P-057 **A biocompatible coating doped with vitamin E microcontainers**

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INTRODUCTION

Many studies have demonstrated a role of vitamin E (VE) as an essential part in healthy skin care. Due to its anti-oxidant activity, VE is important in protecting skin cells from the impact producing free radicals, provides the photoprotective effect via absorption of UVB radiation (wavelength 280-320 nm), the prevention and treatment of the sunburns, anti-aging and wound healing effects (Johnston 2002).

Free VE is unstable and easily decomposes under the influence of oxygen, light, heat, etc. The different delivery systems are used in order to retain VE during the storage, to prevent interactions with other components from the environment, to minimize a side effect of an active component, and to increase a self-penetration of VE into the skin. One possible solution may be encapsulation of VE in a protective biocompatible micro- or nanocontainers.

A new system for a biocompatible film fabrication with entrapped microcontainers of loaded VE is demonstrated in the present study. The containers were produced by emulsification of the VE in an aqueous solution of heteropolysaccharide extruded from *Acacia* trees (GA) by the ultrasound treatment.

MATERIALS AND METHODS

Gum acacia (GA) was purchased by Hopkin & Williams Ltd., UK. α-Tocopherol, poly-L-lysine (PLL) (MW 15000-30000), sodium alginate (SA) (medium viscosity), soybean oil, hexane, cyclohexane, fluorescein isothiocyanate (FITC) and $CaCl_2$ were supplied from Sigma-Aldrich (Germany, Munich). All materials were used without further purification.

Synthesis of the microcontainers: GA microcontainers with entrapped VE were synthesized by the ultrasound treatment as follows (Figure 1). The direct O/W emulsion was formed by mixing 20 ml of 1 wt% aqueous GA solution and 2 ml of vitamin E (α-Tocopherol). The fine emulsion was fabricated by applying high-intensity ultrasound (intensity of 23 $W \cdot cm^{-2}$ and frequency of 20 kHz). The synthesized microcontainers were separated from the reaction mixture by centrifugation (6.708 × g, 5 min) and washed three times with water using centrifugation/resuspension technique. In order to modify the microcontainer surface, they were incubated in PLL-FITC solution (1 ml, 2 $mg \cdot ml^{-1}$, 0.15M NaCl) for 15 min under shaking (Vortex-genie 2, Scientific Industries, Inc., USA).

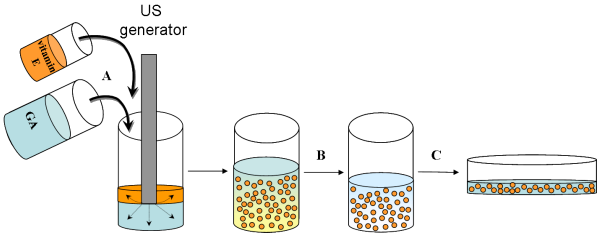

Figure 1 : scheme of the microcontainer fabrication

Fabrication of the film with embedded microcontainers: The polymer film was prepared by using 2 wt% aqueous SA solution containing 5 v/v% of glycerol. One ml of the aqueous microcontainer dispersion (20 wt%) were added to 2 ml of the prepared solution and stirred at room temperature for 15 min. The mixture was poured onto a cellulose filter (Millipore) dipped into 2 wt% $CaCl_2$ solution and covered by the same filter for 15 min. The crosslinked film was washed with physiological solution for 3 times and kept in the refrigerator (4ºC). The control polymer film was prepared by the same method, but the pristine vitamin E was added to the SA solution under stirring instead of the vitamin E-loaded microcontainers.

Vitamin E release: The release behavior of VE from the films was examined in 50 % aqueous ethanol (H_2O/EtOH) solution to establish the sink conditions for VE. The supernatant containing dissolved VE was analyzed by UV–Vis spectroscopy at 295 nm. The experiments were conducted in triple.

Microcontainer characterization: Fourier transform infrared (FTIR) measurements were carried out with a Bruker Hyperion 2000 IR spectrometer equipped with a 158 IR objective and mercury cadmium telluride (MCT) detector at RT in KBr pellets. Spectra between 400 to 4000 cm^{-1} were recorded with 2 cm^{-1} resolution in the transmission mode using a DTGS detector. Confocal images were obtained using a Leica TCS SP confocal scanning system (Leica, Germany) equipped with a 100×oil immersion objective (numerical aperture 1.4). Atomic force microscopy (AFM) images of the film were taken using a Digital Instruments Nanoscope IIIa (Veeco, US) instrument in tapping mode.

RESULTS AND DISCUSSION

Macromolecules such as polysaccharides and glycoproteins possessing various functional groups (amino, carboxyl, sulfhydryl groups etc.) can be cross-linked on the polymer chains by the direct reaction between them (Park 1993). The ultrasound produces cavitation resulting in

highly reactive free radicals (especially $OH^{\cdot}$ and $H^{\cdot}$) (Canselier 2002). In the presence of oxygen, the hydroxyl radicals undergo various reactions including the formation of superoxide ($HO_2^{\cdot}$). Superoxide can crosslink protein molecules adsorbed at the interface between dispersed phase and dispersion medium, and create a permanent structure forming the shell of the microcontainers.

Fourier transform infrared spectroscopy (FTIR) was used to investigate the influence of the ultrasound on the GA solution during the microcontainer fabrication. The changed in the IR spectra of GA demonstrated the chemical interaction between two kinds of GA moieties (C=O stretching band of carboxylic group and N-H stretching band of amide group) via amide linkage formation (data is not shown).

The microcontainers were coated with PLL-FITC in the next step (Figure 1B). Figure 2 shows the confocal images of the VE microcontainers after PLL-FITC adsorption recorded in transmission and fluorescence modes. The confocal fluorescence images of the microcontainers show that PLL-FITC was successfully deposited onto the microcontainer surface.

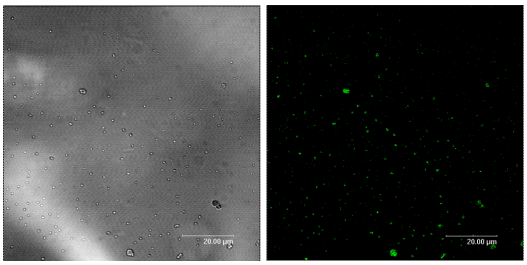

Figure 2 : confocal images of microcontainers coated with PLL-FITC.

In the next step the microcontainers were directly added to SA solution under stirring followed by the Ca-alginate film formation (Figure 1C). The CLSM observation shows a homogeneous, non aggregated placement of the microcontainers in the Ca-alginate film (Figure 3 A). AFM image in Figure 3 B reveals that the microcontainers indeed entrapped within the polymer film. Some of the containers are located on the film surface, some are partly "sunk" within the polymer coating and some are completely covered by the polyelectrolyte matrix (Figure 3 C).

The release profile of VE freely embedded in the film showed the initial burst effect (Figure 4). More than 40 % of VE was released during the first hour. Approximately 70 % of the incorporated vitamin was found in the supernatant after 2 h with 100 % release in 5 h. This behavior can be explained by the higher affinity of vitamin E to the release medium compared with the more hydrophilic environment found inside the polymer film. The vitamin release profile in the case of the polymer film with the embedded microspheres loaded with vitamin E is expanded in time. The reduction in the initial release rate could be ascribed to the isolation of vitamin E from the Ca-alginate film by the sphere shell.The absence of the initial burst effect can be interpreted as a fact that the destruction of SA film does not lead to the direct release of the VE. VE is encapsulated in the microcontainers; therewith the initial release occurs from the microcontainers into the SA matrix followed by the release of the encapsulated compound from the surface of the polymer film.

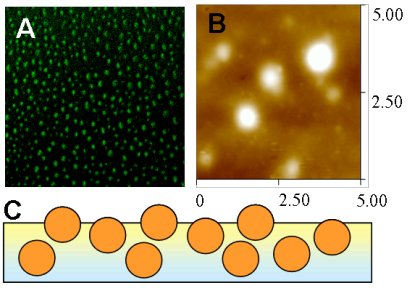

Figure 3 : A. Confocal images of the film with embedded microcontainers.B. AFM observation. C. Schematic demonstration of the film.

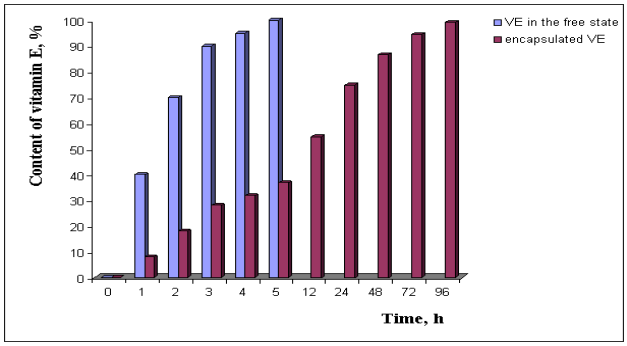

Figure 4 : release profile of the vitamin E.

CONCLUSIONS

We propose a simple procedure for the fabrication of a biocompatible polymer film with the embedded microcontainers loaded with hydrophobic compounds.

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