Biocide-loaded microcontainers obtained by water-based ultrasound chemistry.

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INTRODUCTION

Sustainable development of society requires new materials resistant to environmental degradation effects, such as microbiological degradation. Various biocides are widely used in everyday life to protect product integrity, mainly as a part of protective coatings to prevent their destruction due to bio-induced microorganism's attack.

One of the effective biocide is DCOIT (4,5-dichloro-2-n-octyl-4-isothiazoline-3-one) which possesses bacteriostatic properties against a wide range of microorganisms. However, it is almost impossible to distribute DCOIT homogeneously in the aqueous coating formulations due to its high hydrophobicity. Direct incorporation of biocide influences the efficacy of the protective coating due to its integrity violation, depletion of the barrier properties and rise of the surface roughness. Biocide encapsulation is a big challenge to enhance its efficiency especially for in-door applications, where tendency of gradually exclusion of solvent-based formulations exists. We propose a new class of microcontainers made with chitosan and xanthan gum in one-step procedure applying a low-frequency ultrasound to encapsulate DCOIT.

MATERIALS AND METHODS

Chitosan (20-300 cP), xanthan gum (from Xanthomonas campestris), polyallylamine hydrochloride (MW 70000), fluorescein isothiocyanate (FITC)) were supplied from Sigma-Aldrich. 4,5-dichloro-2-n-octyl-4-isothiazoline-3-one (DCOIT) was purchased from Chemos GmbH, FRG.

Microcontainer formations: Equal volumes of chitosan and xanthan gum solutions (0.25w%, pH 2) were mixed, then layered with heated DCOIT (melting point is 40°C) and exposed to high-intensity ultrasound using a 7-mm diameter titanium sonotrode (56 $W \cdot cm^{-2}$, 20 kHz, 5 min). After centrifugation (6.5 × g, 10 min) and washing with water, the microcontainers were incorporated into a Ca-alginate film or modified with polyallylamine hydrochloride (PAH) and then embedded into the polymer film. We use here alginate as a model polymer which easily forms the film by cross-linking as a result of ion exchange.

Microcontainer characterization: Fourier transform infrared (FTIR) measurements were carried out with a Bruker Hyperion 2000 IR. The microcontainers were characterized by confocal laser scanning microscopy (CLSM) (Leica, Germany), scanning electron microscopy (SEM) (Gemini Leo 1550), dynamic light scattering (Malvern Instruments, UK).

RESULTS AND DISCUSSION

The microcontainers in a wide size range (from 350 nm to 7500 nm) (Figure1A, 1B) were easily obtained by varying an oil/water ratio (from 1:300 to 1:1.5 accordingly). Results showed that the dispersed phase/dispersion medium ratio has an influence on the container size which could be explained by changing an overall emulsion viscosity: An increase of the oil volume fraction results in elevation of the overall emulsion viscosity which leads to the container size increase (Tcholakova 2011).

We analyzed the microcontainers loaded with pure soybean oil to investigate their morphology and structure by cryo SEM.

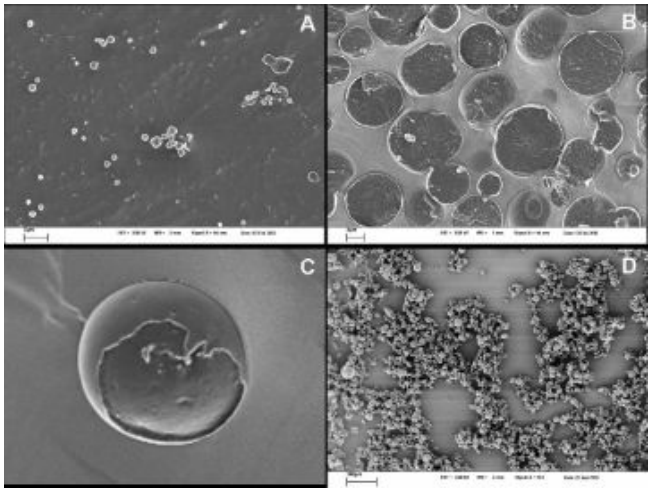

Fig. 1. SEM investigation of the microcontainers: A. smaller fraction and B. bigger fraction. C. Single container. D. DCOIT microcontainers.

Figure 1C reveals a core-shell structure of the containers. The image of the microcontainer with broken shell demonstrates that the inner container core is surrounded by external polymer shell. The thickness of the polymer-shell is around 7-10 nm, which is enough to keep the system stable at least 4 months of storage. Figure 2D shows the microcontainers with encapsulated biocide. SEM

analysis demonstrates surface roughness of the DCOIT containers, that could be explained due to biocide behavior during preparation procedure of SEM probes where drying under vacuum may lead to the deformation of soft polymer shell.

The images in Figure 2 show confocal laser scanning microscopy (CLSM) observation of the containers filled with DCOIT and a fluorescence dye Nile Red, which surface was modified by deposition of PAH-FITC. The microscopy investigations demonstrate a presence of green fluorescence due to the adsorption of PAH-FITC (Fig. 2A1) on the surface of the microcontainers with red inner core (Fig. 2A2). The image 2A3 confirms surface location of the polymer after adsorption. The measurements of electrophoretic mobility showed changes of the surface charge of microcontainers from -50 mV (initial containers) to +52 mV (containers modified with PAH-FITC). Polymer adsorption has no influence on the system stability.

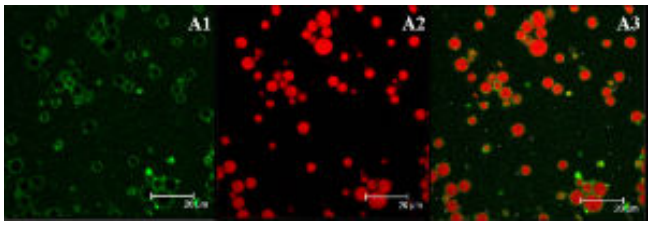

Fig. 2. CLSM observation of the DCOIT containers filled with Nile Red and modified with PAH-FITC.

We applied FT-IR spectroscopy to investigate an ultrasound effect on behavior of the polymers during the container preparation procedure. Comparison of the initial polymer spectra and the spectrum of the polymer mixture extracted from the containers indicate various changes showing the formation of keto ester bonds and amide bonds induced by ultrasound treatment. The mechanism of container shell formation was explained before by influence of the free radicals generated during the sonication process on polymer molecules leading to their cross-linking (Suslick 1990). We suggest that in the case of chitosan/xanthan gum containers, the ultrasound shell formation is a multi-step procedure. The first step consists of hydrogen bridges formations (between the polar groups –COOH, $–NH_2$, -OH and –COOH) which maintain the primary structure of the container shell. The second step involves formation of ester bonding and amide linkage network which contribute to the creation of the containers with permanent core-shell structure.

To demonstrate the advantages of the encapsulated DCOIT, biocide-loaded microcontainers were embedded into polymer coating. We performed comparative tests to evaluate the impact of the encapsulated DCOIT against three representative microorganisms: gram-positive bacteria *Bacillus subtilus* ATCC 6633, gram-negative bacteria *Escherichia coli* ATCC 25922 and mycelial fungus *Aspergillus niger* ATCC 16404 by agar diffusion test. The obtained results indicate a good diffusion of the biocide from the containers and equal bacteriostatic activity compare to the free biocide after 24 hours of incubation. We found more sustained activity of DCOIT entrapped into the microcontainers after 48 hours of incubation which indicates the prolonged action of the encapsulated biocide. Figure 3 demonstrates the sustained bacteriostatic activity of encapsulated DCOIT where number of dead bacterium (red) after 5 hours increases dramatically within next 36 hours of incubation in the presence of DCOIT containers (LIVE/DEAD® *Bac*Light™ Bacterial Viability Kit).

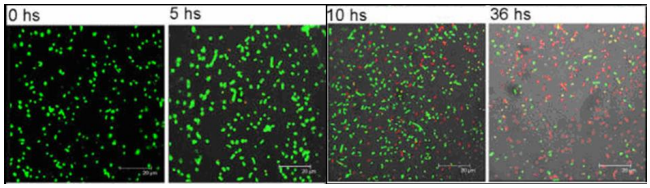

Fig. 3. Bacteriostatic activity of the DCOIT microcontainers against *E. coli* after 5, 10 and 36 hours of their incubation in the presence of the bacterium: red – dead bacterium, green – live bacterium.

CONCLUSIONS

The biocide-loaded chitosan/xanthan gum microcontainers were successfully fabricated in one-step procedure applying a low-frequency ultrasound. The microcontainers have the core-shell structure with permanent shell formed due to cross-linking between chitosan and xanthan gum. The encapsulated DCOIT keeps its bacteriostatic activity and has more sustained bacteriostatic effect compare to the free biocide.

REFERENCES

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