Encapsulation and release of active species for protective coatings and corresponding biological activity

Tedim J.*, Maia F., Fernandes S., Silva A.P., Cunha A., Almeida A., Zheludkevich M.L., Ferreira M.G.S.
CICECO-DEMaC University of Aveiro, Aveiro, Portugal (joao.tedim@ua.pt)

INTRODUCTION

The control over release of active species has been a central topic in several areas of science and engineering, and protective coatings is no exception. The application of coatings for protection of metallic structures in offshore structures deals with two main, inter-related issues: corrosion and (bio)fouling.

One of the most recent strategies to impart anticorrosion and antifouling functionalities to organic coatings has consisted of encapsulation/intercalation of corrosion inhibitors and biocides into the so-called "smart" micro and nanocontainers, structures that store the active species and release them under certain conditions (Zheludkevich, 2012).

In this work, we synthesized and characterized different nanostructured inorganic materials containing an organic corrosion inhibitor also known for its biological activity, 2-mercaptobenzothiazole (MBT) (Maia, 2012). The main objective was to infer the biological activity of (i) empty nanocontainers, (ii) encapsulated species and (iii) free compounds, and correlate it with the release profiles of the encapsulated compounds in solution.

MATERIALS AND METHODS

The active compounds used for encapsulation was 2-mercaptobenzothiazole (MBT). For the capsules preparation, cetyltrimethylammonium bromide (CTAB) (99%) and tetraethoxysilane (TEOS) (99.9%) were purchased from Sigma-Aldrich. Ammonia solution (NH_4OH) (25-28%), sodium hydroxide (NaOH), sodium chloride (NaCl), ethyl ether (99.5%) and buffer solutions were obtained from Riedel-de-Haën. All chemicals were analytic grade and were used without further purification.

Synthesis of Silica Nanocapsules (Si_NC)

Si_NC were prepared via oil-in-water microemulsion approach. The nanocontainers were prepared using CTAB as cationic surfactant, ethyl ether as co-solvent and ammonia solution as catalyst. Firstly, a CTAB solution was prepared (0.25 g in 35 mL of water) and 0.25 mL of ammonia solution (25-28%) was subsequently added (aqueous phase). Afterwards, 0.1 g of MBT dissolved in 25 mL of ethyl ether was added to the aqueous phase, and an oil-in-water microemulsion was obtained. Then, 2.0 mL of TEOS was added to the microemulsion under vigorous stirring and kept in a closed vessel for 24 h. The obtained precipitate was filtered, washed with pure water and dried at room temperature. A small portion of these nanocontainers was calcined at 550ºC during 5 hours, with a heating rate of 10 ºC min^{-1}, in order to determine MBT loading content.

Characterization of silica nanocontainers

The morphology of obtained nanocontainers was characterized by scanning electron microscopy (SEM) coupled with Energy dispersive spectroscopy (EDS), (Hitachi S-4100 system with electron beam energy of 25 keV), and by transmission electron microscopy (TEM) (Hitachi H9000 TEM system with electron beam energy of 300 keV).

Thermogravimetric analysis (TG/DTA) was carried out in a Sataram – Labsys system under air atmosphere, with a heating rate of 10 ºC min^{-1} from room temperature up to 800 ºC.

Release studies of MBT and DCOIT

The release profiles were obtained by UV-Visible spectrophotometry. 1 mL aliquots were collected at every 15 minutes during 4 hours. A calibration curve was obtained for quantification of MBT using 5 standard solutions.

Microbiology studies of Si_NC

The antimicrobial effect of MBT encapsulated in the silica nanocontainers was evaluated on a recombinant bioluminescent strain of *Escherichia coli* (Alves 2008). Bacterial cells suspensions (10^7 cell mL^{-1}) in phosphate saline solution (PBS) were exposed to the nanocontainers, during 6-hour experiments. The inactivation was assessed, in real time, as the decrease in light emission, read in a luminometer (TD-20/20, Turner Designs). In parallel, the independent effect of organic molecule and Si_NC was also assessed as control conditions.

RESULTS AND DISCUSSION

The Si_NC were prepared by o/w microemulsion and results from the alkaline hydrolysis of TEOS and consequent condensation and polymerization. Si_NC show a yellow pale color as a result of the encapsulation of MBT. The resulted Si_NC are spherical and porous, with a narrow size distribution between 100-150 nm, as observed in Figure 1. The encapsulation of MBT does not promote any significant structural or morphological change in the capsules.

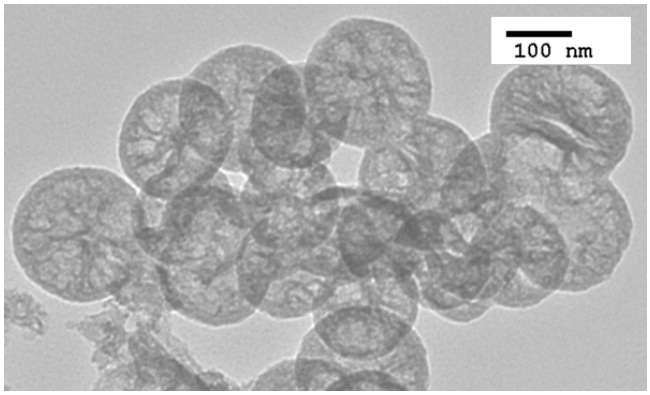

Figure 1: TEM picture of Si_NC

The MBT release profiles (Figure 2) suggest that MBT is released by diffusion through the porous from the core to the outside of the Si_NC.

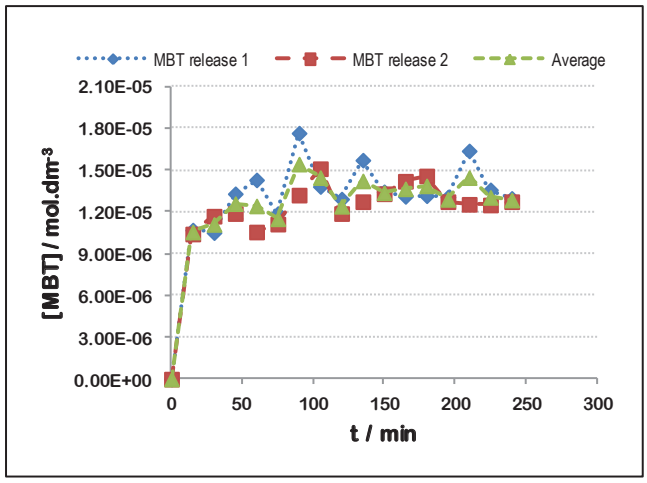

Figure 2: Release profile of MBT

The profile of inactivation of a biolumicescent strain of *Escherichia coli* (Figure 3) indicates that the biocidal effect of the Si_NC loaded with MBT is immediate upon exposure. Light emission is reduced by 5 log within the initial 15 minutes of the experiment, which agrees with the profile of release of biocide from Si_NC nanocontainers (Figure 2). The comparison of light emission profile in the control suspension with that of suspension amended with the nanocontainers, indicates that there is a significant toxic effect (3-4 log reduction of light emission) of the nanocontainer material on the model bacterial strain. This may explain why during the initial 90 minutes of the experiment, the inactivation is enhanced in the suspension amended with encapsulated MBT, in comparison to that amended with the dissolved form of the biocide. In both cases, inactivation to the detection limit was observed after 120 minutes of exposure.

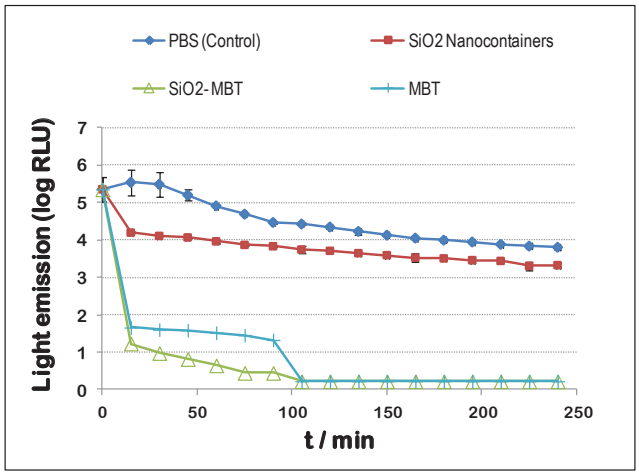

Figure 3: Inactivation of a bioluminescent recombinant strain of *Escherichia coli*.

CONCLUSIONS

In this work organic molecules were encapsulated within silica nanocapsules and the antimicrobial effect correlated with the release profiles of encapsulates into solution.

In addition to the biological activity of organic molecules, some degree of toxicity is also attributed to the silica nanocapsules.

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Address of other authors:
CICECO-DEMaC University of Aveiro, Aveiro, Portugal
CESAM – Biology Department, University of Aveiro, Aveiro, Portugal.