P-013	**Encapsulated oil-in-water emulsion stable to lipid peroxidation**	

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INTRODUCTION AND OBJECTIVES

Wide range of drugs, functional foodstuffs, and consumer care products contains oil-in-water emulsions. Linseed-oil, cod oil, and some marine oils are well-known for healing effect due to high content of polyunsaturated fatty acids. However, instability of polyunsaturated fatty acids to peroxidation often hampers practical use of oil-based products highly shortening their shelf life. Despite the rancid smell, oxidized oils contain carcinogenic and mutagenic end products of lipid peroxidation (Leuratti 1998). Therefore, they should be strictly refused for any kind of bioaplications. Oxidative stability is an essential requirement for oil-based drug delivery systems. Inflammatory centers and cancerous growth are the sources of free radicals which can stimulate the chain reaction of lipid peroxidation. Thus, therapeutic and healing properties of various products containing oil-in-water emulsions depend strongly on ability to preserve intact form of fatty acids.

This paper reports on Layer-by-Layer encapsulation (Sukhorukov 1998) of oil-in-water emulsion of linseed-oil into complex coating shell comprising a layer of antioxidant Tannic acid (TA) for effective protection of emulsified oil against oxidative degradation. The effectiveness of shells with antioxidant TA is discovered in comparison with protective properties of biodegradable multilayer coating shell assembled of Poly-L-Arginine and Dextran sulfate (De Koker 2007). The impact of the antioxidant type and location on oxidative stability of encapsulated emulsion is revealed by the example of two systems: i) encapsulated emulsion with prooxidand scavenging TA located in the coating shell; ii) encapsulated emulsion with chain-breaking antioxidant mixed tocopherols added to linseed-oil in concentration of 10000 ppm.

MATERIALS AND METHODS

Bovine serum albumin (BSA), polyelectrolytes (dextran sulfate (DS, MW. 20000), and poly-L-arginine hydrochloride (PARG, MW. > 70000)), linseed-oil, mixed tocopherols, and Tannic acid (TA) were purchased from Sigma-Aldrich. All chemicals were used as received without further purification. TBARS Assay Kit was purchased from CELL BIOLABS, INC. and used according to enclosed protocol. Deionized water with specific resistivity higher than 18.2 $M\Omega \cdot cm^{-1}$ from a three-stage Milli-Q Plus 185 purification system was used in the experiments.

A primary emulsion of linseed-oil was obtained by sonicating 10 % v/v linseed-oil dispersed in 90 % v/v emulsifier (BSA, 4 mg/ml) water solution for 2 minutes. One part of this primary emulsion was then mixed with two parts of PARG (2 mg/ml) water solution to form a secondary emulsion. The mixture was transferred to 50 ml stirred filtration cell (Millipore Corp.) and kept under vigorous agitation for 15 minutes followed by 3 washing cycles to remove uncoupled polyelectrolyte. To adsorb a next layer of either DS or TA 10 ml of filtered emulsion was toped-up with 20 ml of DS (2 mg/ml) or TA (3 mg/ml) water solutions and vigorous stirred for 15 min followed by 3 washing cycles. The described routine was repeated alternating PARG with DS (or TA) to obtain the desired number of layers in the shell.

Oxidative stability was evaluated by Thiobarbituric Acid (TBA) Reactive Substances (TBARS) assay. TBARS is especially developed to quantify concentration of malondialdehyde (MDA), one of two natural biproducts of lipid peroxidation. TBARS Assay Kit was purchased from CELL BIOLABS, INC. and used according to a protocol provided by supplier. To monitor lipid oxidation during storage, encapsulated emulsions were placed under air in closed microcentrifuge tubes and allowed to oxidize at 37 °C in the dark. Before MDA quantitation each sample was diluted with water. The MDA-TBA adduct formed from the reaction of MDA in samples with TBA was measured colorimetrically reading the microplates by means of Infinite® 200 PRO, Tecan Group Ltd., Switzerland. Absorbance was read at 532 nm, with background subtraction at 570 nm. TBARS levels were determined from a MDA equivalence standard.

RESULTS AND DISCUSSION

Oxidative stability of encapsulated oil-in-water emulsion In order to clarify the influence of the thickness of the coating shell (which depends on the number of adsorbed layers) and the presence of TA on oxidative stability of encapsulated oil, the following samples were obtained: i) linseed-oil/BSA; ii) linseed-oil / BSA / PARG; iii) linseed-oil / BSA/PARG/DS/PARG; iiii) linseed-oil / BSA / PARG/ TA / PARG. Lipid oxidation marker (MDA) was measured as a function of storage time in order to monitor the differences in oxidation kinetics. Figure 1 displays a comparison of oxidation rates in encapsulated linseed-oil-in water emulsions stored over 15 days at 37 °C. Primary, secondary and multilayer coated emulsions demonstrated the same oxidation rate with time as determined with TBARS assay. The values of

MDA concentrations measured in the mentioned samples after the same period of storage fall into the limits of the error bars.

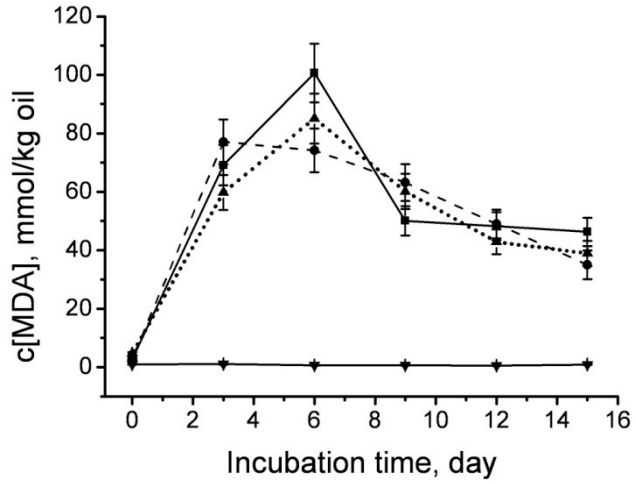

Figure 1: Formation of TBARS in encapsulated linseed-oil-in water emulsions. Shell composition: BSA (—■—), BSA/PARG (--•--), BSA/PARG/DS/PARG (⋯▲⋯), BSA/PARG/DS/PARG (—▼—).

The situation changes drastically if 1 layer of TA was incorporated in the shell. No MDA were detected in the sample of tannic acid coated emulsion over the whole period of observation. Furthermore, the smell of rancidity did not appear as well. Thus, the polyelectrolyte multilayer coating shell comprising antioxidant TA sandwiched in-between of two layers of PARG prevents oxidative degradation in encapsulated linseed-oil.

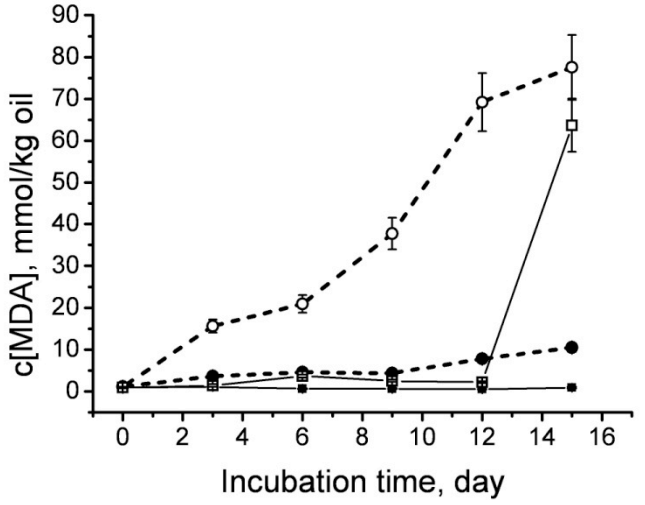

Figure 2: Impact of antioxidant type and location on oxidative stability of encapsulated emulsion. Emulsion with antioxidant shell in H_2O (—■—), in 0.03 mM $FeBr_2$ (—□—). Emulsion with antioxidant core in H_2O (--•--), in 0.03 mM $FeBr_2$ (--○--).

Impact of antioxidant type and location on oxidative stability of encapsulated oil-in-water emulsion Protective efficiency of TA-based encapsulating polyelectrolyte multilayer shell was compared with that of mixed tocopherols (MT) dissolved in oil cores in amount of 10000 ppm. MT containing oil droplets were then encapsulated in BSA/ PARG/DS/PARG shell. The sample with encapsulated emulsion was divided into two parts, which were then placed either in pure water or in prooxidant $FeBr_2$ containing solution and allowed to incubate at 37 ^{o}C over 15 days. The results of comparative study are presented in Figure 2. Although MT dissolved in oil were able to affect oxidation of encapsulated emulsion stored in pure water, antioxidant protection provided by TA containing multilayer shell was sufficiently more reliable in 0.03 mM $FeBr_2$ containing medium. The results suggest that prevention of lipid peroxidation by scavenging of prooxidants by coating shell appears to be an advantageous strategy to protect emulsified oil against oxidation than the use of chain-breaking antioxidant dispersed in oil core.

CONCLUSION

In this paper we demonstrated the effective protection of emulsified linseed-oil against lipid peroxidation. The approach is based on encapsulation of oil droplets inside antioxidant containing polyelectrolyte multilayer shell. Encapsulation was performed by LbL assembly of multilayer coating on aqueous dispersed oil cores preliminary stabilized with protein ionic emulsifier. Protection of encapsulated oil against oxidative degradation was attributed to antioxidant properties of tannic acid used as shell constituent in alternation with Poly-L-Arginine. The water dispersed emulsion encapsulated in multilayer shell comprising tannic acid did not oxidize over 15 days of storage at 37 °C and remained intact during 12 days of incubation in solution with physiological concentration of prooxidant Fe^{2+}. Prevention of lipid peroxidation in encapsulated emulsion by means of prooxidant scavenging by coating shell was found to be more effective approach to preserve the intact form of polyunsaturated fatty acids than breaking of prooxidant-initiated chain reaction with mixed tocopherols dispersed in oil phase. Direct tailoring of antioxidant molecules to emulsion droplets allows a broad range of practical application for the elaborated formulation including food, consumer care and drug delivery.

REFERENCES

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