



Meglumine-enhanced water solubility and stability of quercetin at moderate pH via liposome encapsulation

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ABSTRACT

In the present work, the flavonoid quercetin was encapsulated into liposomes from an aqueous solution using meglumine as solubility enhancer and stabilizer for quercetin. This study was a proof of principle to demonstrate the feasibility of the pH-driven (or pH jump) method even with very oxidation-sensitive polyphenols. We thus investigated meglumine addition together with the standard thin-film hydration method to prepare liposomes. Furthermore, the pH was initially set at 9 to enhance the water-solubility of quercetin and was lowered to 7 after encapsulation. The liposomes were prepared using DPPC, DPPG, cholesterol and DMPE lipids and two different saline buffer solutions containing either sucrose or meglumine. Successful encapsulation of quercetin was confirmed by Differential Pulse Voltammetry. The estimation of the degree of oxidation of quercetin inside the liposomes was performed by High-Performance Liquid Chromatography. It was found that a significant amount of unoxidized quercetin was encapsulated in the liposomes from an aqueous solution, notably in the liposomes prepared with meglumine buffer solution.

1. Introduction

Polyphenols have gained considerable interest in the pharmaceutical and food industries due to their many potential health benefits. Quercetin, in particular, is a well-known and potent antioxidant that has been extensively studied for its anti-inflammatory and promising anti-cancer activities, as well as for the prevention of cardiovascular diseases [1]. However, quercetin (like a vast majority of polyphenols) is poorly water-soluble (<0.05 mM at physiological pH [2]). This limits its widespread use as a simple, orally administered drug or dietary supplement in aqueous formulations.

Numerous attempts have been undertaken to overcome this obstacle, such as the functionalization of target compounds, or the use of carriers to encapsulate and deliver them. For instance, inclusion complexes, such as the well-known cyclodextrins, or lipid carriers, including nanostructured lipid carriers (NLCs) and liposomes [1]. Liposomes are self-

assembled lipidic vesicles which can entrap hydrophilic as well as hydrophobic compounds due to their bilayer structure. They are effective drug delivery systems for many lipophilic compounds such as polyphenols [3,4] and have already proven to be well-suited for quercetin delivery [5–7]. They provide several benefits over other drug delivery systems, such as improved bioavailability (skin or membrane penetration), targeted delivery, controlled drug release, reduced drug concentration, and improved stability (e.g., for storage purposes). However, to load lipophilic polyphenols into the liposome bilayers, organic solvents or solvent mixtures (methanol, acetone, chloroform, etc.) are often used as a first step to solubilize them before they can migrate into the membrane upon solvent removal. In 2014, Pan et al. introduced a pH-driven method (PDM) to load curcumin into casein micelles [8], which eliminates the need for organic solvents and simplifies the process. The PDM has been tested by Peng et al. on resveratrol and quercetin in addition to curcumin [9–12]. Other polyphenols like rutin have also

Abbreviations: DMPE, 1,2-Dimyristoyl-*sn*-glycero-3-phosphoethanolamine; DPPC, 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine; DPPG, 1,2-Dipalmitoyl-*sn*-glycero-3-[phospho-*rac*-(1'-glycerol)] sodium salt; DPV, Differential Pulse Voltammetry; EE, Encapsulation efficiency; HEPES, 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid; HMS, HEPES-meglumine-saline; HPLC, High-Performance Liquid Chromatography; HSS, HEPES-sucrose-saline; NLC, Nanostructured Lipid Carriers; PDM, pH-driven method; PEG, Polyethylene glycol; TFM, Thin-film hydration method.

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been tested using this method [13]. The mentioned authors exploited the fact that (poly)phenates, formed by deprotonation under alkaline conditions, are more water-soluble than polyphenols. Therefore, the drugs can be loaded from an aqueous phase (alkaline medium) into the lipophilic part of liposomes through pH reduction (decrease in solubility with re-protonation).

The major drawback of this technique is the rapid degradation of many polyphenols under alkaline conditions through autooxidation. This is particularly the case for quercetin, which undergoes quick oxidation even at a slightly basic pH, leading to the breakdown of the molecule into a variety of sub-products [14–16]. Consequently, the PDM was considered unsuitable for the encapsulation of quercetin in liposomes, although the oxidation products of quercetin also exhibit interesting biological properties, mainly antioxidant activity [17,18]. Nevertheless, it is desirable to preserve quercetin in its native state until successful delivery to maximize these properties and avoid early oxidative chain reactions.

Based on this idea, we propose the use of meglumine to solubilize quercetin in water at a moderate pH prior to loading it into liposomes. Meglumine, or N-methyl glucamine, is a secondary amino alcohol whose structure is derived from sorbitol. Due to its five hydroxyl groups and one methylated amino group, it is able to connect solvent water molecules through hydrogen bonds and ion pairs with a number of poorly water-soluble compounds, thereby enhancing their solubility and stability [19–25]. Approved by the FDA, it is widely used as an API salt counterion (protonated form) and as a functional excipient in pharmaceutical formulations due to its affordability, high water solubility, and low toxicity [26].

Meglumine has already been suggested as a potential solubilizer in liposomal drug formulations by Aloisio et al. [27]. One of our recent studies showed that an increase in the solubility of quercetin around pH 8–9 was achieved by adding the aminocarbohydrate meglumine [28]. In short, meglumine forms a salt with quercetin, which occurs when quercetin is single or double deprotonated, i.e., negatively charged (Fig. 1). However, the desired solubility-enhancing effect does not require working at a very alkaline pH, which would lead to further deprotonation of quercetin, resulting in its rapid oxidation. A satisfactory compromise between effective solubility and sufficiently slow oxidation, i.e., without significant loss of native quercetin, was found at pH 8–9. We used HPLC to determine the stability of the encapsulated quercetin once the pH was set back to physiological conditions. It should be noted that, since the pH is fixed at slightly alkaline values, the

liposomes that would spontaneously form by the PDM would not have optimal properties (size, size distribution, etc.) due to the lack of cholesterol and phospholipid solubility under these conditions. To overcome this issue, the protocol followed here is an adaptation of the thin-film hydration method (TFM) that uses minimal amounts of organic solvents to solubilize cholesterol and the phospholipids, allowing for the formation of a thin lipid film upon evaporation.

This method offers a notable improvement over other synthesis methods, namely a reduction in time during which quercetin is exposed to elevated pH and temperature. In the widely applied reverse-phase evaporation method, for example, the aqueous encapsulant solution is directly added to the organic lipid solution and the organic solvents are removed by gradually reducing the pressure to form an emulsion. In the TFM, the organic solvents are removed prior to encapsulant addition under nitrogen atmosphere. This prevents quercetin from exposure to atmospheric oxygen. Furthermore, pH and temperature only need to be raised during the extrusion step. Attaining this objective is challenging with other synthesis methods, even when approaches such as ethanol injection or microfluidic techniques may be more scalable and may provide greater control over liposome size and polydispersity than the TFM.

To summarize, in this study liposomes were loaded with quercetin utilizing a meglumine-rich aqueous solution at pH 9 with minimal use of organic solvents. The properties of the synthesized liposomes, the amount of encapsulated quercetin, and their stability towards oxidation were investigated. The chemical structures of quercetin and meglumine are shown in Fig. 1.

2. Experimental part

2.1. Materials

Quercetin monohydrate (HPLC grade), glycine, NaN_3 , HCl (1.0 M, analytical reagent grade), NaOH (0.1 M and 1.0 M, analytical reagent grade), and TFA (analytical reagent grade) were purchased from Sigma-Aldrich/Merck (Germany), 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), NaCl, and sucrose from Carl Roth (Karlsruhe, Germany), and meglumine (>99 %) from TCI Chemicals (Eschborn, Germany). 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-dipalmitoyl-*sn*-glycero-3-[phospho-*rac*-(1'-glycerol)] sodium salt (DPPG) were purchased from Avanti Polar Lipids (Alabama, USA), 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[biotinyl(polyethylene glycol)-2000] (DMPE-PEG2000-biotin) from Nanocs (New York, USA), and cholesterol from Sigma-Aldrich/Merck (Germany). Chloroform and methanol (analytical reagent grade) for liposome synthesis and HNO_3 (65 %, analytical reagent grade) were purchased from Thermo Fisher Scientific (Germany). Methanol (HPLC grade) for HPLC was purchased from VWR International (Ismaning, Germany).

HEPES buffer was prepared from 200 mM HEPES in double distilled water adjusted to pH 7.5. HEPES-sucrose-saline (HSS) buffer was prepared from 10 mM HEPES, 200 mM NaCl, 200 mM sucrose, and 0.01 % (w/v) NaN_3 in double distilled water adjusted to pH 7.5. HEPES-meglumine-saline (HMS) buffer was prepared from 10 mM HEPES, 250 mM meglumine, 70 mM NaCl, and 0.01 % (w/v) NaN_3 in double distilled water adjusted to pH 7.0.

2.2. Liposome preparation

Anionic liposomes were prepared following a modified version of an established TFM protocol [29,30], inspired by the PDM [8–12]. For dialysis, two different buffers were used, a standard sucrose buffer (HSS) and a meglumine buffer (HMS). In the following, liposome samples are referred to as HSS (prepared in sucrose buffer) or HMS (prepared in meglumine buffer).

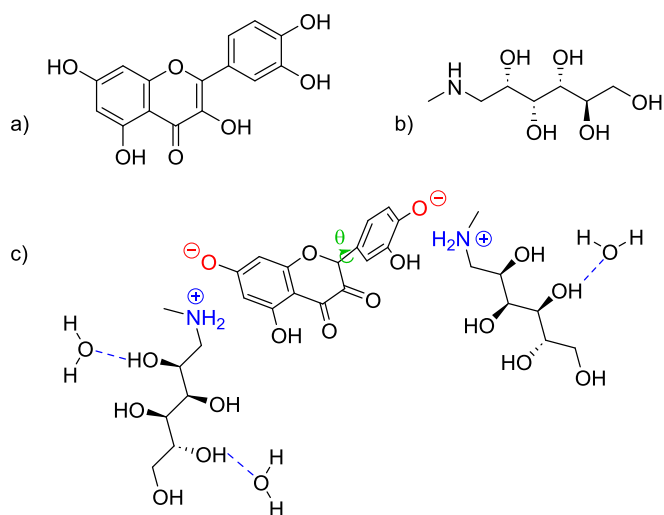


Fig. 1. Structure of (a) quercetin and (b) meglumine. Salt formation of quercetin in its preferential di-anionic keto form at pH 8 and quercetin in its protonated form in aqueous solution (c). Dotted lines represent hydrogen bonds. Reprinted from [28] with permission from Elsevier.

2.2.1. Lipid film and encapsulant preparation

The lipid film was prepared by dissolving DPPC (33.12 mg, 44.12 μmol), DPPG (9.04 mg, 12.14 μmol), DMPE-PEG2000-biotin (3.40 mg, 1.17 μmol), and cholesterol (1.22 mg, 3.16 μmol) in 3 mL chloroform and 0.5 mL methanol in a round glass flask. The solvents were then removed slowly under reduced pressure with a rotary evaporator until a thin lipid film formed on the glass. Concurrently, 7.50 mg of quercetin (5 mM), 219.6 mg of meglumine (250 mM), and 13.15 mg of NaCl (50 mM) were dissolved in 0.45 mL of HEPES buffer (200 mM, pH 7.5) and 3.15 mL of double distilled water. 0.90 mL HCl (1.0 M) was added to adjust the pH to 9. Then 2 mL of quercetin solution was added to the glass flask via syringe and septum, and the flask was vortexed and sonicated until complete emulsification of the lipid film. This procedure was repeated with another 2 mL of quercetin solution. Biotin-conjugated DMPE-PEG2000 (2 %mol biotin) was used as biotin has been described to enhance the cellular permeability and hypoglycemic efficacy of peptides when administered orally [31].

2.2.2. Extrusion

Liposomes were extruded 21 times each successively through 1.0 μm and 0.4 μm polycarbonate membranes (Whatman, New Jersey, USA) using a mini extruder from Avanti Polar Lipids (Alabama, USA). The extrusion temperature was set at 35 °C.

2.2.3. Size exclusion chromatography

Size Exclusion Chromatography was carried out with a Sephadex G-50 (Cytiva, Massachusetts, USA) column (20 cm \times 1.7 cm) with HMS (pH 7.0) or HSS buffer (pH 7.5) as eluent.

2.2.4. Dialysis

Liposomes were dialyzed over two days (buffer exchanged twice) at room temperature with a dialysis membrane from Spectra/Por (molecular weight cutoff: 12–14 kDa, Spectrum Labs, Repligen, California, USA) in 300 mL of HMS (pH 7.0) and HSS buffer (pH 7.5), respectively, to remove residual free quercetin.

2.2.5. Precaution for quercetin oxidation

At the chosen working pH of 9, quercetin undergoes autoxidation and degrades in aqueous solution within a few hours [14,15]. To minimize this effect, liposome preparation was carried out under a nitrogen gas atmosphere and in brown glass containers until the pH was lowered to a physiological value, and quercetin was concurrently encapsulated. The temperature of the method was also reduced from the typical 60 °C to 35 °C, as high temperatures favor the autoxidation reaction of quercetin. It was found that this reduction in temperature had no effect on the properties of the liposomes obtained.

2.3. Liposome characterization

2.3.1. Total lipid concentration

The phospholipid concentration (C_{PL}) of the liposomes was determined by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) using a SPECTROBLUE TI/EOP (SPECTRO Analytical Instruments, Kleve, Germany). Signals of phosphorous were detected at 177.495 nm. After calibrating the measuring device for phosphorous concentrations between 0 μM and 100 μM in 0.5 mM HNO_3 , 1:150 dilutions of the liposomes (3 mL) in 0.5 mM HNO_3 were measured. The total lipid concentration (C_{TL}) was then calculated based on the obtained C_{PL} and the defined lipid composition.

2.3.2. Size, polydispersity, and zeta potential

The size of the liposomes, their polydispersity, and zeta potential were determined through Dynamic Light Scattering (DLS) measurements using a Malvern Zetasizer Nano ZS (Malvern Panalytical, Kassel, Germany). The liposomes were diluted 1:100 (1.0 mL) in either HSS buffer or HMS buffer and transferred (0.7 mL) to semi-micropolyethyl

methacrylate (PMMA) cuvettes (Brand, Germany) for size measurements. Zeta potential measurements were performed using disposable folded capillary zeta cells (Malvern Panalytical, Germany). All buffers were filtered with a 0.2 μm syringe filter (Sartorius, Göttingen, Germany) prior to use. DLS was measured with the following settings: material refractive index of 1.34, material absorbance of zero, and dispersant refractive index of 1.342 (HSS) or 1.337 (HMS). The dispersant viscosity was 1.1185 mPa s (HSS) or 1.0262 mPa s (HMS), and the temperature was set at 25 °C for all measurements. An equilibration time of 60 s was applied before each measurement.

2.3.3. Detection of quercetin

The successful encapsulation of quercetin was assessed using a Differential Pulse Voltammetry (DPV) method adapted from Arvand et al. [32]. The method is based on the controlled redox reaction of quercetin by imposing precise voltage steps. In short, DPV measurements were conducted using a home-made laser-induced graphene (LIG) electrode [33] with minor adjustments from Arvand et al. [32]. The range was set to 0.2–0.5 V, E_{step} to 0.01 V, E_{pulse} to 0.025 V, and t_{pulse} to 0.05 s. The liposomes were diluted 1:2 or 1:4 in HMS buffer containing 0.1 M glycine. DPV measurements were conducted before and after the liposomes were lysed by sonication for 5 min. Samples of each liposome concentration were prepared and measured in triplicate. Solutions containing quercetin oxidation products were prepared by adding NaOH directly into the quercetin solution before neutralizing again with HCl.

2.3.4. Estimation of quercetin and oxidation

To verify the results obtained by DPV, an estimation of encapsulated quercetin was conducted by High-Performance Liquid Chromatography (HPLC). HPLC was performed on a Waters (Massachusetts, USA) system equipped with an RP-18 ACE Equivalent (Advanced Chromatography Technologies, Aberdeen, UK) column (250 mm \times 4.6 mm, 2.5 μm) with a Waters 2487 Dual λ Absorbance Detector set at 210 nm and 370 nm to quantify native and oxidized quercetin. The mobile phase consisted of two solvents, Millipore water with 0.1 % TFA (A) and methanol (B). An isocratic 50:50 mixture was applied for the total duration of the sample analysis (25 min). The mobile phase flow rate was 1.0 mL/min and the injection volume was 10 μL . Liposome samples were each diluted 1:10 or 1:5 in pure methanol and treated by sonication for 3 min to ensure lysis prior to injection. Each sample was analyzed in triplicate. A calibration curve of quercetin in methanol was previously constructed based on the peak area. A sample of quercetin in highly basic aqueous solution (pH > 11 obtained with 0.1 M NaOH) was also prepared and analyzed repeatedly to determine the retention times of quercetin oxidation products. In addition, certain known oxidation products of quercetin (phloroglucinol and phloroglucinic acid) were analyzed in their pure state in aqueous solution using the same method to further validate the ability of the established method to effectively separate native quercetin from degraded derivatives, thus providing an estimation that accounts for the oxidative state of quercetin.

2.3.5. Water solubilization of quercetin and encapsulation

The solubilization of quercetin in water using meglumine as a solubilizer and the concentrations chosen were based on our previous study, which investigated the solubilization mechanism in detail [28]. The solubilization of quercetin in water at pH 9 was achieved with 250 mM meglumine at a concentration of approximately 5 mM [28]. Therefore, we applied these conditions for the experiments presented in this study. After adding quercetin, a small amount remained insolubilized, indicating that the solution had reached saturation. Since less than 1 % quercetin remained insolubilized, the influence on the encapsulation efficiency (EE) was neglected. Nevertheless, insolubilized quercetin was removed by filtration to avoid problems during the liposome extrusion step.

3. Results and discussion

3.1. Liposome characterization and quercetin encapsulation

Liposome samples were analyzed by DLS and ICP-OES to evaluate the impact of the protocol modifications and the presence of meglumine on the liposome properties (size, polydispersity, zeta potential, phospholipid, and total lipid concentration). The properties of the liposomes obtained are shown in Table 1.

Liposomes typically vary in size from 30 nm to a few micrometers, depending on the synthesis method and procedure, with extrusion being the most critical step [34]. Here, liposomes were extruded through a 0.4 μm membrane (i.e., 400 nm), resulting in size and polydispersity values that met expectations. The negative zeta potential values also confirmed the successful synthesis of anionic liposomes. Overall, it can be concluded that the adapted TFM protocol, modified by adjusting the pH and the presence of meglumine, yielded liposomes that are in line with what is expected given the lipid composition used.

3.1.1. Meglumine does not interfere with liposome formation using the TFM

Characterization of liposomes formed by the modified TFM showed that meglumine did not alter their physico-chemical properties. Data for size, polydispersity, and surface charge were within the conventional range for this protocol and this particular lipid composition (Table 1) [35,36]. When comparing the encapsulation of quercetin with and without meglumine, it should be noted that without the addition of meglumine, the solubility of quercetin in water is negligible and consequently no entrapment was found. With NaOH, the solubility of quercetin can be increased, but quercetin is oxidized within seconds to minutes, and thus no unoxidized quercetin could be encapsulated.

3.1.2. Detection of quercetin on LIG electrodes using DPV

After the SEC step in liposome preparation, it was observed that quercetin had been entrapped inside the liposomes, as both samples (HSS and HMS) appeared yellow. Liposomes prepared with HSS buffer appeared slightly darker in color, indicating the possible oxidation of a portion of the quercetin. An attempt was made to confirm the presence of quercetin in both liposome solutions through direct UV-Vis spectroscopy measurements. However, these attempts were unsuccessful (data not shown), likely due to the low concentration of quercetin inside the liposomes. Therefore, a DPV-based electrochemical process using LIG electrodes was carried out instead. The detection method was initially tested on fresh and oxidized quercetin solutions with concentrations ranging from 0.01 μM to 50 μM in HMS buffer (Fig. S1). The results of the DPV measurements of both liposome solutions (HSS and HMS) clearly show that for both liposome samples, quercetin is detected with higher intensity after the liposomes were lysed, thereby demonstrating that quercetin was indeed encapsulated inside (Fig. 2).

It is important to note that this method may not be able to distinguish between native quercetin and several oxidation products of quercetin that contain adjacent hydroxyl groups, such as phloroglucinol, phloroglucinic acid, or protocatechuic acid (Fig. 3). These products could also undergo the same redox reaction and be mistakenly detected as quercetin. Therefore, this method was only used for qualitative detection and

Table 1

Physico-chemical properties of the liposomes obtained: average diameter, polydispersity index (PDI), zeta potential, phospholipid concentration (c_{PL}), and total lipid concentration (c_{TL}).

Sample	Average diameter \pm SD (nm)	PDI \pm SD	Zeta potential \pm SD (mV)	c_{PL} \pm SD (mM)	c_{TL} \pm SD (mM)
HSS	173 \pm 2	0.23 \pm 0.01	-10.6 \pm 1.3	5.2 \pm 0.1	5.5 \pm 0.1
HMS	206 \pm 2	0.27 \pm 0.01	-9.0 \pm 0.9	4.3 \pm 0.1	4.5 \pm 0.1

proof of encapsulation rather than quantification. It is unclear whether these oxidation products generate the same signal intensity, making it difficult to quantify the total concentration of native and oxidized quercetin using this method (Fig. S1). Moreover, the intact liposome sample prepared in HSS also exhibited a signal, albeit significantly weaker than the lysed liposome samples (Fig. 2). It is possible that a portion of quercetin or its oxidation products leaked through the bilayer of the liposomes and were subsequently detected. Based on these measurements, the concentrations of quercetin and its oxidation products were estimated to be in the range of 0.25–2.5 μM per mM c_{TL} of the respective liposomes. Furthermore, liposomes prepared in HMS exhibited a visibly stronger yellow color, typical of a quercetin solution. However, liposomes prepared in HSS showed higher overall signal intensities. This suggests that liposomes prepared in HMS contained more unoxidized quercetin compared to those prepared in HSS.

3.2. Estimation and oxidation state of quercetin

To validate the results obtained with the electrochemical method, an estimation of quercetin with HPLC was carried out. The liposomes were lysed by dilution in pure methanol and brief sonication before being analyzed by HPLC. Fig. 4 displays the chromatograms obtained from both liposome samples (HSS and HMS).

The retention time (R_t) of quercetin was approximately 20 min using the selected HPLC eluents and parameters. Meanwhile, phloroglucinol, phloroglucinic acid, and protocatechuic acid exhibited signals around 2–5 min. Around 20 % of the peak area between 2 and 5 min in elution time is detected for the HMS samples, whereas it is the only signal visible for the HSS samples. This confirms the assumption that quercetin was fully oxidized in the HSS samples, but only to a minor extent in the HMS samples. Finally, the concentration of native quercetin in the liposomes was determined to be approximately 17.9 μM , corresponding to 4.0 μM quercetin per mM c_{TL} , which was higher than expected from the DPV measurements.

3.2.1. Detection and estimation of encapsulated quercetin

Classical protocols for measuring EE in liposomes involve determining the concentration of non-encapsulated product at the end of the process and subtracting it from the initial concentration to determine the amount of encapsulated product. However, detecting and precisely quantifying native liposome-encapsulated quercetin poses a major challenge for two main reasons: (i) Quercetin has a very low solubility in neutral pH solutions, which makes it difficult to detect. Indeed, after the pH is lowered to a physiological value during the entrapment step, the solubility of free quercetin in the solution also decreases, mainly due to precipitation. (ii) The superposition of signals from native and oxidized quercetin leads to uncertainties when quantifying native quercetin with the most common measurement methods, including classical spectroscopic techniques like UV-Vis spectroscopy.

The DPV measurements with LIG electrodes exhibited only semi-quantitative characteristics with respect to native quercetin, with signals being detected at quercetin concentrations exceeding 1–5 μM . Nevertheless, they could still serve as reliable indicators that the encapsulation of quercetin had been successfully achieved (Fig. 2). For more quantitative analysis, HPLC was employed to enable the effective separation of signals from native and oxidized quercetin. The products formed by oxidative degradation of quercetin are considerably smaller and more polar than quercetin itself. Consequently, their retention times are markedly shorter, ranging from 2 min to 5 min, in contrast to approximately 20 min for native quercetin (Fig. 4).

3.2.2. Comparison with other methods to encapsulate quercetin

In this study, we employed a PDM with meglumine as a solubility enhancer and stabilizer to encapsulate quercetin in liposomes. It should be noted that we made one of the rare attempts to distinguish between the actual concentration of quercetin and the concentrations of its

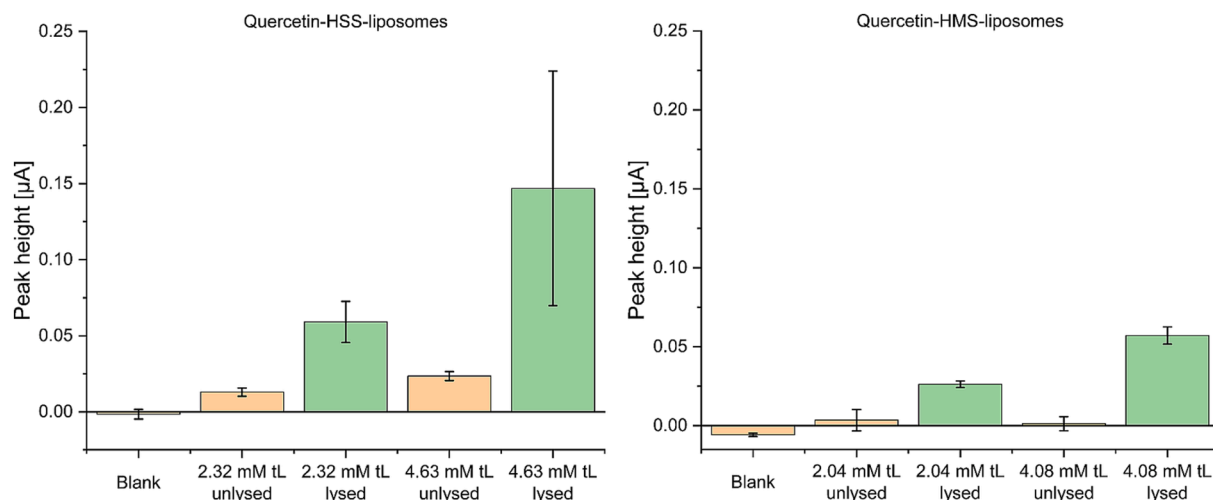


Fig. 2. Peak heights of signals from DPV measurements of lysed and intact quercetin liposomes prepared in HSS buffer (left) and HMS buffer (right).

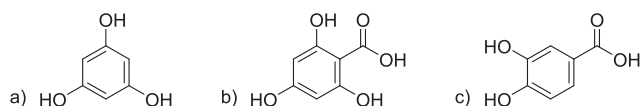


Fig. 3. Structure of (a) phloroglucinol, (b) phloroglucinic acid, and (c) protocatechuic acid.

possible oxidation products inside the liposomes. In general, comparable studies only report the original quercetin concentration, without considering the fate of quercetin. This is often justified arguing that the oxidation products are also still of high value and possess antioxidative properties. This could also explain why other groups, such as Ferreira-Silva et al. were able to achieve higher EE of 31 μg quercetin per μmol lipid, which corresponds to 90 μM per mM c_{TL} [6]. Otherwise, quercetin is usually dissolved in mixtures of chloroform and methanol, which are not comparable to the aqueous systems considered here [37–39]. Our method allows for the encapsulation of unoxidized quercetin, even with the use of a small quantity of organic solvent, thus preserving its antioxidant properties more effectively compared to methods that use alkaline solutions to solubilize quercetin [12].

4. Conclusion

In this work, an alternative protocol was used to investigate the encapsulation of the water-insoluble and unstable flavonoid quercetin in conventional liposomal formulations that maintains the simplicity of the liposome formation strategy and avoids the use of alkaline solutions

leading to quick degradation of quercetin by atmospheric oxygen [40]. Meglumine was successfully used both as a water-solubility enhancer for quercetin to encapsulate it into liposomes from an aqueous solution and as stabilizer against oxidation. The pH was adjusted to 9 to enable the solubilization of quercetin with meglumine and subsequently reduced to 7 after encapsulation to prevent rapid oxidation. This protocol was effective in encapsulating a high ratio of native quercetin to oxidation derivatives for HMS samples. As demonstrated with the HSS samples, the duration of the separation step between encapsulated and free quercetin before resetting the pH back to physiological value is crucial to prevent oxidative degradation of quercetin. The characterization of liposomes revealed that the presence of meglumine in both the encapsulant and buffer solution (for liposomes prepared in HMS buffer) had no significant effect on the size, polydispersity, zeta potential, phospholipid, and total lipid concentration.

We thus suggest that the combination of using meglumine as an additive to aqueous solutions for a “soft” solubilization (i.e., high concentrations at moderate pH) with the encapsulation into liposomes is a versatile strategy to prepare new formulations of concentrated and stabilized antioxidant solutions.

5. Disclaimer

The authors make no claims regarding the drug delivery properties or the biological activities both *in vitro* and *in vivo* of these formulations.

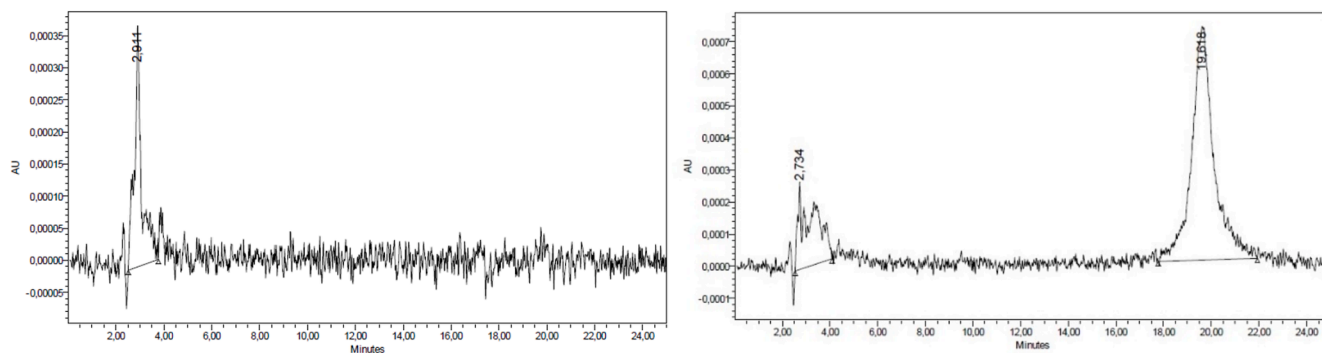


Fig. 4. HPLC chromatograms of lysed liposome samples. Left: quercetin liposomes prepared in HSS buffer diluted in pure methanol (1:10). Right: quercetin liposomes prepared in HMS buffer diluted in pure methanol (1:5).

ORCID iD authorship contribution statement

Ferdinand Holzhausen: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Adrien Fusina:** Writing – original draft, Investigation, Data curation. **Michael Loessl:** Formal analysis, Data curation. **Didier Touraud:** Supervision, Investigation, Conceptualization. **Antje J. Baemner:** Writing – review & editing, Supervision, Methodology. **Véronique Nardello-Rataj:** Supervision, Project administration, Funding acquisition, Conceptualization. **Werner Kunz:** Writing – review & editing, Validation, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.molliq.2025.126864>.

Data availability

Data will be made available on request.

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