Nanoparticle Aggregation and Toxicity

Part (I):

Solvent Mediated Aggregation of Carbonaceous Nanoparticles (NPs)
– Udayana Ranatunga

Part(II):

Dispersibility, Aggregation, and Cytotoxicity of multi-walled carbon nanotubes (MWNTs)
– Dr. Ruhung Wang

Solvent Mediated Aggregation of Carbonaceous Nanoparticles (NPs)

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Overview (part I)

I. Introduction

II. DLVO Colloid Theory

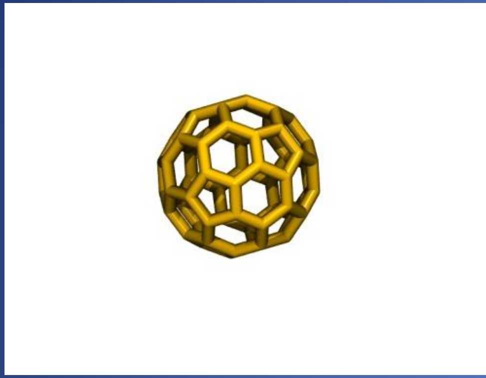
III. Examples of System Which Disobey DLVO Theory

IV. Application to Single Walled Carbon Nanotubes (SWNTs)

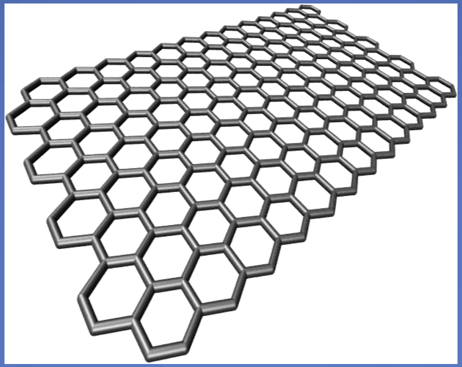
V. Conclusions and Perspectives

Carbonaceous Nanoparticles

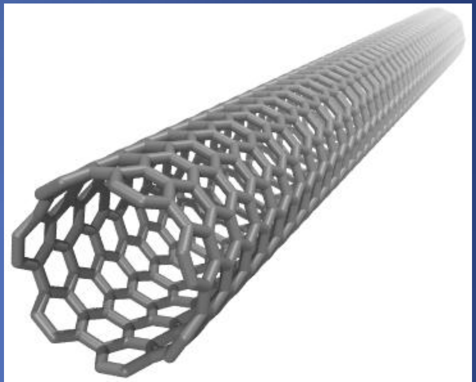
Spherical | Planar | Tubular



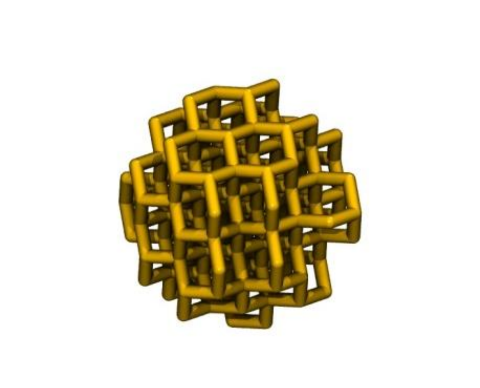
Bucky ball (C_{60})



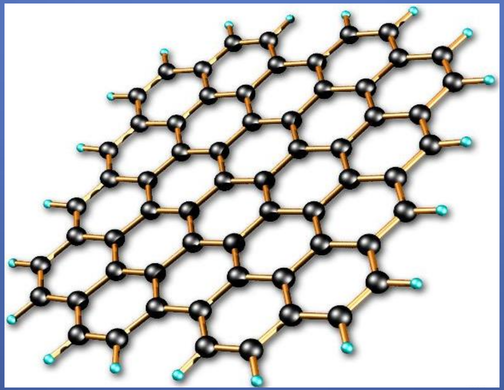
Graphene



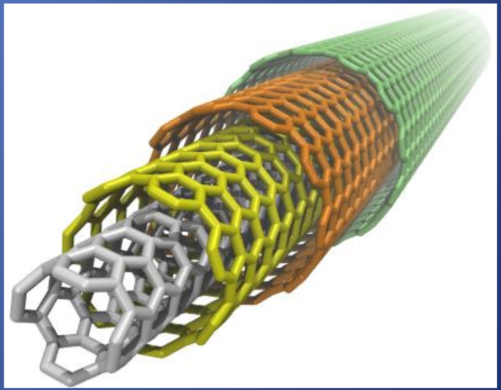
SWNT



Nanodiamond



Graphene Oxide



MWNT

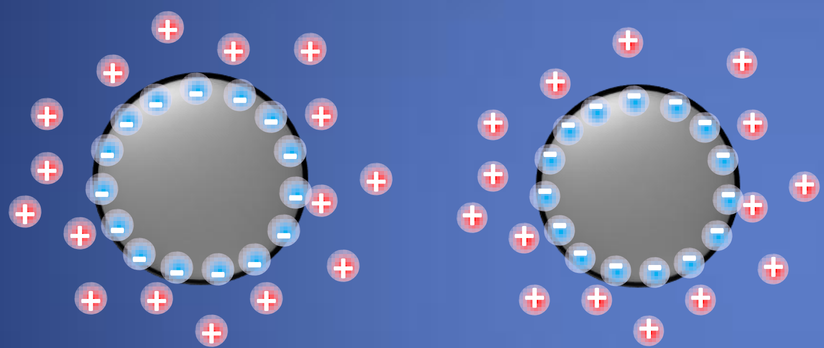
Nanoparticle Aggregation

- Many carbonaceous NPs aggregate in aqueous environments
- Problems with NP aggregates
  - Processability
  - Toxicity
- Require theoretical models which properly treat aggregation
- Conventional theories often adopt macroscopic concepts to the nanoscale
- We use computational methods to gain insight at the molecular scale

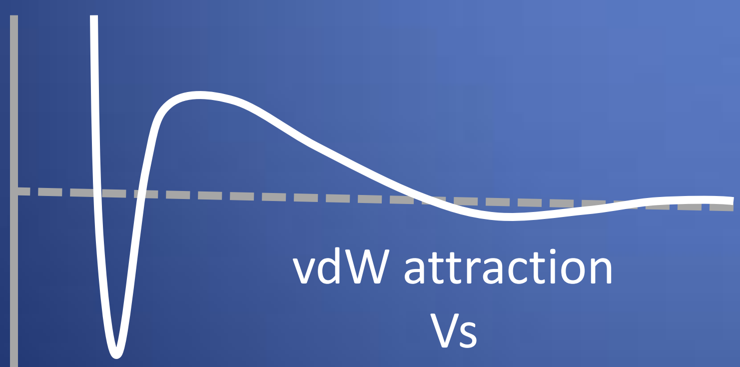
DLVO Theory

- Classical theory of colloidal aggregation

  (**D**erjaguin, **L**andau, **V**ervey, **O**verbeek → DLVO)



Electric double layer



vdW attraction
Vs
electrostatic repulsion

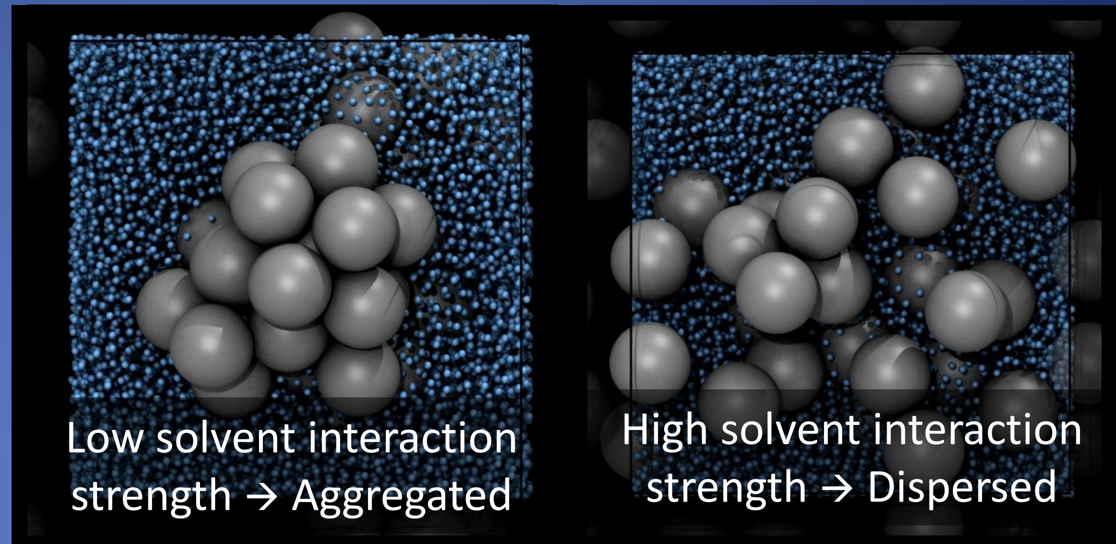
- Known Shortcomings
  - Often fails for nanoparticles
  - Doesn’t acknowledge molecular nature of solvent

Examples of Non-DLVO Systems

Example (1) :

Graphitic particles in bulk water

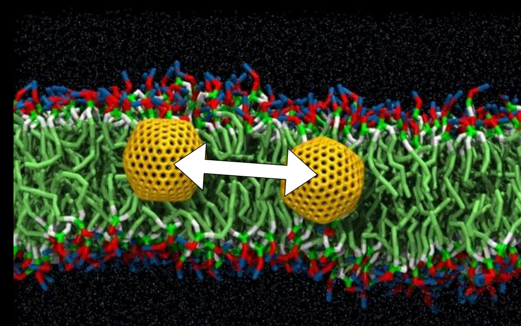
Effect of solvation strength



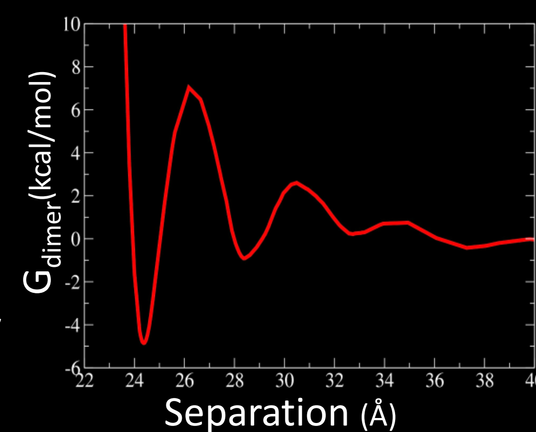
Example (2) :

C_{540} fullerenes in DOPC lipid bilayer

Effect of ‘solvent structuring’



Dimerization free energy profile oscillates due to solvent structuring



Examples of Non-DLVO Systems (Cntd..)

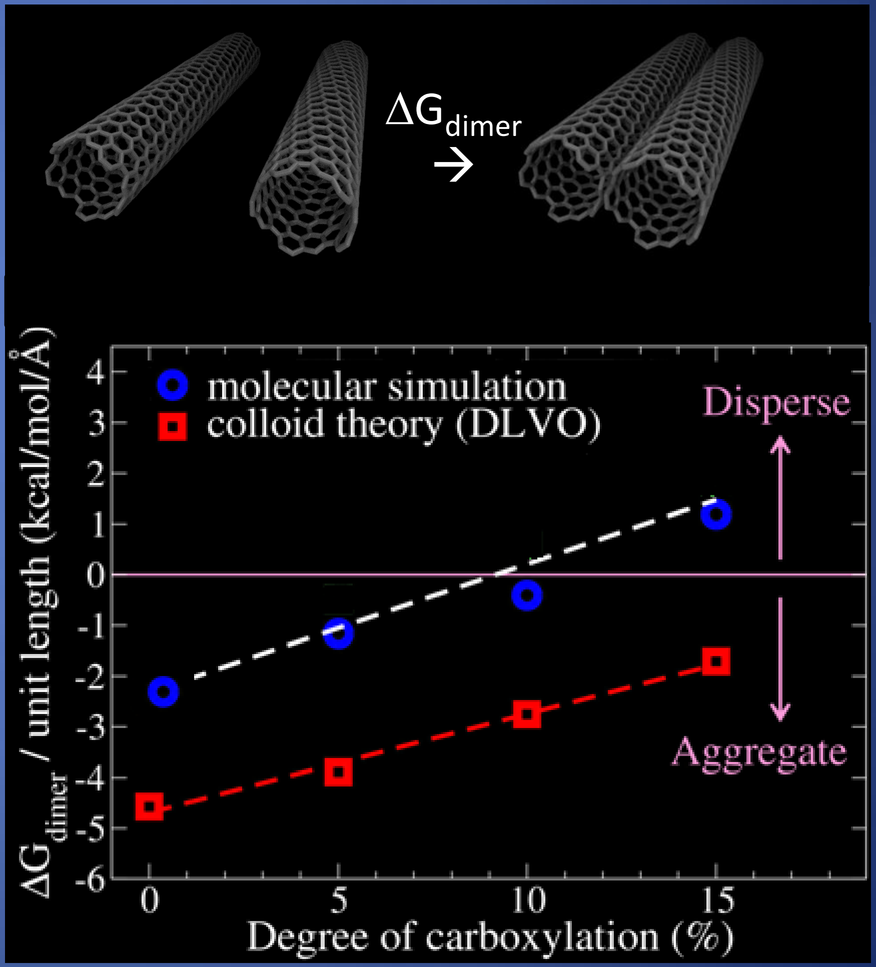
Example (3): Aggregation of carboxylated single walled carbon nanotubes (SWNTs)

Functionalizing SWNT with –COOH

Experimentally found that SWNT do not aggregate above 10%-15% carboxylation

DLVO theory fails

Important implications on toxicity



Nanoparticle Toxicity Often Linked to Aggregation

NANO LETTERS — *Nano Lett.* **2010,** *10,* 1664–1670

Biocompatible Nanoscale Dispersion of Single-Walled Carbon Nanotubes Minimizes in vivo Pulmonary Toxicity

Marc Hersam *et al*, Northwestern University Feinberg School of Medicine,
†Department of Medicine, Division of Pulmonary and Critical Care Medicine,

Thirty days after lung exposure:

- granuloma-like structures observed in mice treated with aggregated SWNTs
- absent in mice treated with nanoscale dispersed SWNTs

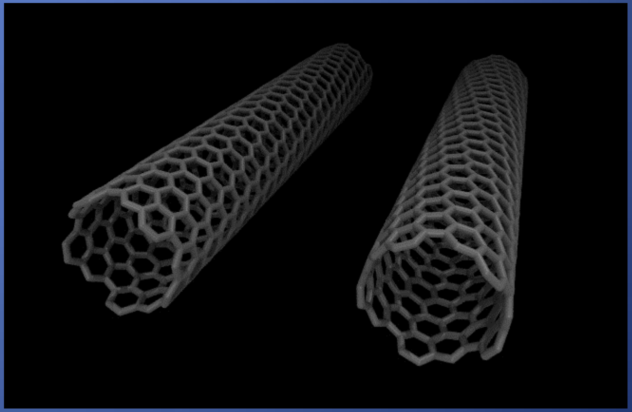
Conclusion: toxicity of SWNTs *in vivo* is attributable to aggregation of the nanomaterial rather than the large aspect ratio of the individual nanotubes.

Applying DLVO Theory to Nanotubes(I)

- Attractive part: van der Waals interactions

- Traditional approaches are continuum models
  - Hamaker summation
  - Lifshitz formulation

- We use direct summation of van der Waals interaction of two nanotubes in vacuum
  - ‘exact’ Hamaker treatment



Applying DLVO Theory to Nanotubes(II)

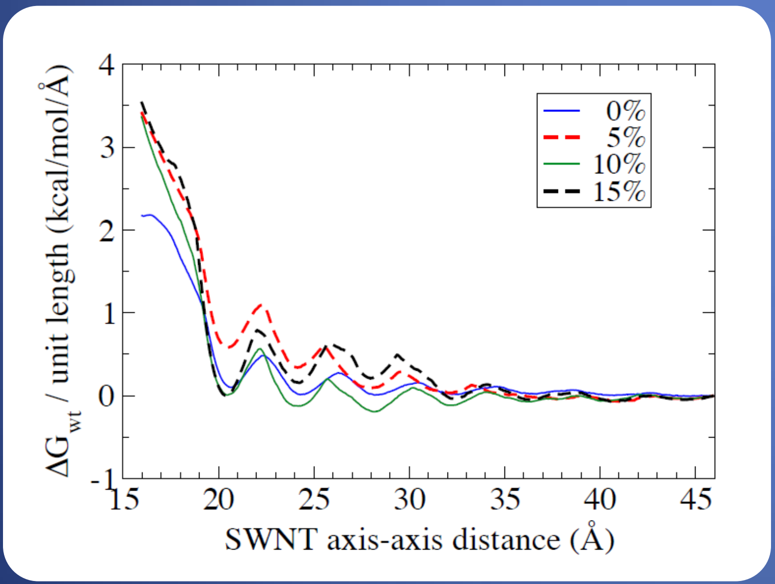
- Repulsive part: electrostatic interactions

- Motivated by the work of McQuarrie & Brenner
  (Biophysical Journal, **13**, 301-331 (1973) and references therein)
  - Use a constant surface site density
  - Dissociation constant for ionizable groups on surface
  - Constant bath T, pH, ionic strength

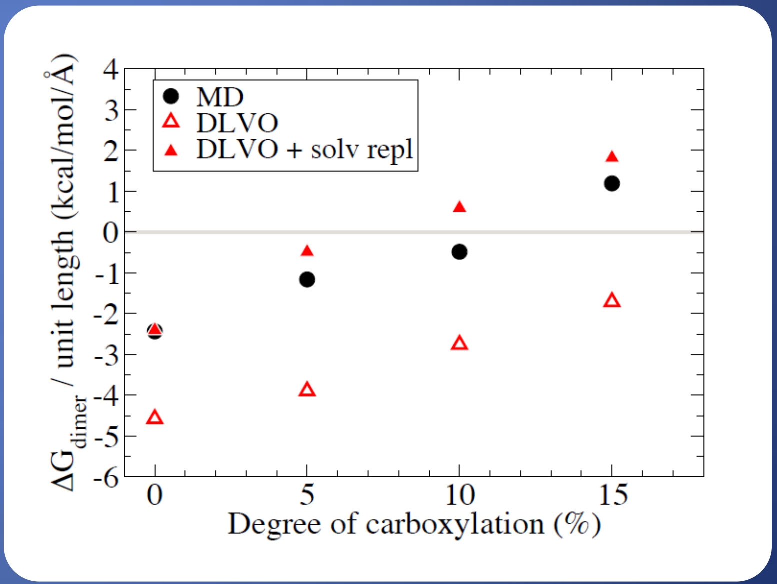
- Self consistent (dynamic) model of dissociation of ionizable surface groups

Applying DLVO Theory to Nanotubes(III)

- Solvent contribution
- Can obtain the solvent contribution by subtracting the dimerization free energy in solvent from that in vacuum



Solvent contribution to the free energy



DLVO theory + solvent contribution, agrees with MD simulations

2078 *J. Phys. Chem. B* **2008**, *112*, 2078–2084

Passive Transport of C_{60} Fullerenes through a Lipid Membrane: A Molecular Dynamics Simulation Study

Dmitry Bedrov,* Grant D. Smith, Hemali Davande, and Liwei Li

Department of Materials Science & Engineering, 122 South Central Campus Drive Room 304, University of Utah, Salt Lake City, Utah 84112

- C_{60} shows strong interaction with water

THE JOURNAL OF CHEMICAL PHYSICS **131**, 115102 (2009)

How hydrophobic hydration responds to solute size and attractions: Theory and simulations

Manoj V. Athawale, Sumanth N. Jamadagni, and Shekhar Garde[a)]

The Howard P. Isermann Department of Chemical and Biological Engineering, and Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, New York 12180, USA

- Hydration energy calculations show that larger spherical fullerenes are hydrophilic (i.e. $G_{wat} < 0$)
- Does not mean fullerenes are soluble in water (particle-particle interactions play a part)

Conclusions and Future Work

- DLVO theory may be used to treat SWNT aggregation if solvent contributions are addressed
- Should be applicable to other carbonaceous NPs
- Studies using dispersal agents
- Extending to MWNTs
- Interaction with biologically relevant environments

Dispersibility, Aggregation, and Cytotoxicity of multi-walled carbon nanotubes (MWNTs)

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University of Texas at Dallas

Overview (Part II)

I. Introduction

II. MWNT product analysis

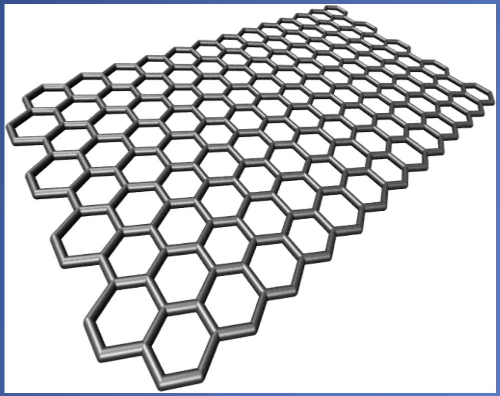
III. Dispersibility of MWNT products in bovine serum albumin (BSA) & copolymer Pluronic F-127 (PF)

IV. Particle size analysis and cytotoxicity assessment

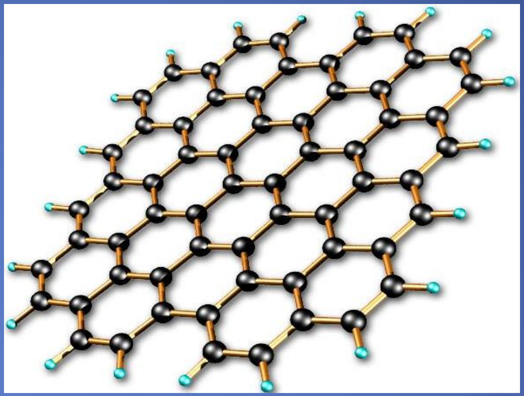
V. Summary

Carbon Based Nano Particles

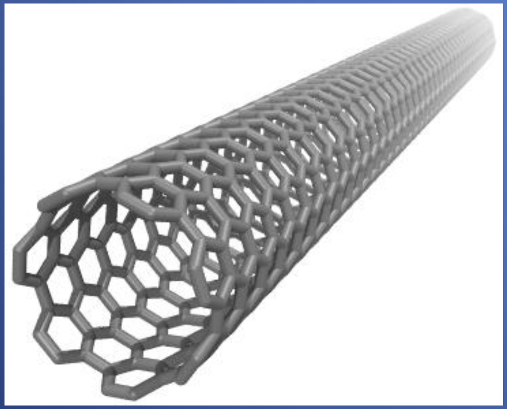
Graphene



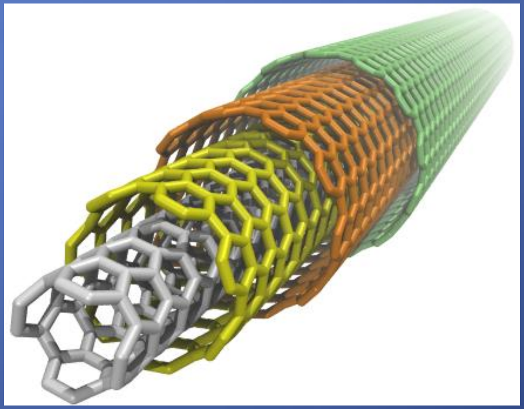
Graphene Oxide



SWNT



MWNT



Current Work on Multi-walled Carbon Nanotubes

Specific Aims:

- Selection of MWNT products and dispersants
- Optimizing MWNT dispersions
- Biological testing

MWNTs: Improving the Dispersant

We have been using the protein bovine serum albumin (BSA)

Ideal dispersant should be:

- ✓ Effective (aggregates may be toxic)
- ✓ Biocompatible (not toxic itself)
- ✓ Defined structure for modeling studies
- ✓ Amenable and scalable for industrial uses
- ✓ Inexpensive

Surveyed a number of dispersants to compare with BSA

Focused on Pluronic F-127 block copolymer

Pluronic F-127 (Poloxamer 407)

POLYOXYPROPYLENE-POLYOXYETHYLENE Tri-block Copolymer

$$H(OCH_2CH_2)_a(OCH_2\underset{|}{\overset{CH_3}{C}}H)_b(OCH_2CH_2)_aOH$$

a = ~ 101, b = ~ 56

Molecular Weight ~ 12,600

Oral LD50 = > 15,000 mg/kg (Rat)
Dermal LD50 = > 5,000 mg/kg (Rabbit)
FDA approved as a component of i.v. injections

BSA: ~$3,000/Kg
Pluronic F-127: ~$120/Kg

2 MWNT Products: Pristine & Carboxylated

Products analysis provided by NanoAmor

Product	OD (nm)	ID (nm)	L (μm)	SSA (m^2/g)	TD (g/cm^3)	Contents (wt%)							
						C	-COOH	Cl	Fe	Ni	S	Co	Al
MWNT	30-50	5-15	0.5-2	90-120	~2.1	97.37	NA	0.20	0.55	1.86	0.02	NA	NA
C-MWNT	30-50	5-12	0.5-2	90-120	NA	> 95	0.69-0.77	< 1.0	< 0.6	< 1.9	< 0.25	< 1.0	< 0.2

OD: Outer Diameter

ID: Inner Diameter

L: Length

SSA: Specific Surface Area

TD: True Density

MWNT Dispersion protocol

- MWNT material –
  - 10 mg purchased MWNT product (MWNT or C-MWNT)
- Solutions –
  - 10 mL dispersant solution
    - HB: 10 mM HEPES, 10 mg/mL BSA, pH 7.4
    - PF: 0.1 % (w/v) Pluronic F-127 in milliQ H_2O
- Sonication –
  - Bath sonication (40K Hz, 120W, cooling coils)
  - 4 hours in $4^{O}C$ cold room (bath temperature: 3-12^{o}C)
  - Up to 8 samples, 10 mL each, prepared per batch
  - → 1 mg/mL MWNT dispersions (Before Centrifugation)
- Centrifugation –
  - 20,000 g, 5 min, collect top 90% supernatant
  - → MWNT dispersions (After Centrifugation)

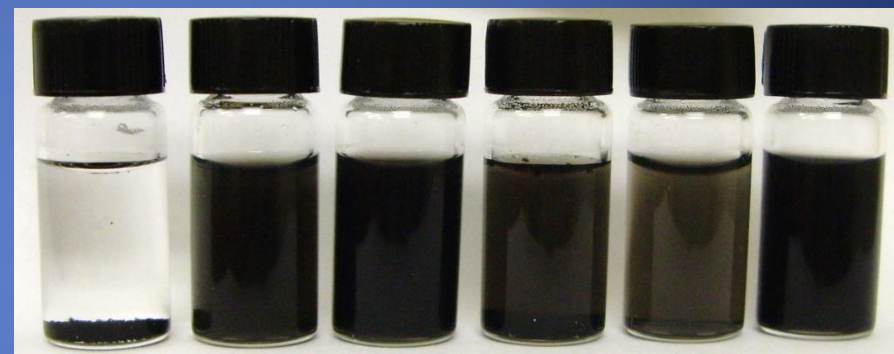
Comparing BSA and Pluronic F-127 in dispersing MWNTs and C-MWNTs

10 mg CNT (MWNT or C-MWNT) + 10 mL Solution (H_2O, BSA, or Pluronic)

Sonication ↓

CNT	MWNT			C-MWNT		
Solution	H2O	BSA	Pluronic	H2O	BSA	Pluronic

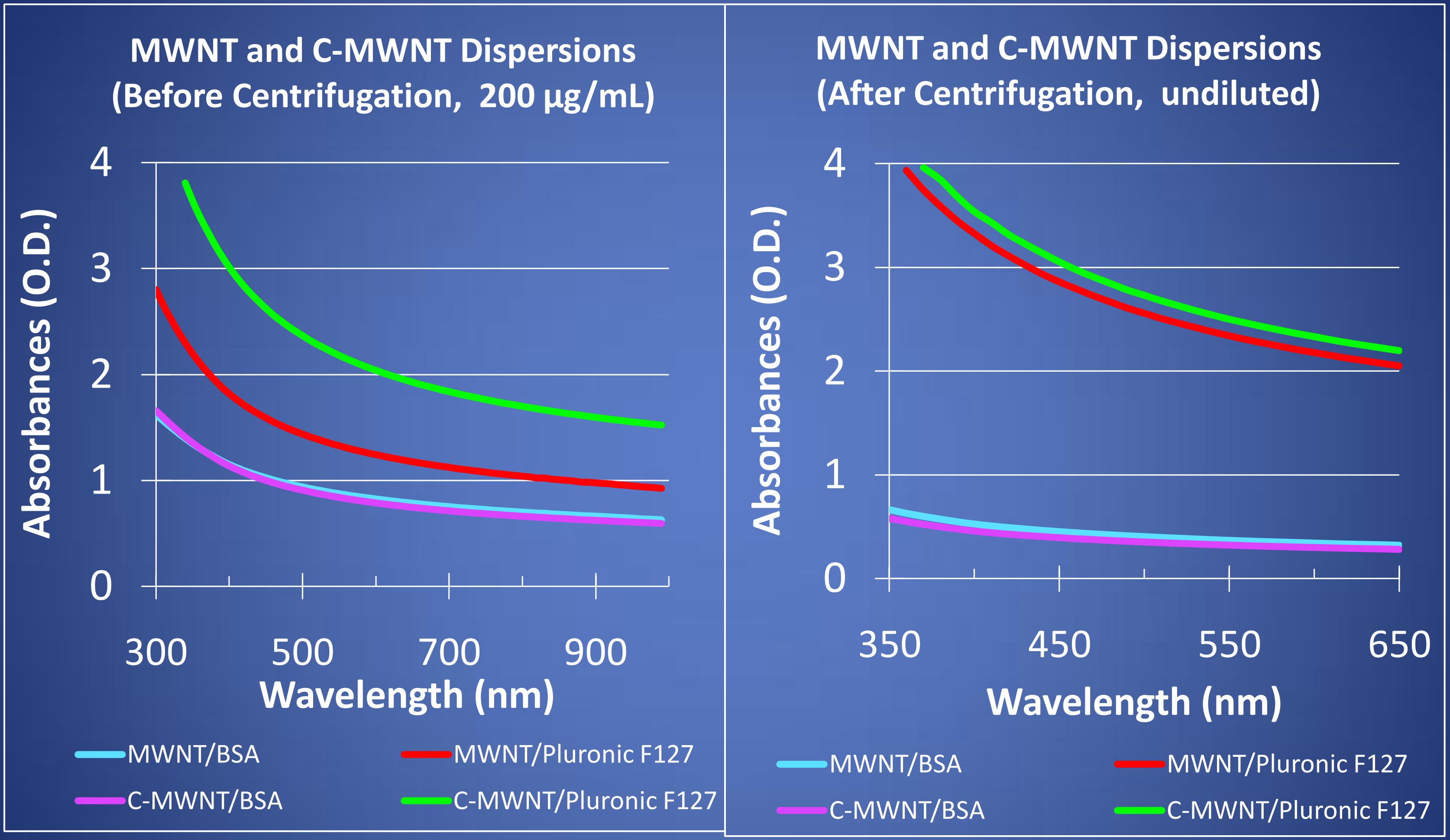
Dispersion (A) (Before Centrifugation) → 1:10 Dilution, 24 h



Centrifugation ↓

Dispersion (B) (After Centrifugation) → 1:10 Dilution, 24 h

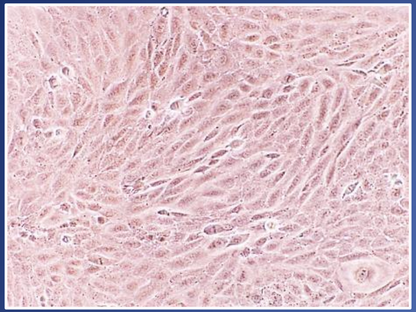
Comparing BSA and Pluronic F-127 in dispersing MWNTs and C-MWNTs



0.1 % Pluronic F127 is more effective than 1% BSA solution

NRK Cells in C-MWNT/HB Dispersions (Before and After Centrifugation)

Control



Before

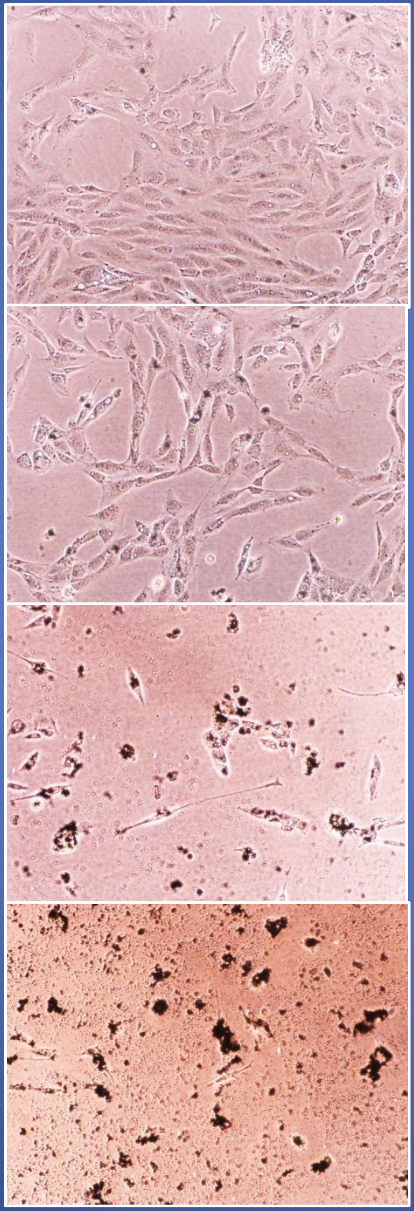
5

10

50

100

(µg/ml)



After

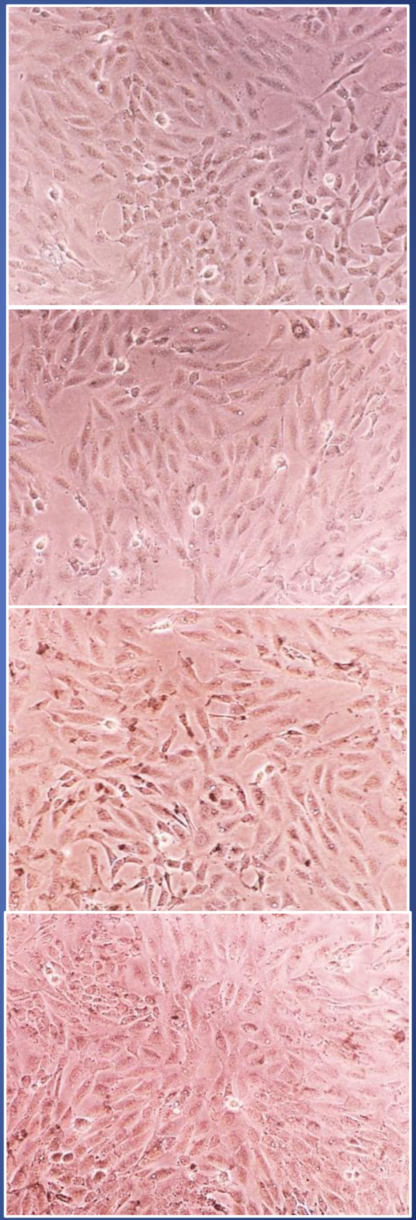
25

50

75

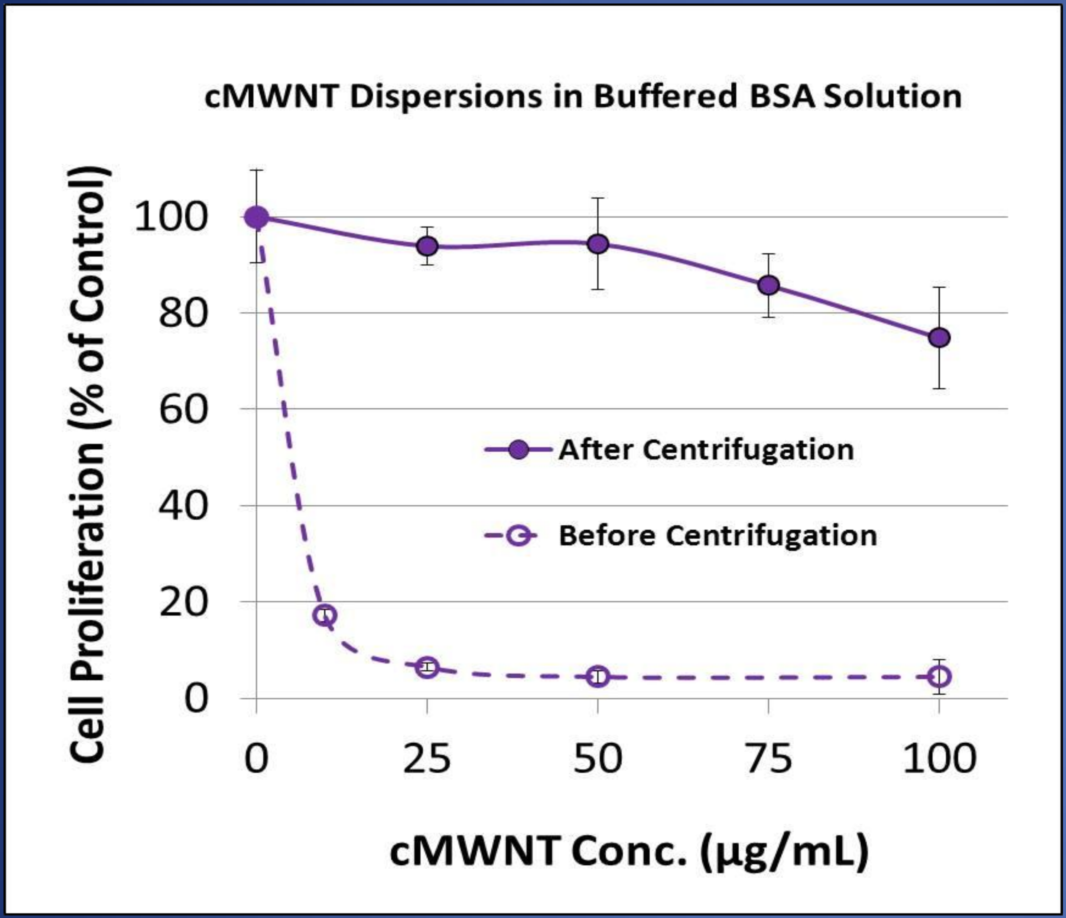
100

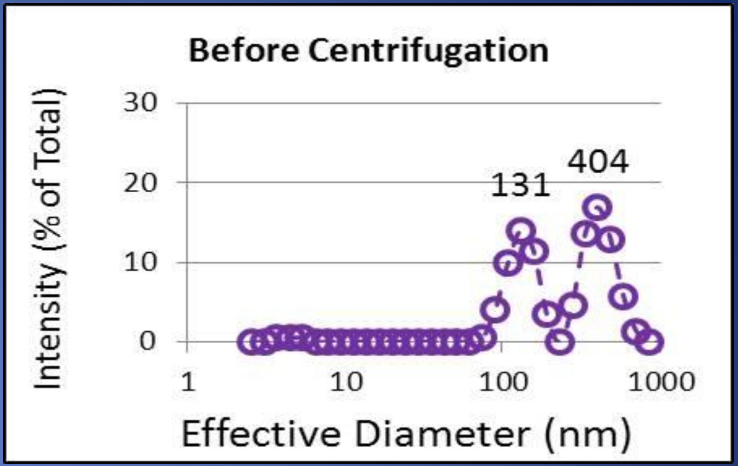
(µg/ml)

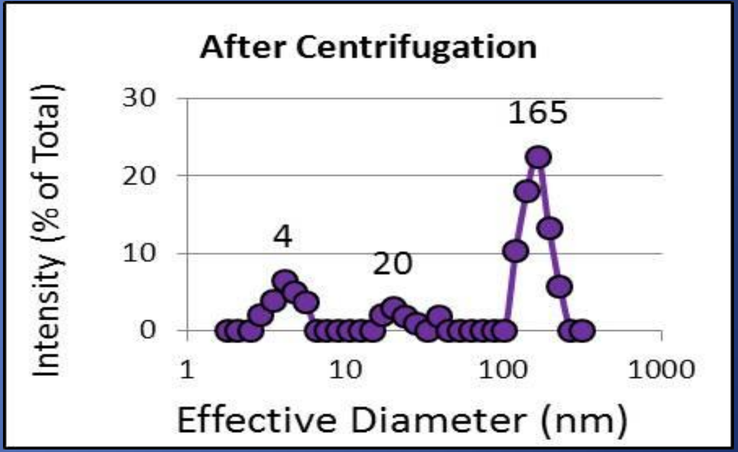


Cytotoxicity of C-MWNT/HB Dispersions (Before and After Centrifugation)

(NRK Cells Proliferation After 3 Days Incubation)





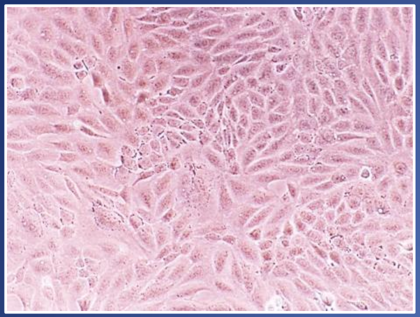


IC50 of C-MWNT/HB:
Before Centrifugation: < 10 µg/mL
After Centrifugation: > 100 µg/mL

PSD of C-MWNT/HB:
Before Centrifugation: 131, 404 nm
After Centrifugation: 165 nm

NRK 2 Days in C-MWNT-PF Dispersions (Before or After Centrifugation)

Control



Before

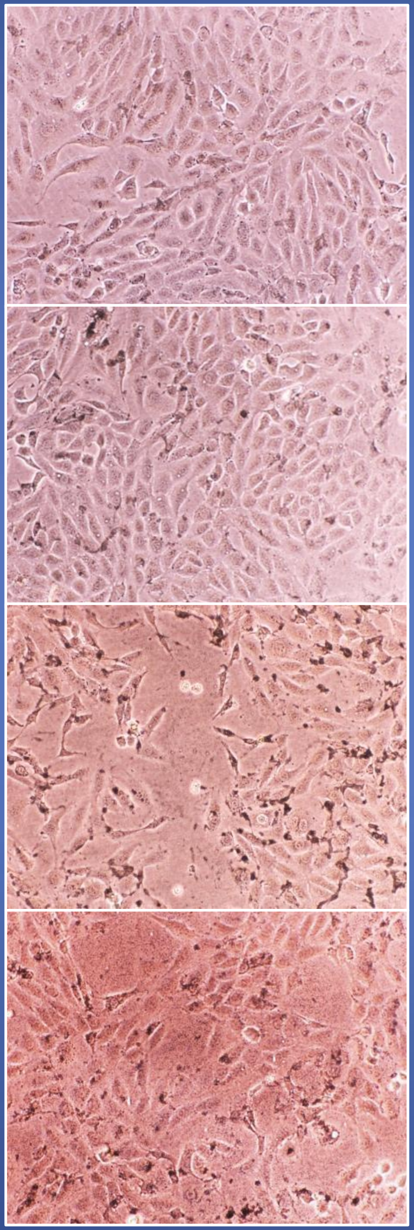
5

10

50

100

(µg/ml)



After

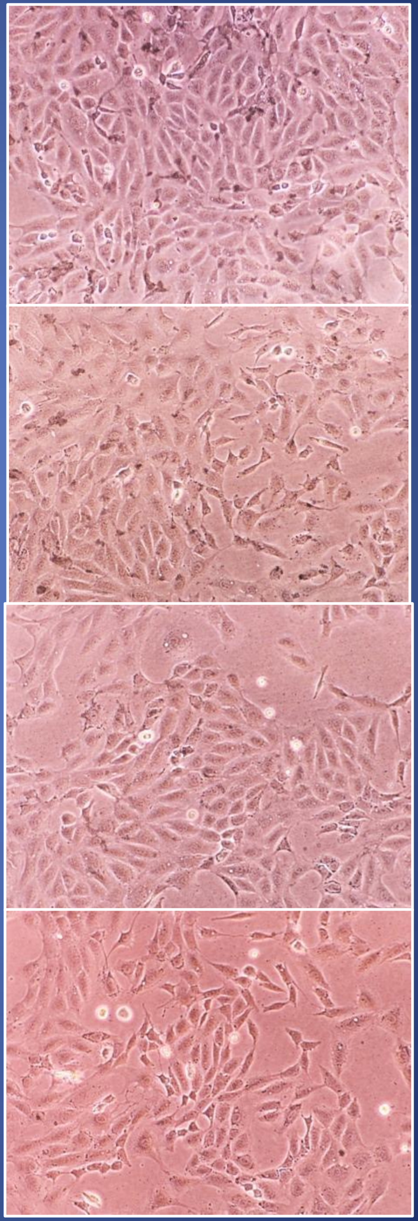
25

50

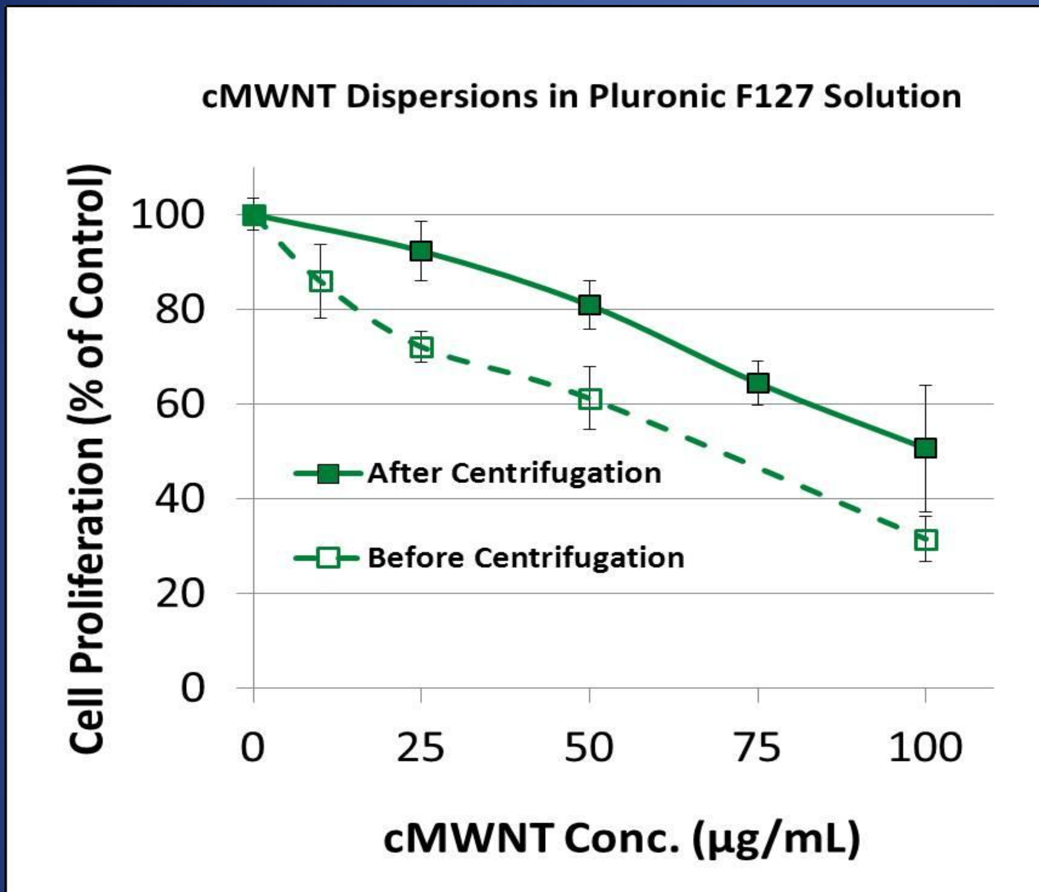
75

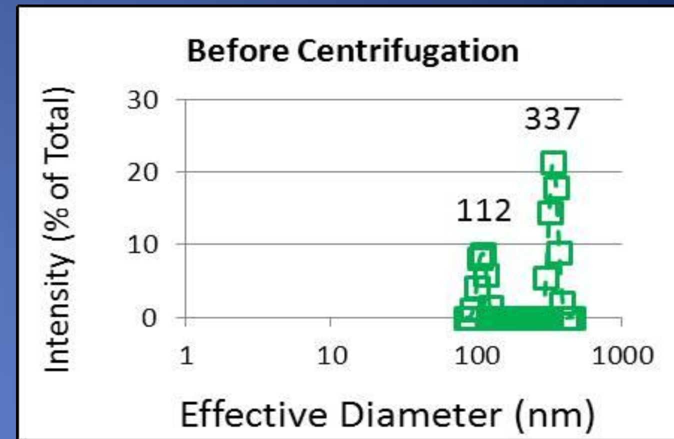
100

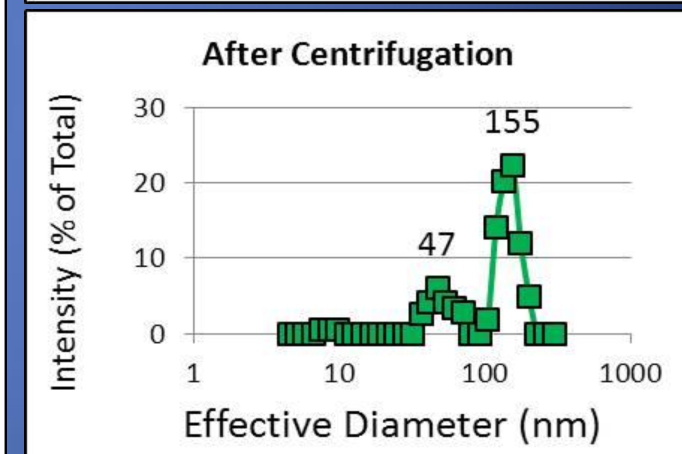
(µg/ml)



Cytotoxicity of C-MWNT/PF Dispersions (Before and After Centrifugation)
(NRK Cells Proliferation After 3 Days Incubation)



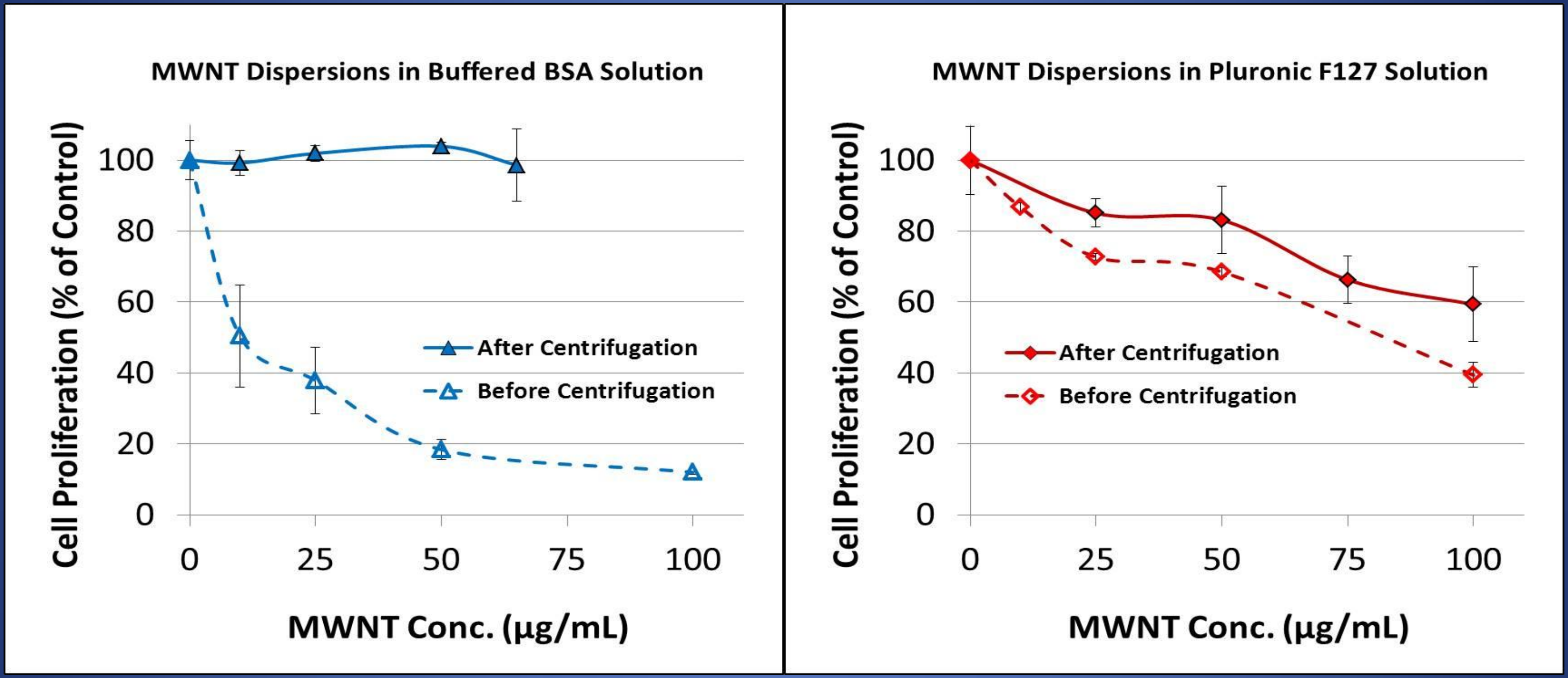




IC50 of C-MWNT/PF:
Before Centrifugation: ~ 70µg/mL
After Centrifugation: ~ 100 µg/mL

PSD of C-MWNT/PF:
Before Centrifugation: 112, 337 nm
After Centrifugation: 155 nm

Cytotoxicity of MWNT Dispersions in BSA and Pluronic F127
(NRK Cells Proliferation After 3 Days Incubation)



IC50 of MWNT/HB:
Before Centrifugation: ~ 10µg/mL
After Centrifugation: > 100 µg/mL

IC50 of MWNT/PF:
Before Centrifugation: ~ 70µg/mL
After Centrifugation: > 100 µg/mL

Summary of Current Work on Multi-walled Carbon Nanotubes

✓ Selection of MWNT products and dispersants

- Commercial MWNT products: Pristine and carboxylated
- Bio-compatible dispersants: BSA and Pluronic F-127

✓ Optimizing MWNT dispersions

- Pluronic F-127 is cheaper and 3 -5 times more effective than BSA

✓ Biological testing (in vitro study using NRK cells)

- Both MWNT and C-MWNT dispersions prepared in BSA solution show adverse effect on cell proliferation
- A simple centrifugation step reduces the adverse effect by ~10 fold
- Both MWNT and C-MWNT dispersions prepared in Pluronic F127 solution show less adverse effect on cell proliferation

Acknowledgements

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Prashant Raghavendran
Synyoung Li
Simon Beck