# Novel Design Of Quorum Sensing Inhibitors Loaded Alginate Nanoparticles As Antimicrobial Agents Against Pathogenic *Pseudomonas aeruginosa*

**Esraa Kamaleldine Eltayeb[1] , Fadilah Sfouq Aleanizy[1] ,Fulwah Yahya Alqahtani[1] , Hamad M Alkahtani [2] , Ibrahim A Alsarra [1]**

[1] Department of pharmaceutics · Department of pharmaceutical chemistry, College of pharmacy ,King Saud University, Riyadh , Saudi Arabia

## INTRODUCTION

*Pseudomonas aeruginosa* is a gram negative widespread common opportunistic pathogen which is versatile in surrounding environment.

➢*P. aeruginosa* caused a wide spectrum of clinical infections including lung disease. It is recognized as the major cause of high morbidity &mortality rates .

➢The emergence of antibiotic-resistant strains of *P. aeruginosa* is a major concern in public health and is associated with many virulence factors.

➢*P. aeruginosa* not only secrete alginate as a major virulence factor but also alginate can be utilized as nutritional material. Another key factor is the cell-to-cell communication known as quorum sensing (QS) that regulates virulence and colonization as well as multidrug resistant behavior.

➢ QS system was inhibited by small molecules known as quorum sensing inhibitors (QSI). Alongside published research regarding development of QSI and their effect against virulent bacteria in particular meta-bromo-thiolactone (mBTL) that showed the maximum efficacy of QS inhibition.

## OBJECTIVES

This study aimed to prepare calcium alginate nanoparticles (CANPs) loaded with QSI specifically mBTL chosen as the test drug, which used as a promise treatment for opportunistic pathogenic infections, characterize their *in vitro* antibacterial activity and Investigate the effect of loaded nanoparticles on wild type PA01 and different pseudomonal QS mutants

## METHODS

**Formulation**

**Preparation of mBTL-loaded CANPs by emulsification method**

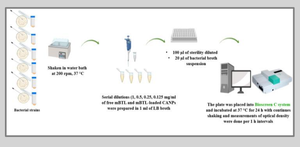
**mBTL-loaded CANPs**

**Characterization**

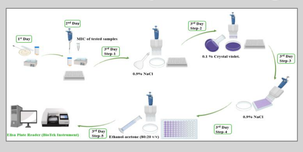
- ❑ Determination of Particle size (PS) and zeta potential analysis(ζ) using zetasizer
- ❑ Morphology of NPs was determined using Transmission Electron Microscope (TEM)
- ❑ Synthesized of mBTL-loaded CANPs was evaluated by *in vitro* release study using dialysis membrane technique
- ❑ Determination of drug encapsulation efficiency (EE%) by UV-visible spectrophotometer

**Microbiological study**

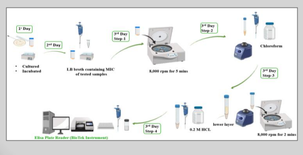
- ❑ Determination Determination of minimum inhibitory concentration (MIC) on bacterial growth using dilution method



- ❑ biofilm formation was done by crystal violet assay staining

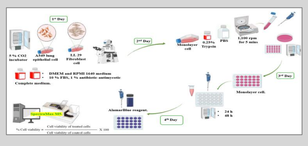


- ❑ Pyocyanin production by chloroform and acidified

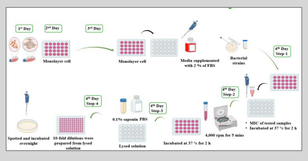


**Biological study**

- ❑ Cytotoxic effect of mBTL-loaded CANPs on A549 lung epithelial cell was assessed using AlamarBlue reagent



- ❑ Effect of mBTLloaded CANPs on bacterial adhesion to on A549 lung epithelial cell was determined by count method



## RESULTS

**Table 1:** Particle size (PS), polydispersity index (PDI), zeta potential (ζ-potential) and encapsulation efficiency (EE%) of mBTL loaded CANPs

| PS nm ±SD | PDI ±SD | ζ-potential ±SD | EE % ±SD |
|---|---|---|---|
| 175.4 nm ± 7.25 | 0.3 ± 0.004 | -45.7± 0.920 | 93.2% ± 0.045 |

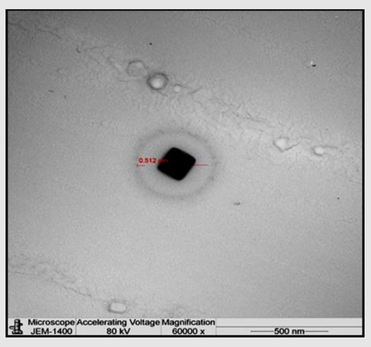



**Figure 1:**TEM image of mBTL loaded CANPs

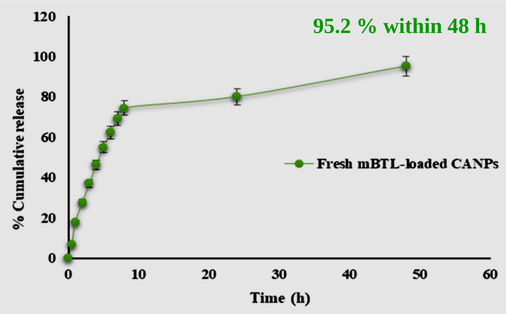
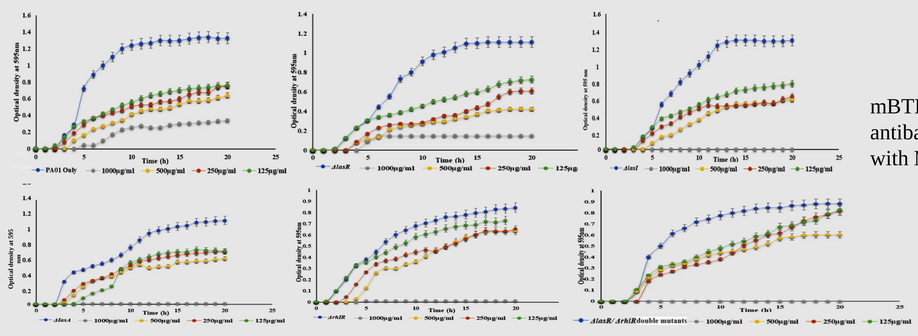



**Figure 2:** *In vitro* release of mBTL from CANPs . The *in-vitro* release profile of mBTL from CANPs was evaluated in PBS buffer of pH 7.4 at 37 ±0.5°C. Points represent averages ±SD



mBTL-loaded CANPs exhibited significant antibacterial activity against *P. aeruginosa* with MIC of 500 µg/ml

**Figure 3:**The effect of mBTL- loaded was investigated using wild type PA01 and different pseudomonal QS mutants PA1430 (*ΔLasR*), PA1432 (*ΔLasI*), PA1871 (*ΔLasA),* PA3477 (*ΔrhlR*) and (*ΔLasR)/ΔrhlR*) double mutants.

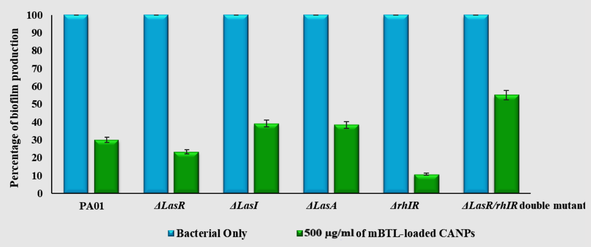



**Figure 4:** Percentage of biofilm production in *P. aeruginosa* at 500 µg/ml of mBTL-loaded CANPs. The biofilm formation was reduced by 69.92 %, 76.79 %, 60.84 %, 61.56 %, 89.22 % and 44.92 % was observed in PA01, PA1430 (*ΔLasR*), PA1432 (*ΔLasI*), PA1871 (*ΔLasA*), PA3477 (*ΔrhlR*) and (*ΔLasR/ ΔrhlR*) double mutant, respectively.

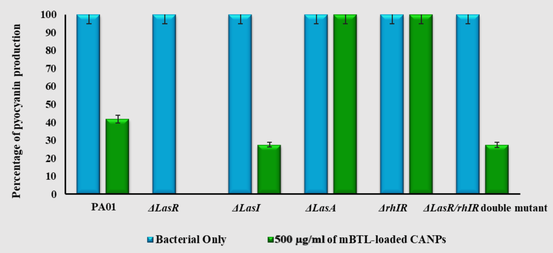



**Figure 5:** Percentage of pyocyanin production in *P. aeruginosa* at 500 µg/ml of mBTL-loaded CANPs. The pyocyanin reduction by 58.20 %, 100 %, 72.45 %, 0.00 %, 0.00 % and 72.59 % were observed in PA01, PA1430 (Δ*LasR*), PA1432 (*ΔLasI*), PA1871 (*ΔLasA*), PA3477 (*ΔrhIR*) and (Δ*LasR/* Δ*rhIR)* double mutant, respectively.

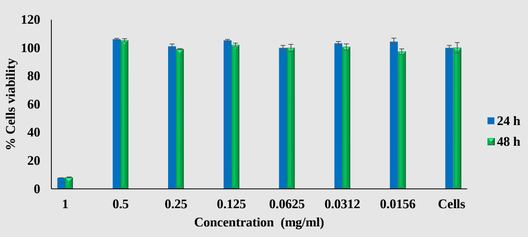
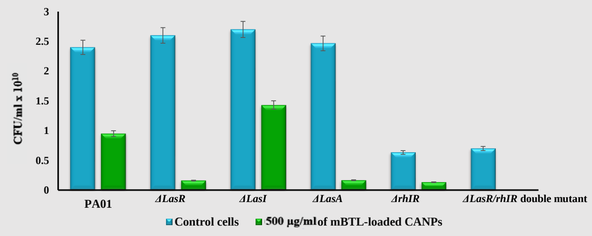



**Figure 6:** Effect of mBTL-loaded CANPs on cell viability was assessed after 24 h and 48 h.. At concentration from 500 µg/ml to 15.6 µg/ml there was no significant dose-dependent decrease in cells viability.



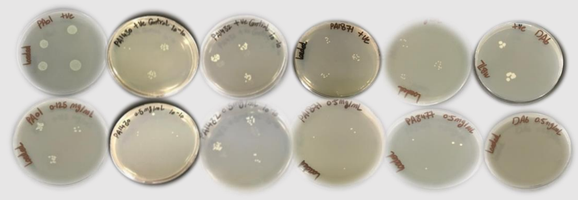



**Figure 7:** Colony- forming unit of wild type and different pseudomonal QS mutants Compared with the control cells, adherence capability of bacteria was reduced by 60.4 %, 93.8 %, 47 %, 93.2 %, 78.9 % and 100 % of wild type, PA1430 *(ΔLasR*), PA1432 *(ΔLasI), PA*1871 *(ΔLasA),* PA3477 *(ΔrhIR) and* double mutant, respectively at 500 µg/ml of mBTL- loaded CANPs .

**Statistical analysis**:

All experiment performed three times in triplicate.
*p < 0.05 in Student's t-test relative to control untreated bacteria.

## DISCUSSION & CONCLUSION

In conclusion, mBTL-loaded-CANPS demonstrated potential antimicrobial drug delivery system against pathogenic *P. aeruginosa* that control virulence factor and overcome resistance by pathogenic strains of serious illness.

## REFERENCES