Release Kinetics and Stability Study of Nanoformulation of Tebuconazole Encapsulated in Polycaprolactone Nanoparticles

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Introduction

One of the main current challenges in the environmental area is to reduce **the over growing application of pesticides** such as **fungicides** which causes **environmental and human health concerns.** While not all the pesticides applied in form of conventional formulations (Pure active ingredient) are effective. Thus, significant **low effectiveness of pesticides applied** against the target organisms (< 0.1%) occurs due to the large amounts of **losing pesticides during application**, stemming from factors like volatilization, degradation, and photolysis, etc. Different carrier systems have been studied with the aim of **controlling the active ingredient release** and **improving the efficacy of formulation** as new formulation. **Controlled release systems** via nanotechnology is offering a **potential solution** to mitigate such problems mentioned above. Thus, in **this work a nanoformulation of tebuconazole (TBZ) encapsulated in nanoparticles of poly(ε-caprolactone) (PCL-TBZ) a biocompatible and biodegradable polymer, was prepared and characterized. Next, the effect of dilution on the release profile and stability of the nanoformulation in reconstituted water (RCW) was studied at** three different concentrations of TBZ with dilution factor 10 including 50000, 5000 and 500 ng TBZ/ ml. From the results we expect to be able to better understand the behaviour of this nanoformulation under more realistic condition of **dilution factor and medium dilution of pesticide formulations when they are used in the field or gardens**.

Materials and method

PCL-TBZ preparation

PCL nanocapsules loaded with TBZ was prepared via interfacial deposition method drawn below.

Organic phase
PCL
Myritol
Sorbitan monostearate surfactant (span 60)
Acetone
TBZ

→ **Aqueous phase**
polysorbate 80 surfactant
water

→ Organic solvent evaporation by rotatory evaporator

Nanocapsule suspension of PCL-TBZ

Characterization

The characterization of PCL-TBZ prepared along with the used methods are provided below.

Parameter	Method
Encapsulation efficiency (EE)	Centrifugal ultrafiltration
Total concentration	Dilution with acetonitrile
Particle size, Polydispersity index (PDI), Zeta potential	Dynamic light scattering
Particle morphology	Scanning electron microscopy

Release and stability experiments

- **Three dilutions of the PCL-TBZ nanoformulation were made with RCW (**50000, 5000 and 500 ng TBZ/ ml)
- **For release kinetic assays,** the dilutions were sampled **at selected times (**0, 4, 8, 24, 48, 240 & 672) and the amount of free TBZ was obtained by centrifugal ultrafiltration and HPLC

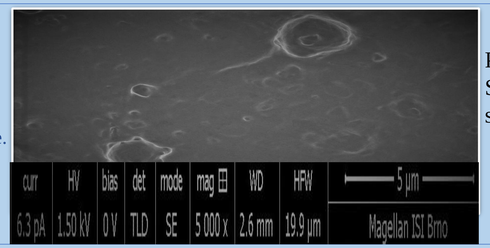
0.5 ml solution to **microcon** (1) then putting under **14000 rcf** centrifugation for **30min** (2) finally extraction sent for measuring **free TBZ** to **LC- MS-MS** (3)

- **For stability study,** the dilutions were sampled at selected times (0,4,24,240 h) and the particle size as well as PDI were determined by dynamic light scattering

Results and discussion

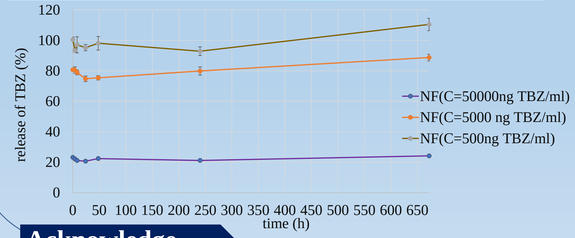
1)Characterization of PCL-TBZ (original NF)

- Total concentration =**313,957 ± 4675** ng/ml
- EE(%) = **95.8 ± 0.2** % , presenting high association of TBZ to polymeric nanocarrier.
- Z-Average: **241.1 ± 0.8 nm**, showing particles are in the range of nano size.
- PDI: **0.242 ± 0.014**, showing great particle homogeneity
- Z-potential: **-37.5 ± 0.5**mV, showing stability of PCL-TBZ and no tend to aggregation

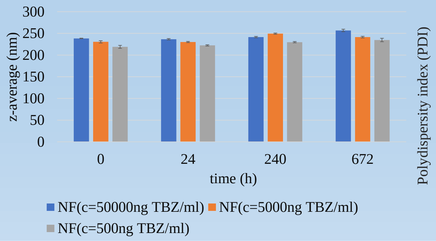


Particle morphology: SEM shows relatively spherical particles

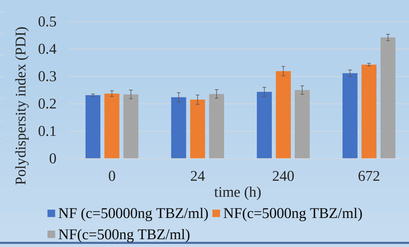
2)Release kinetic of PCL-TBZ in RCW over 672h at 25 °C (n=3)



3)PCL-TBZ Z-average in RCW over 672h(n=3)



4)PCL-TBZ PDI in RCW 672h(n=3)



2) TBZ release from nano carrier in different concentrations showed different pattern. The first burst point in sample 500 ngTBZ/ml (the highest diluted sample) seems as early as introducing PCL-TBZ suspension with medium happened with 100% release, while in 5000 and 50000 ng TBZ/ml were 80 and 20 % release, respectively. **5000 ng TBZ/ml with max. release 91.6% after 48h showed a nice controlled release system**. Low release of TBZ in 50000 ng TBZ/ml sample, can be due to being over the solubility of TBZ in water (TBZ S=32mg/l). **EE for 50000, 5000 and 500 ng TBZ/ml were 84, 41 and 27 % respectively**.

3,4) **Particle size remained in nano range** at different concentrations of TBZ in PCL-TBZ, that could be considered a sign of **stability**. For the concentration of **500 ng** TBZ/ml, a **gradual increase** in PDI (4) was observed, which can be attributed to **starting aggregation or degradation** (PDI as an indication for particle size distribution).

Conclusion

- **PCL-TBZ NF in RCW** showed **stability over 28 days**, all remained in the range of nano **particle size** with no obvious aggregation (**200-250 nm**).
- Release of TBZ from PCL was slower depending on concentration studied, compared to pure TBZ (result for pure is not provided here) so that the **TBZ release in nano formulation followed control release system.**
- Interestingly, the release profile in RCW (including some salts) showed similar behaviour with release kinetic in MilliQ water which can be promising result in real condition (MilliQ test is not provided here).
- Seems, with the increase of dilution the tendency to lose the association with nanocarrier is increasing as sample 500ng TBZ/ml, reached sooner to the burst point compared to sample with 50000ng TBZ/ml attributed to the fact, TBZ diffusion depends on the concentration gradient, so the release occur faster when nanoformulation is diluted.

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