles. In **Delivery and controlled release of bioactives in foods and nutraceuticals** (Ed. N. Garti). Woodhead Publishing. ISBN 9781845691455.

# Chemical Methods




Acosta, E. 2009. Bioavailability of nanoparticles in nutrient and nutraceutical delivery. Current Opinion in Colloid & Interface Science. 14: 3-15.

# Nanoparticle synthesis

- Emulsion evaporation

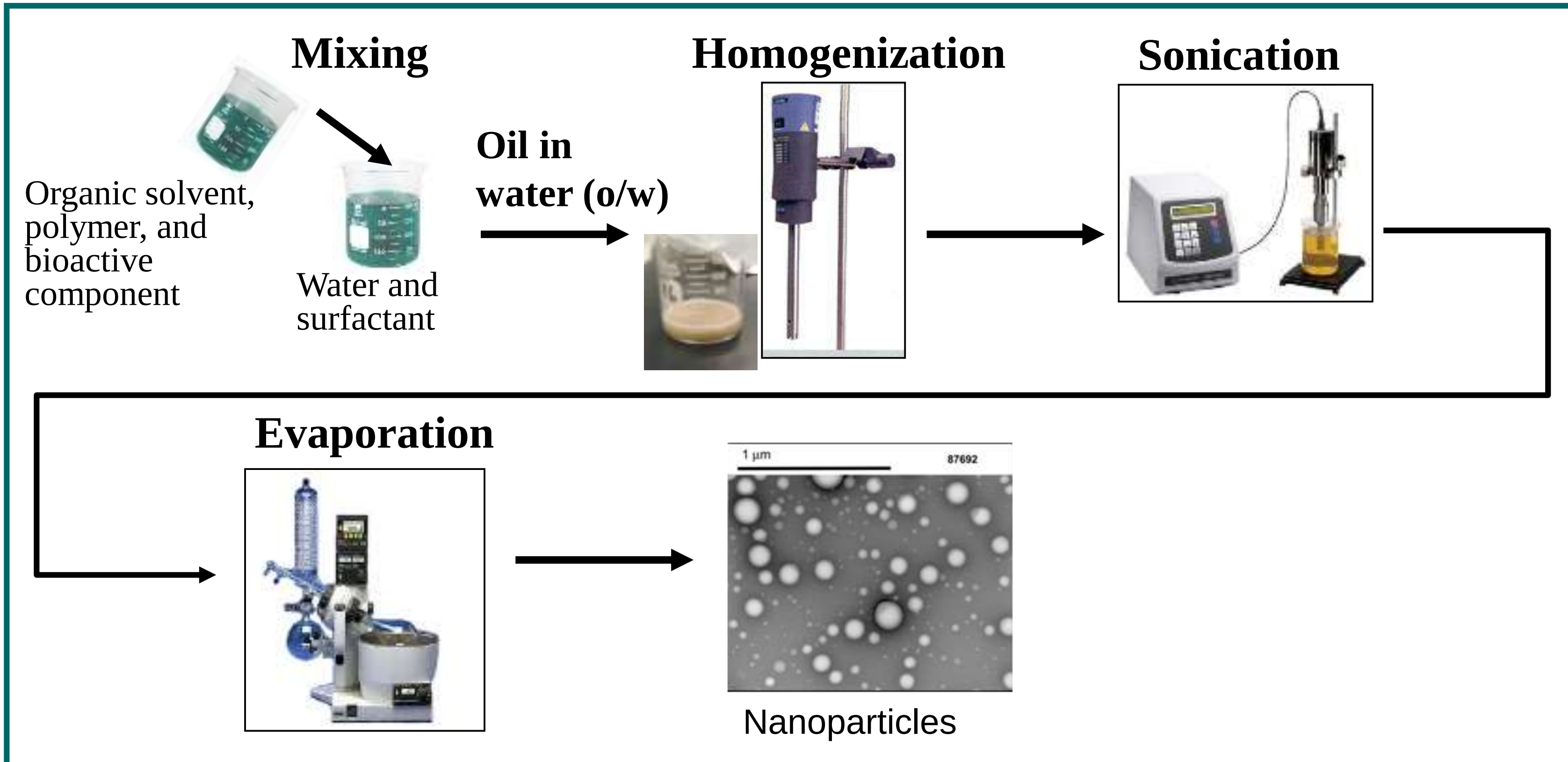


Table 1
Summary of methods used for preparation used for preparation of polymeric nanoparticles

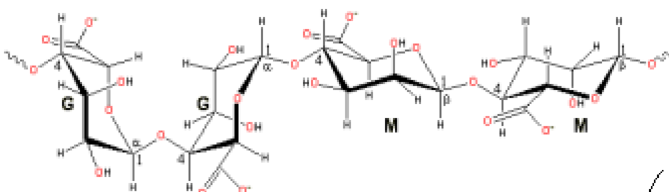
| Method | Polymer | Solvent | Stabilizer | Size (nm) | Reference |
|---|---|---|---|---|---|
| Solvent diffusion | PLGA | Acetone | Pluronic F-127 | 200 | [33] |
| | PLGA | Acetone/DCM | PVA | 200–300 | [19] |
| | PLA-PEG | MC | PVA/PVP | ~130 | [45] |
| | PHDCA | THF | – | 150 | [38] |
| | PLGA | Acetone | Sodium cholate | 161 | [36] |
| | PLGA | Propylene carbonate | PVA or DMAB | ~100 | [16] |
| Solvent displacement | PLA | Acetone/MC | Pluronic F68 | 123±23 | [41] |
| | SB-PVA-g-PLGA | Acetone/ethyl acetate | Poloxamer 188 | ~110 | [32] |
| Nanoprecipitation | PLGA/PLA/PCL | Acetone | Pluronic F68 | 110–208 | [20] |
| | PLGA | Acetonitrile | – | 157.1±1.9 | [18] |
| Solvent evaporation | PLA-PEG-PLA | DCM | – | 193–335 | [48] |
| | PLGA | DCM | PVA | 800 | [10] |
| | PEO-PLGA | MC | PVA | 150±25 | [8] |
| Multiple emulsion | PLGA | Ethyl acetate | – | >200 | [37] |
| | PLGA | Ethyl acetate/MC | PVA/PVP | ~280 | [45] |
| | PLGA | Ethyl acetate/MC | PVA | 335–743 | [34] |
| | PLGA-mPEG | DCM | – | 133.5±3.7–163.3±3.6 | [49] |
| | PLGA | DCM | PVA | 70–160 | [42] |
| | PLGA | DCM | PVA | 213.8±10.9 | [14] |
| | PLGA | DCM/acetone | PVA | 100 | [9] |
| | PLGA | DCM | PVA | ~250 | [6] |
| | PLGA | Ethyl acetate | PVA | 192±12 | [12] |
| | PLGA | Ethyl acetate | PVA | ~300 | [7] |
| | PLGA | DCM | PVA | 380±40–1720±110 | [13] |
| Salting out | PLA | Acetone | PVA | 300–700 | [21] |
| Ionic gelation | Chitosan | TPP | – | 278±03 | [41] |
| Interfacial deposition | PLGA | Acetone | – | 135 | [40] |
| Phase inversion nanoencapsulation | PLGA | MC | – | >5 μm | [15] |
| Polymerization | CS-PAA | – | – | 206±22 | [25] |
| | PECA | – | Pluronic F68 | 320±12 | [31,26] |
| | PE-2-CA | – | – | 380±120 | [30] |

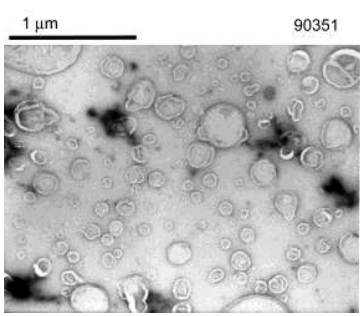
Size is in nm, unless otherwise indicated. DCM, dichloromethane; MC, methylene chloride; PVP, polyvinylpyrrolidone; PHDCA, poly(hexadecylcyanoacrylate); THF, tetrahydrofuran; SB-PVA-g-PLGA, sulfobutylated PVA, graft, PLGA; PCL, poly(epsilon-caprolactone); TPP, sodium tripolyphosphate; PAA, poly(acrylic acid); PECA, polyethylcyanoacrylate; PE-2-CA, polyethyl-2-cyanoacrylate.

# MATERIAL SELECTION- POLYMERS

## Alginic acid

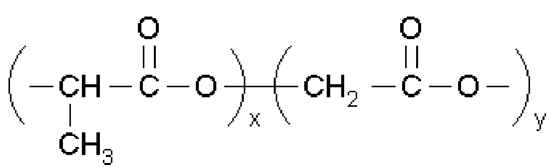
- Polysaccharide with mannuronic and guluronic acid
- Negative charges from carboxylic groups
- Thickening agent

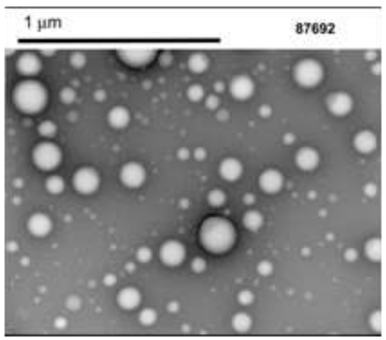



## PLGA

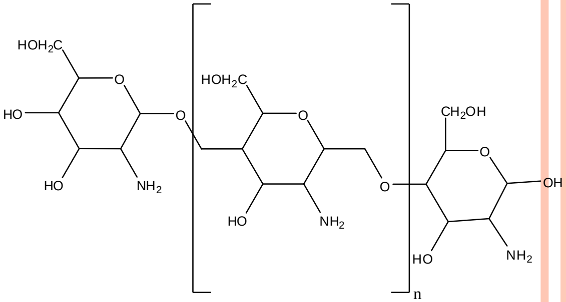
- Poly(lactic-co-glycolic) acid
- Biocompatible and biodegradable
- Mostly used for biomedical applications

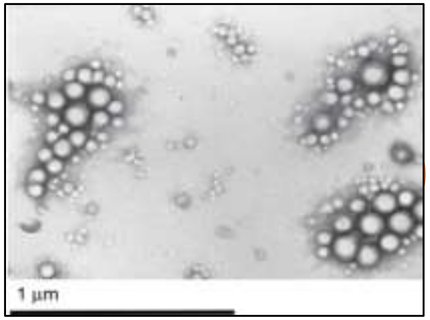



## Chitosan

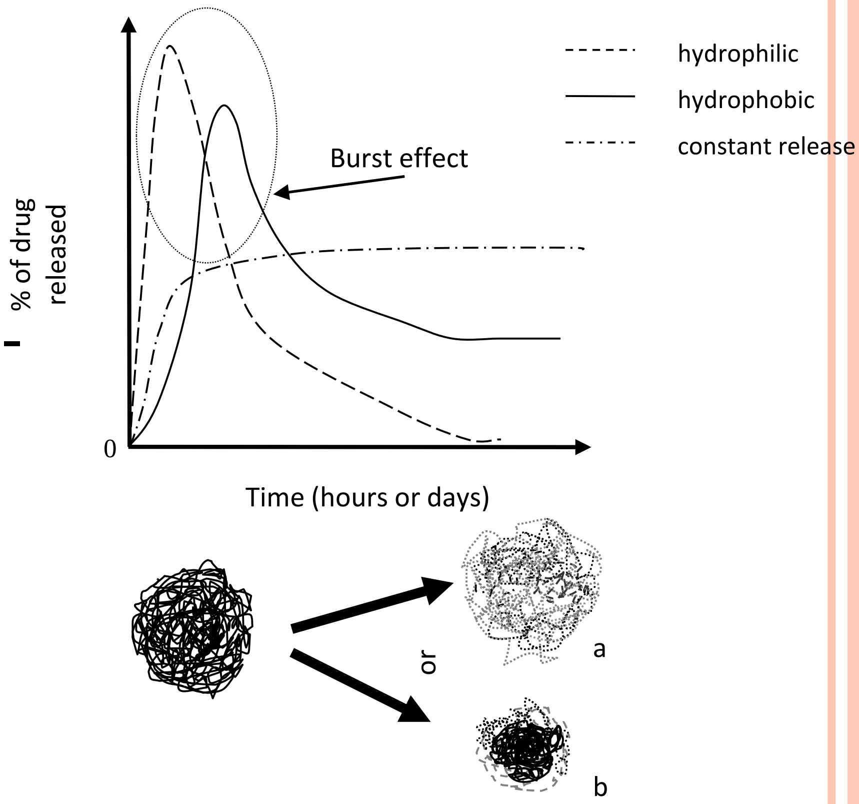
- *N*-deacetylated derivative of chitin
- Degrades to non-toxic compounds
- Positively charged



# Nanoparticle Properties

- Size and size distribution
- Zeta potential (+ or -)
- Drug entrapment efficiency (hydrophobic or hydrophylic)
- Drug release properties (drug-polymer interactions and polymer degradation)
- Degradation properties (bulk eroding or surface eroding)
- Nanoparticle-cell interactions (charge, size)
- Biotoxicity (adhesion, uptake, translocation)



## PROJECT I: IMPROVED DELIVERY OF ANTIOXIDANT LIPOPHILIC VITAMIN

## PROJECT II: ANTIMICROBIAL POLYMERIC NANOPARTICLES

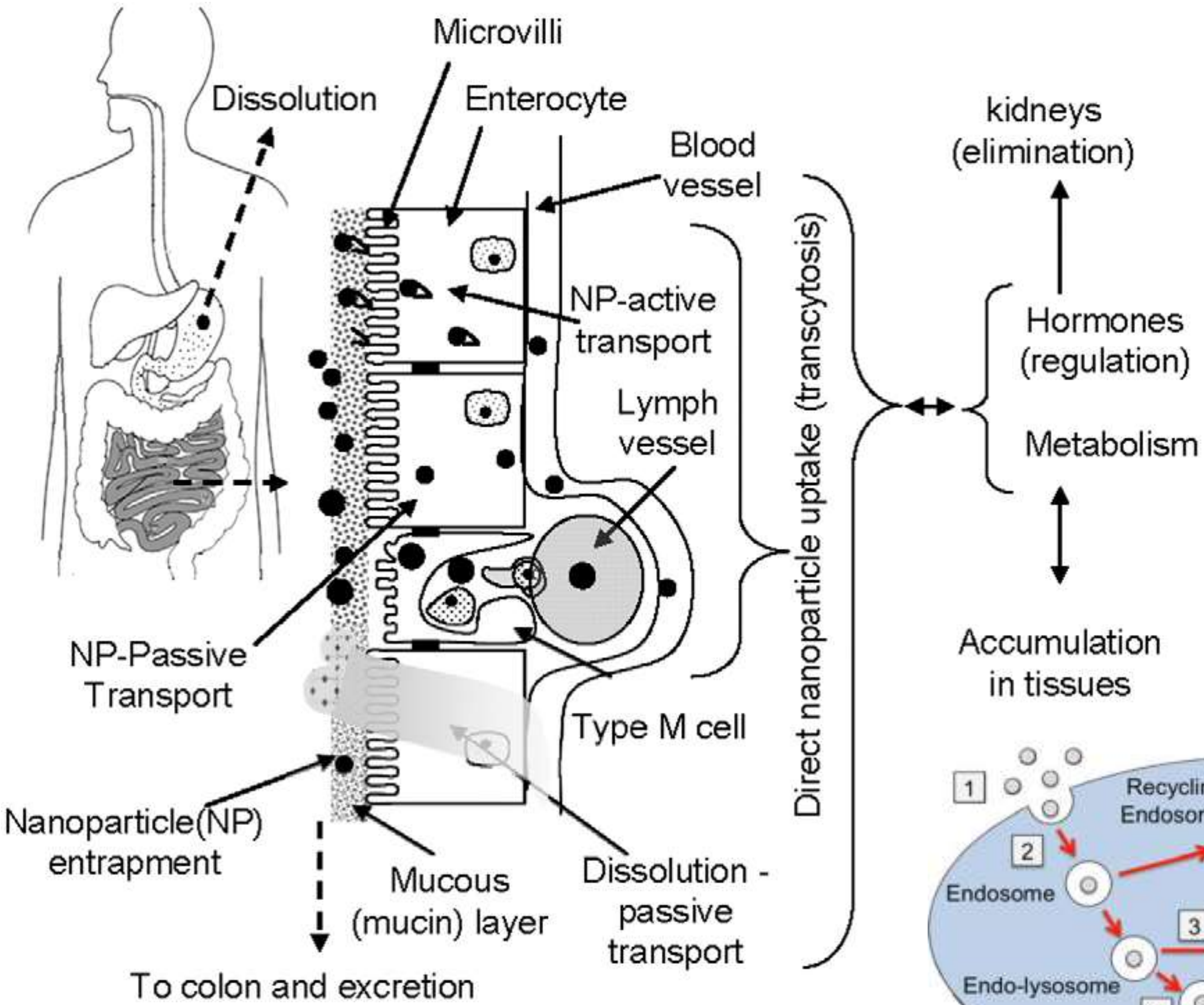
## PROJECT III: IMPROVED FUNCTIONALITY OF HYDROPHOBIC NATURAL COLORANT

# PROJECT I

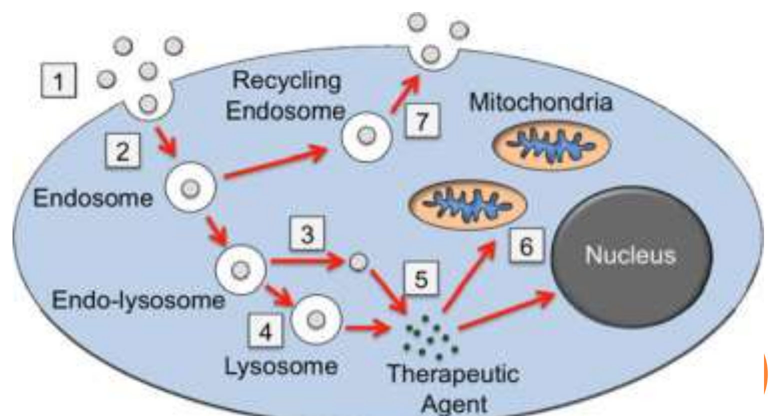
## IMPROVED DELIVERY OF ANTIOXIDANT LIPOPHILIC VITAMIN

Imola Zigoneanu, Carlos Astete, and Abitha Murugeshu

# ORAL DRUG DELIVERY



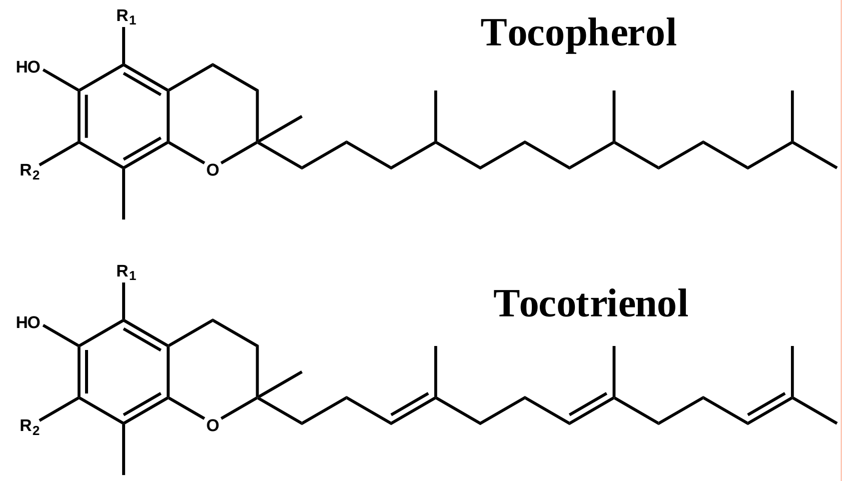

ACOSTA, E. 2009. BIOAVAILABILITY OF NANOPARTICLES IN NUTRIENT AND NUTRACEUTICAL DELIVERY. CURRENT OPINION IN COLLOID & INTERFACE SCIENCE. 14: 3-15.




A. H. FARAKI, P. WIPF. 2009. NANOPARTICLES IN CELLULAR DRUG DELIVERY. BIOORGANIC AND MEDICINAL CHEMISTRY. 17: 2950-2962.

# MODEL LIPOPHILIC BIOACTIVE (ALPHA-TOCOPHEROL)

- Antioxidant lipophilic vitamin
  - Prevents damage from chemical reactions related to cancer, diabetes, cardiovascular disease, inflammatory responses, degenerative diseases, aging, liver injury, cataract, etc.
- Tocopherols +tocotrienols = Vitamin E
  - α- tocopherol is the most biologically active form




| Tocopherol & Tocotrienol | $R_1$ | $R_2$ |
|---|---|---|
| α | $CH_3$ | $CH_3$ |
| β | $CH_3$ | H |
| γ | H | $CH_3$ |
| δ | H | H |

# Vitamin E Transport

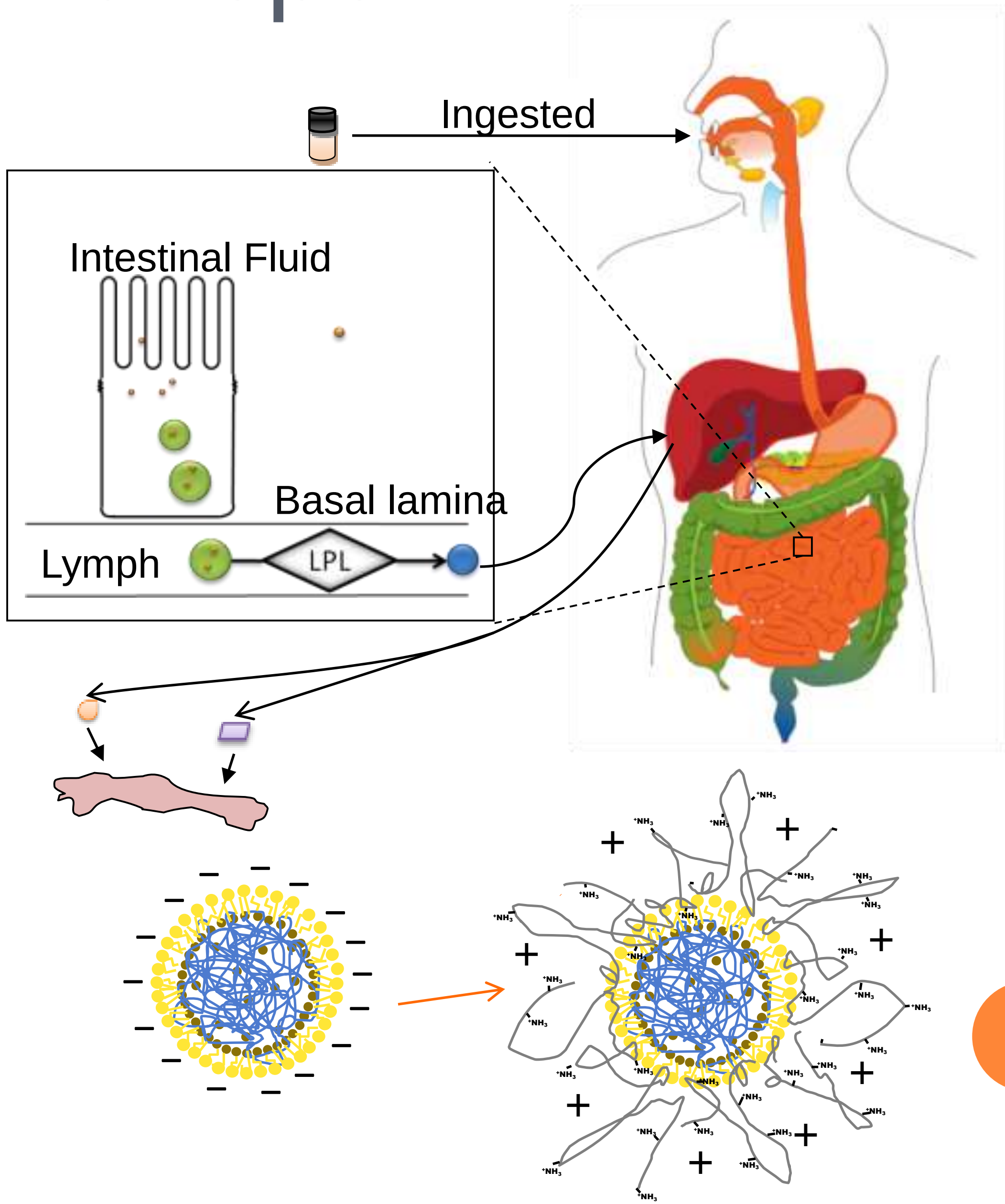
Alpha-tocopherol

Low aqueous solubility

Premature elimination

Entrapment in a delivery vehicle

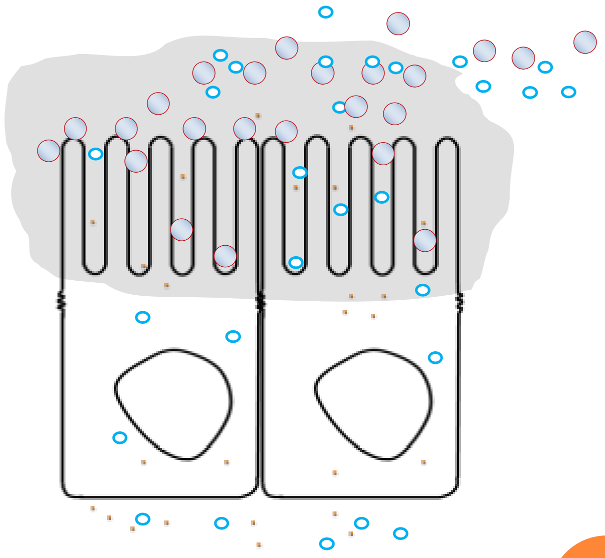
- ensures solubility and transport in aqueous media
- mucoadhesion increases gastrointestinal retention
- controlled release of lipophilic substance from designed matrix



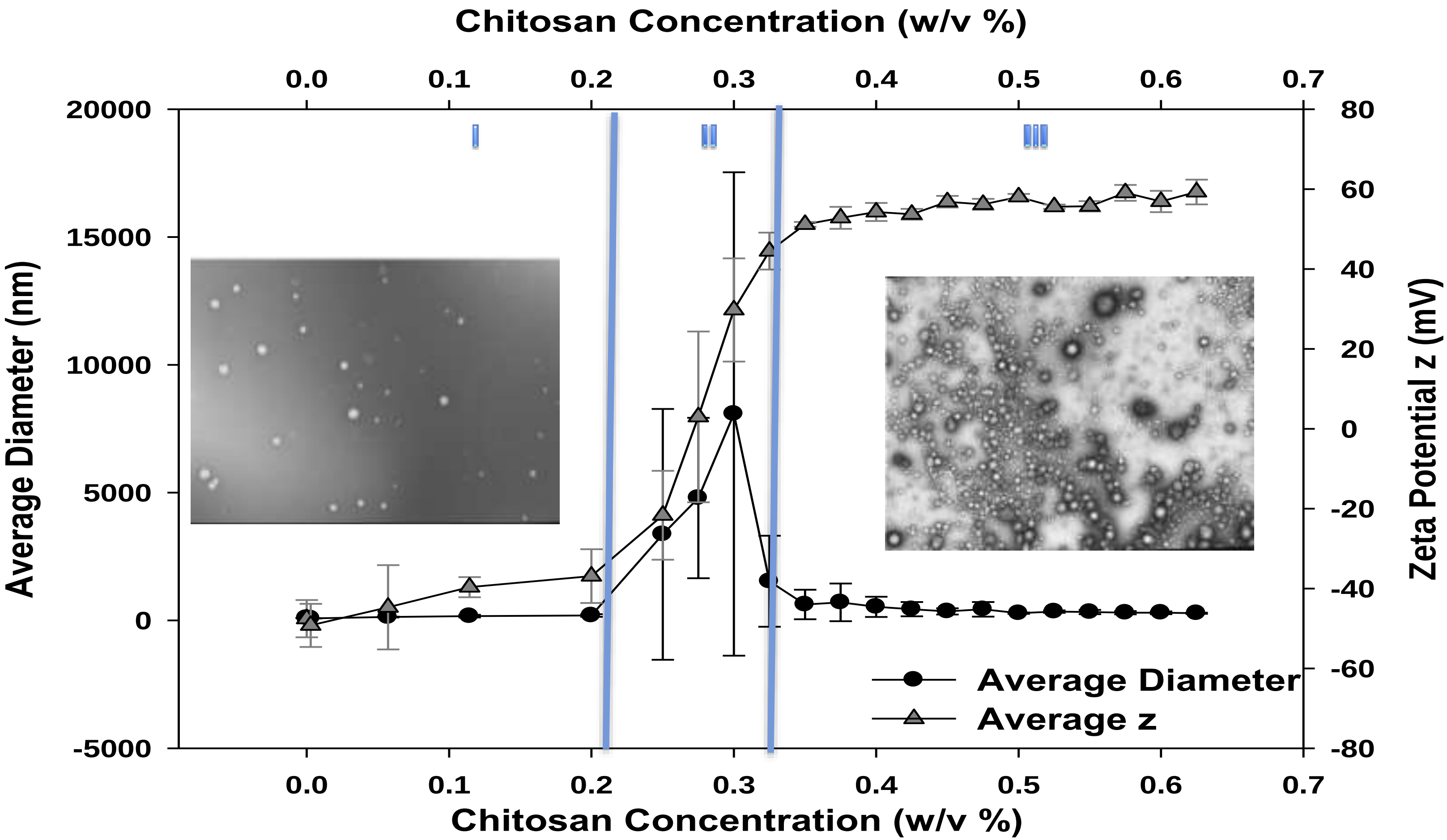
# Rationale

- This project aimed to prolong the gastrointestinal residence time of lipophilic vitamins by entrapping the vitamins in mucoadhesive cationic Chitosan/PLGA nanoparticles.

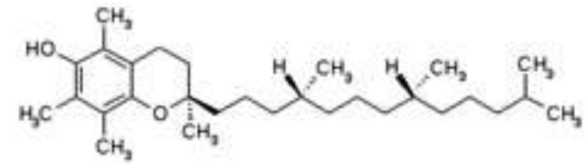
  - Mucoadhesive particle systems were desired because they decrease translocation into the cells, and toxicity
  - Lecithin (FDA approved), Chitosan & PLGA (FDA approved for medical purposes)



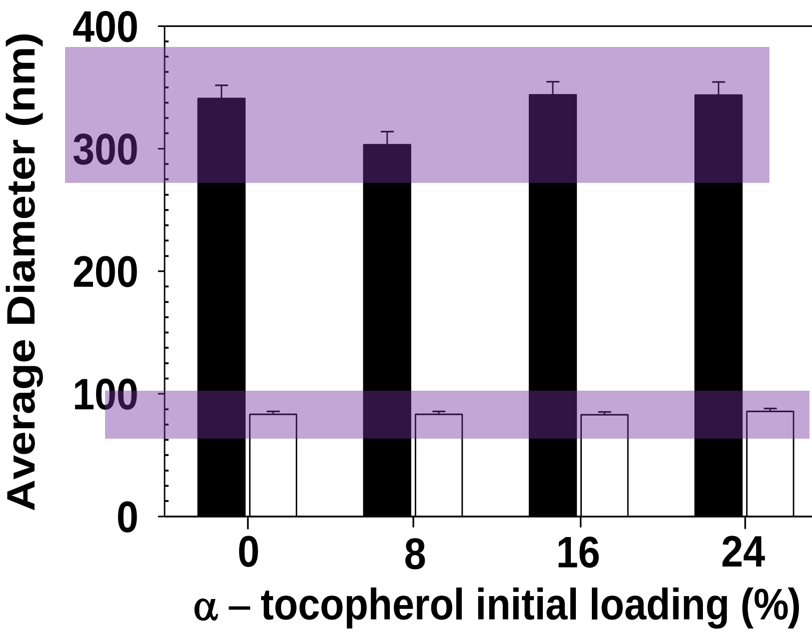
# Chitosan effect on NPs

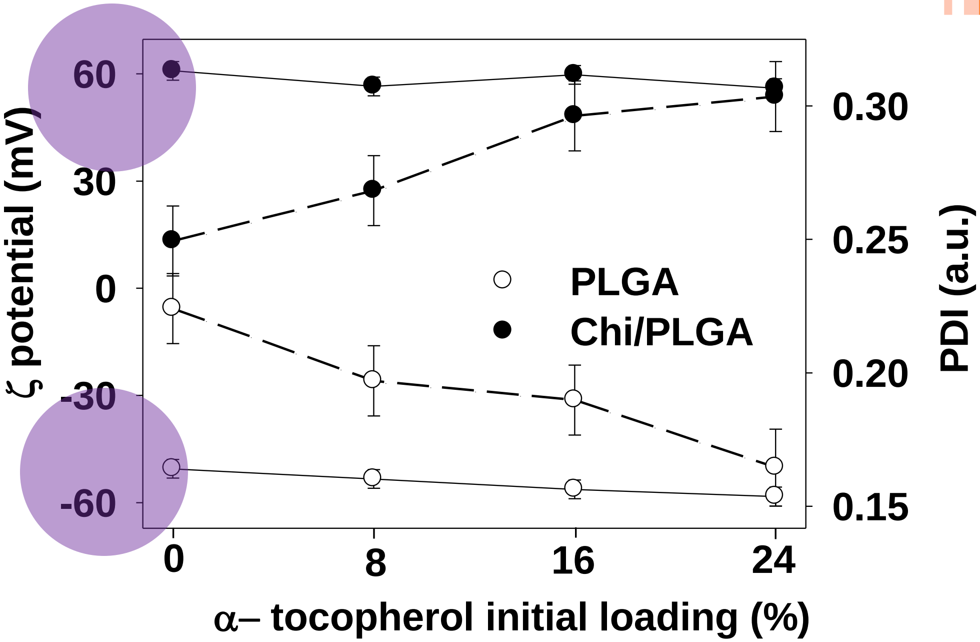



- 3 regions observed
- 0.6 w/v% chitosan concentration selected for positively charges, stable particles



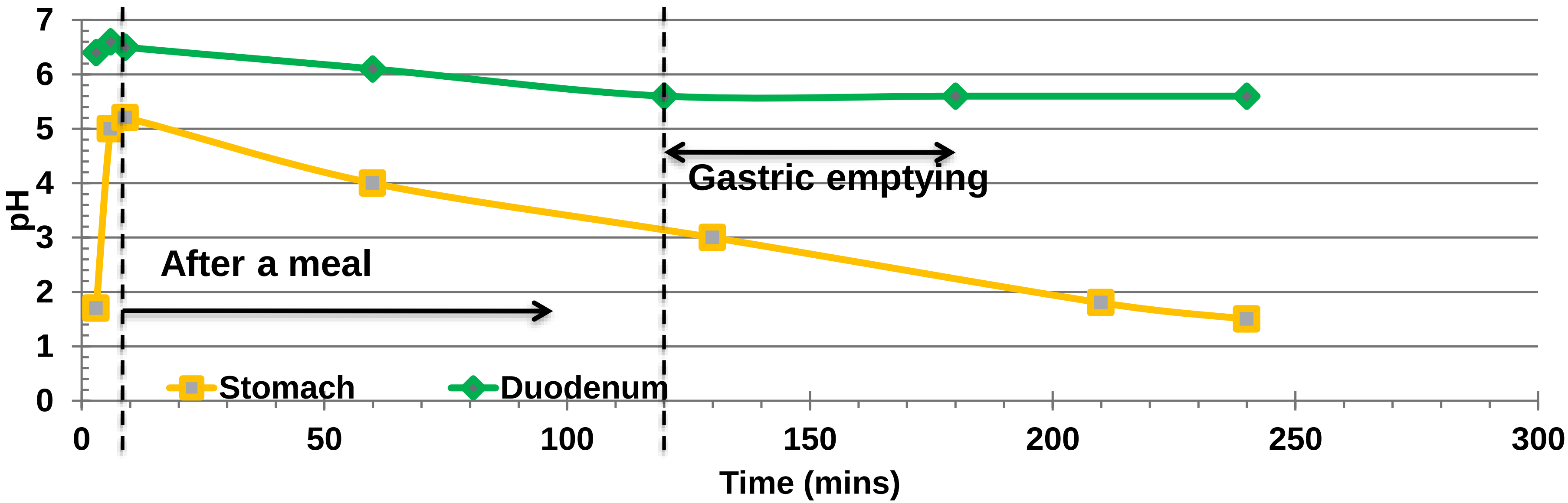

# ALPHA-TOCOPHEROL INITIAL LOADING EFFECT




| αT theoretical loading (% w/w relative to PLGA) | Entrapment Efficiency (% of αT theoretical loading) | PLGA/Lecithin/a-TOC | | Chi/ PLGA/Lecithin/a-TOC | |
|---|---|---|---|---|---|
| | | Size (nm) | Zeta (mV) | Size (nm) | Zeta (mV) |
| 0 | 0 | 83.2 ± 2.35 | -50.6 ± 2.62 | 340.9 ± 10.72 | 60.9 ± 2.62 |
| 8 | 52 ± 0.83 | 83.3 ± 2.35 | -53.4 ± 2.62 | 303.2 ± 10.72 | 56.5 ± 2.62 |
| 16 | 58 ± 2.97 | 82.8 ± 2.35 | -56.3 ± 2.62 | 343.9 ± 10.72 | 59.7 ± 2.62 |
| 24 | 59 ± 1.66 | 85.8 ± 2.35 | -58.3 ± 2.62 | 343.7± 10.72 | 56.0 ± 2.62 |

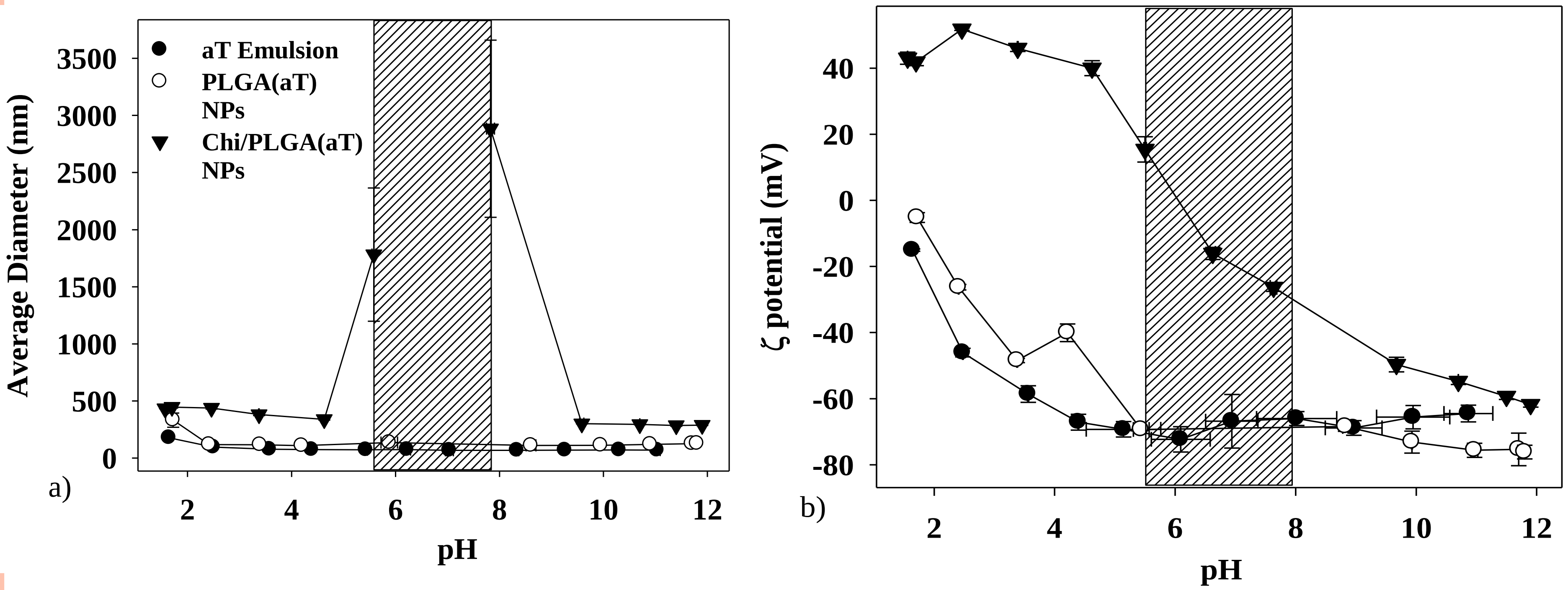
# GI pH change over a feeding cycle




- Gastric
  - Basal = 1.3- 1.7
  - Fed = can rise to ~ 5.0
    - **Average of 1.5 and 4.0**
- Intestinal
  - Basal = 5.9 – 7.4
  - Fed = drop to ~ 5.5
    - **Average of 5.5 and 6.5**

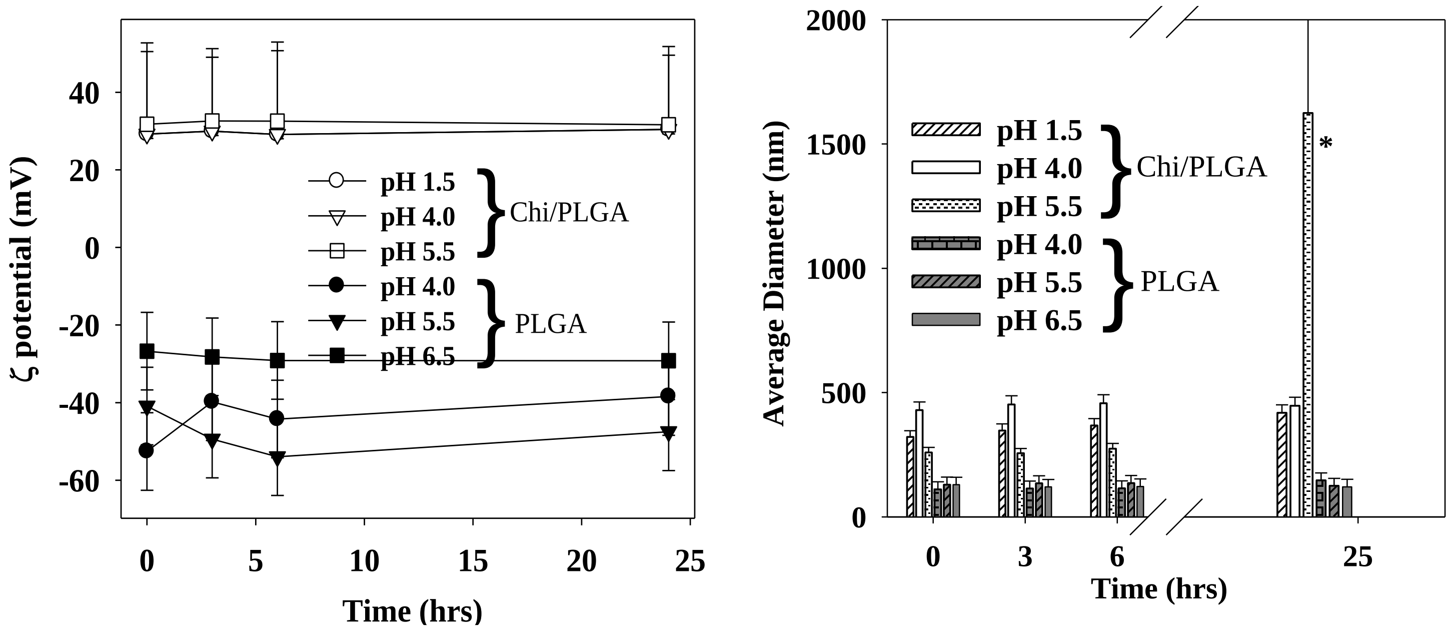
Russell, T., R. Berardi, et al. (1993). "Upper gastrointestinal pH in seventy-nine healthy, elderly, North American men and women." Pharmaceutical Research **10**(2): 187-196.

# pH Stability of Particles




- PLGA particles were negatively charged and approached zero below pH 2
- Chi/PLGA particles approached zero between pHs 5 -8

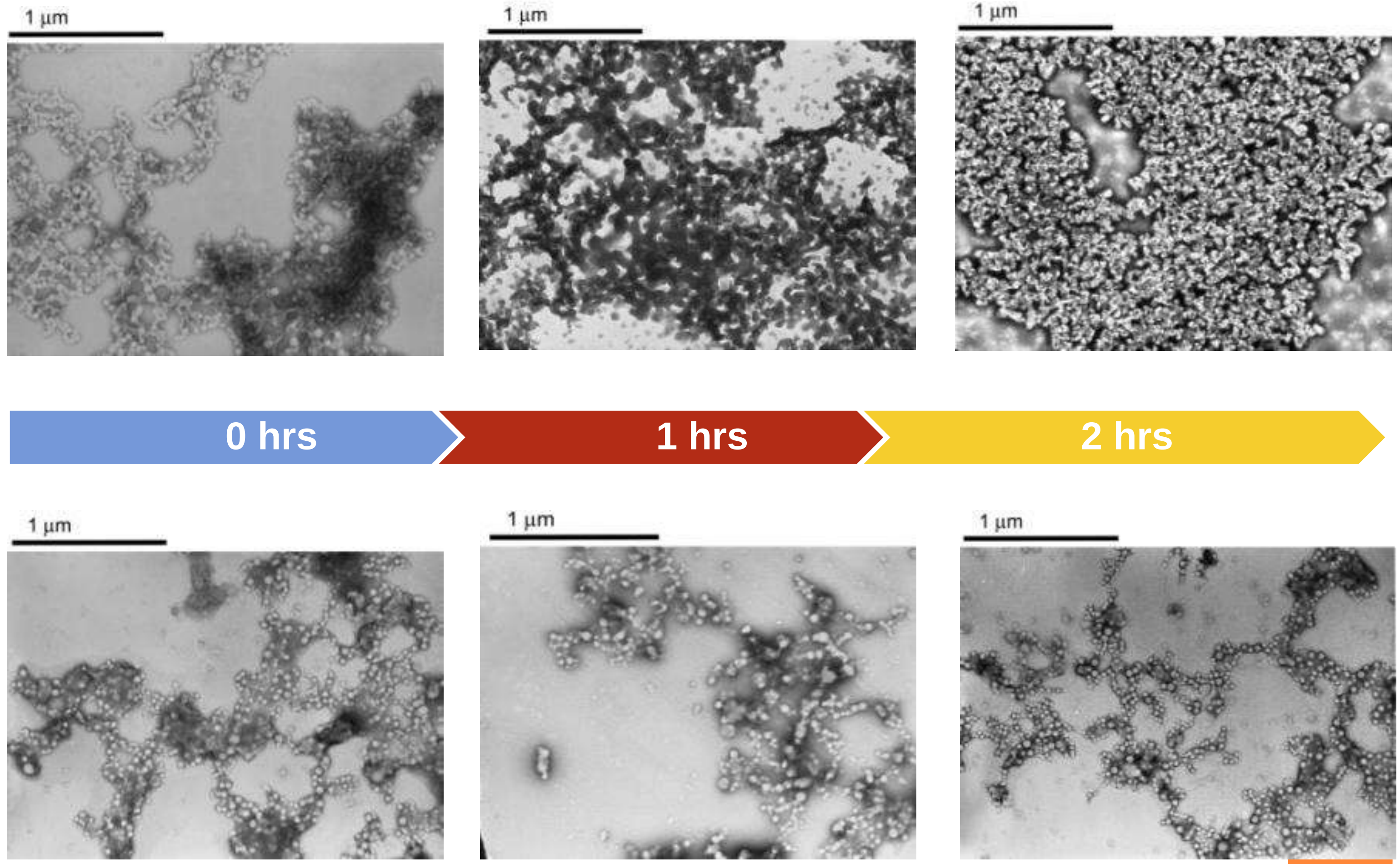
# STABILITY OF PARTICLES OVER TIME




- PLGA particles were stable for 24 hrs under pHs 5.5 and 6.5
- Chi/PLGA particles remained stable for 24 hrs under pHs 1.5 and 4.0
- Chi/PLGA particles aggregated after 24 hrs in pH 5.5, but remained stable for 6 hrs; Chi/PLGA particles precipitated at pH 6.5
- PLGA particles precipitated at pH 1.5,

# PLGA Particle TEM Morphology under gastric pHs

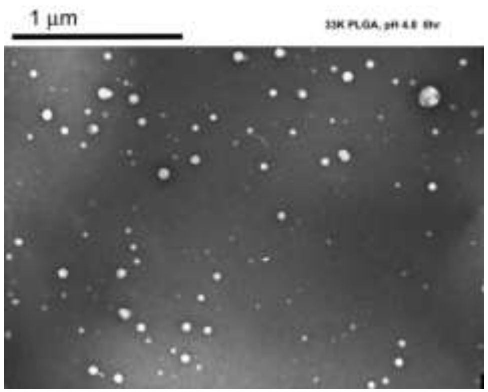
**PLGA 33K pH 1.5**

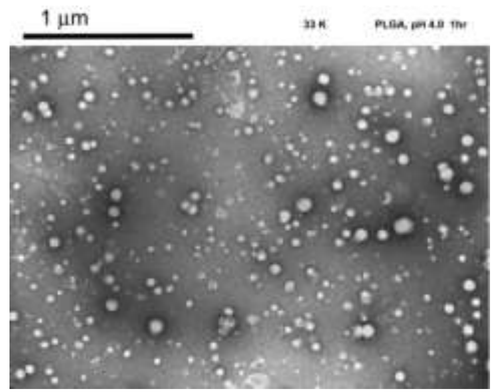


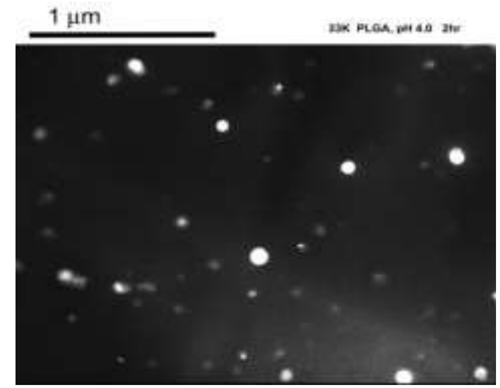

**PLGA 33K pH 4.0**

# PLGA Particle TEM Morphology under Intestinal pHs

**PLGA**
**33K**
**pH 5.5**

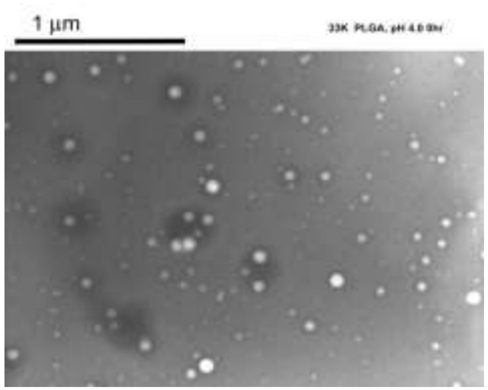


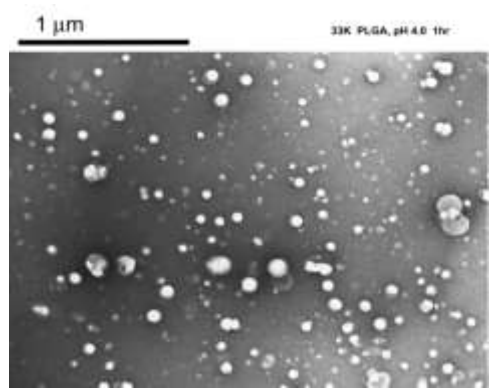



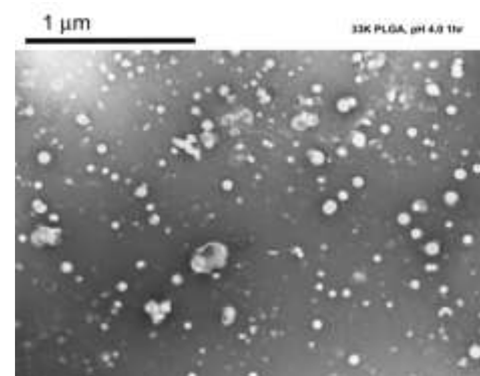




**0 hrs** → **6 hrs** → **24 hrs**

**PLGA**
**33K**
**pH 6.5**

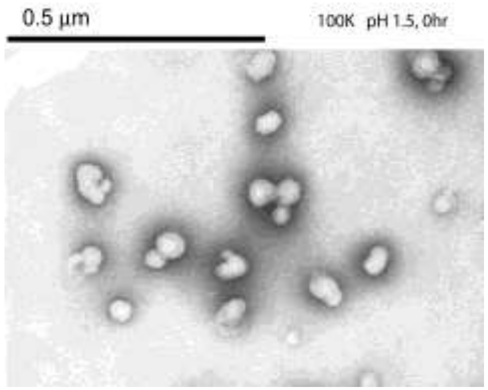


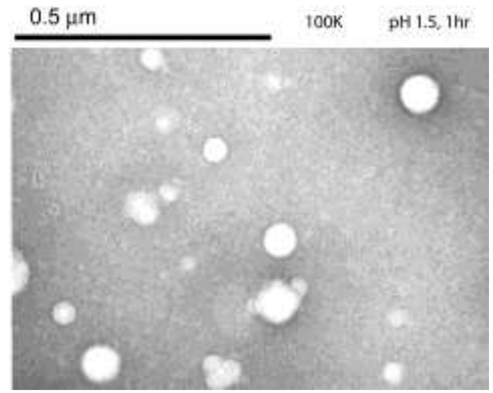



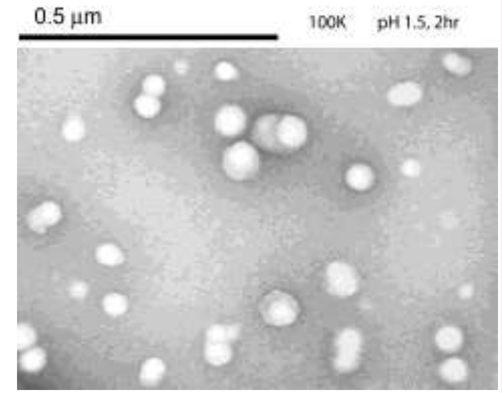



# Chi/PLGA Particle TEM Morphology at under gastric pHs

**Chi/PLGA 100K pH 1.5**

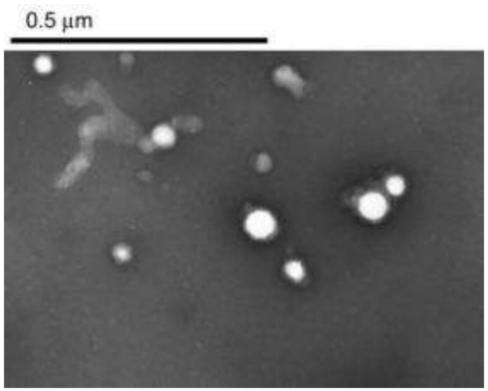


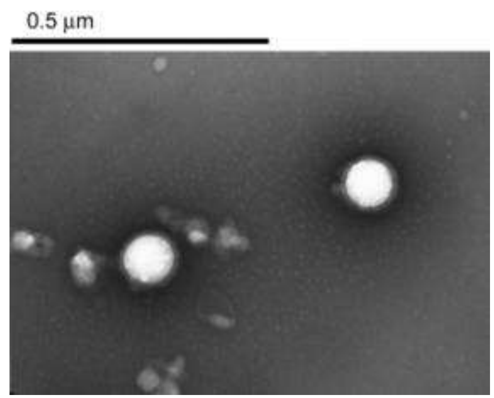



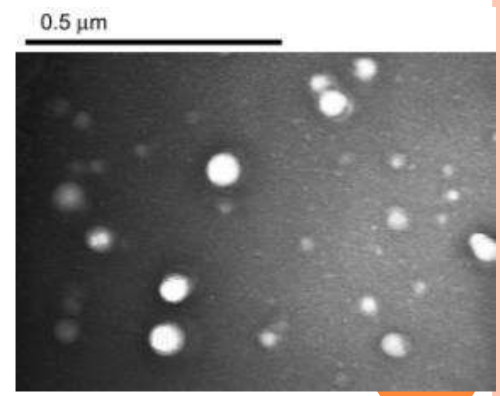




**0 hrs** **1 hrs** **2 hrs**

**Chi/PLGA 100K pH 4.0**

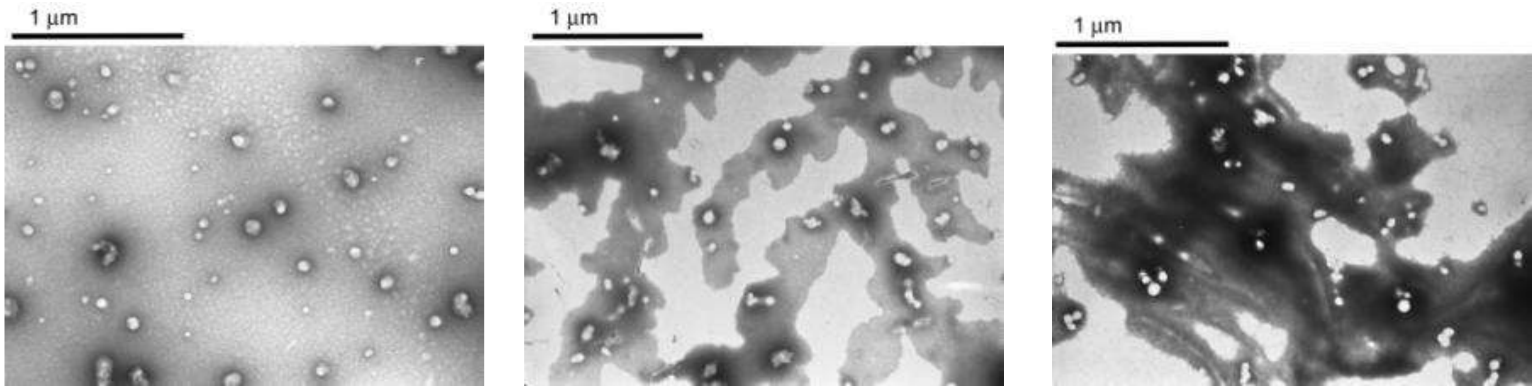


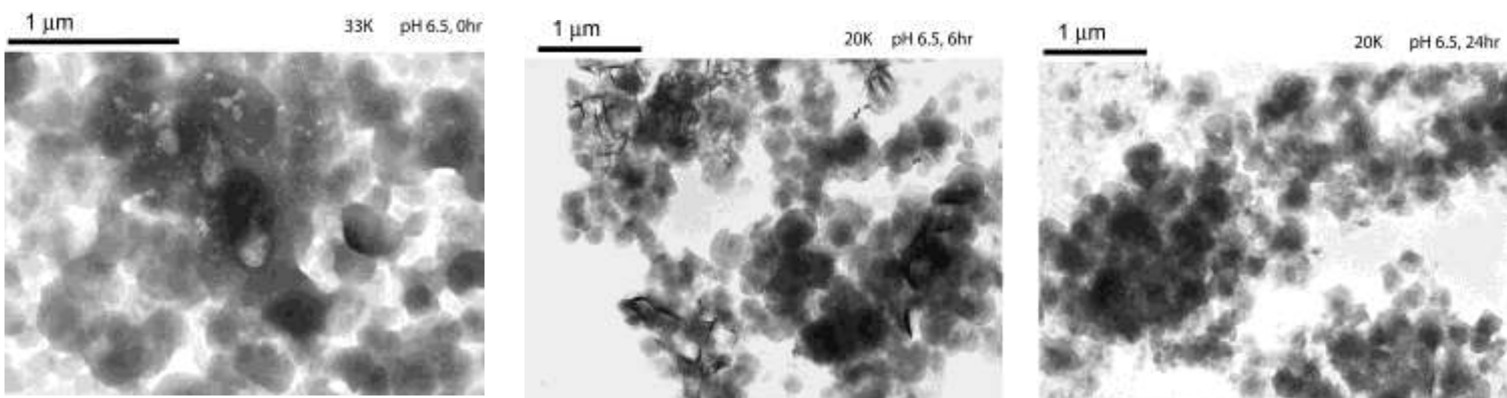
# Chi/PLGA Particle TEM Morphology under Intestinal pHs

**Chi/PLGA**
**33K**
**pH 5.5**

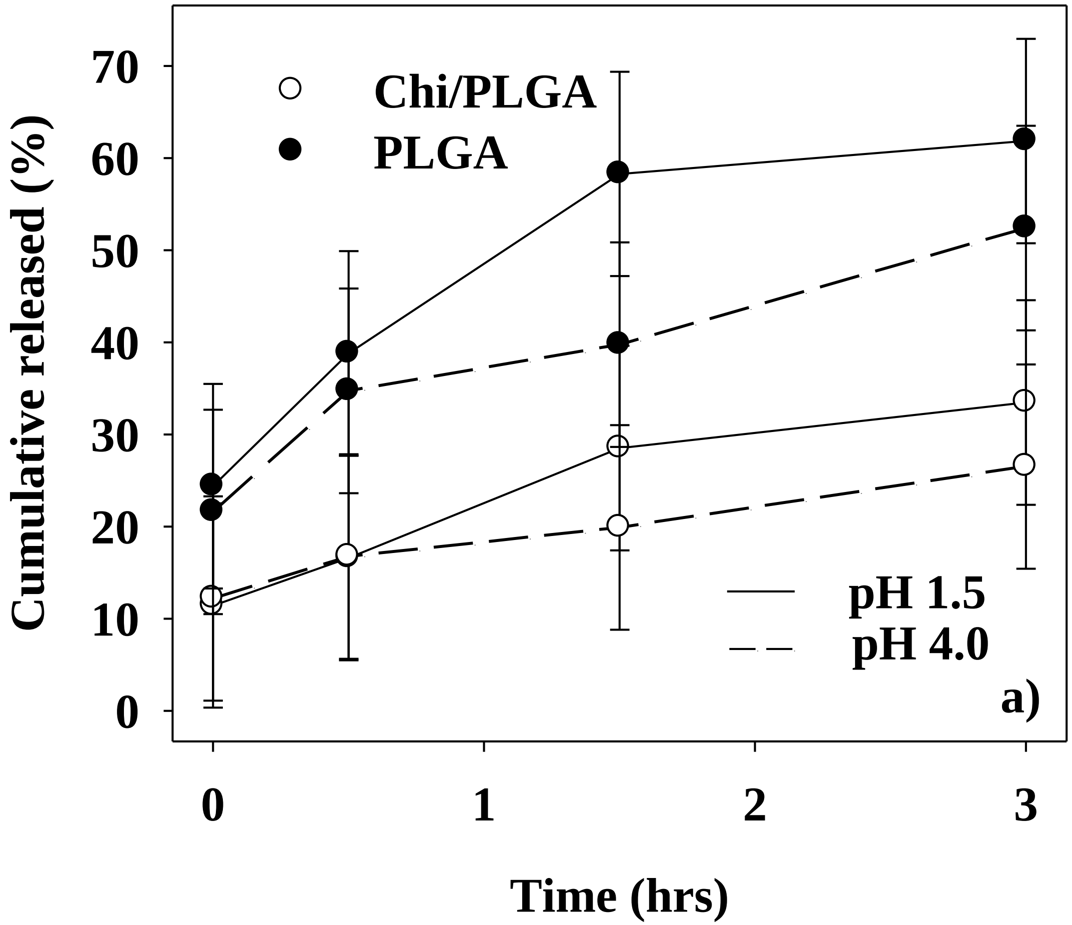



**0 hrs** → **6 hrs** → **24 hrs**

**Chi/PLGA**
**20 K & 33K**
**pH 6.5**

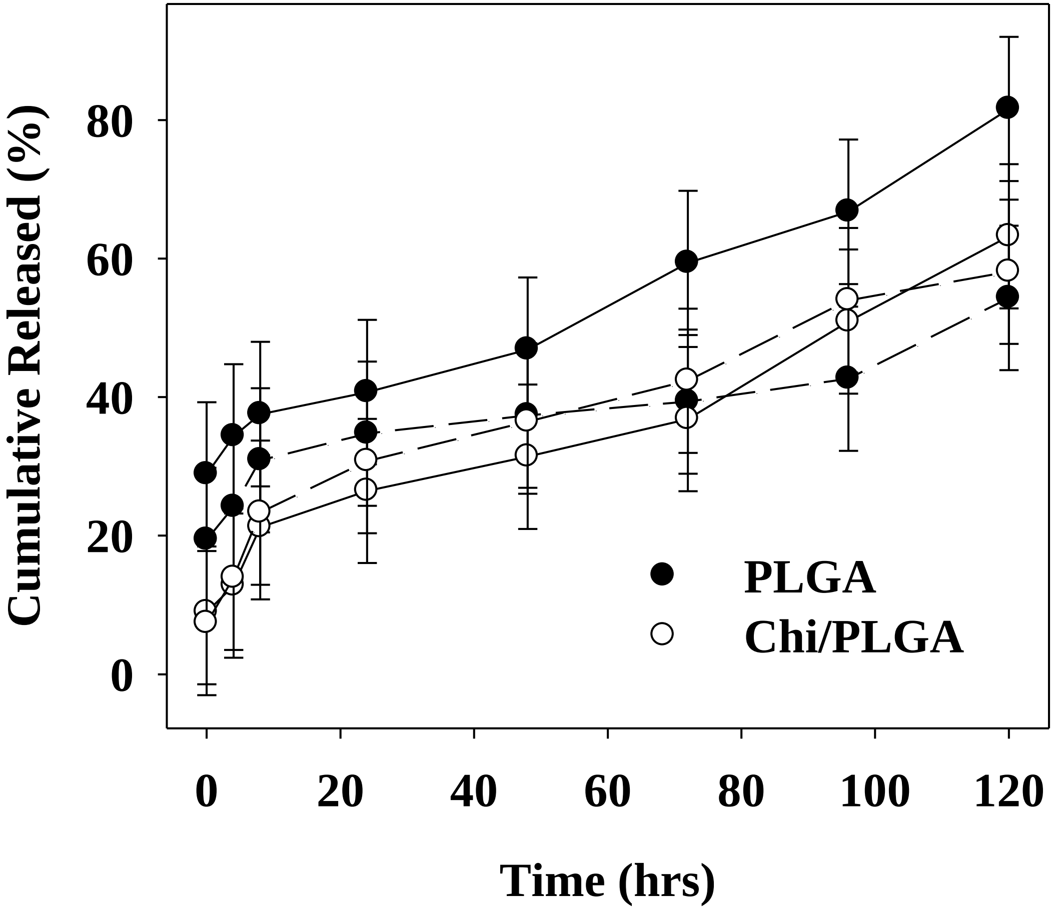


# Alpha-tocopherol gastric release from 16 % Initial loading particles- Effect of system



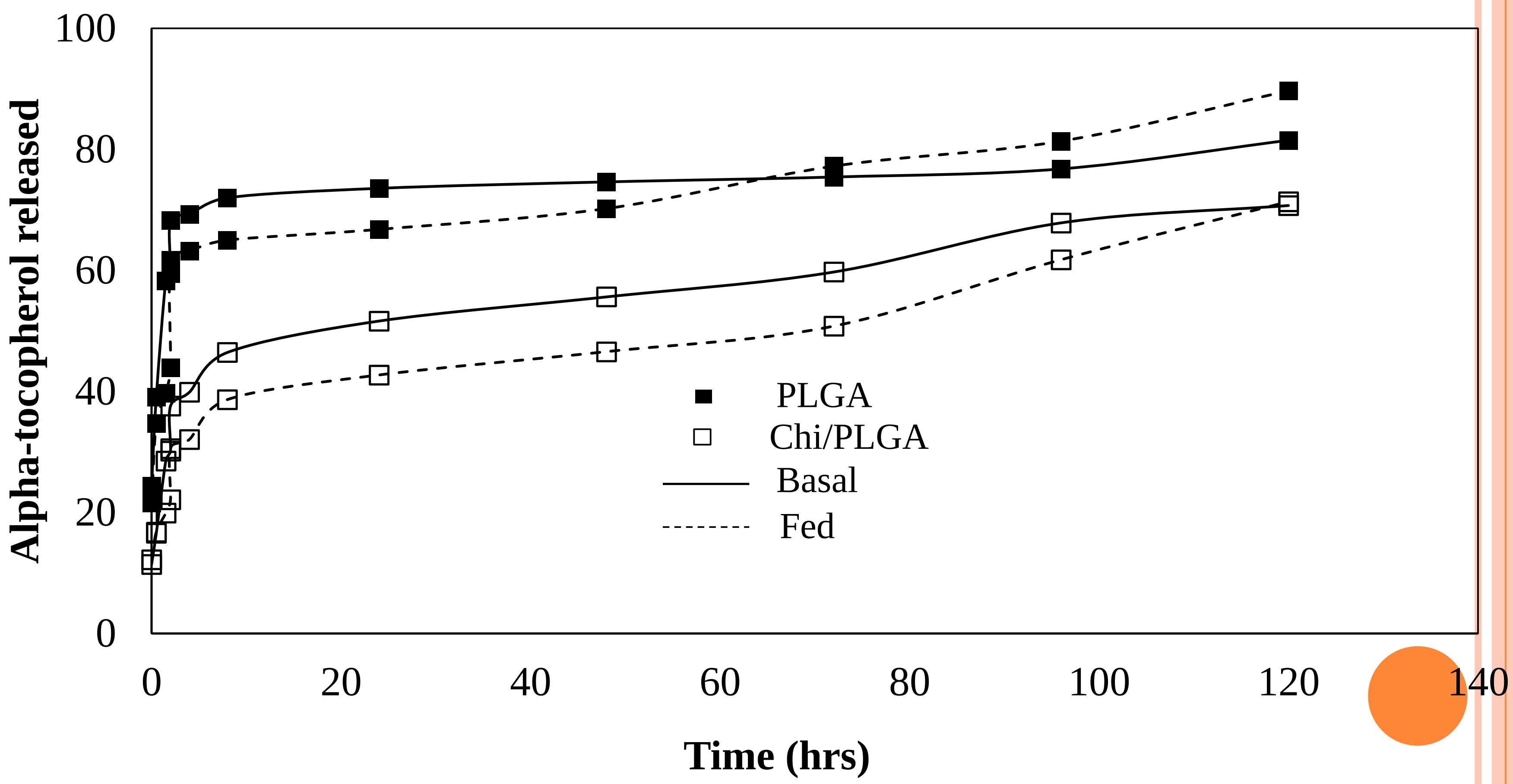
# ALPHA-TOCOPHEROL INTESTINAL RELEASE FROM 16 % INITIAL LOADING PARTICLES- EFFECT OF SYSTEM

- NSD in release rate between pHs and systems
- Slower initial release from Chi/PLGA
  - 19% (P) vs. 9% (CP) pH 5.5
  - 29% (P) vs. 7 % (CP) pH 6.5
  - 54% (P) vs. 58% CP after 5 days




PROJECT I

# GASTROINTESTINAL RELEASE OF ALPHA-TOCOPHEROL FROM 16% INITIAL LOADING PARTICLES



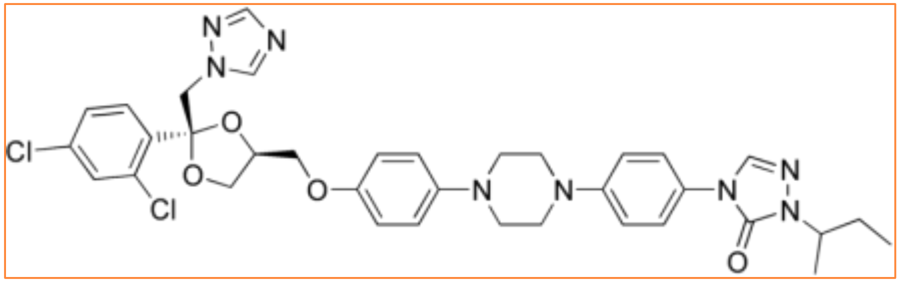
# CONCLUSIONS

- PLGA particles were stable under fed gastric, and all intestinal conditions for 24 hrs (but unstable under basal gastric conditions)
- Chi/PLGA particles were stable under all gastric for 24 hrs, and fed intestinal conditions for 6 hrs (but unstable under basal intestinal conditions)
- Chi/PLGA particles released most of the alpha-tocopherol in the intestine and only half of the amount entrapped was released in 5 days
- Chi/PLGA are better for increasing the particle retention time in the GI tract, AND subsequently expected to increase drug bioavailability

# ANTIMICROBIAL POLYMERIC NANOPARTICLES

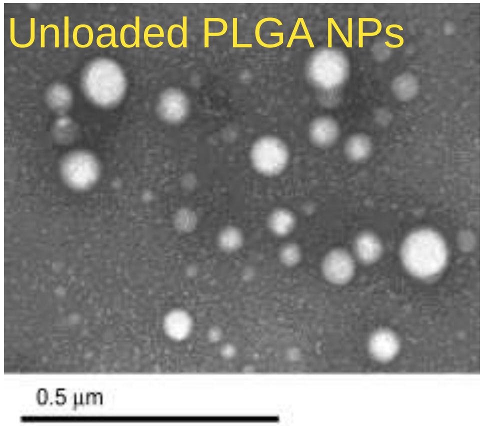
Nipur Patel

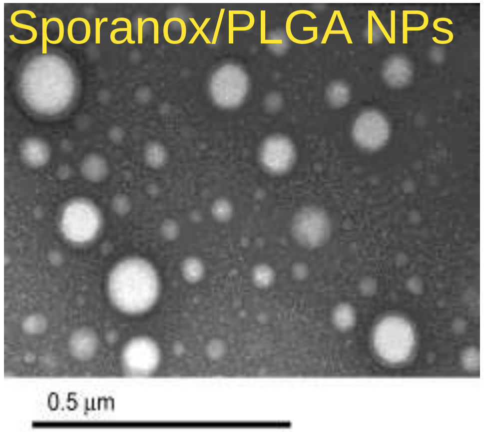
# ITRACONAZOLE (ITZ)



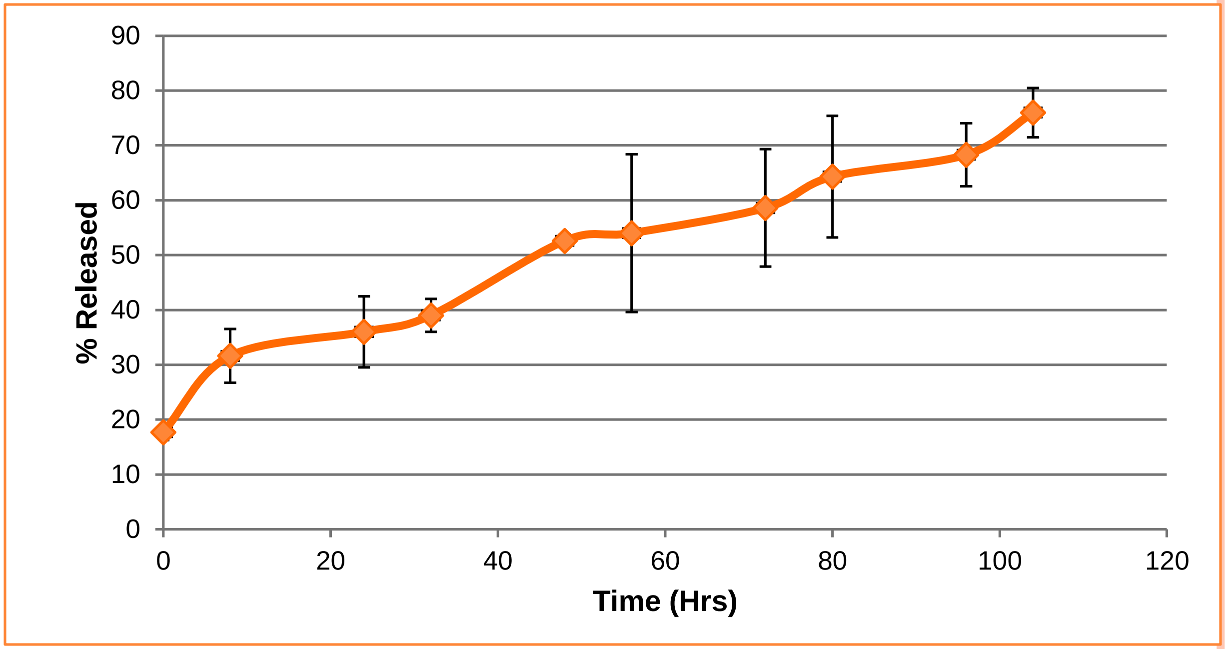
- ITZ acts by impairing the synthesis of ergosterol, essential component of the fungal cell membrane
- Antifungal effect of ITZ is limited due to low bioavailability (60%)
- It is insoluble in aqueous media (*S*~1 ng/mL at neutral pH and S= 4g/mL at pH 1)
- *Hypothesis: entrapping Itraconazole into PLGA nanoparticles improves its solubility, insures a controlled release of the drug over time, and hence improve its antifungal efficacy*

# PLGA NPs WITH ENTTRAPPED ITRACONAZOLE: PROPERTIES




| NP Type | Theoretical Loading (% w/w) | Size (nm) | Pdi (au) | Zeta (mV) |
|---|---|---|---|---|
| **Empty** | 0 | 201.32±5.33 | 0.010±0.031 | -33.14±8.40 |
| **PLGA1** | 12.5 | 232.11±1.76 | 0.213±0.035 | -31.89±5.30 |
| **PLGA2** | 25 | 199.75±4.66 | 0.114±0.042 | -24.28±2.49 |



# PLATE QUALITATIVE ANTIMICROBIAL ACTIVITY

**Itraconazole/water**

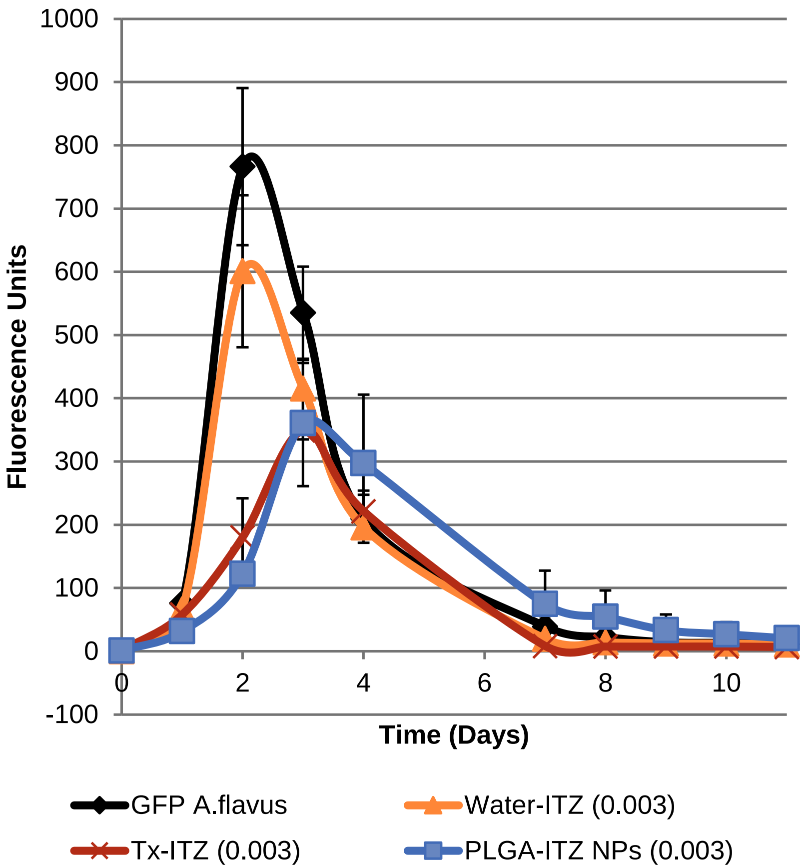

**Itraconazole/NP conc. x**


**Itraconazole/Triton X sol.**

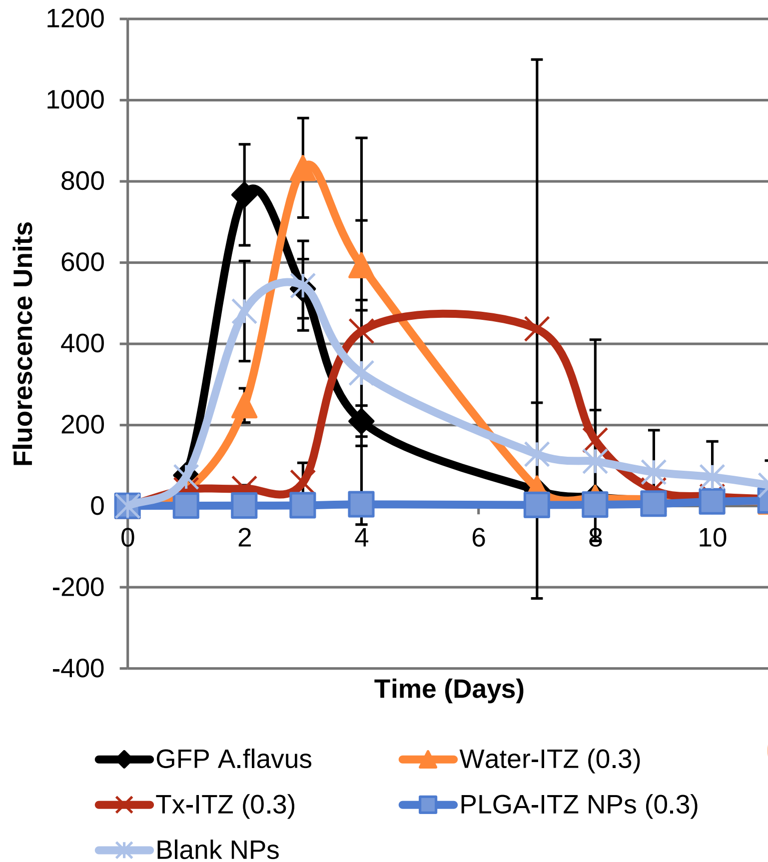

**Itraconazole/NP conc. 10x**

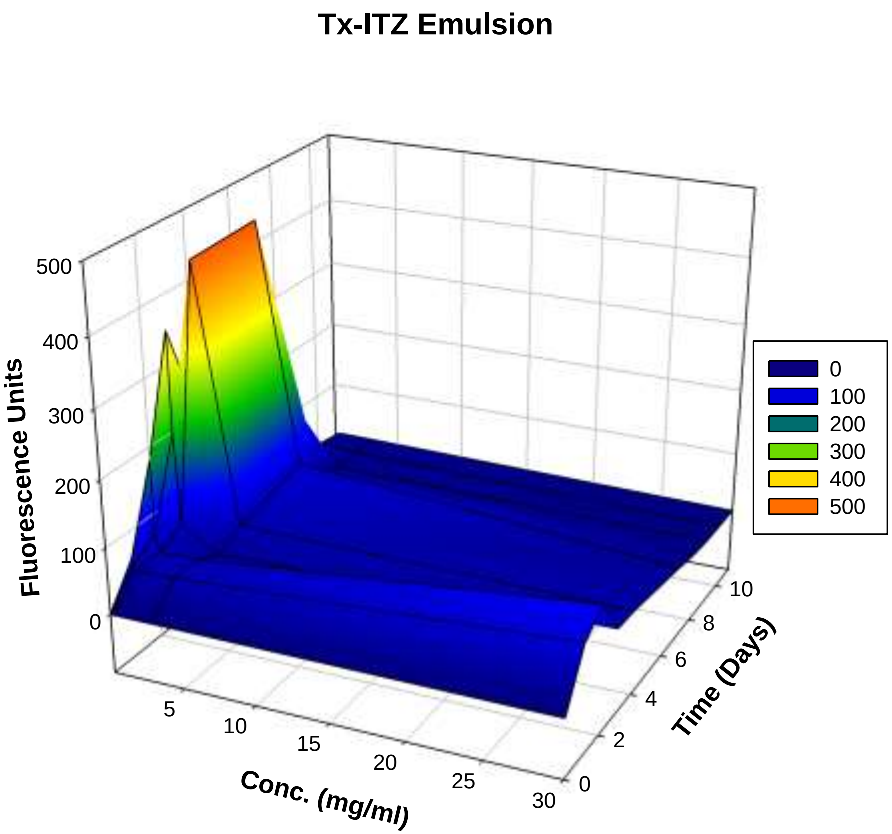
# GFP QUANTITATIVE INHIBITION ACTIVITY

**Fluorescence (low conc)**



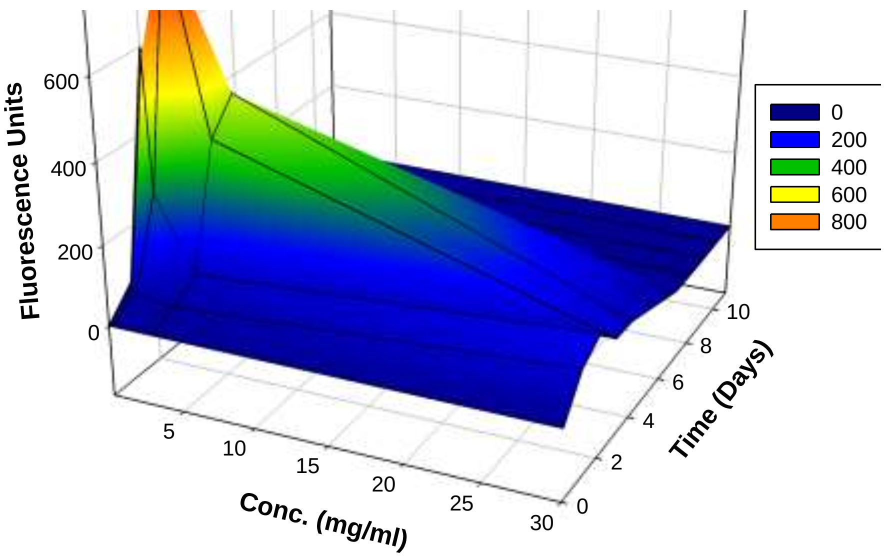

**Fluorescence (higher conc)**

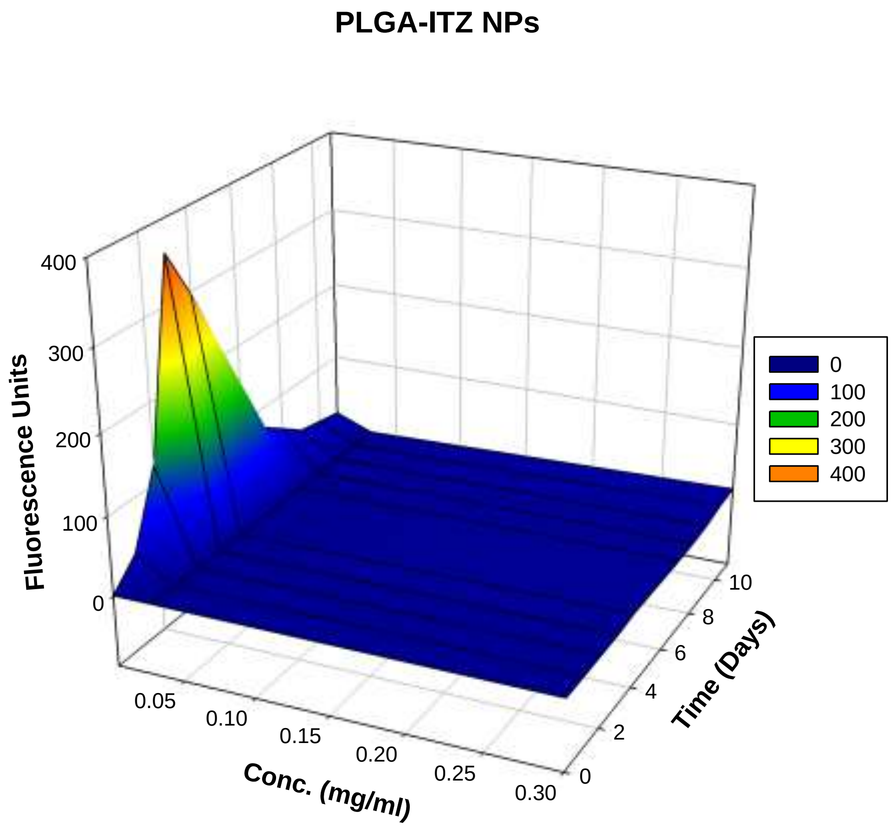





# OF TIME AND

## PLGA-ITZ



# CONCLUSIONS-IMPROVED ANTIMICROBIAL ACTIVITY

- Antifungal particles measuring ~200 nm were synthesized from PLGA with entrapped Itraconazole
- Release of the antifungal was sustained over 100 hrs
- Antifungal properties of the PLGA NPs were superior to those of free Itraconazole or emulsified antifungal
  - *Studies needed to identify mechanism of action*

# Improved functionality of hydrophobic colorant

## Carlos Astete

C. E. Astete, C. M. Sabliov, F. Watanabe, and A. Biris. 2009. $Ca^{2+}$ crosslinked alginic acid nanoparticles for solubilization of lipophilic natural colorants. *Journal of Agricultural and Food Chemistry.* DOI:10.1021/jf900563a.

# The food and pharmaceutical industries mainly use synthetic colorants

## FDA approved synthetic colorants:

| Name | Color | Comments |
|---|---|---|
| FD&C Blue #1 | Bright blue | Allergic reactions |
| FD&C Blue #2 | Royal blue | Poor water solubility |
| FD&C Green #3 | Sea green | Allergic reactions, and it is not used in EU |
| FD&C Red #3 | Cherry red | It may be carcinogenic |
| FD&C Red #40 | Orange-red | Allergic reactions |
| FD&C Yellow #5 | Lemon yellow | Allergic and intolerance reactions |
| FD&C Yellow #6 | Orange | Allergic reaction |

Advantages:
- Water soluble
- Uniform color distribution
- Not expensive
- Color combinations
- Stable over time
- Ease of manipulation

Disadvantages:
- Health issues

G Mazza (2000), Health aspects of natural colorants, In: Natural food colorants: science and technology. Ed by Gabriel Lauro, Marcel and Dekker Inc., New York, 289-314

# Natural colorants can overcome the health issues of synthetic colorants

- Carotenes
  - β-Carotene
  - Capsanthin
  - Lycopene
- Xanthophyls
  - Zaexanthin
  - Bixin
  - Lutein
- Polyphenols
  - Curcumin
- Chlorophylls
  - Porphin
  - Phorbin

| Source |
| --- |
| Carrots |
| Paprika |
| Tomatoes |
| Corn |
| Safron |
| Annatto seeds |
| Green leaves |

Advantages:

- Natural components (low toxicity)
- Health benefits (antioxidants, anticancer, CVD prevention, anti-aging)

Limitations:

- Not uniform color distribution
- Poor water solubility
- Expensive
- Poor stability

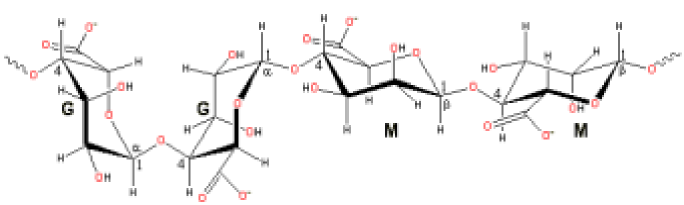
# Hypothesis and Objectives

- Hypothesis
  - Water solubility of a natural oily pigment is improved by entrapment in a natural water soluble polymer (alginic acid) crosslinked with $Ca^{2+}$
- Objectives
  - To synthesize polymeric nanostructures for delivery of natural pigments ($\beta$-carotene)
  - To characterize the nanostructures (size, zeta potential, polydisperse index, morphology) as a function of $Ca^{2+}$ concentration, and alginic acid concentration
  - To study the stability of the formed structures

# Components used are GRAS

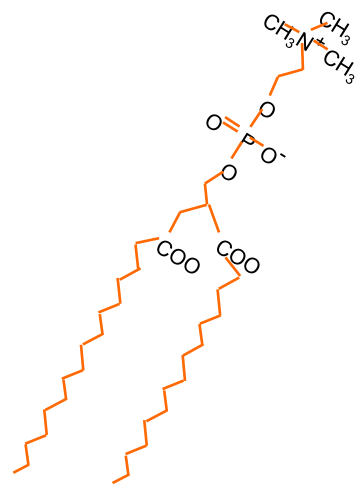
## Alginic acid

- Polysaccharide with mannuronic and guluronic acid
- Negative charges from carboxylic groups
- Thickening agent



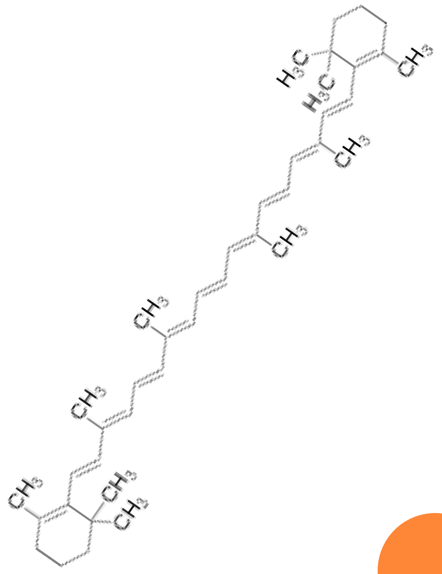
## Lecithin

- Phophatidyl choline
- Surfactant
- Emulsions
- Extracted from egg yolk and soybeans

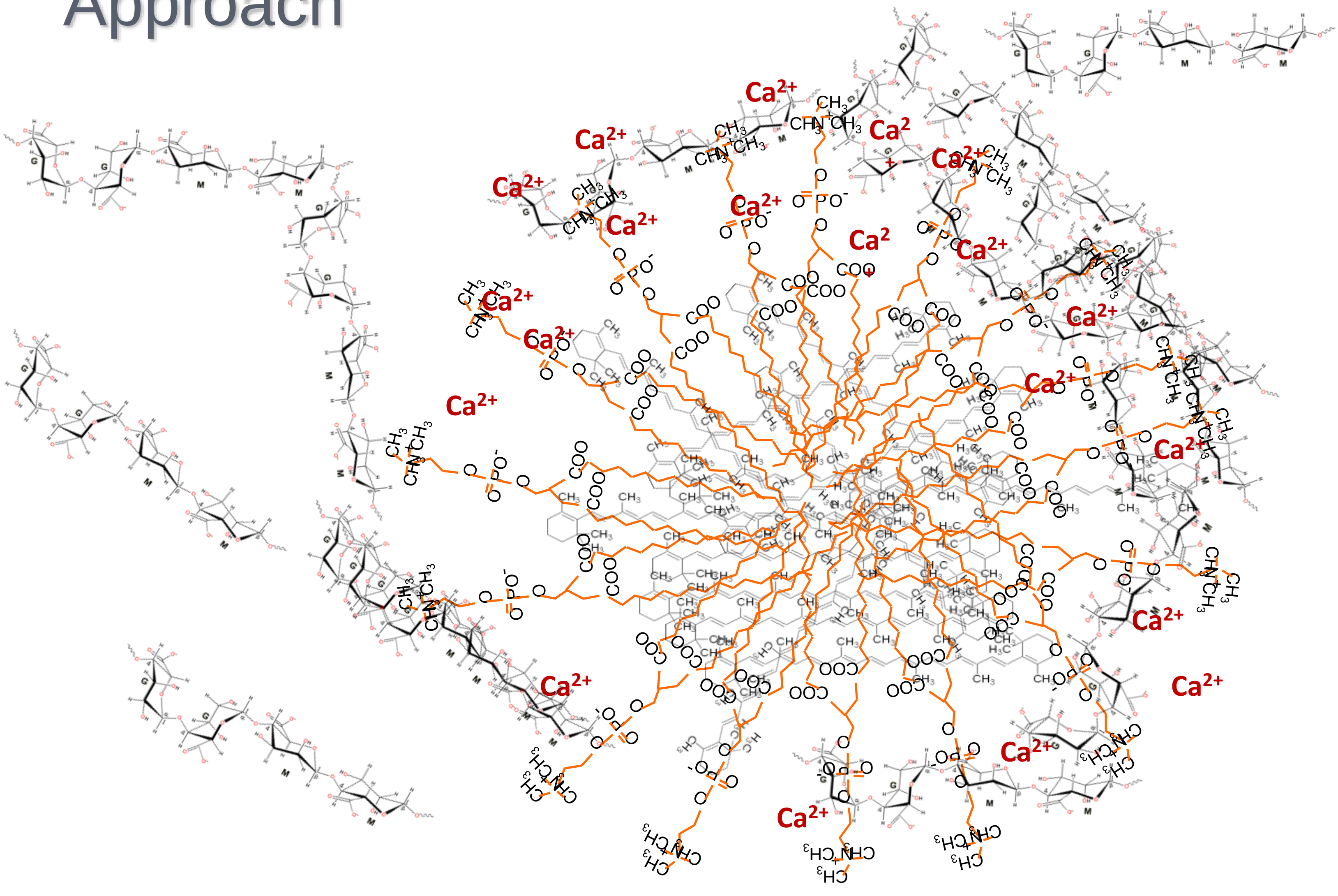


## β-carotene

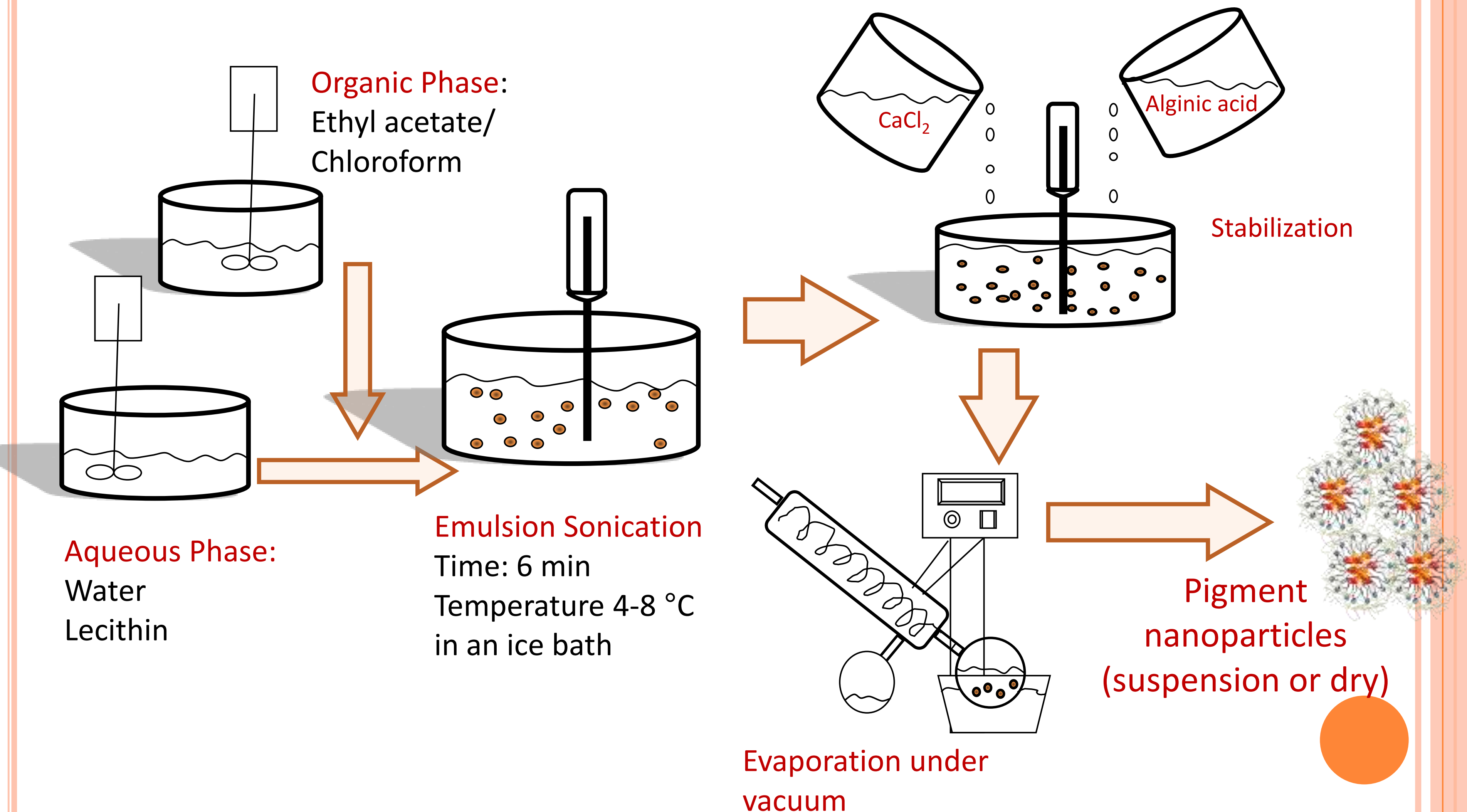
- Natural pigment
- Lipophilic
- Antioxidant

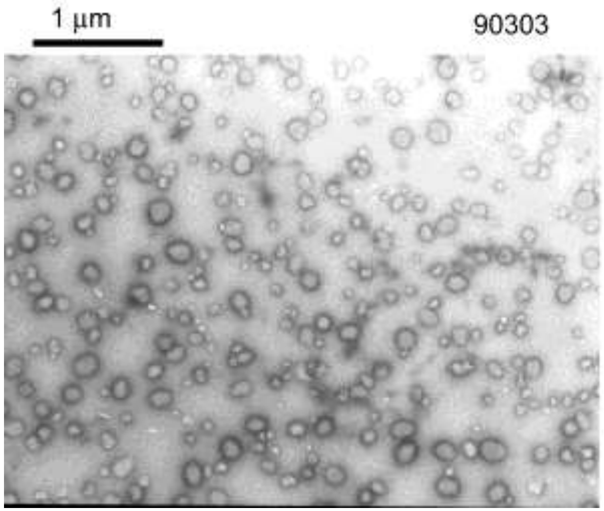


# Approach

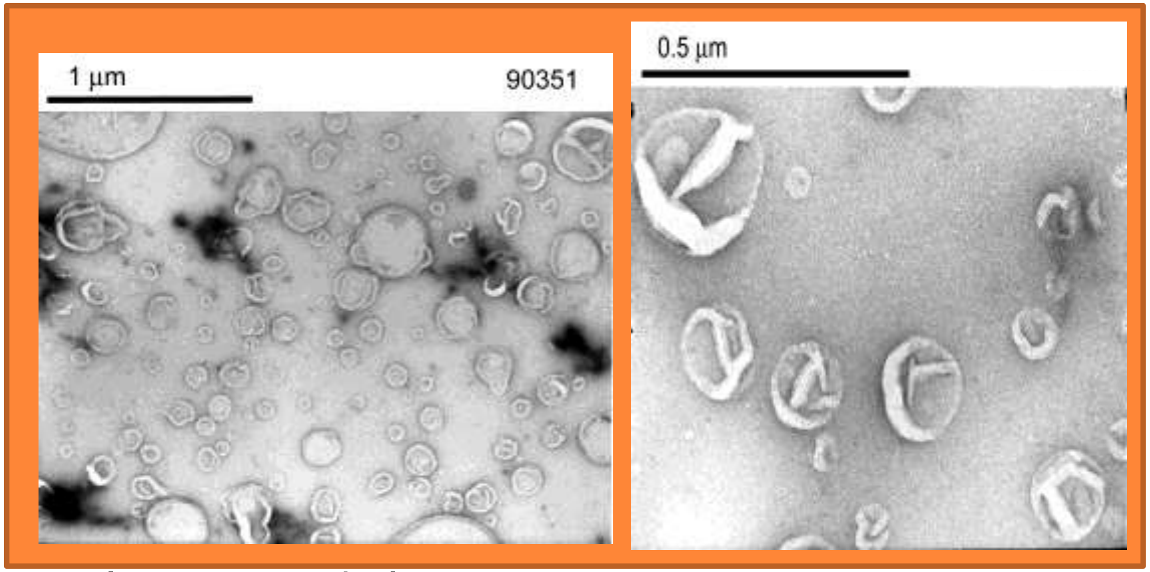


# Nanoparticle synthesis

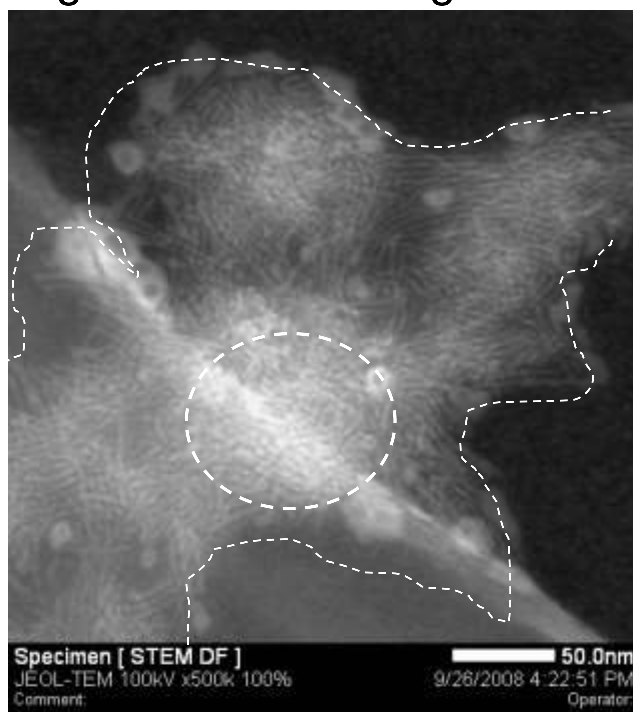


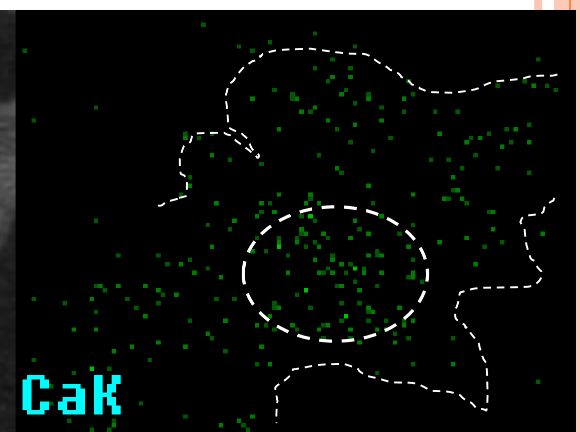



No $CaCl_2$
Alginic acid: 0.35 mg/ml

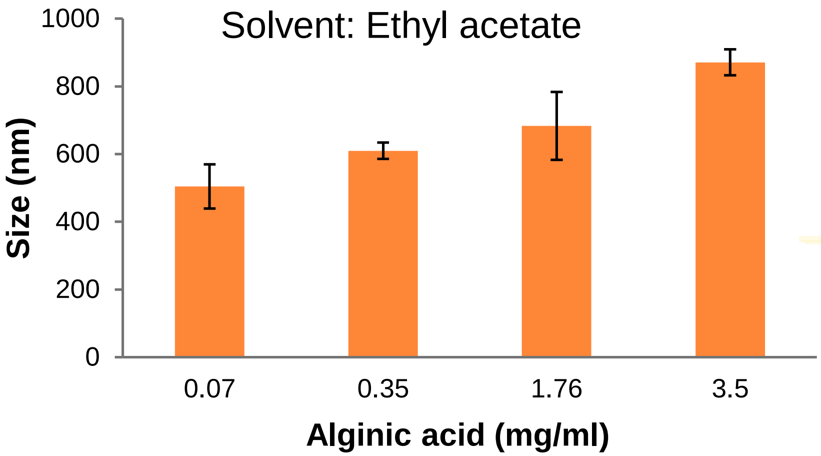


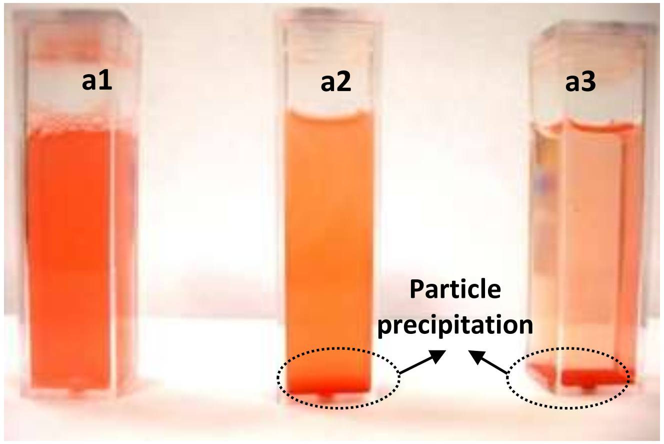

$CaCl_2$: 0.29 mg/ml
Alginic acid: 0.35 mg/ml

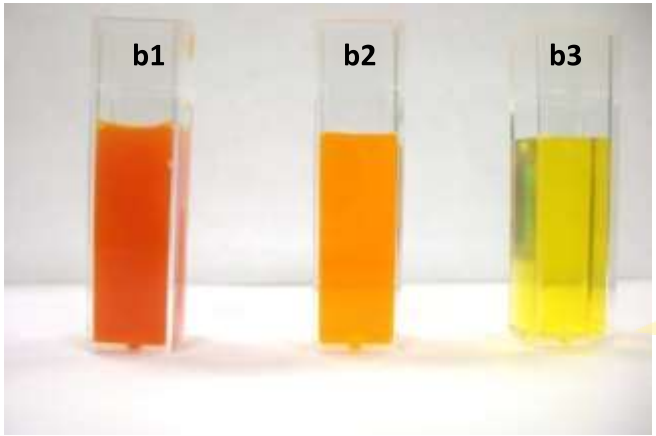


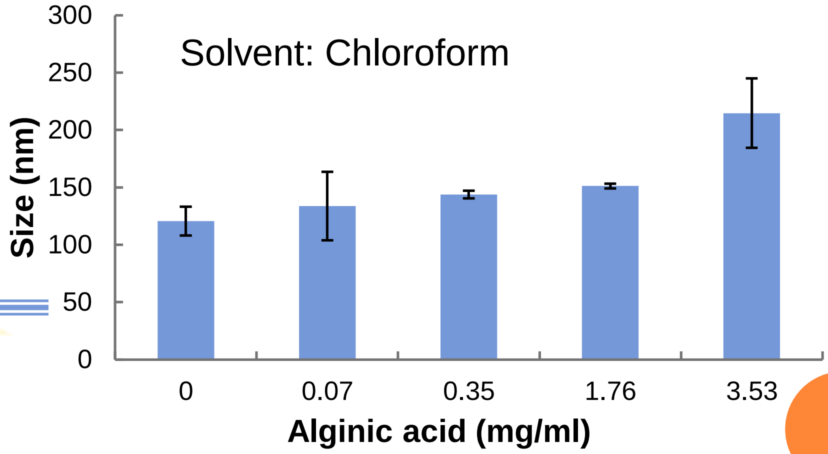



# Nanoparticle stability

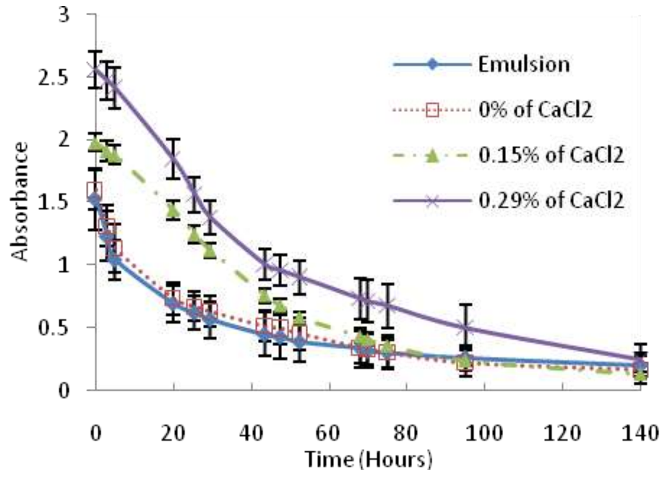


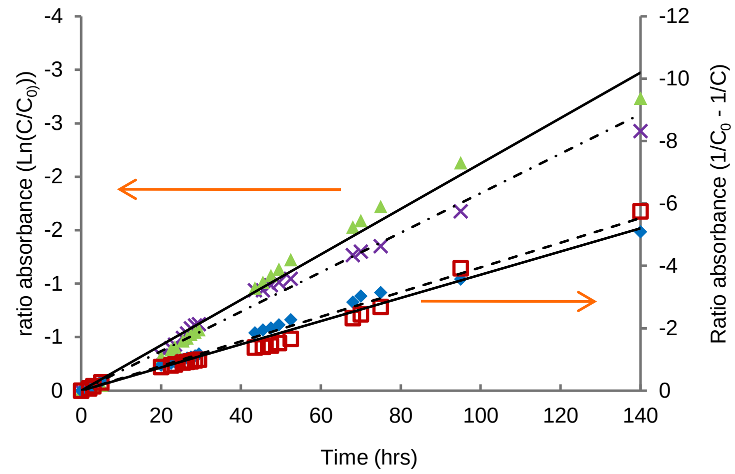



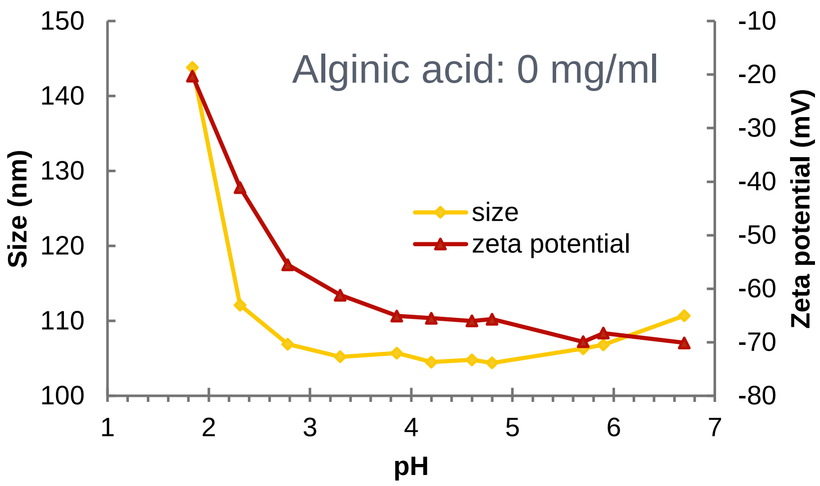



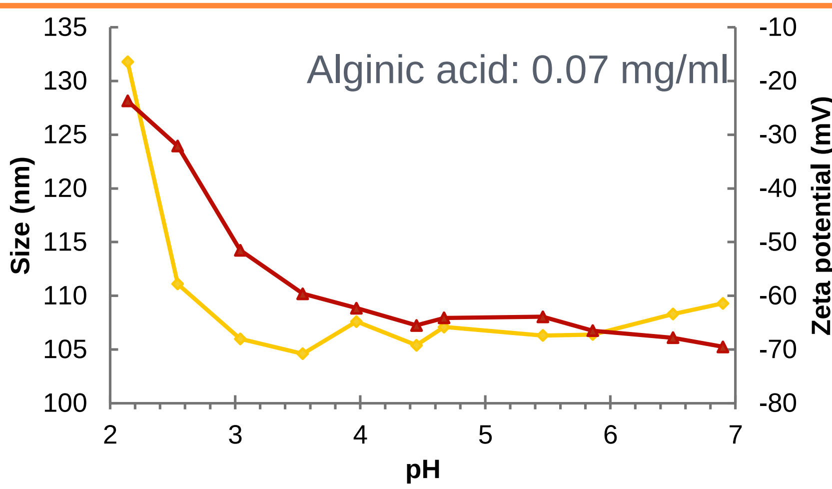



# Antioxidant and pH stability



# Conclusions-improved functionality

- ✓ Water-soluble nanostructures for entrapment of β-carotene were formed with the proposed method
- ✓ The organic solvent used in the synthesis significantly affected the size and stability of the structures by precipitation
- ✓ The nanostructures were stable between pH 3 and 7
- ✓ $Ca^{2+}$ and polymer concentration affected drastically the morphology and functionality of the system, not as much size and size distribution

# ACKNOWLEDGEMENTS

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