In-vitro and *in-silico* studies on curcumin loaded chitin and chitosan nanoparticles from shrimp shells

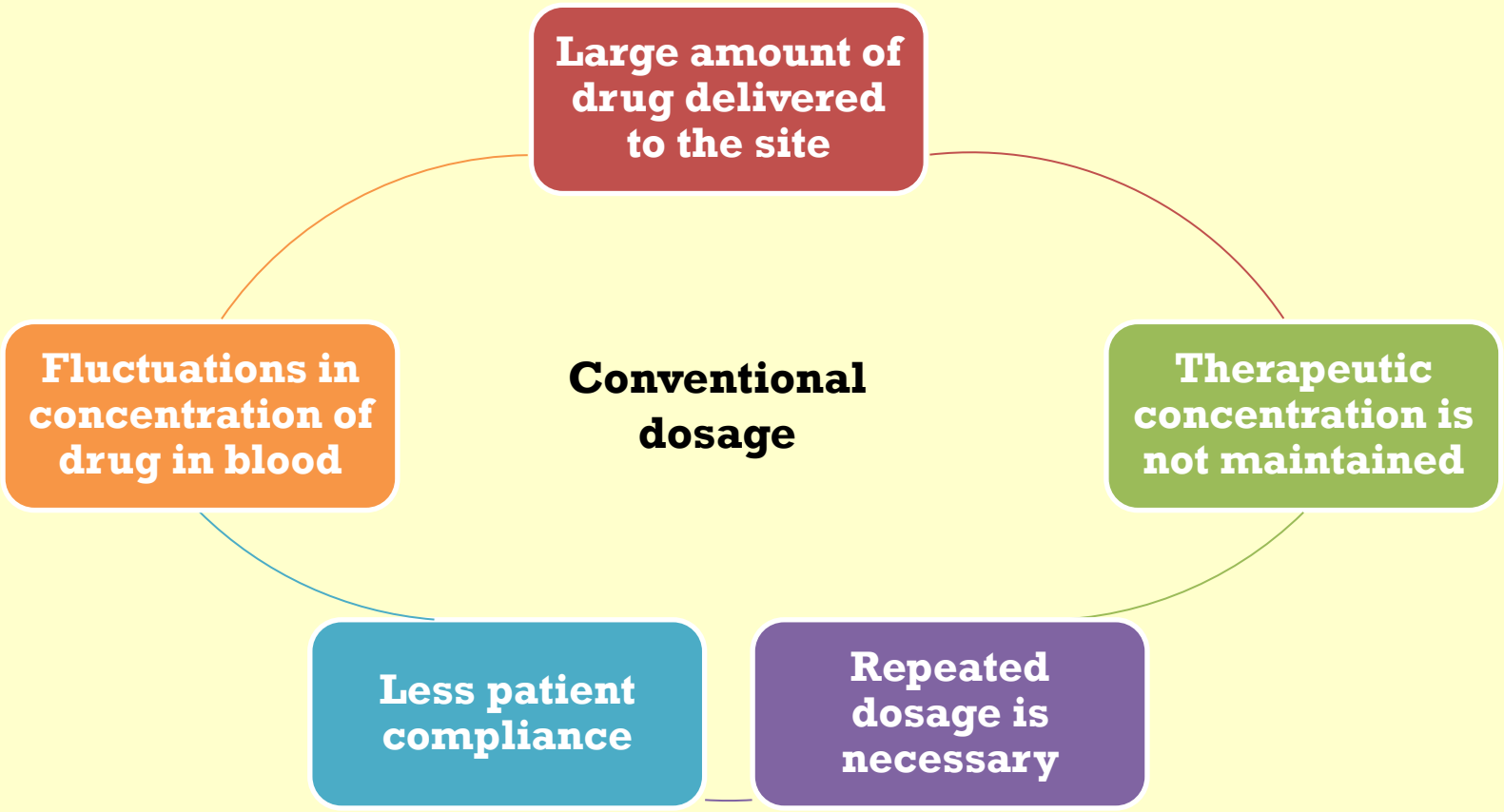
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Objectives

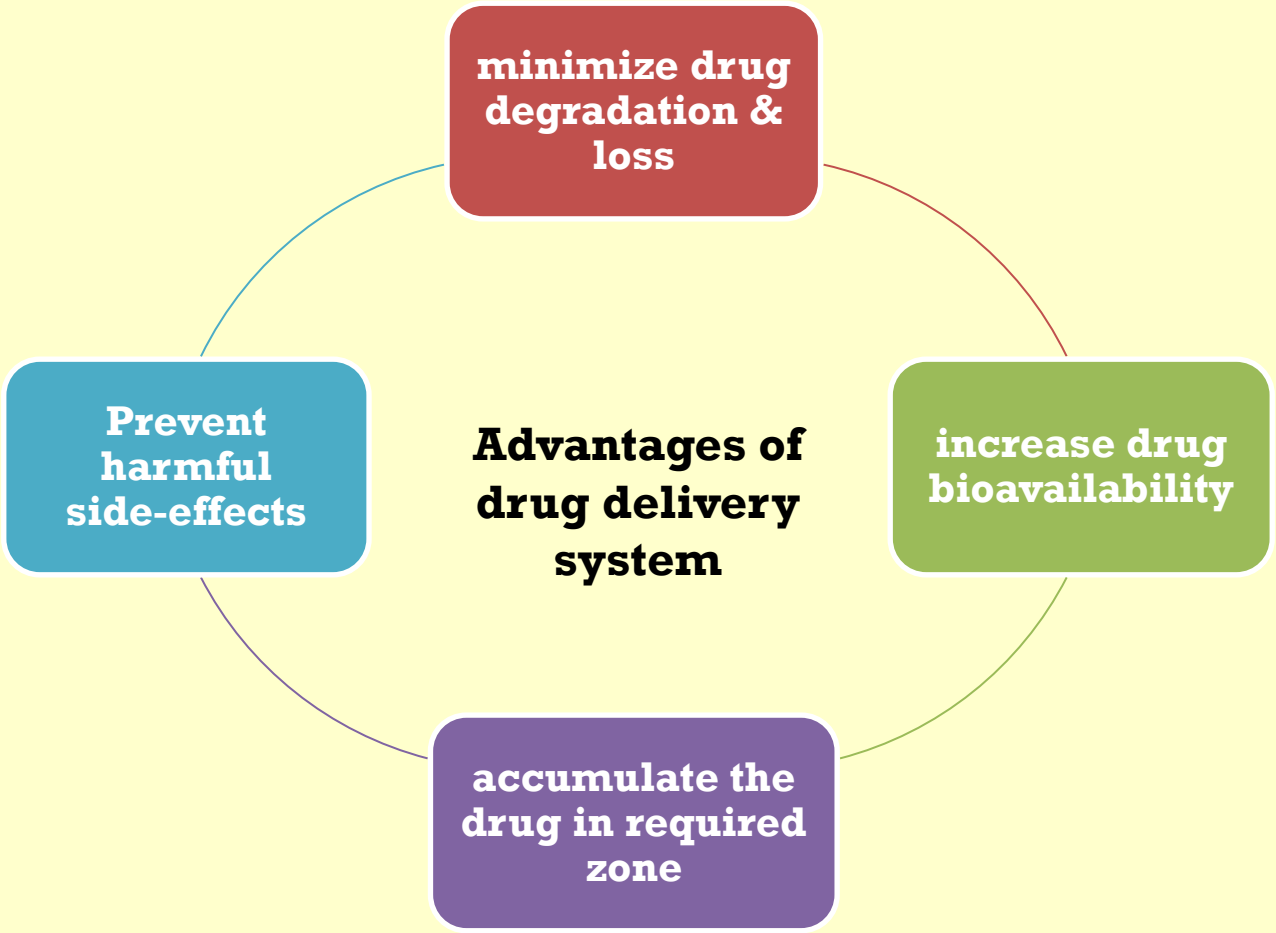
- To evaluate the drug delivery property of the natural biopolymers (chitin and chitosan nanoparticles) by *in-vitro* and *in-silico* approaches using curcumin as a model drug.

Demerits of conventional dosage

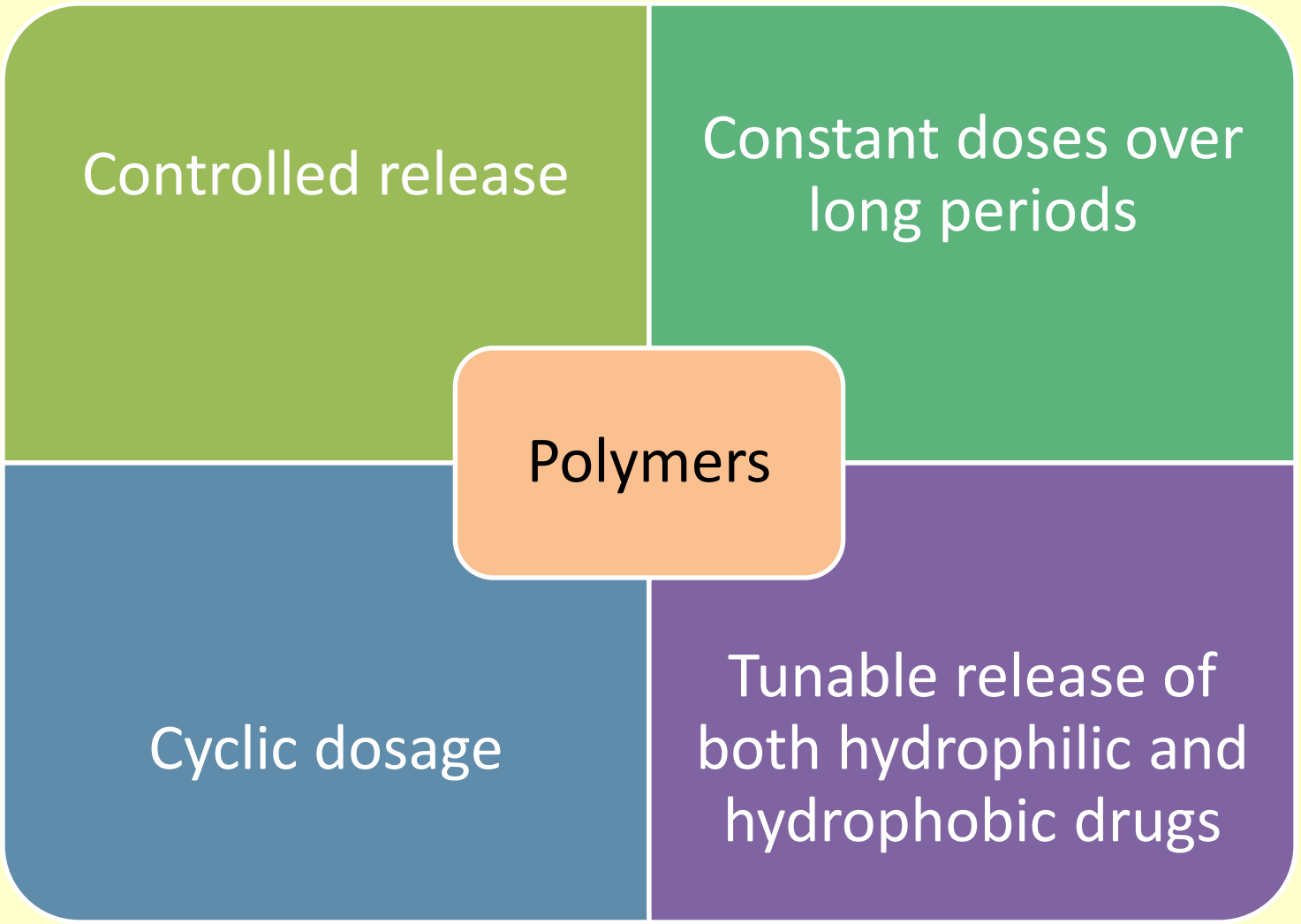


Drug delivery system (DDS) - Advantages

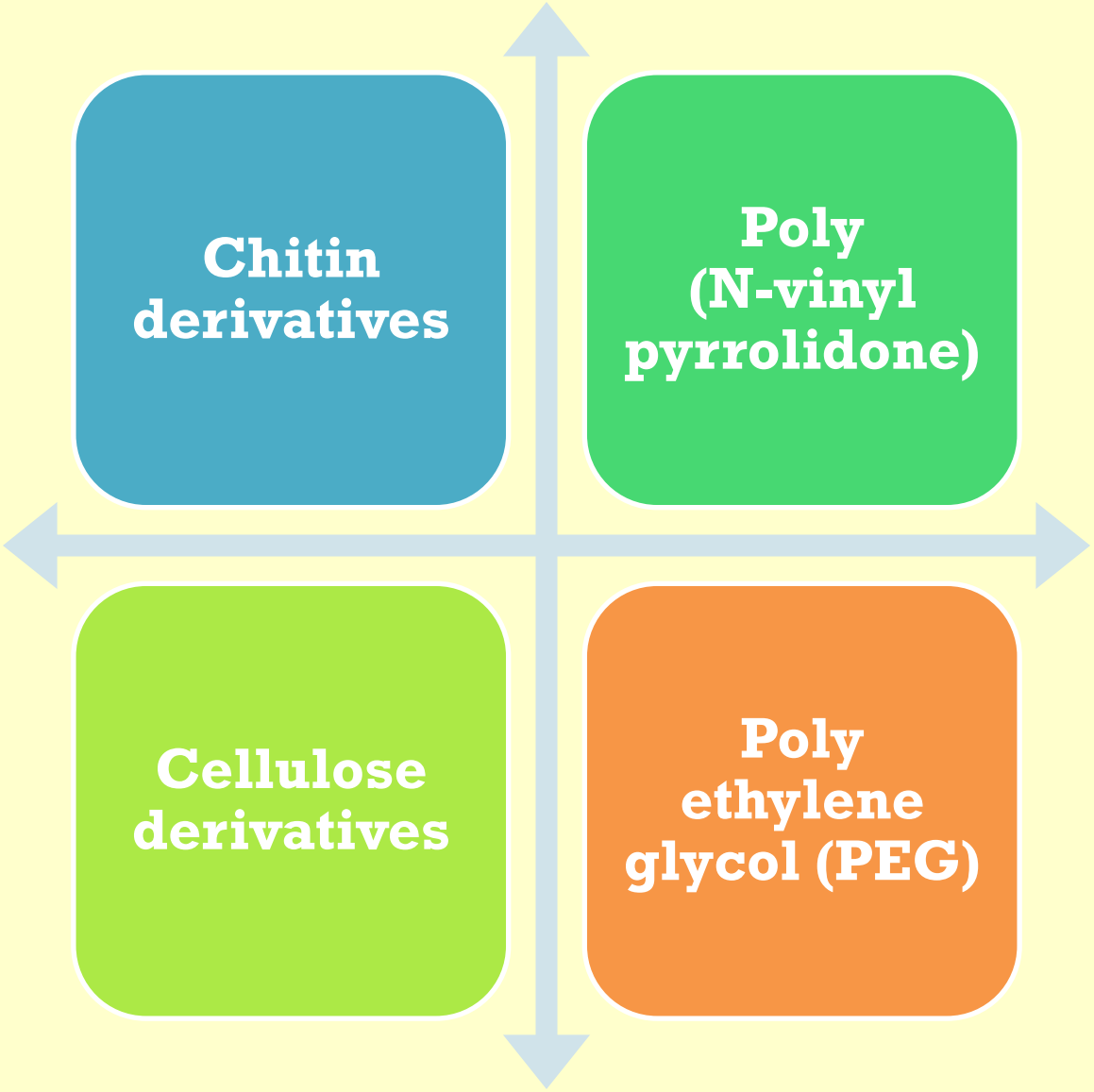
Drug delivery system (DDS) - process of administering a pharmaceutical compounds to achieve a therapeutic effect in humans or animals.



Qualities of polymers as DDS



Commonly used polymers as DDS



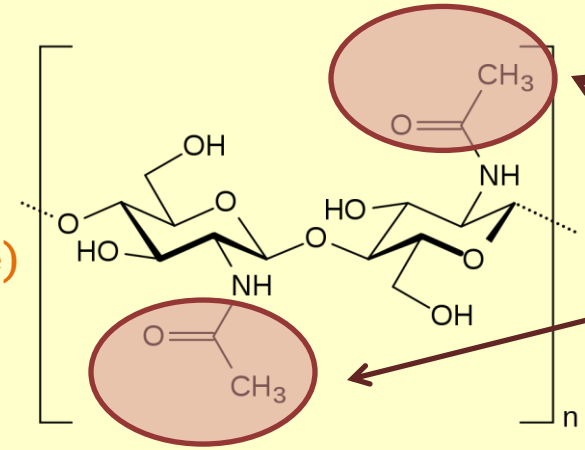
Chitin and chitosan

- Second most abundant polysaccharide
- Mucoadhesivity
- Biocompatibility
- Biodegradability
- Nontoxicity
- Low-immunogenicity

Chitin vs chitosan

- Chitosan is a derivative of chitin

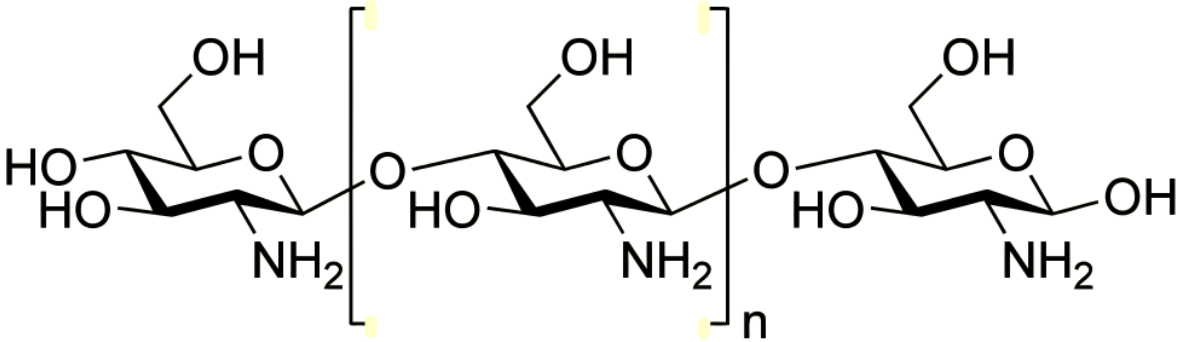
Chitin
(N-acetyl glucosamine)



Acetyl group

Deacetylation

Chitosan
(Glucosamine)

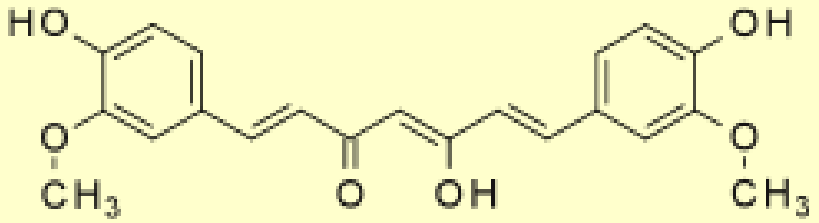


Curcumin

- Hydrophobic polyphenol compound derived from the rhizome of the herb *Curcuma longa* (Turmeric)



Rhizome of turmeric & Curcumin powder



1,7-bis(4-hydroxy-3- methoxyphenyl)-1,6-heptadiene- 3,5-dione)

- Treatment and prevention of a wide variety of human diseases (Heart diseases, cancer, inflammatory diseases etc.)
- Biological activities - antioxidant, anti-inflammatory, antimicrobial, anticarcinogenic, hepato- and nephroprotective, thrombosis suppressing, myocardial infarction protective, hypoglycemic, and antirheumatic effects.
- Model drug to evaluate the drug delivery system

Flowchart of the study



Shrimp (*Penaeus monodon*)

↓

Deshelling

Alkali and acid hydrolysis ↓

Isolation of chitin

Deacetylation ↓

Synthesis of chitosan

Ionic gellation using TPP

Ionic gellation using TPP + Curcumin

chitin and chitosan NP

curcumin loaded chitin and chitosan NP

Characterization

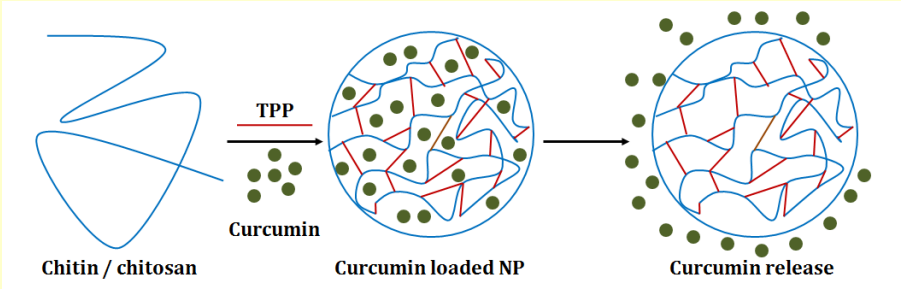
- FTIR
- XRD
- SEM
- DLS

Drug delivery

- pH dependent *(In vitro)*
- Kinetics models (Theoretical)

***In-silico* studies**

- Polymer chain construction
- Docking with Curcumin
- Molecular dynamics



Results

Synthesis of curcumin loaded chitin and chitosan NPs

Weight ratios (mg/ml)		EE (%)	LC (%)
CNP	**Curcumin**		
5	0.5	97.26 ± 0.30	09.72 ± 0.03
5	1	**97.60 ± 0.10**	**19.52 ± 0.02**
5	2	46.30 ± 0.05	18.52 ± 0.02
ChNP			
5	0.5	98.06 ± 0.12	09.80 ± 0.01
5	1	**98.40 ± 0.10**	**19.80 ± 0.02**
5	2	48.07 ± 0.08	19.23 ± 0.02

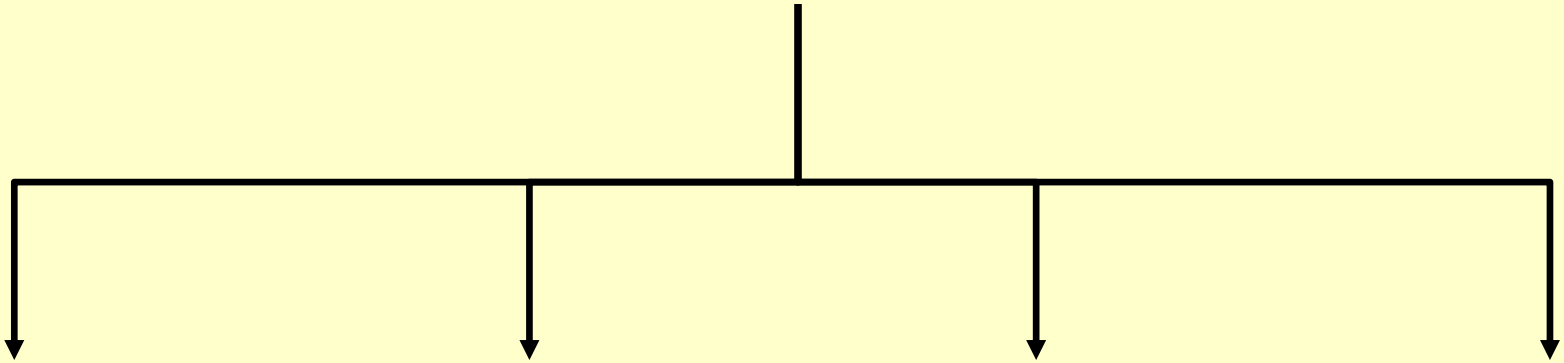
Encapsulation efficiency (EE) = (Total amount of drug – Free drug) / Total amount of drug × 100

Drug loading capacity (LC) = (Total amount of drug – Free drug) / Amount of nanoparticles × 100

5:1 weight ratio selected for further characterization and delivery studies

Characterization

Chitin and chitosan NP
&
Curcumin loaded chitin and chitosan NP
(5:1 weight ratio)



FTIR

Study the chemical interactions and modifications

XRD

Study the binding between the NPs and curcumin

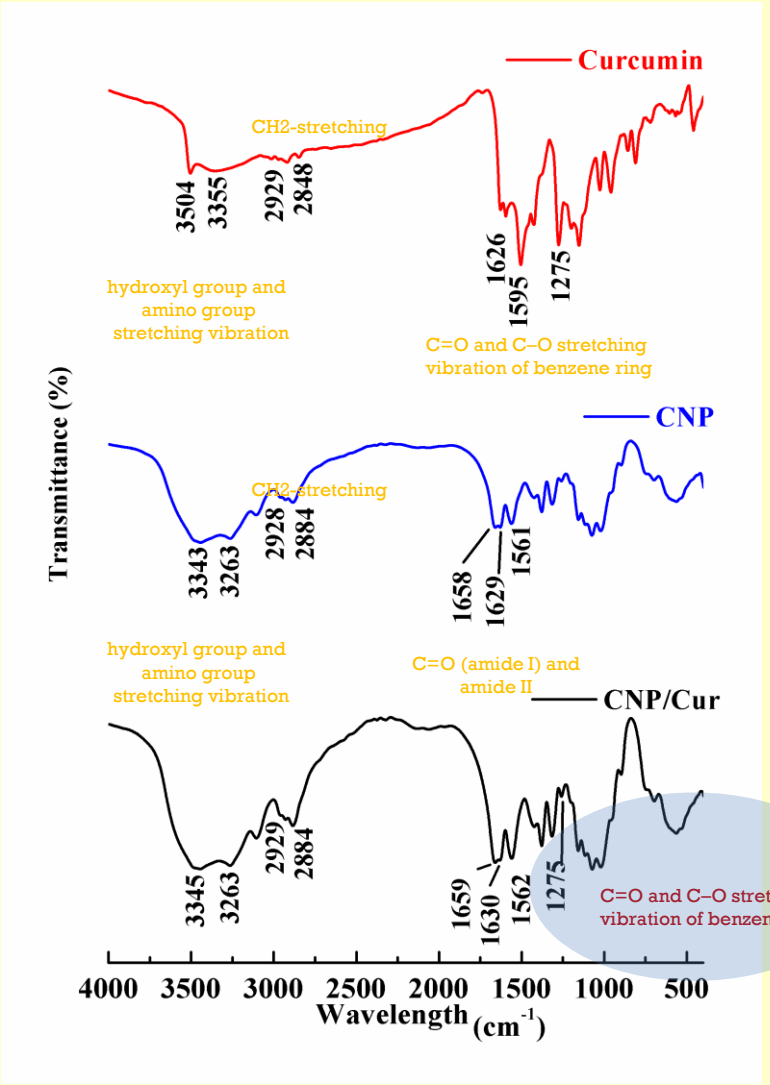
DLS

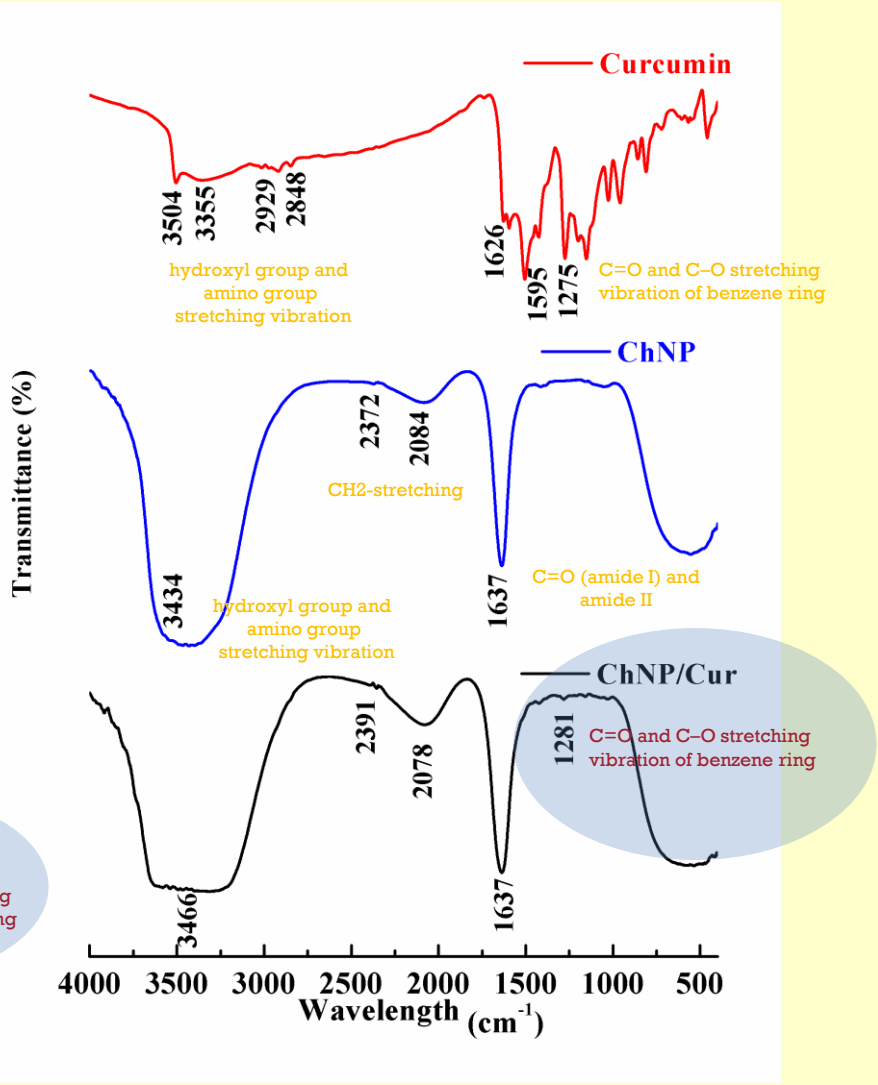
Evaluate the surface charge and average particle size

SEM

Study the morphology and size

FTIR spectroscopy of curcumin loaded NPs





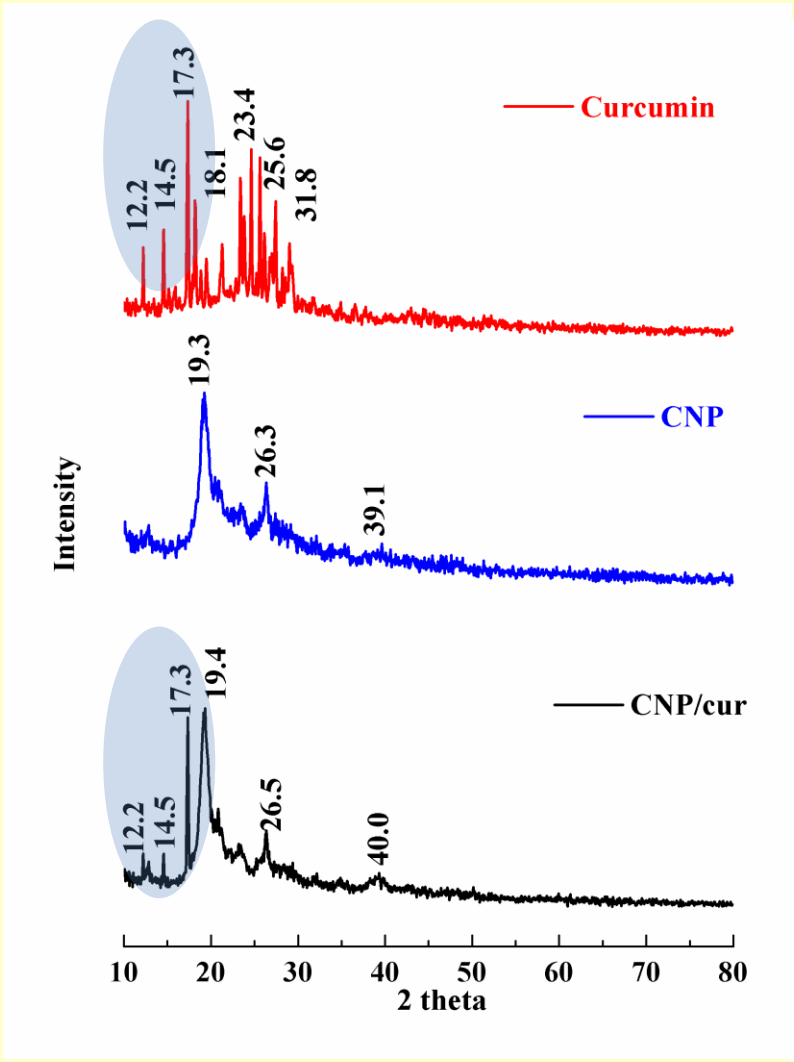
✓Additional peak corresponds to C=O and C–O stretching vibration of benzene ring of curcumin was observed in Curcumin loaded NPs and confirmed the presence of Curcumin.

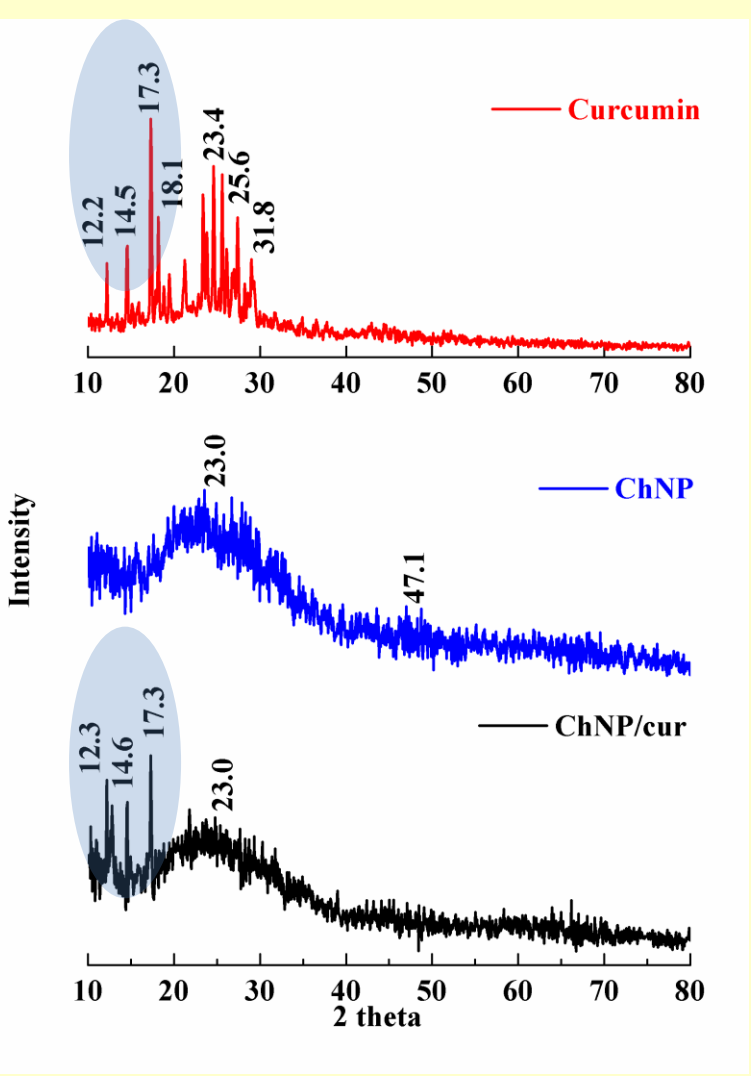
✓Shift has been found about 1 to 10 cm-1 in the curcumin loaded NPs, because of hydrogen bonds, formed between curcumin and NPs

✓Confirmed curcumin binds effectively with NPs

Results

XRD pattern of curcumin loaded NPs





✓Three diffraction peaks corresponds to curcumin was appeared in the Cur/NP complex

✓Remaining peaks of curcumin was disappeared due to formation of an amorphous complex with the intermolecular interaction occurring within NP and curcumin molecules

Results

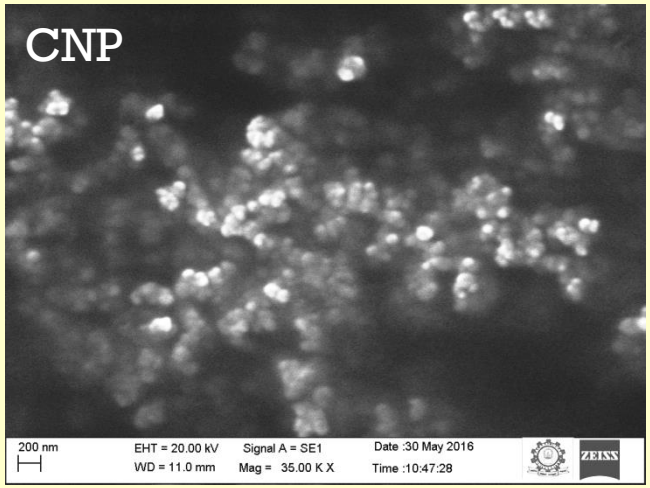
Dynamic light scattering of curcumin loaded NPs

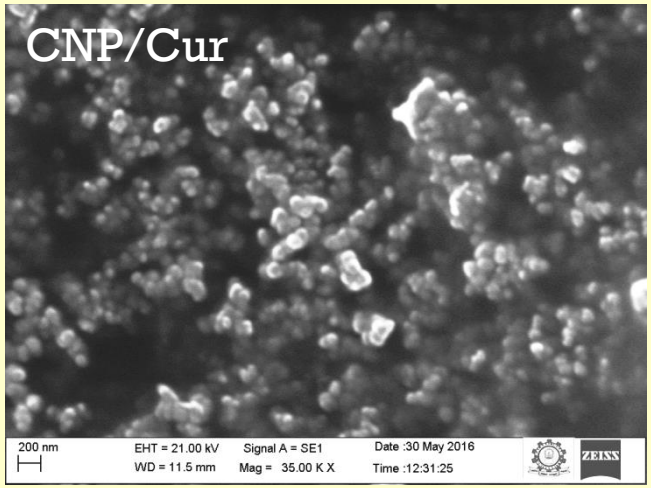
System	Average diameter in DLS (nm)	Zeta potential (mV)
CNP	245.0 ± 10	+ 11.0 ± 1
ChNP	**145.0 ± 5**	**+ 18.4 ± 2**
CNP/Cur	250.9 ± 10	+ 17.9 ± 4
ChNP/Cur	**150.8 ± 20**	**+ 27.6 ± 3**

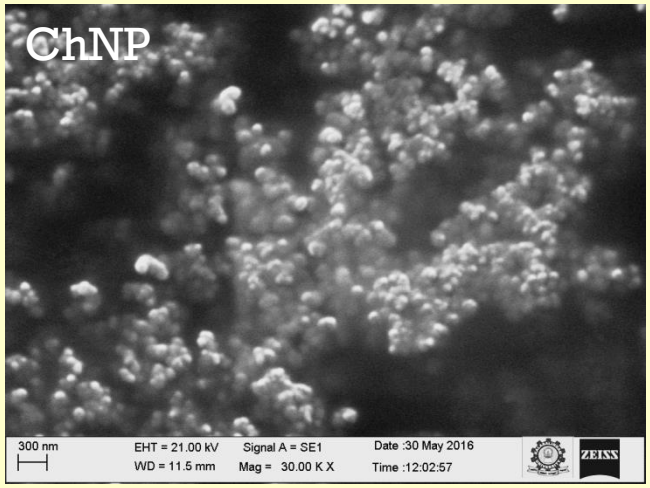
✓ChNP and Curcumin encapsulated ChNP was smaller than CNP, due to lack of acetyl group.

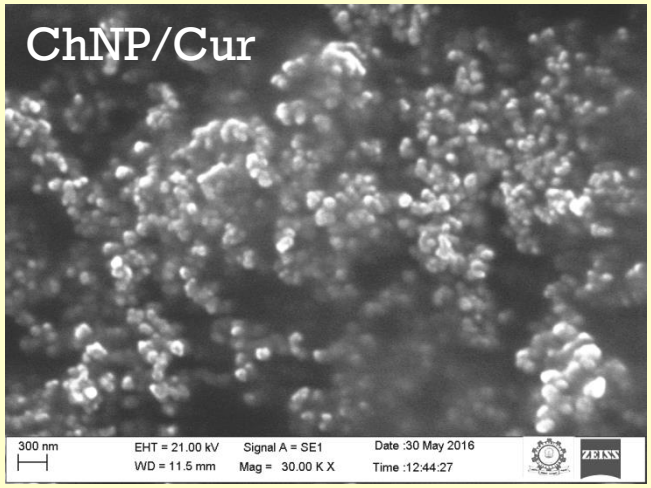
✓ChNP and Curcumin encapsulated ChNP have higher positive charge than CNP, due to the presence of amino groups.

SEM micrograph of curcumin loaded NPs









CNP	126 ± 8
ChNP	69 ± 6
CNP/Cur	135 ± 9
ChNP/Cur	77 ± 12

- ✓Spherical in shape
- ✓ChNP and Curcumin encapsulated ChNP was smaller than CNP
- ✓particle size observed under SEM is approximately half the fold smaller than the average diameter observed in DLS
- ✓Reduction in size is may attributed to that hydrodynamic diameter of freshly prepared nanoparticles measured by DLS

Results

Summary of characterization

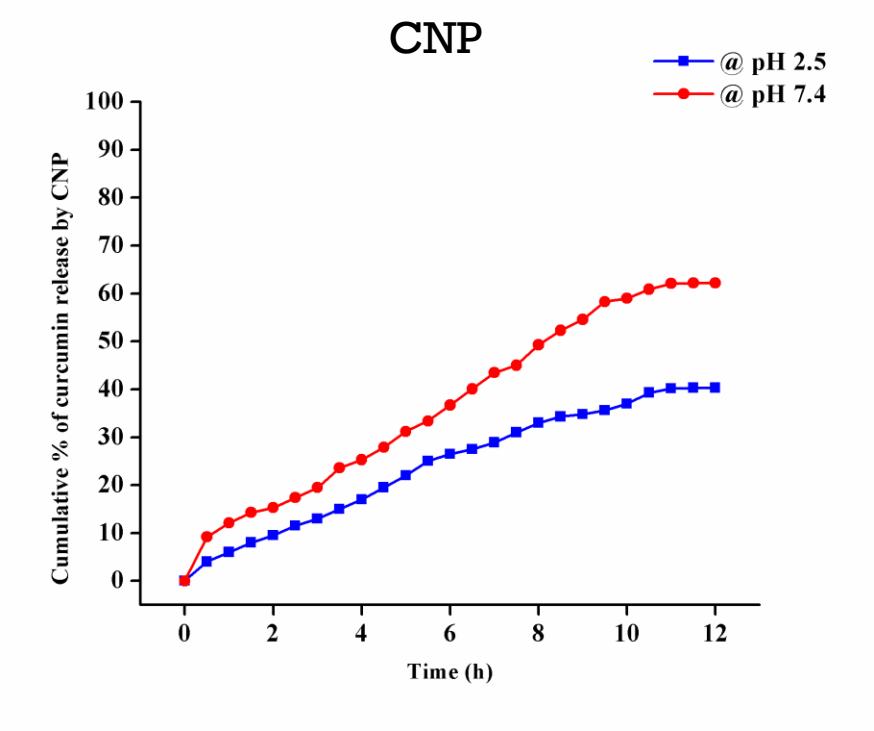
- Curcumin stably encapsulated by CNP and ChNP
- There is no chemical reactions occurred between curcumin and the NPs
- Physical interactions, mostly intermolecular hydrogen bonds occurred between curcumin and the NPs
- DLS and SEM confirmed that the spherical NPs get swelling in the aqueous medium.
- ChNP encapsulated curcumin are smaller in size and have more positive charge.

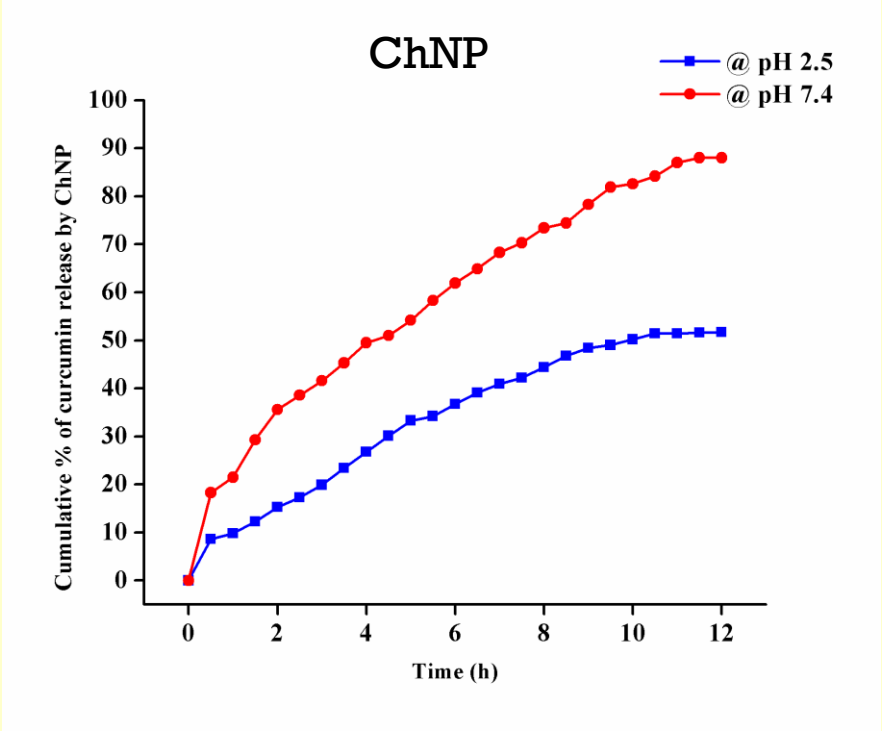
In vitro curcumin release

In vitro curcumin release

- Release of curcumin by CNP and ChNP was tested in two different pHs (2.5 – gastric pH, 7.4 - intestinal pH)
- Four different kinetics models were adapted to study the mechanism by analyzing the experimental data
  - Zero-order model
  - First-order model
  - Higuchi model
  - Korsmeyer-Peppas model

Drug Release Testing





✓The curcumin release was very low at pH 2.5 and very high at 7.4

✓The nanoparticles reaches its maximum swelling effect at higher pH, due to higher number of deprotonated amine groups

✓ChNP has more number of deprotonated amine groups than CNP and the release rate was more in ChNP

Results

Kinetics model

	Zero-order model	**First-order model**	**Higuchi model**	**Korsmeyer-Peppas model**	
	$Q_t = Q_0 + k_0 t$	$\log C_t = \log C_0 - k_1 t/2.303$	$Q_t = k_H.t0.5$	$\log Q_t = \log k + n.\log t$	
	drug release rate is independent from its concentration	drug release from a system is concentration dependent	drug release from an insoluble matrix based on diffusion	n value is $0.45 < n > 0.85$, the release occurred due to diffusion and swelling mechanism	
Complex	**R^2**	**R^2**	**R^2**	**n**	**R^2**
CNP/Cur	0.998	0.979	0.977	0.451	0.984
ChNP/Cur	0.996	0.977	0.966	0.520	0.989

✓The curcumin release from CNP and ChNP fits both Zero order and Korsmeyer-Peppas model

✓Fitting with zero order confirms that the release was independent of its concentration

✓The diffusion exponent (n) determined from the Korsmeyer-Peppas model was also lies between 0.45 and 0.89 and confirms the release occurred due to diffusion and swelling of the nanoparticles

✓Curcumin from CNP and ChNP is controlled by more than one mechanism.

Summary of *In vitro* curcumin release

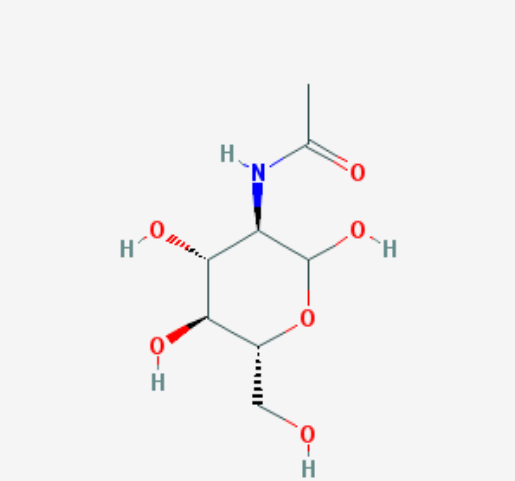
- CNP and ChNP releases more curcumin at pH 7.4
- ChNP releases higher amount of curcumin than CNP
- Kinetics models revealed that the release was controlled by more than one mechanism.

In silico studies

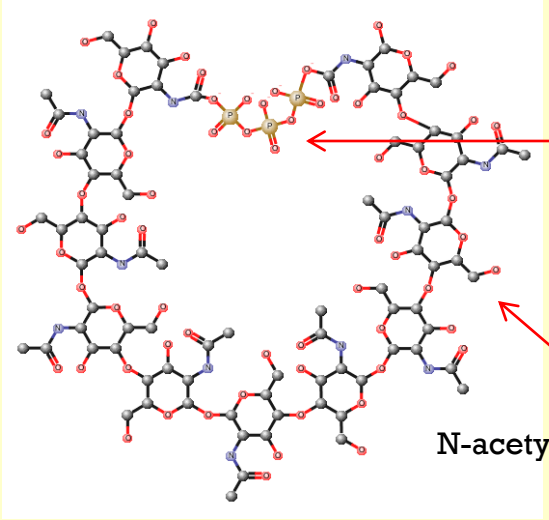
In silico studies of curcumin - NP interaction

- Polymer chain construction
  - Using monomer units and TPP
- Molecular docking
  - To predicts the preferred orientation of curcumin interacted to the NP to form a stable complex.
- Molecular dynamics
  - Curcumin and NP complex were allowed to interact for a fixed period of time (10 ns) to view the dynamical evolution and to study the movement of the atoms.

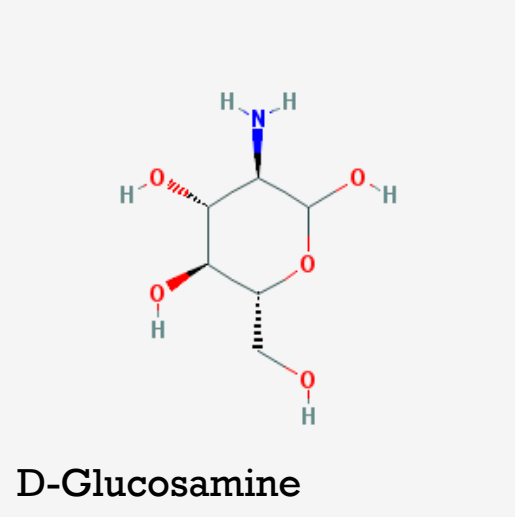
In silico studies – Polymer chain construction



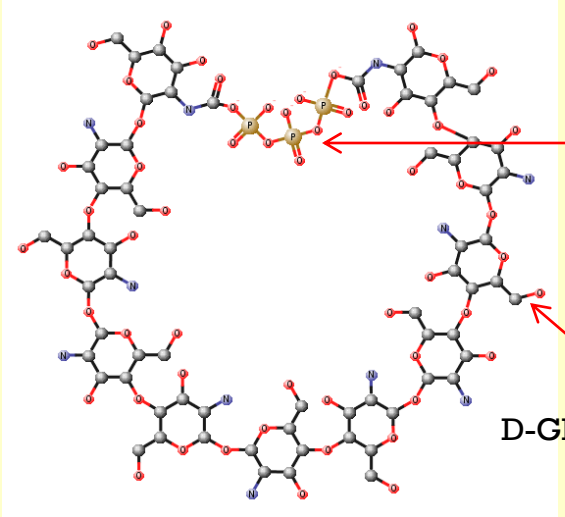
Marvin Sketch



CNP



Marvin Sketch

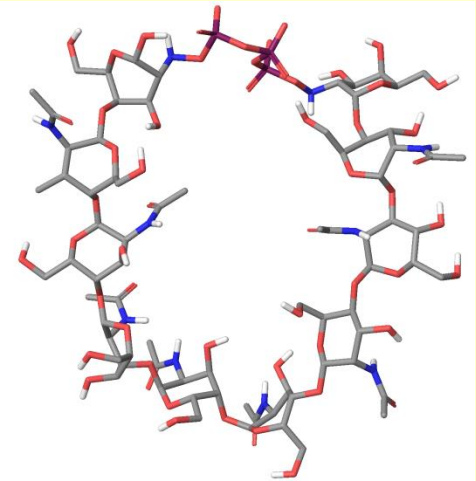


ChNP

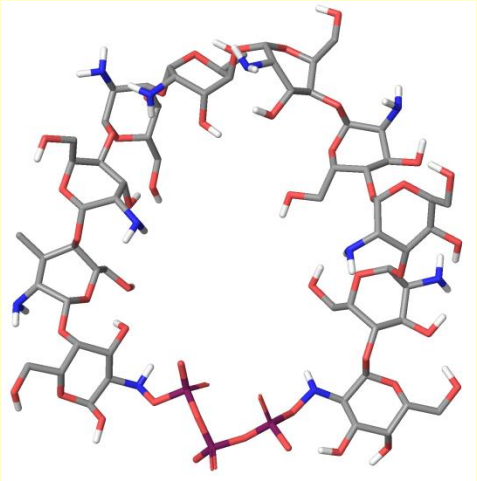
Results

In silico studies – Energy minimization

- To attain a stable confirmation of the polymer chain



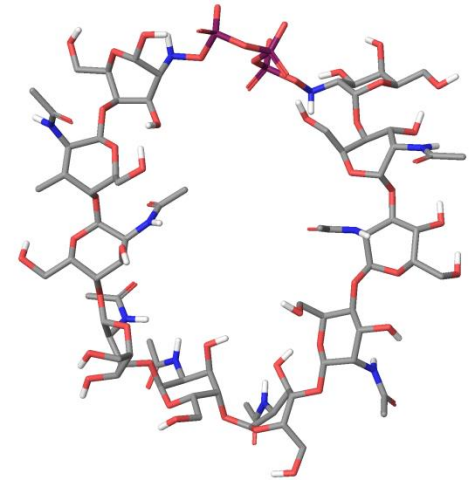
CNP – Energy minimized



ChNP – Energy minimized

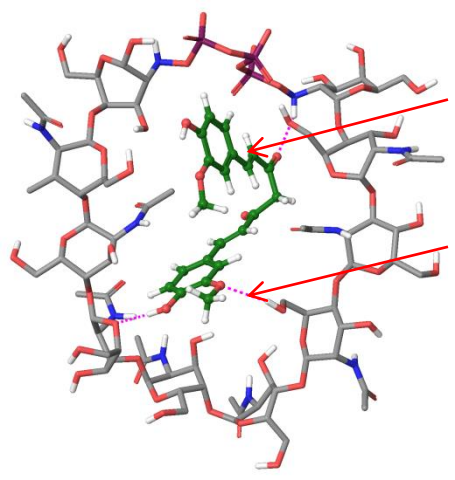
- Schrödinger module Merck Molecular Force Field (MMFF)

In silico studies – Docking

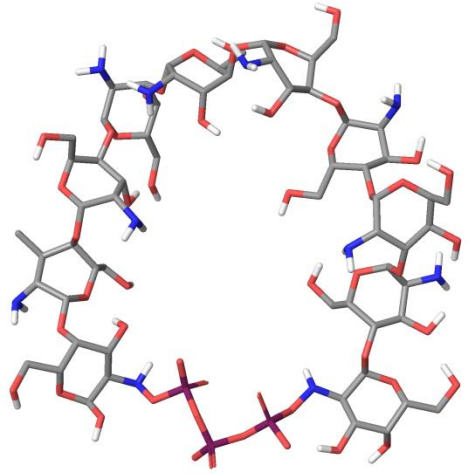


CNP – Energy minimized

Glide module

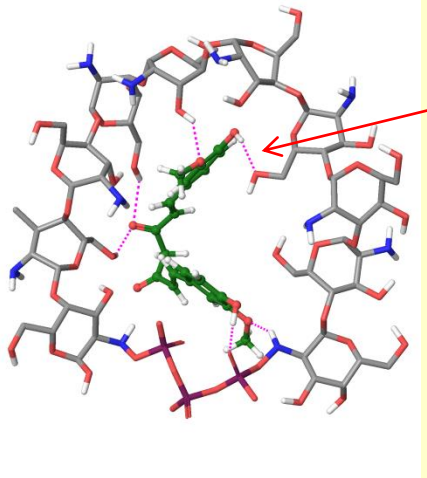


CNP – Docked with Curcumin
Hydrogen bonds – 3
Docking Score – (-2.612)



ChNP – Energy minimized

Glide module



ChNP – Docked with Curcumin

Hydrogen bonds – 6
Docking Score – (-3.305)

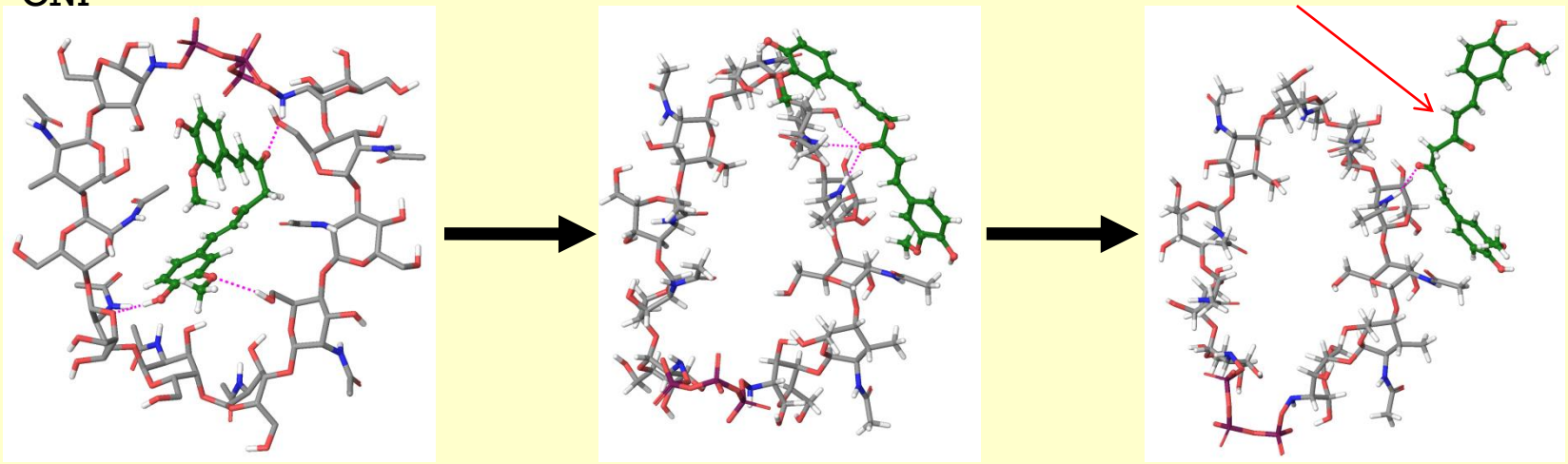
Results

In silico studies – Molecular dynamics

Desmond with Optimized Potentials for Liquid Simulations (OPLS) 2005 force field.

CNP

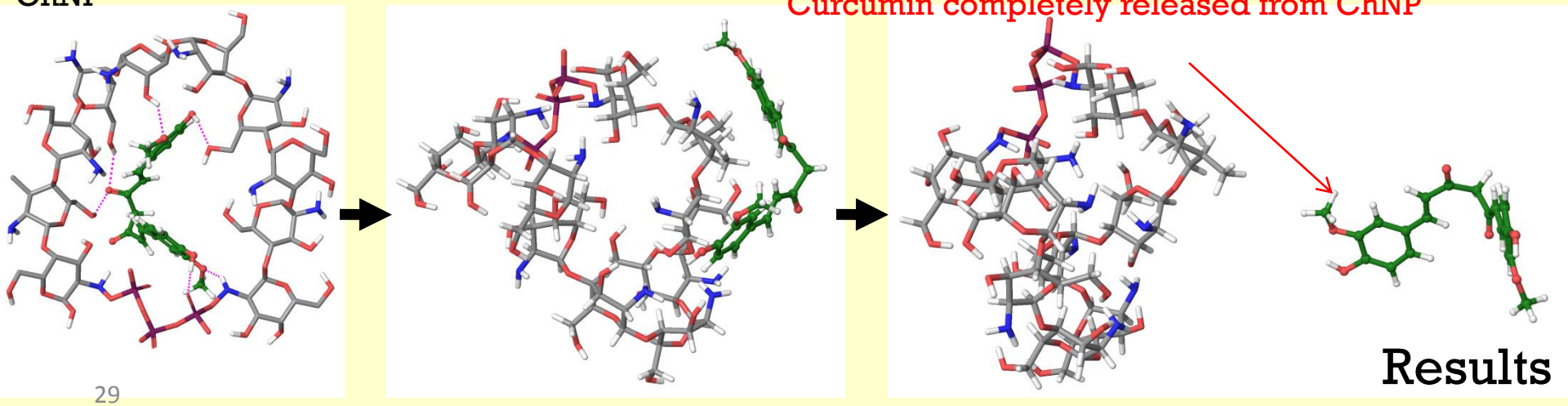
Curcumin still bound with CNP with one H-bond



0 ns 5 ns 10 ns

ChNP

Curcumin completely released from ChNP



0 ns 5 ns 10 ns

Results

Summary of *In silico* studies

- Docking sores of ChNP with curcumin was higher (-3.305) than CNP with curcumin.
- The H-bonds involved between ChNP with curcumin was more (6 Nos) than CNP with curcumin.
- MD revealed Curcumin released very soon from ChNP than CNP.
- ChNP was sensitive to external pH, ions, water molecules and temperature.
- The results obtained in *in silico* studies are well correlated with *in vitro* studies.

Conclusion

✓ChNP shows

- ✓higher encapsulation efficiency
- ✓drug loading capacity

✓7.4 could be the best pH for sustained and controlled release of drugs by CNP and ChNP

✓Drug release by CNP and ChNP was controlled by more than one mechanism more likely by

- ✓swelling of the polymer and diffusion of drug

Conclusion

✓A good correlation was observed between the experimental data and the *in silico* studies, which revealed that the controlled release of curcumin by ChNP and CNP

✓Integrated *in-vitro* and *in-silico* studies can save considerable time during the selection of the best polymer for delivering a particular drug

✓CNP and ChNP could be used for the delivery of hydrophobic drugs

Recent publications from our research group

1. Dhanasekaran Solairaj, **Palanivel Rameshthanga**m; Silver nanoparticle embedded α-chitin nanocomposite for enhanced antimicrobial and mosquito larvicidal activity. *Journal of Polymers and the Environment* (In press) [Springer] (IF-1.97).
2. Chitra Jeyaraj Pandian & **Palanivel Rameshthangam**; Applications of L-arginine functionalised green synthesised nickel nanoparticles as gene transfer vector and catalyst. *Journal of Experimental Nanoscience,* DOI:10.1080/17458080.2016.1204670. [Taylor & Francis] (IF- 0.832).
3. J. P. Chitra, **Palanivel Rameshthangam**, D. Solairaj; Screening antimicrobial activity of nickel nanoparticles synthesized using *Ocimum sanctum* leaf extract. *Journal of Nanoparticles*, DOI:10.1155/2016/4694367 [Hindawi].
4. P. Muthukumaran, Chikkili Venkateswara Raju, C. Sumathi, S. Ravi, D. Solairaj, **Palanivel Rameshthangam**, J. Wilson, Subbiah Alwarappan, Sathish Rajendran; Cerium doped nickel-oxide nanostructures for riboflavin biosensing and antibacterial applications. *New Journal of Chemistry*, DOI: 10.1039/C5NJ03539B. [Royal Society of Chemistry] (IF-3.22).
5. D. Solairaj, **Palanivel Rameshthangam,** P. Srinivasan; Adsorption of Methylene Blue, Bromophenol Blue and Coomassie Brilliant Blue by α-chitin nanoparticles. *Journal of Advanced Research*, 2016, 7(1), 113–124 [Elsevier].
6. J. P. Chitra, **Palanivel Rameshthangam**, D. Solairaj; *Ocimum sanctum* mediated synthesis of nickel nanoparticle as potent adsorbent for dyes and pollutants. *Chinese Journal of Chemical Engineering*, 23(8), 2015, 1307-1315. [Elsevier] (IF-1.23).


Thank
you