Sunflower oil as shell material for oral food delivery systems of micronutrients

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Introduction

Addition of health-related components like enzymes or whole cells and probiotics are interesting for the food industry. However, they are normally decomposed in the mouth or stomach. Encapsulation of these materials could overcome these drawbacks. Materials that have often been used for encapsulation of these components are glassy carbohydrates. These materials work well with dry food stuffs, but for wet food stuffs other wall materials have to be chosen that do not easily dissolve or decompose in water. Polysaccharides (Anal, 2007) such as alginate are known for encapsulation of probiotics. However, these shell materials are porous in nature, which restricts their use as acid resistant shell material for oral delivery systems. Micro-organisms to be encapsulated require a shell material with poor H^+-transporting properties. Shima (2006) encapsulated *Lactobacillus Acidophilus* bacteria in a water-in-oil-in-water (W/O/W) double emulsion to increase the viability of encapsulated microorganism under acidic conditions.
The mechanical properties of the capsules determine the diffusion and release of core material; therefore rigidified oil capsules could be more beneficial than simple fluids. For storage of the capsules based on W/O/W double emulsion in food products like yogurts where the external osmotic pressure fractures the double emulsion, new materials are required to structure the oil phase of the double emulsion. There are several ways by which the oil can be structured, e.g. by structurents like gelators, biopolymers or surfactants. Sunflower oil can be structured in the presence of lecithin and sorbitan tristearate (Pernetti, 2007). In this study a new approach is proposed to prepare hydrophobic microcapsules based on W/O/W double emulsions; the oil phase consists of sunflower oil containing lecithin and sorbitan tristearate as structurent.

Materials and Methods

Emulsion preparation
Sunflower oil was purchased from a local food store. Soya lecithin was purchased from BDH,. Sorbitan tristearate was purchased from Fluka, Tween 20 from Merck, Congo red from Aldrich. A high intensity ultrasonic processor from Sonics and Materials, Danbury, was used for emulsification. A 20 ml solution of oil and structurents (lecithin and STS) was heated to 70˚C and subsequently added to aqueous 3% Tween 20 with sonication for 1 minute, resulting in a milky emulsion that was cooled with magnetic stirring. Water-soluble Congo red was encapsulated in a W/O/W double emulsion. The primary W/O emulsion was made by sonication at 70˚C and the second emulsification step was carried out by simple magnetic stirring at 48˚C.

Measurements
The emulsion was observed under Olympus optical microscope and the particle size distribution was determined using a Coulter LS 230 counter. The measurements were carried out at 20°C; the particle size was determined based on the refractive index of sunflower oil.
Cryo scanning electron microscopy (CryoSEM) was performed as follows: the samples were put on a small copper “shoe nail” Another one was placed up-side-down on top. The double “shoe nails” were immediately and rapidly frozen in liquid propane. The frozen samples were stored in liquid nitrogen and subsequently placed in a brass specimen holder. This holder was placed in a dedicated cryo-preparation chamber (Oxford Instruments CT 1500 HF, Eynsham, England). Here the top

"shoe nail" was removed from the base by a cold scalpel creating a fractured surface of the frozen suspension. This fractured surface was freeze dried for 3 minutes at -90°C at 10^{-4} Pa to remove water and to enhance the surface contrast. After 3 minutes the samples were sputter coated with a layer of 10 nm Pt at the same temperature. The sample was cryo-transferred into the field emission scanning microscope (JEOL 6300F, Japan) on a sample stage at -180°C. The analyses was performed at a working distance of 16 mm, with SE detection at 3.5 kV. All images were recorded digitally (Orion, 6 E.L.I. sprl, Charleroi, Belgium) at a scan rate of 100 seconds (full frame) at the size of 2528 x 2030, 8 bit. The images were optimized and resized by Adobe Photoshop.
Thermal analysis (micro-DSC) was conducted on a Micro-Differential scanning colorimeter (Setaram). Samples were placed in a sealed container. Heating and cooling between 5 and 70°C was performed at a rate of 1°C/min to obtain the melting and crystallization temperatures. The rheological measurements were carried out with an Anton Paar rheometer, Physica MCR 301, with double gap geometry (DG267) having a diameter of 26.66 mm. The encapsulated Congo red in the W/O/W double emulsion was observed by confocal laser scanning microscopy (Zeiss Pascal).

Results and discussion

Formation of gelled oil droplets in water
When sunflower oil containing lecithin and sorbitan tristearate are heated to 70˚C, it forms a clear solution. Addition of this oil phase to water containing 3% Tween 20 with sonication at this temperature, results in the O/W emulsion. The lecithin which is present in the oil phase self assembles to form micelles and Tween 20 enhances the emulsion stability by forming mixed surfactants films at the O/W interface by interaction with sorbitan tristearate (STS) (Sudaxshina, 1999). After cooling the emulsion to room temperature with stirring, three dimensional networks will form in the oil phase through hydrogen bonding of phosphate groups of lecithin with hydroxyl groups of STS. Particles of 1-5 µm were formed, and the emulsion was found to be stable for several months and exhibits normal Brownian motion. With increasing amounts of structurent in the oil phase the volume fraction of micelles in the oil phase increases and more surface area can be stabilized. Therefore, a significant decrease in particle size is observed when the concentration of lecithin and STS (1:1) in the oil phase increases up to 24% and 48%, shown in figure 2b.
The same results were obtained by Cryo-SEM. When the total amount of structurent increases, the particle size decreases. Figure 3. Increase in concentration of structurent up to 48% (lecithin and STS 1:1) gives particles having a size smaller than 1 micron.

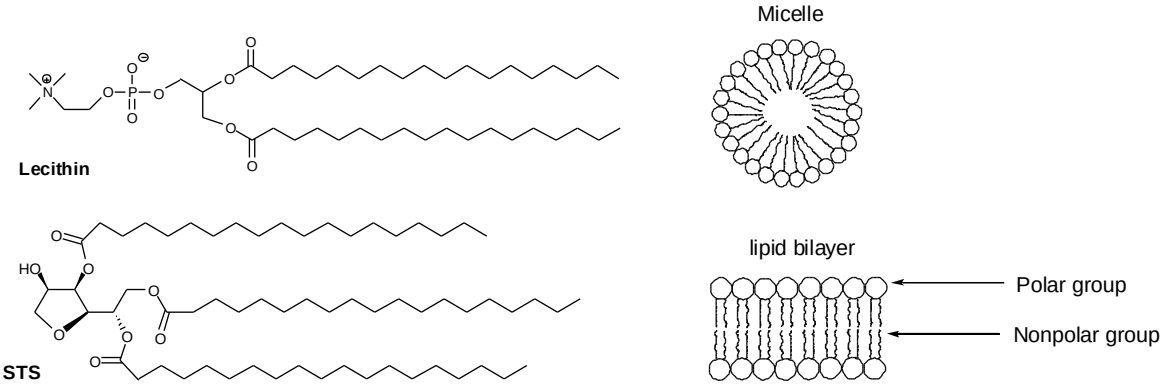


Figure 1 Formation of micelles in the oil phase due to the presence of lecithin. Upon cooling network formation in the oil phase occurs through hydrogen bonding of phosphate groups of lecithin and hydroxyl groups of sorbitan tristearate

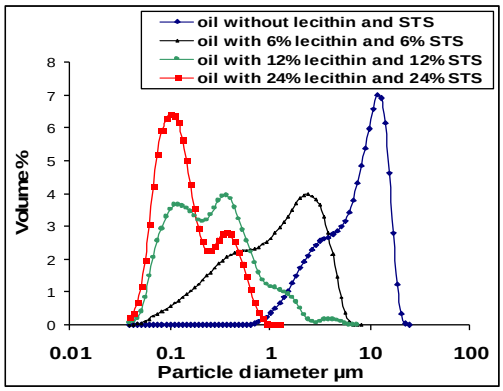


Figure 2. Effect of lecithin and STS concentration on particle size distributions of emulsions at 5% v/v of sunflower oil in water containing 3% Tween 20

a) b)

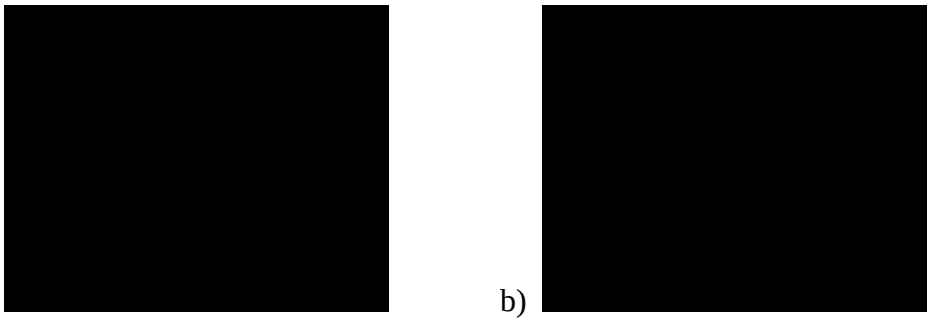
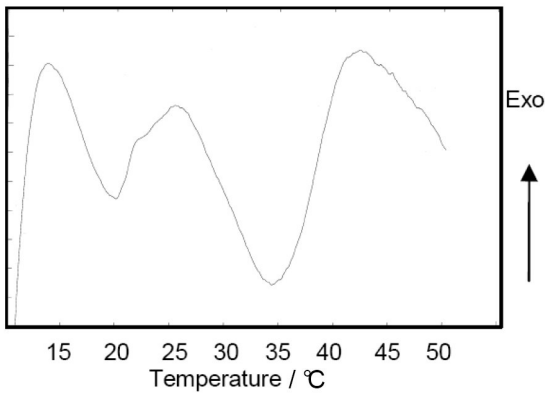
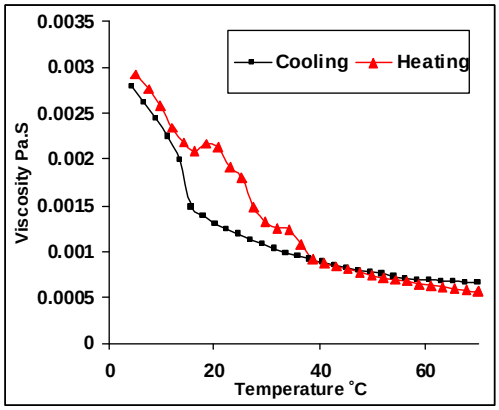


Figure 3. a) CryoSEM images of structured oil particles for 5% v/v oil in water emulsion with oil containing 12% w/w of lecithin and STS (1:1) in the oil phase. b) 24% w/w lecithin and STS (1:1)

Micro DSC measurements for oil in water emulsion with 12% lecithin and STS (1:1) in the oil phase shows a melting temperature of about 35˚C as show in figure 4a. In the rheological measurements (figure 4b), the viscosity decreases suddenly at a temperature corresponding to melting (~35°C) and it suddenly increases at the crystallization temperature (~20°C).



a)



b)

Figure 4. a) Micro DSC heating curve for an emulsion with 5% oil containing 12% lecithin and STS (1:1) (rate 1˚C/min). b) Rheological behavior as a function of temperature of the same sample (heating and cooling rates also 1°C/min)

The melting temperature of a reference gel of sunflower oil containing 12% lecithin and STS (1:1) in the absence of water was also found to be ~40˚C and the crystallization temperature was ~20˚C.

Encapsulation of water-soluble Congo red in a W/O/W double emulsion
The W/O/W double emulsion was prepared as follows: first a primary water-in-oil emulsion was made by sonication at 70˚C of the water phase containing Congo red and the oil phase containing 12% structurent. It was subsequently dispersed into an external aqueous phase containing Tween 20. This second emulsification was carried out by simple stirring. Most of the oil droplets contain encapsulated aqueous Congo red in the oil shell as seen by CLSM (Figure 5). However, the size distribution is found to be rather broad.

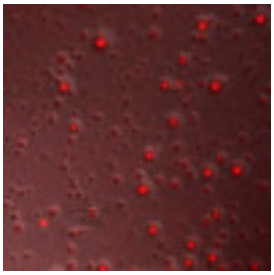


Figure 5 CLSM image of encapsulated Congo red in a W/O/W double emulsion

Conclusions

Gelled sunflower oil droplets can be prepared by structuring the oil phase with lecithin and sorbitan tristearate and stabilized by surface active lecithin and Tween 20 at the O/W interface. The size of gelled droplets can be tuned with increasing structurent concentration in the oil phase. It was feasible to prepare W/O/W double emulsion with encapsulating the water-soluble dye Congo red in to gelled oil droplets. This double emulsion could be very interesting for encapsulation of water-soluble active compounds for oral delivery. Further studies of these double emulsions with respect to monodispersity and low shear emulsification conditions for encapsulation of micronutrients by using microchannels and membranes (van Rijn, 2004) are in progress in our group.

Acknowledgements

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