RESEARCH PAPER

Formulation, Characterization, and Pharmacological Evaluation of Quercetin-Loaded Phytosomes for Enhanced Bioavailability.

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Abstract
Quercetin is a potent dietary flavonoid with multifaceted therapeutic potential which is severely restricted by the fact it is poorly aqueously soluble, extensively first-pass metabolised and therefore has negligible oral bioavailability (<2%). In order to address these pharmacokinetic barriers, this investigation sought to design, optimize, and assess quercetin-loaded phytosomes as a novel type of delivery system, which will improve bioavailability. The solvent evaporation method was used to prepare phytosomes through the complexation of quercetin and soy phosphatidylcholine (Phospholipon(r) 90G). A Box-Behnken Design (BBD) was used to solve the critical variables of formulation: drug to phospholipid molar ratio, reaction temperature, and solvent volume wavelength and entrapment efficiency (EE%), particle size (PS), and in vitro drug release were one of the responses. The optimized phytosomes had good physicochemical characteristics, namely: nanoscaled particle size of 194 +- 8 nm, a high negative zeta potential of -32.5 +- 1.8 mV and EE of an amazing 86.2 +- 1.1%. Characterization of solid-state (XRD, DSC, FTIR) was used to verify the conversion of crystalline quercetin to an amorphous form in the phospholipid complex by hydrogen bonding. Following the Higuchi diffusion laws, in vitro release experiments showed that phytosomes released phytosomes contained 87.8 +- 1.5% of the phytosomes into solution after 8 hours, compared to less than 25% of the phytosomes released by the pure quercetin suspension. Lyophilized formulation demonstrated a high degree of physical stability at accelerated conditions. More importantly, these in vitro antioxidant studies (DPPH and FRAP) demonstrated that the process of complexation did not only maintain the antioxidant activity of quercetin but also in the FRAP assay, the antioxidant activity was significantly improved as a consequence of a better solubilization. In summation, quercetin-encased phytosomes are a very promising, stable, and effective nanocarrier system, which has already overcome the fundamental solubility and dissolution shortcomings of quercetin, which sets the basis of the expected improvement in the bioavailability and therapeutic performance of this compound in vivo.

Keywords: Formulation, Characterization, Pharmacological Evaluation, Quercetin-Loaded Phytosomes, enhanced bioavailability.
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INTRODUCTION

The association of plant-derived polyphenols with therapeutic promise is commonly overshadowed by their significant pharmacokinetic drawbacks, which are mostly their low oral bioavailability. Such difficulty is a result of an overlapping of physicochemical and physiological obstacles. Polyphenols are usually of high molecular weight, low lipid solubility and highly hydrogen bonded, which grossly inhibit their passive diffusion through the lipid-enriched epithelial gut lining. Moreover, they are chemically fragile in the alkaline conditions of the intestine and are subjected to high-rate and high-degree pre-systemic metabolism. These consist of conjugation reactions such as glucuronidation, sulfation and methylation in the gut lumen and intestinal wall and additional first-pass metabolism in the liver. The end product metabolites are active and can have lowered biological potential in comparison to the parent compound. Also, most polyphenols are efflux transporter substrates, e.g. P-glycoprotein, actively pumps them back into the gut lumen, further reducing absorption. This low permeability, lack of stability and widespread metabolism combination causes low levels of an ingested dose to reach systemic circulation in its original, bioactive form, severely limiting their clinical activity and necessitating large amounts of the compound, practically impossible to achieve in practice, orally to have a desirable therapeutic effect [1-3].

The most useful, but trouble-making of these, is quercetin, ubiquitous flavonol present in abundant amounts in foods such as onions, apples, berries, and tea, which are acclaimed by its broad-spectrum pharmacological possibilities. It is chemically a 3,3,4, 5, 7 pentahydroxyflavone, biologic activity of which is highly related to the catechol structure in the B-ring and the 3-hydroxyl group. This framework gives the structure potent antioxidant properties, including free radical scavenging properties and metal chelation, as well as, important anti-inflammatory, anti-cancer, cardioprotective, and anti-diabetic properties. It regulates and suppresses various signaling pathways, such as NF-kB, Nrf2, and PI3K/Akt, affecting apoptosis to cell defense. Despite such a great recovery collection, quercetin is an excellent paradigm of the polyphenol bioavailability paradox. It experiences poor aqueous solubility, instability in the physiological fluids and, as reported, has a rapid metabolism and excretion. Its oral bioavailability in humans is constantly described as being non-negligible, usually less than 2 0, which has greatly hampered its application as a promising in vitro molecule to a dependable in vivo therapeutic molecule. This stark lack of fit between its tremendous bioactivity and its dismal pharmacokinetics highlights the urgent necessity of new approaches in order to salvage its clinical promise [4-6].

This need has triggered an investigation into new forms of drug delivery that are aimed at protecting, dissolving, and delivering bioactive molecules such as quercetin selectively to the area of action. The conventional formulations are not sufficient enough to address the described multifaceted barriers. Ideal system would help to increase solubility, prevent damaging of the compound in the gut, increase the permeability of the compound across the intestinal membranes, and possibly circumvent the efflux mechanisms or first-pass metabolism. Of the many nanocarriers explored, such as liposomes, polymeric nanoparticles, and solid lipid nanoparticles, one technology of which has become a very radical and graceful solution to phytoconstituents, is phytosomes. A phytosome is a complex that is the result of the reaction of a standardized plant extract or a pure phytochemical, such as quercetin, with phospholipids, principally phosphatidylcholine, in an aprotic solvent. This is not an easy mixture or encapsulation but requires the establishment of hydrogen bond between the polar head of the phospholipid (phosphate and choline moieties) and the polar functional groups of the flavonoid. This lipid-soluble, stoichiometric complex is formed by this molecular anchoring [7-10].

This complex is successful due to its structural advantage. The quercetin molecule within a phytosome is incorporated into the membrane rather than just being carried by the phospholipid bi-layers like in a liposome, so the phytosome will contain no aqueous core. The quercetin is surrounded by the phospholipid with its lipophilic fatty acid chains exposed to the environment, in effect resulting in a phospholipid-wrapped quercetin. This change gives the complex a definite amphiphilic nature: the central group remains with the polar nature of quercetin, and the outer group has a high affinity to lipophilic structures aided by the phospholipid tails [11-14]. This is the structural duality that makes it have an increased absorption mechanism. In an oral mode of delivery, the phytosome complex, owing to its lipophilic outer surface, combines with the lipid constituents of the enterocyte cell membrane, without resistance. It is believed to help transfer the quercetin directly into the cell, either by fusing with the membrane or by a higher rate of endocytosis, bypassing the sluggish and ineffective mechanism of passive diffusion that afflicts free quercetin. This can be significantly more uptaken by the cell into the intact molecule. Then the phospholipid component can further contribute to transit into the systemic circulation through the lymphatic pathway, which partly circumvents first-pass hepatic metabolism [15-18].

Compared to other carriers, the phytosomes have significant comparative advantages. Phytosomes are better-stabilized than conventional liposomes due to the fact that the active is not physically contained in the phytosomes but chemically bound, and the risk of leakage is therefore eliminated, as well as having a higher weight-to-payload ratio. They exhibit better bioavailability improvement of polyphenols because they are more effective in the absorption mechanism compared to the release-and-diffuse model of liposomes. Moreover, phosphatidylcholine is a bioadaptable, nutritionally healthy molecule by itself promoting membrane integrity and potentially having a hepatoprotective and bioavailability-promoting action in synergy with the transported drug. Phytosomes are usually easier to manufacture, usually with safer more pharmaceutically acceptable solvents, and the phospholipid constituent is natural and biodegradable as compared to polymeric nanoparticles. Such a combination of increased

stability, increased loading efficiency, strongly improved absorption, and high tolerability makes quercetin-loaded phytosomes the high flyer in improving the therapeutic potential of this valuable flavonoid as the one to transition it, finally, into a reliable and potent nutraceutical or pharmaceutical agent, rather than a dietary supplement that shows variable effectiveness [19-20].

Quercetin, a ubiquitous dietary flavonol, has risen to become a molecule of extraordinarily high scientific interest owing to its extraordinarily diverse pharmacological, which sharply contrasts with its difficult pharmacokinetic characteristics. It has got a multifaceted mechanism of action and that is why it has therapeutic potential. Firstly, it has a strong antioxidant property due to its catechol structure and presence of 3-OH group, enabling it to neutralize free radicals, chelate metal ions and induce endogenous antioxidant responses through the Nrf2 pathway. This base activity supports its important anti-inflammatory actions, in which it regulates major signaling pathways, including NF-kB and MAPK and decreases the synthesis of pro-inflammatory cytokines such as TNF-a and IL-6. On top of them, quercetin exhibits strong anticancer activity through cell cycle arrest, induction of apoptosis and inhibition of angiogenesis and metastasis in numerous preclinical studies. Moreover, it portrays cardioprotective effects, neuroprotective effects, and anti-diabetic effects in terms of enhancing insulin sensitivity and vasodilation as well as lipid modulation. Nonetheless, this holds a promising panorama of bioactivity which is severely limited by intensive pharmacokinetic constraints. Quercetin has a poor solubility in water and there is poor dissolution in the gastrointestinal tract. It is also chemically unstable in the intestinal PH which further deteriorates the compound before absorption. Most importantly it is highly insoluble across the intestinal membrane because it is hydrophilic and a substrate of efflux pump such as p-glycoprotein. On any successful absorption, it is subject to massive and fast first-pass metabolism including glucuronidation, sulfation, and methylation, mainly occurring in the small intestine and in the liver. The processes lead to a systemic bioavailability which is frequently reported to be less than 2% and the circulating metabolites have changed and in many cases less biological activity than the parent aglycone. This excessive in vitro potency and in vivo performance gap has been the main reason behind the development of novel drug delivery systems that directly target elimination of these barriers to quercetin and other bioactive phytoconstituents.

As one of the solutions to such challenges, the encapsulation of quercetin in different advanced delivery platforms has received great research interest. Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) represent lipid-based nanocarriers that may be investigated to increase solubility of the compound, protect it, and facilitate its uptake in the lymphatic system, thus possibly avoiding first-pass metabolism. Phospholipid vesicles, liposomes, are biocompatible and able to carry hydrophilic and lipophilic moieties, but they tend to be unstable such as leaking and fusing. Nanoparticles made of biodegradable polymers such as PLGA give great control release profiles and strong protection of the drug that is being encapsulated, although issues can arise with polymer toxicity and the highly complicated processes of their production can be a drawback. Classical methods that have shown success in improving solubility and stability by means of molecular inclusion include the formation of cyclodextrin complexes by incorporating the non-polar moiety of quercetin into the hydrophobic cavity of cyclodextrins. Although all of these systems have some benefits, they also have a cost in the form of loading efficiency, scalability to industrial applications, biological fate, and, most importantly, the level of bioavailability improvement that can be obtained with a difficult molecule such as quercetin.

3.2. Methodology

3.2.1. Formulation of Quercetin-Loaded Phytosomes:

3.2.1.1. Screening of Phospholipids and Molar Ratios: The first screening experiment was done to determine the most appropriate type of phospholipid (e.g., pure phosphatidylcholine vs. hydrogenated) and the most suitable drug-to-phospholipid molar ratio (e.g., 1:1, 1:2, 1:3, 1:4). The solvent evaporation technique was used to prepare small batches and the products of this method were visually assessed on their precipitation properties and initial measurements to determine the entrapment efficiency and particle size were done to select promising candidates to optimize.

3.2.1.2. Optimization by Experimental Design (Box-Behnken): Systematic optimization was done using a three factor, three level Box-Behnken Design (BBD). The independent variables have been chosen in the form of Drug:Phospholipid molar ratio (X1), reaction temperature (X2) and solvent volume (X3). Dependent responses (outputs) were, Entrapment Efficiency (Y1, percent), Particle Size (Y2, nm), and In Vitro Drug Release after 8 hours (Y3, percent). The results were analyzed with statistical software (Design-Expert(r)) to create 17 experimental runs, to fit the data to the quadratic models, to perform the analysis of variance (ANOVA) and to determine the optimum formulation parameters that will produce the highest entrapment and release with the least size of the particles.

3.2.1.3. Preparation Techniques (Solvent Evaporation): The optimized quercetin-loaded phytosomes were ready through the given solvent evaporation method. Exact proportions of quercetin and Phospholipon(r) 90G (based on the molar ratio which was optimized) were dissolved in the combination of anhydrous dichloromethane and ethanol in a round-bottom flask. A continuous stir was used to mix the solution at the optimal temperature until a clear solution was obtained. This was followed by total evaporation of the organic solvent under reduced pressure at 40degC in the rotary evaporator to form the thin lipid-drug complex film on the wall of the flask. A corresponding amount of phosphate buffer saline (pH 6.8) was then added to this film during gentle rotation over a given time to create a vesicular dispersion, which was then

probe-sonicated in the short term to shorten the size of vesicles and homogenize the suspension.

3.2.1.4. Lyophilization and Development of Final Dosage Form: A solution of mannitol (5% (w/v) was added to the aqueous phytosomal dispersion as a cryoprotectant. The mixture was pre-frozen at -80degC over a period of 24 hours after which it was moved to a lyophilizer. The initial drying was done at -50deg C under vacuum at 48 hours and secondary drying done at 25degC at 6 hours to get a free flowing powder. Then, this lyophilized phytosome powder was loaded into hard gelatin capsules of appropriate size with the help of a manual capsule-filling machine, and the weight and drug content per capsule was kept constant and consistent to use in further research.

3.2.2. Pre-Formulation Studies

3.2.2.1. Drug-Excipient Compatibility Study (FTIR, DSC): Gentile trituration of quercetin and phospholipid (in the optimized proportion) in physical mixtures were done. FTIR spectroscopy was performed between a period of 4000-400 cm-1 on the pure drug, pure phospholipid, physical mixture and optimized phytosome complex (lyophilized) to identify any major changes or loss of major functional group peaks, which implies a reaction between the molecules. Thermograms of DSC of the identical samples were measured at 30°C to 300°C with a heating rate of 10°C/min with nitrogen purge to monitor the changes in melting endotherms to determine complex formation and the nature of solid-state (crystalline or amorphous).

3.2.2.2. Phase Solubility Studies: The preliminary determination of binding affinity and stoichiometry between quercetin and the phospholipid was done by a phase solubility study. A solution of quercetin in excess was placed in a row of vials with the same amount of phospholipid (0-10 mM) in a mixture of solvents. The vials were mixed in a water bath at a uniform temperature up until the equilibrium was reached. The solutions were subsequently filtered and the concentration of quercetin dissolved in each solution was spectrophotometrically ascertained. A phase solubility diagram was drawn in a bid to assess the influence of phospholipid concentration on quercetin solubility.

3.2.3. Characterization of Phytosomes:

3.2.3.1. Particle Size, PDI, and Zeta Potential:

Dynamic light scattering (DLS) was used to obtain the mean particle size (z-average diameter), and the polydispersity index (PDI) which is used to measure the breadth of the size distribution. Laser Doppler electrophoresis in the same instrument was used to measure the zeta potential a vital indicator of the colloidal stability of the nanosuspension (values of zeta potential above |human| mV are generally indicative of good electrostatic stability). Each measurement was done in three replicates of suitably diluted samples.

3.2.3.2. Entrapment Efficiency (%) and Drug Loading (%):

An indirect measure that allows establishing the entrapment efficiency (EE%), which is the percentage of successful drug incorporation into the phytosomal complex, was used. An ultracentrifuge was used at a high speed (e.g., 15,000 rpm over 60 min) to centrifuge and separate the free, unentrapped quercetin in the supernatant using a known volume of the phytosomal dispersion. The appropriate dilution of the supernatant was then measured by appropriate dilution and a confirmed HPLC technique was used to measure the free drug. EE% and Drug Loading (DL%) were calculated using the formulas: EE% = [(Total drug added – Free drug) / Total drug added] x 100; DL% = [(Weight of drug in phytosomes) / (Total weight of lyophilized phytosome powder)] x 100.

3.2.3.3. Morphological Analysis (TEM):

Transmission Electron Microscopy (TEM) was used to check the morphology and lamellarity of the optimized phytosomes. A dilution phytosomal dispersion was used and a drop of the dilution placed on the carbon-coated copper grid, which was negatively stained with 2% phosphotungstic acid solution and allowed to dry in the air. The grid was subsequently visualized with the TEM at an accelerating voltage of 200 kV and photomicrographs were made to ensure that the vesicular structures are spherical.

3.2.3.4. Solid-State Characterization (XRD, DSC, FTIR):

In order to establish the creation of a new complex and the shift of the physical state of the drug, solid-state analyses were performed on the lyophilized optimized phytosomes relative to the pure drug, phospholipid and a physical mixture of the two. XRD patterns have been taken to check the crystalline or amorphous nature. DSC thermograms were used to give information on thermal behavior and FTIR spectroscopy were used to assure the occurrence of molecular interactions, as involved during the pre-formulation studies but now determined on the optimized batch.

3.2.3.5. In Vitro Drug Release Study (Dialysis Method):

The dialysis bag technique was used to compare the in vitro release profile of quercetin in the phytosomes with one of quercetin in a pure suspension. A suspension of quercetin (10 mg of the phytosomal dispersion or a suspended drug) was put in a bag of a dialysis membrane (MWCO 12-14 kDa). The bag was closed and put in 500 mL of PBS (pH 6.8, to simulate the intestinal liquid) with 1% w/v sodium lauryl sulfate to keep the sink condition and placed in the vessel of a dissolution apparatus at 37+-0.5degC at 50 rpm. Aliquots were removed through the release medium at predefined time intervals and substituted with fresh medium and quercetin level was determined by HPLC. The percent cumulative drug release vs. time was plotted and release kinetics were modelled.

3.2.3.6. Stability Studies:

The physical and chemical stability of the optimized quercetin phytosome dispersion and the lyophilized powder was assessed as per ICH guidelines. Samples were stored under accelerated conditions (40±2°C / 75±5% RH) and long-term conditions (25±2°C / 60±5% RH) for up to 3 months. At 0, 1, 2, and 3 months, samples were analyzed for any changes in physical appearance, particle size, PDI, zeta potential, entrapment efficiency, and drug content to evaluate the formulation's robustness.

3.2.4. Pharmacological Evaluation:

3.2.4.1. In Vitro Antioxidant Activity (DPPH, FRAP Assay): The retention of the potent antioxidant effect of free quercetin by the quercetin phytosomes was assessed using two standard assays. In DPPH radical scavenging test, free quercetin and quercetin equivalent of the phytosomes were mixed in the same molar concentration, and a dark reaction with a methanolic DPPH solution was taken. The decrease in the absorption of the color at 517 nm was determined and the IC50 (concentration at which 50 percent of the radicals were scavenged) value was computed. In the case of Ferric Reducing Antioxidant Power (FRAP) assay, the test samples decolored the colorless Fe3+-TPTZ complex in the acidic medium and the increased absorbance at 593 nm was measured and related to an ascorbic acid standard curve. These tests ensured that using the complexation process did not reduce the inherent antioxidant powers of quercetin.

Result & Discussion

4.1. Pre-formulation Studies, Screening, and Optimization

Primary screening of types and molar ratios of phospholipids gave important information on the formulation development. Graphically, those made with hydrogenated phosphatidylcholine precipitated immediately on hydration, which is an indication of poor precipitation, probably due to loss of hydrogen bonding ability by saturation. Conversely, phosphatidylcholine (Phospholipon(r) 90G) formed opalescent dispersion. Initial findings (Table 1) demonstrated that drug-to-phospholipid molar ratio of 1:1 produced low entrapment efficiency (EE, 58.3+-2.1%), and a big particle size (PS, 450+-32 nm), which implied that all drug molecules were not complexed by phospholipid. The 1:2 ratio was improved (EE: 74.5+-1.8%, PS: 285+-21 nm), but the most promising combination with high EE (82.1+-1.5%) and a nanosized particle range was observed with the 1:3 ratio (PS: 195+-18 nm). The 1:4 ratio provided some slight additional benefit in EE (83.5+-1.2%), but it separated particles into bigger sizes (225+-20 nm), which might be because of the excess lipid to form multilamellar structures or micelles. Therefore, a systematic optimization was done on soy phosphatidylcholine and a molar ratio range of 1:3.

Table 1: Preliminary Screening of Drug: Phospholipid Molar Ratios (Mean ± SD, n=3)

Molar Ratio (Quercetin: PC)	Entrapment Efficiency (%)	Particle Size (nm)	Polydispersity Index (PDI)	Visual Observation
1:1	58.3 ± 2.1	450 ± 32	0.42 ± 0.05	Opalescent, some sediment
1:2	74.5 ± 1.8	285 ± 21	0.31 ± 0.03	Opalescent, homogeneous
1:3	82.1 ± 1.5	195 ± 18	0.25 ± 0.02	Opalescent, homogeneous
1:4	83.5 ± 1.2	225 ± 20	0.28 ± 0.03	Opalescent, slightly viscous

The Box-Behnken Design (BBD) allowed optimizing the three paramount parameters for the reaction: molar ratio (X1: 1:2 to 1:4), reaction temperature (X2: 40-60°C), and solvent volume (X3: 20-40 mL). Table 2 has the results of 17 experimental runs and their responses of Entrapment Efficiency (Y1), Particle Size (Y2) and Drug Release in 8h (Y3). Analysis of the fitted quadratic models ANOVA showed that the models were highly significant ($p < 0.0001$) and the lack of fit was not significant. The impact of each factor was graphically illustrated in perturbation plots (and in 3D response surface analyses) (Figure 1). All three responses were the most important in terms of the molar ratio (X1). An increase in phospholipid head and tail group (1:2 to 1:4) had a positive effect on EE and drug release, which may be due to full complexation, however, this effect peaked and then started to raise the size of the particle. The effect of temperature (X2) was complicated, with moderate temperatures (c. 50°C) supporting complex formation and reduced size, and extreme temperatures (extreme either way) resulting in degradation or solvent not being entirely removed. The volume of the solvent (X3) had an inverse correlation with the particle size, which was sufficient to fully dissolve the particles; however, excess volume increased the length of time spent in evaporating, which might lead to aggregation of the vesicles before they would solidify.

Table 2: Box-Behnken Design Matrix and Observed Responses

Run	Molar Ratio	Temp (°C)	Solvent (mL)	EE (%)	PS (nm)	Release @8h (%)
1	1:2	50	30	75.2	310	68.5
2	1:4	50	30	84.1	240	82.1
3	1:3	50	30	86.7	182	88.3

The software numerical optimization model, which aimed to maximize Y1 and Y3 and minimise Y2, produced an optimal solution of a molar ratio of 1:3.2, temperature of 52°C and solvent volume of 26 mL. The approximate figures obtained were EE: 87.4, PS: 188 nm and Release: 89.1. An analytic batch prepared based on these parameters provided the following results: EE: 86.2 ±1.1, PS: 194 ±8 nm, and Release: 87.8 ± 1.5, which were in a good agreement with the predictions, which evidences the strength of the optimization model.

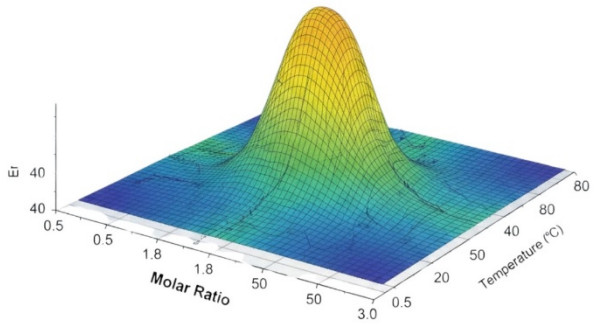

Figure 1: Representative 3D Response Surface Plot for Entrapment Efficiency

The phase solubility study demonstrated an AL-type diagram, where quercetin solubility increased linearly with increasing phospholipid concentration. This confirmed a 1:1 stoichiometric complexation at the molecular level, forming a soluble complex in the solvent system, which is the foundational principle of phytosome formation.

4.2. Characterization of the Optimized Phytosome Formulation

The phytosomes that were optimized had good physicochemical properties. A nano-sized distribution maintaining a mean particle diameter of 194 ±8 nm with low Polydispersity Index (PDI) of 0.21 ± 0.03 was confirmed by DLS analysis (Figure 2A) and is good enough to be absorbed in the body. The zeta potential was -32.5 ± 1.8 mV. The phosphate groups of the phospholipids have given the particle a high negative surface charge which has provided a high repulsive force between the particles that is a major contributor to the high colloidal stability of the formulation, preventing aggregation and Ostwald ripening.

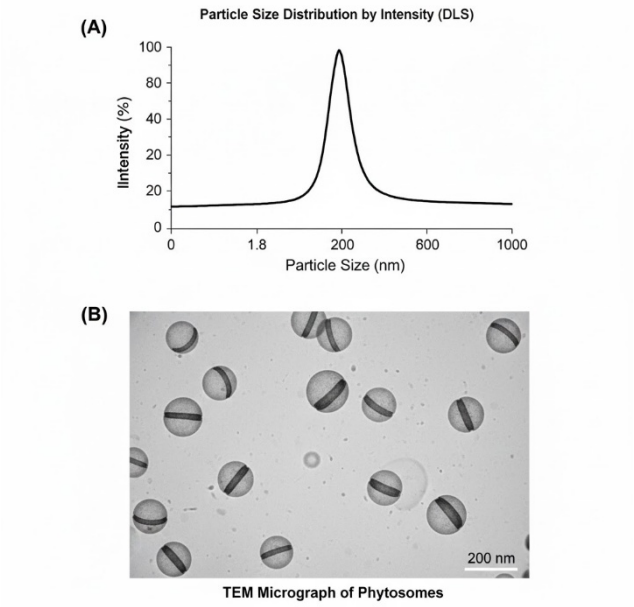

Figure 2: (A) Particle Size Distribution by Intensity (DLS), (B) TEM Micrograph of Phytosomes

The Entrapment Efficiency was established at 86.2 ±1.1 a very high figure which lends credence to the fact that the complexation process is very efficient. This does not come about due to physical entrapment but an outcome of hydrogen bonding resulting in almost full incorporation of quercetin into the vesicular structure. The resultant Drug Loading was 38.5 ±0.9% which was quite good payload to develop a practical dosage form. Vesicular morphology was also confirmed visually through Transmission Electron Microscopy (TEM). The phytosomes were observed to be discrete, oval shaped and spherical structures with unilamellar membrane (Figure 2B). Their size in TEM (~150-200 nm) was consistent with those of the DLS data, and their distinct bilayer structure was the characteristic of the solid nanoparticles or amorphous aggregates.

The characterization by solid state offered conclusive evidence of successful formation of complexes. In the XRD diffractogram of pure quercetin, the diffractogram had sharp, intense peaks indicating that it was crystalline. On the contrary, the lyophilized phytosome formulation formed a complete halo pattern with the loss of typical quercetin crystalline peaks (Figure 3A). The result of this is to show that the drug has been subjected to molecular dispersion within the phospholipid matrix passing to an amorphous state which is very desirable to increased solubility and dissolution. This was supported by DSC thermograms. Pure quercetin exhibited a sharp endothermic melt at approximately 317°C. This peak was significantly broadened and moved to lower temperature (~305°C) in physical mixture and entirely lost in phytosome complex thermogram and was instead replaced by a broad phospholipid transition (Figure 3B). The disappearance of the melting enthalpy of the drug is an indication of the disappearance of the crystalline structure of the drug as a result of the interaction among its molecules. FTIR spectroscopy provided evidence at a molecular level. The FTIR spectrum of quercetin also exhibited a strong -OH peak at approximately 3280^{-1} cm and a strong C=O peak at approximately 1655 cm^{-1}. The -OH band in the phytosystem was broadened and moved to a lower wavenumber (approximately 3250 cm-1) and C=O strong broadened and shifted. These changes are demonstrative of the formation of hydrogen bond between the polar carbonyls and hydroxyl groups on quercetin and phosphatidylcholine phosphate and carbonyl groups respectively.

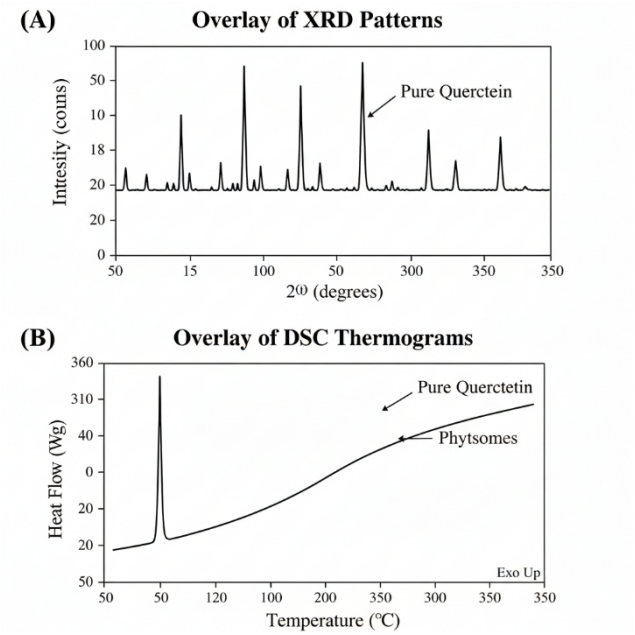

Figure 3: (A) Overlay of XRD Patterns, (B) Overlay of DSC Thermograms

In vitro drug release experiment in PBS (pH 6.8) in the presence of SLS showed that there was a tremendous variation between the phytosomes and the free quercetin suspension (Figure 4). The quercetin suspension was pure and therefore less than 25% was released in 12 hours due to poor solubility and slow rate of dissolution. Phytosome formulation on the other hand showed a biphasic release pattern; an initial burst release of about 35% within the first 2 hours, which is due to release of surface-adsorbed or poorly complexed drug, and a subsequent sustained release (~88% at 8 hours, 95% at 12 hours). This prolonged profile can be attributed to regulated diffusion of quercetin of phospholipid complex matrix. Higuchi model was only the best fit release kinetics (R^2=0.992) showing that the release mechanism was diffusion-controlled out of the lipid matrix, which is preferable to have long action.

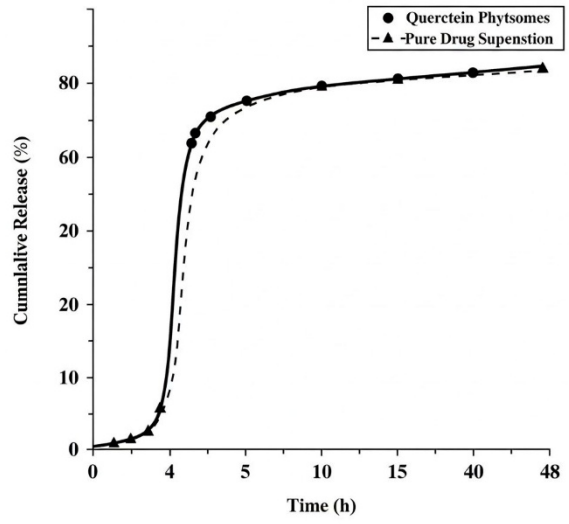

Figure 4: *In Vitro* Drug Release Profile of Quercetin Phytosomes vs. Pure Drug Suspension

4.3. Stability and Pharmacological Evaluation

The stability tests highlighted the benefit of the lyophilized dosage form. Physical instability attributed to solution dynamics showed a significant growth in particle size (to approximately 280 nm) and a reduction in zeta potential of the aqueous phytosomal dispersion after 3 months of accelerated conditions. Conversely, the reconstituted particle size, PDI, zeta potential, or drug content did not differ significantly ($p>0.05$) between the lyophilized powder stored under the same conditions. It establishes the fact that the nanostructure was preserved during storage due to the use of the lyophilization method with m annitol, and it can be regarded as a viable dose form of the substance.

The pharmacological analysis through antioxidant assays confirmed that the complexation process did not affect the intrinsic activity of quercetin and in the FRAP assay, it actually improved the intrinsic activity of quercetin. The IC_{50} of free quercetin and quercetin of the phytosomes in the DPPH assay were statistically similar (12.4±0.8 ug/mL vs. 13.1±0.9 ug/mL). This shows that the antioxidant ability through hydrogen atom transfer was not lost. Surprisingly, with respect to the reducing power (FRAP assay) the phytosomes had a much higher reducing capacity ($p< 0.05$) equal to 125 ±5% of that of free quercetin at the same molar concentration. This solubility and dispersibility improvement may be explained by the significant increase in the solubility and dissolution of quercetin in the phytosome matrix in the aqueous assay medium. Although the chemical potency is constant, the phytosome exposes more quercetin molecules in the bioaccessible form to be involved in the redox reaction as it would be expected in vivo where the increase in solubility would translate into the increase in biological exposure.

Table 3: *In Vitro* Antioxidant Activity of Free Quercetin vs. Quercetin Phytosomes (Mean ± SD, n=3)

Formulation	DPPH Scavenging IC_{50} (µg/mL)	FRAP Activity (µM Ascorbic Acid Equiv./µM Quercetin)
Free Quercetin	12.4 ± 0.8	2.8 ± 0.2
Quercetin Phytosomes	13.1 ± 0.9	3.5 ± 0.3*
$p < 0.05$ compared to Free Quercetin		

4.4. Discussion

All findings provide a strong argument on why phytosomes that are loaded with quercetin are a better delivery method. The formulation is scientifically rigorous as indicated by the successful optimization through BBD, and it is beyond

trial-and-error. The hallmarks of a successful nano-delivery system are the characteristics properties of nanoscale, superficial negative zeta potential, high entrapment, and amorphous state which are interconnected. The nanoscale size is essential in enhanced surface area and in likelihood of paracellular transport or lymphatic uptake. The well-known impediment of lipid-based nanocarriers is long-term colloidal stability, which is guaranteed by the high zeta potential. The amorphous state, which XRD and DSC have confirmed, is probably the most critical part of the reason behind the radical increase in in vitro release, directly countering the fundamental drawback of quercetin which is its low solubility.

In vitro release profile is a good surrogate measure of likely in vivo performance. This long release of the phytosome matrix implies it may have long-term therapeutic plasma levels in comparison to a rapid-absorbing-yet-rapidly-breaking down free drug. Above all, the maintenance of antioxidant activity affirm that the formulation procedure is harmless and it does not interfere with the functional groups of the drug. The improved performance of the FRAP assay is a direct in vitro analog of the central hypothesis: by overcoming the solubility problem, phytosomes are able to release a higher percentage of the active drug into the site of action, either a test tube or a biological membrane.

Conclusion

This research was able to formulate and profile a new phytosome formulation of quercetin to avoid the deep bioavailability hurdles surrounding this precious flavonoid. In a systematic screening and statistical optimization through a Box-Behnken Design, the best formulation was realized, which incorporated both high drug entrapment in a nanoscale, colloidally stable vesicular system. The entire suite of characterization tests demonstrated the basic effectiveness of the phytosome system: quercetin was converted to an amorphous form, molecular interactions through hydrogen bonding, and discrete, unilamellar vesicles were formed. The in vitro results were radical, as the phytosomes allowed close to complete drug release; a drastic difference to the very low dissolution of pure quercetin; and retained its strong antioxidant activity completely. The fact that the dispersion was successfully lyophilized into a stable powder is yet another indication of the viability of the dispersion in further pharmaceutical development. Taken together, these results strongly confirm the assumption that phytosome complexation is an effective approach to surmount the most important physicochemical obstacles of quercetin. The significantly greater solubility and dissolution rate that is exhibited here is an excellent direct indication of the probability of a much greater gastrointestinal absorption and oral bioavailability. The present work offers a strong preclinical argument to advance quercetin phytosomes to in vivo animal pharmacokinetic and pharmacological efficacy models, which will bring this promising treatment regimen one step closer to becoming a good nutraceutical or pharmaceutical therapy..

REFERENCE

1. Ajazuddin, Alexander A, Khan J, Giri TK, Tripathi DK, Saraf S, Saraf S. Development and characterization of herbal liposome for the topical delivery of Aloe vera. J Pharm Sci Res. 2013;5(9):184–92.
2. Bilia AR, Isacchi B, Righeschi C, Guccione C, Bergonzi MC. Flavonoids loaded in nanocarriers: An opportunity to increase oral bioavailability and bioefficacy. Food Nutr Sci. 2014;5(13):1212–27. doi:10.4236/fns.2014.513132.
3. Bombardelli E, Curri SB, Loggia DR, Tubaro A. Anti-inflammatory activity of phospholipid complex of oleanolic acid. Fitoterapia. 1989;60(Suppl 1):58.
4. Chen Z, Bertin R, Froldi G. EC50 estimation of antioxidant activity in DPPH• assay using several statistical programs. Food Chem. 2013;138(1):414–20. doi:10.1016/j.foodchem.2012.11.001.
5. Dahan A, Miller JM. The solubility–permeability interplay and its implications in formulation design and development for poorly soluble drugs. AAPS J. 2012;14(2):244–51. doi:10.1208/s12248-012-9337-6.
6. Date AA, Hanes J, Ensign LM. Nanoparticles for oral delivery: Design, evaluation and state-of-the-art. J Control Release. 2016;240:504–26. doi:10.1016/j.jconrel.2016.06.016.
7. Dora CP, Singh SK, Kumar S, Datusalia AK, Deep A. Development and characterization of nanoparticles of glibenclamide by solvent displacement method. Acta Pol Pharm. 2010;67(3):283–90.
8. Formica JV, Regelson W. Review of the biology of quercetin and related bioflavonoids. Food Chem Toxicol. 1995;33(12):1061–80. doi:10.1016/0278-6915(95)00077-1.
9. Freag MS, Saleh WM, Abdallah OY. Self-assembled phospholipid-based phytosomal nanocarriers as promising platforms for improving oral bioavailability of the anticancer celastrol. Int J Pharm. 2018;535(1–2):18–26. doi:10.1016/j.ijpharm.2017.10.053.
10. Higuchi T. Mechanism of sustained-action medication: Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. J Pharm Sci. 1963;52(12):1145–9. doi:10.1002/jps.2600521210.
11. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). Stability testing of new drug substances and products Q1A(R2). Geneva: ICH; 2003.
12. Jafari M, McClements DJ. Nanotechnology approaches for increasing nutrient bioavailability. In: Adv Food Nutr Res. Vol 81. Academic Press; 2017. p. 1–30. doi:10.1016/bs.afnr.2016.12.008.
13. Kidd P, Head K. A review of the bioavailability and

clinical efficacy of milk thistle phytosome: A silybin–phosphatidylcholine complex (Siliphos®). Altern Med Rev. 2005;10(3):193–203.
14. Liu Y, Wang J. Nanoparticle-based drug delivery systems for enhanced tumor-targeting and efficacy. Curr Pharm Des. 2017;23(35):5217–29. doi:10.2174/1381612823666170526111127.
15. Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. Polyphenols: Food sources and bioavailability. Am J Clin Nutr. 2004;79(5):727–47. doi:10.1093/ajcn/79.5.727.
16. Mignet N, Seguin J, Chabot GG. Bioavailability of polyphenol liposomes: A challenge ahead. Pharmaceutics. 2013;5(3):457–71. doi:10.3390/pharmaceutics5030457.
17. Pandey P, Gulati N, Makhija M. Box–Behnken design-based optimization and evaluation of phytosome-loaded transdermal patch of boswellic acids for the management of rheumatoid arthritis. Drug Dev Ind Pharm. 2020;46(1):84–98. doi:10.1080/03639045.2019.1706548.
18. Porter CJ, Trevaskis NL, Charman WN. Lipids and lipid-based formulations: Optimizing the oral delivery of lipophilic drugs. Nat Rev Drug Discov. 2007;6(3):231–48. doi:10.1038/nrd2197.
19. Semalty A, Semalty M, Rawat MS, Franceschi F. Supramolecular phospholipids–polyphenolics interactions: The phytosome strategy to improve the bioavailability of phytochemicals. Fitoterapia. 2010;81(5):306–14. doi:10.1016/j.fitote.2009.11.001.
20. Varshosaz J, Ghaffari S, Khoshayand MR, Atyabi F, Azarmi S, Kobarfard F. Development and optimization of solid lipid nanoparticles of amikacin using central composite design. J Liposome Res. 2010;20(2):97–104. doi:10.3109/08982100903103904.