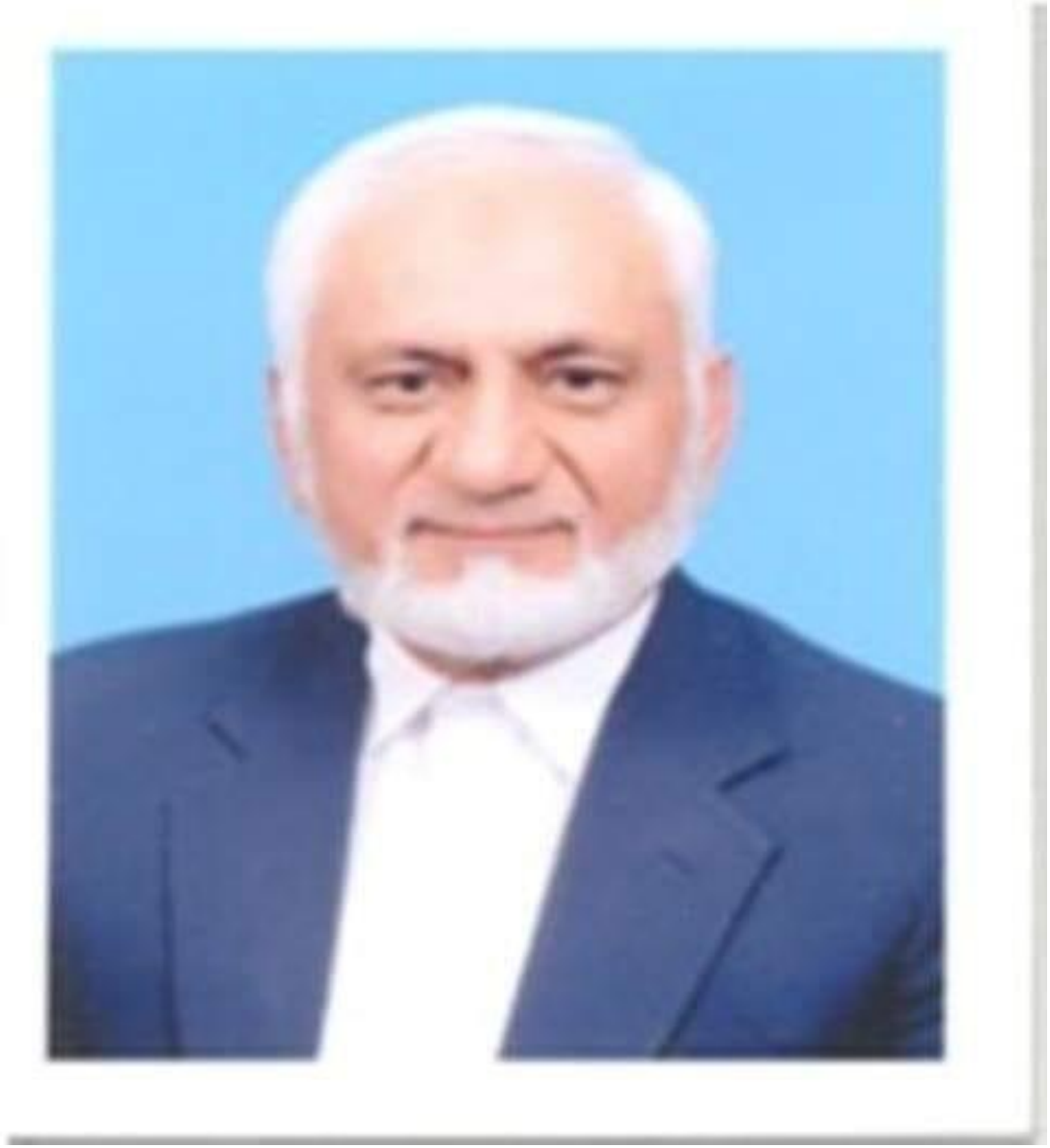


# Dr. Din Mohammad Memorial Lecture

# Nano Drug Delivery Systems: Recent Developments and Challenges

**Prof. Dr. M. Raza Shah (T.I)**

**H.E.J Research Institute of Chemistry, International Center for Chemical and Biological Sciences (ICCBS), University of Karachi, Pakistan**

# Oral drug administration

Oral drug administration is preferred for the following reasons

- More safe,
- Higher patient compliance
- Non-invasiveness
- Easy to counter over dose and adverse event

# Problem Associated with Oral drug administration

- Poor aqueous solubility
- Gastrointestinal tract serves as a barrier for drug absorption.
- Drug instability in gastric environment and mucous layer prevents drug penetration and absorption across intestinal membrane.
- Low Permeability
- First Pass metabolism
- P-gp mediated Efflux
- Enzymatic Degradation
- Low circulation half-life
- Systematic toxicity

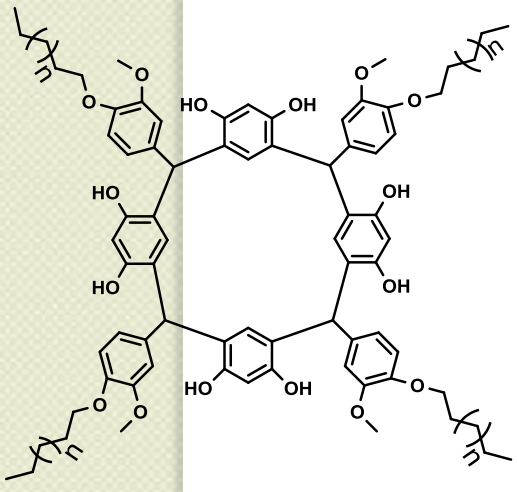
# Nanocarrier Based Drug Delivery System

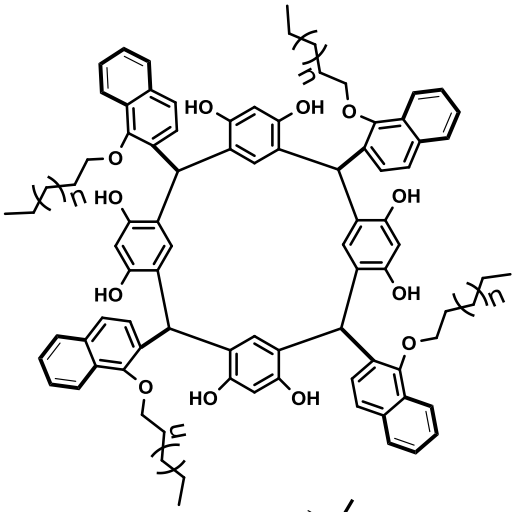
- **Large surface area to volume ratio**
- **Tunable size and Shape**
- **Easy design and Preparation.**
- **Biocompatible and Biodegradable**
- **Increased Stability**
- **Can be used for Lipophilic and Hydrophilic Drugs**
- **Target Delivery**

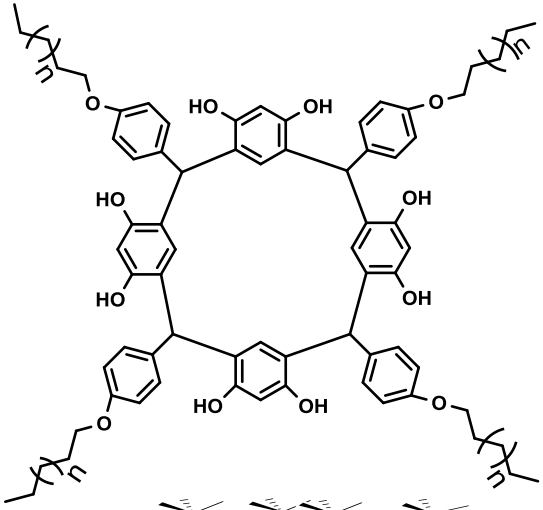
# Nanocarrier Based Drug Delivery System Developed in our Group

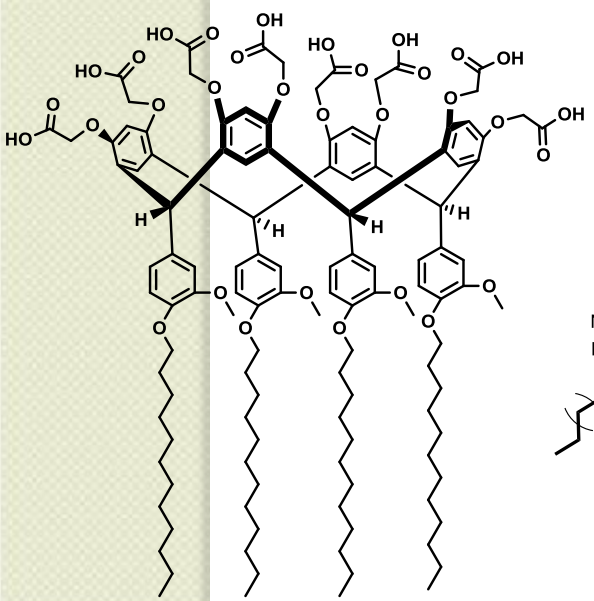
- **Enhanced  Bioavialability**
- **Reversing Multi-Drug Resistance**
- **Target Specific Drug Delivery**
- **Enhanced Therapeutic Efficacy**

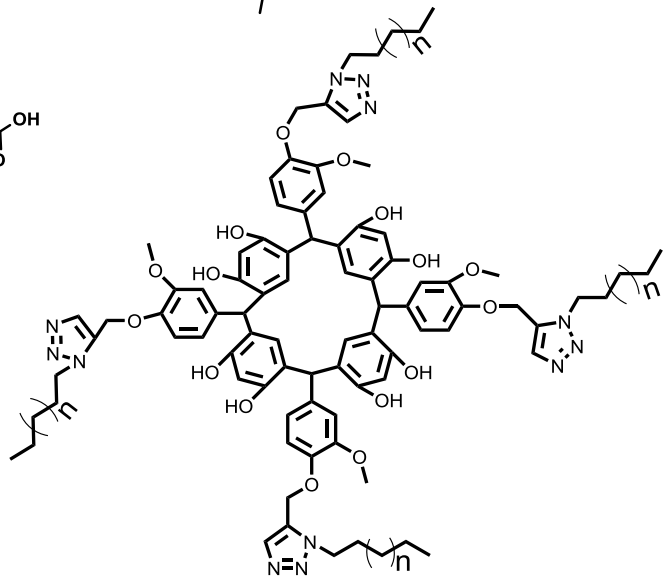
# NIOSOMES for Drug delivery in our group

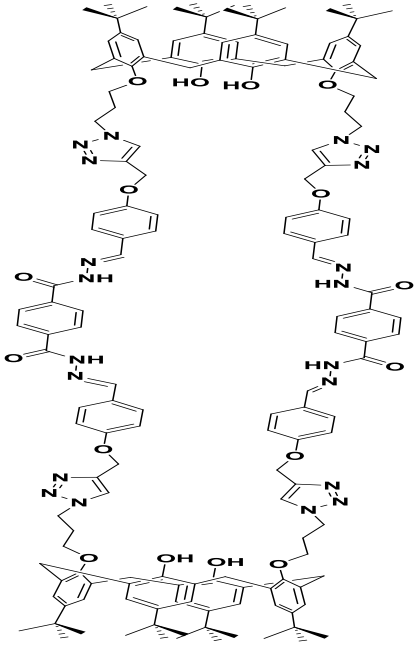




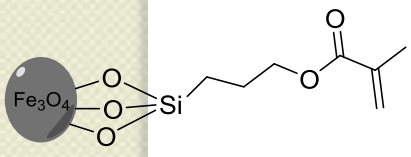


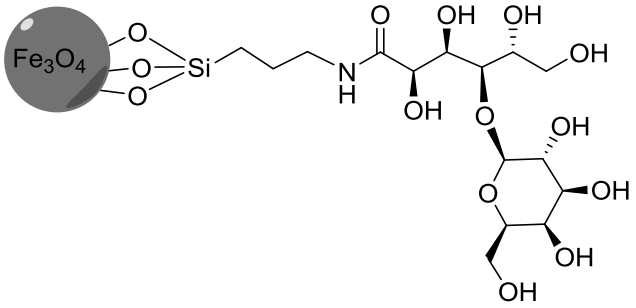




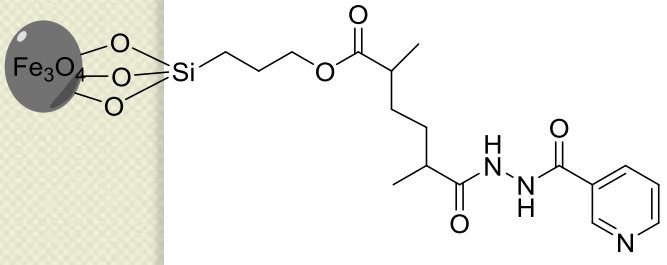


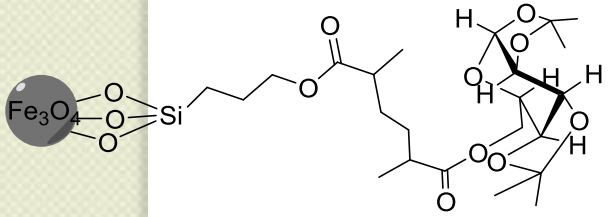
# Nanoparticles for Drug delivery in our group




POLYMERIC NANOPARTICLES



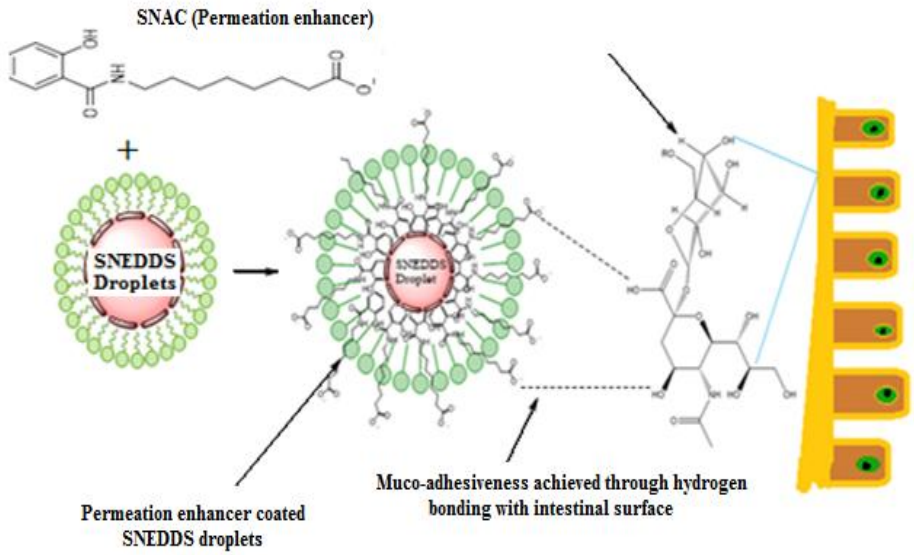

SILVER AND GOLD NANOPARTICLES COATED WITH BIODEGRADABLE AND BIOCOMPATABLE MATERIALS

# ENHANCED ORAL BIOAVAILABILITY

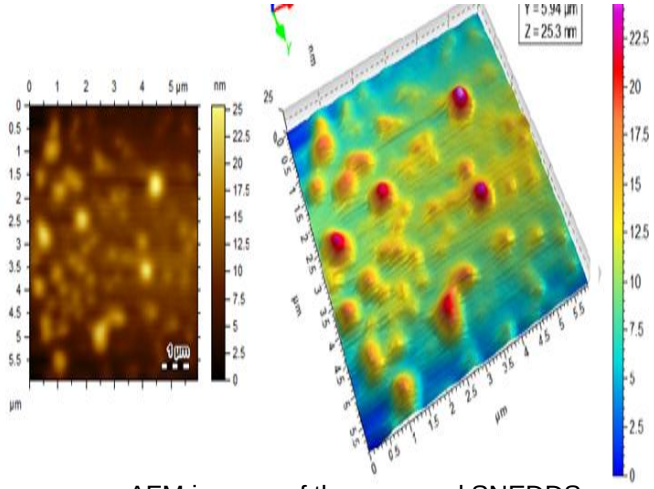
# Nanocarrier for Ceftriaxone.

- **Ceftriaxone** oral bioavailability is limited due to its poor membrane permeability and gastric instability.
- Self-nanoemulsifying drug delivery system (SNEDDS) for Ceftriaxone, having a permeation enhancer (SNAC) decorated on the droplet surfaces. The nanoformulation increased 2.4 times the oral bioavailability of the drug as compared to simple solution of the drug. Moreover, the drug retention in the plasma time was also significantly increased (Tasmina kanwal et al., 2019, J. Mol. Liquids).

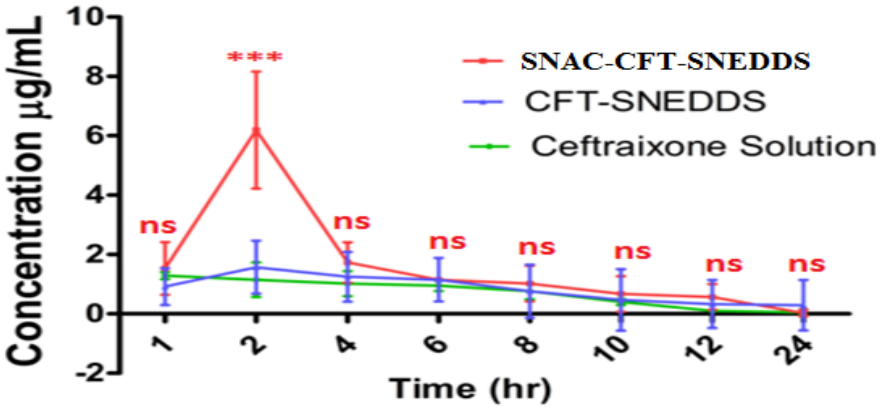
# Enhancing Oral Bioavailability via Nanocarriers



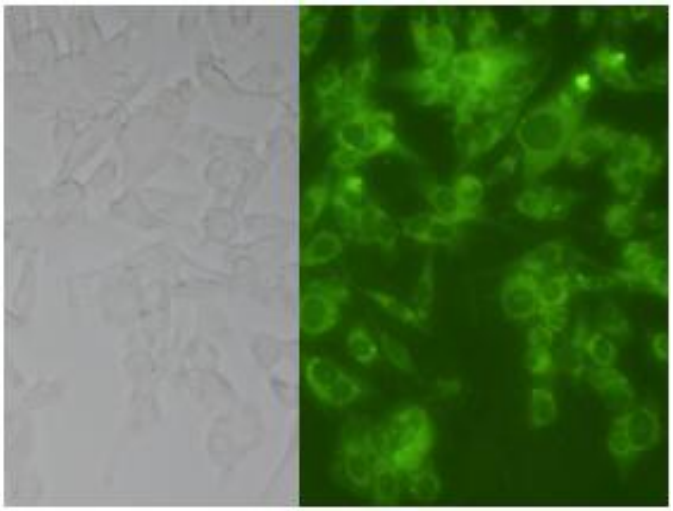

Graphical presentation of the study



AFM images of the prepared SNEDDS




Drug plasma concentration versus time curve of SNAC-CFT-SNEDDS, CFT-SNEDDS and ceftraixone solution



Cellular uptake study of SNEDDS containing SNAC

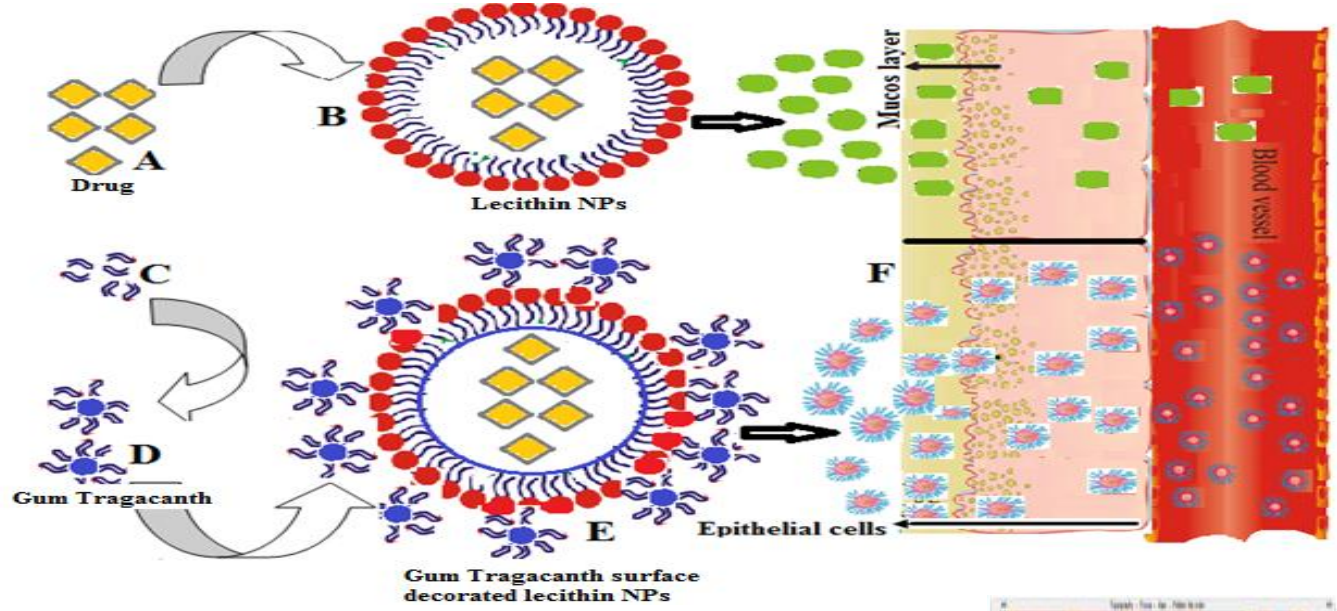
Kanwal, T., Kawish, M., Maharjan, R., Ghaffar, I., Ali, H. S., Imran, M., M. Raza Shah, (2019).. *Journal of Molecular Liquids*, 111098.

# AMPHOTERICIN B

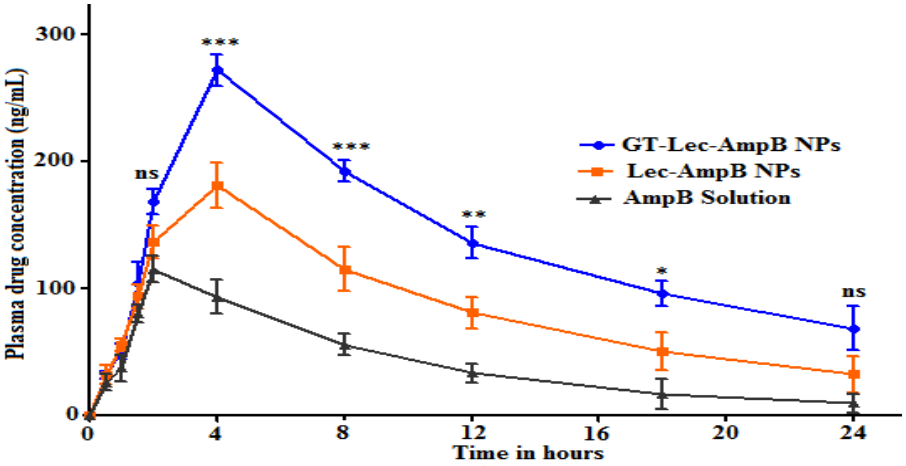
**Lecithin-gum tragacanth based muco-adhesive hybrid nano drug delivery system**

- BCS class IV drug, which has poor solubility and permeability, thus its oral delivery results in low bioavailability.
- Lecithin-gum tragacanth based muco-adhesive hybrid nano drug delivery system that enhanced solubility and permeability of **Amphotericin B** across biological membrane.
- The nano drug delivery system increased the oral bioavailability of the drug two time more than the drug solution.
- Moreover, the drug clearance from the body decreased upon loading and delivering in the novel hybrid nanocrrier system.
- The drug retention in the biological system increased due to sustain release of the drug from the nano drug delivery system (Tooba, shah et al., 2018, Carbohydr Polym).

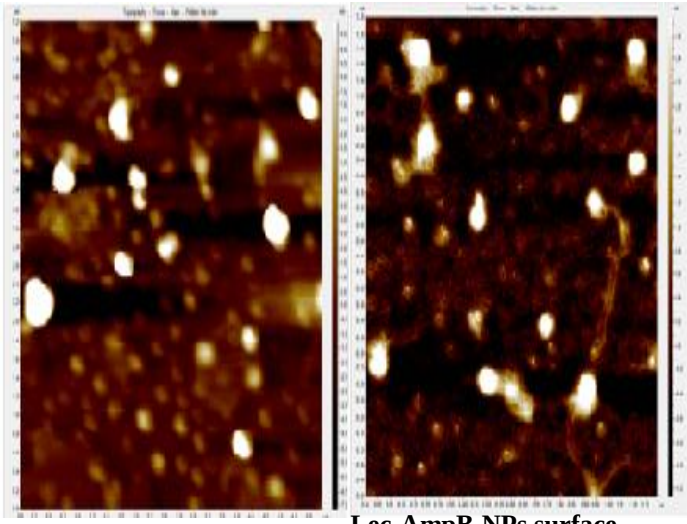
# Enhancing Oral Bioavailability via Nanocarriers




Graphical presentation of the study




In-vivo drug plasma concentration curves



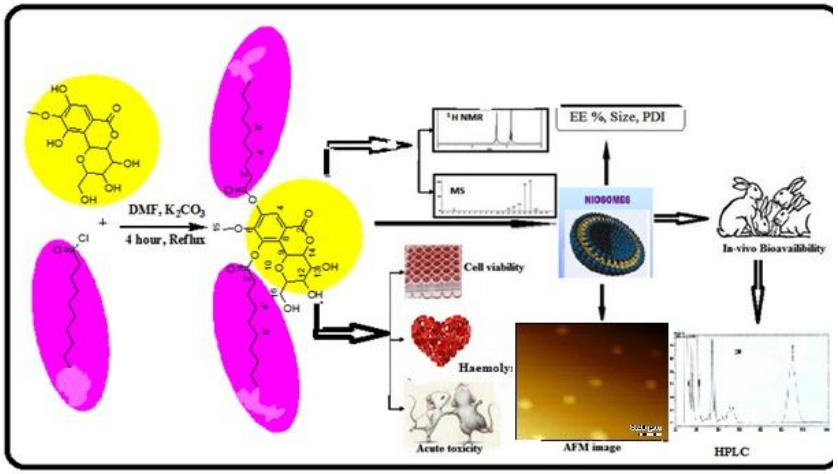
**Lec-AmpB NPs** **Lec-AmpB NPs surface decorated with Gum Tragacanth**

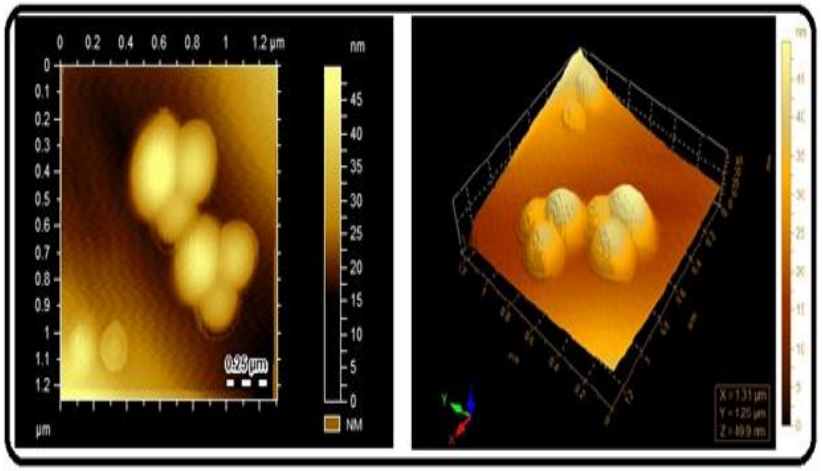
Tooba., Imran, M., Rao, K., Ali, I., Arfan, M., M. Raza Shah. (2018). *Carbohydrate polymers*, *194*, 89-96.

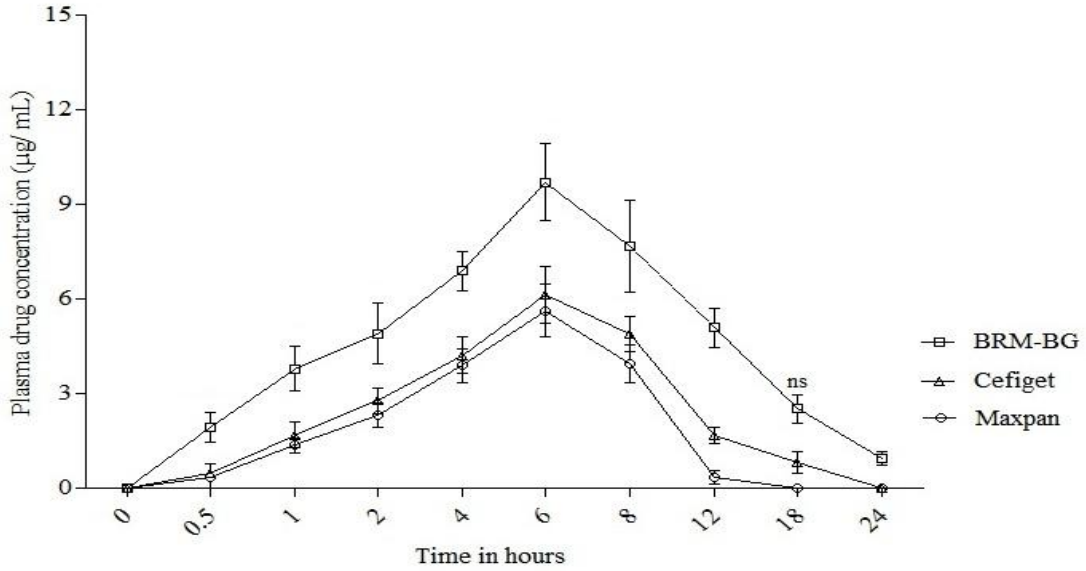
# CEFIXIME

- It is a weak acid with poor solubility after oral administration,
- 40-50% of the drug is excreted from the body through the biliary and renal routes
- Our niosomal nano formulation for loading and delivering of Cefixime, thus enhancing its aqueous solubility and oral bioavailability.
- The niosomal nano formulation was prepared through our own synthesized nonionic double tail surfactant from a biocompatible and renewable source.
- The drug oral bioavailability increased significantly as compared to commercial suspension and capsules (Imran

# Enhancing Oral Bioavailability via Nanocarriers



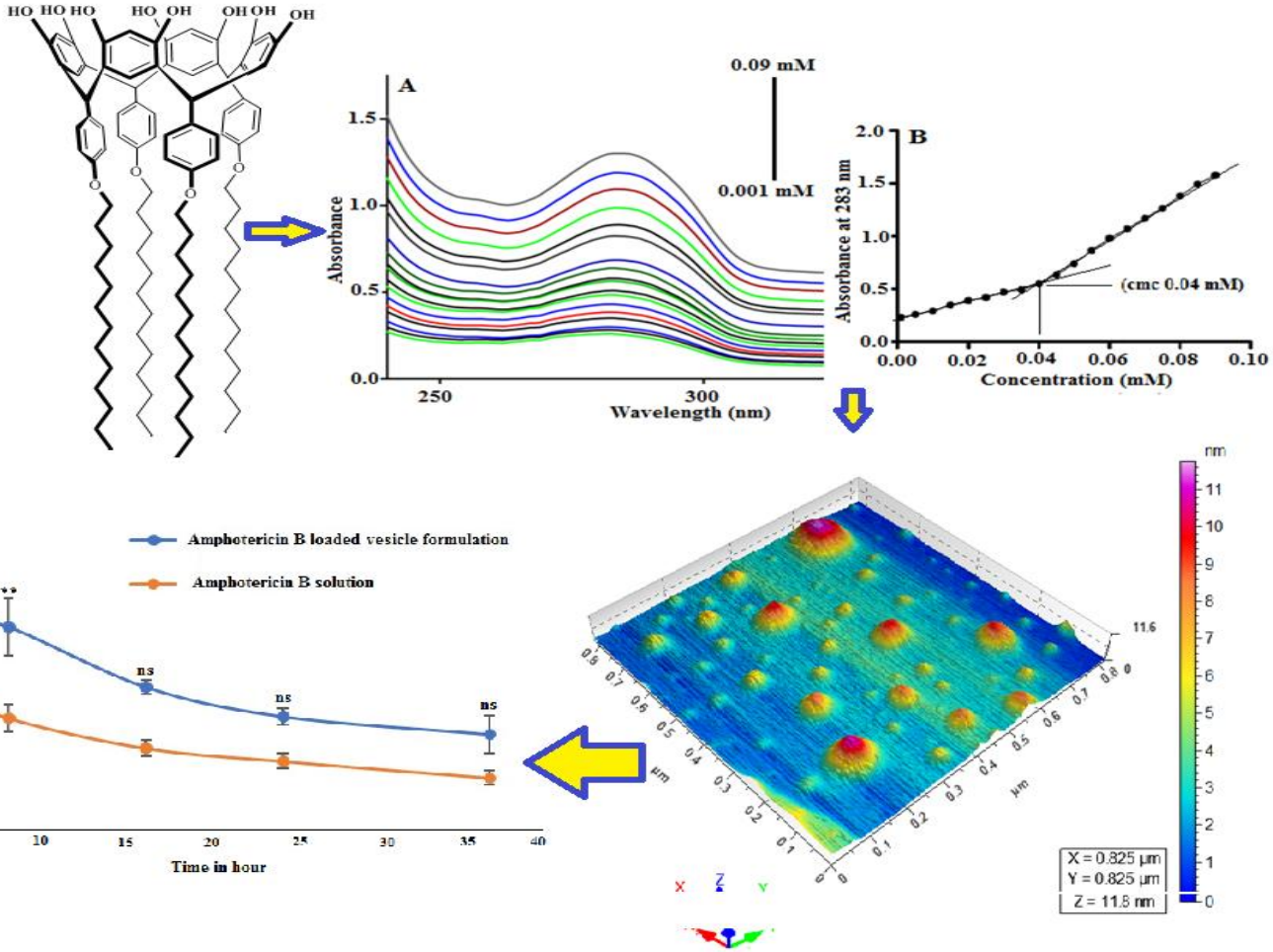




Imran, M., Raza Shah.,.. & Ali, I. (2016). *International Journal of Pharmaceutics*, *505*(1-2), 122-132.

# Enhancing Oral Bioavailability via Nanocarriers

## Various novel self-assembling amphiphiles niosomal nanocarriers synthesized by our group




Ali, I., Rehman, J. U.,, Imran, M., Javed, I.. M. Raza Shah (2018). *Artificial cells, nanomedicine, and biotechnology*, *46*(sup3), S1204-S1214.

# REVERSING MULTIDRUG RESISTANCE VIA NANOCARRIERS

# Reversing Multidrug resistance via Nanocarriers

Bacteria and cancer cells develop resistance against antibiotics and chemotherapeutic agents through various pathways, thus reduce their therapeutic efficacy.

- Nanocarriers can reverse MDR
- Via co-delivery of drugs,
- efficient delivery of drug s cargo across bacterial cell wall or cancer cell membrane,
- inhibition of P-gp efflux, thus enhancing the drugs clinical efficacy against the target cells or bacteria.
- Moreover, nanocarriers can effectively deliver the loaded drugs across memebrane due to their nano range size.

Shah et al, 2019, Bactericidal potentials of silver nanoparticles: Novel aspects against mutli-drug resistance bacteria. In: Metal nanoparticles for drug delivery and diagnosis. , Elsevier

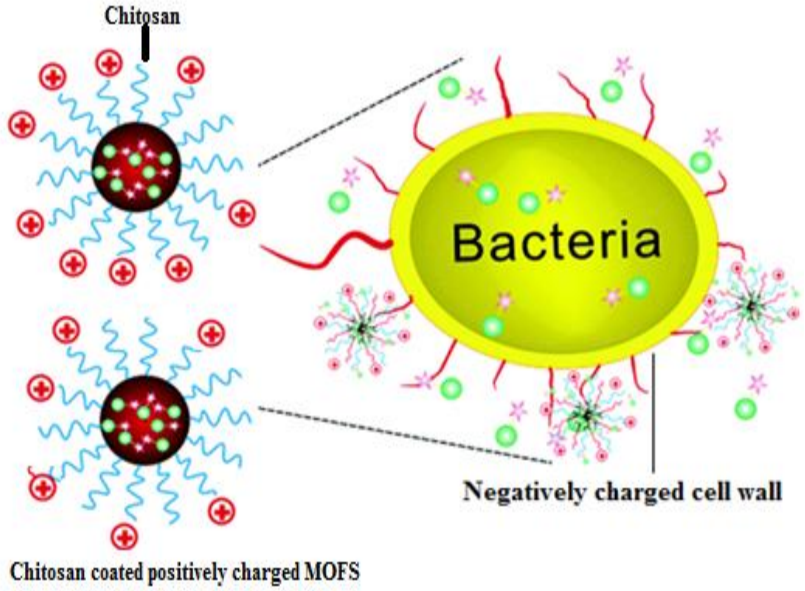
# Reversing MDR via Nanocarriers

- *S. aureus* strains exhibiting increased resistance to vancomycin, known as vancomycin intermediate-resistant *S. aureus* (WA et al., 2017, Yale, J Biomed), thus resulting in the failure of infections treatment with vancomycin.

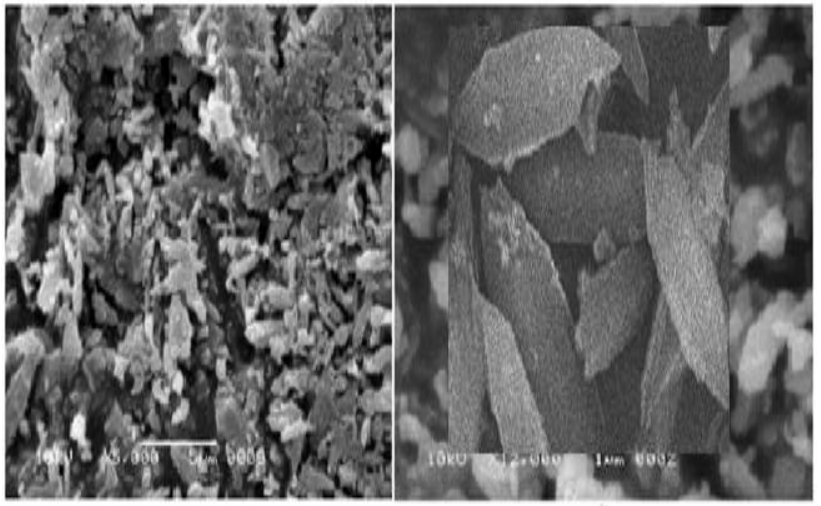
## Reversing MDR via Nanocarriers

- Our group recently ' reported metal organic frameworks **(**MOFs) surface coated with chitosan bearing positive charge that showed greater interaction towards *S. aureus* having negative charge, due to electrostatic interactions.
- The synthesized MOFs were capable of loading Vancomycin and exhibited higher stability.
- This in turn enabled the intracellular delivery vancomycin, thus reversing *the S. aureus* resistant against vancomycin.
- Chitosan coated NMOFs significantly enhanced the drug bactericidal activity against the resistant and sensitive *S. aureus* strains.
- This was further authenticated through surface morphological analysis of the target strains using AFM (Iqra, Raza Shah et al., 2019, J, Matt, Chem C).

# Reversing MDR via Nanocarriers




Graphical representation of the study

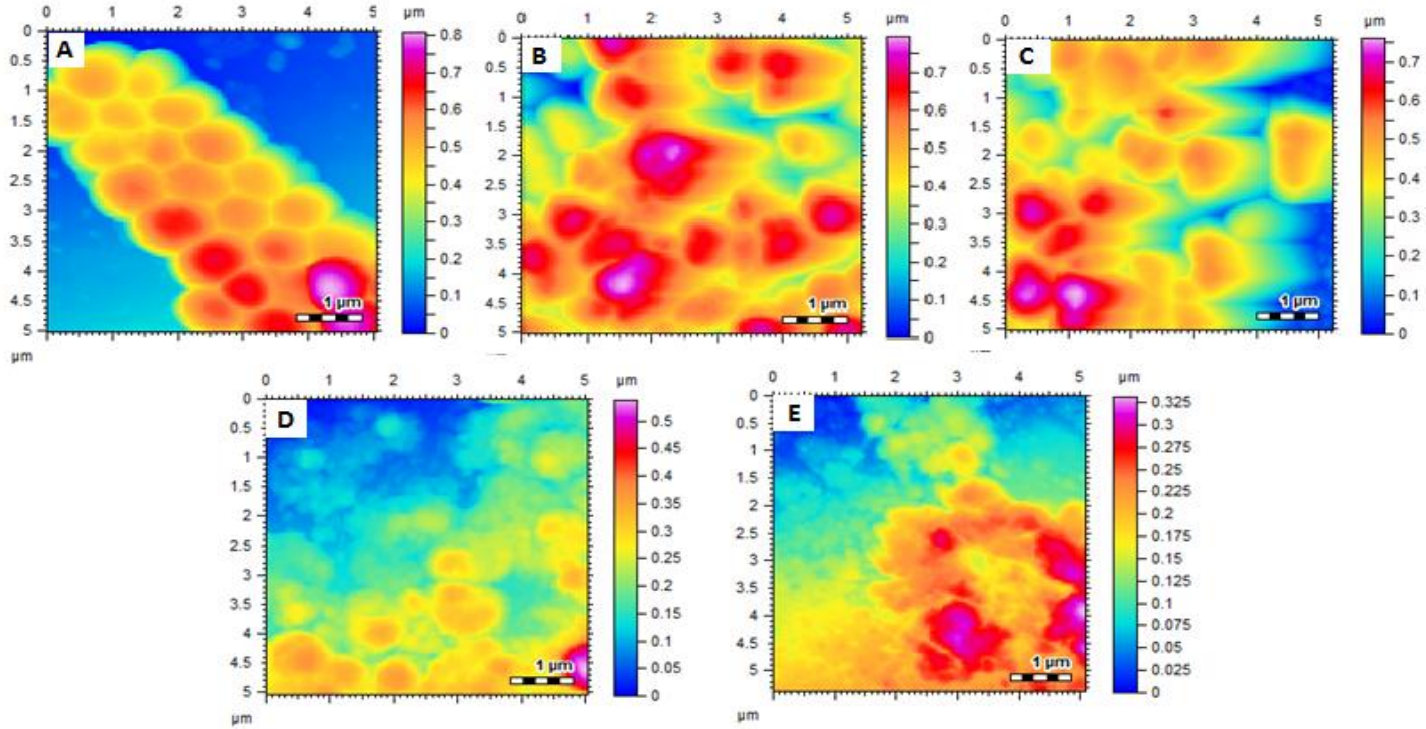


SEM images of drug loaded chitosan coated MOFS

| Test Sample | Sensitive *S. aureus* | Resistant *S. aureus* |
|---|---|---|
| Vancomycin | 29.39 ±0.67 | 69.48 ±1.77 |
| NMOF | 89.27 ±1.51 | 132.65 ±0.81 |
| Van-NMOF | 21.48 ±1.92 | 55.35 ±0.70 |
| CS-Van-NMOF | 16.73 ±0.88 | 24.06 ±1.18 |

Table 2: IC50 values (µg/mL) of testes samples against both sensitive and resistant strains

# Reversing MDR via Nanocarriers




**Surface morphological analysis:** (A) *S. aureus* (Vancomycin resistant) Control (B) *S. aureus* vancomycin treated(C) *S. aureus* NMOF treated (D)*S. aureus* Van-NMOF treated (E*) S. aureus* CS-Van-NMOF treated

Ghaffar, I., Imran, M., M. Raza Shah. (2019). *Materials Science and Engineering: C*, *105*, 110111.

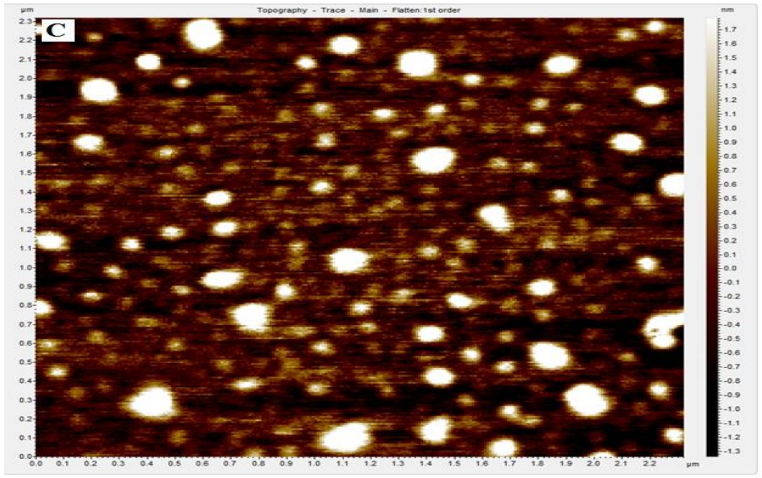
# Reversing MDR via Nanocarriers

- **Paclitaxel** potent against lung cancer, breast cancer and refractory ovarian cancer.
- Poor absorption and low bioavailability due to its lower water solubility.
- Studies in mice have demonstrated that P-gp efflux systems limit the uptake of Paclitaxel from the intestinal tract.
- **Naringin** is a flavonoid and is extracted from citrus fruits and has been reported as as P-gp inhibitor. Thus, co-delivery of anticancer drug with Naringin can reverse MDR in cancer cells.

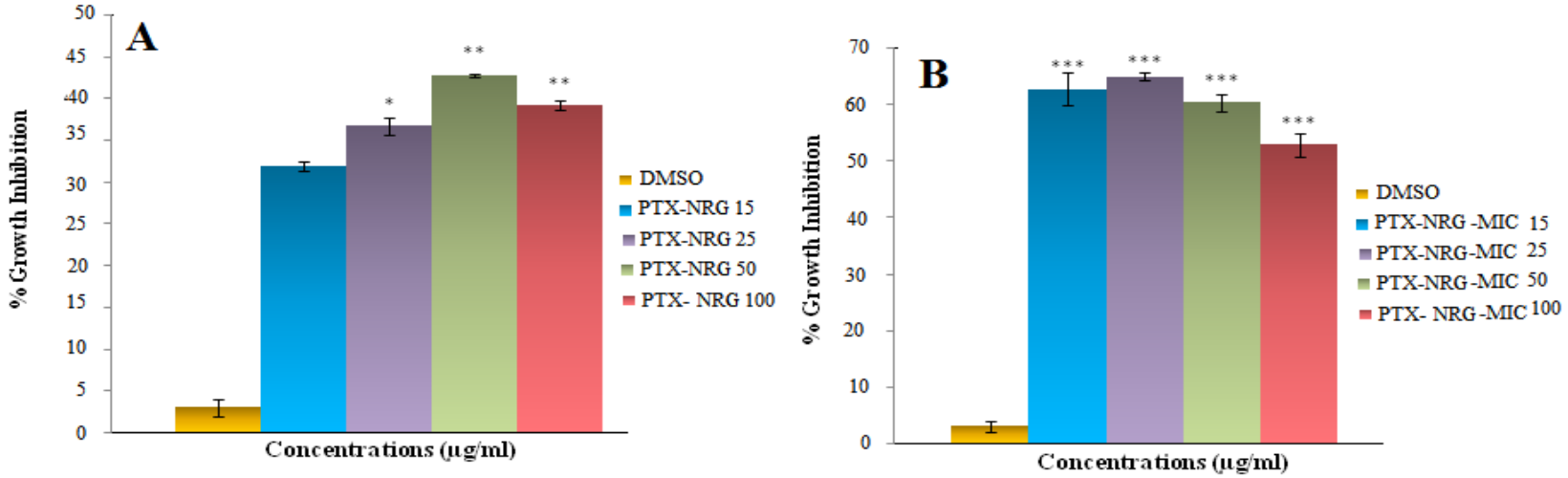
# Reversing MDR via Nanocarriers

- Recently our group (Tooba , Raza Shah et al., 2019, Drug Dev Indus Pharm).) reported polymeric micelles for co-delivery of paclitaxel with Naringin in order to achieved increased anticancer activity of the drug.
- Nano-size micelles with spherical morphology and negative charge encapsulated 76.52 ±0.94% and 32.87 0.61% Paclitaxel and Naringin respectively.
- The micelles were thermally stable and retained 87.05 ±0.69% and 92.88 ±2.17% Paclitaxel and Naringin upon one month storage. Maximum drug release was achieved at $4^{th}$ h of the study for both the loaded drugs.
- Paclitaxel co-encapsulation with Naringin synergistically improved its intracellular uptake and 65% *in vitro* cytotoxicity against breast cancer cells was achieved at its lower dose of 15 µg/mL .

# Reversing MDR via Nanocarriers



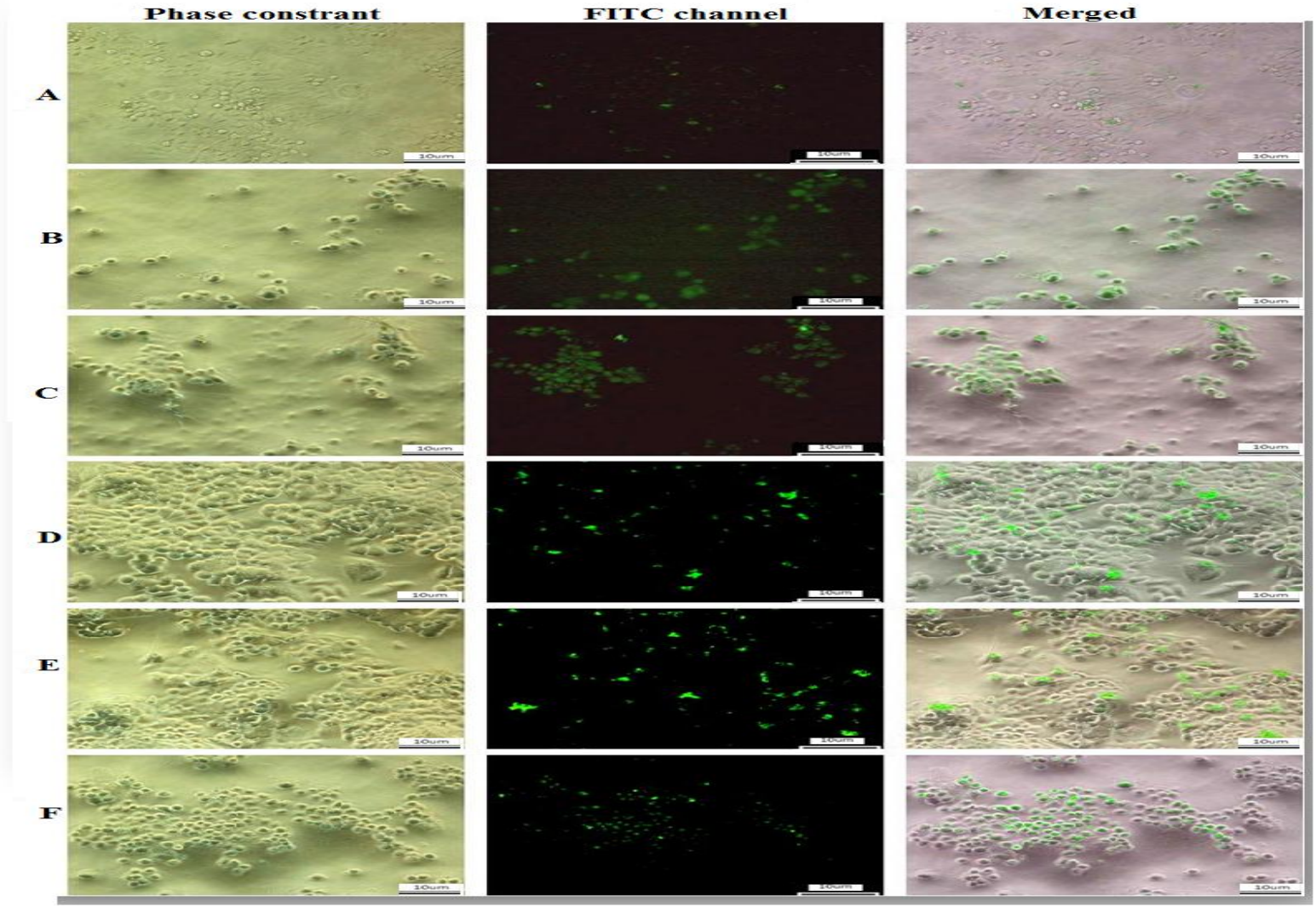

Polymeric micelles used for co-delivery of drug and Naringin




Anticancer activity against breast cancer cells

Jabri, T., Imran, M., Raza Shah, (2019). *Drug development and industrial pharmacy*, *45*(5), 703-714.

# Reversing MDR via Nanocarriers




Fluorescent images of the cells treated with FIT C labeled micelles where **(A), (B)** and **(C)** show cells treated with Paclitaxel Micelles (TPX-MIC) at 25, 50 and 100 concentrations µg/mL respectively, while **(D)**, **(E)** and **(F)** show cells treated with Paclitaxel and Naringin Micelles (PTX-NRG-MIC) at 25, 50 and 100 µg/mL concentrations respectively.

Jabri, T., Imran, M., Raza Shah, (2019). *Drug development and industrial pharmacy*, *45*(5), 703-714.

# Reversing MDR via Nanocarriers

- **Clarithromycin** clinical efficacy against *S. pneumonia* has been reduced due to emergence of resistance in *S. pneumonia* against macrolides.

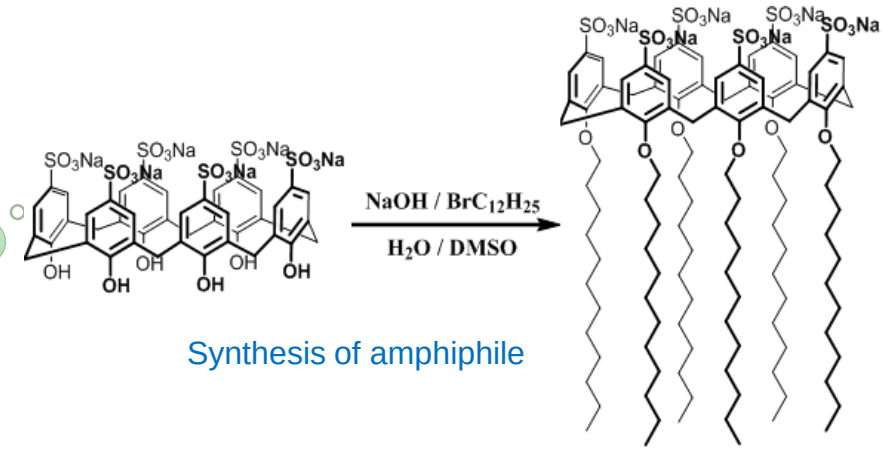
- Moreover, Clarithromycin belongs to class II drugs of Biopharmaceutical Classification System (BCS), thus its lower aqueous solubility results in its lower therapeutic efficacy

# Reversing MDR via Nanocarriers

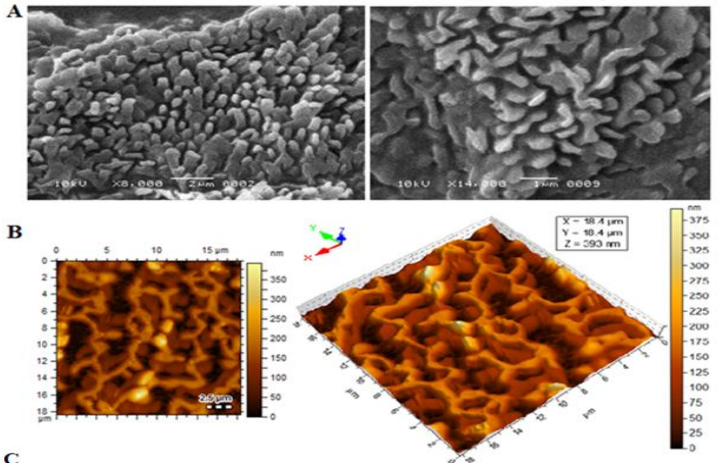
- Our group recently reported the synthesis of water soluble amphiphilic *p*-sulfonatocalix[6]arene that was capable of self-assembling into nano-fibers and loaded increased amount of **Clarithromycin.**
- The resulting nano-fibers wound highly stable and released the drug in a controlled release manner.
- The nano-fibers formulation significantly increased the drug bactericidal potential against the resistant *S. pneumonia* strain.
- Surface morphological further authenticated the drug enhanced activity against the resistant strain after loading in the amphiphile based nanofibers.

A. Imdad, M. Imran, S. M. Raza Shah, 2019 Colloid SurfB BioInterfaces.

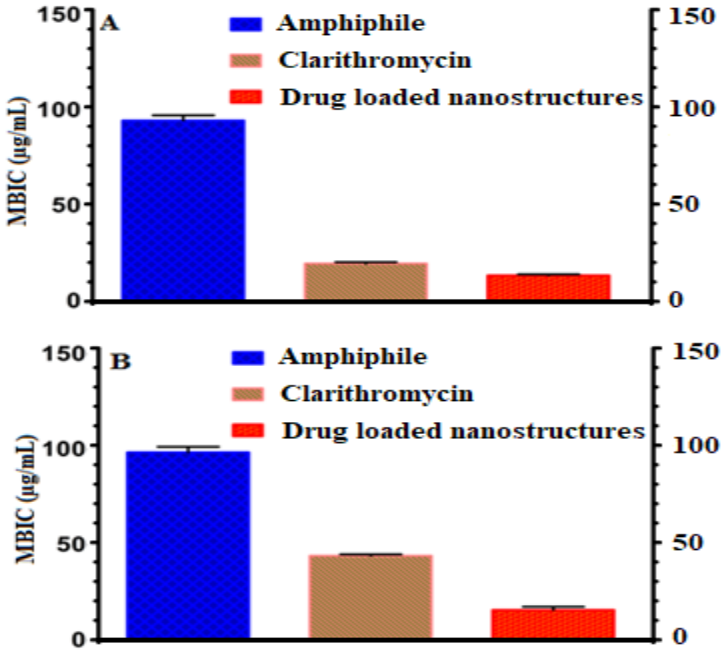
# Reversing MDR via Nanocarriers



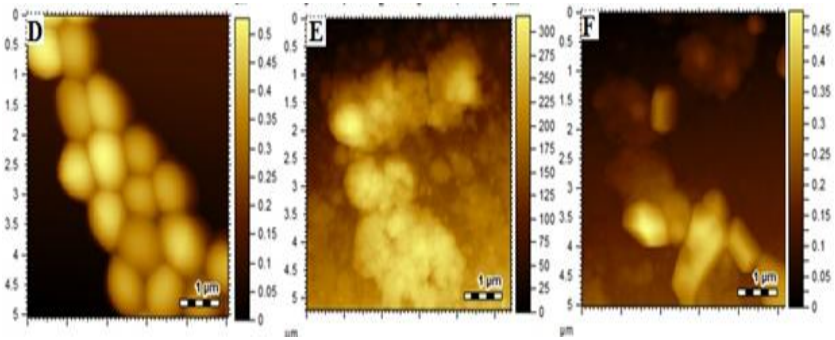

Synthesis of amphiphile



Drug loaded amphiphile based nanofibers




Antibacterial activity of drug against resistant strain



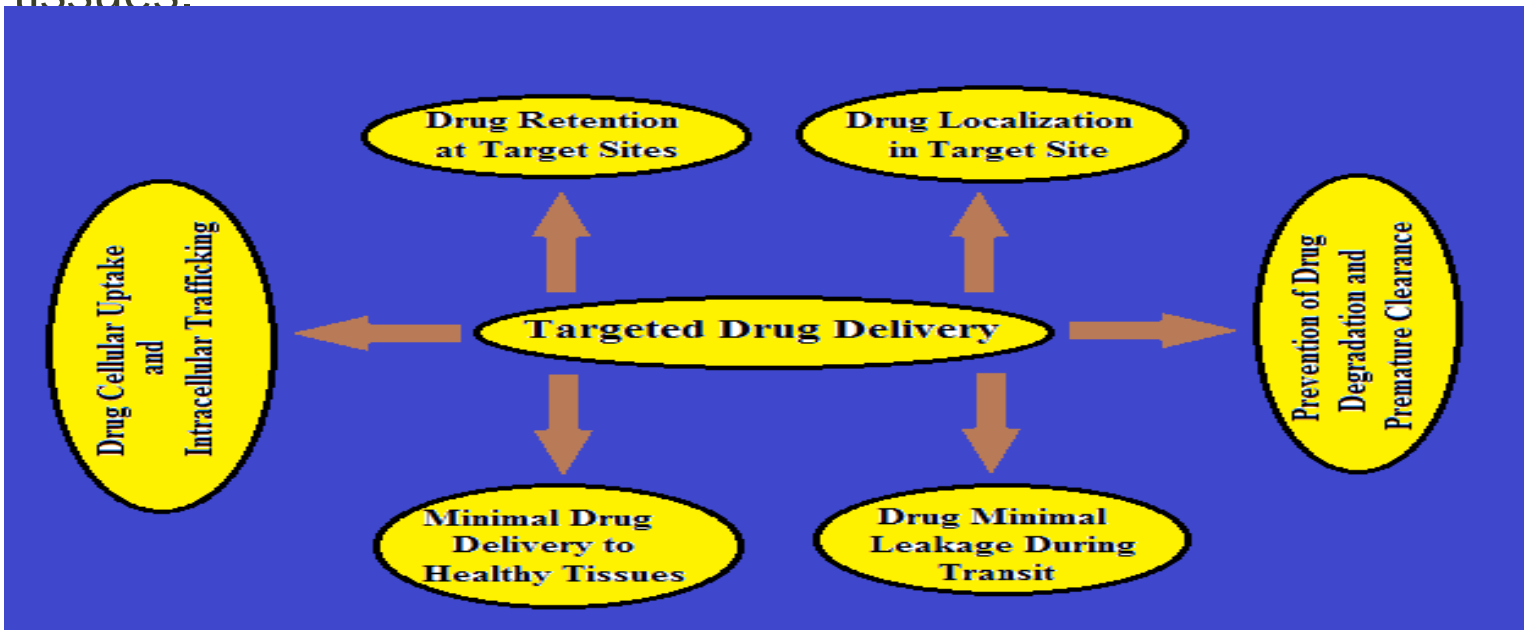

Surface morphological analysis

A. Imdad, M. Imran, S. Salim, M. Raza Shah, 2019 “Amphiphilic *p*-Sulfonatocalix[6]arene Based Self-assembled Nanostructures for Enhanced Clarithromycin Activity Against Resistant *Streptococcus Pneumoniae,* Colloid SurfB BioInterfaces.

# TARGETTED DELIVERY OF DRUGS THROUGH NANOCARRIER

# Drug Targeting via Nanocarriers

- Targeted delivery can be achieved through surface engineering of the nanocarriers with targeting ligands that recognize the target tissues selectively due to overexpression of certain receptors.
- Drugs can also be selectively delivered to the target tissues by making the nanocarriers responsive to temperature, pH and redox changes occurred in the microenvironment of the target tissues.




Applications of targeted drug delivery

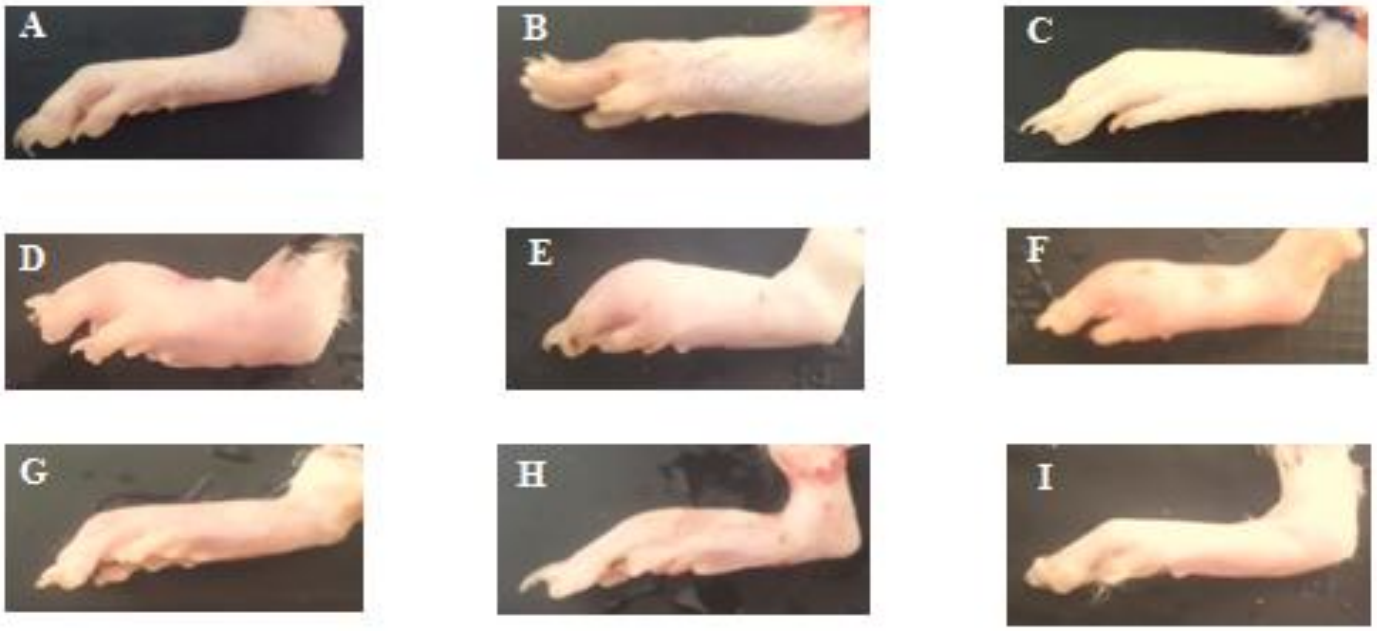
# Drug Targeting via Nanocarriers

- Our group has recently delivered Hesperidin, an anti-inflammatory flavonoid, to the inflammed tissues in the arthritic animals.
- Hesperidin loaded lecithin nanoparticles were surface engineered with folic acid as targeting ligand can recognize folate receptors overexpressed in inflamed tissues.
- Results revealed significant recovery of the bone degeneration in inflamed joints due to targeted delivery of the pharmacological molecule to the target tissues in arthritic animals.

Komal Rao, Sabahat Aziz, Talat Roome, & Muhammad Raza Shah; *Artificial Cells, Nanomedicine, and Biotechnology,* 46 (2018) 597-607.

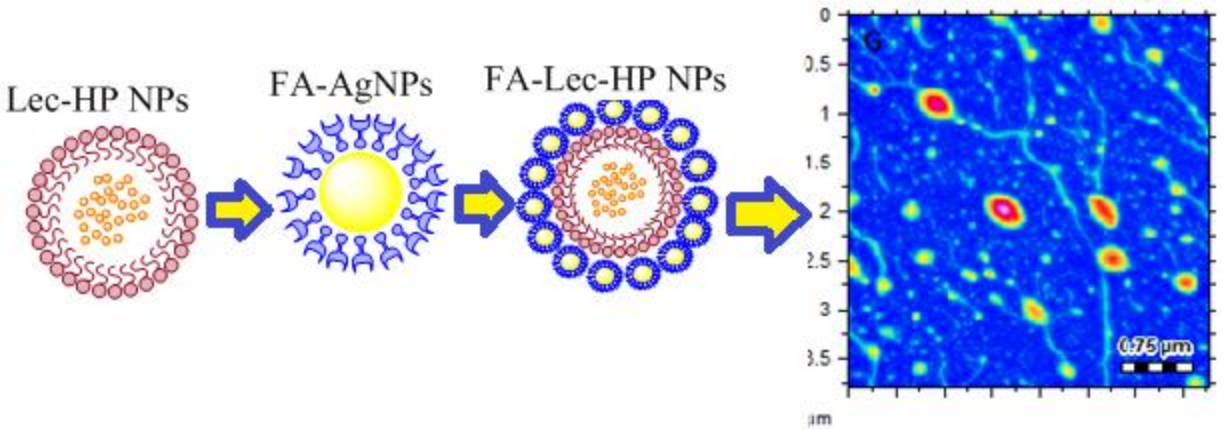
# Macroscopic Evaluation

- Treatment with FA-Ag NPs, Lec-HP NPs and FA-Lec-HP NPs shows remarkable reduce in joint swelling and erythema.

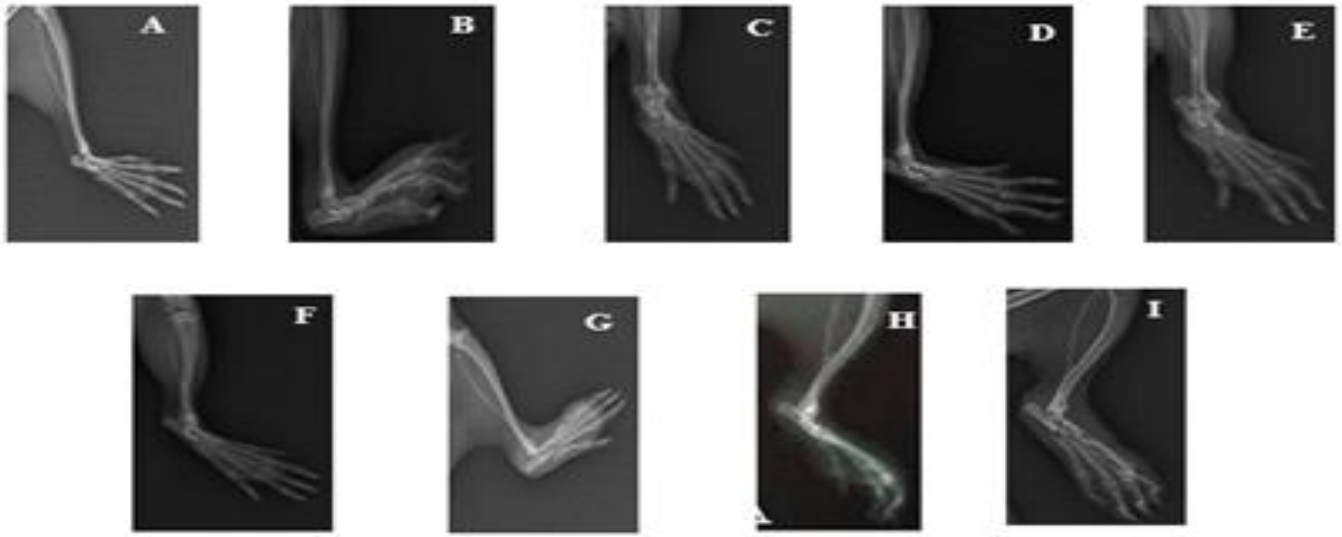



A) normal (B) Control (C) Indo 5 mg/kg (D) FA-Ag Nps1 mg/kg (E) FA-Ag NPs 3 mg/kg (F) Lec-HP NPs 1 mg/kg (G) Lec-HP NPs 3 mg/kg (H) FA-Lec-HP NPs 1 mg/kg (I ) FA-Lec-HP NPs 3 mg/kg.

# Drug Targeting via Nanocarriers




Surface decoration of drug loaded lecithin NPs with folic acid



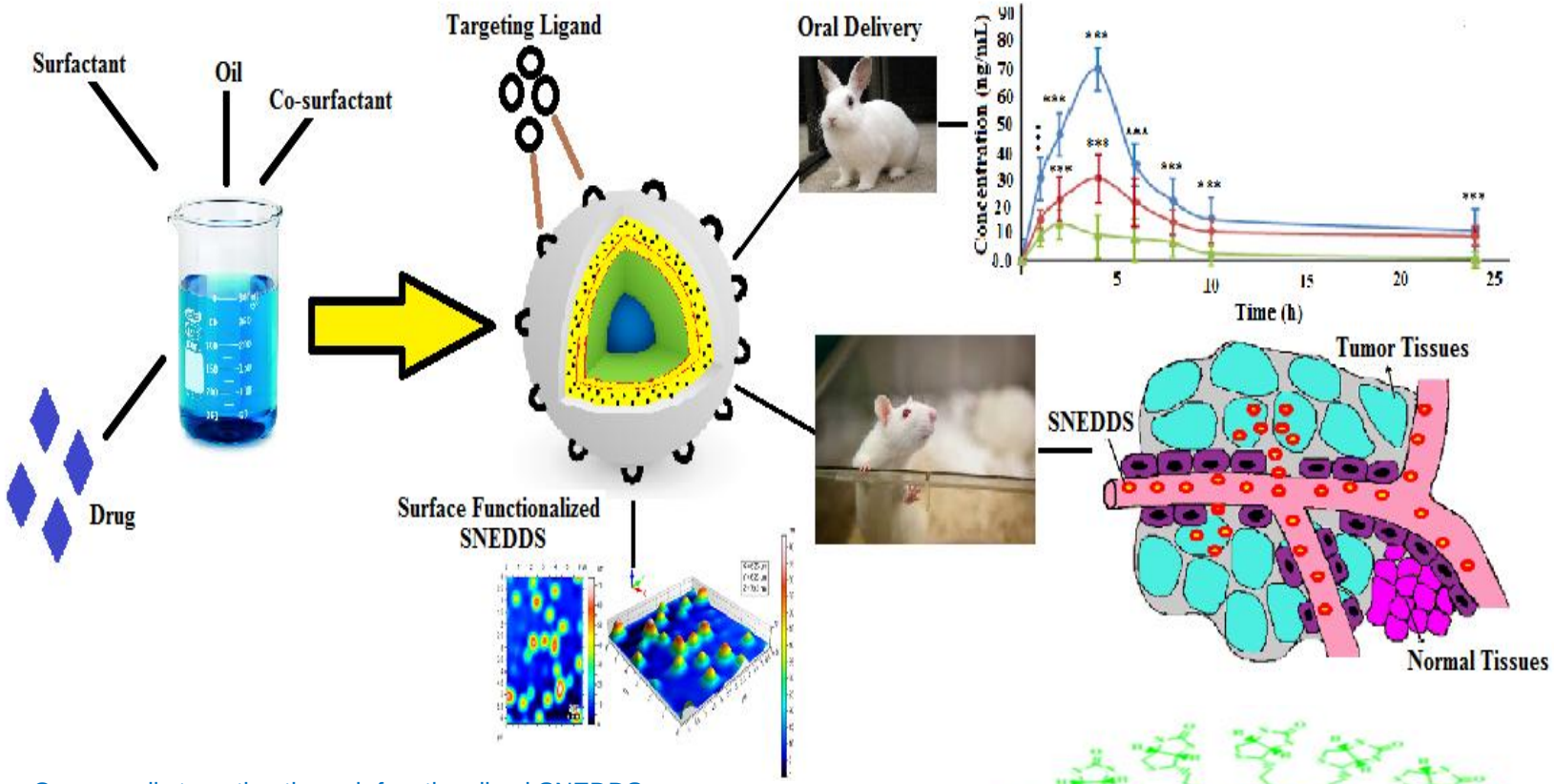

X-ray analysis of the inflamed joints after targeted delivery of the molecule

(A) normal (B) Control (C) Indo 5 mg/kg (D) FA-Ag NPs 1 mg/kg (E) FA-Ag NPs 3 mg/kg (F) Lec-HP Nps 1 mg/kg (G) Lec-HP NPs 3 mg/kg (H) FA-Lec-HP NPs 1 mg/kg (I ) FA-Lec-HP NPs 3 mg/kg.

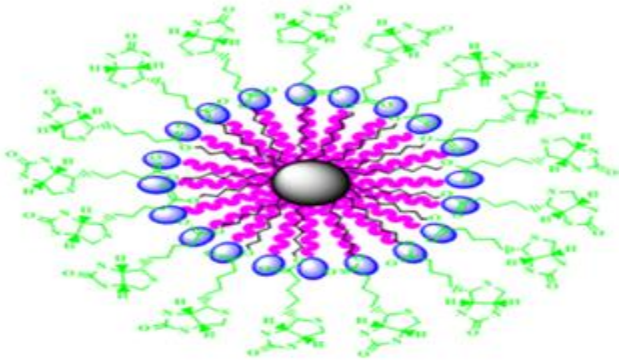
# Drug Targeting via Nanocarriers

- Self-nanoemulsifying drug delivery system (SNEDDS) surface decorated with biotin as a ligand for biotin receptors over expressed on cancer cells.
- The formulation was used for selective targeting of curcumin to cancer cells in hepatocellular carcinoma induced in mice.
- The biotinylated SNEDDS were capable of targeting the cancer cells and delivered increased amount of the drug to target, resulting in enhanced anticancer activity of the drug as compared to simple drug solution.

# Drug Targeting via Nanocarriers




Cancer cells targeting through functionalized SNEDDS



SNEDDS surface decorated with biotin

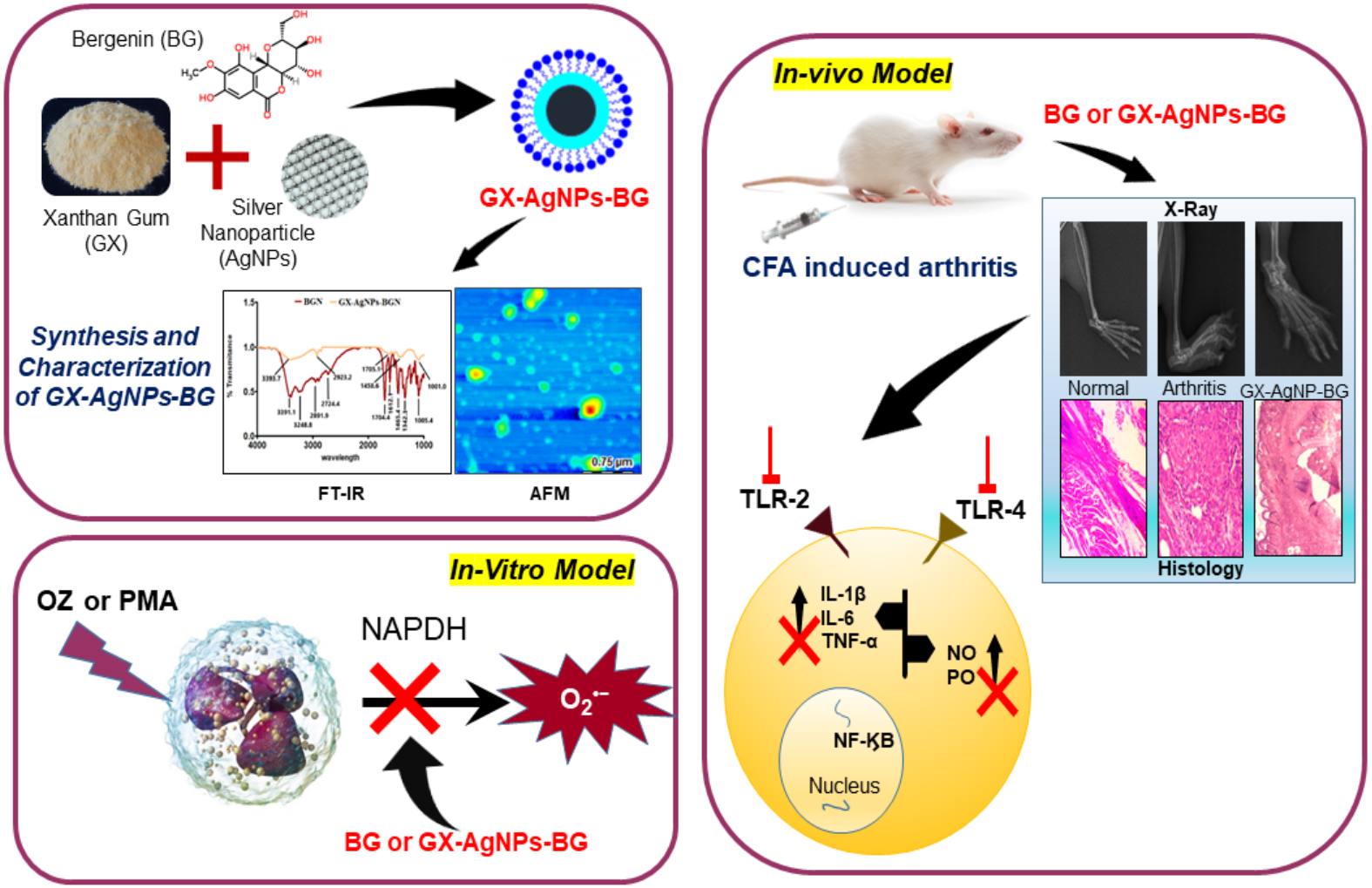
Tasmina et al., 2019, ready for submission

# Drug Targeting via Nanocarriers

- Our group has reported targeted delivery of Bergenin to the arthritic tissues via gum xanthan (GX) stabilized silver nanoparticles.
- Upon delivery in the designed nano system, Bergenin exhibited potent anti-arthritic activity with minimal arthritic score, mild to moderate paw tissue swelling, reduced degenerative changes along with mild articular changes and lesser influx of inflammatory cells in macroscopic X-Ray and histological examination.
- The molecules further suppressed the levels of reactive oxygen species (ROS) significantly as compared to the arthritic control group upon delivery in the designed system.

Rao, K., Roome, T., Aziz, S., Razzak, A., Abbas, G., Imran, M., .M. R. Shah. (2018).. *Journal of Materials Chemistry B*, *6*(27), 4486-4501.

# Drug Targeting via Nanocarriers




Graphical presentation of Bergenin targeting arthritic tissues

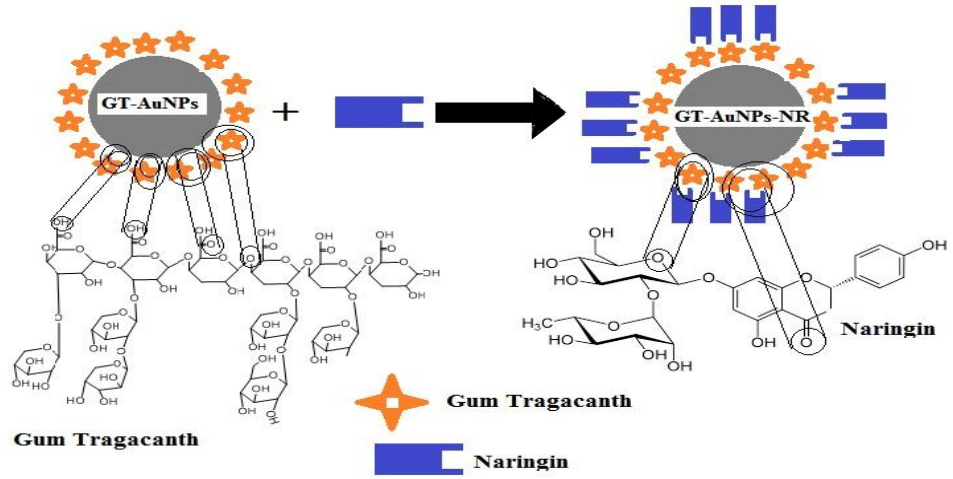
Rao, K., Roome, T., Aziz, S., Razzak, A., Abbas, G., Imran, M., .M. R. Shah. (2018).. *Journal of Materials Chemistry B*, *6*(27), 4486-4501.

# Drug Targeting via Nanocarriers

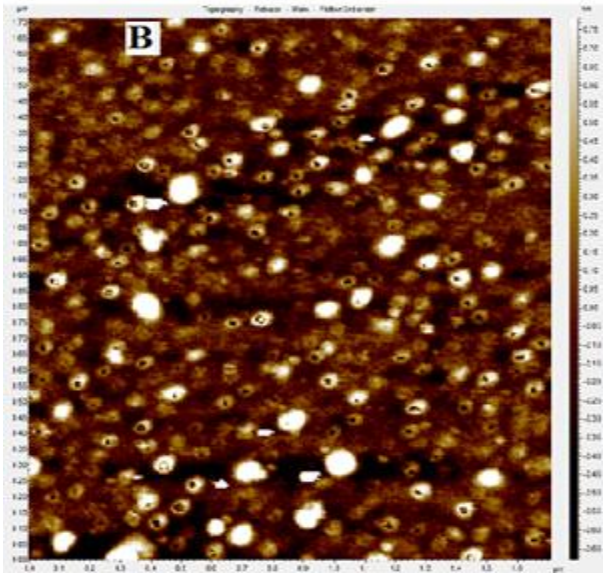
- Naringin, a flavonoid from citrus fruits, has been a versatile pharmacological molecule. It has been selected as a model flavonoid because of its interesting antioxidant, anti-cancer and anti-inflammatory potentials.
- Therapeutic efficacy of flavonoids is retarded by their degradation in acidic pH, susceptibility to oxidation, poor water solubility and aqueous dissolution rates.
- Upon loading in gum tragacanth (GT) stabilized green AuNPs, Naringin bactericidal potentials got enhanced against various tested bacterial strains.
- Surface morphological analysis, showing enhanced membrane destabilizing effects of loaded Naringin.

Komal, Imran, Jabri, Ali, Perveen, M. Raza Shah (2017). Gum tragacanth stabilized green gold nanoparticles as cargos for Naringin loading: A morphological investigation through AFM. *Carbohydrate polymers*, *174*, 243-252.

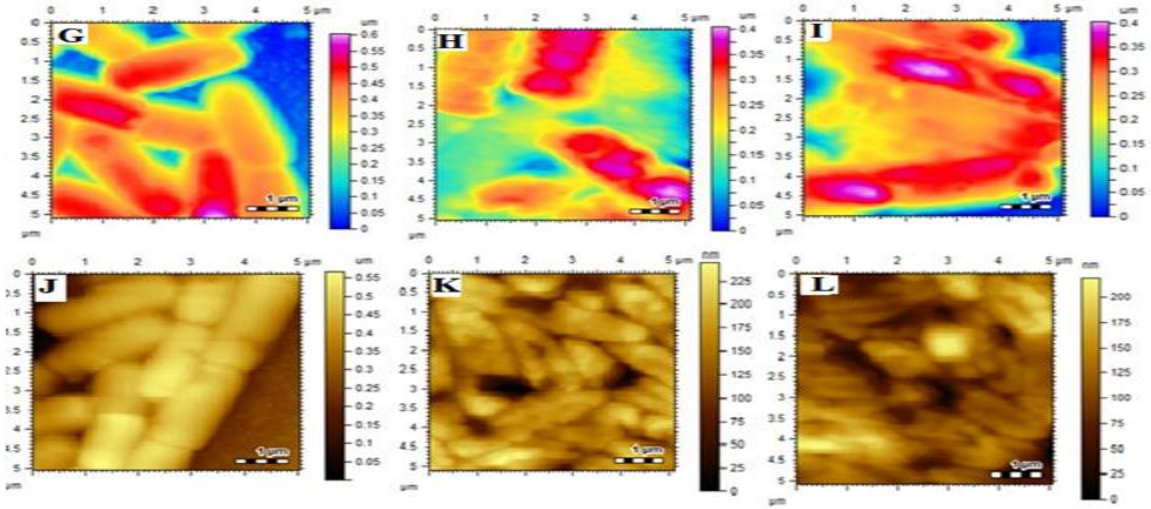
# Drug Targeting via Nanocarriers




Gum tragacanth stabilized AuNPs and its Naringin loading capability




Naringin loaded gum tragacanth stabilized NPs




*E. coli* after incubation (G) without test sample, (H) with Naringin and (I) with GT-AuNPs-NR, *P. aeruginosa* after incubation (J) without test sample, (K) with Naringin and (L) with GT-AuNPs-NR

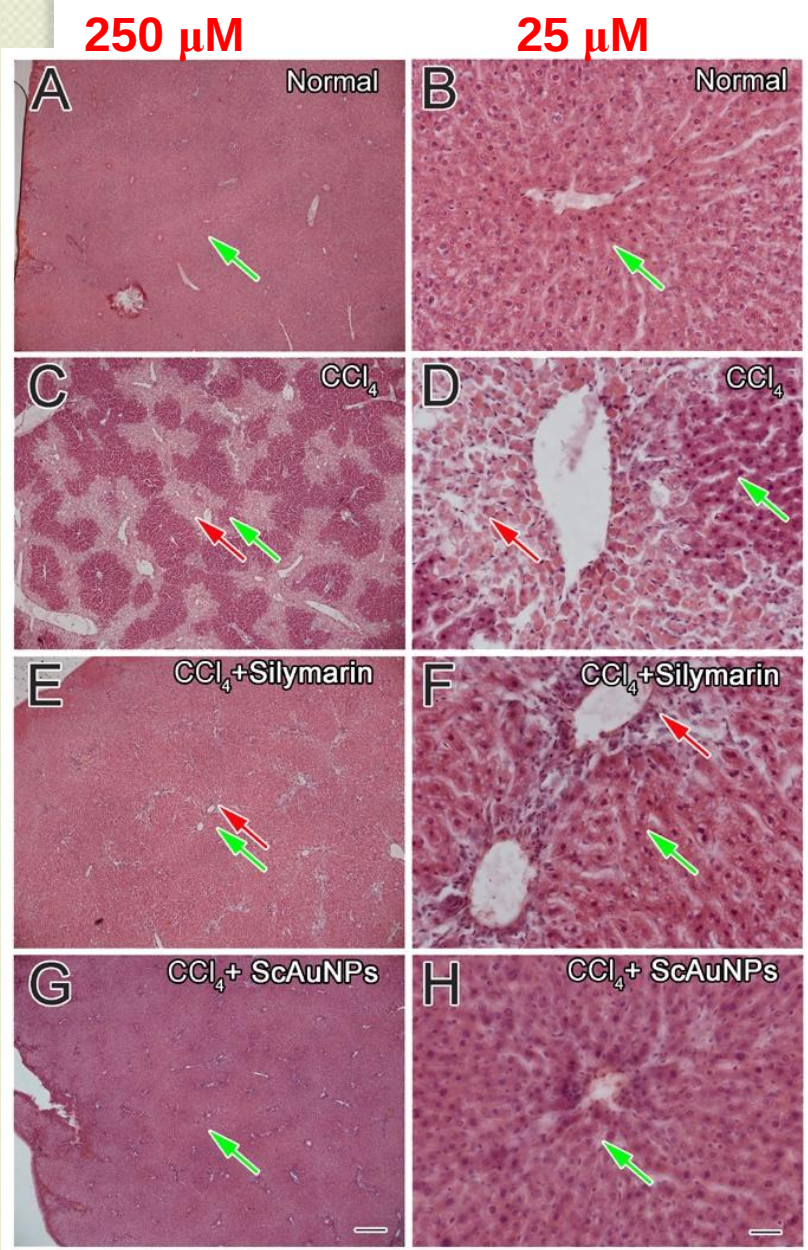
Komal, Imran, Jabri, Ali, Perveen, M. Raza Shah (2017). Gum tragacanth stabilized green gold nanoparticles as cargos for Naringin loading: A morphological investigation through AFM. *Carbohydrate polymers, 174*, 243-252

# Protection from Liver Injury by Silymarin-coated Gold Nanoparticles

1) CCl4 induces the production of several types of Reactive oxygen species (ROS) via cytochrome P450, thereby causing liver injury

*RSC Advances*., **2014**, 4, 9012

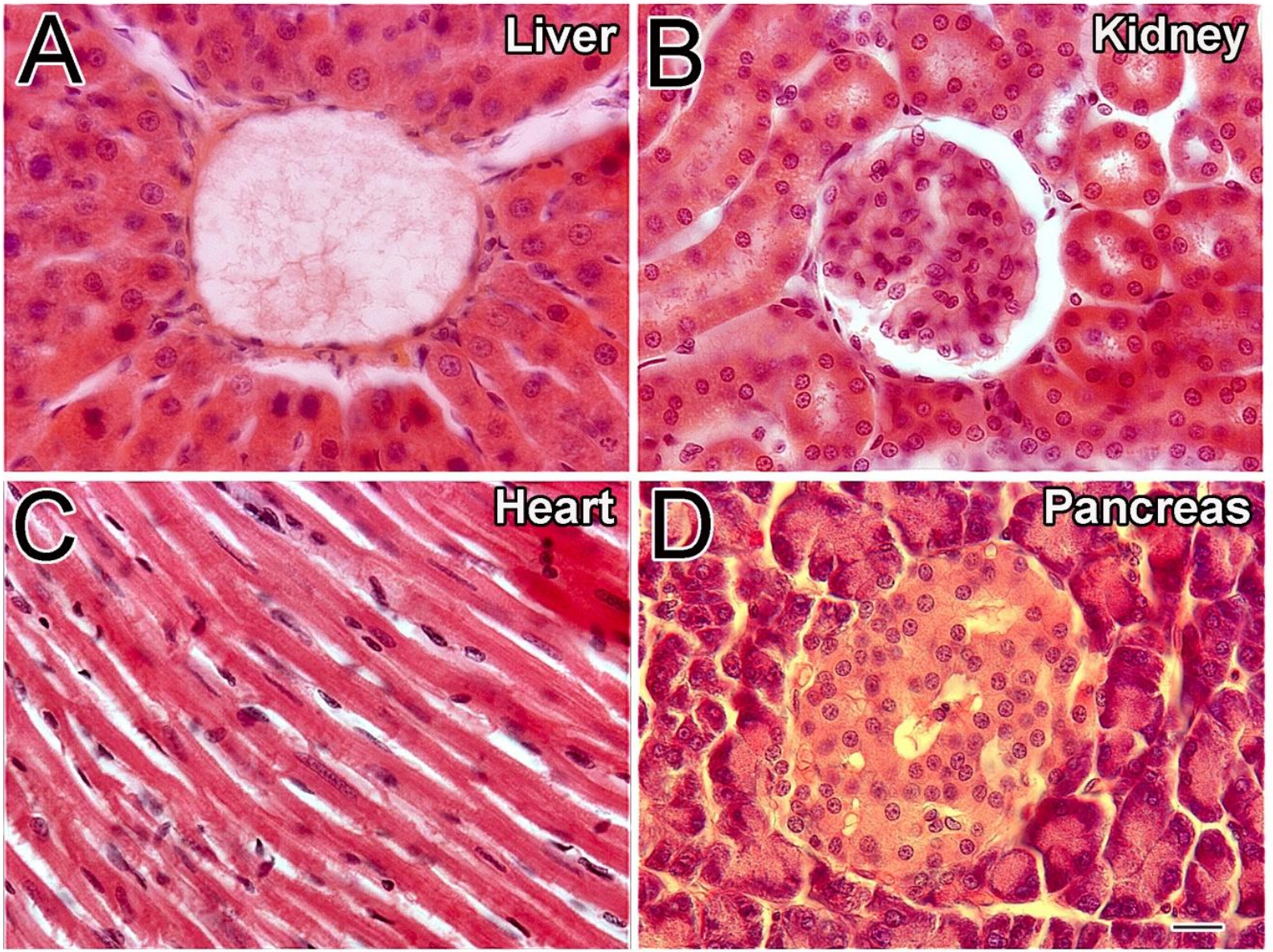
# Histological analysis of CCl4-induced acute liver injury




1) (A,B) Control group;
2) (C,D) group injected with CCl4;
3) (E,F) positive control group (CCl4 and silymarin);
4) (G,H) group injected with CCl4 and treated with ScAuNPs.

Necrotic or injured marked by red arrows. Healthy areas are marked by green arrows

# Histological analysis of organs of animals injected for 14 weeks with ScAuNPs




**No abnormalities were observed in these tissues**

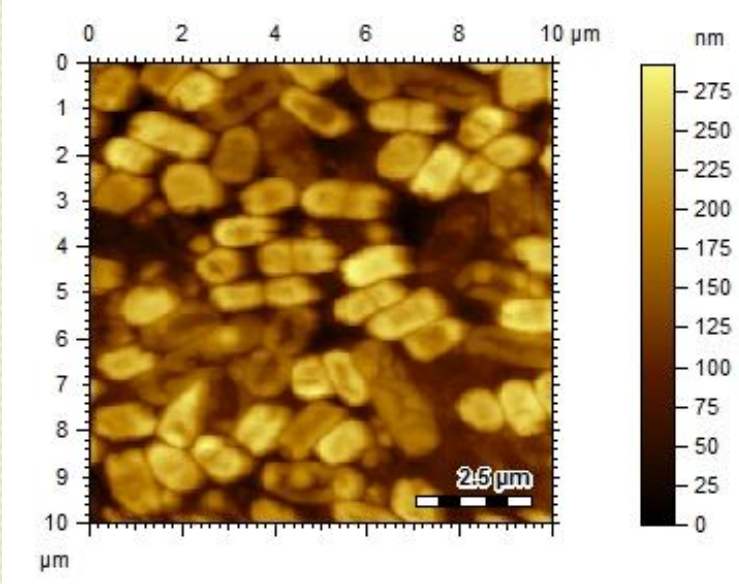
# ANTIMICROBIAL ACTION OF SILVER AND GOLD NANOPARTICLES

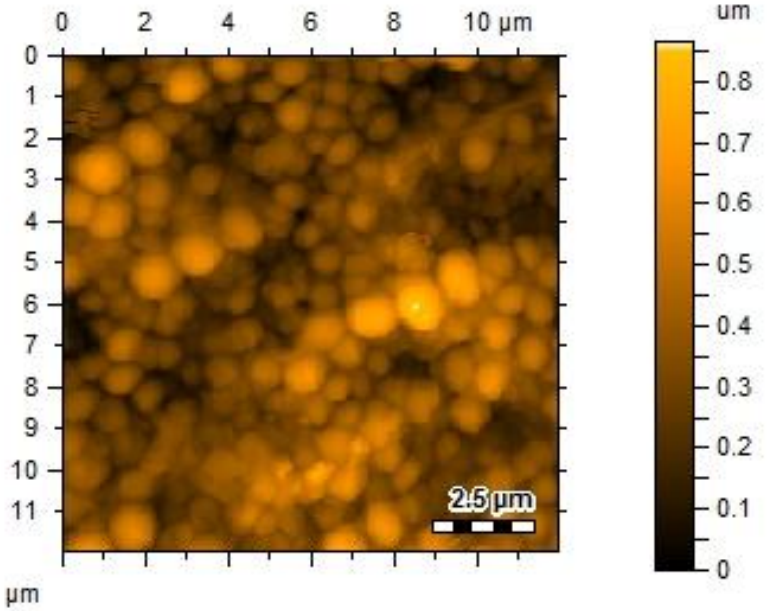
*Journal of Nanobiotechnology* **2013**, *11*, 1-9

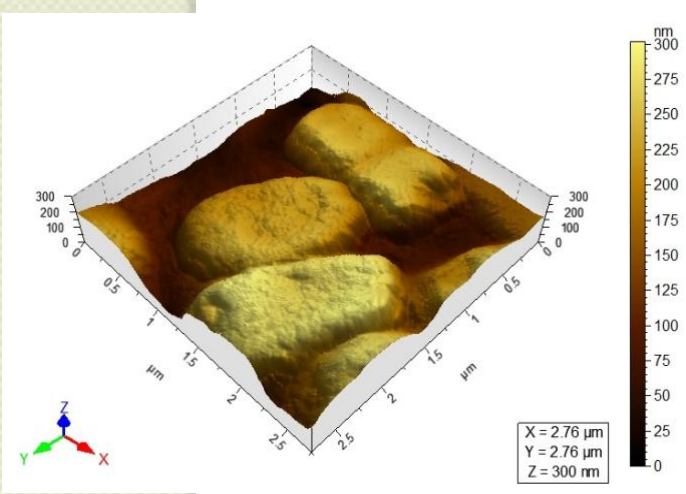
*New Journal of Chemistry* **2014**, *38*, 5633-5640

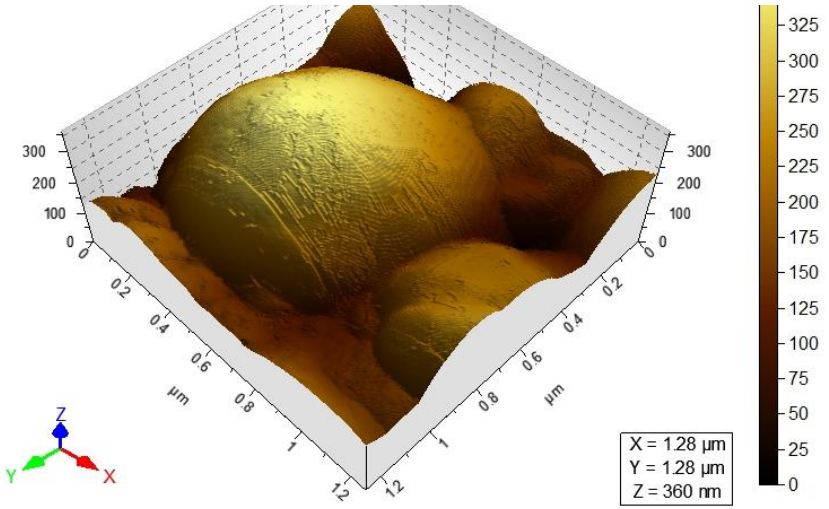
*Journal of Nanobiotechnology* **2014**, *12*, 34

# Controlled AFM Images of *E. Coli and S. Aureus in liquid*



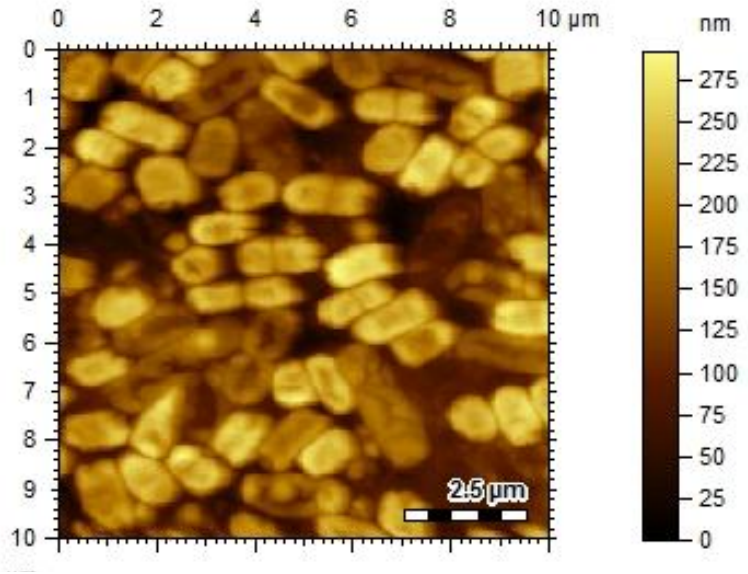



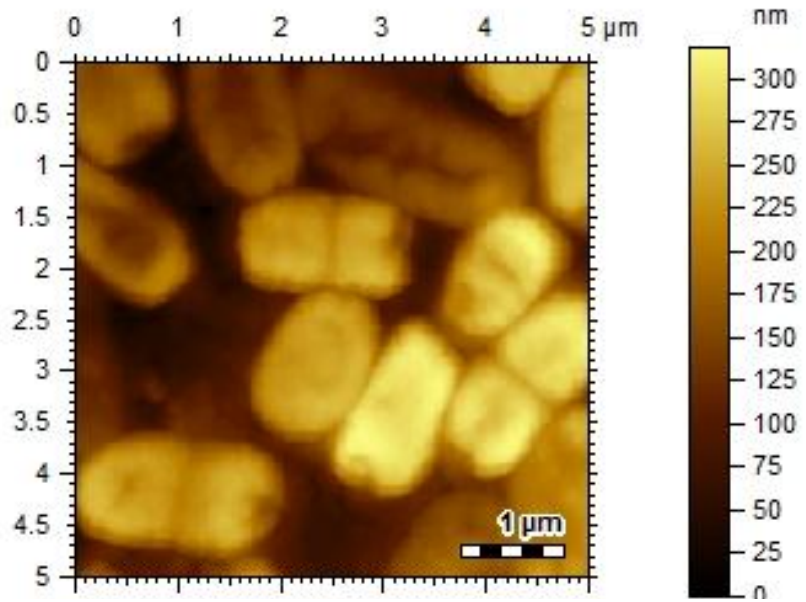



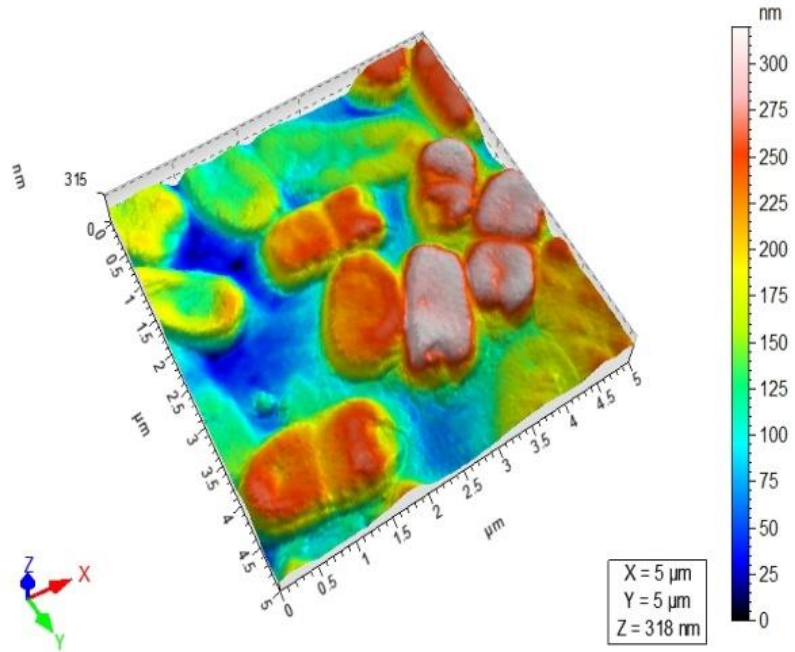



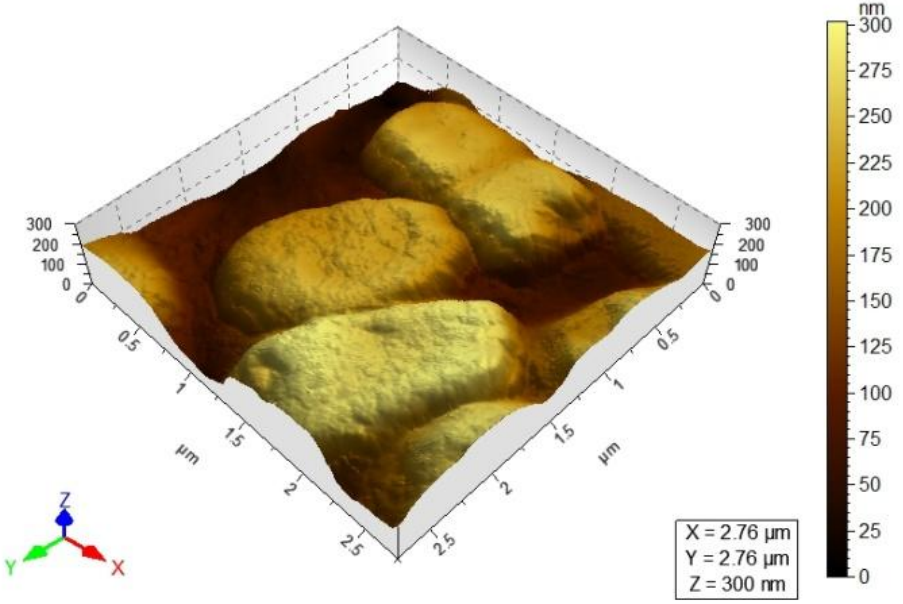

*New Journal of Chemistry* **2014**, *38*, 5633-5640

# *E.coli* Images of controlled sample in liquid



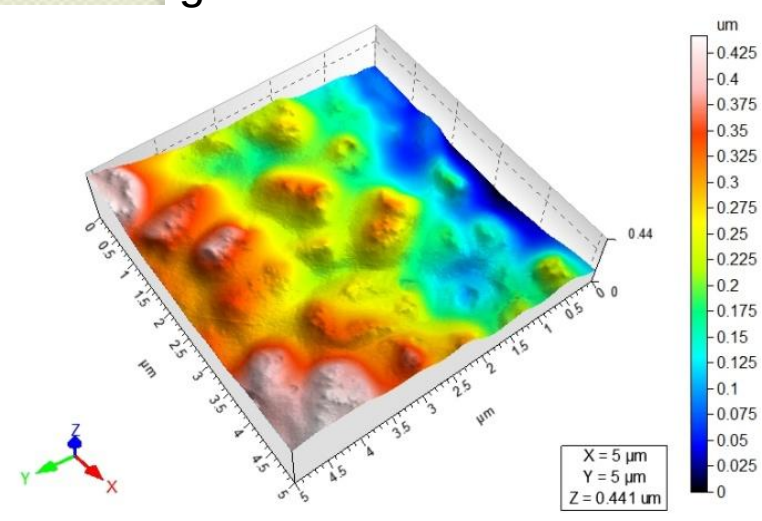



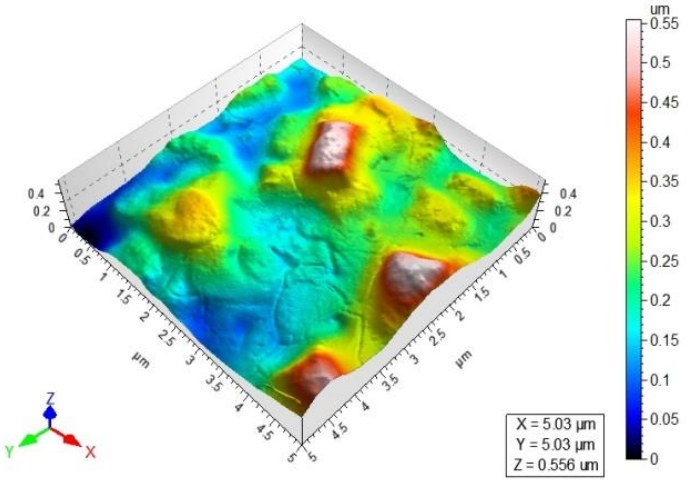



# *E.coli* treated with ceftriaxone in liquid

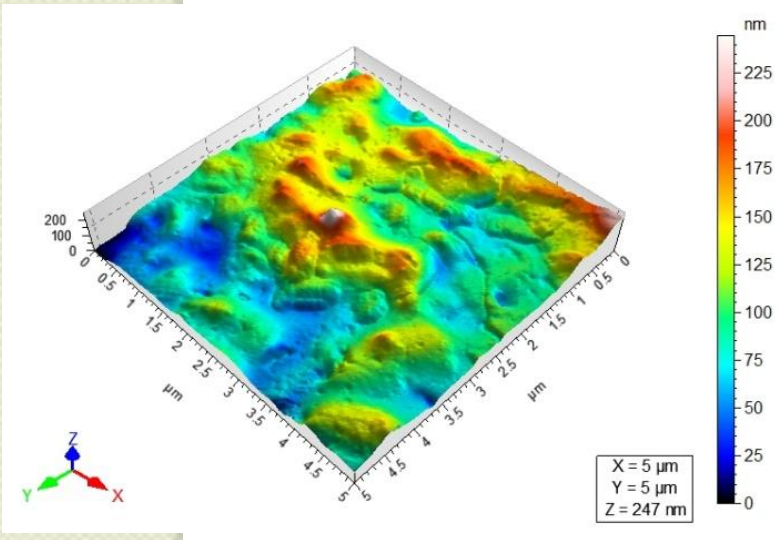
5 mg/mL 2hours



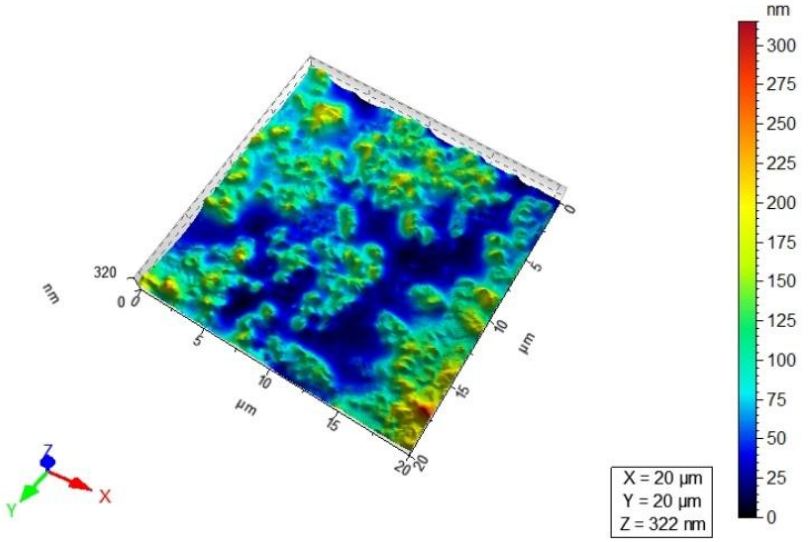

5 mg/mL 6hours




5 mg/mL 8hours

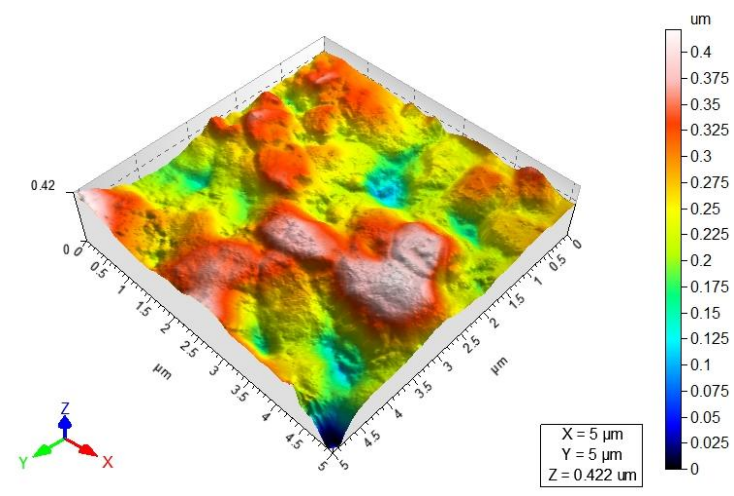



5 mg/mL 8hours larger area

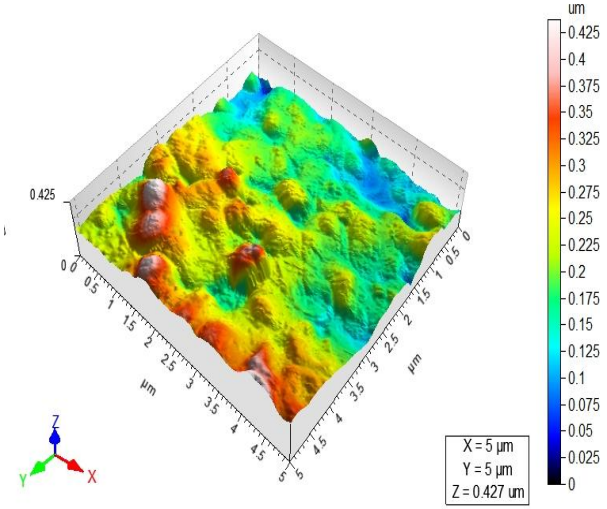


# *E.coli* treated with Cef_GNP in liquid

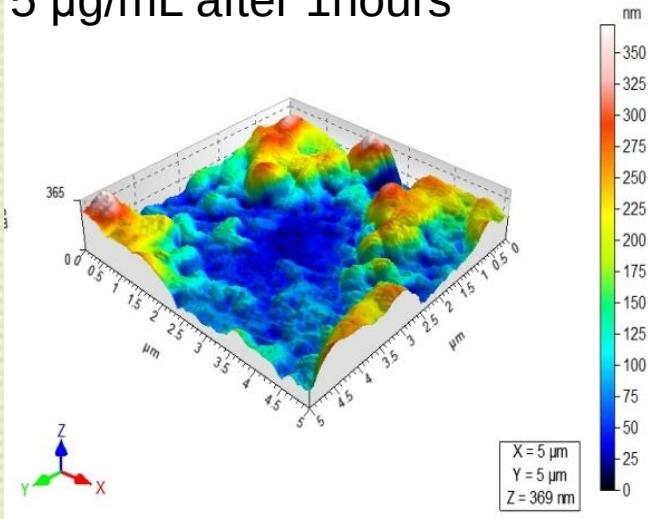
1 µg/mL after 1hours



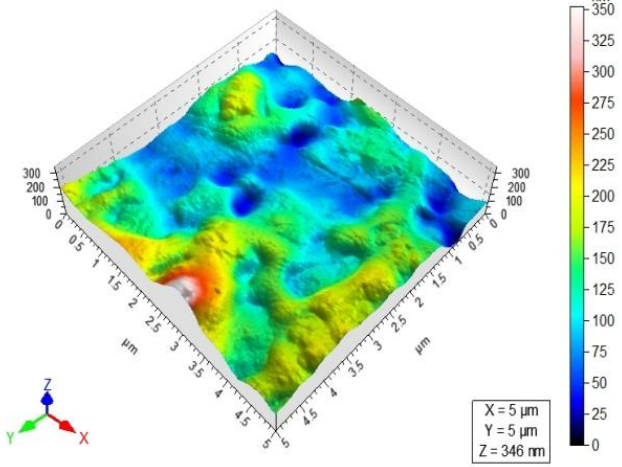

1 µg/mL after 2hours




5 µg/mL after 1hours




5 µg/mL after 2hours

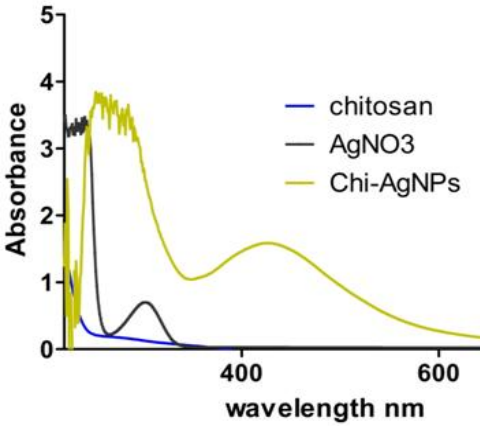



*New Journal of Chemistry* **2014**, *38*, 5633-5640

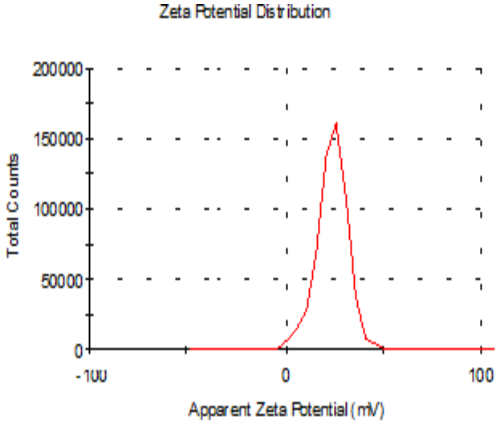
## Nano Systems for Diagnostic Applications

- Our group has recently invstigated chitosan green AgNPs for lipopolysaccharides found on the cell wall surfaces of Gram-negative bacteria.
- The NPs have positive zeta potential on their surfaces, thus interacting electrostatically with negatively charged lipopolysaccharides.
- The same nano systems was ultimately used for selective and sensitive detection of pathogenic *E. Coli.*
- The system was highly stable and reproducible results were produced employing principles of electrochemistry (Imran et al., 2019, paper is ready for submission).

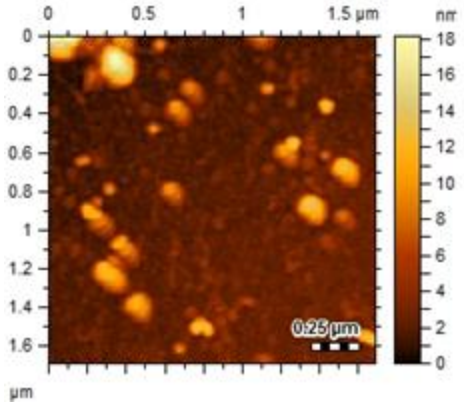
# Nano Systems for Diagnosis Applications



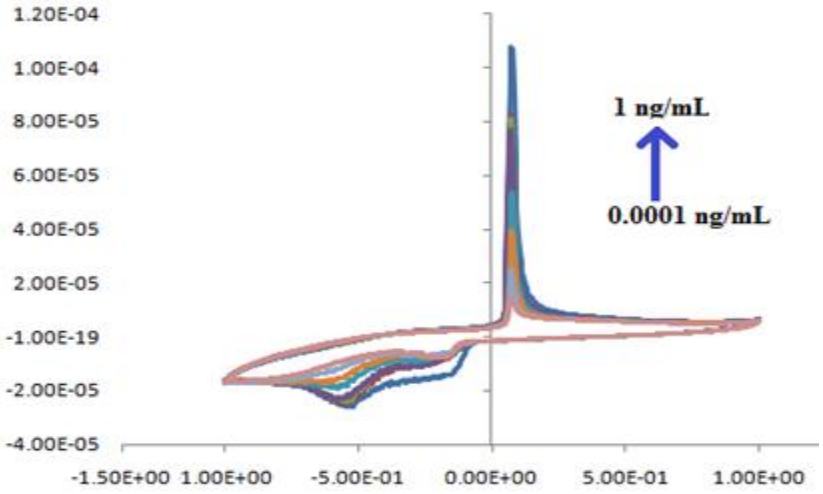

Chitosan stabilized green AgNPs



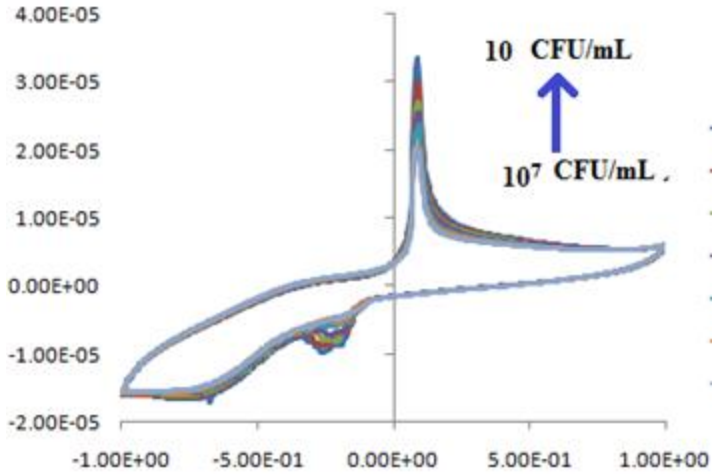

Positive zeta potential of NPS




Morphology of NPs




Detection of LPS in 0.0001-100 ng/mL range by NPs




Detection of E. coli in 10- **$10^7$** CFU/mL range by NPs

(Imran et al., 2019, paper is ready for submission)

## Nano Systems for Diagnosis Applications

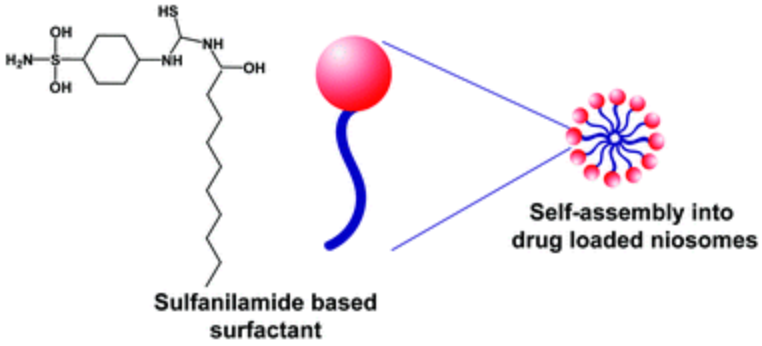
- Currently used diagnostic tests for H. pylori are based on detection of the function of urease, i.e conversion of urea to $CO_2$ and ammonia. Thus often leads to wrong results, thus leading to wrong diagnosis and economic burden on patients.
- Our group is designing nanotechnological strategies to detect urease directly in a sensitive and selective manner while employing electrochemical principles.

# Challenges in Nano Drug Delivery

Toxicity is one of the main challenges in nano drug delivery.

Our group is working on evaluation of toxicities associated with nano drug delivery.

We have reported toxicological profile of nano self-assembling sulfanilamide-based nonionic amphiphile.




Self-assembly of the amphiphile in nano size


**This article was selected as part of the themed collection: Toxicology Research Recent HOT articles**

Ali, I., Shah, M. R.,& Javed, I. (2018). Hemolytic and cellular toxicology of a sulfanilamide-based nonionic surfactant: a niosomal carrier for hydrophobic drugs. *Toxicology research*, *7*(5), 771-778.

## Challenges in Nano Drug Delivery

- Scalability is another major issue related nano drug delivery systems.
- Our group is trying to design methods for preparing nano formulations on large scale using lab level instruments.
- We have optimized SNEDDS and Lecithin NPs on large scale.

# Spectrum of journals

**International Journal of Pharmaceutics**

**Drug Delivery**

**Journal of Molecular liquids**

**RSC Journal of Material Chemistry B**

**Journal of Material Chemistry and Engineering C**

**Toxicology Research**

**Artificial cells nanomedicine and biotechnology**

**Drug Development and Industrial Pharmacy**

**Carbohydrate polymers**

**Journal of Drug Delivery Science and Technology**

**Journal of Applied polymer**

**Biomedicine and Pharmacotherapy**

**Scientific Reports**

**Antimicrobial Agents and Chemotherapy**

**Colloids and Surfaces B Biointerfaces**

**Journal of Liposome Research**

**Chemistry and Physics of lipids**

**Journal of peptide science**

**ACS Chemical neuroscience**

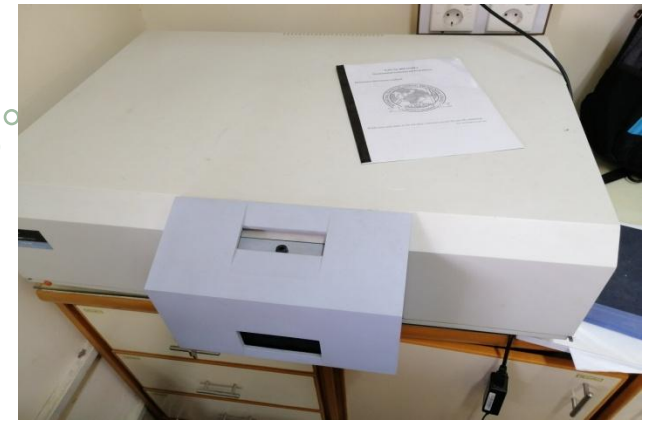
**ACS parasitology research**

**RSC New Jiurnal of Chemistry**

**RSC Advances**

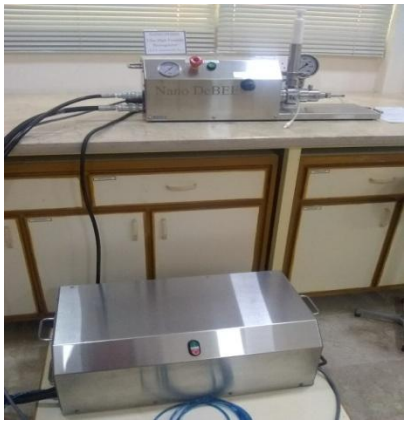
**Journal of Naoparticles Researcn**

# Facilities Available



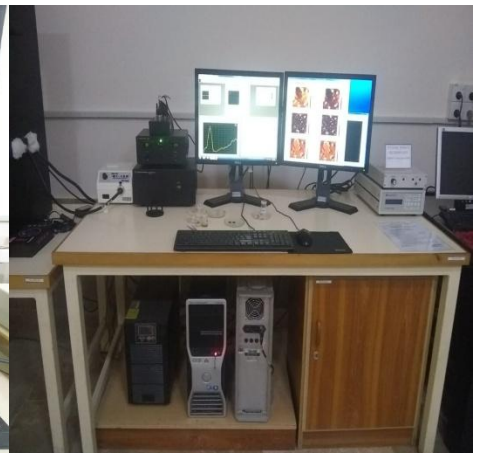
Fluorescence Spectrometer

Multipurpose Varioskan Lux



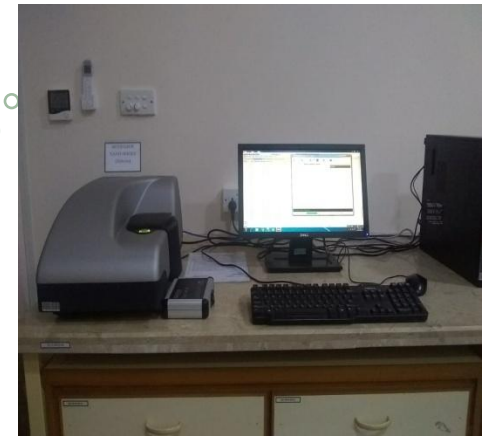
Nano DeBEE

Continuum Laser



Atomic Force Microscope

# Facilities Available



Zetasizer

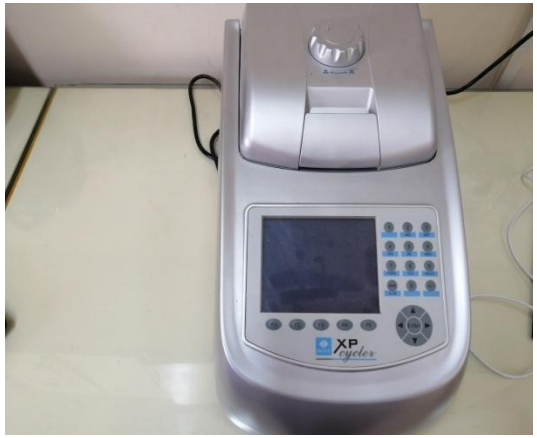
Zyophilizer


Ultrasonic Processor

Inverted Microscope

TGA

# Facilities Available




Thermocycler

UV-vis Soecrophotometer

HPLC

FT-IR


Water bath sonicator

# Acknowledgments

**Imran, Iqra Ghaffar,**

**Shafi, Jawad**

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**Tooba Jabri, Talat Rome,**

**Anam, Sabahat**

**Komal Rao**

**Kawish Iqbal,**

**Technical and Non-technical staff of HEJ**

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