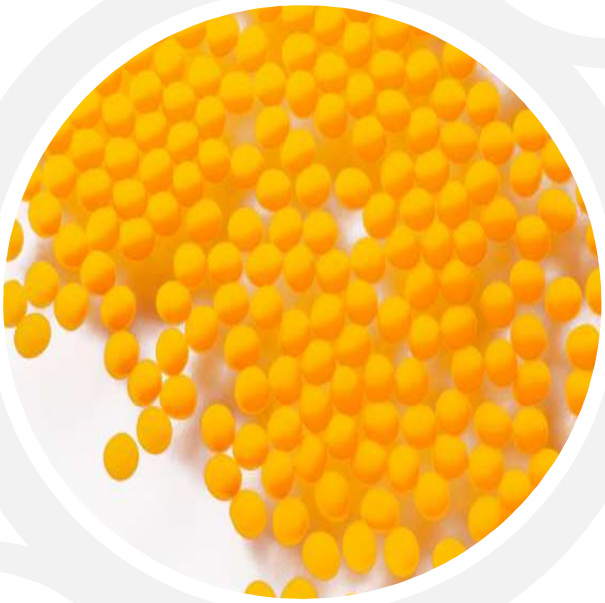
Application Note No. 145
Encapsulation of Methotrexate in Alginate & Hyaluronic acid

Encapsulator B-390 / B-395 Pro: Encapsulation of methotrexate in alginate & hyaluronic acid microbeads for controlled release in cancer treatment



1. Introduction

In recent years it has being shown in numerous studies how microbeads produced by the BUCHI Encapsulator can be used as an effective delivery system for the controlled release of drugs and bioactives [1,2,3].

In the presented study of Genc & Butuktiryaki, Methotrexate (MTX) was encapsulated in alginate-hyaluronic acid microbeads which were prepared using the Inotech Encapsulator (pre-cursor of the BUCHI Encapsulators) [4]. MTX is an anti-metabolite and anti-folate drug used in treatment of cancer, autoimmune diseases, ectopic pregnancy, and for the induction of medical abortions. It acts by inhibiting the metabolism of folic acid.
Encapsulation of MTX provides a mechanism for the controlled release of the drug; enabling the release of the required dose while also preventing interaction of the drug with healthy tissue (reduces toxic side-effects) [1]. Encapsulation also protects the drug from oxygen, pH changes and other unfavorable conditions which can cause the drug to degrade before reaching its target area.
The effect of different nozzle diameters and MTX concentrations on encapsulation efficiencies and the characteristics of the produced capsules were examined as well as the ability of the microcapsules to effectively deliver MTX to a cancer cell line (5RP7).

Aim: Development of a microencapsulation system for the controlled delivery of MTX to cancer cells.

2. Equipment

- Instrument: Inotech Encapsulator IE-50R*
- Set up: Single nozzle system
- Pumping: Syringe pump

*Precursor model to the BUCHI Encapsulator models B-390/B-395 Pro.

3. Chemicals and Materials

Chemicals:
- Polymer: Sodium alginate & hyaluronic acid in deion. water
- Gelling sol: 100 mM $CaCl_2$
- Encapsulant: Methotrexate

4. Procedure and Parameters

4.1 Procedure

Suspend the required amount of MTX in water and then add to a solution of 1.5% sodium alginate and 100 ppm hyaluronic acid. This solution is is pumped and extruded through the selected nozzle size to produce droplets, which are converted into MTX-loaded microbeads after landing in a solution of $CaCl_2$.

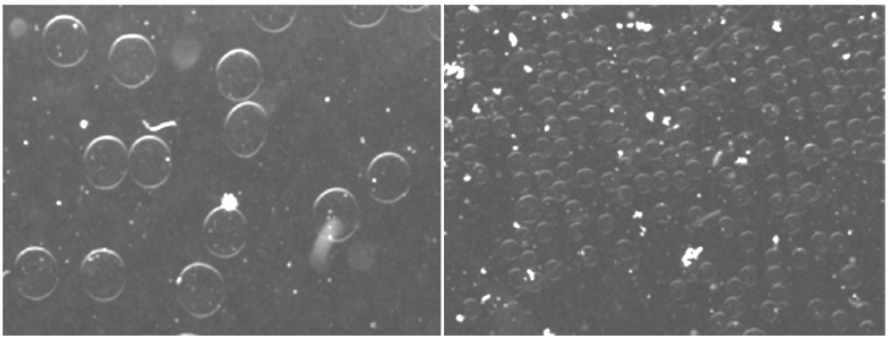
Begin bead production after obtaining a stable chain of droplets and use the electrostatic charge to disperse the droplets and prevent coalescence. After droplets have landed in gelling bath allow to harden for 30 min. After hardening remove from $CaCl_2$ and wash several times with HPLC.

4.2 Process parameters

- Nozzle sizes 200 & 400 µm
- Flow rate -
- Frequency 400 900 Hz
- Amplitude 1-4
- Charge 1000 - 2500 V

5. Results

5.1 MTX-loaded microbeads



Picture 1: Light microscope image displaying MTX-loaded microbeads which were produced in this study [1].

- Sizes (nozzle size) 400 (200) & 800 (400) µm
- Morphology Spherical
- Encapsulation efficiency 76-89%

5.2 Encapsulation efficiency

The encapsulation efficiency (% of drug remaining in capsule after production) of MTX in microcapsules as a function of nozzle size and initial drug concentration was examined to determine optimal conditions to obtain maximum drug loading. From Figure 1 it can be observed that the best encapsulation efficiencies for MTX loading were achieved using bigger nozzles and higher concentrations of MTX. For the biggest nozzle size (400 µm) used in this study it was possible to achieve an encapsulation efficiency off up to 89% in the alginate/hyaluronic acid microcapsules.

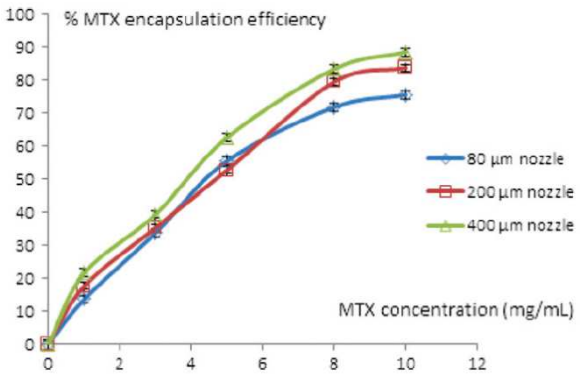


Figure 1. MTX-loading efficiencies in microbeads as a function of nozzle size and initial drug concentration [1].

5.3 In vitro release of MTX

The in vitro release profile of encapsulated MTX from microcapsules was examined and the results are shown in Figure 2. From the results it can be seen that it was possible to control the release of MTX from microcapsules over 30 hours, which wasn't possible for un-encapsulated MTX. Using the developed encapsulation system it was possible to deliver between 68-79% of the encapsulated drug in a controllable manner, with bigger particles delivering higher amounts.

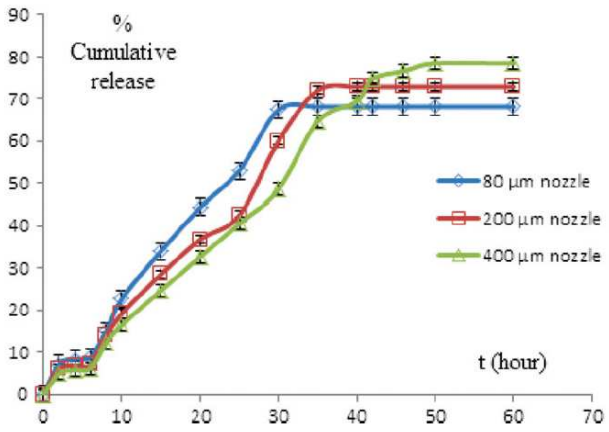


Figure 2. In vitro release of MTX from microcapsules as a function of size [1].

5.4 Cytotoxicity on a cancer cell line

In vitro cytotoxicity effects of MTX-loaded microbeads formulations on 5RP7 (rat fibroblast cancer cell line) was also examined. From the results it was observed that increasing the dose of microcapsules resulting in higher cell death of the cancer cell line.

6. Conclusion

This study has shown how microencapsulation can be used to control the release of MTX to cancerous cells and reduce its toxic effect on healthy tissue. Using the microcapsule system developed on the Encapsulator it was possible to obtain an MTX encapsulation efficiency of up to 89% while also effectively controlling the release rate of the drug from the microbeads over a 30 h period. In cell culture studies delivery of the encapsulated MTX reduced cancer cell viability by up to 88.5% compared to 49.7% for un-encapsulated MTX.

7. References

[1] Doherty S.B., et. al. (2011). Development and characterization of whey protein micro-beads as potential matrices for probiotic protection. Food Hydrocolloids 25:1604-1617.
[2] Dorati, R et. al. (2013). Microencapsulation of a hydrophilic model molecule through Vibration nozzle and emulsion phase inversion technologies 30(6):559-570.
[3] Hoad, C. (2011) Encapsulation of lipid by alginate beads reduces bio-accessibility: An in vivo 13C breath test and MRI study. Food Hydrocolloids 25:1190-1200.
[4] Genc, L. & Butuktiryaki S (2014). Preparation and characterization of methotrexate-loaded microcapsules. Pharmaceutical development & technology 19(1): 42-47.