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ORIGINAL ARTICLE

Characterization and stability study of a water-in-oil microemulsion incorporating quercetin

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Abstract

Background: The effectiveness of a water/oil (w/o) microemulsion containing quercetin against ultraviolet B radiation (UVB) induced damage was recently demonstrated by our group. However, during the development of new pharmaceutical products, the evaluation of percutaneous absorption and in vivo effectiveness should be accompanied by evaluation of stability parameters as an integral part of the process. *Objective*: The aim was to investigate the stability of the final microemulsion formulation considering the temperature ranges of storage and application. *Methods*: The physical, chemical, and functional stability of this formulation under different conditions of storage during 12 months and the photostability of quercetin incorporated into this system over UVB exposure for 7 days were evaluated. *Results*: Although the results indicated a notable physical stability of the w/o microemulsions during the experimental period under all employed conditions, in both, the chemical and functional studies, a significant loss of quercetin content and antioxidant activity was found after 6 months of storage at 30°C/70% relative humidity and after 2 months at 40°C/70% relative humidity. The photostability study results demonstrated that the incorporation of quercetin into the w/o microemulsion maintained the previously demonstrated photostability of this flavonoid under forced exposure to UVB irradiation. *Conclusion*: Thus, this work demonstrates that special storage conditions (at 4 ± 2°C) are necessary to maintain the functionality of the w/o microemulsion containing quercetin and mainly emphasizes the importance of studying physical, chemical, and functional parameters at the same time during stability evaluation of active principles.

Key words: Characterization, physical, chemical, and functional aspects, quercetin, stability, w/o microemulsion

Introduction

Microemulsions are defined as 'a system of water, oil and amphiphile which is a single optically isotropic and thermodynamic stable liquid solution'[1]. The microemulsion system combines the properties of emulsions (such as scattered laser light measurability of tiny particles) with those of solutions (drugs show saturation solubility in microemulsions and, unlike in macroemulsions, no partition coefficient; no measurable interfacial tension between the oil and water components besides thermodynamic stability)[2].

Microemulsion vehicles are formed spontaneously when appropriate quantities of the components are admixed without requiring additional mechanical energy, and they are 'infinitely' physically stable because of their thermodynamic nature. Furthermore, they are transparent and have low viscosity, which facilitates filtration and visual inspection for particles. The characteristics of microemulsions make them straightforward components for pharmaceutical formulations, and the wide range of oil–water–surfactant compositions forming microemulsions enable solubilization of a wide range of both lipophilic and hydrophilic drugs—potentially even in

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the same vehicle. Favorable cutaneous drug delivery and solvent properties, together with the ease of formulations and the 'infinite' physical stability of these unique oil–water–surfactant mixtures, make microemulsions very promising vehicles for future topical formulations[3].

The use of water/oil (w/o) microemulsion as a strategy to increase the penetration into the skin of the well-known antioxidant quercetin, and thus optimize its effects against damage induced by ultraviolet B Radiation (UVB) irradiation exposure, was previously investigated by this research group. The results demonstrated that w/o microemulsion increased skin penetration of quercetin both in vitro and in vivo, being effective against UVB-induced decrease of endogenous reduced glutathione levels and increase of cutaneous proteinase secretion/activity[4].

Nevertheless, stability evaluation represents a crucial part of the testing program during the study of new pharmaceutical products that could be useful in the treatment of UVB-induced oxidative skin damage. Product instability modifies its three essential requisites, that is, quality, efficacy, and safety[5,6]. Moreover, temperature conditions influence the formation and maintenance range of microemulsions and may change the hydrophilic–lipophilic balance of the surfactants and destabilize the surfactant interface. Then, the stability of the final microemulsion formulation should always be examined within the temperature ranges of storage and application[3].

In the present study, the physical, chemical, and functional stability of quercetin-loaded w/o microemulsion under different conditions of storage during 12 months and the photostability of quercetin incorporated into w/o microemulsion over UVB exposure for 7 days were evaluated.

Materials and methods

Materials

Quercetin dihydrate 99% ($C_{15}H_{10}O_7{\cdot}2H_2O$, M_w = 338.26) was purchased from Acros Organics (Morris Plains, NJ, USA), propylene glycol and polyoxyethylene (80) sorbitan monolaurate (Tween 80®) were from Synth (Diadema, SP, Brazil), and sorbitan monolaurate (Span 80®) and 2,2-diphenyl-1-picryl-hydrazyl (DPPH•) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Methanol (MeOH) and glacial acetic acid, both of high-performance liquid chromatography (HPLC) grades, were from J.T. Baker (USA) and Merck (Darmstadt, Germany), respectively. All other chemicals were of reagent grade and were used without further purification.

Microemulsion containing quercetin

Microemulsion was obtained by adding the following components to the final stated percentages (w/w): 15% of a mixture of propylene glycol and water (3:1) as water phase, 46.75% of a mixture of Span 80® and Tween 80® (3:1) as the surfactant/cosurfactant system, and 38.25% of canola oil as the external phase. This and other microemulsion compositions were identified from the constructed pseudo-ternary phase diagram (F. Rossetti, unpublished data).

Oil phase dissolved quercetin (0.3%, w/w) was added to the mixture of surfactant and cosurfactant followed by the aqueous phase. After vortex mixing microemulsions formed spontaneously.

Microemulsion characterization

The unloaded (ME) and quercetin-loaded (0.3%, w/w) (ME + Q) microemulsions were characterized as described below.

Physical characterization

Polarized light microscopy. To determine optical isotropy, formulations were examined in a polarized light microscopy (Carl Zeiss, Oberkichen, Germany). Rotation of the plane of polarized light is a very useful tool to distinguish isotropic ME from anisotropic lamellar and hexagonal mesophases[7].

Centrifugation assay. To eliminate metastable systems, the formulations were centrifuged at 5000 rpm for 15 minutes at room temperature (27 ± 2°C).

Conductivity measurements. Electrical conductivity was measured by a conductivity meter model CD-20 (Digimed, SP, Brazil) calibrated at 200 μΩ/cm scale with a standard solution of KCl (1.412 μΩ/cm) at 26 ± 0.1°C.

Rheological measurement. Rheological determinations were performed at 27 ± 2°C in a model DV-III Brookfield rheometer using an SC4-18 spindle. Rheogram curves constructed with ascendant and descendant segments were obtained with rotation speeds progressively increasing (2–14 rpm) and decreasing (14–2 rpm).

Refractive index measurement. The refractive index was measured by a digital refractometer calibrated with deionized water.

pH measurements. One gram of each formulation was weighted, diluted with distilled water to 10 mL, and homogenized. A DMPH-2 Digimed pH meter measured the samples' pH at 27 ± 2°C.

Particle size analysis. Mean diameter and particle size distribution of formulations were determined by using a dynamic light-scattering system (Zetasizer Nano system ZS, Malvern, UK) at 173°C with a He–Ne laser. Samples were not diluted and the measurements were performed at 25°C.

Zeta potential measurements. Samples diluted in 10 mM aqueous sodium chloride had the zeta potential determined by a Zetasizer Nano system ZS equipment.

Chemical characterization

The ME + Q and the raw material were diluted in methanol–water (60:40, v/v) and methanol, respectively, to final quercetin concentrations equivalent to 10.0, 25.0,

50.0, 75.0, and 100.0 μg/mL and evaluated for quercetin content by HPLC method.

HPLC analyses were performed using a Shimadzu (Kyoto, Japan) liquid chromatograph, equipped with an LC-10 AT VP solvent pump unit and an SPD-10A VP UV-Visible detector. Samples were injected manually through a 20 μL loop with a Rheodyne injector. The separation was performed in a C18 Hypersyl BDS-CPS ciano (5 μm) 250 × 4.6 mm column with a mobile phase of methanol–water (60:40, v/v) containing 2% acetic acid (flow rate of 1 mL/min), and quercetin was detected at 254 nm. Data were collected using a Chromatopac CR8A integrator (Shimadzu, Kyoto, Japan). Values obtained for methanolic quercetin showed linearity over the concentration range of 0.1–200 μg/mL with a correlation coefficient (r) of 0.999. The quantification limit in the HPLC assay was 0.03 μg/mL and the average for relative standard variation and error was no more than 4.68% in all concentrations tested, which is considered adequate for analytical assays[8]. No unidentified peaks were seen in the HPLC chromatograms.

Functional characterization

Quercetin and the ME + Q were diluted in methanol and methanol–water (60:40, v/v), respectively, to final quercetin concentrations equivalent to 5.0, 25.0, 37.5, 50.0, and 75.0 μg/mL, and their antioxidant activities were determined by the DPPH• assay.

Quercetin H-donor ability was evaluated using an ethanol solution of DPPH•, a stable nitrogen-centered free radical. Briefly, for radical scavenging measurements, 1 mL of acetate buffer (pH 5.5; 0.1 M), 1 mL of ethanol, and 0.5 mL of 250 μM ethanolic solution of DPPH• were mixed with 50 μL of the test samples, and light absorbance was measured after 10 minutes at 517 nm[9]. The following controls were included in the test: (i) one positive control was prepared in the absence of the raw material and (ii) another by adding the quercetin-free formulation. The positive control indicates maximum DPPH• odd electrons, considered as the 100% free radical reference in the calculation of the quercetin hydrogen-donating ability (%). Samples containing the formulation added with quercetin as well as its positive control (ME) were centrifuged at 3500 rpm for 30 minutes before the absorbance determination. The blank was the reaction mixture without DPPH• solution. All measurements were performed in triplicate.

Values obtained for standard methanolic quercetin showed linearity over the concentration range of 0.1–2.0 μg/mL with a correlation coefficient (r) of 0.996. The average for relative standard variation and error was not more than 6.43% in all concentrations tested, in agreement with literature recommendations[8].

Stability study

ME and ME + Q were stored at 4 ± 2°C, 30 ± 2°C/70 ± 5% relative humidity (RH), and 40 ± 2°C/70 ± 5% RH, conditions relative to Zones III and IV, for 12 months. At predetermined times (immediately after preparation and at 1, 2, 3, 6, 9, and 12 months), samples were collected for the evaluation of physical, chemical, and functional stability[10,11].

To determine physical stability, formulations stored at different conditions were macroscopically characterized by visual inspection, and determinations of pH, particle size, and zeta potential were performed as described above.

Quercetin content was determined by UV–HPLC analysis, as described in Chemical Characterization section, for which the ME + Q was diluted in methanol–water (60:40, v/v) to a final quercetin concentration equivalent to 50 μg/mL.

DPPH• assay (described in Functional Characterization section) was used to evaluate the antioxidant activity of quercetin incorporated in formulations submitted to different storage conditions. ME + Q was diluted in methanol–water (60:40, v/v) to a final quercetin concentration equivalent to 50 μg/mL. The positive control was prepared by adding the quercetin-free formulations (ME) submitted to the same storage conditions.

Photostability of w/o microemulsion containing quercetin

ME+Q was exposed for 7 consecutive days to UVB irradiation. The UVB source of irradiation consisted of a Phillips TL-40W/12 RS lamp (Medical, Eindhoven, Holand), emitting a continuous light spectrum between 270 and 400 nm with a peak emission at 313 nm. UVB output (80% of the total UV irradiation) was measured using a model IL-1700 Research Radiometer (International Light, Newbury, MA, USA; calibrated by IL service staff) with a radiometer sensor for UV (SED005) and UVB (SED240).

The UVB irradiation rate was 0.40 mW/cm^2, and the dose used was 241.92 J/cm^2. A control ME + Q that was prepared under the same conditions was not exposed to the irradiation. The photostability of the formulation was evaluated by measuring the content of quercetin and its antioxidant activity.

Quercetin content was determined by UV–HPLC analysis, as described in Chemical Characterization section, for which the nonirradiated and irradiated ME + Q were diluted in methanol–water (60:40, v/v) to a final quercetin concentration equivalent to 50 μg/mL.

DPPH• assay (described in Functional Characterization section) was used for the evaluation of ME + Q functional photostability; the raw material and the nonirradiated and irradiated ME + Q were diluted in methanol and methanol–water (60:40, v/v) solutions, respectively, to final quercetin concentrations equivalent to 5.0, 25.0, 37.5, 50.0, and 75.0 μg/mL. The following controls were included in the test: (i) one positive control prepared in the absence of the raw material and (ii) another prepared by adding the ME.

Statistical analyses

Data were statistically analyzed by one-way analysis of variance (ANOVA), followed by Bonferroni's multiple comparisons *t*-test. The evaluations were made using the GraphPad Prism® software and the results were considered significantly different when $P < 0.05$ was obtained.

Results and discussion

Microemulsion characterization

Assigning the correct structure to a particular system is a complex procedure, which requires more than one characterization technique[12]. Microemulsions are easily prepared but it is far from a trivial matter to characterize its microstructure, although such knowledge is essential for successful commercial exploitation. Microemulsions have been evaluated using a wide range of different techniques over the years, but complementary methods are generally required to fully characterize these systems[1].

In view of the fact that the entrapped drug may participate in the formation of the vehicle and, consequently, influence its release, it is very important to investigate the drug impact on the physicochemical properties of the vehicle[13]. Therefore, the present study performed a physicochemical analysis on both unloaded and quercetin-loaded microemulsions.

First, to determine the suitable concentration of quercetin to be incorporated into the microemulsion, a short-term (7 days) physical stability study was previously conducted (data not shown). In this study, the microemulsion system was incorporated with 0.1%, 0.3%, 0.5%, and 1% (w/w) of quercetin. The systems containing up to 0.5% (w/w) of quercetin were isotropic, transparent dispersions, and after centrifugation no phase separation could be observed. However, the quercetin quantification in the microemulsion by HPLC showed that, in the system incorporated with 0.5% (w/w) of quercetin, only the equivalent to 0.3% (w/w) was solubilized in it, and the other part precipitated. The formulation containing 1.0% (w/w) of quercetin was a cloudy dispersion and showed phase separation after centrifugation. After 7 days, such features were maintained, which suggests that an excess of quercetin was incorporated into the system, changing the hydrophilic–lipophilic balance of the surfactants and destabilizing the surfactant interface to lead to system break. For this reason, the final percentage of 0.3% (w/w) of quercetin was selected for further studies.

Table 1 shows the values for the physicochemical properties evaluated in the microemulsion characterization. Results of electrical conductivity for both ME and ME + Q showed low values, indicating that these are w/o microemulsions and that quercetin incorporation did not change the structure of the system. Considering the low water solubility of quercetin (0.48 ± 0.072 μg/mL)[14] it can be assumed that it is contained in the external phase of the w/o microemulsion system.

Table 1. Physicochemical properties of unloaded and quercetin-loaded microemulsions.

	ME	ME + Q[a]
Electrical conductivity (μΩ/cm)	0.63	0.45
Viscosity (cP)	312.40	315.80
Refractive index	1.46 ± 0.0003	1.47 ± 0.0005
pH value	5.32 ± 0.02	5.51 ± 0.01
Particle size (nm)	11.10 ± 0.26	15.90 ± 0.45
Polydispersity	0.08 ± 0.04	0.12 ± 0.05
Zeta potential (mV)	-5.21 ± 0.47	-6.19 ± 0.14

Results are represented as mean ± SD ($n = 3$).
[a]The concentration of quercetin in the ME was 0.3% (w/w).

Similarly, the refractive index, pH, and viscosity values did not change after quercetin incorporation into the microemulsion system, and both the ME and the ME+Q systems exhibited Newtonian rheological behavior (data not shown).

Physical stability of vehicle dispersions depends on particle size and particle size distribution, important parameters in in-process control and particularly in quality assurance[15]. Dynamic light-scattering measurements (Table 1 and Figure 1) revealed that the vehicle possessed a peak droplet size of 11.1 nm, which increased to 15.9 nm when quercetin was incorporated into the formulation at a concentration of 0.3% (w/w). It may be hypothesized that quercetin accumulated on the droplets' interfacial layers, thereby staying in the continuous phase[16]. In addition, the polydispersity index (PdI < 0.2) showed that the microemulsions were constituted by homogeneous populations as indicated by the narrow size distributions. In the case of zeta potential, the incorporation of the flavonoid did not significantly influence the surface charge of the microemulsion droplets, as shown in Table 1.

Chemical characterization of the w/o microemulsion did not show interfering peaks in the chromatographic patterns (data not shown), and the HPLC method was considered adequate to determine quercetin in the concentration range employed (10–100 μg/mL) (Table 2). This further confirms that the present formulation could adequately be assayed by HPLC in the performance of a chemical stability study.

Quercetin antioxidant activity has been demonstrated by different in vitro methods such as lipid peroxidation induced by Fe^{2+}/citrate[17,18]), chemiluminescence using H_2O_2/luminol/HRP system[18,19], and DPPH• assay[18,20,21]). The last one was chosen to be used in the present study because besides being an easy, inexpensive, fast, and precise methodology to measure the antioxidant activity[22], the validation of this method using methanolic solutions of quercetin showed its sensitivity, adequacy, and reproducibility to measure the antioxidant activity of this flavonoid[8].

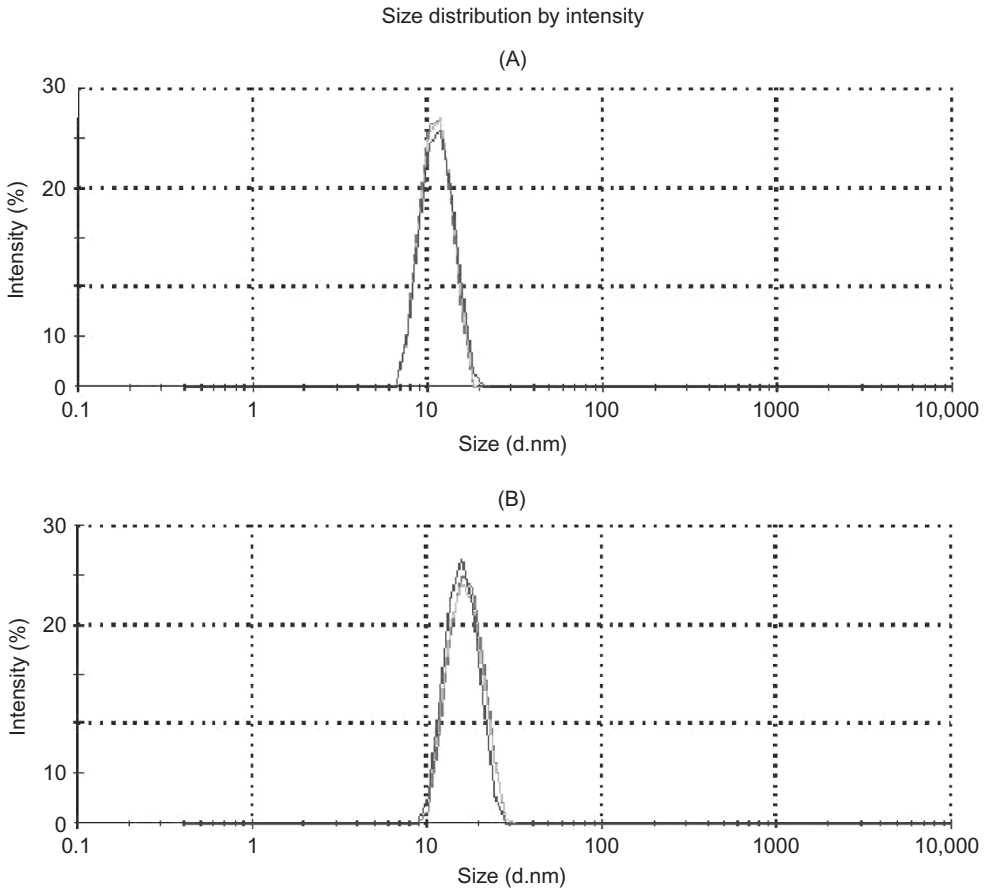

Figure 1. Particle size distribution of unloaded (A) and quercetin-loaded (B) microemulsions as determined by light-scattering analysis. Thirty-six measurements were carried out at 25°C for each sample. Both unloaded and quercetin-loaded microemulsions showed polydispersity index values lower than 0.2.

Table 2. HPLC determination of quercetin as the raw material and in quercetin-loaded microemulsion.

Real concentration (μg/mL)	Quercetin concentration (raw material) (μg/mL)	Quercetin concentration in ME + Q[a] (μg/mL)
10.0	14.79 ± 0.29	14.63 ± 1.02
25.0	23.79 ± 0.49	22.99 ± 0.62
50.0	45.36 ± 1.26	44.09 ± 1.12
75.0	79.78 ± 2.41	65.19 ± 0.83
100.0	89.19 ± 0.57	88.89 ± 0.93

Results are represented as mean ± SD ($n = 3$). No statistically significant difference was detected.
[a]The concentration of quercetin in the ME was 0.3% (w/w).

Therefore, to verify whether the DPPH• assay was effective for determining the functional activity of quercetin incorporated into the w/o microemulsion, several dilutions of the formulation were made and compared to standard quercetin solutions. The results showed that ME + Q and raw material dilutions had similar antioxidant activity against the DPPH• radical up to the concentration of 50 μg/mL (final concentration of 1 μg/mL in the reaction medium). At higher concentrations, there was interference of formulation components in the measurement of quercetin hydrogen-donating ability, because DPPH• scavenging is measured by spectroscopy (Figure 2).

Stability study

An in vivo study recently showed the effectiveness of w/o microemulsion containing quercetin against damage induced by UVB irradiation[4]. However, during the development of new pharmaceutical products, the evaluation of percutaneous absorption and in vivo effectiveness should be accompanied by evaluation of stability parameters as an integral part of the process. Therefore, the present study is intended to evaluate the physical, chemical, and functional stability of this quercetin-loaded microemulsion under different conditions of storage during 12 months.

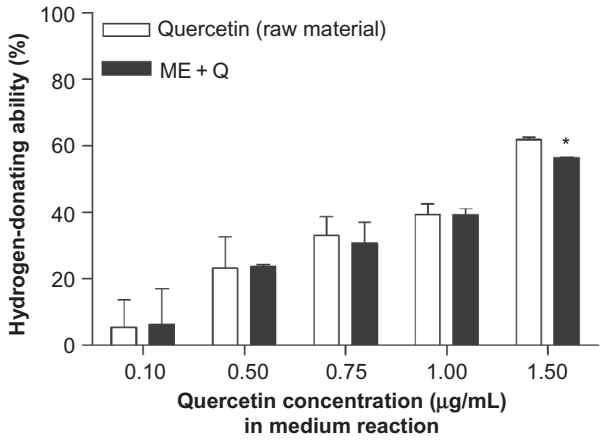

Figure 2. Hydrogen-donating ability of different concentrations of quercetin as a raw material and incorporated into the quercetin-loaded microemulsion. Statistical analysis was performed by one-way ANOVA followed by Bonferroni's test of multiple comparisons. *Significant statistical difference compared to the respective control ($P < 0.05$).

Phase transitions are known to occur in microemulsion microstructures as a result of changes in pH, temperature, dilution, and so on, as well as because of the presence of the guest molecules, the solubilized drugs. These tend to precipitate and crystallize as large crystals on storage or dilution, hindering delivery. Therefore, stability examinations of drug-loaded microemulsions should be performed[13].

To determine physical stability, the formulations were macroscopically characterized by visual inspection, and determinations of pH, particle size, and zeta potential were performed. The organoleptic study showed that, during storage, the homogeneity of the microemulsions was broken down at 4 ± 2°C, but easily recovered by shaking. However, the ones containing quercetin and stored at 40 ± 2°C/70 ± 5% RH were slightly darkened after 2 months of storage. During the 12 months of study, the ME and ME + Q showed pH ranges of 5.10–5.82 and 5.01–5.79, respectively.

Phase inversion of microemulsions upon addition of excess dispersed phase or in response to temperature is an interesting property of these systems that may affect drug release both in vivo and in vitro[23]. During phase inversion drastic physical changes occur, including changes in particle size and surface charge of microemulsion droplets.

Figure 3 shows the particle size and zeta potential for ME and ME + Q during the physical stability study. During 12 months, these microemulsions showed particle size ranges of 8.72–16.37 and 11.97–25.80 nm, respectively. The highest value was found after 9 months at 30°C/70% RH. However, in this instance the particle size does not seem to mean physical instability of the systems, because particle homogeneity, as determined by low values of polydispersity, was maintained during all of the stability study. Besides this, a physical instability of microemulsions is usually determined by phase inversion; during this phenomenon, drastic physical changes occur, such as changes in the surface charge of the microemulsion droplets. In the present study, there was no drastic change in the zeta potentials of the systems

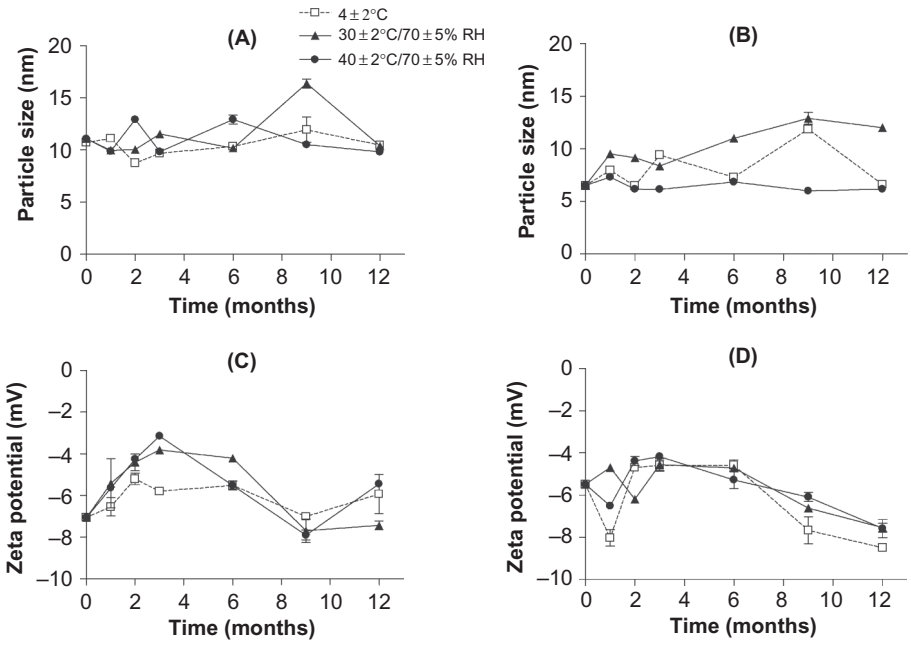

Figure 3. Particle size distribution and zeta potential analysis of unloaded and quercetin-loaded microemulsions stored at 4°C, 30°C/70% RH, and 40°C/70% RH for 12 months. Panels (A) and (B) show the particle size distribution of ME and ME + Q, respectively. Both microemulsions showed polydispersity index values lower than 0.5. Panels (C) and (D) show the zeta potential of ME and ME + Q, respectively. Results are represented as mean ± SEM ($n = 3$).

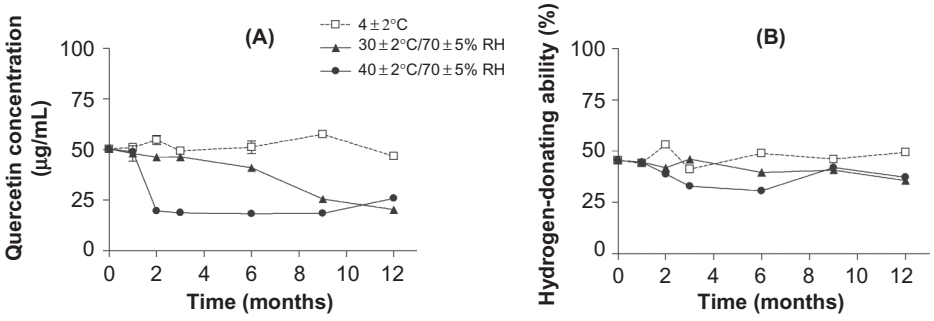

Figure 4. Chemical and functional stability of a w/o microemulsion containing quercetin stored at 4°C, 30°C/70% RH, and 40°C/70% RH for 12 months. (A) Concentration of quercetin determined by HPLC. Chromatographic conditions: C18 Hypersyl BDS-CPS ciano (5 μm) 250 × 4.6 mm column attached to a precolumn, mobile phase: methanol–water (60:40, v/v) containing 2% acetic acid, 1 mL/min. Detection at 254 nm. (B) H-donor ability of quercetin in the w/o microemulsion using stable radical DPPH• in 250 μM ethanolic solution. Results are represented as mean ± SEM ($n = 3$).

stored under different conditions. Therefore, these results seem to indicate a notable physical stability of these microemulsions during the full experimental period under all the employed conditions.

Combined analytical techniques, including HPLC methodology, are currently used to define chemical stability of formulations over time[24]. In the present study, the chemical stability of the ME + Q was determined based on its concentration upon storage at different temperatures (4°C, 30°C/70% RH, and 40°C/70% RH) for 12 months.

The results, plotted in Figure 4A, show the mean values of three samples for each time and temperature of storage. Samples stored at 4°C were chemically stable over the time of the experiment (1 year). However, the samples stored at 30°C/70% RH showed a significant ($P < 0.05$) decrease in the quercetin concentration (18.5%) after 6 months of storage, and the samples stored at 40°C/70% RH showed a significant ($P < 0.001$) loss of quercetin concentration (60.8%) after only 2 months of storage. Significant changes during the first 3 months of the analysis of samples submitted to the accelerated stability study (extreme conditions of temperature and humidity) indicate that care must be taken during transport and handling of these formulations[25].

The functional stability of quercetin incorporated into microemulsion was evaluated by its H-donor capability, using the free radical DPPH• assay originally developed by Blois[26]. Functional stability guarantees the efficacy of a product with a specific function and has been proposed as a different approach to evaluate the stability of quercetin as an active pharmaceutical ingredient and in different topical formulations[17,27].

Corroborating the chemical stability results, the functional study demonstrated that the w/o microemulsion maintained its antioxidant activity when stored at 4°C during the 12 months of analysis. However, the samples under the other conditions (30°C/70% RH and 40°C/70% RH) had a significant decrease of the antioxidant activity after 6 and 2 months of storage, respectively, just as in the chemical study. After 6 months, at 30°C/70% RH, the loss concerning the initial activity was 12.9%, and after 2 months at 40°C/70% RH, it was 14.7% (Figure 4B).

The discrepant percentages (mainly for samples stored at 40°C/70% RH) when comparing quercetin concentration (chemical stability) and antioxidant activity (functional stability) might be explained by the fact that some of the quercetin degradation products may have antioxidant activity, with an ability to donate H^+ to the DPPH• radical.

In contrast to our results, Smith et al.[28] demonstrated the chemical stability of quercetin, and Casagrande et al.[17] showed that after 6-month storage at different temperatures (4°C, room temperature, and 40°C/70% RH), quercetin as raw material and incorporated into nonionic and anionic emulsions maintained its hydrogen-donating ability as on the first day of experiments, suggesting its functional stability. However, it is known that the stability of a substance is a function of its vehicular system. Previously study had reported that the vehicle might change the heat of activation in a degradation reaction, bringing about a different decomposition mechanism[24].

For the w/o microemulsion evaluated in the present study, both chemical and functional stability studies demonstrated that this formulation needs special storage conditions (at 4 ± 2°C) to maintain its functionality and possible usefulness as a topical formulation to prevent oxidative stress-induced skin damage.

Photostability of w/o microemulsion containing quercetin

Besides the physical, chemical, and functional stability of the studied formulation, to prevent or decrease the oxidative damage in the skin induced by UV irradiation, the flavonoid quercetin has to maintain its photochemical stability after being incorporated into the w/o microemulsion. A light-induced degradation can result in a decreased efficacy and sometimes also involve significant adverse side effects after formulation administration[29].

A previous work, recently published by our group, has demonstrated the photostability of quercetin raw

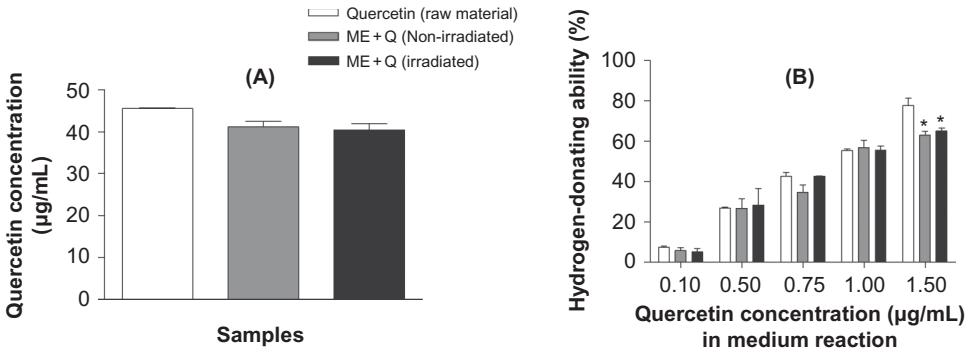

Figure 5. Photostability of a w/o microemulsion containing quercetin. (A) Concentration of quercetin in raw material methanolic solution, nonirradiated and irradiated ME + Q determined by HPLC. (B) H-donor ability of quercetin in raw material methanolic solution, nonirradiated and irradiated ME + Q, using stable radical DPPH$^{\bullet}$ in 250 µM ethanolic solution. Results are represented as mean ± SEM ($n = 3$). Statistical analysis was performed by one-way ANOVA followed by Bonferroni's test of multiple comparisons.
*Significant statistical difference compared to control (raw material solution) ($P < 0.05$).

material under forced exposure to UV irradiation[8]; however, this property might be affected by the vehicular system in which quercetin was incorporated. For this reason, the present work evaluated the photostability of quercetin incorporated into the w/o microemulsion system under forced exposure to UVB irradiation.

The results demonstrate that the irradiated ME + Q showed no loss in quercetin concentration after UV exposure, when compared with both quercetin methanolic solution and the nonirradiated ME + Q (Figure 5A). In the same way, as demonstrated by Figure 5B, the antioxidant activity of quercetin incorporated into the microemulsion was maintained after UV exposure. A very similar profile of dose–response curve was obtained for nonirradiated and irradiated ME + Q, with both of them being concentration-dependent as with the quercetin raw material. Besides this, the concentration of antioxidant needed to decrease the initial DPPH$^{\bullet}$ concentration by 50% (IC_{50}) was 0.91 µg/mL for the raw material solution, 1.05 µg/mL for the nonirradiated ME + Q, and 0.95 µg/mL for the irradiated ME + Q. However, for the final quercetin concentration of 1.5 µg/mL, a statistically significant difference was observed in the quercetin antioxidant activity determined for both nonirradiated and irradiated microemulsions when compared with the raw material solution. This difference can be explained by the fact that the DPPH$^{\bullet}$ method is adequate in evaluating the H-donor ability of the quercetin incorporated into the w/o microemulsion until the final concentration of 1.0 µg/mL, as demonstrated above.

Then, the photostability study results demonstrated that the incorporation of quercetin in the w/o microemulsion maintained the previously demonstrated[8] photostability of this flavonoid under forced exposure to UVB irradiation. Considering the fact that the maximum UVB dose used in the in vivo studies was approximately 80 times lower than the one applied in the photostability study, these results confirm the viability of using this formulation against the skin damages induced by UVB irradiation.

Conclusions

The present study initially demonstrated that the addition of quercetin (0.3%, w/w) to the w/o microemulsion did not significantly influence the microstructure of this vehicle. The analytical methods employed to evaluate quercetin concentration and antioxidant activity were successful in assessing the chemical and functional stability of the w/o microemulsion, confirming their adequacy to be applied in this type of screening.

Regarding the stability study, although the results indicated a notable physical stability of the w/o microemulsions during the experimental period under all employed conditions, the chemical and functional studies demonstrated the necessity of special storage conditions for maintaining functionality. The photostability study confirms the viability of using this formulation against the skin damages induced by UVB irradiation, because ME + Q was chemically and functionally stable under forced exposure to UVB irradiation. Finally, these results also emphasize the importance of studying physical, chemical, and functional parameters at the same time during stability evaluation of active principles.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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