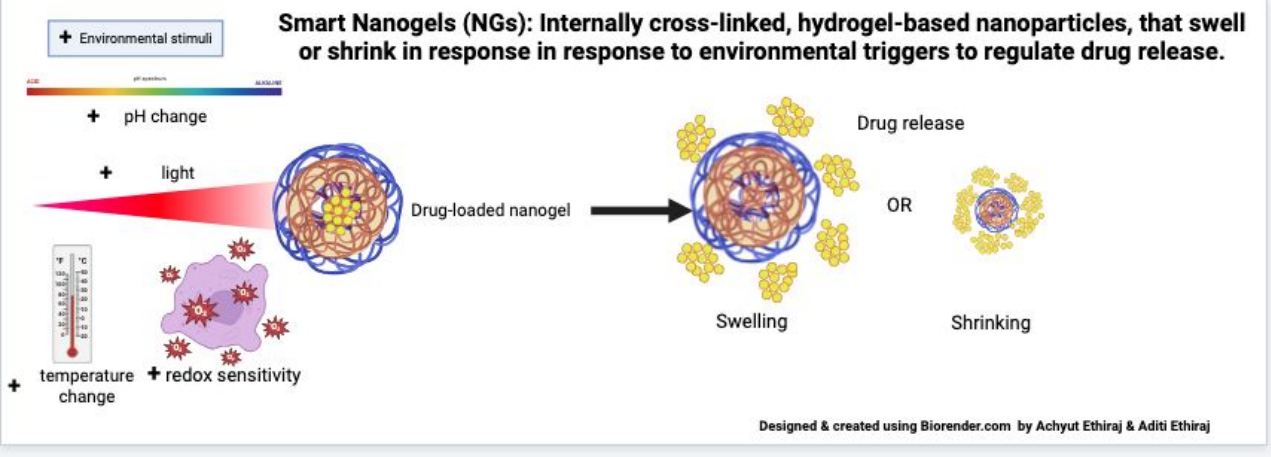
Synthesis and Characterization of Novel pH-Responsive Dual-Layer Chitosan-Alginate Nanogels for Curcumin Delivery with Relevance to Colorectal Cancer and Biological Validation in Drosophila melanogaster

Aditi & Achyut Ethiraj

Challenge & Approach

- Colorectal cancer (CRC) is the 2nd largest cause of cancer deaths worldwide
- Curcumin (CUR) shows anti-cancer, anti-inflammatory, & antioxidant properties
- CUR has low bioavailability gets degraded by stomach acid before reaching the colon
- No existing delivery system targets the colon with pH- responsive release efficiently

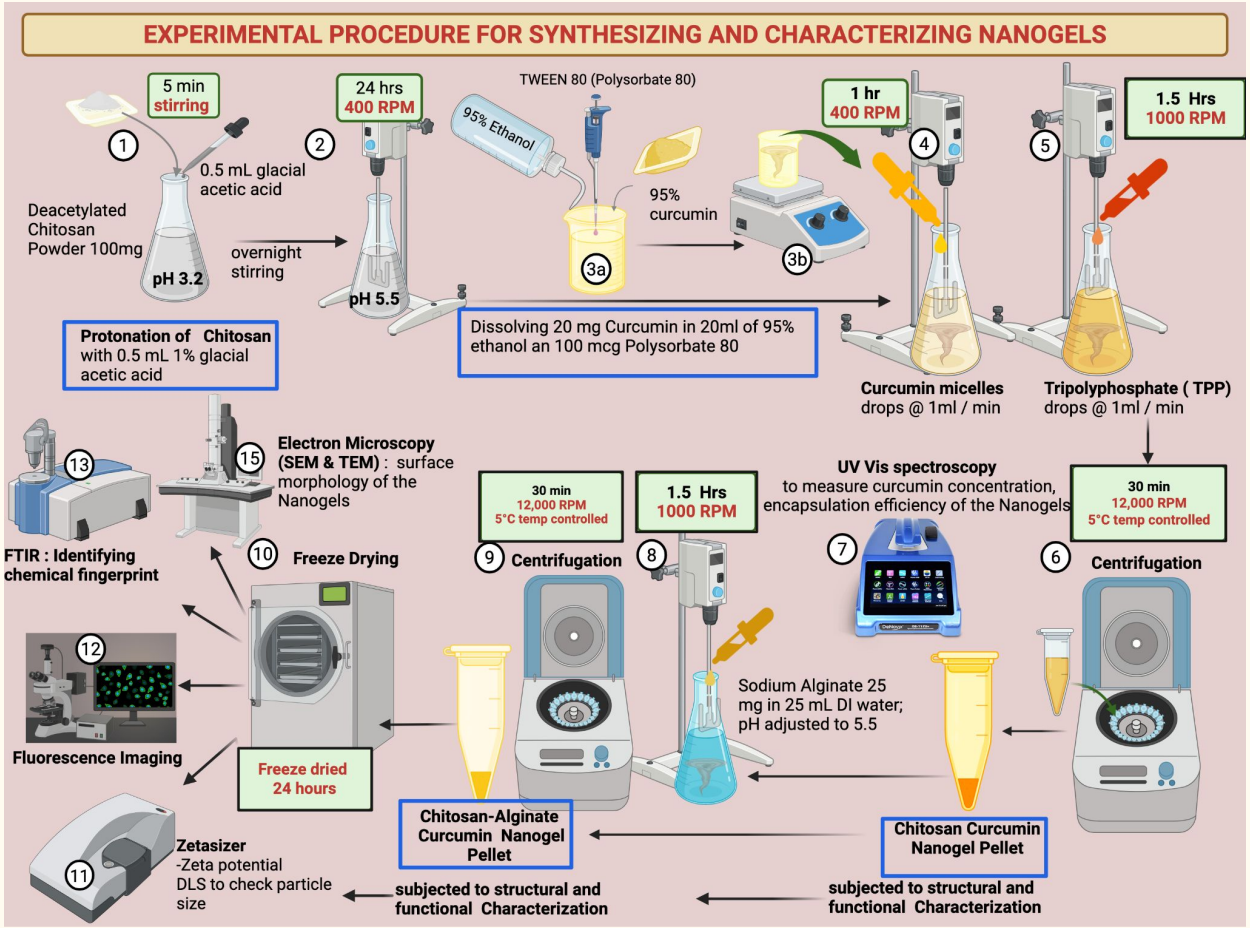


- Designed a dual-layer nanogel (NG) using chitosan (core) & sodium alginate (outer)
- Ionic crosslinking with TPP for controlled encapsulation
- Alginate coating remains intact at gastric pH, dissolve at colonic (7.4)
- Targeted, sustained CUR release directly at the tumor site

Methods/Materials

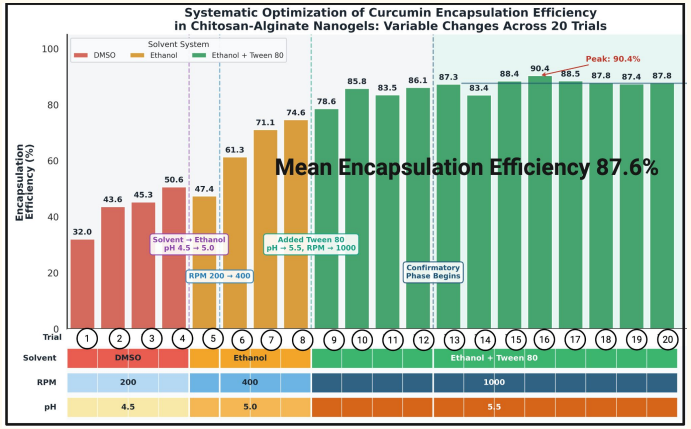
Ionic gelation protocol and optimized synthesis parameters

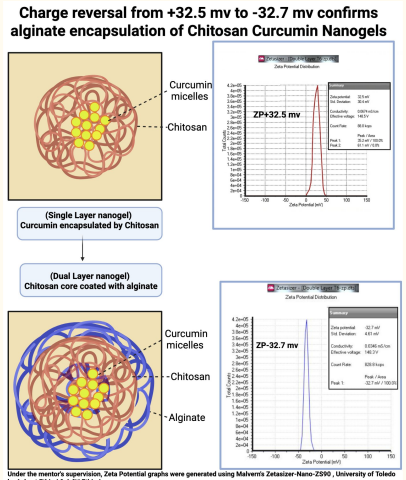
- Chitosan Core
- Dissolving Curcumin
- TPP Crosslinking
- Alginate coating
- Characterization



Characterization of NG

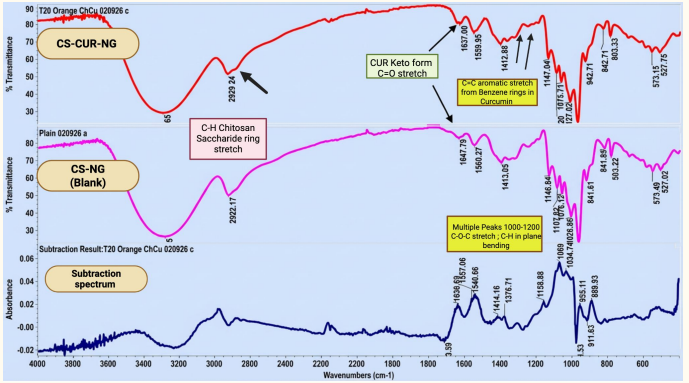
Encapsulation efficiency of the NG to hold curcumin is 87.6%

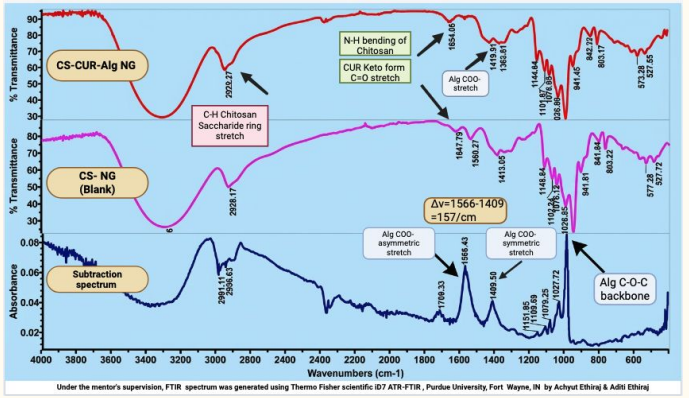




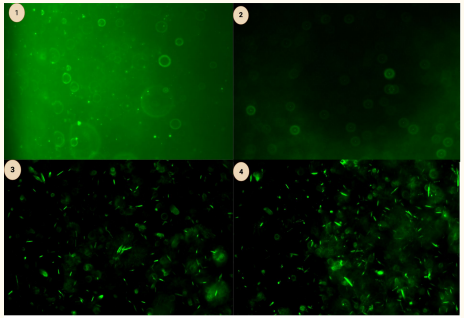
ATR-FTIR findings

Chitosan: Saccharide ring stretching (2929)
CUR: Multiple peaks C=C aromatic ring stretching (1500- 1600);
Peak shifts at 1637-1647: interaction between CUR C=O and NH of CS
Alginate: COO- stretch (symmetric: 1566 and asymmetric: 1409)
C-O-C stretch (1027)
TPP: O-P-O stretching at 800-1200





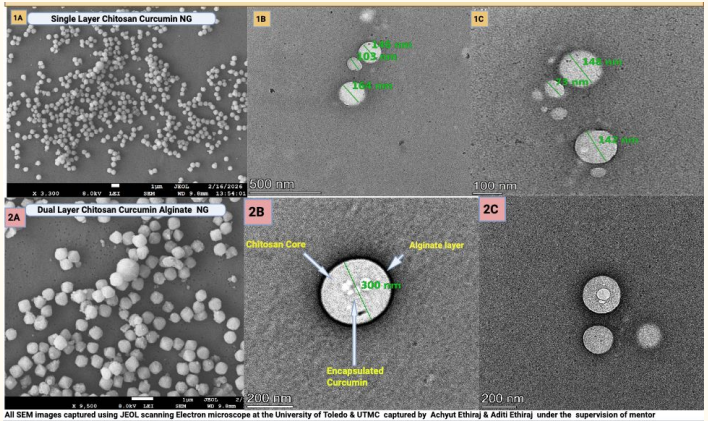
Fluorescent Microscopy



FLUORESCENT MICROSCOPY

Sample	Solvent	Microscopic Observation	Results
Single-Layer NG (1)	EtOH & T80	• Distinct green spheres • No aggregation	CUR is evenly distributed in the CS core
Dual-Layer NG (2)	EtOH & T80	• Green core • "Ring-like" alginate shell	CUR is confined to the CS core
Single-Layer NG (3)	DMSO	Bright green/rod shaped structures	CUR crystallized outside the matrix
Dual-Layer NG (4)	DMSO	Bright green/rod shaped structures	CUR crystallized outside the matrix

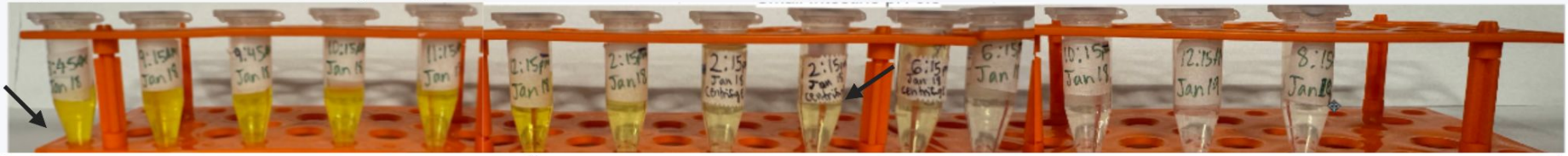
SEM/TEM imaging: spherical, uniform
150 nm (single NG) and 350 nm (Dual layer NG) with PDI<0.06



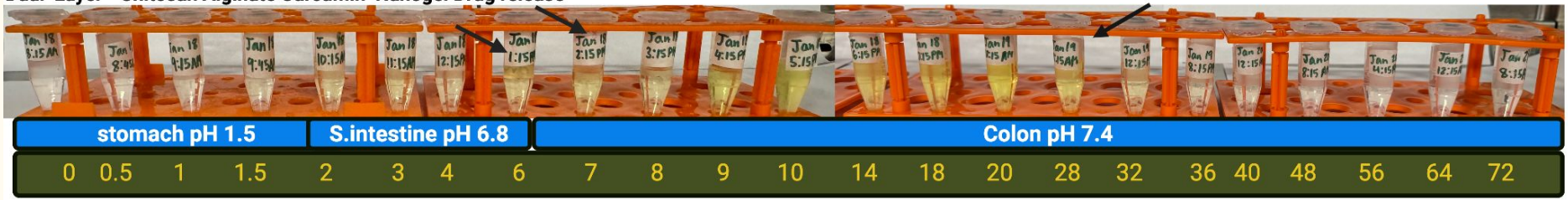
In Vitro Validation

pH responsive drug release kinetics across simulated Gastrointestinal environment

Single Layer - Chitosan Curcumin Nanogel Drug release



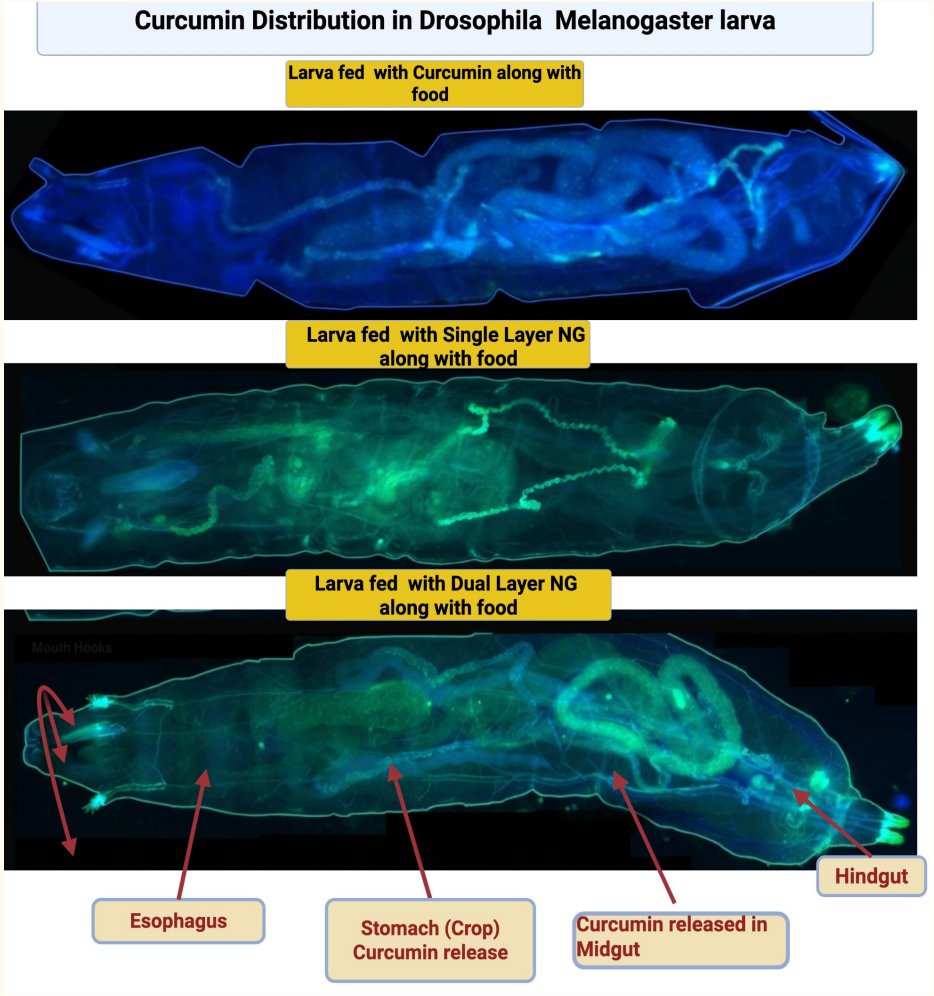
Dual Layer - Chitosan Alginate Curcumin Nanogel Drug release



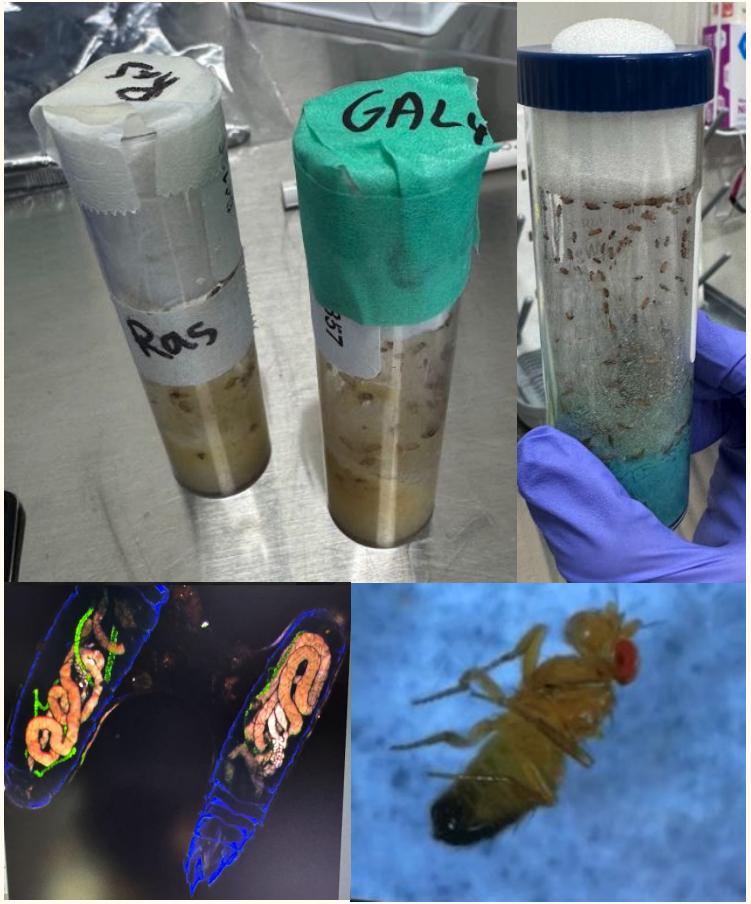
Drug release behavior of Single -layer CS-Cur NG and Dual-Layer CS-CUR-ALG NG was evaluated under gastrointestinal tract: Stomach (pH1.5), small intestine (pH6.8), and colon (pH 7.4). At each time point, supernatant samples were collected and curcumin concentration was quantified by UV - Vis spectroscopy (λ = 425nm) against a pre generated standard calibration curve.

(Top) Single layer Nanogels exhibited premature drug release beginning at gastric pH, indicating limited protection in the upper GI tract.

(Bottom) Dual layer Nanogels demonstrated significantly suppressed release at stomach and small intestinal pH, with sustained curcumin release occurring preferentially at colonic pH 7.4.



Drosophila Validation



Discussion/Analysis

- Dual-layer NG architecture is validated with SEM/TEM , Zeta potential reversal confirms successful layer by layer assembly. PDI showed uniform particle size distribution.
- Korsmeyer-peppas Kinetics release exponent (n) indicated super case II super transporter - explains that Curcumin release by the NG is by swelling and contraction rather than simple diffusion.
- Phase 3 formulation was optimized: Ethanol + Tween 80 as a solvent system for curcumin, pH of 5.5 to the chitosan mixture, 1000 RPM stirring speed for cross linking –maximizing ionic gelation and curcumin entrapment.

Conclusion & Significance

- **Optimization & Synthesis**: High encapsulation efficiency was achieved by using EtOH/Tween 80 as the CUR solvent, maintaining chitosan solution pH of 5.5, and using 1000 RPM for TPP cross-linking.
- **Structural Characterization**: SEM/TEM and PDI confirmed a uniform dual-layer architecture. Zeta potential reversal validated the successful dual-layer of the NG system
- **Delivery Performance**: Compared to single-layer NGs, the dual-layer reduced premature gastric release and significantly improved colonic CUR delivery
- **Drug Release Kinetics**: Korsmeyer-Peppas modeling revealed Super Case ll transport, confirming that the release is driven by pH-responsive swelling and relaxation.
- The dual-layer chitosan-alginate NG effectively targets the colon

Future Works

- Implement microfluidic assisted synthesis to reduce the NG particle size
- Conduct NG cytotoxicity tests on HCT-116 CRC cell lines (MTT and Clonogenic Assays)
- Extend the research to include in vivo mouse studies
- Investigate cellular uptake efficiency of NG by HCT-116 cells using flow cytometry
- Understand internalization mechanisms using specific endocytosis inhibitors to identify the biological pathways of NG entry.

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