

Article

Gemini Surfactant as a Template Agent for the Synthesis of More Eco-Friendly Silica Nanocapsules

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Abstract: Silica mesoporous nanocapsules are a class of “smart” engineered nanomaterials (ENMs) applied in several fields. Recent studies have highlighted that they can exert deleterious effects into marine organisms, attributed to the use of the toxic cationic surfactant *N*-hexadecyl-*N,N,N*-trimethylammonium bromide (CTAB) during the synthesis of ENMs. The present study reports the successful synthesis and characterization of novel gemini surfactant-based silica nanocapsules. The gemini surfactant 1,4-bis-[*N*-(1-dodecyl)-*N,N*-dimethylammoniummethyl]benzene dibromide (QSB2-12) was chosen as a more environmentally-friendly replacement of CTAB. Nanocapsules were characterized by scanning electron microscopy (SEM), Fourier-transformed infrared spectroscopy (FTIR), dynamic light scattering (DLS), thermogravimetric analysis (TGA) and N₂ adsorption-desorption isotherms. Short-term exposure effects of new ENMs were evaluated in four marine species (*Nannochloropsis gaditana*, *Tetraselmis chunii* and *Phaeodactylum tricorutum*) and the microcrustacean (*Artemia salina*). The replacement of the commercial cationic surfactant by the gemini surfactant does not change the structure nor the environmental behaviour in seawater of the newly synthesised silica nanocontainers. Additionally, it is demonstrated that using gemini surfactants can reduce the toxicity of novel silica nanocapsules towards the tested marine species. As a result, environmentally-friendly ENMs can be obtained based on a safe-by-design approach, thereby fitting the concept of Green Chemistry.

Keywords: cationic surfactants; microemulsion; mesoporous silica; encapsulation; ecotoxicity

1. Introduction

Engineered nanomaterials (ENMs) have been proposed as new solutions for encapsulation of active compounds, such as corrosion inhibitors, antifouling agents, pH indicators, drugs or dyes [1–7]. Nanocapsules are core–shell structures with diameter generally ranging between 100–500 nm [8], and one of the most used nanocapsules are based on mesoporous silica, typically with a diameter of 100–200 nm [9]. This cutting-edge immobilization technique has assumed a relevant role in the field of smart coatings, by providing a controlled release of encapsulated molecules, reducing their toxicity (compared to the free forms), preventing direct interaction with coating matrices, reducing leakages and ultimately increasing the coating’s service life [3,10,11]. However, state-of-the-art information shows that even empty silica nanocapsules show some degree of toxicity [10]. They have been found to be more toxic towards selected marine species than other engineered nanomaterials, namely Zn–Al layered double hydroxides (LDH) [12,13]. Recent studies have revealed that the silica nano-form or the formation of agglomerates in artificial saltwater, over time, is not necessarily the

source of silica nanocapsules toxicity [10–12,14]. Indeed, it is related to the presence of a cationic surfactant, *N*-hexadecyl-*N,N,N*-trimethylammonium bromide (CTAB) which is used as a template during nanomaterials' synthesis. Replacing CTAB with a greener surfactant has been suggested previously in order to reduce capsules' toxicity [10]. There are strategies that allow the complete removal of CTAB from capsules' surface such as extraction with solvents solutions, washing with a solution of ethanolic HCl or thermal treatment (calcination) [9,15,16]. Although these steps are effective for removal of synthesis remnants from empty silica nanocapsules, they may be undesirable if the entrapment of active molecules inside the nanomaterials is the main goal: calcination leads to complete degradation of all organic molecules, while using other solvents may lead to leaching of the encapsulated agents as well. Therefore, replacing CTAB with a more eco-friendly surfactant would prevent toxicological issues and may avoid the implementation of additional steps.

Cationic gemini surfactants are a group of innovative quaternary ammonium salts that consist of two monomeric moieties linked by a spacer. Each moiety is made of a hydrophilic part (positively charged nitrogen atom) and a hydrophobic part (alkyl chain) [17]. Due to this dimeric structure, gemini surfactants show unique surface properties, such as critical micelle concentrations (CMC), which is lower comparing with conventional cationic surfactants, with higher efficacy in lowering surface tension (for surfactants with the same number of carbon atoms in hydrophobic parts). CMC is the concentration at which micelles start forming [18], and the ability of cationic surfactants to form stable oil in water emulsion allows their use as a template in silica nanocapsules synthesis [14]. For a conventional monomeric surfactant, *N*-dodecyl-*N,N,N*-trimethylammonium bromide (DTAB), the CMC is 16 mM whereas for a dimeric surfactant, with a rigid spacer, such as 1,4-bis-*[N*-(1-dodecyl)-*N,N*-dimethylammoniummethyl]benzene dibromide (QSB2-12) the CMC is 1.21 mM [18]. For CTAB the CMC is 0.907 mM [19], lower than for QSB2-12. It has been reported that by modifying the surfactants' structure, aggregation behavior may be changed. Increasing the number of hydrophilic parts decreases the CMC (when the hydrophobic part is fixed), which is also observed when an increase in the number of carbon chains of the hydrophobic part occurs (when hydrophilic part is fixed) [17].

Gemini surfactants are known to be less toxic to freshwater organisms [20]. As an example, and looking at the key freshwater species *Daphnia magna*, CTAB was found to be 28 times more toxic than QSB2-12: LC₅₀ of 0.026 mg/L [21] vs. 0.73 mg/L [20], respectively. Comparing QSB2-12 with its monomeric analogue DTAB, the gemini surfactant is also less toxic than the conventional one (LC₅₀ for DTAB towards *Daphnia magna* is 0.35 mg/L [20]). It has been already reported that elongating an alkyl chain leads to an increase in toxicity [22,23]. As an example, for betainate cationic dimeric surfactants, LC₅₀ values towards *Daphnia magna* decreases from 50 to 7.5 mg/L [22] when the number of carbon atoms in the hydrophobic parts increases from 8 to 10. It can be also observed for the above-mentioned monomeric surfactants DTAB (C12) and CTAB (C16). The compound with 12 carbon atoms is classified as very toxic toward *Daphnia magna* [20,24] whereas the one with 16 carbon atoms as extremely toxic [21]. Nevertheless, when we look at marine or estuarine organisms, not many information is available regarding gemini surfactant toxicity. This represents a serious gap in marine hazard information available, as nanocapsules synthesized using gemini surfactants are often used in maritime infrastructures. One of the few examples is a study where gemini surfactants were shown to be 20-times less toxic when looking at marine microalgae than CTAB [25].

Taking into consideration the relation between surfactant structure, aggregation properties and ecotoxicity, environmentally-friendly silica nanocapsules may be designed. This approach, named safe-by-design (SbD), focuses on setting, at minimum, any sources of possible hazards for the environment and humans. SbD aims at ecologically oriented design since the beginning, meaning that all possible safety issues will be identified and replaced with non-toxic or less toxic alternatives [26,27].

Therefore, the aim of this study was to develop a safe-by-design approach to silica nanocapsules synthesis. In order to achieve it, CTAB was replaced by the gemini surfactant QSB2-12 as a template for the nanocapsules' synthesis. The nanocontainers based on both cationic surfactants

were fully characterized by scanning electron microscopy (SEM), Fourier-transformed infrared spectroscopy (FTIR), dynamic light scattering (DLS), thermogravimetric analysis (TG/DTA) and N₂ adsorption–desorption isotherms, as well as, in terms of their ecotoxicity towards chosen marine microalgae and crustaceans. The marine environment is the compartment where most of these ENMs will be applied and, therefore, looking at effects to marine biota is crucial and it will help to fill in the gap on the few available information regarding hazard assessment.

2. Materials and Methods

2.1. Materials

Ammonia solution (NH₄OH) (25%) was purchased from Merck (Portugal). Hexadecyltrimethylammonium bromide (CTAB) (≥98%), tetraethyl orthosilicate (TEOS) (≥99%), diethyl ether and sodium chloride (NaCl) were purchased from Sigma-Aldrich (Oeiras, Portugal).

Cationic gemini surfactant 1,4-bis-[N-(1-dodecyl)-N,N-dimethylammoniummethyl]benzene dibromide (QSB2-12) was synthesised according to the methods described in the literature [20].

The structures of cationic surfactants are presented in Figure 1.

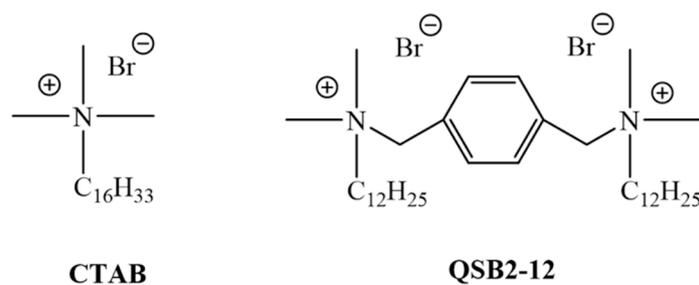


Figure 1. Structure of the cationic surfactants.

2.2. Methods

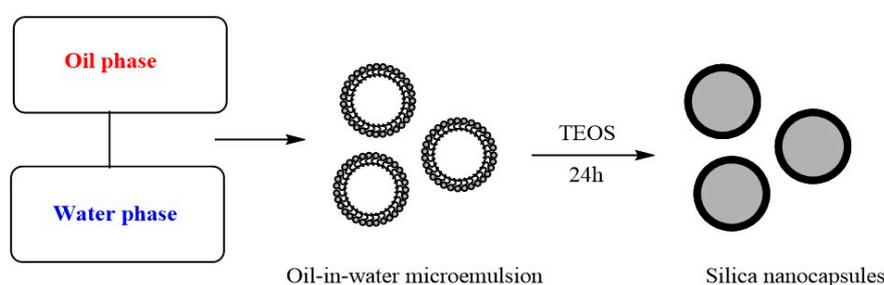
2.2.1. Nanocapsules Synthesis and Characterization

Silica nanocapsules (SiNC) were synthesized in a one-step process through an oil-in-water microemulsion (Figure 2) based on a published procedure, with minor alterations [9]. Diethyl ether was used as a co-solvent, ammonia as a catalyst and TEOS as a silica precursor. The water phase consisted of different amounts of cationic surfactant (Figure 2) dissolved in 35 mL water and 0.25 mL of ammonia solution. Diethyl ether (25 mL) was then added (dropwise) to the water phase and an oil-in-water microemulsion was obtained. Subsequently, 2 mL of TEOS was added dropwise to the microemulsion under controlled stirring and kept in a closed vessel for 24 h. The obtained products were filtered, washed with warm water (~40 °C) and dried at 60 °C.

Capsules morphology was characterized by scanning electron microscopy (SEM) coupled with energy dispersive spectroscopy (EDS) (Hitachi SU-70 electron microscope) and by scanning transmission electron microscopy (STEM) with secondary electron (SE) imaging capability (Hitachi STEM/SEM HD-2700).

Fourier-transformed infrared spectroscopy (FTIR) was used to identify characteristic peaks of the synthesized nanomaterials. FTIR analysis was performed on a Bruker tensor 27 spectrophotometer coupled with an ATR device.

A Malvern Zetasizer Nano-ZS instrument was used to perform dynamic light scattering (DLS) and zeta potential (ζ) measurements. The measurements were performed in deionized water and the concentration of silica nanocapsules was 10 mg/L.



Surfactant	CTAB	QSB2-12
Mass used	0.25 g (0.67 mmol)	0.20 g (0.29 mmol)

Figure 2. Scheme for the silica nanocapsules synthesis and a quantity of surfactants used.

Thermogravimetric analysis (TG/DTA) was conducted on a *Sataram-Labsys* system under air atmosphere, with a heating rate of $10\text{ }^{\circ}\text{C min}^{-1}$ from room temperature up to $800\text{ }^{\circ}\text{C}$. A portion of these nanocapsules was calcined at $550\text{ }^{\circ}\text{C}$ for 5 h, with a heating rate of $10\text{ }^{\circ}\text{C min}^{-1}$.

Textural properties of nanocapsules were evaluated based on the adsorption–desorption isotherms of N_2 at $196\text{ }^{\circ}\text{C}$, performed on the equipment Quantachrome NOVA 4200e. Samples were previously degassed at $180\text{ }^{\circ}\text{C}$ for 6 h. The specific area (S_{BET}) was calculated by the BET method (Braunauer, Emmett and Teller), the total pore volume ($V_{\text{Pp}/p_0=0.98}$) was obtained from the volume of N_2 adsorbed at $p/p^0 = 0.98$. The most frequent pore diameters (ϕ_{pores}) was calculated by the BJH method (Barrett–Joyner–Halenda), applied to the desorption branch of the isotherm [14].

2.2.2. Ecotoxicity Tests

The short-term toxic effects of the two silica nanocapsules were evaluated on marine microalgae species (*Nannochloropsis gaditana*, *Phaeodactylum tricornerutum*, *Tetraselmis chuii*) and on a microcrustacean species (*Artemia salina*), following the standard protocols OECD 201 (2011) [28] and ISO 10253 (2016) [29], respectively, with some adaptations fully described by Kaczerewska et al. [25] Briefly, tests were run with $0.45\text{ }\mu\text{m}$ filtered artificial seawater (ASW) and for each compound, five concentrations plus one negative control (ASW only) were tested ($n = 4$ for microalgae; $n = 3$ for crustaceans). Range-finding tests were run for exposure concentrations ranging from 0.01 mg/L to 100 mg/L . Definitive exposure tests included the following exposure concentrations: *Nannochloropsis gaditana* and *Tetraselmis chuii*: 2 mg/L , 4 mg/L , 6 mg/L , 8 mg/L , 10 mg/L ; *Phaeodactylum tricornerutum*: 2 mg/L , 4 mg/L , 6 mg/L , 8 mg/L , 10 mg/L for SiNC_QSB2-12 and 0.5 mg/L , 1 mg/L , 2 mg/L , 4 mg/L , 6 mg/L for SiNC_CTAB; *Artemia salina*: 6.25 mg/L , 12.5 mg/L , 25 mg/L , 50 mg/L , 100 mg/L . Microalgae growth inhibition was monitored for 72 h through fluorescence daily measurements whereas crustaceans mortality or immobilization was checked after 48 h of exposure. The median lethal (LC_{50}) and median growth inhibition (IC_{50}) concentrations were determined by a non-linear regression model with the software *Graphpad Prism v.6.0*. Then, toxicity endpoints were categorized according to the EC Directive 93/67/EEC scheme adapted by Blaise et al. [30] for nanomaterials: non-toxic ($L/\text{IC}_{50} > 100\text{ mg/L}$), harmful ($10 > L/\text{IC}_{50} \geq 100\text{ mg/L}$), toxic ($1 > L/\text{IC}_{50} \geq 10\text{ mg/L}$), very toxic ($0.1 > L/\text{IC}_{50} \geq 1\text{ mg/L}$) and extremely toxic ($L/\text{IC}_{50} \leq 0.1\text{ mg/L}$).

L/IC_{50} values estimated for both nanomaterials were statistically compared. A table containing the $\log L/\text{IC}_{50}$, associated standard error and degrees of freedom (data extracted from each non-linear regression report) for both SiNC (QSB2-12) and SiNC (CTAB) was prepared for each tested species. Then, the null hypothesis that both best fitting datasets were similar was tested ($p < 0.05$) through a *t*-test, followed by an F-test to compare the data variance (total of 4 independent tests).

3. Results and Discussion

3.1. Synthesis

As mentioned in the Materials section, silica nanocapsules have been obtained in a one-step process with minor adjustments. Both SiNC_CTAB and SiNC_QSB2-12 were obtained as white powders. CTAB concentration used for the synthesis was 19 mM, which is definitely above its CMC (0.907 mM). The number of moles used for QSB2-12 was reduced by half (11.6 mM) due to its dimeric structure. Zeta potential of CTAB and QSB2-12 microemulsions were +58.3 mV and +41.9 mV, respectively. Values of ζ for the surfactants differ slightly suggesting similar stability of the obtained microemulsions. Micelles made of cationic surfactants were a template for silica shell formation after adding TEOS [10].

3.2. Characterization of Silica Nanocapsules

SEM and STEM pictures of synthesised silica nanocapsules are presented in Figure 3. Obtained nanocontainers have a spherical shape with a diameter ranging between 100 nm and 200 nm. In STEM images (Figure 3c,d), a distinction (red arrow) between the wall and the core of the nanocapsules can be noticed, which confirms that obtained nanomaterials are capsules.

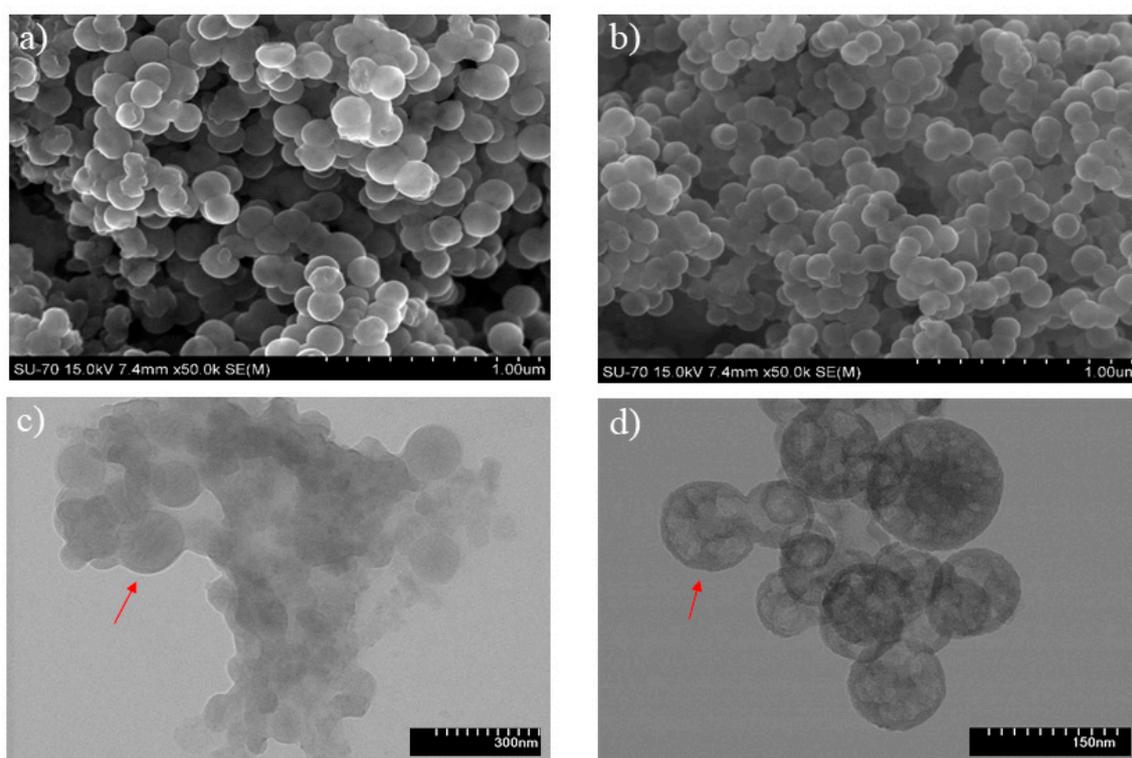


Figure 3. SEM (a,b) and scanning transmission electron microscopy (STEM) (c,d) images of silica nanocapsules based on *N*-hexadecyl-*N,N,N*-trimethylammonium bromide (CTAB) (a,c) and QSB2-12 (c,d).

In the FTIR spectra, presented in Figure 4a–c, some characteristic bands for silica capsules can be observed, such as Si–O–Si stretching at 1049 cm^{-1} , Si–OH stretching at 935 cm^{-1} and Si–O–Si bending at 800 cm^{-1} . By overlapping the spectra of SiNC_CTAB and SiNC_QSB2-12 (Figure 4c) it is possible to observe their similarity. FTIR spectra for CTAB and QSB2-12 show characteristic wavenumbers values of the bonds: C–H stretching absorption vibrations of long alkyl chains between 2840 cm^{-1} to 3000 cm^{-1} , C–H bending vibration of the $(\text{CH}_3)_4\text{N}^+$ cation at around 1490 cm^{-1} and rocking vibrations of $(\text{CH}_2)_n$, when $n \geq 4$, at around 720 cm^{-1} (present only in case of long alkyl chains) [25]. In the silica capsules' spectra, lower intensity of signals associated with CTAB and QSB2-12 are observed

suggesting that traces of cationic surfactants remained on the capsules' surface, as can be seen in the overlapped spectra of silica nanocapsules and surfactants (Figure 4a,b). Washing silica nanocapsules with an ethanolic solution of hydrochloric acid (1.5 mL of HCl in 150 mL of ethanol) at 60 °C allows the removal of cationic surfactants completely [16]. This route can be used for empty nanomaterials but applying it to nanocapsules loaded with active molecules may cause the unwanted release of the active molecules during washing steps. Another possibility to remove traces of cationic surfactants is calcination. However, this process removes all the organic compounds and cannot be applied to nanomaterials loaded with organic molecules [9,11].

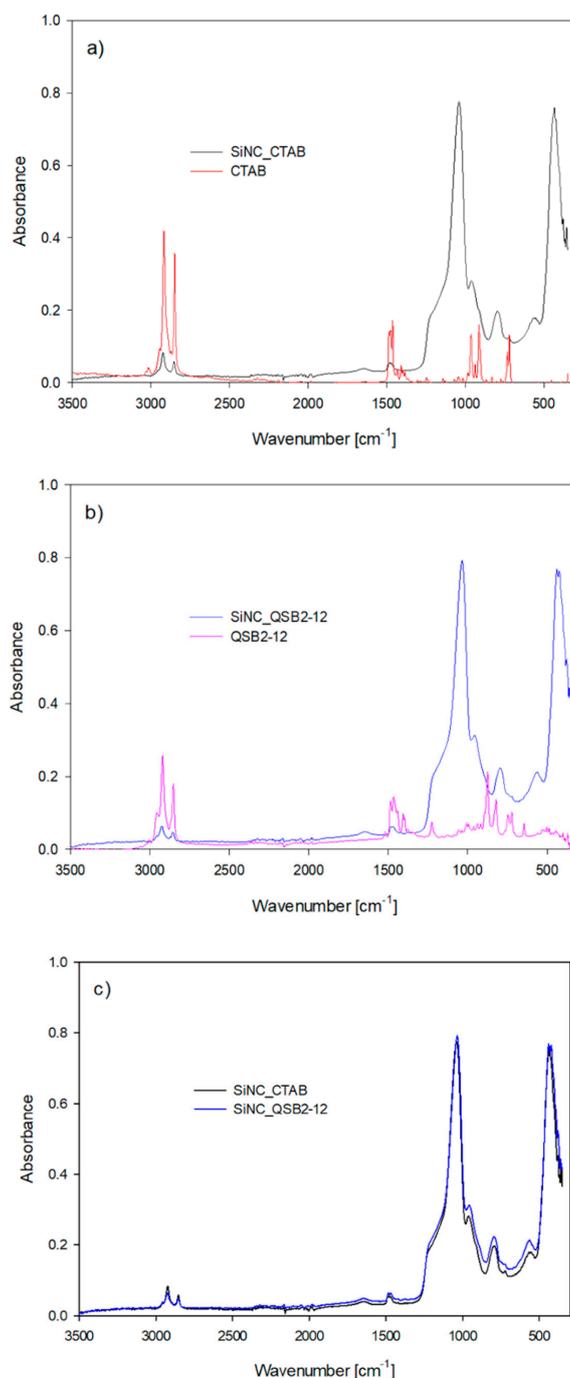


Figure 4. Overlapped Fourier-transformed infrared spectroscopy (FTIR) spectra of (a) SiNC_CTAB and CTAB, (b) SiNC_QSB2-12 and QSB2-12, and (c) SiNC_CTAB and SiNC_QSB2-12.

Regarding the size distribution of the synthesised capsules in distilled water (Table 1), determined by DLS, peaks centred at 157.1 nm and 191.6 nm were observed for SiNC_CTAB and SiNC_QSB2-12, respectively (Figure 5). These data agree with the size determined by SEM, although polydispersity index (Pdl) shows values higher than 0.5, indicating heterogeneity of the samples and the presence of larger aggregates. For both SiNC_CTAB and SiNC_QSB2-12 larger particles are observed probably due to the presence of polymer residues (TEOS polymerization) [14]. DLS data also confirm this assumption by the presence of secondary peaks, indicating the agglomeration of the nanocapsules and the polymer residues into larger particles [14]. The zeta potential associated with SiO₂ is typically −28 mV [31], so the recorded positive values of Z-potential for the synthesised silica nanocapsules in this work (cf. Table 1) are associated with the traces of cationic surfactants remaining in the capsules, which is consistent with FTIR spectra. Data for SiNC_CTAB are also in agreement with the literature where the hydrodynamic size for that conventional nanomaterial range from 80 nm to 180 nm [10,11,14].

Table 1. Dynamic Light Scattering (DLS) data for synthesized SiNC_CTAB and SiNC_QSB2-12.

	Hydrodynamic Size [nm]	ζ [mV]	PdI
SiNC_CTAB	157.1 ± 18.3 731.2 ± 134.3	32.8 ± 1.1	0.68
SiNC_QSB2-12	191.6 ± 37.1 662.1 ± 160.0	40.9 ± 6.8	0.56

ζ —Zeta potential; PdI—Polydispersity index.

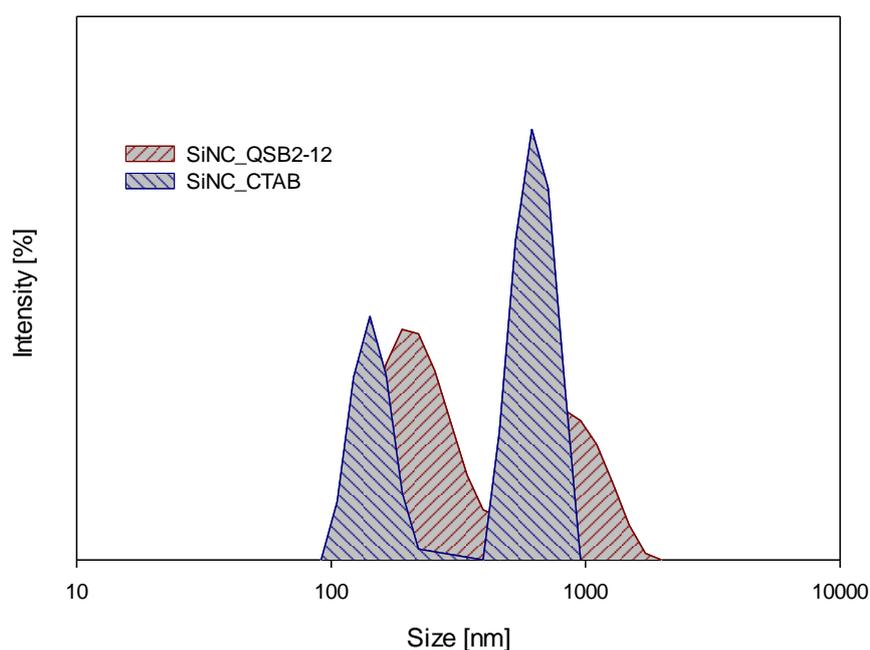


Figure 5. Size distribution of silica nanocontainers (SiNC) synthesized with CTAB and QSB2-12.

Thermogravimetric (TG) experiments were performed in order to verify and compare the thermal stability of synthesized nanocapsules. Tests with calcined samples (after thermal treatment) were carried out as a reference. TG profiles are presented in Figure 6. The degradation temperature for both SiNC_CTAB and SiNC_QSB2-12 was found to be 105 °C, which can be assigned to the process of dehydration of water adsorbed in the interlayer structure [32]. As expected for inorganic silica materials, curves for calcined nanomaterials show no variation in temperature, which suggests good thermal stability. Comparing curves for calcined and as-synthesized nanocapsules, a mass loss of approximately 80% can be observed for the as-synthesized ones. This may be due to the degradation

of non-hydrolysed TEOS as well as some residues of cationic surfactants used for the synthesis [14]. These results suggest that replacing CTAB with QSB2-12 will not change the thermogravimetric profile of silica nanocapsules.

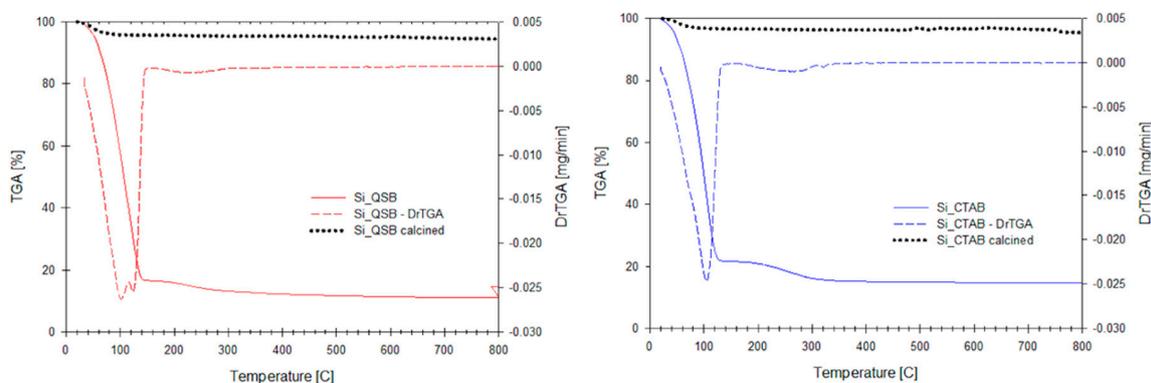


Figure 6. TG profile of SiNC_CTAB and SiNC_QSB2-12.

Textural properties of silica nanocontainers (SiNC_CTAB and SiNC_QSB2-12) were evaluated by adsorption–desorption isotherms N₂ at -196 °C. In Figure 7a it is possible to observe that both samples show a typical type IV adsorption–desorption, according to IUPAC classification, with symmetric adsorption–desorption pathway isotherm indicating the presence mesoporous materials. A characteristic type H3 hysteresis loop is observed. This type is typical for mesoporous materials with slit-shaped pores [33]. Table 2 presents the main textural parameters of the silica nanocontainers: specific surface area (SBET) and the most frequent diameter of pores (mode of distribution of pore diameter). Data obtained for SiNC_CTAB are in agreement with what is reported in the literature when using diethyl ether as co-solvent [9]. When CTAB is replaced with QSB2-12 a slight increase in pore size was observed. Additionally, changing surfactants during synthesis results in a decrease in surface area from 719 m²/g to 603 m²/g.

Table 2. Textural properties of SiNC_CTAB and SiNC_QSB2-12.

	S _{BET} [m ² /g]	Pore Size [nm]
SiNC_CTAB	719	4.4
SiNC_QSB2-12	603	5.9

3.3. Ecotoxicity

Ecotoxicity data (I/LC₅₀) are summarized in Table 3. Respective dose–response curves supporting these results are presented in a Supplementary Material (Figure S1). Both nanomaterials were toxic towards the tested microalgae and harmful towards the tested crustaceans [30]. However, SiNC_CTAB was more toxic than the novel SiNC_QSB2-12, for all tested species, statistically significant ($p < 0.05$) in the case of the diatom *P. tricornutum* (up to 3.5-times more toxic) and *T. chuii* (+25%) (Table 3). It has been suggested that the toxicity of silica nanocapsules may be associated with traces of cationic surfactants used during synthesis [10]. This is in accordance with FTIR data which confirm the presence of cationic surfactant residues in both nanomaterials. Previous studies by researchers from the University of Aveiro have shown that CTAB has 20-times higher toxicity towards *P. tricornutum* than gemini surfactant QSB2-12 [25]. These results prompted the decision to replace CTAB with QSB12, leading to the development of the work presented in this paper.

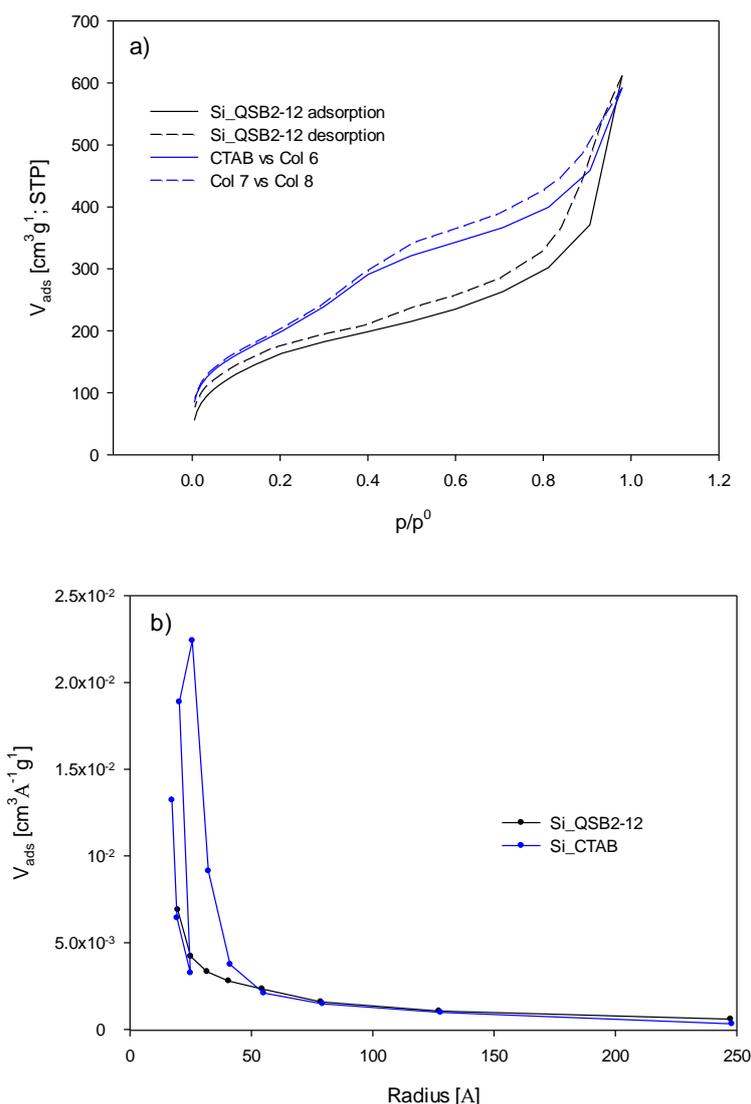


Figure 7. (a) Nitrogen adsorption–desorption isotherms and (b) pore size distribution of silica nanocapsules.

Table 3. Toxicity of synthesized reference (SiNC_CTAB) and newly developed (SiNC_QSB2-12) silica nanocapsules towards marine species and statistical comparison of I/LC₅₀ through *t*-tests and F-tests per tested species.

Marine Species	I/LC ₅₀ [mg/L] (95% CI)		<i>t</i> -Test	<i>p</i> -Value	F-Test to Compare Variances DFn, DFd	<i>p</i> -Value
	SiNC_CTAB	SiNC_QSB2-12				
<i>Nannochloropsis gaditana</i>	8.51 (6.76–10.7)	9.69 (9.48–9.91)	1.2230	0.2282	94.91 20, 22	<0.0001
<i>Tetraselmis chuii</i>	7.41 (7.37–7.45)	9.26 (8.97–9.55)	3.3437	0.0014	17.28 19, 22	<0.0001
<i>Phaeodactylum tricorutum</i>	2.37 (1.75–3.23)	8.30 (2.69–15.61)	2.1350	0.0386	14.84 22, 20	<0.0001
<i>Artemia salina</i>	23.0 (20.6–25.7)	25.4 (18.4–35.0)	0.6126	0.5451	8.42 14, 14	0.0003

Data are presented as median growth inhibition concentration (72 h-IC₅₀) and median lethal concentration values (48 h-LC₅₀), and respective 95% confidence intervals (CI). DFn—degrees of freedom numerator. DFd—degrees of freedom denominator.

There are some ecotoxicity data available in the literature for CTAB-based silica nanocapsules. For the diatom *P. tricornutum* IC₅₀ ranges from 2.03 mg/L [10] to 3.67 mg/L [13], for microalgae *N. gaditana* IC₅₀ is 1.15 mg/L [10], while for the crustacean *A. salina* SiNC_CTAB are harmful (LC₅₀ = 12.2 mg/L [34]) or non-toxic (LC₅₀ > 100 mg/L [10]). The results obtained in the present work agree with the literature data, particularly in the case of diatoms and crustaceans. There is a difference for the microalgae *N. gaditana*, which may be associated with different amounts of the cationic surfactant still adsorbed to the silica nanocapsules surface.

Sustainability is, nowadays, one of the main challenges in materials science. Designing nanomaterials by a proper selection of raw materials seems to be the way to obtain environmentally-friendly nanocontainers. Since it has been reported that the encapsulation of active molecules reduces their toxicity due to a controlled release mechanism [10], non-toxic nanocarriers are needed. To the best of our knowledge, there is no information published on the use of different cationic surfactants in order to prepare silica nanocapsules. However, comparing data of the toxicity profile of SiNC_CTAB with another nanomaterial namely, layered double hydroxides (LDHs) the silica nanocarriers have been found as exerting some degree of toxicity towards marine species [12]. Other eco-friendly alternatives have been reported in the literature, such as natural polymercarriers based on gelatine or chitosan microcapsules. These materials are non-toxic, biocompatible and biodegradable [35,36]. However, due to some limitations, such as size or swelling in aqueous solutions, their use is not always possible. In those cases, silica nanocapsules are often a solution, however current versions are still toxic.

This study demonstrates that developing greener silica mesoporous nanocapsules by replacing cationic surfactants used during SiNC synthesis, may lead to less toxic alternatives. In the present study, this was not fully achieved, however, the statistical differences between the toxicity on two ecologically relevant marine species demonstrate this approach is in the right direction to obtain less toxic materials. Considering the potential application of such nanomaterials as coating additives for maritime applications, improvements in their design and further ecotoxicological studies on other marine species may confirm the present findings.

4. Conclusions

Silica nanocapsules based on gemini surfactant QSB2-12 were successfully synthesised and characterized.

The synthesis is performed in a one-stage process where the toxic commercial cationic surfactant CTAB is replaced by a less toxic gemini surfactant QSB2-12. Changing surfactants allows the reduction, by 50%, of the number of moles used to obtain a stable microemulsion and does not hinder the formation of the nanomaterials. Moreover, the present results show that replacing the surfactant does not significantly change the structure and properties of silica nanocapsules.

Ecotoxicity tests show that nanocapsules based on CTAB are significantly more toxic than nanocontainers prepared with gemini surfactant for two (out of four) species and slightly more toxic for the other two. Both nanomaterials are classified as toxic (microalgae) and harmful (crustacean). The present study shows that by choosing a template surfactant carefully, environmentally safer silica nanocapsules can be designed. Nonetheless, further research is still needed.

To the best of our knowledge, the safe-by-design approach for silica nanocapsules synthesis is addressed in this work for the first time. Following this approach allows us to obtain not only environmentally friendly nanomaterials but also prevent the use of toxic surfactants and decrease the number of used chemicals, thus being aligned with the concept of green chemistry [37].

Supplementary Materials: The following are available online at <http://www.mdpi.com/2076-3417/10/22/8085/s1>, Figure S1. Dose–response curves of the microalgae *Nannochloropsis gaditana* and *Tetraselmis chunii*, the diatom *Phaeodactylum tricornutum* and the crustacean *Artemia salina* exposed to tested cationic surfactants.

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References

1. Mirmohseni, A.; Akbari, M.; Najjar, R.; Hosseini, M. Self-healing waterborne polyurethane coating by pH-dependent triggered-release mechanism. *J. Appl. Polym. Sci.* **2019**, *136*, 47082. [[CrossRef](#)]
2. Zhao, D.; Liu, D.; Hu, Z. A smart anticorrosion coating based on hollow silica nanocapsules with inorganic salt in shells. *J. Coat. Technol. Res.* **2017**, *14*, 85–94. [[CrossRef](#)]
3. Galvão, T.L.P.; Sousa, I.; Wilhelm, M.; Carneiro, J.; Opršal, J.; Kukačková, H.; Špaček, V.; Maia, F.; Gomes, J.R.B.; Tedim, J.; et al. Improving the functionality and performance of AA2024 corrosion sensing coatings with nanocontainers. *Chem. Eng. J.* **2018**, *341*, 526–538. [[CrossRef](#)]
4. Zheludkevich, M.L.; Tedim, J.; Ferreira, M.G.S. “Smart” coatings for active corrosion protection based on multi-functional micro and nanocontainers. *Electrochim. Acta* **2012**, *82*, 314–323. [[CrossRef](#)]
5. Wang, J.-X.; Wang, Z.-H.; Chen, J.-F.; Yun, J. Direct encapsulation of water-soluble drug into silica microcapsules for sustained release applications. *Mater. Res. Bull.* **2008**, *43*, 3374–3381. [[CrossRef](#)]
6. Samadzadeh, M.; Boura, S.H.; Peikari, M.; Kasirha, S.M.; Ashrafi, A. A review on self-healing coatings based on micro/nanocapsules. *Prog. Org. Coat.* **2010**, *68*, 159–164. [[CrossRef](#)]
7. Jain, A.K.; Thareja, S. In vitro and in vivo characterization of pharmaceutical nanocarriers used for drug delivery. *Artif. Cells Nanomed. Biotechnol.* **2019**, *47*, 524–539. [[CrossRef](#)] [[PubMed](#)]
8. Pathak, C.; Vaidya, F.U.; Pandey, S.M. Mechanism for Development of Nanobased Drug Delivery System. In *Applications of Targeted Nano Drugs and Delivery Systems*; Elsevier: Amsterdam, The Netherlands, 2019; pp. 35–67, ISBN 978-0-12-814029-1.
9. Chen, H.; He, J.; Tang, H.; Yan, C. Porous Silica Nanocapsules and Nanospheres: Dynamic Self-Assembly Synthesis and Application in Controlled Release. *Chem. Mater.* **2008**, *20*, 5894–5900. [[CrossRef](#)]
10. Figueiredo, J.; Oliveira, T.; Ferreira, V.; Sushkova, A.; Silva, S.; Carneiro, D.; Cardoso, D.; Goncalves, S.; Maia, F.; Rocha, C.; et al. Toxicity of innovative anti-fouling nano-based solutions in marine species. *Environ. Sci. Nano* **2019**, *6*, 1418–1429. [[CrossRef](#)]
11. Maia, F.; Silva, A.P.; Fernandes, S.; Cunha, A.; Almeida, A.; Tedim, J.; Zheludkevich, M.L.; Ferreira, M.G.S. Incorporation of biocides in nanocapsules for protective coatings used in maritime applications. *Chem. Eng. J.* **2015**, *270*, 150–157. [[CrossRef](#)]
12. Avelelas, F.; Martins, R.; Oliveira, T.; Maia, F.; Malheiro, E.; Soares, A.M.V.M.; Loureiro, S.; Tedim, J. Efficacy and Ecotoxicity of Novel Anti-Fouling Nanomaterials in Target and Non-Target Marine Species. *Mar. Biotechnol.* **2017**, *19*, 164–174. [[CrossRef](#)] [[PubMed](#)]
13. Gutner-Hoch, E.; Martins, R.; Oliveira, T.; Maia, F.; Soares, A.; Loureiro, S.; Piller, C.; Preiss, I.; Weis, M.; Larroze, S.; et al. Antimicrofouling Efficacy of Innovative Inorganic Nanomaterials Loaded with Booster Biocides. *J. Mar. Sci. Eng.* **2018**, *6*, 6. [[CrossRef](#)]
14. Maia, F.; Tedim, J.; Lisenkov, A.D.; Salak, A.N.; Zheludkevich, M.L.; Ferreira, M.G.S. Silica nanocontainers for active corrosion protection. *Nanoscale* **2012**, *4*, 1287–1298. [[CrossRef](#)] [[PubMed](#)]
15. Lang, N.; Tuel, A. A Fast and Efficient Ion-Exchange Procedure to Remove Surfactant Molecules from MCM-41 Materials. *Chem. Mater.* **2004**, *16*, 1961–1966. [[CrossRef](#)]

16. Hofmann, C.; Duerkop, A.; Baeumner, A.J. Nanocontainers for Analytical Applications. *Angew. Chem. Int. Ed.* **2019**, *58*, 12840–12860. [CrossRef]
17. Brycki, B.E.; Kowalczyk, I.H.; Szulc, A.; Kaczerewska, O.; Pakiet, M. Multifunctional Gemini Surfactants: Structure, Synthesis, Properties and Applications. In *Application and Characterization of Surfactants*; Najjar, R., Ed.; InTech: London, UK, 2017; ISBN 978-953-51-3325-4.
18. Garcia, M.T.; Kaczerewska, O.; Ribosa, I.; Brycki, B.; Materna, P.; Drgas, M. Hydrophilicity and flexibility of the spacer as critical parameters on the aggregation behavior of long alkyl chain cationic gemini surfactants in aqueous solution. *J. Mol. Liq.* **2017**, *230*, 453–460. [CrossRef]
19. Goronja, J.; Janosevic-Lezaic, A.; Dimitrijevic, B.; Malenovic, A.; Stanisavljev, D.; Pejic, N. Determination of critical micelle concentration of cetyltrimethylammonium bromide: Different procedures for analysis of experimental data. *Hem. Ind.* **2016**, *70*, 485–492. [CrossRef]
20. Garcia, M.T.; Kaczerewska, O.; Ribosa, I.; Brycki, B.; Materna, P.; Drgas, M. Biodegradability and aquatic toxicity of quaternary ammonium-based gemini surfactants: Effect of the spacer on their ecological properties. *Chemosphere* **2016**, *154*, 155–160. [CrossRef]
21. European Chemicals Agency. *Cetrimonium Bromide*—Registration Dossier (EC Number: 200-311-3). 2020. Available online: <https://echa.europa.eu/brief-profile/-/briefprofile/100.000.283> (accessed on 11 November 2020).
22. Garcia, M.T.; Ribosa, I.; Kowalczyk, I.; Pakiet, M.; Brycki, B. Biodegradability and aquatic toxicity of new cleavable betainate cationic oligomeric surfactants. *J. Hazard. Mater.* **2019**, *371*, 108–114. [CrossRef]
23. Zhang, C.; Cui, F.; Zeng, G.; Jiang, M.; Yang, Z.; Yu, Z.; Zhu, M.; Shen, L. Quaternary ammonium compounds (QACs): A review on occurrence, fate and toxicity in the environment. *Sci. Total Environ.* **2015**, *518–519*, 352–362. [CrossRef]
24. European Chemicals Agency. *Dodecyltrimethylammonium Chloride*—Registration Dossier (EC Number: 203-927-0). 2020. Available online: <https://echa.europa.eu/substance-information/-/substanceinfo/100.003.570> (accessed on 11 November 2020).
25. Kaczerewska, O.; Martins, R.; Figueiredo, J.; Loureiro, S.; Tedim, J. Environmental behaviour and ecotoxicity of cationic surfactants towards marine organisms. *J. Hazard. Mater.* **2020**, *392*, 122299. [CrossRef] [PubMed]
26. Van de Poel, I.; Robaey, Z. Safe-by-Design: From Safety to Responsibility. *Nanoethics* **2017**, *11*, 297–306. [CrossRef] [PubMed]
27. Nymark, P.; Bakker, M.; Dekkers, S.; Franken, R.; Fransman, W.; García-Bilbao, A.; Greco, D.; Gulumian, M.; Hadrup, N.; Halappanavar, S.; et al. Toward Rigorous Materials Production: New Approach Methodologies Have Extensive Potential to Improve Current Safety Assessment Practices. *Small* **2020**, *16*, 1904749. [CrossRef] [PubMed]
28. OECD Test No. 201: Freshwater alga and cyanobacteria, growth inhibition test. In *OECD Guidelines for the Testing of Chemicals*; Organization for Economic Co-Operation and Development: Paris, France, 2011; ISBN 978-92-64-06992-3.
29. ISO. *ISO 10253 Water Quality—Marine Algal Growth Inhibition Test with Skeletonema sp. and Phaeodactylum tricornutum*; International Organization for Standardization: Geneva, Switzerland, 2016.
30. Blaise, C.; Gagné, F.; Féraud, J.F.; Eullaffroy, P. Ecotoxicity of selected nano-materials to aquatic organisms. *Environ. Toxicol.* **2008**, *23*, 591–598. [CrossRef]
31. Sousa, I.; Maia, F.; Silva, A.; Cunha, A.; Almeida, A.; Evtuyugin, D.V.; Tedim, J.; Ferreira, M.G. A novel approach for immobilization of polyhexamethylene biguanide within silica capsules. *RSC Adv.* **2015**, *5*, 92656–92663. [CrossRef]
32. Kozak, M.; Domka, L. Adsorption of the quaternary ammonium salts on montmorillonite. *J. Phys. Chem. Solids* **2004**, *65*, 441–445. [CrossRef]
33. Alothman, Z. A Review: Fundamental Aspects of Silicate Mesoporous Materials. *Materials* **2012**, *5*, 2874–2902. [CrossRef]
34. Gutner-Hoch, E.; Martins, R.; Maia, F.; Oliveira, T.; Shpigel, M.; Weis, M.; Tedim, J.; Benayahu, Y. Toxicity of engineered micro- and nanomaterials with antifouling properties to the brine shrimp *Artemia salina* and embryonic stages of the sea urchin *Paracentrotus lividus*. *Environ. Pollut.* **2019**, *251*, 530–537. [CrossRef]
35. Hiwale, P.; Lampis, S.; Conti, G.; Caddeo, C.; Murgia, S.; Fadda, A.M.; Monduzzi, M. In Vitro Release of Lysozyme from Gelatin Microspheres: Effect of Cross-linking Agents and Thermoreversible Gel as Suspending Medium. *Biomacromolecules* **2011**, *12*, 3186–3193. [CrossRef]

36. Sousa, I.; Quevedo, M.C.; Sushkova, A.; Ferreira, M.G.S.; Tedim, J. Chitosan Microspheres as Carriers for pH-Indicating Species in Corrosion Sensing. *Macromol. Mater. Eng.* **2019**, 1900662. [[CrossRef](#)]
37. Saleh, H.E.-D.M.; Koller, M. Introductory Chapter: Principles of Green Chemistry. In *Green Chemistry*; Saleh, H.E.-D.M., Koller, M., Eds.; IntechOpen: London, UK, 2018.

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