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Biocompatible nanodispersions as delivery systems of food additives: A structural study

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ABSTRACT

Nanodispersions based on food grade biocompatible materials were developed and structurally characterized to be used as carriers of bioactive compounds with specific nutritional value. The main idea was to formulate concentrated solutions of specific food components at the nanoscale to be consumed either on their own or as integrating parts of classic foods, upon aqueous dilution. For this purpose microemulsions consisting of (R)-(+)-limonene/ethanol/Tween 40/water/propylene glycol were formulated in the presence and in the absence of squalene, gallic acid and octyl gallate. The limits of the single-phase region as described by pseudo-ternary phase diagrams were related to the nature of the food additive. The more extended monophasic region was obtained when octyl gallate was added in the system. Interfacial properties of the microemulsions were studied by electron paramagnetic resonance (EPR) spectroscopy employing the nitroxide spin probe 5-doxylstearic acid (5-DSA). In general guest molecules decreased the flexibility of the surfactant monolayer as manifested from the calculation of rotational correlation time (τ_R) and order parameter S of 5-DSA. Particle size measurements were performed using dynamic light scattering (DLS) and oil droplet diameters in the range of 11.7 to 17.4 nm were observed. The addition of squalene resulted in the formulation of larger oily droplets whereas octyl gallate formed smaller ones. Finally SAXS experiments provided qualitative information of o/w microemulsions showing squalene solubilization in the dispersed oily phase, octyl gallate localization on the membrane and gallic acid solubilization in the continuous aqueous phase.

1. Introduction

Recent research reveals that certain bioactive compounds, mainly from natural origin, exhibit important health benefits beyond nutrition and have been linked to the prevention of important chronic diseases (Liu, 2003; Hooper & Cassidy, 2006). Incorporation of compounds such as antioxidants, vitamins, and bioactive peptides into food systems can provide novel nutritional products, in fact delivery systems, with special beneficial effects on human health and wellbeing (Palzer, 2009; Sagalowicz & Leser, 2010).

The main aim of the present study is to obtain stable liquid nanodispersions based exclusively on materials approved for human consumption to be used as carriers of food ingredients of specific nutritional value. In this respect, microemulsions based on safe, food grade components were preferably selected among other types of liquid nanodispersions, mainly due to their unique physicochemical properties. Unlike other types of emulsions, microemulsions are thermodynamically stable and can form spontaneously. Depending on the relative ratios of their constituent components, microemulsions can be oil continuous dispersions of water nanodroplets (w/o), oil confined nanodomains in an aqueous environment (o/w) or bicontinuous with almost equal amount of oil and water. In all cases there is a coexistence of distinct domains of opposite polarity offering the unique possibility to solubilize both hydrophilic and lipophilic substances in a macroscopically homogeneous solution (Attwood, 1994; Augustin & Hemar, 2009; Flanagan & Singh, 2006; Rao & McClements, 2011).

Until recently, most of research interest and consequently most of the published work on the domain of microemulsions was focused on the application of hydrocarbons as the oily component and ionic surfactants as emulsifiers. Furthermore in order to lower interfacial tension and stabilize the nanodispersions, a co-surfactant was usually added in the system. However, it is obvious that the development of microemulsions as carriers of functional materials with potential application in the food industry is related to the compatibility and acceptability of the constituents. In this respect, during the past few years there has been a growing research interest towards the application of oils from natural origin as the oily phase and non-ionic surfactants as emulsifiers, whereas the use of toxic co-surfactants was gradually eliminated (Klossek, Marcus, Touraud, & Kunz, 2013; Klossek, Touraud, & Kunz, 2012). Biocompatible microemulsions based on non-ionic surfactants often include co-solvents to stabilize the dispersed phase and extend

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the limits of the monophasic area. In this regard, an oil of natural source such as limonene and safe nonionic surfactants such as polysorbates were chosen in the present investigation. Limonene is a cyclic monoterpene obtained from renewable resources that has been associated with diverse biological activities, including antimicrobial and antitumor activities (Miller et al., 2012).

To facilitate oil solubilization and reduce the polarity of the aqueous phase, ethanol and propylene glycol (PG) were added in the oil and water phases respectively (Garti, Yaghmur, Leser, Clement, & Watzke, 2001).

The main idea of the present study was to formulate concentrated solutions of specific bioactive compounds to be consumed either on their own or as integrating parts of classic foods, upon aqueous dilution. In this respect, the concentration of the specific neutraceutical ingredient encapsulated in the system had to be at a level that guarantees their efficiency. Moreover, the incorporation within the microemulsion should not alter the structural characteristics of the system in a way that could affect its stability. Diluted colloidal formulations based on microemulsions seemed very challenging for future functional food applications since protection against chemical oxidation, phase separation or precipitation of the enclosed nutrients during digestion were highly ensured (Sagalowicz & Leser, 2010).

The followed procedure included the development of a biocompatible microemulsion system and the incorporation of specific bioactive compounds with different hydrophilicities, namely squalene (hydrophobic), gallic acid (hydrophilic) and octyl gallate (amphiphilic). The chemical structures of the compounds are shown in Fig. 1. Although the incorporation of a variety of bioactive substances in similar systems would be of interest, our research efforts were focused on the micronutrients mentioned above for specific reasons. Both squalene and gallic acid are natural antioxidants that can be extracted from virgin olive oil, a staple food for the people living in the Mediterranean countries, and also known worldwide for its unique nutritional properties (Boskou, 2009). Squalene is a natural lipid belonging to the terpenoid family and a biochemical precursor of cholesterol and other steroids. Squalene exhibits antioxidant and chemopreventive activities whereas due to its significant dietary benefits is extensively used as an excipient in pharmaceutical formulations (Reddy & Couvreur, 2009). Gallic acid, apart from its use as food antioxidant was shown to exhibit a variety of pharmacological and biological activities, including antimicrobial, anti-inflammatory and anticancer activities (Giftson, Jayanthi, & Nalini, 2010; Raina et al., 2008). Octyl gallate is a water insoluble synthetic antioxidant based on gallic acid which is widely used in various food products for prevention of lipid oxidation (Tzika et al., 2011). Similar to several flavonoid compounds, octyl gallate has been shown to possess anti-inflammatory and antibacterial properties in animal studies (Törmäkangas et al., 2005).

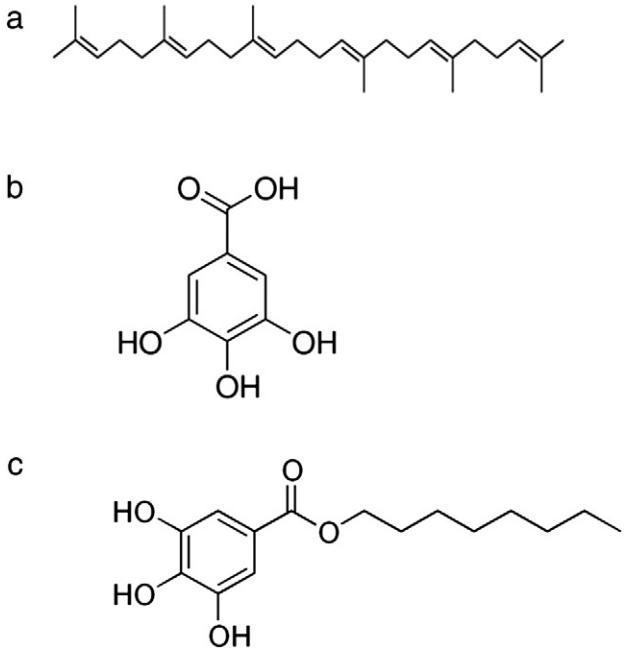

Fig. 1. Chemical structures of a) squalene, b) gallic acid and c) octyl gallate.

A structural characterization of microemulsions made by biocompatible, food grade surfactants and oils is very important when investigating the potential applications of these systems as model carriers of bioactive compounds (Astray, Gonzalez-Barreiro, Mejuto, Rial-Otero, & Simal-Gándara, 2009; Cid et al., 2013). Multiple instrumental techniques based on spectroscopy and scattering were undertaken to elucidate the structural details of the proposed microemulsions. Interfacial properties of the surfactant monolayer in w/o, bicontinuous and o/w microemulsions at given aqueous weight fractions, were studied by electron paramagnetic resonance (EPR) spectroscopy using adequate spin probes (Papadimitriou, Sotiroudis, & Xenakis, 2007; Papadimitriou et al., 2008). Dynamic light scattering (DLS) is a well-established scattering technique for studying self-organizing amphiphilic systems like microemulsions. This technique provided valuable information on the size and size distribution of the dispersed domains both in the absence and in the presence of active ingredients (Shukla, Janich, Jahn, & Neubert, 2003). Finally, to fully elucidate the structure of the systems, small angle X-ray scattering (SAXS) was applied to free and loaded o/w microemulsions (Tomsic, Podlogar, Gasperlin, Bester-Rogac, & Jamnik, 2006).

2. Materials and methods

2.1. Materials

(R)-(+)-Limonene 97% and 1,2-propanediol (propylene glycol) 98% were purchased from Alfa Aesar, Karlsruhe, Germany. Tween 40 (Polyoxyethylenesorbitan monopalmitate) was from Sigma-Aldrich Corporation, USA. Squalene, gallic acid and octyl gallate were purchased from Sigma-Aldrich, Germany. 5-Doxyl stearic acid [5-(1-oxyl-2,2-dimethyl-oxazolidin) stearic acid] (5-DSA) was obtained from Sigma-Aldrich, Germany. High-purity water was obtained from a Millipore Milli Q Plus water purification system.

2.2. Pseudo-ternary phase diagrams

The phase behaviour of a system consisting of (R)-(+)-limonene, ethanol, Tween 40, water and propylene glycol, at a constant temperature of 25 °C, was described on a pseudo-ternary phase diagram. The pseudo-ternary phase diagram was constructed in the following way: First, stock solutions of the oily phase and the aqueous phase were prepared separately. The oily phase consisted of (R)-(+)-limonene and ethanol at a weight ratio of 1:1. The aqueous phase consisted of water and propylene glycol at a weight ratio of 2:1. Then mixtures of oily phase and Tween 40 with varying weight ratios (from 1:9 to 9:1) were prepared and allowed to equilibrate in a water bath at 25 °C. Finally the mixtures were titrated with the aqueous phase to the solubilization limit, which was detected visually by the appearance of cloudiness or sharply defined separated phases. The time for equilibrium between additions of successive aliquots was typically from a few minutes to 24 h. The microemulsion region (monophasic area) was identified as shown in Fig. 2.

The final weight ratio of ethanol in the microemulsions varied depending on the composition. At a given initial alcohol concentration the final overall percentage of alcohol depended on the degree of aqueous dilution. For example, along the dilution line D73 (70% surfactant–15% oil–15% ethanol) the percentage of ethanol in the final microemulsions varied from 1.5 to 15% w/w.