| P-008 | **Tigecycline encapsulation into poly(methymethacrylate) drug delivery** |  |
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## INTRODUCTION AND OBJECTIVES

The development of drug delivery systems to release drugs in a controlled manner is one of the main goals of nanopharmaceutical researchers. This is of particularly importance in the case of biomaterial-associated infections where different methods for antibiotics encapsulation, to be local delivered without invasive procedures, are under investigation (Kreuter 2007). Being a polymer with biocompatible properties with recognized use in arthroplasty, poly(methylmethacrylate) (PMMA) was considered for use in particulate carrier systems for controlled antibiotic release. The selected antibiotic - tigecycline - is a semi-synthetic glycylcycline known to be active against Gram-positive cocci and Gram-negative rods, including methicillin-resistant *Staphylococcus aureus* (MRSA) (Sorlózano 2006). In this work we aimed to compare two different particle preparation techniques, namely, the double emulsion solvent evaporation (w/o/w) and Supercritical Fluids methodologies (SCF) for items such as particle characterization, entrappment efficiency, yield of production, *in vitro* release profiles and microbiological activity.

## MATERIALS AND METHODS

### *Materials*

PMMA powder (Sigma Aldrich). Tigecycline (Wyeth); Dichloromethane (DCM; Merck). Phosphate buffer saline (PBS; Gibco). Tetrahydrofuran (THF; Riedel-de Haen). Nitrogen N45 (Air Liquide, Portugal).

### *Particle Preparation*

Plain PMMA particles and tigecycline loaded PMMA particles-Tige(PMMA)-were prepared by two different methods: 1) a modified double-emulsion (w/o/w) solvent evaporation method (Florindo 2009) and 2) Supercritical Fluids (SCF) methodology using Supercritical Enhanced Atomization (SEA) (Padrela 2010). Considering the SEA technique, a 0.2%(w/w) polymer solution prepared in THF and a 5.0% ($w/w_{PMMA}$) of tigecycline prepared in an appropriate amount of THF were pumped through a coaxial nozzle allowing mixing with the compressed supercritical nitrogen ($SC\text{-}N_2$) in a small mixing chamber prior to its depressurization into a precipitator vessel at atmospheric pressure. Plain and loaded particles were collected in a filter at the precipitator exit.

### *Scanning Electron Microscopy and Particle Size Analysis*

Particle morphologies were analyzed by a Scanning Electron Microscope (SEM) Hitachi S2400. Samples were coated prior to measurement with a gold film by electrodeposition in vacuum. Particle size distributions were obtained by analysis of SEM images using the Sigma Scan 4.01 software.

### *Antibiotic Entrappment Efficiency*

Entrapped tigecycline was determined: a) indirectly, for double emulsion method, measuring the antibiotic amount present in the supernatant phase after particles centrifugation. Supernatant was adequately diluted before analysis; b) directly, for SEA method, measuring the antibiotic amount present in the aqueous phase, after a liquid-liquid extraction with water from DCM where Tige(PMMA) particles have been dissolved. Tigecycline content was measured by UV-spectrophotometer (Hitachi U-2001) at 345 nm using a calibration curve obtained with tigecycline standard aqueous solutions.

### *In vitro Release Studies*

Tigeycline released from the antibiotic/PMMA particles obtained by SEA method was determined by suspending particles in PBS buffer (pH 7.4) in a propylene tube under soft stirring at 37ºC. At predetermined intervals, tubes were collected and centrifuged, 2.0 mL of the supernatants were measured for tigecycline content using the UV method described above. The withdrawn sample was then replaced with equal volumes of fresh PBS and stirring continued until next collection.

### *Microbiological Testing*

Antibacterial activity was performed by the Mueller-Hinton agar diffusion method against reference antibiotic-susceptible isolates of *Escherichia coli* (ATCC 25922) and of *Staphylococcus aureus* (ATCC 25923). Oxoid CT0998B discs containing 15 μg/mL of each antibiotic formulation tested sample were orderly in the bacterial culture on the nutrient. After incubation of the culture plates at 37ºC for 24 h, the zones of growth inhibition were observed around the dried disks and diameters were measured in mm, indicating the microbiological activity for the antibiotic efficacy.

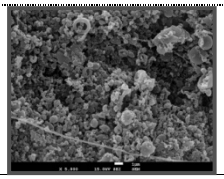
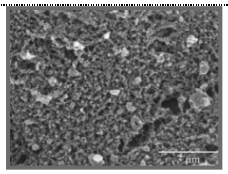
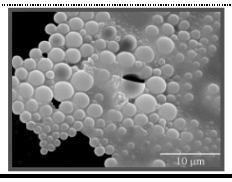
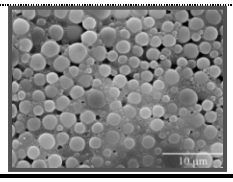
## RESULTS AND DISCUSSION

### *SEM and particle size analysis*

Particles present spherical shape with a smooth surface (Table 1) for both production methods. For the double emulsion method it is visible a sucrose film embedding particles, probably due to a poor liofilization. The yield of production (YP %) it is much higher for the double emulsion method than for the supercritical one. This is

related with particle recovery at the precipitator exit where losses to the atmosphere may occur. Concerning particle size, particles obtained with SEA method are much smaller while particles obtained by double emulsion method tend to be microparticles. This may be related with the solvent evaporation rate which is much higher for supercritical fluids technology than for conventional methods, leading to a sudden growth of polymer concentration and subsequent precipitation with smaller sizes. The different size between plain and loaded particles, obtained by SEA, may be due to tigecycline entrappment that causes particle swelling and an increase in diameter.

**Table 1: Physicochemical characteristics of particles**

| Particles / Method | Plain PMMA | | Tige(PMMA) | |
|---|---|---|---|---|
| SEA | YP (%) | $\phi_m$ (μm) | YP (%) | $\phi_m$ (μm) |
| | 45 | 0.08±0.09 | 25 | 0.16±0.10 |
| |  | |  | |
| Double Emulsion | YP (%) | $\phi_m$ (μm) | YP (%) | $\phi_m$ (μm) |
| | 96.1±2.2 | 1.63±0.67 | 80.8±1.31 | 1.77±1.05 |
| |  | |  | |

***Antibiotic Entrappment Efficiency***

The entrappment efficiency for SEA particles was 35.8±1.0% while for the double-emulsion particles tigecycline was hardly entrapped due to its high water solubility.

***In vitro Release Studies***

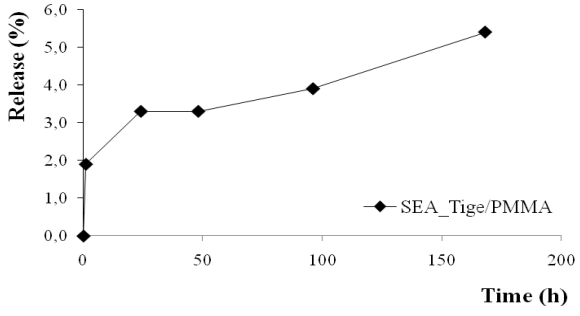


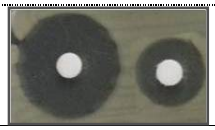
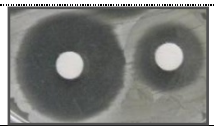
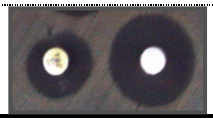
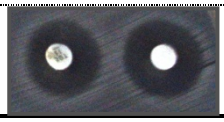

**Fig.1-In vitro antibiotic release profile for SEA particles.**

SEA Tige(PMMA) particles showed a faster release during the first 24h, corresponding to a concentration of 1.8 mg/L. There was a sustained release during one week of PBS immersion after what particle samples appeared with an orange dark colour suggesting tigecycline oxidation. At this moment tigecycline released in the medium was 1.6 mg/L, well above tigecycline $MIC_{90}$ 0.25 mg/L (Garrison, 2009) for *E. coli* and *S. aureus*. For double emulsion Tige(PMMA) particles release study was not performed given the negligible amounts of tigecycline entrapped.

***Microbiological Testing***

Growth inhibition zones observed around the dried disks indicated that tigecycline has maintained its microbiological activity after particles processing.

**Table 2: Bacteria inhibition zones**

| Bacteria / Method | *E. coli* | | *S. aureus* | |
|---|---|---|---|---|
| SEA | $\phi_{Std}$ | $\phi_{Tige/PMMA}$ | $\phi_{Std}$ | $\phi_{Tige/PMMA}$ |
| | 26 mm | 18 mm | 27 mm | 17 mm |
| |  | |  | |
| Double Emulsion | $\phi_{Std}$ | $\phi_{Tige/PMMA}$ | $\phi_{Std}$ | $\phi_{Tige/PMMA}$ |
| | 18 mm | 23 mm | 20 mm | 21 mm |
| |  | |  | |

## CONCLUSIONS

Particle preparation techniques have no effect on the microbiological activity of tigecycline. In spite of YP values, the SEA method has produced antibiotic loaded nanoparticles with suitable values for entrappment efficiency and release profile. Improvements must be done on particles harvesting procedure.

## REFERENCES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