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Exploitation of amaranth oil fractions enriched in squalene for dual delivery of hydrophilic and lipophilic actives

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ABSTRACT

The study describes an ideal approach to increase the co-encapsulation of two water and lipid-soluble drugs in the same delivery system. The main purpose is represented by the exploitation of oil fractions isolated from amaranth seeds for the development of squalene-based nanocarriers able for a dual release of one antitumor drug, pemetrexed and one bio-flavonoid, hesperidin. The co-encapsulated nanocarriers presented unique nanoassembly morphology and showed excellent stability against aggregation. A delimited repartition of both actives, mainly in oily nanoconfinements of lipid nanocariers has been indicated by scanning calorimetry. The entrapment efficiency study revealed a great encapsulation effect with values reaching 94% for hesperidin and 89% for pemetrexed. These values are associated with a high ability of squalene-nanocarriers to capture free radicals. The greatest antioxidant activity was determined for nanocarriers that co-encapsulate 1.4% drugs, *e.g.*, 97.3 and 98.2%. *In vitro* co-release tests demonstrated that pemetrexed and hesperidin were gradually released despite of their different lipophilicity. The most concentrated squalene fraction assures a slower release of both actives. The cumulative results showed that the applied strategy is a promising approach to improve the performance of medical treatments used to prevent and treat diseases associated with tumor and oxidative stress.

1. Introduction

With the increasing public perception of a strong correlation between drugs—safety—and disease prevention, new approaches should be introduced to enrich the biomedical application field with *"health promoting substances"*. Usually, in the treatment of various diseases it is not sufficient to use highly active drugs, the effectiveness of any active being mainly dependent upon its delivery in an appropriate concentration to target cells and tissue (Borrelli et al., 2014). A great challenge comes from the difficulty to design functional delivery systems which combine low toxicity, lack of immunogenicity and biodegradability, does not accumulate in cells or tissues, and could also bring supplementary biological effects (Jyoti et al., 2015; Lacatusu et al., 2012).

In the soft matter field, nanostructured lipid carriers (NLCs) are highly suitable drug carriers that can be readily formulated in water-based systems and provide one of the most convenient colloidal carrier systems as alternative materials to polymeric nanoparticles, microemulsions, nanoemulsions and liposomes (Barbinta-Patrascu et al., 2014a; Kraft et al., 2015). The lipid nanocarriers are modified solid lipid nanoparticles in which the lipid core contains both solid and liquid lipids at body temperature with a less-ordered crystalline structure or an amorphous solid structure and their surfactants can be selected from low-cost and GRAS materials (Barbinta-Patrascu et al., 2014b; Puglia et al., 2012). Many studies demonstrated that NLCs increase the encapsulation efficiency and drug loading and are valuable option for improving the chemical stability, bioavailability and controlled release of lipophilic compounds (Liu et al., 2014; Wun How et al., 2013). In general, the conventional lipid nanoparticles delivery systems have manifested high encapsulation efficiency for hydrophobic drugs but low encapsulation efficiency for hydrophilic drugs (Severino et al., 2014; Vrignaud et al., 2012, 2011).

Recently, the co-encapsulation of drugs into nanostructured systems has been proposed as a means to promote synergic therapeutic effects. Co-encapsulation studies of lipid nanoparticles and nanocarriers started only few years ago and their benefits clearly emerge from the latest research, *e.g.*, high effi-

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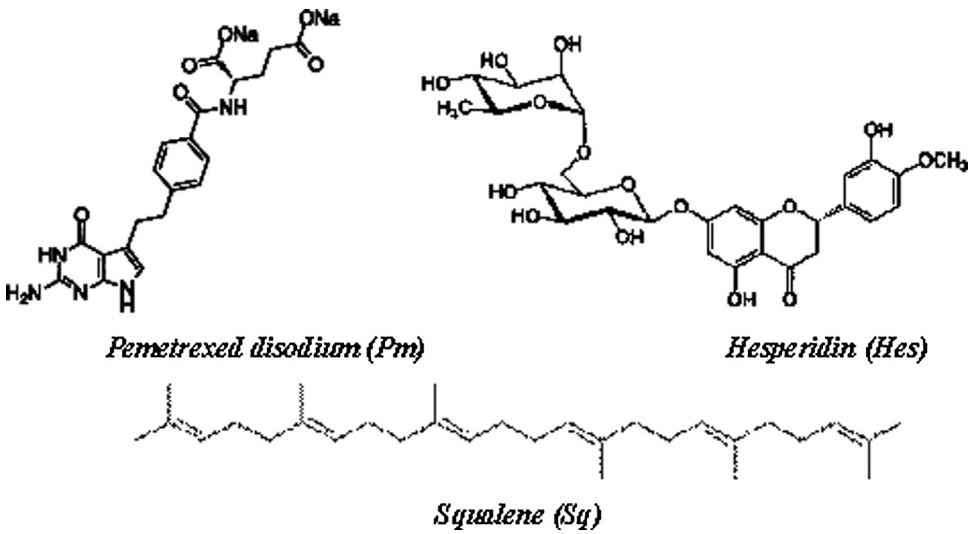

Fig. 1. The active compounds used for the development of lipid nanocarriers.

ciency for tumor targeted local delivery (Taratula et al., 2013), great cytotoxicity potential and cellular uptake against breast cancer therapy (Baek and Cho, 2015), provide an efficient reservoir system for long-term transdermal administration (Vitorino et al., 2013), improve the antioxidant activity and photostability (Coradini et al., 2014).

However, despite the great potential of NLCs in many biomedical applications, limited data are available on their association with vegetable oils that may manifest valuable therapeutic effects. For instances, the dominant role of vegetable oils on the lipid nanocarriers efficacy to improve several properties (*e.g.*, antibacterial and antioxidant activities, photoprotection, release behavior) has been already demonstrated in several studies achieved by our group (Lacatusu et al., 2014; Niculae et al., 2014).

The exploitation of plant resources to develop vegetable lipid-based nanocarriers has a high potential to strengthen the ongoing research, in order to identify the targeted delivery systems that present superior therapeutic efficacy. In this context, the present study aimed to prove that both water- and lipid-soluble actives can be converted into optimal vegetable-based nanocarriers prepared with various vegetable fractions enriched in squalene (Sq) (*e.g.*, 5.8, 34.7 and 83.4% Sq) and further exploit it as a combinatorial drug therapy.

Pemetrexed disodium (*Pm*) and hesperidin (*Hes*) were the active candidates for co-encapsulation into nanocarriers based on vegetable Sq fractions (Fig. 1). *Pm* is a multi-targeted antifolate with broad in vivo antitumor activity. It has been used for the treatment of meduloblastoma (Morfouace et al., 2014), for non-small cell lung cancer (Gautschi et al., 2015; Liang et al., 2015) and demonstrated apoptosis and cytotoxicity against osteosarcoma cells (Zhu et al., 2014). In addition, *Hes* is a bioflavonoid from citrus species that has various biological properties, particularly those for the prevention of cancer and cardiovascular diseases (Roohbakhsh et al., 2015). Studies have shown both anti-cancer and cancer chemopreventive effects, *e.g.*, induces apoptosis in colon carcinogenis (Saiprasad et al., 2014) and protective effects against oxidative stress (Javed et al., 2015; Pari et al., 2015). Beside the selected drugs, the focus is given on the prominent role of vegetable Sq fractions for improving the entire pharmacokinetic profile. Sq is a natural isoprenoid compound, a key intermediate in cholesterol synthesis and with ability to act as a sink for lipophilic molecules (Kelly, 1999).

Given the established link between Sq and its primary therapeutic adjunctive in several cancer therapies (Rao et al., 1998), their association with the dual antitumor and antioxidant actives in the same vegetable-based NLCs for specific targeting purpose, proves the sustainability and advanced research achieved in the present study.

2. Material and methods

2.1. Materials

The surfactants, Sodium colate, Synperonic PE/F68 (block copolymer of polyethylene and polypropylene glycol) and Tween 20 were obtained from Merck and Sigma Aldrich Chemie GmbH (Germany). The solid lipids, glycerol monostearate (GM) and cetyl palmitate (CP) were obtained from Cognis GmbH (Germany) and Acros Organics (USA), respectively. *Pm* was purchesd from ScinoPharm Taiwan Ltd. (Taiwan). *Tris*[Hydroxymethyl] aminomethane, 5-amino-2,3-dihydro-1,4-phthalazinedione (Luminol) and *Hes* were purchased from Sigma–Aldrich Chemie GmbH and hydrogen peroxide was obtained from Merck (Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,2 azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) and potassium persulfate were purchased from Sigma–Aldrich.

The vegetable fractions with different amounts of Sq have been obtained by cold pressed method from amaranth seeds and have been further processed by supercritical and Soxhlet extractions and analysed by liquid and gas cromatography (Merck-Lachrom High Pressure Liquid Chromatograph with UV–vis detector and variable wavelength; 6890N Agilent Gas chromatograph equipped with a flame ionization detector (FID) and 7683B auto sampler). The con-

Table 1
Main components of amaranth oily fractions.

Composition	Sq 1 fraction[a] (%)	Sq 2 fraction (%)	Sq 3 fraction (%)
C14:0 (myristic acid)	0.12	–	–
C16:0 (palmitic acid)	15.84	10.34	3.11
C16:1 (palmitoleic acid)	0.16	–	–
C18:0 (stearic acid)	2.91	2,01	0.59
C18:1 (oleic acid)	18.69	15,20	3.67
C18:2 (linoleic acid)	38.85	27.91	7.05
C18:3 (α-linolenic acid)	0.84	0.71	0.17
C20:0 (arachidic acid)	0.64	–	–
Squalene	5.8	34.67	83.39

[a] Sq—squalene.