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Effect of vegetable oils on obtaining lipid nanocarriers for sea buckthorn extract encapsulation

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ABSTRACT

The principal aim of the present study was to develop new safe and highly antioxidant nanostructured lipid carriers loaded with sea buckthorn extract. Three vegetable oils — grape seed oil, sea buckthorn oil and St. John's wort oil (*Hypericum perforatum* oil) — were used as matrix components and the modified high shear homogenization technique has been employed for the synthesis of nanostructured materials. The effect of these oils on the antioxidant and antimicrobial activities of loaded sea buckthorn extract — nanostructured lipid carriers — has also been studied. For this purpose, a combination of two solid lipids: cetyl palmitate with glyceryl stearate and lecithin/block copolymer has been used. The obtained nanostructured lipid carriers have been characterized for the particle size and zeta potential by means of dynamic light scattering measurements. The nano-dimension morphology of loaded nanostructured lipid carriers was confirmed by transmission electron microscopy. Their crystallinity measured by differential scanning calorimetry has revealed a high disordered lipid matrix. The properties of sea-buckthorn-extract-loaded nanoparticles have been evaluated by an appropriate *in vitro* analysis (chemiluminescence method). The presence of the three vegetable oils influences extensively the antioxidant properties of the developed nano-formulations, as has been demonstrated using the chemiluminescence technique. The antimicrobial activity of the studied nanostructured lipid carriers, analyzed by the diffusion disc method, shows in most of the samples a high efficiency against *Escherichia coli* bacteria.

1. Introduction

Sea buckthorn (SB) is a plant that grows widely in various regions of Asia, Europe, and North America. All parts of the plant are considered to be a good source of a large number of bioactive substances, which have proved some medicinal and nutritional properties [1,2]. Sea buckthorn berries are orange/red in color, 10–15 mm in diameter. The berries are rich in vitamins, minerals and antioxidant components, including ascorbic acid, tocopherols, polyphenols and carotenoids [3,4]. These fruits are richer in β-carotene than carrots. Sea buckthorn leaves are small and narrow, 2–6 cm in length. Leaves are very rich in polyphenolic compounds and are reported to have antioxidant properties [5]. The major flavonoids in the extract of sea buckthorn are: catechin (CA), quercetin (QU), kaempferol (KA) and isorhamnetin (IS) [1]. These flavones have similar molecular structures, as shown in Fig. 1.

Nanostructured lipid carriers (NLCs) based on a mixture of solid and liquid lipids are a new type of lipid nanoparticles, offering the advantage of having improved drug-loading capacity and release properties. There are several methods to produce nanostructured lipid carriers (NLCs), such as solvent evaporation in oil, in water microemulsion systems [6,7], solvent diffusion technique [8], high-pressure homogenization (HPH) technique [9,10], oil in water microemulsion technique [11], ultrasonic

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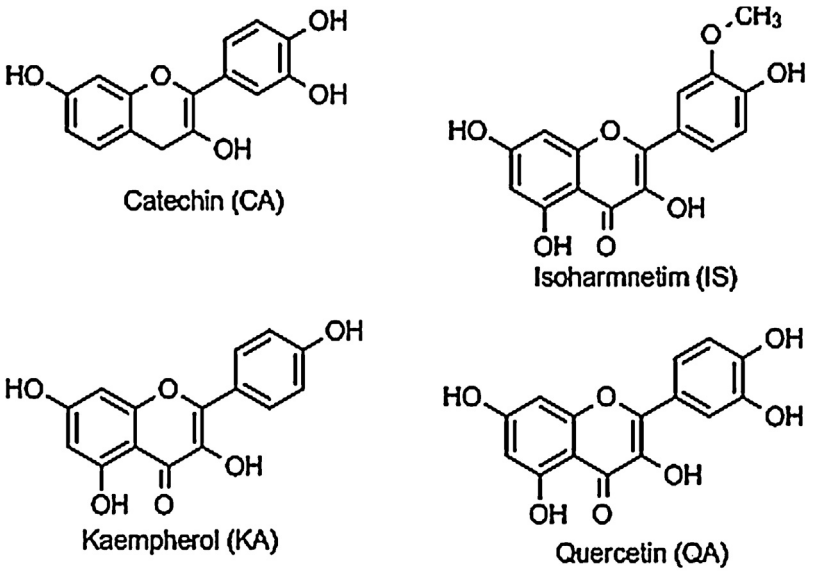

Fig. 1. Chemical structures of flavonoids present in sea buckthorn extract.

technique [12]. However, in comparison with other methods, the high shear homogenization (HSH) technique shows many advantages. It is a versatile technique that avoids the use of organic solvents and excessive energy or of other methods. It requires also a short production time.

The main objective of the present work was to obtain NLCs systems by the melt emulsification method coupled with high shear homogenization (HSH), with a view to the encapsulation of sea buckthorn extract (SBE) into the appropriate lipidic matrix with improved antioxidant and antimicrobial properties, with the purpose of developing potential applications as an efficient antioxidant delivery system. The choice of the lipid matrix and of the type of surfactant (with pharmacological use) is essential for obtaining safe and stable nanocarriers [13,14]. There exists a lot of plants that contain bioactive compounds with therapeutic effects in the treatment of various diseases. For this reason, three types of vegetable oils were used: grape seed oil (GSO), sea buckthorn oil (SBO), and St. John's wort oil (HPO) as liquid components of nanostructured lipid nanocarriers. They are known as efficient antioxidants [1,15], acting also as therapeutic agents that can prevent cardiovascular diseases, diabetes, and cancer [16]. The developed NLCs were characterized from the physico-chemical point of view: average particle size, zeta potential, morphological characteristics, loading capacity. Therefore, SBE-loaded NLCs prepared with these three vegetable oils could be used as food supplements and could be considered as interesting alternatives to conventional antioxidant formulations.

2. Experimental part

2.1. Materials

Polyethylene glycerol sorbitan monolaurate (Tw20), polyethylene glycol sorbitan monooleate (Tw80), were obtained from Merck (Germany). Synperonic PE/F68 (block copolymer of polyethylene and polypropylene glycol), L-α-phosphatidylcholine, (lecithin) and tris[hydroxymethyl] aminomethane (luminol) were purchased from Sigma Aldrich Chemie GmbH (Munich, Germany). *n*-Hexadecyl palmitate (CP) 95% was purchased from Acros Organics (USA), while glycerol stearate (GS) was supplied by Cognis GmbH. Grape seed oil was purchased from S.C. Manicos SRL (Romania). Sea buckthorn oil and St. John's wort oil (*Hypericum perforatum* oil) were provided by Hofigal (Romania). Spectroscopic-quality chloroform (Fluka) and ethanol (Sigma Aldrich) were used as solvents. The sea buckthorn extract was obtained by us using the procedure described in a paper under preparation.

2.2. Preparation of NLCs

NLCs were prepared by a modified HSH method. This method was already described in a previous study [17,18]. An aqueous phase containing a surfactant mixture of Tw20 or, separately, Tw80:SynperonicF68:lecithin with a mass ratio of 1:0.25:0.25, was heated at 85 °C. The lipid-phase mixture, consisting of CP and GS either with GSO, SBO or with HPO in the weight ratio of 35:35:30, was heated at the same temperature for 30 min. Various amounts of SBE were added in the lipid phase to form a clear molten solution. Before mixing the two phases, the aqueous phase was stirred at high speed for 2 min at 15,000 rpm. By adding gradually the lipid phase into the aqueous one, different NLCs pre-emulsions were obtained (Table 1). After stirring 1 h at 85 °C, the pre-emulsions were processed by HSH with a Lab High-Shear Homogenizer PRO250 apparatus, at 25,000 rpm for 10 min. The lipid dispersions were cooled down to room temperature under stirring. The water excess was removed by lyophilization, by using an Alpha 1-2 LD Freeze Drying System. The NLCs suspensions were frozen for 24 h at –25 °C, and then the