Microencapsulation of biocides: a new strategy for biofouling control

R. B Feio[a], O. Ferreira[a], J. C. M. Bordado[a], A. C. Marques[a, c*], E. R. Silva[a, b*]

[a] *Centro de Recursos Naturais e Ambiente, Departamento de Engenharia Química, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal*
[b] *Centro de Química e Bioquímica, Departamento de Química e Bioquímica, Faculdade de Ciências das Universidade de Lisboa, Campo Grande C8, 1749-016 Lisboa, Portugal*
[c] *Greeseal Research Ltd, Parque Industrial de Vendas Novas, Rua 4 Lote 48, 7080-341 Vendas Novas, Portugal*

* am@greensealchem.com; elisabetesilva@tecnico.ulisboa.pt

Abstract

In this work biocidal polyurethane-polyurea microcapsules (MC's) were successful synthesized by a microemulsion method combined with interfacial polymerization, following two main strategies for biocide (Econea) microencapsulation: a) the encapsulation of biocide in the microcapsules core, thus acting by a controlled releasing mechanism; b) the immobilization of biocides in the shell of microcapsules by chemical binding, thus acting by contact, thus, avoiding at same time the releasing of toxic agents into the environment. Yields as high as 98% have been achieved. From the developed biocidal microcapsules, the ones with encapsulated Econea (20 wt.%) evidenced the best morphologic properties, i.e., were characterized by uniform and relatively well-defined and smooth polymeric membrane (shell). However, microbiological analysis demonstrated that all developed biocidal MC's possess bioactivity. In addition, leachates analysis of those MC's evidenced a biocide releasing mechanism. Nevertheless, both followed strategies can provide either a biocide releasing mechanism and/or biocidal action by contact, since due to the biocide functionally (NH or NCO), its chemical immobilization can occur.

As ultimate goal, the most promising biocidal MC's were incorporated in a biocide free polyurethane based marine paint, the resulted formulations were used to coat PVC plates, in order to be further assessed in terms of bioactivity and/or antifouling effect. Leaching tests in artificial seawater of the coated plates, performed for about 30 days, didn't evidence any biocide releasing. This behavior was associated to low solubility of Econea in water, which can lead to very low biocide concentrations in the leachate waters, becoming undetectable by the used characterization techniques. Nonetheless, microbiological analysis on paint films of those prepared formulation evidenced bioactivity, proving the biocidal MC's concept.

Keywords: Antifouling coatings; biofouling; immobilized biocides; microencapsulation; biocidal microcapsules

1. Introduction

Marine biofouling, defined as the adhesion and growth of organisms on surfaces in contact with water, is one of the most serious problems for the shipping industry. The accumulation of biofouling on ships' hulls promotes their deterioration, and the introduction of invasive species, which can have a negative impact on the aquatic environment [1, 2]. This deterioration becomes the ships' hulls rougher and heavy, leading to an increase of frictional resistance resulting in higher fuel consumption. This also leads to increases on the emissions of greenhouse gases (CO_2, SO_2 and NO_x), which could have a major impact on the environment [2, 3]. The International Maritime Organization (IMO) estimates that emissions of CO_2 associated with high fuel consumption and under extreme scenarios could double by 2030 [1, 4]. Therefore, several efforts have been made to combat and prevent marine biofouling. The most common way of preventing biofouling is made using the addition of biocides in the formulation of paints. The most revolutionary generation was around 1960 through the appearance of Tributyltin based paints (TBT) due to its high antifouling effectiveness and versatility. However its toxicity proved to be extremely harmful to the marine ecosystem due to its slow degradation. As a result, the use of TBT in antifouling paints has been banned by the IMO in 2008 [3]. Since this prohibition, various efforts have been made to fill the gap left by this biocide, which led to the development of a new generation of antifouling coatings free of TBT derivatives, such as compounds of copper, which is characterized by being effective against barnacles, tubeworms and most algae. Nevertheless, the isolated action of these copper agents has not proven effective for the marine biofouling, becoming necessary to develop antifouling coatings with a wider spectrum, leading to the use of biocides as a reinforcement, such as Diuron and Irgarol 1051 [4-6].

However, a major problem of these antifouling coatings is the poor control biocidal agents releasing into the aquatic environment, and the potential degradation of these agents in the paint matrix, which may result in a loss of antifouling function of the coating before the lifetime intended. An alternative for achieving the required lifetime is to use of high quantities of biocide in the coating, but this may not be economically or environmentally acceptable because of its toxicity and accumulation in aquatic environments, leading to adverse side effects [7]. Thus, various efforts have been made in order to create new antifouling methods as potential alternatives. A relatively recent method, which involves the microencapsulation of biocidal agents, thereby preventing their degradation and allowing a minimum inhibitory concentration on the coating surface to be maintained for a relative longer period of time, thus conducting to a more controllable biocide releasing into the environment, thereby increasing the antifouling effect [8-10]. It should be noted that a controlled releasing of biocides from microcapsules ensures the presence of a constant amount of biocide in the paint matrix, ensuring the maintenance of the desired biocidal effect [11, 12].

Therefore, microencapsulation emerges as a very promising technology and can immobilize single substances which may be in a solid, liquid or gas state within a polymeric membrane. This technology involves a specific set of techniques that enable the manufacture of functional particles, consisting of an encapsulating polymeric based material (contained in the shell), and an encapsulated material (contained in the core). The microencapsulation process results in the formation of microcapsules. Microcapsules (MC´s) are particles comprising an inner core containing the active substance and which is surrounded by a polymer layer (polymer membrane). These can be mononuclear, when they are constituted by a single core, or polynuclear, if it is in the presence of more than one core within the shell [13-15].

The purpose of this work is to perform the microencapsulation of commercial biocides with recognized antifouling effect (Fig.1 a)), namely Econea, in order to promote not only a greater control of its further releasing, but also to explore their potential immobilization in the constitution of the microcapsule (Fig.1 b)) following a newly strategy. This recent approved commercial biocide has never been immobilized by encapsulation.

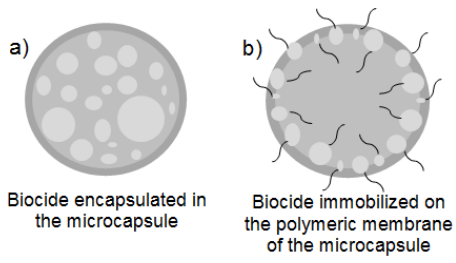

Figure 1 – Strategies for biocide immobilization in microcapsules: a) in the microcapsules core; b) in the microcapsules shell.

Microencapsulation/immobilization of biocides appears as a promising approach, able to overcome some problems associated with more conventional strategies for the incorporation of antifouling agents into a polymeric matrix. In particular, their potential immobilization in the constitution of the microcapsule shell is an innovation in the area, because it remains chemically fixed, thus avoiding the releasing of biocide into the environment, also becoming a potential, non-toxic and "environmentally friendly" alternative.

The microencapsulation method employed in this work for the microcapsules synthesis was the microemulsion method combined with interfacial polymerization, since it promotes the formation of microcapsules with high mechanical strength and stability, then becoming difficult to break, and since the ultimate goal is the incorporation of those developed MC´s into polymeric coatings, it makes perfect sense to be sturdy and stable.

The developed biocidal MC's were further incorporated in a biocide free polyurethane based marine paint (provided by HEMPEL, SA) and the resulted formulations were used to coat PVC plates, in order to be further assessed in terms of bioactivity or antifouling effect.

2. Experimental procedures

2.1. Materials

The active compound used for encapsulation was the marine antifouling agent Econea, supplied by *Janssen PMP* (95%). For the preparation of microcapsules, toluene (T) (99.8%) and N-methylpyrrolidone (NMP) (99%) were purchased from Sigma-Aldrich. Ongronat 2500® (O) (98%) was supplied by BorsodChem. Diethylene glycol (DEG) (99%) was obtained from Resiquímica. The surfactant DC193 was obtained from Dow Corning. Etanol (E) (99%) was purchased from Aga. Dimethyl sulfoxide (DMSO) (99.5%) was supplied by Lab-Scan, Analytical Sciences. All the chemicals were used as received and without further purification.

2.2. Biocidal microcapsules synthesis

Briefly the preparation of MC´s with biocidal properties involves the mixing of two different composed solutions (organic and aqueous), which results in a microemulsion water-in-oil (W/O), in accordance with the interfacial polymerization, and in order to obtain polyurethane-polyurea microcapsules.

2.2.1. Microencapsulation of commercial biocide (Econea)

Confidential

2.2.2. Immobilization of biocide (Econea-m)

Confidential

2.3. Microcapsules characterization

The methods/techniques used for the characterization of biocidal microcapsules include: optical microscopy, scanning electron microscopy (SEM), *Fourier* transform infrared spectroscopy (FTIR), leaching tests and microbiological tests.

2.3.1. Optical microscopy

The obtained biocidal microcapsules were observed on a zoom stereo microscope – *A. Kruss, MSZ 5600*.

For the observation under an optical microscope, it was collected small samples of microcapsules and placed on a glass coverslip (1x3 cm). Then the MC´s were dispersed in ethanol and preceded of its observation with a 20x objective.

2.3.2. Scanning electron microscopy (SEM)

The morphology of the synthesized biocidal microcapsules was characterized by scanning electron microscopy (SEM), using a *JEOL 7001F* (JEOL, Tokyo, Japan) SEM-FEG (Field Emission Gun) microscope.

The samples were placed in the sample holder using conductive adhesive tape with double face. Thereafter, were covered with a conductor film of Au/Pd, of about 20 nm thick. All observations were made with electrons beams of 15 kV.

2.3.3. Fourier transform infrared spectroscopy (FTIR)

The FTIR spectroscopy analyses were performed on: a Nicolet 5700 (Thermo Electron Corporation), with a kBr beam splitter and a DTGS-TEC detector in the middle-infrared region, using an ATR accessory with a diamond crystal with a 4 cm^{-1} resolution and 128 scans.

The FTIR analyses were performed in a frequency range of 600-4000 cm^{-1}, and recorded on a Nicolet Magna FTIR 550 spectrometer.

2.3.4. Biocide leaching assessment of microcapsules

Leaching tests were performed intending to determine the occurrence of biocide release through the microcapsule into the surroundings, in this case, artificial sea water.

The tested amount of microcapsules was such to provide the same biocide content or as much close as possible in all MC´s samples, this is, about 2-3 wt.% in 7.5 mL of artificial sea water (0.1 L distillated water + 3.25g of salt [*sera marin salt*, pH=8.3]). Then the mixture was placed on a stirring plate with a rotation speed of 120 rpm. Samples of leaching waters were collected after the first 24 hours and after 30 days, to be further assessed in terms of bioactivity from microbiological tests.

Preliminary leaching tests were also performed in dimethyl sulfoxide (DMSO) for some microcapsules.

2.3.5. Bioactivity assessment

Microbiological tests were performed to evaluate the biocidal activity of microcapsules and leaching waters obtained from microcapsules, by using the Well Diffusion Method.

Initially, the microorganisms were cultured on Muller-Hinton agar for bacteria *Staphylococcus aureus*. Then, 100 µL of a standardized microorganism suspension, corresponding to 0.5 McFarland, was used to inoculate a Petri dish of solid Mueller-Hinton medium. These suspensions were spread over the medium surface using a sterile swab. Subsequently, agar wells were made of approximately 5.0 mm in diameter with a Pasteur pipette. Then, 50 µL of each sample, negative control (DMSO) and positive control (Econea) for Gram positive bacteria (*Staphylococcus aureus*), were added on each of the wells. Plates were incubated at 37ºC for 24 hours. After this period, the diameters of the inhibition zones were measured (no growth) and the results were expressed in millimeters (mm). The assay was performed under aseptic conditions and in triplicate [17, 18]. Different mediums were used to assess the bioactivity of microcapsules, which were artificial sea water, distillated water and DMSO.

2.4. Incorporation of microcapsules in a polymeric matrix (marine paint)

The biocidal MC´s were incorporated in a biocide free polyurethane based marine paint (provided by HEMPEL, SA) and the resulted formulations were used to coat PVC plates (6x3 cm and 2x2 cm), in order to be further assessed in terms of bioactivity or antifouling effect.

Previously to the incorporation of the MC´s in marine paint, these were subjected to dispersion to promote its deagglomeration. Required amounts (in order to achieve a content of 2-3 wt.% of biocide in paint formulation) of MC´s (dry at room temperature) were dispersed in ethanol using an Ultraturrax with a rotation speed of 4000 rpm. It was allowed to evaporate part of ethanol, before re-dispersing the MC´s in a solvent (in this case DMSO) with a content of approximately 40 wt.%. This procedure allows incorporation of the microcapsules into the polymer matrix in a more homogeneous possible way, avoiding or reducing subsequent problems, such as formation of lumps and roughness in the obtained polymeric films. Finally, the dispersion (MC´s in DMSO) was added to the paint base (polyurethane) and curing agent.

The ratio proportion of polyurethane base (F0038 Base - F0032) and curing agent (F0038 Cure – 95580), recommended by the paint manufacturer (HEMPEL, SA) and used in this work was 11:1.

2.4.1. Leaching tests on coated plates

Leaching of the biocide through the paint films of the coated plates containing the developed biocidal MC´s was assessed by leaching tests. Those tests consisted on the coated plates (6x3 cm) immersion in 100 mL glasses containing artificial seawater (0.1 L distillated water + 3.25g

salt [*sera marin salt*, pH=8.3]) under controlled conditions along of the time. These were placed on a stirring plate with a rotation speed of 120 rpm for about 30 days. Finally, the leaching waters were analyzed on microbiological tests to detect any bioactivity (section 2.4.2).

2.4.2. Bioactivity and biocide leaching assessment on polymeric paint films

Microbiological tests were performed to evaluate the bioactivity of:

- Coating films with incorporated biocidal MC´s;
- Leaching waters obtained from tested coated plates with marine paint containing biocidal MC´s.

The followed procedure was similar to the one described in section 2.3.5.

Different mediums were used to assess the bioactivity of paint films: artificial sea water and DMSO.

3. Results and discussion

3.1. Biocidal Microcapsules

In order to succeed with the biocidal microcapsules synthesis, several experimental parameters were studied and optimized, such as, stirring speed, reaction temperature, volume ratio of the organic and aqueous phases, support solvent and type and amount of surfactant. Despite the optimized conditions/parameters have been already described in the experimental section, here it is briefly discuss some of this parameters optimization in order to provide an assessment of their effects on the microencapsulation steps.

Assays for the microemulsion preparation were performed with stirring speed ranging from 3200 rpm and 7000 rpm. It was set a speed of 5000 rpm, since for this speed was found the formation of a more stable and fairly uniform microemulsion. Moreover, and for MC´s formation, magnetic stirring speeds ranging from 400 rpm to 1000 rpm were tested. The MC´s formation was only identified, from optical observation, at speeds between 600 rpm and 800 rpm. Nonetheless, 600 rpm was considered the best speed, since it leads to the formation of MC's possessing a more uniform and well-defined shell. Regarding the reaction temperature, tests were conducted between 50ºC and 70ºC. However, the formation of uniform and well-defined microcapsules, and therefore, possessing a good morphology was only found in a temperature around 55-60ºC. In turn, at 70ºC early polymerization occurs, which hampered the MC's formation, and instead, poorly defined polymer agglomerates were observed. The support solvent (solvent used to store the MC´s obtained after filtration) was also accurately selected. Two support solvents were tested: ethanol and toluene. Ethanol, despite being a solvent that avoids aggregation of microcapsules, it is hydrophilic and characterized by small molecules, relatively to toluene, and thus, can promote the leaching out of the core content from the microcapsules. For this reason toluene was the selected support solvent, also because it is an inert solvent for this kind of systems. Regarding the amount and type of surfactant, it was observed that for the higher tested quantities, irregular MC´s were obtained. For higher contents of surfactant in the aqueous phase, the encapsulation capacity of the intended substance (in this case, the biocide) decreases as a result of the greater volume occupied by surfactant. DC193 is a high HLB surfactant, i.e., with more affinity to hydrophilic environments, and, as expected, it was the one which provided a better performance, when compared for instance with SPAN 80 and TWEEN 85. This is associated to the fact that the surfactant is incorporated in the aqueous (disperse) phase, and thus, surfactants with a higher HLB, such as the DC193 are preferred in order to provide stable and uniform microemulsions and subsequently, good quality microcapsules. The yield of microencapsulation is a parameter which expresses the efficiency of MC´s synthesis, taking into account the obtained amount of MC´s and the amount of reactants involved in the reaction. The yield has been calculated by using the following equation [19]:

$$\eta(\%) = \frac{m_{microcapsules}}{m_{reative\ compounds}} \times 100$$

Where, the ratio was defined between the MC´s mass and the sum of the reactive compounds the shell formation.

The obtained average yield was found to be around 97-98%. However, it should be noted that the microcapsules have not been totally dried, otherwise they would have been degraded and, therefore, the mass value of the microcapsules is a bit overestimated.

3.2. Microcapsules characterization

3.2.1. Optical microscopy

Optical microscopy was a characterization technique used to monitoring MC´s formation at different conditions, and therefore contributing to the selection of the best synthesis conditions. Optical microscope imagens of the MC´s with encapsulated Econea at a content of 20 wt.%, and obtained with different aqueous phase compositions, can be found in Fig. 2. In Fig. 3 can be also find the correspondent MC's references, i.e., without any biocidal agent for both tested conditions in terms of aqueous phase composition.

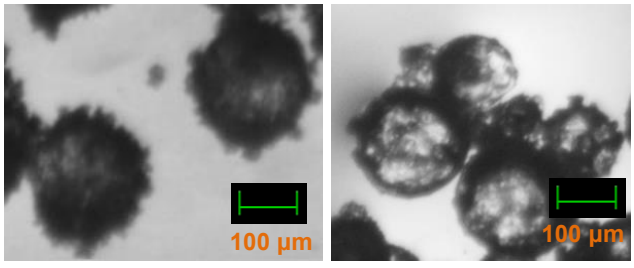

Figure 2 – Microcapsules with encapsulated Econea (20 wt.%) obtained from: (left) an aqueous phase: distillated water; (right) an aqueous phase: DEG and distillated water).

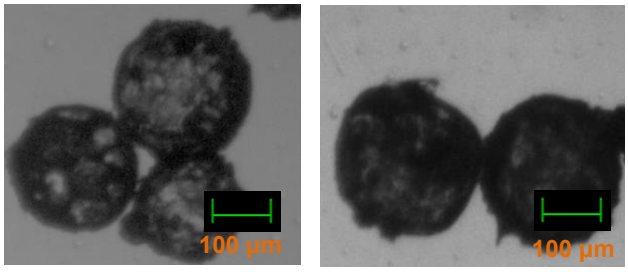

Figure 3 – Reference microcapsules (free of biocide), obtained from: (left) an aqueous phase: distillated water; (right) an aqueous phase: DEG and distillated water).

The MC's with encapsulated Econea (aqueous phase: distilled water) evidenced a uniform structure with a good morphology (spherical) and a well-defined shell, yet with some surface roughness. The average diameters of these biocidal MC´s were ranging from 100 to 200 µm. MC´s with encapsulated Econea (aqueous phase: DEG and distilled water) revealed a similar morphology to the previous obtained MC´s, in terms of spherical shape and well-defined shell. However they revealed to possess a smoother and transparent surface, but maintained the average diameters, ranging from 100 to 200 µm. Comparing the biocidal MC´s with their reference counterparts, it appears that the first have a less spherical and uniform shape. This behavior was expected, since a novel compound in the solution was introduced (in this case, the biocide), which can affect MC´s properties, mainly in terms of its morphology. Another approach of this work was the MC´s synthesis with chemical immobilized biocide in its shell. It is a very promising approach, since the immobilization of the biocide by chemical bonds, can avoid any biocide releasing, being the bioactivity provided by contact. In Fig. 4, representative optical images of MC´s containing immobilized biocide in their shell are shown.

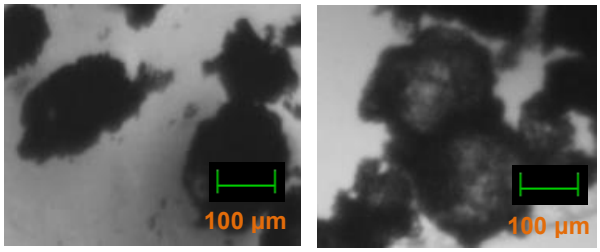

Figure 4 – Microcapsules with immobilized Econea-m (20 wt.%) (left) and its reference counterpart free of biocide (right)

These MC´s with chemical immobilized Econea-m and when compared with the MC´s with encapsulated Econea evidenced a less uniform and spherical shape, with lower average size, ranging from 100-150 µm. These effects are associated to the chemical binding of the biocide, which can affect not only the morphology of the shell by itself, but also, provide smaller MC´s cores, reducing its size. Nonetheless, it should also be mentioned, that in the first approach, the chemical immobilization of the biocide in these systems is also possible, due to the NH functionality of the biocide, but in a less extension, and thus, its effect in MC's morphological properties is not evidenced as in this case, were it is promoted the chemical immobilization.

3.2.2. Scanning electron microscope (SEM)

SEM images obtained for the biocidal microcapsules are shown on the following figures.

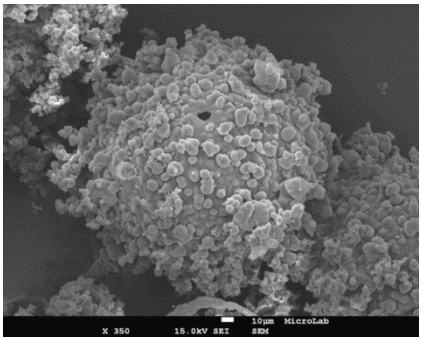

Figure 5 – Microcapsules with encapsulated Econea (20 wt.%) obtained from an aqueous phase composed by distillated water.

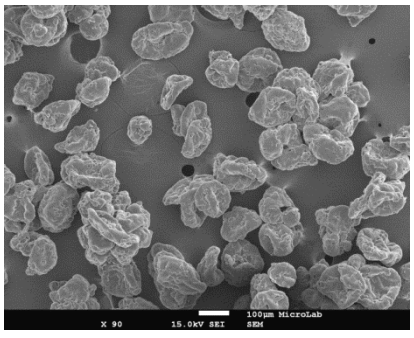

Figure 6 – Microcapsules with encapsulated Econea (20 wt.%) obtained from an aqueous phase composed by DEG and distillated water.

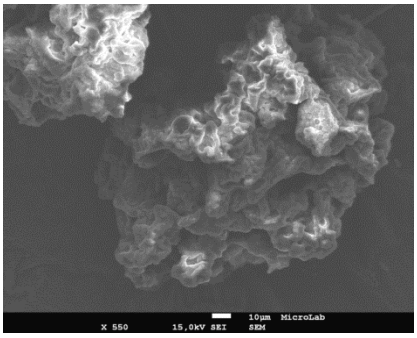

Figure 7 – Microcapsules with immobilized Econea-m (20 wt.%).

Fig. 5 evidence that MC´s with encapsulated Econea (aqueous phase: distillated water) have a spherical and uniform shape. However, it seems that occurs the agglomeration of smaller microcapsules on the surface of larger microcapsules. In addition, a small detected hole in a particular MC's, this is, a lack of polymeric material in the microcapsule surface, can suggest that an extraction of a small microcapsule was promoted during samples preparation for SEM observation. The average MC's diameter, range from 100 to 200 µm, which is in agreement

with the sizes determined from optical microscopy (Fig. 2, on the left). Nevertheless, changes in the average size of the MC´s are expected and are associated to the content of polyurethane-polyurea polymers which constitutes the MC´s shell, and the amount of encapsulated biocide.

For the MC's with encapsulated Econea obtained from an aqueous phase composed by DEG and distillated water, SEM images (Fig. 6) revealed less spherical and uniform shaped MC´s, when compared with the previous described MC's (Fig. 5). These microcapsules are slightly deformed, probably due to the passaging through the vacuum chamber in the electronic microscope, which on the other hand, indicates that those MC's are weaker, in terms of its mechanical resistance. In addition, those MC's were also smaller, ranging from 100-150 µm.

In the case of MC's with immobilized Econea-m rough and irregular shaped MC's were obtained (Fig. 7), which is in accordance with the previous optical observation (Fig. 4, on the left). This irregular MC's morphology is associated to the isocyanate functionality of the applied modified biocide (Econea-m), which is able to react with the shell of the microcapsule, which can reflects in morphologic MC's properties degradation.

3.2.3. Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of the commercial Econea, MC´s with encapsulated Econea either obtained from an aqueous phase composed by distillated water or by DEG and distillated water can be found on Fig. 8.

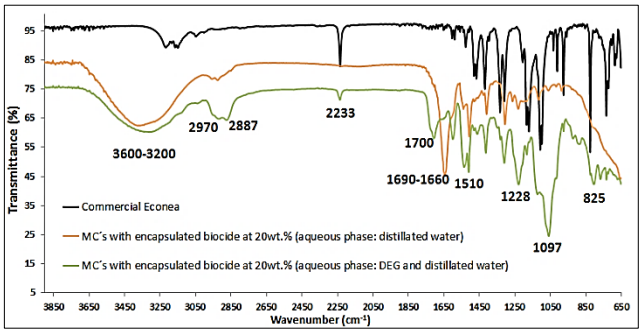

Figure 8 –Infrared spectrum (FTIR) of microcapsules with encapsulated Econea.

From these spectra it is clearly identified the presence of water (OH stretching) by the presence of the band ranging from at 3600 to 3200 cm^{-1}. In the case of the MC's (aqueous phase: DEG + distilled water) the presence of DEG is also identify through the band maximum at 2970 cm^{-1} (CH_2 stretching asymmetric) and 2887 cm^{-1} (CH_2 stretching symmetric), as well as the Econea presence, due to the appearance of the maximum at 2233 cm^{-1} (C≡N stretching), at 1097 cm^{-1} (C-F stretching) and at 825 cm^{-1} (C-Cl stretching). On the other hand, and for the MC's which aqueous phase composed only by water, the biocide is not detected [20].

It should also be noted that, there are no traces of the peak of Ongronat 2500® at 2237 cm^{-1} assigned to the isocyanate N=C=O bonds, reflecting its complete reaction in the interfacial polymerization to form a MC's shell. It can also be confirmed the presence of the N-H stretching band at 3400 to 3200 cm^{-1} and the maximum at 1510 cm^{-1} of the N-H bending, which can be associated to the presence of both polyurethane and polyurea, also confirmed by the characteristic bands of these polymers. For instance the urethane bonds presence is evidenced by the maximum at 1228 cm^{-1} (C-O stretching), and carbonyl groups at 1700 cm^{-1} (C=O from urethane). Whereas urea presence is identified by the characteristic band of urea at 1690-1660 cm^{-1} (C=O stretching) [20, 21]. Therefore, the obtained MC's spectra clearly confirmed that MC´s of polyurethane-polyurea were achieved.

As can also been depicted from those spectra, when the core of the biocidal microcapsules is consisted of water and DEG, the MC´s are mostly composed by polyurethane, since the C=O stretching band displaced to be close to the polyurethane characteristic maximum at 1700 cm^{-1}. This result can be associated to the fact that the core is composed mainly by DEG and thus, the predominant reaction is the one between the diisocyanate of the NCO groups and the OH group (DEG) to form polyurethane. Nevertheless, and since water is also present, some polyurea is also formed.

In the case of biocidal microcapsules containing only water in its core (no DEG), the corresponding spectrum suggests that its shell is mainly composed by polyurea. This is associated to the reaction between NCO group (ongronat 2500®) and OH group from water to form polyurea, which is promoted by the main presence of water in the original aqueous phase composition. On the other hand, and when there is the presence of biocide, it appears that the shell is also essentially consist of polyurea, maximum of its characteristic band located in the range of 1690-1660 cm^{-1}. This result can be justified by the possible biocide reaction with the ongronat 2500®, through the N-H group of Econea with the NCO group of Ongronat 2500®, thus favoring the formation of polyurea.

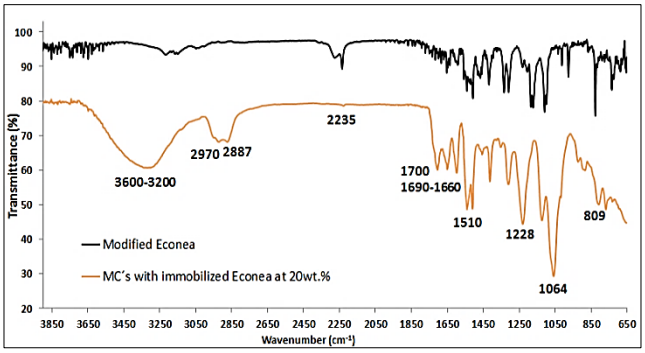

Figure 9 – Infrared spectrum (FTIR) of microcapsules with immobilized Econea-m.

From the spectrum of the biocidal MC´s with immobilized biocide (Fig. 9) it can be also identified the

presence of water (OH stretching) in the range of 3600-3200 cm^{-1}, thus proving that it is encapsulated. The presence of DEG was also confirmed through the band with a maximum at 2970 cm^{-1} (CH_2 stretching asymmetric) and 2887 cm^{-1} (CH_2 stretching symmetric). The Econea-m is identified due the presence of the bands with maximums at 1097 cm^{-1} (C-F stretching), and at 809 cm^{-1} (C-Cl stretching) [20].

It can also be confirmed the presence of both polyurethane and polyurea, from the characteristic N-H stretching band at 3400 to 3200 cm^{-1} and the maximum at 1510 cm^{-1} of the N-H bending. Polyurethane presence is also evidenced by the maximum at 1228 cm^{-1} (C-O stretching), and carbonyl groups at 1700 cm^{-1} (C=O from urethane [20, 21]. Moreover, with immobilized Econea-m in the MC´s, it is also verified that the MC´s are mainly composed by of polyurea, since the band of the C=O bond is mostly pronounced in the range of 1690-1660 cm^{-1}. This may be due to the fact that the Econea-m leads to a higher content of NCO functional groups that reacts more actively with the OH groups of water than with OH groups of DEG.

3.2.4. Bioactivity and leaching of biocide from microcapsules

- Microcapsules

Microbiological tests on MC´s were performed in three different mediums: artificial seawater, DMSO and distilled water. In Table 1 can be found the inhibition zones (mm) for the *Staphylococcus aureus* bacterium, obtained from the different tested MC´s. It should also be noted that when the inhibition zone is 5 mm, this means that the sample does not show bioactivity, since this value is equal to the negative control (reference MC´s without biocide).

Table 1 – Inhibition zones for the *Staphylococcus aureus* bacterium, obtained from the tested microcapsules at different mediums.

Sample	Medium		
	Artificial seawater	Distilled water	DMSO
	Inhibition zone for *S. aureus* (mm)		
1	5	5	5
2	9	12	14
3	14	12	18
4	13	11	15
E	20	23	19

E-commercial Econea (positive control); 1 – Reference MC´s without biocide, 2 - MC´s with encapsulated Econea at 20 wt.% (aqueous phase: distillated water); 3 - MC´s with encapsulated Econea at 20 wt.% (aqueous phase: DEG and distillated water); 4 – MC´s with immobilized Econea-m at 20 wt.%.

From Table 1, MC's with encapsulated biocide (samples 2 and 3), exhibit bioactivity for all the three studied mediums. Another observation that can be depicted from the bioactivity results is that the biocidal MC´s have a higher bioactivity when in a DMSO medium, expressed by the higher inhibition zone areas, suggesting that in this medium the biocide is likely to leach out through the microcapsule. This is an expected behavior, since the Econea biocide is poorly soluble in water, but is reasonable soluble in DMSO.

- Leaching waters

Table 2 – Inhibition zones for the *Staphylococcus aureus* bacterium obtained for the leachates from the microcapsules after 24 hours and 30 days.

Sample	Artificial seawater 24 hours/30 days	DMSO 24 hours/30 days
	Inhibition zones for *S. aureus* (mm)	
1	5/5	-
2	5/5	19/17
3	5/5	21/20
4	5/5	-
E	21/20	

E-commercial Econea (positive control); 1 – Reference MC´s without biocide, 2 - MC´s with encapsulated Econea at 20 wt.% (aqueous phase: distillated water); 3 - MC´s with encapsulated Econea at 20 wt.% (aqueous phase: DEG and distillated water); 4 – MC´s with immobilized Econea-m at 20 wt.%.

Leaching waters obtained from the biocidal microcapsules exhibit bioactivity only when the used medium is DMSO. Once again the results suggest that the highest solubility of the biocide towards DMSO solvent promotes its leaching.

On the other hand, in artificial seawater the absence of bioactivity even after 30 days, can be associated to the lowest biocide solubility in this solvent, which even for longer test periods the biocide content can be low enough to be undetectable by this kind of analysis.

3.3. Incorporation of microcapsules in a polymeric matrix (marine paint)

Representative coated plates with polyurethane based marine paint,containing the developed biocidal MC's can be found in Fig. 10.

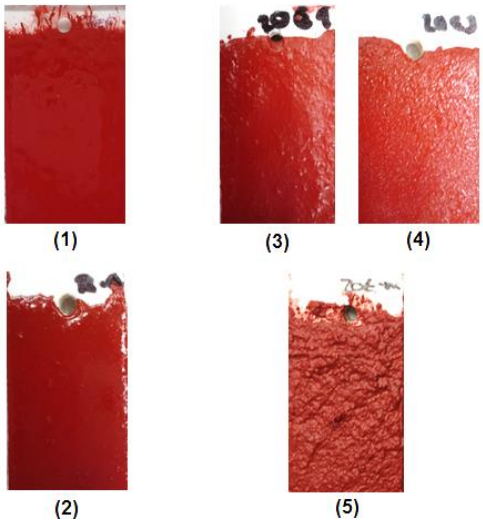

Figure 10 – Coated PVC plates with polyurethane based marine paint: **(1)** Polyurethane coating without microcapsules; **(2)** Reference (MC´s without biocide); **(3)** Microcapsules with encapsulated Econea at 20 wt.% (aqueous phase: distillated water); **(4)** Microcapsules with encapsulated Econea at 20 wt.% (aqueous phase: DEG and distillated water); **(5)** Microcapsules with immobilized Econea-m at 20 wt.%.

From Fig. 10, it can be observed that the plates containing biocidal MC´s have different surface textures when compared with the reference plate **(1)**. With the incorporation of MC´s in the paint formulations the polymeric paint films lost brightness and showed some roughness against the reference. This behavior seems to increase with MC's content and for biocidal MC's, in particular for the ones containing immobilized biocide. This result is somehow expected, since any additive addition in paint formulations is expected to degrade paint properties, reason why its content is limit in those formulations. On the other hand, for rougher MC's (see SEM and optical images), such as the ones with immobilized biocide those effects are expected to be more effective, since MC's dispersion is worst in this particular case, reason why this formulation was not considered for the next characterization tests.

3.3.1 Bioactivity and biocide leaching from polymeric films

Leaching tests performed on coated plates with marine paint containing biocidal MC´s were intended to examine the occurrence, or not, of biocide releasing into the aqueous medium, in this case, artificial seawater. After 30 days, leaching water samples were collected and analyzed by microbiological tests, which will demonstrate, or not, if these waters have any bioactivity. Additional microbiological analyses were performed on paint films removed from the coated plates in two different mediums: artificial seawater and DMSO.

In Table 3 and 4 are presented the obtained inhibition zones (mm) for the *Staphylococcus aureus* bacterium and for both tests types. It should be also noted that when the inhibition zone is 5mm, this means that the sample shows no bioactivity, since it is equal to the negative control (plate coated with polyurethane based marine paint without MC´s or even with MC´s without biocide).

- Paint films containing biocidal microcapsules

Table 3 – Halos of inhibition for the bacterium *Staphylococcus aureus* obtained for marine paint films based polyurethane containing biocidal microcapsules.

Sample	Medium	
	Artificial seawater	**DMSO**
	Inhibition zone for *S. aureus* (mm)	
1	5	5
2	5	5
3	5	11
4	5	10
E	18	20

E-commercial Econea (positive control); 1 - Plate containing MC´s without biocide; 2 – Plate containing MC´s with encapsulated Econea (20 wt.%), aqueous phase: distillated water; 3 - Plate containing MC´s with encapsulated Econea (20 wt.%), aqueous phase: DEG and distillated water; 4 – Plate containing MC´s with immobilized Econea-m (20 wt.%).

From Table 3, it can be observed that when the used medium is artificial seawater, the samples didn't evidence bioactivity. This result is associated to the low biocide concentration in the films, together with the very low solubility of the biocide in such medium. When the medium used is DMSO, the films evidenced encouraging results, showing bioactivity for polymeric films containing MC's with encapsulated Econea, as well as, for films containing MC's with immobilized Econea-m. In this case, the solubility of the biocide in DMSO is the driving factor, which promotes the leaching of the biocide from the paint film to its surroundings. In the case of films containing immobilized Econea-m, it is not exclude the hypothesis of some encapsulated biocide, being the founded bioactivity associated to the release of this encapsulated agent.

- Leaching waters

In Table 4, inhibition zones obtained for the *Staphylococcus aureus* bacterium on the tested leaching waters from coated plates can be found.

Table 4 – Inhibition zones obtained for the *Staphylococcus aureus* bacterium on the tested leaching waters of the coated plates.

Leaching water sample after 30 days of exposure	Inhibition zones for the *S. aureus* (mm)
1	5
2	5
3	5
4	5
E	20

E-commercial Econea (positive control); 1 - Plate containing MC´s without biocide; 2 – Plate containing MC´s with encapsulated Econea (20 wt.%), aqueous phase: distillated water; 3 - Plate containing MC´s with encapsulated Econea (20 wt.%), aqueous phase: DEG and distillated water; 4 – Plate containing MC´s with immobilized Econea-m (20 wt.%).

As can be seen, the tested leaching waters didn't reveal any biocidal activity, meaning that after about 30 days of exposure no biocide releasing was detected. This is an expected result, which is associated to the very low biocide solubility in water and subsequently if any biocide leaching occurs, the amount is so low that the total biocide concentration is not detectable by the microbial test. On the other hand, and for the polymeric films containing MC's with immobilized biocide, no leaching out is expected.

4. Conclusions

In this work biocidal polyurethane-polyurea microcapsules (MC's) were successfully synthesized by following a microemulsion method combined with interfacial polymerization. Two different strategies were followed: - the microencapsulation of commercial biocide (Econea) in the MC's' core and the chemical immobilization of modified biocide (Econea-m) in MC's' shell. For both strategies, and in order to achieve well-structured microcapsules possessing a good biocide encapsulation capacity, several experimental parameters were assessed and optimized, such as stirring speed, the reaction temperature, the volume ratio between the dispersant and dispersed phase, support solvent and the type and amount of surfactant. Yields as high as 98%, were achieved for the obtained biocidal MC's.

SEM and optical images evidenced that MC's with encapsulated biocide (Econea) originates more uniform MC's possessing a better defined shell, when compared with MC's containing immobilized modified biocide (Econea-m) in their shell. The rough and irregular shaped MC's in this last case was associated to the isocyanate functionality of the modified biocide, since their high reactivity with OH groups from water or DEG to form the microcapsules shell, can reflect in the morphologic MC's properties degradation. FTIR analysis were very conclusive, allowed to realize that when the core is composed only by water and biocide, the shell is mainly composed of polyurea in turn, when the core is composed by water, DEG and biocide, the shell is mainly composed of polyurethane. Finally, microbiological analysis confirmed that the obtained MC´s containing biocide are bioactive. But the leaching of biocide from MC's is only clearly evidenced in a DMSO medium. For an aqueous medium the low biocide solubility is the main associated reason to lead to a very slow leaching out of biocide from the MC's, and therefore undetected in the used test conditions.

As ultimate goal, biocidal MC's (namely MC's with encapsulated Econea (20 wt.%) and MC's with immobilized Econea (20 wt.%) were incorporated in a biocide free polyurethane based marine paint (provided by HEMPEL, SA), and used to coat PVC plates. The coated plates revealed changes in texture and brightness with MC's inclusion, meaning that in order to avoid paint degradation limit MC's contents should be defined. For the studied systems, such content only allow to include about 2-3 wt.% of biocide in the polymeric formulations. Nevertheless, the microbiological tests carried out on paint films containing MC's with encapsulated Econea proved to be bioactive, followed by MC's with immobilized Econea on the polymeric shell. On the other hand, leaching tests with artificial seawater also performed on coated plates, and after about 30 days, didn't reveal any biocide releasing. This result was associated to the low biocide concentration in those leaching waters. As a whole, the results were very promising, not only bioactive MC's were obtained from two different strategies, but also bioactive polymeric films. In addition, and in order to prove the concept in real and/or simulated conditions, coated plates will be in a near future tested in seawater aquariums and in Atlantic sea.

References

[1] E. R. Silva, O. Ferreira, J. C. Bordado, M. J. Calhorda, *Revestimentos poliméricos para o controlo da bioincrustação industrial: novas estratégias amigas do ambiente,* Boletim da Sociedade Portuguesa da Química*,* 134 (**2014**) 43-49.

[2] C. Shan, W. JiaDao, C. HaoSheng and C. DaRong, *Progress of marine biofouling and antifouling technologies*, Chinese Science Bulletin*,* Vol. 56 (**2011**) 598-612.

[3] D. M. Yebra, S. Kiil and K. Dam-Johansen, *Antifouling technology-past, present and future steps towards efficient and environmentally friendly antifouling coatings*, Progress in Organic Coatings*,* Vol. 50 (**2004**) 75–104.

[4] N. Voulvoulis, M. D. Scrimshaw and J. N. Lester, *Comparative environmental assessment of biocides used in antifouling paints*, Chemosphere*,* Vol. 47 (**2002**) 789–795.

[5] K. A. Dafforn, J. A. Lewis and E. L. *Johnston, Antifouling strategies: History and regulation, ecological impacts and mitigation*, Marine Pollution Bulletin*,* Vol. 62 (**2011**) 453–465.

[6] M. Löschau and R. Krätke, *Efficacy and toxicity of self-polishing biocide-free antifouling paints*, Environmental Pollution*,* Vol. 138 (**2005**) 260 – 267.

[7] M. A. Trojer, L. Nordstierna, J. Bergek, H. Blanck, K. Holmberg, M. Nydén, *Use of microcapsules as controlled release devices for coatings*, Advances in Colloid and Interface Science, Vol. 222 (**2014**) 18-43.

[8] S. Jämsä, R. Mahlberg, U. Holopainen, J. Ropponen, A. C. Ritschkoff, *Slow release of a biocidal agent from polymeric microcapsules for preventing biodeterioration*, Progress in Organic Coatings, Vol. 76 (**2013**) 269-276.

[9] M. Masuda, *Microencapsulation of pesticides for controlling release from coatings*, Master Thesis, Chalmers University of Technology, Suécia, pp. 16-39, **2011**.

[10] M. A. Trojer, *Modification of microcapsules for controlled release,* PhD Thesis, Chalmers University of Technology, Suécia, pp. 13-17, **2011.**

[11] G. Sørensen, A.L. Nielsen, Morten Møller Pedersen, S. Poulsen, H.Nissen, M. Poulsen, S.D. Nygaard, *Controlled release of biocides from silica microparticles in wood paint*, Progress in Organic Coatings, Vol. 68 (**2010**) 299-306.

[12] L. Nordstierna, A. A. Abdalla, M. Masuda, G. Skarnemark, M. Nydén, *Molecular release from painted surfaces: Free and encapsulated biocides*, Progress in Organic Coatings, Vol. 69 (**2010**) 45-48.

[13] J. S. L. Brasileiro, *Microencapsulação de compostos bioactivos: Inovação em diferentes áreas*, Master Thesis, Universidade Fernando Pessoa, Porto, pp. 16-39, **2011**.

[14] J. Suave, E. C. Dall'agnol, A. P. T. Pezzin, D. A. K. Silva, M. M. Meier, V. Soldi, *Microencapsulação: Inovação em diferentes áreas*, Revista Saúde e Ambiente/Health and Environment Journal, Vol. 7 (**2006**) 12-20.

[15] T. Szabó, L. Molnár-Nagy, J. Bognár, L. Nyiko, J. Telegdi, *Self-healing microcapsules and slow release microspheres in paints*, Progress in Organic Coatings, Vol. 72 (**2011**) 52-57.

[16] E. R. Silva, O. Ferreira, J. C. Bordado, *Processo de funcionalização de biocidas para imobilização em matrizes poliméricas*, Patente PT nº108096, **2015**.

[17] P. Rijo, D. Matias, A. S. Fernandes, M. F. Simões, M. Nicolai, C. P. Reis, *Antimicrobial Plant Extracts Encapsulated into Polymeric Beads for Potential Application on the Skin*, Polymers, Vol. 6 (**2014**) 479-490.

[18] M. Pereira, D. Matias, F. Pereira, C. P. Reis, M. F. Simões, P. Rijo, *Antimicrobial screening of Plectranthus madagascariensis and P. neochilus extracts*, Biomedical and Biopharmaceutical Research, Vol. 12 (**2015**) 127-138.

[19] S. Lu, J. Xing, Z. Zhang, G. Jia, *Preparation and Characterization of Polyurea/Polyurethane Double-Shell Microcapsules Containing Butyl Stearate Through Interfacial Polymerization*, Vol. 121 (**2011**) 3377-3383.

[20] R. M. Silverstein, F. X. Webster, D. J. Kiemle, *Spectrometric Identification of Organic Compounds*, John Wiley & Sons, INC., Seventh edition, **2005**.

[21] C. S. Wong, K. H. Badri, *Chemical Analyses of Palm Kernel Oil-Based Polyurethane Prepolymer*, Materials Sciences and Applications, Vol. 3 (**2012**) 78-86.