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Formulation optimization and topical delivery of quercetin from solid lipid based nanosystems

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ABSTRACT

The presence of large amounts of reactive oxygen species (ROS) leads to oxidative stress that can damage cell membranes, lead to DNA breakage and cause inactivation of free radical scavenger enzymes, eventually resulting in skin damage. Quercetin is a natural flavonoid that has been shown to have the highest anti-radical activity, along with the ability to act as a scavenger of free radicals and an inhibitor of lipid peroxidation. In this research work, a solvent-free solid lipid based nanosystem has been developed and evaluated for topical delivery of quercetin. Systematic screening of the formulation and process parameters led to the development of a solid lipid (glyceryl dibehenate) based nanosystem using a probe ultrasonication method. The selected variant demonstrated good physical stability for up to 8 weeks at 2–8 °C. Transmission electron microscopy (TEM) images showed spherical particles in the nanometer range. *In vitro* release studies showed biphasic release of quercetin from the SLN formulation, with an initial burst release followed by prolonged release for up to 24 h. *In vitro* permeation studies using full thickness human skin showed higher amounts of quercetin to be localized within the skin compared to a control formulation with particles in the micrometer range. Such accumulation of quercetin in the skin is highly desirable since the efficacy of quercetin in delaying ultra-violet radiation mediated cell damage and eventual necrosis mainly occurs in the epidermis.

1. Introduction

Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is classified as a flavonol, a sub-class of flavonoids, that is commonly found in the plant kingdom. It is present in numerous edible fruits and vegetables such as onions, apples, berries and red grapes. Quercetin has been shown to promote a wide range of pharmacological activities related to the anti-oxidant systems of the skin, including the scavenging of oxygen radicals (Bors et al., 1990, 1994), protection against lipid peroxidation (Laughton et al., 1991) by reducing the amount of malondialdehyde (Erden Inal et al., 2001) and complexation of transition metal ions to form inert chelate complexes (Afanas'ev et al., 1989; Cao et al., 1997). In a comparative study of three flavonoids, catechin, quercetin and diosmetin, quercetin was shown to exhibit a higher antiradical activity toward hydroxyl radicals, peroxyl anions and superoxide anions due to the presence of three active functional groups in its structure (Morel et al., 1993). The expression of matrix metalloproteinase-1, responsible for skin wrinkling and loss of elasticity in both healthy and photoaged skin, has also been observed to be reduced by quercetin at both the mRNA and protein levels (Sim et al., 2007). In addition, quercetin has been shown to be a potent inhibitor of UVB-induced oxidative skin damage following topical application to the skin (Casagrande et al., 2006; Gonzalez et al., 2008). These facts coupled with its safety profile and its natural origin make quercetin a very attractive candidate for incorporation into formulations intended for topical delivery to the skin.

The aqueous solubility of quercetin has been reported to be as low as 0.55 μM (Azuma et al., 2002) with an octanol–water partition coefficient (log *P*) around 1.82 ± 0.32 (Rothwell et al., 2005). Although the log *P* of quercetin is theoretically adequate to permeate the skin, its rather limited solubility in water is believed to hinder permeation through the skin and limit bioavailability (Bonina et al., 1996). This has been confirmed by *in vitro* skin permeation studies where limited permeation of quercetin through the stratum corneum into the deeper layers of the skin has been reported (Kitagawa et al., 2009; Tan et al., 2011).

Various strategies have been attempted to improve the permeability of quercetin through the skin to facilitate topical/transdermal delivery. These include the use of permeation

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enhancers like dimethylformamide and L-menthol (Olivella et al., 2007), synthesis of ester prodrugs of quercetin (Montenegro et al., 2007), microemulsion based approaches (Censi et al., 2012; Vicentini et al., 2008) and the use of lecithin–chitosan nanoparticles (Tan et al., 2011). Most of these studies have shown some degree of quercetin permeation into the skin but no transdermal delivery. In an *in vitro* study using a Transcutol® P based microemulsion, about 0.9% of the administered dose of quercetin was detected in the receptor compartment of a Franz diffusion cell after 12 h (Censi et al., 2012). The enhancement in quercetin permeation through the skin was attributed to the ability of Transcutol® P to easily permeate the skin thereby facilitating the diffusion of the solubilized quercetin.

Occlusion of the skin surface leads to an increased hydration of the stratum corneum and reduction in the packing of corneocytes, thereby facilitating drug penetration into the deeper layers of the skin (Cevc, 2004; Zhai and Maibach, 2001). These occlusive effects have been reported to be related to the particle size, with nanoparticles (~200 nm) showing 15 times higher occlusivity than microparticles (~4 μm) (Wissing et al., 2001). Lipid based nanosystems that have been investigated for topical applications include solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC) and nanoemulsions (NE) (Schafer-Korting et al., 2007). SLN are composed of lipids that are solid at ambient temperature whereas NLC are mixtures of solid and liquid lipids. Some of the advantages of SLN and NLC include the use of physiological lipids in the composition, the avoidance of organic solvents, the possibility to produce concentrated lipid suspensions and the availability of established scale-up processes (Mehnert and Mader, 2001). Topical application of SLN and NLC based systems have been studied with various active compounds such as Vitamin E (Dingler et al., 1999), Vitamin A (Jenning et al., 2000a), clotrimazole (Souto et al., 2004), triptolide (Mei et al., 2003), retinoic acid (Castro et al., 2009) and tretinoin (Shah et al., 2007).

SLNs containing quercetin have been previously evaluated for oral delivery (Li et al., 2009) and brain delivery (Dhawan et al., 2011). Recently, nanostructured lipid carriers of quercetin have been developed using a solvent (chloroform/acetone) based emulsification technique and evaluated for topical delivery (Chen-yu et al., 2012). One of the major disadvantages of a manufacturing method involving the use of organic solvents can be toxicological issues arising from solvent residues. The aim of our study was to develop a solvent free solid lipid based nanosystem of quercetin using a probe ultrasonication process. The beneficial pharmacological action of quercetin on the skin coupled with the various advantages of SLN and NLC systems in delivering drugs topically make such a system very attractive. Compritol® 888 and Precirol® ATO 5 were selected as the solid lipids and various non-ionic and anionic surfactants were evaluated to determine their effect on SLN stability. Systematic screening of the formulations and process parameters were carried out for the development of a physically and chemically stable nanosystem. Characterization of the systems was performed and included particle size, zeta potential, morphology, crystallinity, physical stability, *in vitro* release rates of quercetin and evaluation of the formulation as a topical delivery system using full thickness human skin. All excipients evaluated in this study were deemed as safe for human use in skin delivery systems.

2. Materials and methods

2.1. Materials

Highly pure (>99%) quercetin was obtained from Merck (Darmstadt, Germany). Compritol® 888 (glyceryl dibehenate) and Precirol® ATO 5 (glyceryl palmitostearate) were donated by Gattefossé (Paramus, NJ, USA). Poloxamer 188 and sodium lauryl sulfate (SLS) were purchased from BASF (Florham Park, NJ, USA) and TensaChem S.A. (Ougree, Belgium) respectively. Tween 20 (polyoxyethylene derivative of sorbitan monolaurate), Tween 80 (polyoxyethylene derivative of sorbitan monooleate) and dioctyl sodium sulfosuccinate (DOSS) were purchased from Sigma–Aldrich Corporation (St. Louis, MO, USA). All other solvents and reagents used were of HPLC or analytical grade.

2.2. Surface tension measurement of surfactants

The surface tension of various non-ionic (Poloxamer 188, Tween 20 and Tween 80) and anionic (sodium lauryl sulfate (SLS) and dioctyl sodium sulfosuccinate (DOSS)) surfactants in deionized water were measured using the pendant drop method (Semmler and Kohler, 1999) on a contact angle goniometer (Ramé-Hart Instrument Co., Succasunna, NJ, USA). This method measures the profile of a drop hanging at the tip of a capillary. The instrument uses a proprietary edge tracing technology to precisely capture and analyze the drop dimensions and profile characteristics in order to accurately calculate the surface tension of the liquid using the Young–Laplace equation. All measurements were performed in triplicate at 25 °C.

2.3. Preparation of lipid nanoparticles

Quercetin nanoparticles were prepared using the probe ultrasonication method, which has been used previously for the production of lipid nanoparticles (Delmas et al., 2011). 0.5 g of Compritol® 888 ATO (glyceryl dibehenate) or Precirol® ATO 5 (glyceryl palmitostearate) either alone or in combination (3:2 ratio of compritol:precirol) with 0.025 g of quercetin was melted at 85 °C using a water bath. The heated mixture of solid lipid and quercetin was then mixed using sonication with 20 mL of pre-heated surfactant solution (compositions discussed in subsequent sections) at a specific speed for a pre-determined time interval (speed and sonication times described in subsequent section) at 85 °C using a Sonic Dismembrator Model 550 (Fisher Scientific, Pittsburgh, PA, USA). Since this homogenization step is carried out a temperature that is at least 10 °C greater than the melting point of the lipid, the primary product at this stage was a nanoemulsion due to the liquid state of the lipid. At the end of sonication, the mixture was dispersed into 30 mL of an ice-cold surfactant solution maintained in an ice bath. The final mixture was then sonicated at a specific speed for a predetermined time interval (speed and sonication times described in subsequent section) immersed in the ice-bath. This cooling step promoted the formation of the solid lipid nanoparticles. All bulk formulations were stored in the refrigerator at 2–8 °C till further analysis.

2.4. Freeze drying of nanoparticles

In order to evaluate the crystallinity of the quercetin nanoparticles, it was essential to convert the liquid to a dried form. Samples were dried using a freeze drying process in a Usifroid Freeze Dryer (Elancourt, France). Pre-cooling from room temperature to −50 °C was achieved at a cooling rate of 1 °C/min. The sample was then maintained at −50 °C for 1 h, followed by primary and secondary drying using a cooling rate of 1 °C/min. Since the primary purpose of drying the nanoparticles was to obtain a powder for further solid state characterization, no matrix formers were added to the solution prior to freeze drying.