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Development and evaluation of lipid nanocarriers for quercetin delivery: A comparative study of solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), and lipid nanoemulsions (LNE)

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ABSTRACT

To understand the effect of the physical state and composition of the lipid materials on the formation and performance of lipid nanocarriers, three types of carriers namely, solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC) and lipid nanoemulsions (LNE) were prepared and compared. Quercetin was used as a model nutraceutical compound to evaluate the potency of these nanocarriers to increase bioaccessibility. Among the developed nanocarriers, quercetin loaded and free NLC showed the smallest particle size (~34 and 47 nm) compared to SLN (~103 and 127 nm) and LNE (~82 and 83 nm). Encapsulation efficiency of quercetin in these nanocarriers was >90%. Stability of these nanocarriers in simulated stomach conditions was proved by their unaffected size and size distribution after incubation in simulated gastric fluid. Maximum bioaccessibility was observed with NLC and LNE (~60%) compared to SLN (~35%) and free quercetin solution (~7%). Controlled release was observed in enzyme free simulated intestinal fluid with maximum release being obtained with LNE compared to SLN and NLC. This study showed that by optimally controlling the lipid physical state and composition, it is possible to fabricate the lipid nanocarriers with desired properties.

1. Introduction

As quoted by Hippocrates, the father of modern medicine "*Let food be thy medicine and medicine be thy food*", in the last couple of decades, human beings are more aware of the pivotal link between diet, health and life style diseases like obesity, certain types of cancer, diabetes, cardiovascular diseases etc. (Kim & Park, 2011). Life style diseases are mainly caused due to unhealthy alteration on our living pattern and also by harmful effect of industrialization (air pollution, water pollution etc). These diseases can be prevented up to maximum extent by adopting healthy diet, living style and living in pollution free environment. Functional foods based on nutraceutical interventions are currently being investigated on a large-scale basis as potential treatments for these life style diseases. In recent years, there has been a growing demand and interest in the food industry to incorporate quercetin flavonols with desired health promoting and disease preventing properties into food products. Quercetin can be used as a nutritional molecule to maintain the general health in healthy people and also can be used as nutraceutical to cure disease in diseased once like obesity, diabetes, cardiovascular diseases (Hollman & Katan, 1999).

Although some bioactive compounds can be added directly into foods using extracts of plant or animal origin, hydrophobic molecules like quercetin cannot be easily incorporated into food products due to their hydrophobicity and crystallinity which results in

Abbreviations: SLN, solid lipid nanoparticles; NLC, nano structured lipid carriers; LNE, lipid nanoemulsions; EE, encapsulation efficiency; LC, loading capacity; MCT, medium chain triglyceride; HPH, high pressure homogenizer; T80, Tween 80; PDI, poly dispersity index; ELS, electrophoretic light scattering; ZP, zeta potential; SGF, simulated gastric fluid; SIF, simulated intestinal fluid; FFAs, free fatty acids; MAG, mono-acylglycerols; SOL, solution.

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reduced quantity or fraction of the ingested quercetin that is available for absorption (low bioaccessibility and bioavailability) finally results in reduced biological activity. Due to its bitter taste, direct addition into food products compromises with consumer's acceptance which in turn reduces product's commercial value. In addition, quercetin degrades quickly in alkaline condition and it is also sensitive to heat exposure. Hence maximum amount of quercetin gets degraded during product processing and shelf life (Gugler, Leschik, & Dengler, 1975; Kumari, Kumar, & Yadav, 2012; Pool, Mendoza, Xiao, & McClements, 2013).

To overcome these problems, the use of colloidal delivery system has been proposed. Colloidal delivery system, in fact, has proven to be a competent pathfinder to decipher the practical problems associated with functional or fortified food product development (Acosta, 2009; McClements, Decker, Park, & Weiss, 2009). For lipophilic molecules, various types of lipid based delivery systems like SLN, NLC, emulsions, and liposomes received considerable attention (Sagalowicz & Leser, 2010). Among the lipid based delivery systems, SLN, NLC and LNE are extensively studied. The feasibility for large scale production, nontoxicity and easy availability of excipients made these systems industrially viable. In addition, by maintaining suitable hydrophilic and lipophilic balance it is possible to fabricate nanosized carriers (Chen et al., 2010; Severino et al., 2012). Among these nanocarriers SLNs are produced using lipids which are solid at room and body temperature. If we interchange these solid lipids with liquid lipid it results in emulsions. Further if these lipid nanocarriers are produced by partially interchanging solid lipid with liquid lipid it forms nanoparticles with more internal defects called as NLC (Severino et al., 2012). This favors accommodation of more guest molecules within the core region compared to SLN (Severino et al., 2012). Thus even though SLN, NLC and LNE are composed of lipids, they differ in their physical state and composition. Although several attempts have been made to use these carriers for delivery of nutraceuticals (Muller, Shegokar, & Keck, 2011), quite less attention has been paid to compare SLN, NLC and LNE completely and comprehensively regarding their efficiency in protecting and delivering nutraceuticals.

Thus, the key purposes of this study were to produce SLN, NLC and LNE and to study the effect of difference in physical state and composition of each delivery system on nanocarrier formation and biological activity of quercetin. Effects of these critical parameters on produced delivery system were evaluated by taking following criteria as indicators: size, zeta potential, quercetin loading and encapsulation efficiency, redispersability and transparency for product development and stability in simulated gastrointestinal condition, controlled release and bioaccessibility for product performance.

2. Materials and methods

2.1. Materials

Imwitor 900 K, medium chain triglyceride (MCT) were received as free sample from Sasol Germany GmbH (Witten, Germany). Lipoid® SPC-3, pure soybean phosphatidylcholine, was purchased from Lipoid GmbH (Ludwigshafen, Germany). Tween®80 (polysorbate 80) and Span 20 were purchased from Sigma–Aldrich (Madrid, Spain). Quercetin (>95% pure) was purchased from Sigma–Aldrich (Madrid, Spain). Lipase, pepsin, bile salts were purchased from Sigma–Aldrich (Madrid, Spain). All other chemicals were of analytical grade.

2.2. Fabrication of quercetin loaded lipid nanocarriers

Three different lipid based nano carriers SLN, NLC and LNE were produced using HPH as described earlier with slight modification (Junyaprasert, Teeranachaideekul, Souto, Boonme, & Muller, 2009). Briefly, an aqueous surfactant phase (double distilled water) consisting of a hydrophilic surfactant Tween 80 (T80) was prepared and heated upto 65–68 °C before adding to the oil phase. Simultaneously, the lipid phase constituting lipophilic surfactant (lecithin and Span 20), lipophilic nutraceutical (quercetin) along with lipids of choice (SLN 100% (w/w) solid lipid (Imwitor 900 K), LNE 100%(w/w) liquid lipid (MCT) and NLC mixture of solid and liquid lipids (20:80% w/w)] was prepared and heated up to 65–68 °C prior to addition of the water phase. Hot aqueous phase was added to lipid phase and homogenized using Ultra-Turrax T25 homogenizer (IKA Labortechnik, Staufeni, Germany) at 8000 rpm for 120 s. The formed emulsion was further processed by using HPH for 10 cycles at 1200 bar (Emulsiflex C-3, Avestin, Mannheim, Germany) and the resulted emulsion was cooled to room temperature resulting in formation of SLN, NLC or LNE depending on the physical state of the used lipids. Blank lipid nanoparticles were also produced as stated above without adding quercetin to the formulation. Trehalose (1%) was added to the lipid nanoparticle dispersion and uniformly mixed and kept at −70 °C overnight. Later lyophilization was done at 0.40 mbar and −30 °C for 24 h using Labconco 6 L Plus manifold lyophilization system (Labconco Corporation, Kansas, USA). All samples were produced at least in triplicate. The compositions of the formulations were presented in Table 1.

2.3. Particle size and zeta potential

The average diameter and polydispersity index was measured on an intensity basis by means of photon correlation spectroscopy (PCS) using Nano Zeta Potential and Submicron Particle Size Analyzer (Delsa nano C, Beckman Coulter Inc., Fullerton, CA, USA) at pH 7.2 ± 0.1 and 25 °C with a scattering angle of 165° after suitable dilution (1:100) in Milli Q water. Zeta potential (ZP) of nanocarriers was assessed by determining the particle electrophoretic light scattering (ELS) using the same Nano Zeta Potential and Submicron Particle Size Analyzer at 25 °C.

In case of lyophilized samples, SLN, NLC and LNE were resuspended in deionized water prior to measurements. Both size and zeta potential measurements were performed at least in triplicate ($n \geq 3$).

Table 1
Composition of blank and quercetin loaded lipid nanocarriers.

Formulation		Solid lipid (%)	Liquid lipid (%)	Tween 80 (%)	Lecithin (%)	Span 20 (%)	Water (%)	Mono/di/tri glycerides (%)*	Quercetin (mg)
Quercetin free	BLK-SLN	5	0	4	0.5	0.5	90	55/45	–
	BLK-NLC	1	4	4	0.5	0.5	90	44/36/18	–
	BLK-LNE	0	5	4	0.5	0.5	90	>90	–
Quercetin loaded	QSLN	5	0	4	0.5	0.5	90	55/45	15
	QNLC	1	4	4	0.5	0.5	90	44/36/18	15
	QLNE	0	5	4	0.5	0.5	90	>90	10

*Mono, di- and triglyceride content in NLC was calculated by taking % of different lipids used for fabrication: BLK-SLN: blank solid lipid nanoparticles; BLK-NLC: blank nanostructured lipid carriers; BLK-LNE: blank lipid nanoemulsions; QSLN: quercetin loaded solid lipid nanoparticles; QNLC: quercetin loaded nanostructured lipid carriers; QLNE: quercetin loaded lipid nanoemulsions.