BJPS

Brazilian Journal of
Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902020000118744

Article

Nanoformulations of quercetin: a potential phytochemical for the treatment of uv radiation induced skin damages

Nancy Tripathi[1], Surajpal Verma[1,3*], Manish Vyas[1], Narendra Singh Yadav[2], Subhajit Gain[1], Gopal Lal Khatik[1]

[1]School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India, [2]French Associates Institute for Agriculture and Biotechnology of Drylands, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Midreshet Ben Gurion 84990, Israel, [3]School of Pharmaceutical Sciences, Delhi Pharmaceutical Sciences & Research University (DPSRU), Mehrauli-Badarpur Road, Puspvihar, Sector-3, New Delhi-110017, India

The continuous prolonged exposures of sun light especially the ultra violet (UV) radiation present in it, cause not only the risk of skin cancer but also it may cause premature skin aging, photodermatoses and actinic keratoses. Flavonoids (including Flavane, Flavanone, Flavone, Flavonol, Isoflavone, Neoflavone *etc.*) having potent antioxidant activity, used as topical applications for protection against UV induced skin damages as well as for skin care. Most commonly used flavonoid is quercetin (Flavonol), which is present in fruits, vegetables, and herbs. We aim to review the research focused on development of different novel formulations to treat UV radiations induced skin diseases. In this review, several formulations of flavonoid quercetin were discussed and their outcomes were compiled and compared in context to solubility, stability and efficiency of application. On the basis this comparative analysis we have concluded that three formulations, namely glycerosomes, nanostructured lipid carriers and deformable liposomes hold good applications for future aspects for topical delivery of quercetin. These formulations showed enhanced stability, increased quercetin accumulation in different skin layers, facilitated drug permeation in skin and long-lasting drug release.

KEYWORDS: Photodermatoses. Keratoses. Flavonoids. Quercetin and reactive oxygen species.

ABBREVIATIONS

ROS - Reactive oxygen species
UV radiations – Ultraviolet radiations
AP-1 - Activator Protein-1
NFkB - Nuclear Factor Kappa B
DNA - Deoxyribonucleic acid
REF - Radiations exposure
µg – Microgram
mL – Milliliter
mg- Milligram
log P- Partition coefficient
NLCs - Nanostructured lipid carriers
SLNs- Solid lipid nanoparticles
TEM - Transmission electron microscopy
QT-NLCs - Quercetin-loaded nanostructured lipid carriers
MSNs - Mesoporous silica nanoparticles
pH - Potential of hydrogen
poly-NIPAM - Poly-N-isopropylacrylamide

***Correspondence:** S. Verma. School of Pharmaceutical Sciences. Lovely Professional University. Phagwara, Punjab, India. E-mail: surajpal_1982@yahoo.co.in

HaCaT - Human keratinocytes
DPPH - 2,2-diphenyl-1-picrylhydrazyl
SCF- Supercritical fluid
EPAS - Evaporative precipitation into aqueous solution
HPH- High press homogenization
MCM-41 - Mobil Composition of Matter No. 41
TGA - Thermo gravimetric analysis
XRD - X-ray diffraction
HRTEM - High-resolution transmission electron microscopy
FT-IR Spectroscopy - Fourier-transform infrared spectroscopy
DSC - Differential scanning calorimetry
EE - Eudragit® E
PVA - Polyvinyl alcohol
1H NMR - 1H nuclear magnetic resonance
MDA - Malondialdehyde

INTRODUCTION

Sun emits radiation in the form of visible light and ultraviolet radiation (UV radiations) which reaches the Earth surface. UV radiations consists of three regions of wavelength named as UV-A (315-400 nm), UV-B (280-315 nm) and UV-C (100-280 nm) with share of 90%, 5% and 5%, respectively. The shortwave UV radiation (UV-C) has potential to cause the maximum risk. However, the UV-C does not reach to the earth surface because it gets absorbed by the earth's atmosphere. The medium wave UV-B has much more damaging effect on skin. The continuous prolonged exposures cause the risk of skin cancer (Manca *et al*., 2014a). Being the largest human organ, skin performs an important barrier function that allows the body to be protected against harmful environmental effects (Casagrande *et al*., 2006). Skin is exposed continuously to a combination of environmental agents including visible radiation, UV radiation and high oxygen concentration, which constantly threatens the integrity of cellular oxidizable structures of the skin (Vicentini *et al*., 2011). Exposure of human cells to UV radiation gives rise to two major changes that lead to harmful outcomes: chemical modifications to DNA, and generation of reactive oxygen species (ROS) (Wu *et al*., 2008). These generated ROS; include singlet oxygen, superoxide anion, hydroxyl radical, and hydrogen peroxide. These ROS have a causal relationship with oxidative stress and are capable of damaging cutaneous tissues. These also may activate transcription factors, such as AP-1 (Activator Protein-1) and NF*k*B (Nuclear Factor Kappa B), which may contribute to cell proliferation and/or apoptotic cell death. These enormous changes in skin lead to many acute and chronic effects on the skin. Out of them sunburn (erythema) is the best-known acute effect of excessive UV radiation exposure. Over the longer exposure, UV radiation not only induces degenerative changes in cells of the skin but also induces same type of changes in cells of fibrous tissue and blood vessels leading to premature skin aging, photodermatoses and actinic keratoses. In the most serious cases, skin cancers can occur (Ichihashi *et al*., 2003).

Mechanism of UV radiation induced skin damages

UV radiations mainly act on the epidermal basal layer of the skin. It leads to several direct and indirect adverse biological effects, including formation of pyrimidine photoproducts, isomerization of *trans*- to *cis*-uronic acid, induction of ornithine decarboxylase activity, stimulation of DNA synthesis, free radical production in skin, arrest of cell cycle growth, photocarcinogenesis, and photoaging. All these effects lead to reduction in antioxidant's concentration in skin which further causes reduction of skin's ability to protect itself against the free radicals generated by exposure to sunlight. This is responsible for inducing skin cancer due to damage in DNA and it also lowers the skin's efficiency system (Heim, Tagliaferro, Bobilya, 2002).

The exposure of UV radiations for about 2 hrs to the epidermal skin causes damages that can be indicated by decrease in keratinosomes, which result in formation of dyskerotic cells. After 16-18 hrs of exposure intracellular edema can be seen, this is followed by intercellular edema around keratinocytes after 30-48 hrs of UV radiations exposure (Spiclin *et al*., 2003).

Sunburn is a classic example of apoptosis. UV radiations induced apoptotic cells are phagocytized by macrophages and their number increases dramatically after UV radiations exposure.

Chemotherapy for UV radiations induced skin damages

The homeostasis between ROS and antioxidant concentration is a major mechanism in preventing damage by oxidative stress. In recent years, naturally occurring compounds such as Vitamins (e.g. vitamin A, C, and E), Polyphenolic compounds (e.g. flavonoids),

Carotenoids (e.g. β-carotene), Coenzyme Q10, Alpha-lipoic acid [(R)-5-(1,2-dithiolan-3-yl) pentanoic acid], have gained considerable attention as antioxidants. These substances can be used in diet or added to preparation for topical application. In this review, we have discussed only flavonoids, especially quercetin as potential antioxidant to improve the antioxidant machinery in skin system to cope with UV radiation induced skin damages (Ichihashi *et al.*, 2003).

Flavanoids

Flavonoids are polyphenolic compounds with potent antioxidant activity and commonly used for topical applications for protection against skin damages viz. sunburn, photoaging, skin cancer as well as for skin care (Manca *et al.*, 2014a). Flavonoids are derived from plant kingdom and are widely present in the human diet in the form of edible fruits and vegetables Table I (Ichihashi *et al.*, 2003). Chemically, flavonoids are benzo-γ-pyrone derivatives consisting of phenolic and pyran ring Figure 1 (Heim, Tagliaferro, Bobilya, 2002).

TABLE I - Classification of flavonoids, their chemical constituents and sources (Panche et al. 2016); (Ugazio *et al*. 2016)

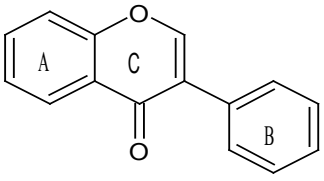
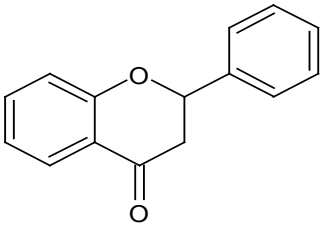
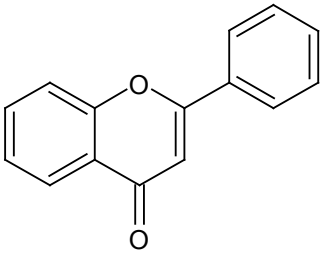
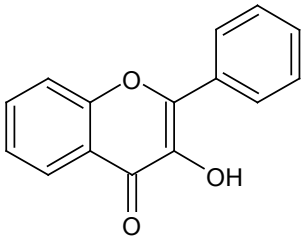
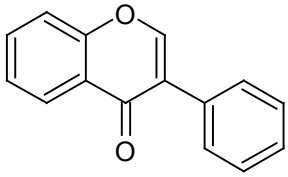
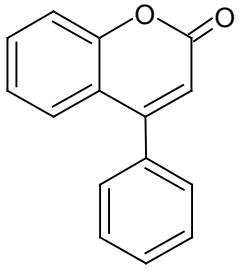
Generic structure	Flavonoid	Dietary Source	Applications
Flavane 	(+)-catechin (-)-epicatechin Epigallocatechin gallate		Anioxidant and antimicrobial agent
(continuing)			
Flavanone 	Narigin Narigenin Taxifolin Eriodictyol Hesperidin	Citrus, grapefruit Lemons Oranges	Antioxidant, anti-inflammatory, and cholesterol-lowering effect
Flavone 	Chrysin Apigenin Luteolin Luteolin glucosides Diosmetin	Fruit skins Parsley, celery Red wine, buckwheat Citrus, tomato skin, Red pepper	Gout and kidney stones treatment as xanthine oxidase modulators

TABLE I - Classification of flavonoids, their chemical constituents and sources (Panche et al. 2016); (Ugazio *et al*. 2016)

Generic structure	Flavonoid	Dietary Source	Applications
Flavonol 	Kaempferol	Leek, broccoli, endives	Antioxidant potential, lower dementia and reduced risk of vascular disease
	Rutin	grapefruit, black tea Onion, lettuce, broccoli,	
	Quercetin	tomato, tea, red wine, apples, *Ginkgo biloba*	
	Myricetin	Berries, olive oil, apple skin	
	Tamarixetin	Cranberry grapes, red wine	
Isoflavone 	Genistin Genistein Daidzin Daidzein	Soyabean	Estrogenic activity, metabolic diseases
Neoflavone 	Calophyllolide	*Calophyllum inophyllum* seeds	Estrogen-like activity

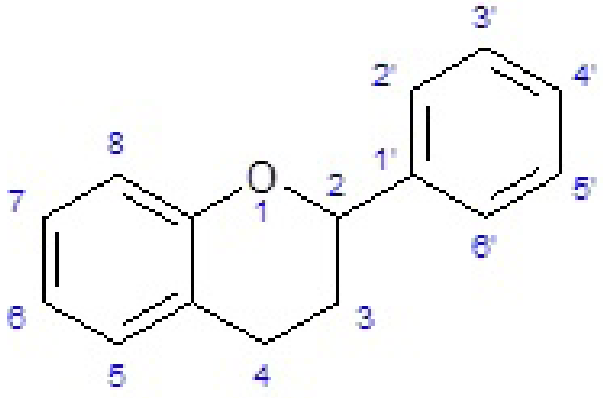


FIGURE 1 - General structure of flavonoid.

Mechanism of action of flavonoids

The general mechanism of the antioxidant activity of flavonoids involves their reactivity with the ROS and chelation of transition metal ions responsible for oxygen activation via redox reaction (Ichihashi *et al.*, 2003). Besides being exogenous efficient free radical scavengers, these can also improve the endogenous antioxidant system, suppress oxidative and nitrosative stress, decrease macrophage oxidative stress by inhibiting cellular oxygenase enzyme as well as by interfering with various signal transduction pathways (Ugazio *et al.*, 2016). Figure 2.

Most of the flavonoids are generally administered orally, but they can also be applied topically in the form of creams, lotions, gels etc. The topical use of flavonoids is very useful because it allows a synergic combination of three important advantages: the hydration of the affected skin area, the massage action that takes place during the application, the active compound release (Njeri *et al.*, 2014). Likewise, flavonoids extract also having antioxidant properties, UV protection, and anti-inflammatory properties which can enrich skin care products, adding valuable benefits to the formulations (Ugazio *et al.*, 2016).

However topical applications of flavonoids are limited because of their poor solubility, low stability and slow release after application. Moreover, chemical changes in flavonoids due to degradation may decrease the effectiveness and safety of skin care products (Miyake *et al.*, 2000). Thus, to ensure that topically administered antioxidants are effective upon skin application, an important point in the product formulation is their stabilization. Under this point of view, antioxidants are very unstable and may be converted to inactive forms before reaching the target. On the other hand, to exert the desired effect antioxidants must be properly absorbed into the skin, reach their target tissue in the active form and remain at the site of action for long period of time (Ugazio *et al.*, 2016). Table-I.

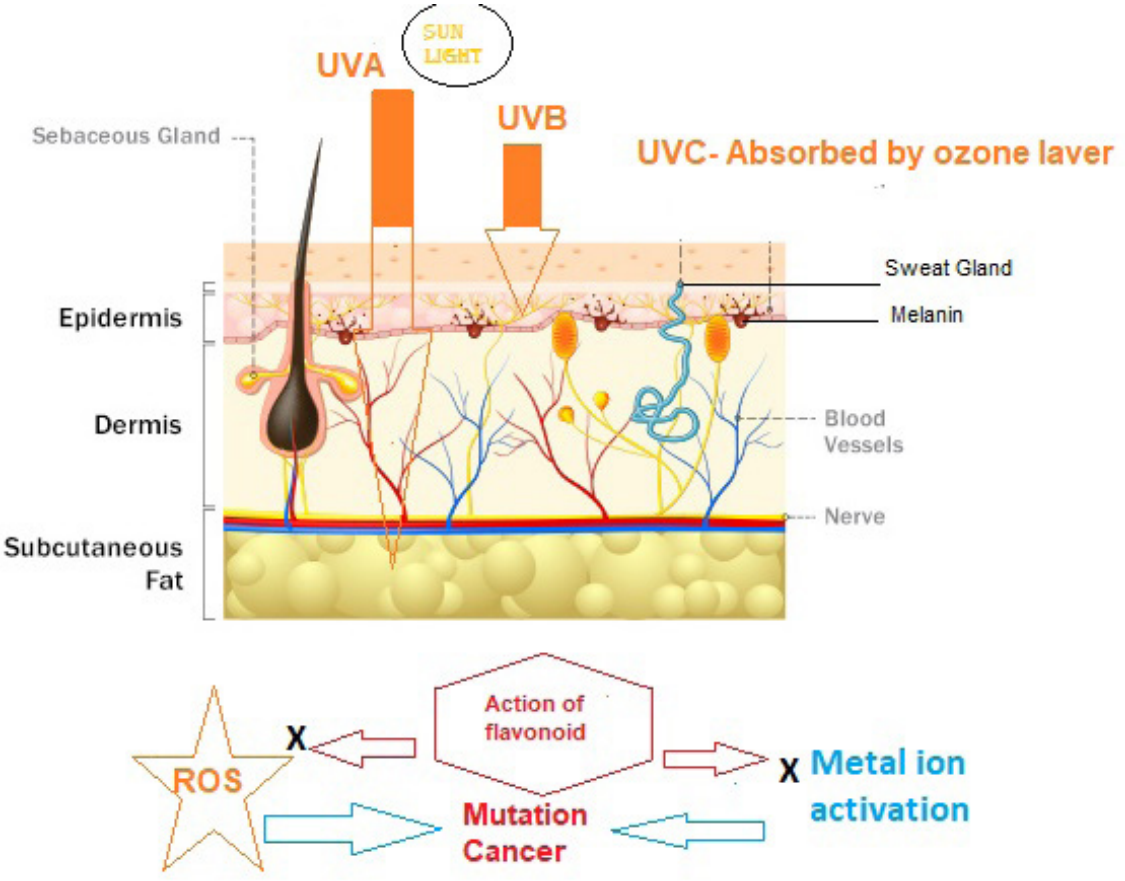


FIGURE 2 - General mechanism of action of flavonoids.

Quercetin

Quercetin is a well-known flavonoid present in fruits, vegetables, and herbs (Table I) which contains flavanol nucleus (Wu *et al*., 2008). Among flavonols, quercetin reveals a strong antioxidant activity because it can chelate transition metals, scavenge oxygen free radicals, inhibition of lipid peroxidation and *in vitro* inhibits xanthine oxidase. In addition, quercetin is also able to counteract UV-induced oxidative skin damages following topical application to the skin. In addition, its safety and natural origin make the molecule an attractive candidate for incorporation into skin care formulations (Lopez *et al*., 2001).

Quercetin is able to reduce redness, itching, and inflammation of damaged skin; it may also help restore skin barrier function, increasing hydration, and reducing water loss. (Maramaldi *et al*., 2016).

The high antioxidant activity shown by quercetin has been credited to the presence of three active functional groups in its structure the ortho-dihydroxy (catechol) moiety in the B ring, the C2 – C3 double bond in conjunction with a 4-oxo function, and the hydroxyl substitution at positions 3, 5 and 7, Figure 3 (Ichihashi *et al*., 2003). The various physicochemical properties of quercetin are shown in the Table II.

TABLE II - Physicochemical properties of quercetin (Kumar et al. 2014)

Quercetin physicochemical properties	Values
Chemical formula	$C_{15}H_{10}O_7$
Chemical name (IUPAC)	2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one
Solubility in MilliQ water	0.48 ± 0.1 µg/mL
Solubility in PBS[1] pH 3	0.44 ± 0.1 µg/m
Solubility in DMSO[2]	30 mg/mL
Solubility in ethanol	2 mg/mL

(continuing)

TABLE II - Physicochemical properties of quercetin (Kumar et al. 2014)

Quercetin physicochemical properties	Values
Partition coefficient (log P)	1.82 ± 0.3
Polymorphism	Three polymorphic forms

[1] Phosphate buffered saline; [2] Di-methyl sulphoxide

Besides these promising activities, quercetin suffers from poor water solubility and inability to penetrate skin. Quercetin shows water solubility less than 0.5µg/mL but has higher solubility in polar organic solvents (2 mg/mL in ethanol). Due to the presence of nonpolar groups in its structure, quercetin has a partition coefficient of 1.82 ± 0.32. But despite this log P value, polar hydroxyl groups in quercetin hinder its skin penetration capacity (Kumar, Kushwaha, Sharma, 2014).

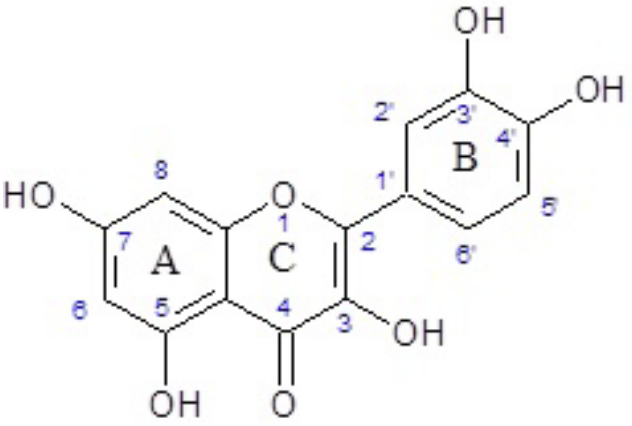


FIGURE 3 - Structure of quercetin.

The extremely low hydrophilicity and solubility of quercetin combined with its extensive metabolism by the gut microflora result in minimal absorption from the gastrointestinal tract (Ichihashi *et al*., 2003) and a very low bioavailability (less than 17% in rats and even 1% in human) with no measurable plasma concentration following its oral administration. Due to these limitations the beneficial effects of quercetin observed

in *in vitro* studies can't be directly transferred to the *in vivo* or clinical level with an oral delivery approach. In addition, quercetin is chemolabile and thermolabile and rapidly degraded and discolored when exposed to alkaline media, light and warm temperature, due to its parent skeleton of flavonoids. Therefore, the quercetin solution formulation containing solubilizer or co-solvent may undergo degradation during the production and storage (Gao *et al*., 2011). Hence, topical application of quercetin can be a very attractive formulation approach, considering the fact that topical application of quercetin has been shown to result in potent inhibition of UV-induced oxidative skin damages (Ichihashi *et al*., 2003).

Novel formulations of quercetin

Various approaches have been tried to improve the penetrability and enhance the bioavailability of quercetin are discussed here. These are:

1. Lipid based nanosystems (NLCs and SLNs) (Ichihashi *et al*., 2003; Guo *et al*., 2012)
2. Nanovesicles (Manca *et al*., 2014a)
3. Thermoresponsive mesoporous silica nanoparticles (Ugazio *et al*., 2016)
4. Nanosuspension (Gao *et al*., 2011)
5. Aminopropyl functionalized mesoporous silica nanoparticle (NH2-MSN) (Sapino *et al*., 2015)
6. Ceramide liposome-in-hydrogel complex system (Park *et al*., 2013)
7. Polymeric nanoparticles (Wu *et al*., 2008)
8. Deformable liposomes (Kim *et al*., 2015)

1. Lipid based nano-systems

Lipid based nano-systems such as solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) have been earlier show enhanced and improved delivery of several agents like glucocorticoids, Vitamin A and betamethasone (Maia, Mehnert, Schäfer-Korting, 2000 and Jenning, Thünemann, Gohla, 2000) to the specific skin layers. This formulation approach has also been used to prepare solid lipid based nano-systems of quercetin and check their practicability for topical delivery.

As compared to the control formulation with particle size in the micrometer range, the optimized SLN formulation showed better topical delivery of quercetin with statistically significant differences. However, the samples placed on stability at 2-8 ºC showed an increase in the particle size after 8 weeks. This increase in particle size could be credited to lipid transformation of the solid lipid (glyceryl dibehenate) used in these nanoparticles over time, which leads to the formation of a highly ordered lipid structure resulting in drug discharge from the SLN system. The morphology of the nanoparticles visualized using TEM and X-ray diffraction patterns confirmed the lipid transformation. In order to minimize the rigidity, ordered structure of the lipid matrix and insert imperfections in the matrix to reduce drug expulsion upon storage, nanostructured lipid carriers (NLCs) were prepared. These are second generation lipid-based nanoparticles and are prepared by substituting the solid lipid used in the SLN formulation with a liquid lipid (Muller, Radtke, Wissing, 2002).

Based on solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLCs) were developed which are second generation lipid-based nanoparticles (Muller, Radtke, Wissing, 2002). Liquid lipid introduced into NLCs can disturb the highly regular lattice structure, form an imperfect matrix structure and further increase the space for accommodating drugs (Saupe, Gordon, Rades, 2006). Thus, it overcomes the drawbacks of SLN including relatively low drug payloads and potential drug expulsion. For the unique properties of NLCs many studies focused on their topical application in recent years (Pardeike, Schwabe, Müller, 2010; Mitri *et al*., 2011; and Nikolić *et al*., 2011). Moreover, NLCs can increase the apparent solubility of incorporated drugs that can form high concentration gradient on skin to facilitate drug permeation (Castangia *et al*., 2015).

The nano-sized particles can firmly stick to the surface of skin and transport the drugs in a more controlled manner. The NLCs can improve skin hydration and promote drug penetration by exerting their occlusive effect. Further, the components of NLCs like lipid and surfactants can act as permeation enhancers and loses the lipid bilayers of stratum corneum (Ruktanonchai *et al*., 2009). NLC has been also considered as a safe and novel carrier for skin application of antioxidant (Jia *et al*., 2010). The aim of the study was to design and characterize Quercetin-loaded nanostructured lipid carriers (QT-NLCs). Evaluation of *in vitro* drug permeation through mouse skin and *in vivo* drug distribution in epidermis and dermis of mice was done. Based on the observation under light microscope, the effect of application of quercetin NLCs on the skin

surface was also discussed. In addition, the potential use of NLCs for topical delivery of quercetin for anti-oxidant effects was investigated. In the study, the quercetin, a poorly soluble drug was successfully incorporated, into NLCs by an emulsion evaporation solidification method at low temperature. The results obtained confirmed the potential of NLCs as carriers for topical administration. *In vitro* and *in vivo* skin permeation studies displayed that quercetin NLCs could enhance the amount of drug retention in epidermis and dermis as compared to quercetin propylene glycol solution. Studies on effect of quercetin NLCs on skin surface confirmed that quercetin NLCs could facilitate drug permeation in skin by weakening the barrier function of stratum corneum. This study also provides additional evidences that NLCs have a targeting and prolonged release effect with better potentials in dermal delivery (Li *et al*., 2009).

The formulations belong to NLCs can provide sustained release of quercetin also provide stability for a longer period of time. But formulations containing SLNs were not stable for longer period of time and having problems of increase particle size and drug discharge on storage.

2. Nanovesicles

In this study, to improve the antioxidant activity of quercetin against skin damages, it was entrapped in glycerosomes (new phospholipid-glycerol vesicles) (Santoso *et al*., 2002). The physico-chemical study showed that with increase in glycerol concentration, there is a strong, dose-dependent, interaction of glycerol with the polar portions of the phospholipid molecules, with enhanced vesicle stability as the glycerol concentration increased. Furthermore, glycerosomes were able to promote the accumulation of quercetin in the different skin layers. The vesicles with the highest glycerol concentration increased quercetin deposition in the deeper skin layers. 30–50% glycerosomes, which have been studied, displayed the best potential features as cutaneous delivery system of quercetin. All quercetin loaded vesicles showed a marked free radical-scavenging ability and protected human keratinocytes *in vitro* from damage due to oxidative stress. In addition, 40-50% glycerosomes were rapidly internalized in these cells. Therefore, results demonstrate that quercetin-loaded 40-50% glycerosomes are the most promising carriers for the treatment of skin problems associated with oxidative damage (Castangia *et al*., 2015).

Nanovesicles can provide an improved technique to deliver quercetin as topical application because these formulations having long time period stability as well as better antioxidant activity.

3. Thermoresponsive mesoporous silica nanoparticles (MSN)

Currently, mesoporous silica nanoparticles (MSNs) have been proposed as carriers of active ingredients in the dermo cosmetic field (Hudson *et al*., 2006; Berlier *et al*., 2013a; Sapino *et al*., 2015). They are denoted by an ordered mesoporous structure and high biocompatibility. Moreover, it is possible to regulate the delivery of a bioactive agent in response to different stimuli including light, temperature, pH, electric fields, or chemicals (e.g. enzymes) by grafting functional moieties on their surface. Functional moieties used for this purpose are usually smart polymers which can protect the drug until it reaches the site of action and can then regulate its release to obtain the desired release profile (Doadrio *et al*., 2015). Smart polymers are materials that respond in a fast and considerable way to very slight changes in the environment. An example of such polymer is Poly-(N-isopropylacrylamide) (poly-NIPAM) which is a temperature-responsive polymer that was first synthesized in 1950s. Usually, for potential *in vivo* applications, carriers must retain active molecules tightly before usage, but should release them in the application site as per individual requirements. Recently, variable stimuli-responsive systems based on MSNs have been designed for drug delivery. This technique can be very useful in order to retain the effectiveness of antioxidants in skin care products during shelf-life and the period-after-opening. In this study, MSNs functionalized with a thermoresponsive co-polymer of NIPAM were developed and used for delivery of flavonoid quercetin which is characterized by poor water solubility, low stability and a short half-life, effectively to skin (Gloria *et al*., 2013). MSNs have often been proposed to control the release of drugs and active compounds, but to our knowledge their use in cutaneous preparations has not been much documented so far. This work aims to realize an innovative delivery system able to preserve the physico-chemical and biological properties of labile active ingredients of dermo cosmetic interest until their release in the skin, and to trigger the release according to the temperature condition of the application site (Zarzyka,

Lorenzo, Pyda, 2014). The antioxidant quercetin has been chosen as a model molecule, while copolymer-grafted MSNs with two different mesoporous sizes have been successfully prepared: both these systems displayed biocompatibility with immortalized human keratinocytes (HaCaT), particularly within 24 h. *In vitro* release profiles of quercetin /copoly-MSNs complexes demonstrated that thermoresponsive properties, more evident in the case of larger pores, could be further improved by optimizing the size of the grafted chains. DPPH and Fe^{2+}-ferrozine assays demonstrated that the antioxidant efficacy of quercetin was maintained upon immobilization in the siliceous nanoparticles and it was likely improved by the intrinsic radical scavenging capability of the carrier. Finally, ex vivo studies of quercetin accumulation and permeation through the skin from a hydrogel formulation underlined a certain role of the copolymer in hindering quercetin release and its interaction with the skin barrier. The research presented here obviously needs further studies, especially as for the relationship between surface properties of the copolymer-grafted MSNs and the physico-chemical characteristics of the overall formulation. However, based on these preliminary results, this innovative system can be considered as a promising and strategic approach to control the skin delivery of antioxidants and could be implemented to increase the efficacy of other active compounds (Manca *et al*., 2014b).

The data shown that MSN big having particle size in the range of 295-361 nm, 32.4% loading for quercetin and more than 53% antioxidant activity. The MSN small formulation having particle size in the range of 181-219 nm, 33.5% loading for quercetin and more than 72% antioxidant activity. Hence, the formulation containing small MSN having better antioxidant efficiency as compared to the formulation containing big MSN.

4. Nanosuspension

Currently, to tackle the formulation issue of the poorly soluble drugs, the nanosuspension technology has been successfully applied. Nanosuspension is a carrier-free colloid drug delivery system containing only minimum stabilizers and pure drug particles with a mean particle size in the nanometer range, typically between 10 and 1000 nm (Keck, Muller, 2006). The nanosuspension offer various advantages like enhanced drug solubility, high dispersity and homogenization, intravenously injectable, simple production process, universal adaptivity that enable its applications in the formulation of poorly soluble compounds. Moreover, the formation of suspensions is one of effective approaches for the stabilization of chemolabile molecules that are insoluble in aqueous solution (Moschwitzer *et al*., 2004). Generally, according to the differences of the production principle, the nanosuspension techniques are classified as bottom up processes and top down processes (Keck, Muller, 2006).

In the bottom up processes, the poorly water-soluble drug is first dissolved in an organic solvent and then precipitated through addition of a non-solvent in the presence of stabilizers, as in supercritical fluid (SCF) technology, evaporative precipitation into aqueous solution (EPAS), spray-freezing into liquid process, and emulsion-solvent evaporation (Rabinow, 2004). These processes were simple and cost effective without any high energy input. However, the following prerequisites should be met: (i) the drug should be soluble at least in one solvent and (ii) the solvent should be miscible with a non-solvent (Junyaprasert, Morakul, 2015).

The top down processes involve the mechanical comminution processes of larger drug particles, as in media milling, micro fluidization and high press homogenization (HPH). These methods don't require harsh solvents but involve high energy input and low power efficiency (Gutierrez-Villanueva *et al*., 2009). Since there is generation of heat during comminution process so, some additional measures are required to minimize the degradation of heat sensitive drugs.

This study aimed at comparing the EPAS process (bottom-up) and the HPH process (top-down) for the preparation of quercetin nanosuspension. The characterization of quercetin nanosuspension was done in terms of particle size, size distribution, thermal properties and X-ray powder diffraction. The comparison of solubility and dissolution behavior of quercetin before and after EPAS and HPH process was also done. At last, the chemical stability and photostability of nanosuspension were determined.

The nanosuspension formulations developed by both the methods that is EPAS and HPH were not provided good results for antioxidant activity although these having appropriate particle size for topical formulations.

5. Aminopropyl functionalized mesoporous silica nanoparticles

Mesoporous silica is expected to have relatively good biocompatibility and stability, even if its fate in physiological fluids and the actual effect on cells remains the object of many investigations, especially when nanoparticles are employed (Fontecave, 2012).

The immobilization of quercetin in aminopropyl-functionalized MCM-41 mesoporous silica nanoparticles were also reported. By spectrophotometry or thermo gravimetric analysis (TGA), the quercetin-loading capacity was determined (Berlier *et al*., 2013b). A particular attention was devoted to the physico-chemical properties of the inclusion complex. Therefore, to characterize the nanoparticles before and after complexation, several analytical techniques (XRD, HRTEM, N_2 adsorption analysis, FT-IR spectroscopy, zeta potential measurements and DSC) were employed. The quercetin diffusion through a cellulose membrane was studied, and the photostability of the included molecule was analyzed upon UV radiation. With a view to a possible use of quercetin in topical formulations, an *ex vivo* study using Franz diffusion cells was performed to investigate the influence of the inclusion in silica nanoparticles on the skin uptake of quercetin. Further, in order to determine the chemoprevention potential of this innovative nanosized complex compared to that of free quercetin, an *in vitro* cytotoxicity on JR8 human melanoma cells was tested (Doadrio *et al*., 2015; Jadhav *et al*., 2017).

The percent drug loading by this method was very less. Hence, it is not suitable to formulate the Aminopropyl functionalized mesoporous silica nanoparticles for the quercetin.

6. Ceramide liposome-in-hydrogel complex system

Hydrogels are three-dimensional networks which are composed of hydrophilic polymers that retain a large amount of water without dissolving by swelling in aqueous solution. Currently, the development of cellulose-based hydrogels has been actively explored. Hydrogels based on cellulose have high permeation of active constituents, good biodegradable properties, high degree of swelling, and no irritation or toxicity is associated with them. Hydrogels are extensively used in the pharmaceutical and medical fields as drug delivery vehicles due to their high biocompatibility (Peppas *et al*., 2000).

Liposomes have a phospholipid composition which resembles the lipid bilayer of cell membranes in the body. Moreover, within the internal space of liposomes both hydrophilic and hydrophobic drugs can be loaded. They are also associated with low toxicity and high biocompatibility. Due to these reasons, liposomes are extensively used as a drug delivery system (El Maghraby, Barry, Williams, 2008). Quercetin is reported to act as strong antioxidant (Tusevski *et al*., 2014). It has been widely used as antioxidant in cosmetics, but due to poor aqueous solubility their use is limited. Thus, investigation has been done to improve their solubility in water (Kreilgaard, 2002). Another study includes designing of a two-step delivery system containing ceramide liposomes composed of biocompatible membranes and porous cellulose hydrogel to enhance transdermal permeation of quercetin (Fry, White, Goldman, 1978). The entrapment efficiency from this formulation was 41.5% but the antioxidant activity was not reported.

7. Polymeric nanoparticles

Due to poor solubility of quercetin, the clinical studies investigating different programs of its administration have been limited. For the drug delivery of water-insoluble compounds, nanoparticles are particularly useful. Therefore, using a simple nanoprecipitation technology with Eudragit® E (EE) and polyvinyl alcohol (PVA) as carriers, the novel quercetin nanoparticles system was prepared The quercetin nanoparticles were evaluated for physico-chemical characterization by transmission electron microscopy (TEM), differential scanning calorimetry (DSC), powder X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), 1H nuclear magnetic resonance (1H NMR), and dissolution study (Rabinow 2004; *Moschwitzer et al*., 2004) Also, the antioxidant effects of pure quercetin and of its nanoparticles were also evaluated by free radical scavenging, anti-superoxide formation (Ugazio *et al*., 2016), anti-lipid peroxidation, and scavenging superoxide studies (Sahil *et al*., 2011). The results obtained from this formulation were good as entrapment efficiency was about 99% and particle size is about 82nm. But the antioxidant activity was not reported.

8. Deformable liposomes

As drug carriers, liposomes have several advantages like high solubility, better stability and enhancement of cellular uptake. For the last decades, topical delivery of drugs by liposomal formulations has evoked a considerable interest. But contrary to the previous findings, it became evident that classic liposomes are of either little or no value as carriers for transdermal drug delivery as they do not deeply penetrate skin and remain confined to upper layers of the stratum corneum. Confocal microscopy studies showed that intact liposomes were not able to penetrate into the granular layers of the epidermis (Junyaprasert, Morakul, 2015).

Current approaches in modulating drug delivery through skin have resulted in the design of deformable liposomes. They consist of phospholipids and an edge activator. An edge activator is generally a single chain surfactant that destabilizes lipid bilayers of the vesicles and enhances deformability of the bilayers. sodium cholate, span 80, tween 80, dipotassium glycyrrhizinate were employed as edge activators. Deformable liposomes are able to improve the skin delivery of a variety of chemical agents as compared to conventional liposomes. They are also able to penetrate intact skin, *in vivo*, transferring therapeutic amounts of drugs, with efficiency comparable with that of subcutaneous administration (El Maghraby *et al*., 2008; Rakesh, Anoop, 2012). It was suggested that deformable liposomes could respond to external stress by rapid shape transformations requiring low energy (Gangwar *et al*., 2012). Currently, they are used as a carrier for protein, peptides (Kulkarni *et al*., 2011) and non-steroidal anti-inflammatory agents

(Cagdas, Sezer, Bucak, 2014). But, very few studies have been performed to show that deformable liposomes with a flavonoid antioxidant can protect against UV-radiation damages (Dong *et al*., 2016).

This study was carried out to investigate the potential application of deformable liposomes for delivery of quercetin for treating UV radiation-induced cellular damages. Here, the preparative method and physico-chemical properties of quercetin deformable liposomes were discussed. In addition, the protective effects of deformable quercetin liposomes against UV (280–320 nm)-induced reactive oxygen species-generation, the malondialdehyde (MDA) level, and cell death in human skin keratinocyte HaCaT were investigated. Finally, it's *in vivo* effect was observed using mice exposed to UV-radiation (Pignatello *et al*., 2006).

The formulation as deformable liposomes containing quercetin is very effective for the treatment of UV radiation-induced skin disorders, this having proof of pre-clinical studies. These finding further required to evaluation under clinical trials.

CONCLUSION

Quercetin, a well-known and extensively studied flavonoid which present in fruits, vegetable, and herbs, and has been reported as benchmark discovery in treatment of UV- induced skin damages. Our skin is in continuous exposure to UV radiations which induce harmful modifications in skin such as chemical modification of DNA, and generation of ROS. These ROS are capable of damaging cutaneous tissues. Quercetin structure has ortho-dihydroxy (catechol) moiety, the C2–C3 double bond in conjunction with a 4-oxo function, and the hydroxyl substitution at positions 3, 5 and 7 which has imparted high antioxidant property to quercetin. Beside these promising activities, quercetin has poor water solubility (< 0.5µg/mL) and inability to penetrate skin which was a major problem in delivering the drug to the skin. To combat this problem extensive research was done by various research groups to develop a preparation which has effective delivery to the skin.

In this report several formulations of quercetin were discussed and their outcomes were compiled to find a suitable formulations Table III. From this study, it can be suggested that three formulations, namely nanovesicles, nanostructured lipid carriers and deformable liposomes holds good applications for the future for topical delivery of quercetin.

TABLE III - Comparative study of different formulations of Quercetin for topical application (Ichihashi *et al*. 2003); (Guo *et al*. 2012); (Manca *et al*. 2014a); (Ugazio *et al*. 2016); (Gao *et al*. 2011); (Sapino *et al*. 2015); (Park *et al*. 2013); (Wu *et al*. 2008) and (Yuri *et al*. 2015)

Formulation	Preparation technique	Excipients	Particle size (nm)	PDI	Surface charge (mV)	Entrapment Efficiency (%)	Antioxidant activity (%)
Lipid based nanosystems	Probe Ultra Sonication	Compritol 888, Glyceryl dibehenate, Olic acid	281.9 ± 2.9	0.31± 0.1	-36.57 ± 2.67	NS	NS
Nanovesicles Glycerosomes	Thin film hydration method	Lecithin, Glycerol	80±3.0	0.26± 0.2	-67.0 ± 3.0	81.0 ± 1.0	95 ± 1.0
Liposomes			102 ± 3.0	0.32± 0.1	-78.0 ± 2.0	88.0 ± 3.0	87 ± 2.0
Thermoresponsive mesoporous silica nanoparticles (MSN)	Q/ copoly-MSN Big	CTAB, TEOS, NaOH TMB	328 ± 33.0	NS	-20.6 ± 0.7	32.4 (Q-loading)	53 ± 4.0
	Q/ copoly-MSN small		200 ± 19.0	NS	-21.3 ± 0.5	33.5 (Q-loading)	72 ± 2.0
Nanosuspension	EPAS	Ethanol, Pluronic F68, Lecithin	251.56±24.6	0.23± 0.1	-21.12 ± 2.7	NS	NS
	HPH		192.47±31.8	0.21 ± 0.1	-22.48 ± 4.6	NS	NS
Aminopropyl functionalized mesoporous silica nanoparticles (NH_2-MSN)	Sol-gel method	N-cetyl-trimethylammonium bromide, Tetraethyl orthosilicate	250 ± 50.0	NS	+13.6 ± 0.2	8 (drug loading %)	NS
Ceramide liposomes-in-hydrogel complex system	Thin film hydration method	Egg PC, Ceramide, Cholesterol, Oleic acid	NS	NS	NS	41.5	NS
Polymeric nanoparticle	Nanoprecipitation technique	PVA, Eudragit-E	82 ± 5.0	0.22± 0.1	NS	99.9 ± 0.6	NS
Deformable liposomes	Ethanol injection method	Lecithin, Cholesterol, Tween-80	132 ± 14.0	NS	21.1 ± 8.0	80.4 ± 4.2	NS

CTAB -Cetyl trimethylammonium bromide, TEOS- Tetraethoxysilane, TMB- 3,3',5,5-tetramethylbenzidine, NS- Not specified

The glycerosomes and liposomes showed enhanced stability, increased quercetin accumulation and deposition in different skin layers. The vesicles showed a marked free radical-scavenging activity and protection against oxidative stress induced damages having antioxidant activity of 95%.

Nanostructures lipid carriers enhanced the drug retention in epidermis and dermis, facilitated drug permeation in skin by weakening the barrier function of stratum corneum, and also had a targeting and prolonged release effect with better potentials in dermal delivery. Its encapsulation efficiency (EE) was found to be 89.95%.

Deformable liposomes showed high EE about 80.4%, small particle size around 132 nm, negative charge, and long-lasting drug release. The deformable liposomes also suppressed the levels of ROS and MDA induced by UV-radiation. It enhanced the flux of quercetin in the skin and also protected the skin from photo damage caused by UV-radiation.

The formulations containing SLNs were not stable for longer period of time and having problems of increase particle size and drug discharge on storage. Similarly, thermoresponsive mesoporous silica nanoparticles (MSN) as big size having particle size in the range of 295-361nm, 32.4% loading for quercetin and more than 53% antioxidant activity. The smaller size formulation having particle size in the range of 181-219nm, 33.5% loading for quercetin and more than 72% antioxidant activity. Hence, the formulation containing small MSN having better antioxidant efficiency as compared to the formulation containing big MSN. But stability is the main issue with these types of formulations. The nanosuspension formulations developed by both the methods that is EPAS and HPH were not provided good results for antioxidant activity although these having appropriate particle size for topical formulations.

Aminopropyl functionalized mesoporous silica nanoparticles has very less drug loading. The ceramide liposome-in-hydrogel complex system and polymeric nanoparticles had good entrapment efficiency but the antioxidant activity was not reported for these formulations.

Despite achieving extremely small particle size with nano-dosage forms, still the lipid content and the type of lipid seem to be the main determinant for the extent of quercetin present in the depth of skin layers. Therefore, advance studies should be performed to get detail understanding about the exact depth that a quercetin formulation can achieve. At the same time, more research should be made to investigate other possible applications for quercetin in other skin disorders such as psoriasis or atopic dermatitis.

CONFLICT OF INTEREST

Authors declared that no conflicts of interest.

REFERENCES

Berlier G, Gastaldi L, Sapino S, Miletto I, Bottinelli E, Chirio D, Ugazio E. MCM-41 as a useful vector for rutin topical formulations: Synthesis, characterization and testing. Int J Pharm. 2013a;457(1):177-186. doi:10.1016/j.ijpharm.2013.09.018.

Berlier G, Gastaldi L, Ugazio E, Miletto I, Iliade P, Sapino S. Stabilization of quercetin flavonoid in MCM-41 mesoporous silica: Positive effect of surface functionalization. J Colloid Interface Sci. 2013b;393(1):109-118. doi:10.1016/j.jcis.2012.10.073.

Cagdas M, Sezer AD, Bucak S. Chapter 1-liposomes as potential drug carrier systems for drug delivery. *Nanotechnol Nanomater »* "Application Nanotechnol Drug Deliv. IntechOpen. 2014:1-50. doi: http://dx.doi.org/10.5772/58459.

Casagrande R, Georgetti SR, Verri WA Jr., Dorta DJ, dos Santos AC, Fonseca MJ. Protective effect of topical formulations containing quercetin against UVB-induced oxidative stress in hairless mice. J Photochem Photobiol B Biol. 2006;84(1):21-27. doi:10.1016/j.jphotobiol.2006.01.006.

Castangia I, Nacher A, Caddeo C, Diez-Sales O, Catalán-Latorre A, Fernàndez-Busquets A, et al. Therapeutic efficacy of quercetin enzyme-responsive nanovesicles for the treatment of experimental colitis in rats. Acta Biomater. 2015;13(January):216-227. doi:10.1016/j.actbio.2014.11.017.

Doadrio A, Salinas A, Sánchez-Montero J, Vallet-Regí M. Drug release from ordered mesoporous silicas. Curr Pharm Des. 2015;21(42):6213-6819. doi:10.2174/1381612822666151106121419.

Dong Y, Mosquera-Giraldo LI, Taylor LS, Edgar KJ. Amphiphilic Cellulose Ethers Designed for Amorphous Solid Dispersion via Olefin Cross-Metathesis. Biomacromolecules. 2016;17(2):454-465. doi:10.1021/acs.biomac.5b01336.

El Maghraby GM, Barry BW, Williams AC. Liposomes and skin: From drug delivery to model membranes. Eur J Pharm Sci. 2008;34(4-5):203-222. doi:10.1016/j.ejps.2008.05.002.

Fontecave T, Sanchez C, Azais T, Biossere C. Chemical Modification As a Versatile Tool for Tuning Stability of Silica Based Mesoporous Carriers in Biologically Relevant Conditions. Chem. Mater. 2012; 24 (22):4326–4336. doi:10.1021/cm302142k.

Fry DW, White JC, Goldman ID. Rapid separation of low molecular weight solutes from liposomes without dilution. Anal Biochem. 1978;90(2):809-815. doi:10.1016/0003-2697(78)90172-0.

Gangwar M, Singh R, Goel RK, Nath G. Recent advances in various emerging vescicular systems: An overview. Asian Pac J Trop Biomed. 2012;2(SUPPL.):S1176-S1188. doi:10.1016/S2221-1691(12)60381-5.

Gao L, Liu G, Wang X, Liu F, Xu Y, Ma J. Preparation of a chemically stable quercetin formulation using nanosuspension technology. Int J Pharm. 2011;404(1-2):231-237. doi:10.1016/j.ijpharm.2010.11.009.

Guo CY, Yang CF, Li QL, Tan Qi, Xi YW, Liu WN, et al. Development of a Quercetin-loaded nanostructured lipid carrier formulation for topical delivery. Int J Pharm. 2012;430(1-2):292-298. doi:10.1016/j.ijpharm.2012.03.042.

Gutierrez-Villanueva JL, Martin-Martin A, Pena V, Iniguez MP, de Celis B, de la Fuente R. Calibration of a portable HPGe detector using MCNP code for the determination of137Cs in soils. J Environ Radioact. 2008;99(10):1520-4. doi:10.1016/j.jenvrad.2007.12.016.

Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. J Nutr Biochem. 2002;13(10):572-584. doi:10.1016/S0955-2863(02)00208-5.

Hudson S, Tanner DA, Redington W, Magner E, Hodnett K, Nakahara S. Quantitative TEM analysis of a hexagonal mesoporous silicate structure. Phys Chem Chem Phys. 2006;8(29):3467. doi:10.1039/b605581h.

Ichihashi M, Ueda M, Budiyanto A, Bito T, Oka M, Fukunaga M, et al. UV-induced skin damage. Toxicology. 2003;189(1-2):21-39. doi:10.1016/S0300-483X(03)00150-1.

Jadhav SA, Scalarone D, Brunella V, Ugazio E, Sapino S, Berlier G. Thermoresponsive copolymer-grafted SBA-15 porous silica particles for temperature-triggered topical delivery systems. Express Polym Lett. 2017;11(2):96-105. doi:10.3144/expresspolymlett.2017.11.

Jenning V, Thünemann AF, Gohla SH. Characterisation of a novel solid lipid nanoparticle carrier system based on binary mixtures of liquid and solid lipids. Int J Pharm. 2000;199(2):167-177. doi:10.1016/S0378-5173(00)00378-1.

Jia L, Zhang D, Li Z, Duan C, Wang Y, Feng F, et al. Nanostructured lipid carriers for parenteral delivery of silybin: Biodistribution and pharmacokinetic studies. Colloids Surfaces B Biointerfaces. 2010;80(2):213-218. doi:10.1016/j.colsurfb.2010.06.008.

Junyaprasert VB, Morakul B. Nanocrystals for enhancement of oral bioavailability of poorly water-soluble drugs. Asian J Pharm Sci. 2015;10(1):13-23. doi:10.1016/j.ajps.2014.08.005.

Keck CM, Muller RH. Drug nanocrystals of poorly soluble drugs produced by high pressure homogenisation. Eur J Pharm Biopharm. 2006;62(1):3-16. doi:10.1016/j.ejpb.2005.05.009.

Kim YA, Tarahovsky YS, Yagolnik EA, Kuznetsova SM, Muzafarov EN. Integration of Quercetin-Iron Complexes into Phosphatidylcholine or Phosphatidylethanolamine Liposomes. Appl Biochem Biotechnol. 2015;176(7):1904-913. doi:https://doi.org/10.1007/s12010-015-1686-z.

Kreilgaard M. Influence of microemulsions on cutaneous drug delivery. Adv Drug Deliv Rev. 2002;54(SUPPL.). doi:10.1016/S0169-409X(02)00116-3.

Kulkarni R, Yadav JD, VaidyaKA and Gandhi PP. Transferosomes: an emerging tool for transdermal drug delivery. Int J Pharm Sci Res. 2011;2(4):735-741. doi: http://dx.doi.org/10.13040/IJPSR.0975-8232.2(4).735-41.

Kumar A, Kushwaha V, Sharma PK. Pharmaceutical microemulsion: Formulation, characterization and drug deliveries across skin. Int J Drug Dev Res. 2014;6(1):1-21.

Li HL, Zhao X Bin, Ma YK, Zhai GX, Li LB, Lou HX. Enhancement of gastrointestinal absorption of quercetin by solid lipid nanoparticles. J Control Release. 2009;133(3):238-244. doi:10.1016/j.jconrel.2008.10.002.

Lopez M, Martinez F, Del Valle C, Orte C, Miro M. Analysis of phenolic constituents of biological interest in red wines by high-performance liquid chromatography. J Chromatogr A. 2001;922(1-2):359-363. doi:10.1016/S0021-9673(01)00913-X.

Maia CS, Mehnert W, Schäfer-Korting M. Solid lipid nanoparticles as drug carriers for topical glucocorticoids. Int J Pharm. 2000;196(2):165-167. doi:10.1016/S0378-5173(99)00413-5.

Manca ML, Castangia I, Caddeo C, Pando D, Escribano E, Valenti D, et al. Improvement of quercetin protective effect against oxidative stress skin damages by incorporation in nanovesicles. Colloids Surfaces B Biointerfaces. 2014a;123:566-574. doi:10.1016/j.colsurfb.2014.09.059.

Manca ML, Castangia I, Matricardi P, Lampis S, Fernandez-Busquets X, Fadda AM, et al. Molecular arrangements

and interconnected bilayer formation induced by alcohol or polyalcohol in phospholipid vesicles. Colloids Surfaces B Biointerfaces. 2014b;117:360-367. doi:10.1016/j.colsurfb.2014.03.010.

Maramaldi G, Togni S, Pagin I, Giacomelli L, Cattaneo R, Eggenhoffner R, et al. Soothing and anti-itch effect of quercetin phytosome in human subjects: a single-blind study. Clinical, Cosmetic and Investigational Dermatology. 2016; 9:55-62. doi:10.2147/CCID.S98890.

Mitri K, Shegokar R, Gohla S, Anselmi C, Müller RH. Lipid nanocarriers for dermal delivery of lutein: Preparation, characterization, stability and performance. Int J Pharm. 2011;414(1-2):267-275. doi:10.1016/j.ijpharm.2011.05.008.

Miyake Y, Shimoi K, Kumazawa S, Yamamoto K, Kinae N, Osawa T. Identification and antioxidant activity of flavonoid metabolites in plasma and urine of Eriocitrin-treated rats. J Agric Food Chem. 2000;48(8):3217-3224. doi:10.1021/jf990994g.

Moschwitzer J, Achleitner G, Pomper H, Müller RH. Development of an intravenously injectable chemically stable aqueous omeprazole formulation using nanosuspension technology. Eur J Pharm Biopharm. 2004;58(3):615-619. doi:10.1016/j.ejpb.2004.03.022.

Muller RH, Radtke M, Wissing SA. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. Adv Drug Deliv Rev. 2002;54(SUPPL.):131-155. doi:10.1016/S0169-409X(02)00118-7.

Nikolic S, Keck CM, Anselmi C, Murutller RH. Skin photoprotection improvement: Synergistic interaction between lipid nanoparticles and organic UV filters. Int J Pharm. 2011;414(1-2):276-284. doi:10.1016/j.ijpharm.2011.05.010.

Njeri R, Njogu E, Kariuki DK, Kamau DM, Wachira FN, Njogu RNE. Effects of Foliar Fertilizer Application on Quality of Tea (Camellia sinensis) Grown in the Kenyan Highlands. Am J Plant Sci. 2014;5(5):2707-2715. doi:10.4236/ajps.2014.518286.

Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. J Nutr Sci. 2016;5: e47. doi:10.1017/jns.2016.41.

Pardeike J, Schwabe K, Müller RH. Influence of nanostructured lipid carriers (NLC) on the physical properties of the Cutanova Nanorepair Q10 cream and the in vivo skin hydration effect. Int J Pharm. 2010;396(1-2):166-173. doi:10.1016/j.ijpharm.2010.06.007.

Park SN, Lee MH, Kim SJ, Yu ER. Preparation of quercetin and rutin-loaded ceramide liposomes and drug-releasing effect in liposome-in-hydrogel complex system. Biochem Biophys Res Commun. 2013;435(3):361-366. doi:10.1016/j.bbrc.2013.04.093.

Peppas NA, Bures P, Leobandung W, Ichikawa H. Hydrogels in pharmaceutical formulations. Eur J Pharm Biopharm. 2000;50(1):27-46. doi:10.1016/S0939-6411(00)00090-4.

Pignatello R, Ricupero N, Bucolo C, Maugeri F, Maltese A, Puglisi G. Preparation and characterization of Eudragit Retard nanosuspensions for the ocular delivery of cloricromene. AAPS PharmSciTech. 2006;7(1):E192-E198. doi:10.1208/pt070127.

Rabinow BE. Nanosuspensions in drug delivery. Nat Rev Drug Discov. 2004;3(9):785-796. doi:10.1038/nrd1494.

Rakesh R, Anoop KR. Ethosomes for transdermal and topical drug delivery. Int J Pharm Pharm Sci. 2012;4(SUPPL.3):17-24. doi:10.1081/DDC-120025458.

Ruktanonchai U, Bejrapha P, Sakulkhu U, Opanasopit P, Bunyapraphatsara N, Junyaprasert V, et al. Physicochemical Characteristics, Cytotoxicity, and Antioxidant Activity of Three Lipid Nanoparticulate Formulations of Alpha-lipoic Acid. AAPS PharmSciTech. 2009;10(1):227-234. doi:10.1208/s12249-009-9193-6.

Sahil K, Premjeet S, Ajay B, Middha A, Bhawna K, Bihani SGL. Stealth Liposomes: a Review. Int J Res Ayurveda Pharm. 2011;2(5):1534-1538.

Santoso S, Hwang W, Hartman H, Zhang S. Self-assembly of Surfactant-like Peptides with Variable Glycine Tails to Form Nanotubes and Nanovesicles. Nano Lett. 2002;2(7):687-691. doi:10.1021/nl025563i.

Sapino S, Ugazio E, Gastaldi L, Miletto I, Berlier G, Zonari D, et al. Mesoporous silica as topical nanocarriers for quercetin: Characterization and in vitro studies. Eur J Pharm Biopharm. 2015;89(December):116-125. doi:10.1016/j.ejpb.2014.11.022.

Saupe A, Gordon KC, Rades T. Structural investigations on nanoemulsions, solid lipid nanoparticles and nanostructured lipid carriers by cryo-field emission scanning electron microscopy and Raman spectroscopy. Int J Pharm. 2006;314(1):56-62. doi:10.1016/j.ijpharm.2006.01.022.

Spiclin P, Homar M, Zupancic-Valant A, Gasperlin M. Sodium ascorbyl phosphate in topical microemulsions. Int J Pharm. 2003;256(1-2):65-73. doi:10.1016/S0378-5173(03)00063-2.

Tusevski O, Kostovska A, Iloska A, Trajkovska L, Simic SG. Phenolic production and antioxidant properties of some Macedonian medicinal plants. Cent Eur J Biol. 2014;9(9):888-900. doi:10.2478/s11535-014-0322-1.

Ugazio E, Gastaldi L, Brunella V, Scalarone D, Jadhav SA, Oliaro-Bosso S, et al. Thermoresponsive mesoporous silica nanoparticles as a carrier for skin delivery of quercetin. Int J Pharm. 2016;511(1):446-454. doi:10.1016/j.ijpharm.2016.07.024.

Vicentini FT, He T, Shao Y, Fonseca MJ, Verri WAJr, Fisher GJ, et al. Quercetin inhibits UV irradiation-induced inflammatory cytokine production in primary human keratinocytes by suppressing NF-κB pathway. J Dermatol Sci. 2011;61(3):162-168. doi:10.1016/j.jdermsci.2011.01.002.

Wu TH, Yen FL, Lin LT, Tsai TR, Lin CC, Cham TM. Preparation, physicochemical characterization, and antioxidant effects of quercetin nanoparticles. Int J Pharm. 2008;346(1-2):160-168. doi:10.1016/j.ijpharm.2007.06.036.

Zarzyka I, Lorenzo MLD, Pyda M. Phase Diagrams of Smart Copolymers Poly (N-isopropylacrylamide) and Poly (sodium acrylate). Sci World J. 2014;1-8. doi:http://dx.doi.org/10.1155/2014/516076.

Received for publication on 06th June 2018
Accepted for publication on 15th February 2019