colloids and interfaces

MDPI

Article

Synthesis of Submicrocontainers with "Green" Biocide and Study of Their Antimicrobial Activity

Saule B. Aidarova [1,2], Altynay A. Sharipova [1,*], Assem B. Issayeva [1], Botagoz Zh. Mutaliyeva [3], Aiym B. Tleuova [4,5], Dmitry O. Grigoriev [6], Dariga Kudasova [3], Madina Dzhakasheva [3] and Reinhard Miller [7]

1 Industrial Engineering Department, Kazakh National Research Technical University after K. Satpayev, Almaty 050013, Kazakhstan
2 Department of Science and Innovation, Kazakh-British Technical University, Almaty 050000, Kazakhstan
3 Biotechnology Department, M. Auezov South Kazakhstan State University, Shymkent 160012, Kazakhstan
4 Chemical Technologies of Inorganic Chemistry Department, M. Auezov South Kazakhstan State University, Shymkent 160012, Kazakhstan
5 Department of Chemical Engineering, University of Chemistry and Technology Prague, 166 28, 6 Prague, Czech Republic
6 Fraunhofer Institute for Applied Polymer Research, D-14476 Potsdam, Germany
7 Max Planck Institute of Colloids and Interfaces, D-14476 Potsdam-Golm, Germany
* Correspondence: a_sharipova85@mail.ru; Tel.: +7-7017476719

Received: 27 September 2018; Accepted: 28 November 2018; Published: 3 December 2018

Abstract: The synthesis and properties of submicrocontainers with a shell of nanoparticles of silicon dioxide and a core of polymerized 3-(Trimethoxysilyl) propyl methacrylate loaded with 5-Dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT) are considered. The resulting containers were characterized by scanning electron microscopy SEM, laser correlation spectroscopy and thermogravimetric analysis. The obtained submicrocontainers show low polydispersity with a small increase in size in comparison with the initial droplet size of the Pickering emulsion. The Zeta potential of the final containers was sufficiently negative at pH7 to be stable. The maximum release of encapsulated biocide was observed over approximately 24–27 h with a lease of about 78% of the encapsulated biocide during 3.5 h. The effectiveness of the encapsulated biocide by the Pickering emulsion technique was studied by tests on the growth rate of a microfungi colony (*Aspergillus niger, Aspergillus awamori*) and the growth rate of the bacteria *Bacillus cereus*. The test shows that the submicrocontainers of DCOIT facilitate a growth inhibition of 70% against 52% for the free biocide after 5 days; this is due to the fact that free biocide loses its activity promptly, while the encapsulated biocide is released gradually, and thus retains its effectivity for a longer time.

Keywords: Pickering emulsion; submicrocontainers; microencapsulation; antifouling; biocide; release; antimicrobial activity; growth inhibition

1. Introduction

Great attention is paid to the formation of stable emulsions, as such systems have a wide range of applications, for example, for the microencapsulation of various active agents. Microcapsules can be prepared by a single step emulsion method for hydrophobic agents, and by a double emulsion method for hydrophilic agents. Thus, many works were devoted to the development of systems for producing micro-and nanoparticles and for this purpose different techniques were used, such as microemulsion extrusion [1,2], precipitation [3], complexation of hydrogels [4], spray-drying [5].

Microencapsulation solves problems such as storage of antimicrobial and other important properties, protection of unsustainable substances from the effect of an external environment, surface functionalization allowing targeted delivery, and keeping a prolonged and controlled release during the application [1,3].

Recently, Pickering emulsions have became attractive because they are simple and bear strong similarities with the well-known surfactant-based emulsions [6]. Their 'surfactant-free' characteristic makes them attractive to several application fields, in particular cosmetics and pharmaceutical applications where surfactants often show adverse effects (irritancy, hemolytic behavior, etc.). The enhanced stability of Pickering emulsions in comparison with classical emulsions stabilized by emulsifiers is a supplementary advantage. The droplet coating by solid particles makes a rigid barrier acting against coalescence; therefore, concentrated emulsions are efficiently stabilized. Solid particles attached to the oil–water interface are partly immersed in water and partly in the oil medium, i.e., both oil and water wet their surface sufficiently. Both o/w and w/o emulsions can be prepared depending on the wetting conditions and/or the type of the emulsification process [7–17].

The colloid-chemical approach based on Pickering emulsions can be used for the encapsulation of inhibitors [18]. Polyurea micro- and nanocontainers loaded with the corrosion inhibitors 8-HQ and MeBT were prepared via an emulsion route (starting from oil-in water "O/W" emulsions) by interfacial polyaddition. Such corrosion protection additives based on inhibitor loaded polymeric micro- and nanocontainers can be considered as a valuable "green" alternative to conventional anticorrosive pigments [18]. This approach can serve for the encapsulation of other active substances as well as substances with antimicrobial activity.

Nowadays, there is global concern regarding the widespread use of substances with antimicrobial activity, which have significant drawbacks including increased costs, handling hazards, the risk of respective residues in food, and threats to human health and the environment [19]. Public awareness of these risks has increased interest in finding safer substances or alternative protectants to replace synthetic chemicals. Therefore, interest in products that have an antimicrobial activity and low toxicity, less environmental affects and wide public acceptance has increased [20,21].

One of the substances used in the present studies, 4,5-Dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT) is an antifoulant and antimicrobial preservative. For example, it is widely used in marine applications as an alternative biocide to tributyltin (TBT), which is now prohibited because of its toxic effects on the marine environment. Compared with TBT, DCOIT shows better environmental behavior [22] and more efficient microbial corrosion inhibition [23].

DCOIT is a chlorinated isothiazolone used as a broad-spectrum booster biocide in antifouling paints, where it affects both soft- and hard-fouling [24]. DCOIT is easily biodegraded with a reported half-life time in natural seawater between 24 h and 3 days [24].

The use of DCOIT in external [25,26] and internal [27] antimicrobial coatings, as well as in coatings against biofouling [28] is constantly growing. On the other hand, the addition of this biocide into environmentally less harmful varnishes and water-based paints and the uniform distribution in them is complicated by its substantial hydrophobicity. In addition, even in hydrophobic antimicrobial coatings, its concentration in the outer layers is rapidly reduced due to the high biodegradability of DCOIT. This leads to a fairly rapid absorption of the biocide at the outer layer of the coating and to the partial or complete loss of its antimicrobial properties.

A general solution to the problem of premature depletion of antimicrobial coatings by a biocide was proposed on the basis of micro- and nano-encapsulation of the active ingredients before their addition into the paint mixture and then into the coating matrix [29]. This approach is applicable for DCOIT as a biocide, and for other "green" biocides and can lead to the elimination of some of the shortcomings in their application, which are described above. Thus, DCOIT microencapsulation for subsequent addition into antimicrobial hydrophobic coatings used for residential building facades [30] led to a significant reduction in the use of these materials.

DCOIT is approved in the European Union as an active substance in biocidal products of type 21—antifouling agents (No 437/2014 of 29 April 2014) [31]. It diffuses easily through cell membranes and cell walls [32], and causes oxidative stress in the cell followed by necrosis [33].

Abiotic degradation of DCOIT through hydrolysis and photolysis is considerably slower and the main route of dissipation in the environment is therefore of biological nature [34]. Although considered as readily biodegradable, DCOIT peak concentrations of 9 to 13 nmol/L were reported from marinas on the Spanish coast [35].

Despite the antimicrobial activity of some substances in free form, their commercial application could be limited, for example due to loss by evaporation of the active components or degradation at high temperatures, oxidation and UV light [36]. Therefore, the formulation of substances with antimicrobial activity such as DCOIT involves their preparation in liquid forms (emulsions, micelles, liquid solutions etc.), semi-liquid forms (gels, liposomes, etc.) or solid forms (microcapsules or microspheres), and they have to be employed for controlling the release of active ingredients and protecting them from the external environment. Thus, they allow us to reduce the dosing frequency and provide improved efficiency [37,38].

In the present work, the synthesis of submicrocontainers with DCOIT was carried out for their subsequent use as an additive in antimicrobial internal coatings on a water basis, as well as in external coatings against biofouling. The obtained submicrocontainers were characterized by their physico-chemical parameters, the rate of biocide release and also by a test of antimicrobial activity to use it in various coating matrices. The submicrocontainers' influence on the growth of microorganisms was studied, and their practical value substantiated.

2. Materials and Methods

2.1. Materials

An aqueous suspension of hydrophilic non-aggregated amorphous silica particles with a primary diameter of 20-25 nm having a pH of 9.1 (Ludox AS-40, Sigma-Aldrich Co., 40% by weight in aqueous dispersion) was used as a stabilizing agent for the preparation of the O/W emulsion. As the basis of the oil phase, 3- (Trimethoxysilyl) propyl methacrylate (TPM, Alfa Aesar, 97%) was used, into which the oil-soluble biocide 4,5-Dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT) was introduced. Milli-Q water with a specific resistance of 18 MΩ cm at 25 °C was used in the synthesis.

Four microbial strains were used for the assessment of antimicrobial activity of microspheres with and without loaded DCOIT: *Aspergillus niger, Aspergillus awamori,* as representatives of fungi, and *Bacillus cereus* as a representative of bacteria. The cultures of filamentous fungi, *Aspergillus niger* and *Aspergillus awamori*, isolated from the soil of southern Kazakhstan, were obtained as a result of screening, multistage selection and mutagenesis [39]. A new mutant strain of *A. awamori* was deposited in the Republican State Enterprise "Republican Collection of Microorganisms" of the Ministry of Education and Science of the Republic of Kazakhstan; it was assigned by the registration number *A. awamori F-RKM 0719*. The microorganism *Bacillus cereus* was provided by the Republican Veterinary Laboratory of Shymkent.

2.2. Methods

Pickering emulsions were prepared in the following way: the initial concentrated suspension of silica (2.0 g) was diluted with deionized water about 15 times, the oil phase was added at a ratio of 1:19 to the aqueous phase, and then the aqueous phase was further increased by 25–30%. This mixture spontaneously emulsified at room temperature over 24 h. It was previously found that the mass ratio of the oil phase to silicon dioxide, at which a spontaneous emulsification takes place leading to a practically monodisperse size distribution, should be not lower than 1.3 [40,41]. Based on these data, the mass ratio of the amount of oil phase to silica in the suspension was taken as 2.23. The composition of the oil phase used for the preparation of submicrocontainers via emulsion polymerization was

as follows: 1.6 g of TPM, 0.16 g of 4,5-Dichloro-2-n-octyl-4-isothiazolin-3-one, 2 g of Ludox AS-40, i.e., the concentration of biocide in containers was close to 10 wt%."

In the second stage, the polymerization process was carried out under an ultraviolet light using the photoinitiator Irgacure 651 (2,2-Dimethoxy-2-phenylacetophonone). The polymerization reaction proceeded with stirring at 400 rpm for 20 min. The submicrocontainers thus obtained, were separated from the reaction mixture by centrifugation at 18000 rpm and washed with Milli-Q water 2 times with intermediate centrifugation under the same conditions. The finished submicrocontainers were dried for 12 h at 35 °C.

The morphology of the submicrocontainers was studied using scanning electron microscopy (SEM, Control LEO 1550). Samples for SEM were prepared by drying droplets of the diluted emulsions on special substrates.

To study the size distribution and zeta potential of submicro-particles, the laser correlation spectroscopy method (Zetasizer Nano ZS ZEN3500, Malvern Instruments) was used at 25 °C, and the zeta potential averaged particle diameters and the polydispersity index were calculated. Before measuring, the device was tested by the Malvern Zeta Potential Transfer Standards with potential values of −42 mV or −68 mV, respectively.

The thermogravimetric analysis (TGA) was used to quantify the effectiveness of encapsulation. The measurements were carried out using a Netzsch TG 209 F1 (Germany) instrument at a heating rate of 10 K·min^{-1} under a nitrogen atmosphere.

The spectrophotometric method was used to study the rate of release of DCOIT from the submicrocontainers with a shell of silicon dioxide nanoparticles and a core of polymerized TPM loaded with DCOIT.

The biological tests were performed using Petri dishes with a Czapeks-Dox agar medium for studying the antifungal activity against *Aspergillus niger*, and a modified Czapek medium, to which the filtered liquid of the broth of wheat was added to study the antifungal activity against *Aspergillus awamori*, and a Meat-Peptone Agar medium to study the antibacterial activity against *Bacillus cereus*. All nutrient media were preliminarily mixed with empty submicrocontainers and loaded with biocide, respectively, and the active agent in free form, and then were inoculated with 100 µL suspension of the tested fungi or with suspension of the tested microorganism *Bacillus cereus* [42]. Filter paper discs impregnated with biocide in free and encapsulated form were used for the assessment of their antimicrobial activity against fungi *Aspergillus niger* [42]. Petri dishes were incubated at 25 °C ± 3 °C for 5 days. The storage period for observation of an inhibition zone was from 5 until 15 days. The growth inhibition was determined by measuring the diameter of microbial growth zones. The growth inhibition was calculated by Equation (1):

$$\text{Growth inhibition (\%)} = [(D_c - D_t)/D_c] \times 100 \quad (1)$$

where D_c is the diameter of the colony of microorganisms in the control series, and D_t is the diameter of the colony of microorganisms in the test series.

3. Results and Discussion

3.1. Formation of Submicrocontainers

The main process (Figure 1) leading to the spontaneous formation of silica stabilized O/W Pickering emulsions is the TPM hydrolysis and polycondensation [41,43]:

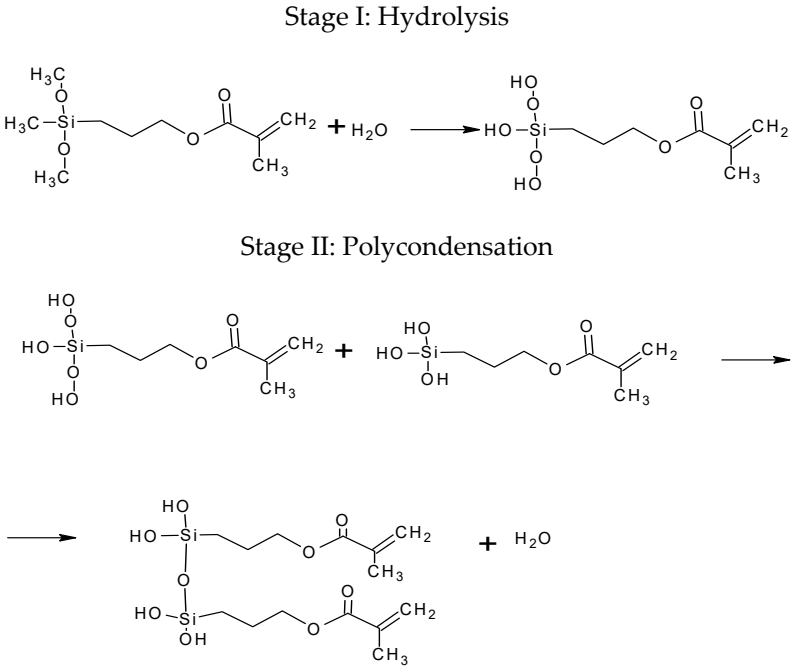

Figure 1. Reactions leading to spontaneous formation of O/W Pickering emulsions in the water/TPM + DCOIT system.

The product of the hydrolysis reaction of TPM silanols interact with other silanol groups (Figure 1, stage II) as well as with silanol groups on the surface of the silicon dioxide particles and the latter become partially hydrophobic as a result of the polycondensation reaction on their surface. Such particles contribute to the formation and subsequent stabilization of the O/W Pickering emulsion, formed in the system spontaneously.

Polycondensation of TPM leads to the formation of a network that allows to stabilize emulsion droplets, however, experiments showed that emulsions formed with silica particles modified by polycondensed aggregates of TPM do not have a high stability and tend to coagulate. On the other hand, polymerization of TPM allows forming solid particles. In addition, the reaction can be easily controlled by mean of temperature, initiator concentration, and duration of the process. Polymerization leads to the formation of such containers, which can be re-dispersed in water even after drying.

At the next stage, when the polymerization process takes place, the polycondensed TPM molecules on the silicon dioxide surface polymerize through double bonds in the methacrylic group. That leads to a strengthening of the core/shell structure required to remove the water phase from the emulsion and obtain the submicrocontainers in powder form.

3.2. Properties of Submicrocontainers

The obtained containers were characterized by dynamic light scattering and a low polydispersity was observed (Figure 2A). It is shown that the particle size after polymerization only slightly increased (about 1.3 times) as compared to the initial droplet size (before and after filtration). The polymerization process leads to a swelling of the particles. The final particle size depends on the type and concentration of the monomer, the medium, the amount and type of both the stabilizer and the initiator, and the temperature of polymerization.

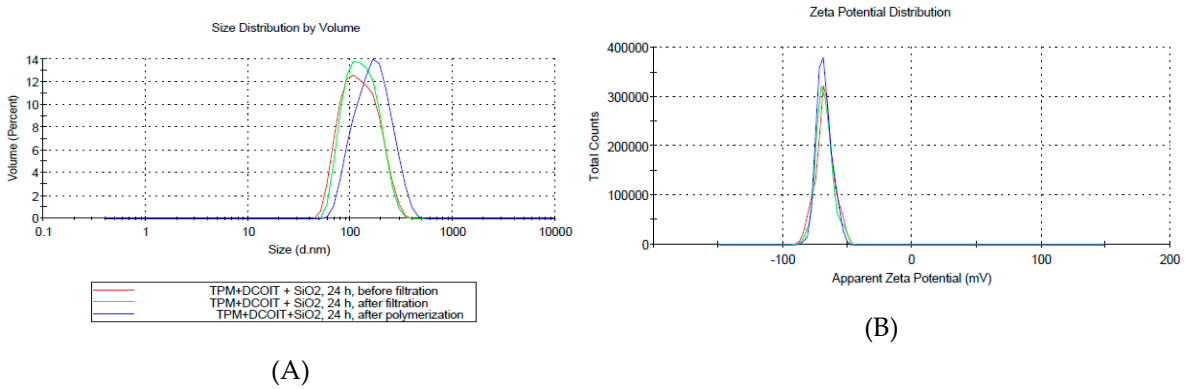

Figure 2. Characteristics of O/W submicrodrops of Pickering emulsion submicrocontainers with a shell of silicon dioxide nanoparticles and a core of substituted polymerized TPM with incorporated DCOIT before and after polymerization, respectively: (**A**) Size distribution curves; (**B**) Zeta potential at pH 7.

Due to the fact that the submicrocontainer shells are formed by only partially hydrophobized silica nanoparticles, the Zeta potential of the final containers remained sufficiently negative at pH 7 (Figure 2B).

Data on the size distribution of the submicrocontainers, obtained by dynamic light scattering, are consistent with SEM micrographs (Figure 3).

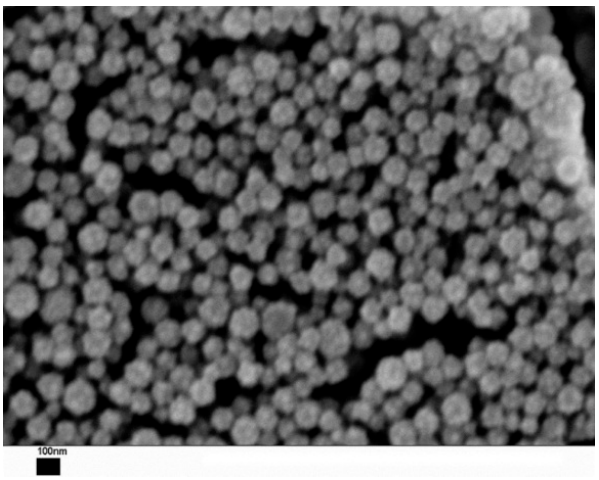

Figure 3. SEM images of submicrocontainers with a shell of nanoparticles of silicon dioxide and a core of polymerized TPM loaded with DCOIT.

The biocide content in the obtained submicrocontainers loaded with 10% by weight of DCOIT was determined by the TGA method.

The rate of release of the biocide DCOIT from submicrocontainers was studied spectrophotometrically in a model water-ethanol medium (1:1) to ensure sufficiently high concentrations of the biocide to also be detected in the first stage of the release process. For pure aqueous media, the release rate of DCOIT is extremely slow due to its very low solubility in water. Therefore, it can be disturbed by the processes of its photo- and biodegradation, which take place simultaneously with the release. Thus, an aqueous medium containing 50 wt% ethanol was chosen as a model release medium.

The maximum possible release of encapsulated biocide was observed after approximately 24–27 h (Figure 4).

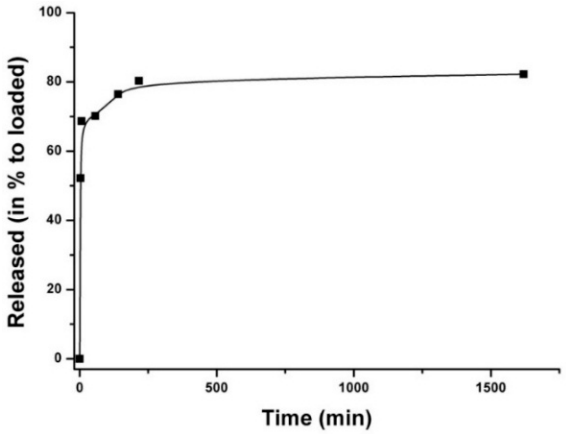

Figure 4. Rate of release of biocide in a model water-ethanol (1:1 wt) release medium from submicrocontainers with a shell of nanoparticles of silicon dioxide and a core of polymerized TPM loaded with DCOIT (approx. 10% by weight).

According to Figure 4, the beginning of the plateau in the kinetics release of DCOIT was achieved after the release of about 78% of the encapsulated biocide after more than 3.5 h. At the same time, the biocide concentration necessary for its effective action against the majority of microorganisms was achieved in the water-ethanol medium.

Obviously, for submicrocontainers embedded in a hardened matrix of antimicrobial coating with a thickness of several tens or hundreds of micrometers, or for an aqueous environment where the solubility of DCOIT is much lower than in the used water-ethanol model medium, the DCOIT concentration may differ markedly from that required for an effective antimicrobial action.

Therefore, studies on the antimicrobial activity of coatings containing submicrocontainers with DCOIT were carried out. The antimicrobial activities of the biocide in microencapsulated and non-micron - encapsulated form were evaluated against pathogenic fungi species: *Aspergillus niger*, *Aspergillus awamori*, and bacteria *Bacillus cereus*.

A poisoned food method and impregnated disk method were used to evaluate the antimicrobial activity of submicrocontainers loaded with biocide. A full inhibition zone was measured for all systems containing submicrocontainers with biocide. In the case of material carriers without biocide, no antimicrobial activity was observed. The optimized microparticles showed a sustained in vitro release profile (50% of the antifungal activity was maintained after 5 days of the study).

The differences in inhibiting the growth of microfungi colonies are shown in Figure 5A, and for bacteria in Figure 5B.

As can be seen, the addition of an encapsulated biocide and free biocide into the system markedly decreases the growth rate of the microfungi colony, but after 10 days as a comparison the same amount of biocide added into the system in a free form is less effective.

The use of an encapsulated biocide to suppress the growth of colonies of *Bacillus cereus* bacteria also showsequally positive results, but after 10 days a free biocide has shown less efficiency (Figure 5B).

From Figure 5B, it can be seen that in the control dish on meat-peptone agar with empty submicrocontainers (without biocide) the *B. cereus* forms a continuous white scurf, sometimes with a mealy surface. In dishes with free and encapsulated biocide no growth of microorganisms is observed, the surface of dishes remains transparent, but after 10 days the growth of microorganisms can be observed in the Petri dishes with free biocide.

Therefore, an unambiguous positive effect of encapsulation of biocidal DCOIT in submicrocontainers, confirmed by statistically reliable tests of biological activity was established.

From Figure 5A,B, it can be seen that encapsulated biocide is characterized by a prolonged time of antimicrobial activity in comparison with free biocide. The same results were obtained in tests with other fungi (*Aspergillus niger*).

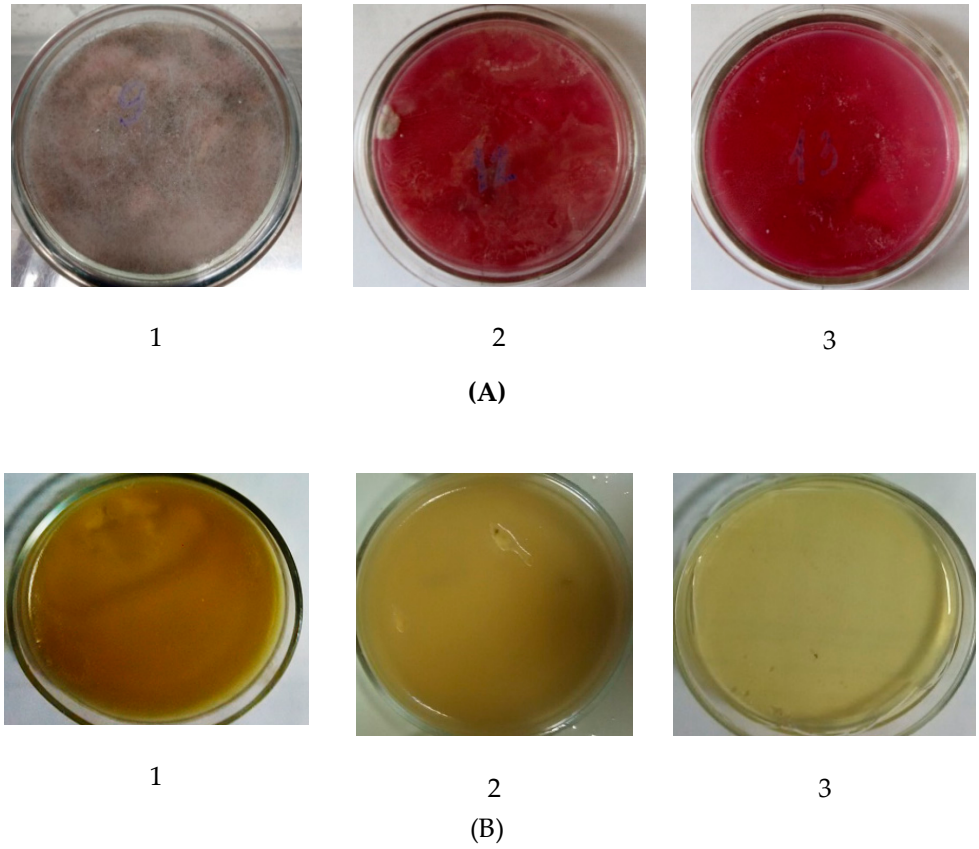

Figure 5. Comparison of the effectiveness of suppression of growth of microfungi after 10 days with empty submicrocontainers (1) (similar to control with free growth of microorganisms); biocide in free form (2);encapsulated biocide (3): (**A**) *Aspergillus awamori;* (**B**) *Bacillus cereus.*

The characterization of the inhibition of the microorganisms' growth was determined by measuring the diameter of inhibition zones, the results obtained in this study are expressed in terms of a growth inhibition value and presented in Figure 6. From the obtained results it can be concluded that the use of biocide in free and encapsulated form affect their antimicrobial activity, also, the encapsulation in submicrocontainers may effectively reduce the decreasing rate of the amount of the DCOIT, thus increase the potential antifungal activity.

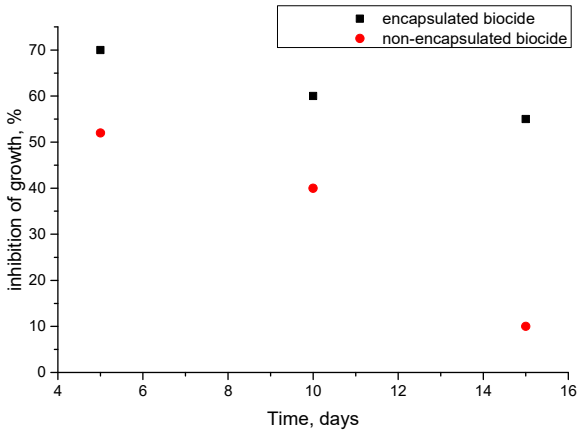

Figure 6. Antifungal activity profile of free and encapsulated DCOIT against *Aspergillus niger.*

To more visually show the difference between the antimicrobial activity of encapsulated and free biocide, the method of impregnated disks was used (against fungi *Aspergillus niger)*. The interpretation of the activity profile of free and encapsulated tested biocide for the first 5 days of the test, as well as for a longer period of up to two and four weeks, as shown in Figure 6, clearly confirms the significant increase in the effectiveness of the biocide achieved by its encapsulation.

According to the obtained results, the antifungal activity of free DCOIT was decreased by about 48% after a storage period of 5 days. Note that after a storage period of 15 days, as shown in Figure 6, the antifungal activity of this biocide continues to weaken while the encapsulated biocide still maintains its antifungal activity for a longer time. The same effect was also observed for the bacteria *Bacillus subtilis*.

By using the poisoned food method, it was established that the DCOIT submicrocontainers had a strong inhibition effect against microorganisms (A. awamori, *A. niger, B. cereus)* causing a complete inhibition at a dose of 10 mg/dish. However, the most obvious difference in the degree of inhibition is shown by tests with impregnated disks, where the disks were impregnated by solutions containing beads of DCOIT, and free DCOIT: a growth inhibition of 70% and 52%, respectively. From Figure 6it can be seen that the biocide in submicrocontainers (1.6 g TPM, 0.16 g DCOIT, 2 g Ludox) showed the best results in the inhibition of microorganism growth of up to 69–70% in comparison with the free form of biocide where the inhibition makes up to 52–55% after 5 days of inoculation. The optimal concentration ratio for substituents of submicrocontainers would be 1.6 g TPM, 0.16 g DCOIT, 2 g Ludox. Any further increase in the capsule materials can worse the antimicrobial activity of the submicrocontainers, which is apparently associated with a deterioration in the release of the biocide from the capsule. From the plate with empty submicrocontainers, a free growth of microorganisms without an inhibition zone is seen, similar to the growth of microorganisms in the control dish.

The diameter of the inhibition zone of microorganism growth in the test by using the impregnated disk method after 5 days of inoculation is shown in the Table 1.

Table 1. Diameter of suppressed microorganism growth (impregnated disk method).

No.	Sample	Diameter of inhibition zone of microorganism growth, cm: *Aspergillus niger (diameter of the full inhibition zone 5 cm)*
1	Control nutrient medium without biocide	+
2	Biocide in free form	2.0–2,6
3	Submicrocontainer with biocide (1.6 g TPM, 0.16 g DCOIT, 2 g Ludox)	3–3.5
4	Empty capsule without biocide (TPM and Ludox)	+

Biocide in free form is characterized by a smaller inhibition zone in comparison with the encapsulated form, which can be explained by the fact that free biocide soon loses its activity, while the encapsulated biocide is released gradually, and thus retains its effect for a longer time.

The results shown in Figure 7 confirm that the submicrocontainers with biocide keep the zone of inhibition after 15 days of seeding (60%), and the free biocide gradually loses its activity, whereas the empty submicrocontainers could not inhibit the growth of microorganisms. The effect remains for a long time. After 15 days, the submicrocontainers with biocide, produced by optimal composition of substituents, constantly keep their activity and the zone of microorganism growth inhibition is decreased slower because of the gradual release of the active ingredient from the submicrocontainers. It was established that this is the optimal correlation ratio for producing the most effective submicrocontainers.

Thus, it can be concluded that encapsulated biocide as an additive in media and coatings has a high implementation potential for industrial applications.

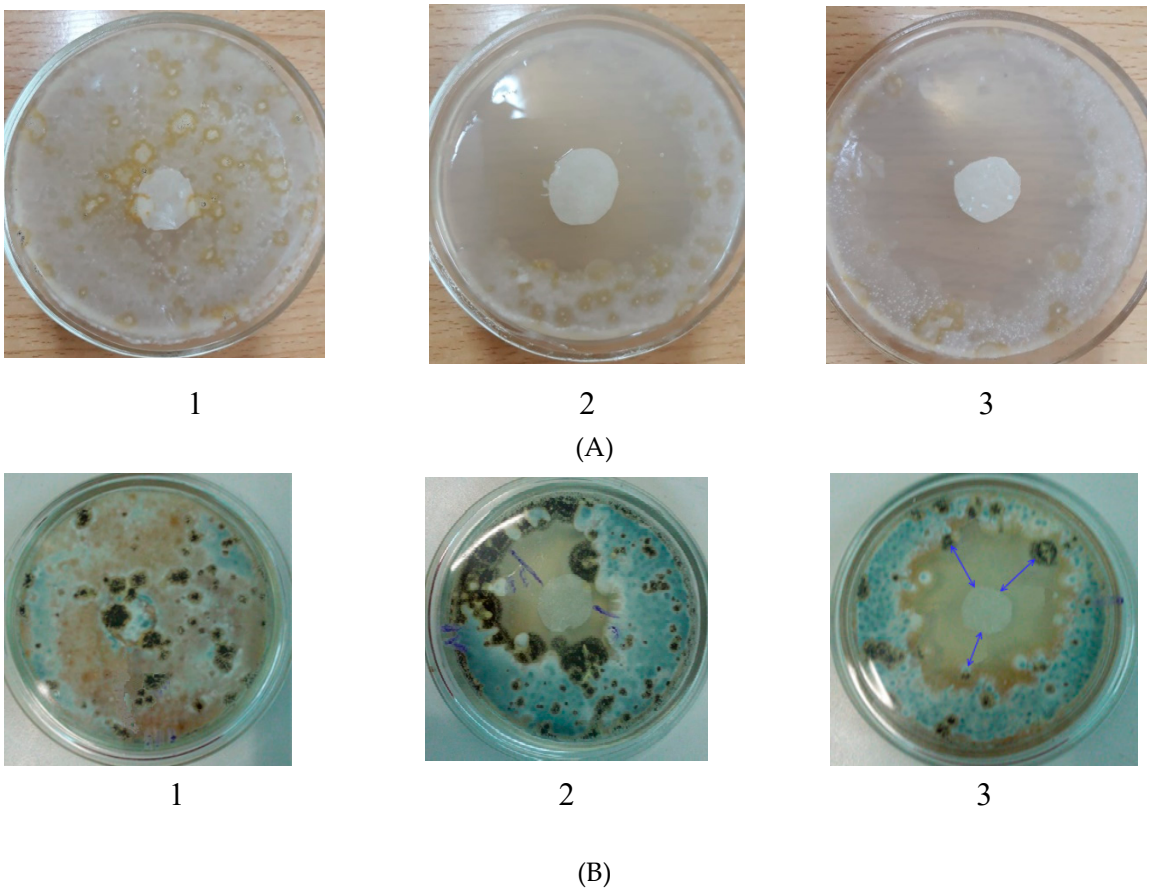

Figure 7. Comparison of the effectiveness of inhibiting the growth of *Aspergillus niger* after: (**A**) 5 days by empty submicrocontainers (1) (similar to control with free growth of microorganisms), biocide in free form (2), encapsulated biocide (3); (**B**) the same as A but after 15 days.

4. Conclusions

The possibility of encapsulating biologically active substances, such as the biocide DCOIT, which has a broad antifungal and antimicrobial effect, was experimentally investigated using nanoparticles of silicon dioxide for forming spontaneous Pickering O/W emulsions. The optimal parameters of this process and the composition were determined by scanning electron microscopy SEM, laser correlation spectroscopy, and thermogravimetric analysis. Biological tests of the antifungal and antimicrobial activity of DCOIT encapsulated in submicrocontainers were carried out using Petri dishes filled with Czapek-Dox agar, and meat-peptone agar media.

The obtained submicrocontainers show a narrow size distribution with a small increase in size in comparison with the initial Pickering emulsiondroplets. The Zeta potential of the final containers was sufficientlynegative at pH7 for a stable system. The maximum possible release of encapsulated biocide was observed in such systems for approximately 24–27 h with arelease of about 78% of the encapsulated biocide after 3.5 h.

Biological tests confirm the significant increase of the effectiveness of the encapsulated biocide which is shown by aninhibition of the microorganism growth (*Aspergillus niger, Aspergillus awamori* and *Bacillus cereus),* even in comparison with biocide in free form. The submicrocontainers with DCOIT gave a growth inhibition of 70% against 52% of free DCOIT after 5 days of storage, which is confirmed by the fact that free biocide loses its activity more quickly, while the encapsulated biocide is released gradually, and thus retains its effect for a long time.

The effect of the encapsulated biocide additive in the media and coating has a high implementation potential for industrial applications. Further development of basic scientific knowledge in this area

will enable the application of the anencapsulation method based on spontaneous O/W Pickering emulsions to extend to a variety of other fields such as biotechnology, the production of additives for paints, varnishes, protective coatings of various types, etc.

Author Contributions: All authors (S.B.A., A.A.S., A.B.I., B.Z.Mu. 3a, A.B.T., D.O.G., D.K., M.D. and R.M.) contributed in equal parts to the search and analysis of literature data, writing the text and designing the graphs. A.A.S. is responsible for the coordination of work and correspondence with the journal.

Acknowledgments: The work was supported by projects of the Ministry of Education and Sciences of the Republic of Kazakhstan grant number AP05131968, AP05131984, AP05132810.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Soliman, E.A.; El-Moghazy, A.Y.; El-Din, M.S.M.; Massoud, M.A. Microencapsulation of Essential Oils within Alginate: Formulation and in vitro Evaluation of Antifungal Activity. *J. Encapsul. Adsorpt. Sci.* **2013**, *3*, 48–55. [CrossRef]
2. de Jesus, M.B.; Radaic, A.; Zuhorn, I.S. Microemulsion extrusion technique: A new method to produce lipid nanoparticles. *J. Nanopart. Res.* **2013**, *15*, 1960. [CrossRef]
3. Kim, B.; Peppas, N.A. In vitro release behavior and stability of insulin in complexation hydrogels as oral drug delivery carriers. *Int. J. Pharm.* **2003**, *266*, 29–37. [CrossRef]
4. Elzoghby, A.O.; Elgohary, M.M.; Kamel, N.M. Chapter Six—Implications of Protein- and Peptide-Based Nanoparticles as Potential Vehicles for Anticancer Drugs. *Adv. Protein Struct. Biol.* **2015**, *98*, 169–221.
5. Song, L.; Zhi, Z.; Pickup, J.C. Nanolayer encapsulation of insulin- chitosan complexes improves efficiency of oral insulin delivery. *Int. J. Nanomed.* **2014**, *9*, 2127–2136.
6. Chevalier, Y.; Bolzinger, M. Emulsions stabilized with solid nanoparticles: Pickering emulsions. *Colloids Surf. A Physicochem. Eng. Asp.* **2013**, *439*, 23–34. [CrossRef]
7. Zhou, H.; Shi, T.; Zhou, X. Preparation of polystyrene/SiO_2 microsphere via Pickering emulsion polymerization: Synergistic effect of SiO_2 concentrations and initiator sorts. *Appl. Surf. Sci.* **2013**, *266*, 33–38. [CrossRef]
8. Yin, D.; Ma, L.; Liu, J.; Zhang, Q. Pickering emulsion: A novel template for microencapsulated phase change materials with polymer–silica hybrid shell. *Energy* **2014**, *64*, 575–581. [CrossRef]
9. Frelichowska, J.; Bolzinger, M.; Chevalier, Y. Pickering emulsions with bare silica. *Colloids Surf. A Physicochem. Eng. Asp.* **2009**, *343*, 70–74. [CrossRef]
10. Sharipova, A.; Aidarova, S.; Mucic, N.; Miller, R. Dilational rheology of polymer/surfactant mixtures at water/hexane interface. *Colloids Surf. A Physicochem. Eng. Asp.* **2011**, *391*, 130–134. [CrossRef]
11. Sharipova, A.; Aidarova, S.; Cernoch, P.; Miller, R. Effect of surfactant hydrophobicity on the interfacial properties of polyallylamine hydrochloride/sodium alkylsulphate at water/hexane interface. *Colloids Surf. A Physicochem. Eng. Asp.* **2013**, *438*, 141–147. [CrossRef]
12. Kaewsaneha, C.; Tangboriboonrat, P.; Polpanich, D.; Eissa, M.; Elaissari, A. Preparation of Janus colloidal particles via Pickering emulsion: An overview. *Colloids Surf. A Physicochem. Eng. Asp.* **2013**, *439*, 35–42. [CrossRef]
13. Li, C.; Li, Y.; Sun, P.; Yang, C. Pickering emulsions stabilized by native starch granules. *Colloids Surf. A Physicochem. Eng. Asp.* **2013**, *431*, 142–149. [CrossRef]
14. Zou, S.; Yang, Y.; Liu, H.; Wang, C. Synergistic stabilization and tunable structures of Pickering high internal phase emulsions by nanoparticles and surfactants. *Colloids Surf. A Physicochem. Eng. Asp.* **2013**, *436*, 1–9. [CrossRef]
15. Santini, E.; Guzmán, E.; Ferrari, M.; Liggieri, L. Emulsions stabilized by the interaction of silica nanoparticles and palmitic acid at the water–hexane interface. *Colloids Surf. A Physicochem. Eng. Asp.* **2014**, *460*, 333–341. [CrossRef]
16. Schmitt, V.; Ravaine, V. Surface compaction versus stretching in Pickering emulsions stabilised by microgels. *Curr. Opin. Colloid Interface Sci.* **2013**, *18*, 532–541. [CrossRef]
17. Zhang, L.; Shi, T.; Wu, S.; Zhou, H. Graphene/polystyrene nanocomposites synthesized via Pickering emulsion polymerization. *J. High Perform. Polym.* **2014**, *26*, 156–165. [CrossRef]

18. Grigoriev, D.; Shchukina, E.; Tleuova, A.; Aidarova, S.; Shchukin, D. Core/shell emulsion micro- and nanocontainers for self-protecting water based coatings. *Surf. Coat. Technol.* **2016**, *303*, 299–309. [CrossRef]
19. Paster, N.; Bullerman, L.B. Mould Spoilage and Mycotoxin Formation in Grains as Controlled by Physical Means. *Int. J. Food Microbiol.* **1988**, *7*, 257–265. [CrossRef]
20. Paster, N.; Menasherov, M.; Ravid, U.; Juven, B. Antifungal activity of oregano and thyme essential oils applied as fumigants against fungi attacking stored grain. *J. Food Prot.* **1995**, *58*, 81–85. [CrossRef]
21. Hamilton-Kemp, T.R.; Archbold, D.D.; Loughrin, J.H.; Andersen, R.A.; McCracken, C.T.; Collins, R.W.; Fallik, E. Stimulation and Inhibition of Fungal Pathogens of Plants by Natural Volatile Phytochamicals and Their Analogs. *Curr. Top. Photochem.* **2000**, *4*, 95–104.
22. Bixler, G.D.; Bhushan, B. Biofouling: Lessons from nature (review). *Philos. Trans. R. Soc. A* **2012**, *370*, 2381–2417. [CrossRef] [PubMed]
23. Borenstein, S.W. *Microbiologically Influenced Corrosion Handbook*; Woodhead Publishing Ltd.: Cambridge, UK, 1994.
24. Jacobson, A.H.; Willingham, G.L. Sea-nine antifoulant: An environmentally acceptable alternative to organotin antifoulants. *Sci. Total Environ.* **2000**, *258*, 103–110. [CrossRef]
25. Schonknecht, U.; Gruycheva, J.; Mathies, H.; Bergmann, H.; Burkhardt, M. Leaching of Biocides Used in Façade Coatings under Laboratory Test Conditions. *Environ. Sci. Technol.* **2009**, *43*, 9321–9328. [CrossRef] [PubMed]
26. Atarijabarzadeh, S.; Strömberg, E.; Karlsson, S. Inhibition of biofilm formation on silicone rubber samples using various antimicrobial agents. *Int. Biodeterior. Biodegrad.* **2011**, *65*, 1111–1118. [CrossRef]
27. Markarian. Rethinking Biocides for Plastics, Compounding World. July 2013, pp. 16–22. Available online: http://www.nanobiomatters.com/wordpress/wp-content/uploads/2013/07/CWJuly2013.pdf (accessed on July 2013).
28. Negroni, A.; Zanaroli, G.; Ruzzi, M.; Fava, F. Biological fate of Diuron and Sea-nine® 211 and their effect on primary microbial activities in slurries of a contaminated sediment from Venice Lagoon. *Ann. Microbiol.* **2010**, *60*, 321–327. [CrossRef]
29. *Marine Paint Annual Report 2008*; University of Gothenburg, Plant and Environmental Sciences: Gothenburg, Sweden, 2008.
30. Breuer, K.; Mayer, F.; Scherer, C.; Schwerd, R.; Sedlbauer, K. Wirkstoffauswaschung aus hydrophoben Fassadenbeschichtungen: Verkapselte versus unverkapselte Biozid Systeme. *Bauphysik* **2012**, *34*, 19–23. [CrossRef]
31. European Union. Commission implementing regulation (EU) No. 437/2014 approving 4,5-dichloro-2-octyl-2h-isothiazol-3-one as an existing active substance for use in biocidal products for product-type 21. *Off. J. Eur. Union L* **2015**, *128*, 64–67.
32. Morley, J.O.; Kapur, A.J.O.; Charlton, M.H. Kinetic studies on the reactions of 3-isothiazolones with 2-methyl-2-propanethiol. *Int. J. Chem. Kinet.* **2007**, *39*, 254–260. [CrossRef]
33. Arning, J.; Dringen, J.; Schmidt, M.; Thiessen, A.; Stolte, S.; Matzke, M.; Bottin-Weber, U.; Ceasar-Geertz, B.; Jastorff, B.; Ranke, J. Structure-activity relationships for the impact of selected isothiazol-3-one biocides on glutathione metabolism and glutathione reductase of the human liver cell line Hep G2. *J. Toxicol.* **2008**, *246*, 203–212. [CrossRef]
34. Norwegian Climate and Pollution Agency. *Competent Authority Report 4,5-Dichloro-2-octyl-2H-isothiazol-3-one (DCOIT) PT21*; Climate and Pollution Agency: Oslo, Norway, 2010.
35. Martinez, K.; Ferrer, I.; Hernando, M.D.; Fernandez-Alba, A.R.; Marce, R.M.; Borrull, F.; Barcel, D. Occurrence of antifouling biocides in the Spanish Mediterranean marine environment. *J. Environ. Technol.* **2001**, *22*, 543–552. [CrossRef]
36. Ayala-Zavala, J.F.; del Toro-Sanchez, L.; Alvarez-Parrilla, E.; Gonzalez-Aguilar, G.A. High Relative Humidity In-Package of Fresh-Cut Fruits and Vegetables: Advantage or Disadvantage Considering Microbiological Problems and Antimicrobial Delivering Systems. *J. Food Sci.* **2008**, *73*, 41–47. [CrossRef] [PubMed]
37. Xiong, M.-H.; Bao, Y.; Yang, X.-Z.; Zhu, Y.-H.; Wang, J. Delivery of antibiotics with polymeric particles. *Adv. Drug Deliv. Rev.* **2014**, *78*, 63–76. [CrossRef] [PubMed]
38. Cheow, W.S.; Hadinoto, K. Factors affecting drug encapsulation and stability of lipidpolymer hybrid nanoparticles. *Colloids Surf. B Biointerfaces* **2011**, *85*, 214–220. [CrossRef] [PubMed]

39. Dzhakasheva, M.A.; Rysbaeva, G.S.; Esimova, A.M.; Narymbaeva, Z.K.; Elemanova, Z.R. *Selection of Aspergillus Awamori Strain for Reception of the Highly Active Producer of the Complex of Pectolytic Enzyme*; News of NAS RK, Biological and Medical Series; No. 5; Academy of Science of Republic of Kazakhstan: Almaty, Kazakhstan, 2017; pp. 165–173.
40. Sacanna, S.; Kegel, W.K.; Philipse, A.P. Spontaneous oil-in-water emulsification induced by charge-stabilized dispersions of various inorganic colloids. *Langmuir* **2007**, *23*, 10486–10492. [CrossRef] [PubMed]
41. Tleuova, A.; Aidarova, S.; Sharipova, A.; Bekturganova, N.; Schenderlein, M.; Grigoriev, D. Using profile analysis tensiometry for monitoring auto-oscillations caused by the hydrolysis of 3-(trimethoxysilyl) propyl methacrylate when contacting water. *Colloids Surf. A Physicochem. Eng. Asp.* **2016**, *505*, 18–22. [CrossRef]
42. Balouiri, M.; Sadiki, M.; Ibnsouda, S.K. Methods for in vitro evaluating antimicrobial activity: A review. *J. Pharm. Anal.* **2016**, *6*, 71–79. [CrossRef] [PubMed]
43. Pantoja, M.; Velasco, F.; Broekema, D.; Abenojar, J.; del Real, J.C. The Influence of pH on the Hydrolysis Process of γ-Methacryloxypropyltrimethoxysilane, Analyzed by FT-IR, and the Silanization of Electrogalvanized Steel. *J. Adhes. Sci. Technol.* **2014**, *24*, 1131–1143. [CrossRef]