Trop J Nat Prod Res, August 2024; 8(8):8081 - 8086 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Formulation and Characterisation of pH-Sensitive Eudragit L-100 Nanoparticles for Quercetin Delivery in Lung Carcinoma

Firas F. Al-Mamoori[1,2*], Habibah A. Wahab[1], Waqas Ahmad[1]

[1]*School of Pharmaceutical Science, Universiti Sains Malaysia*
[2]*School of Pharmaceutical Science, University of Babylon, Iraq*

ARTICLE INFO

Article history:
Received 16 June 2024
Revised 17 June 2024
Accepted 23 July 2024
Published online 01 September 2024

ABSTRACT

Quercetin is a flavonoid found abundantly in many vegetables and fruits. Quercetin is known for its antioxidant, anti-inflammatory, and anti-proliferative properties. The present study aims to formulate quercetin nanoparticles (Qu-NPs) for targeting lung carcinoma cells. Qu-NPs were formulated using the pH-sensitive polymer Eudragit® L-100. The physicochemical properties of the Qu-NPs were characterized by dynamic light scattering (DLS), Fourier transform infrared (FT-IR) spectroscopy, X-ray diffraction (XRD), differential scanning calorimetry (DSC), and scanning electron microscopy (SEM). *In vitro* drug release of Qu-NPs was assessed using the dialysis bag method. Cytotoxic activity of Qu-NPs against non-small cell lung cancer cell line (NCI-H460), and normal human lung fibroblasts cell line (CCD-19Lu) was assessed using the 3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) assay. DLS results indicated a mean particle size of 242.86 ± 20.61 nm, zeta potential of -18.2 ± 2.97 mV and polydispersion index of 0.266 ± 0.008 for Qu-NPs. FT-IR spectra confirmed the presence of hydrogen bonds between quercetin and the polymer, which is crucial for drug entrapment and release. XRD and SEM analysis demonstrated the amorphous nature of quercetin within the nanoparticles. *In vitro* drug release studies revealed a sustained drug release across different pH conditions including simulated gastric fluid (pH 1.2), and intestinal fluid (pH 6.8, and pH 7.4). Cytotoxicity assay revealed a potent cytotoxic effect of Qu-NPs against NCI-H460. This study underscores the potential of Eudragit® L-100 nanoparticles (NPs) as carriers for quercetin in cancer therapy. It also highlights the potential of Qu-NPs for improved drug efficacy through targeted and controlled release.

***Keywords*:** Quercetin, Nanoparticles, Lung cancer, Eudragit®L-100, Cytotoxic test, pH-sensitive polymer.

Introduction

Cancer remains a leading cause of death worldwide. According to the World Health Organisation, cancer is projected to become the top cause of death globally by 2025, resulting in approximately 12.7 million deaths. This staggering number accounts for nearly one in six deaths.[1,2] The most common types of cancer include breast, lung, colon, rectum, and prostate cancer.[3] The main factors contributing to about one-third of cancer-related deaths are tobacco use, alcohol consumption, a diet lacking in fruits and vegetables, and insufficient physical activity.[4] Non-small cell lung cancer is the most common form of lung cancer, impacting both smokers and non-smokers, including individuals under the age of 45 years. In male smokers, adenocarcinoma accounts for roughly 30% of primary lung tumors, while in female smokers, it accounts for 40%. Among non-smokers, these rates are approximately 60% in males and 80% in females.[5] The most common cancer treatment approaches include chemotherapy, radiation, and surgery.

*Corresponding author. E mail: ferasfalih2@gmail.com
Tel: +96417825212336

Citation: Al-Mamoori, FF, Wahab, HA, Ahmad, W. Formulation and Characterisation of pH-Sensitive Eudragit L-100 Nanoparticles for Quercetin Delivery in Lung Carcinoma. Trop J Nat Prod Res. 2024; 8(8): 8081 – 8086. https://doi.org/10.26538/tjnpr/v8i8.23

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

These approaches can be used individually or in combination. The major challenges associated with these treatment approaches include unwanted side effects, tumor recurrence, and resistance to chemotherapy or radiation therapy.[6] The low water solubility of most chemotherapeutic agent could also pose significant therapeutic challenges. Drug candidates such as curcumin, hesperetin (from citrus fruits), cyanidin (from red fruits and berries), daidzein (from soybean), proanthocyanidins (from apple, grape, and cocoa), are poorly water soluble, meaning that intravenous administration could cause complications like embolism and respiratory system failure due to drug precipitation, while extravascular administration could lead to poor absorption.[7,8] Since chemotherapeutic drugs given intravenously are distributed to all tissues, including healthy ones, severe systemic side effects are possible with intravenous administration of adjuvant chemotherapy.[9] Pharmaceutical industries frequently use Eudragit® polymer, a family of commercially available acrylic acid and its derivatives, and film coating to slow the rate of drug release from tablets and capsules. Eudragit® L-100, the most popular form of this polymer, was developed as an enteric coating. It is a Food and Drug Administration (FDA)-approved cationic polymer with a high solubility above pH 5 (pH 5.5-6.7). It is a synthetic polymer for film coating that outperforms natural products such as sugar and shellac. It has higher contents of methyl methacrylate and methacrylic acid in comparison to other Eudragit® polymers (e.g. Evonik, and Eudragit®polymer). Carboxyl groups attached to the side chains of polymers are susceptible to protonation in an acidic environment, and polymers do not dissolve in acids (such as stomach acid). Nevertheless, carboxyl groups undergo ionization at neutral or basic pH.[9] It has been found that the polymer's payload is released when it becomes more soluble in water and when the negative charges between the carboxylate groups make them repel

each other. The amount of carboxyl or other substituent groups on the polymer can be varied to fine-tune the pH value that governs their water solubility. The ratio of carboxyl groups to ester groups in polymethacrylic acid-co-methyl methacrylate can be varied to modify the polymer.[8] Quercetin (Qu) is a well-known flavonoid that has been shown to have antiproliferative activity against several types of cancer such as gastrointestinal, brain, skin and ovarian cancer through multiple mechanisms involving free radical scavenging and chelation of transition metal ions.[10] However, quercetin has a very low water solubility (log P = 0.35) compared to the majority of other flavonoids in its category.[11] As a result of its potential activity against lung cancer, the present study proposes to produce a novel quercetin-based nanoparticle that would enable oral administration and facilitate delivery to the lungs. In this study, a polymeric nanoparticle formulation of quercetin was developed as a potential nanomedicine against lung cancer. The nanoparticles were produced to specifically target the lungs after being administered orally. This is possible due to the variation in the solubility of Eudragit® L-100 at different pH along the gastrointestinal tract (GIT). It was hypothesised that the pH sensitivity of the polymer would protect the drug as it passes through the GIT until it reaches the lungs. Once at the target site, the drug can then be released to produce its therapeutic effect while minimizing any potential side effects.

Materials and Methods

Chemicals and Reagents

Quercetin was a product of Sigma-Aldrich chemicals, Pluronic® (F-68) (average MW = ~8350 g/mol) was selected as a surface-active agent and obtained from Molekula (UK). Eudragit®L-100 (MW 13100) was a product of Evonik (Germany). Polysorbate 80 (Tw2een 80) was of Eva Chem (Ohio, USA), Potassium dihydrogen phosphate (KH_2PO_4) and absolute ethanol (99.5-99.8%) were supplied by J.T. Baker (Avantor Performance Materials, Phillipsburg, NJ). Ulltrapure water was produced using Milli-Q purification system (EMD Millipore, Billerica, MA, USA). Dialysis bag (analytical grade) (8.000–14.000) MWCO USA.

Cell line and culture media

Lung adenocarcinoma cell line [non-small cell lung cancer cell line (NCI-H460)], and normal human lung fibroblasts cell line (CCD-19Lu) were purchased from the American Type Culture Collection (ATCC), 3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) was purchased from HiMedia, India. Cell culture medium (RPMI-1640 Medium), Minimum Essential Medium (MEM), Fetal Bovine Serum, Penicillin-Streptomycin (antibiotic), Sodium Pyruvate, and MEM non-essential amino acids were all products of GIBCO.

Preparation of quercetin nanoparticle

Single emulsion (w/o) solvent evaporation technique (solvent diffusion method) was used for the preparation of quercetin nanoparticles according to previously described procedures.[12,13] Briefly, 50 mg of Eudragit®L-100 was dissolved in 5.0 mL of absolute ethanol, and quercetin (0.75 mg/mL) was dissolved in the Eudragit®L-100 solution. The polymer-drug solution was then added to 10 mL of ultrapure water in a drop wise manner while vigorously stirring at 1000 rpm, this was followed by the addition of 0.5% v/v Pluronic® F-68 in ultrapure water as a stabilizer at room temperature while stirring gently. When organic solvents diffuse spontaneously into aqueous phase, they cause interfacial turbulence, which results in the formation of nanoparticles. The nanoparticle dispersion was stirred for about 3 - 4 h to remove the organic phase. This was done by sonication at 60% voltage efficiency at 25ºC for 1 min using a probe sonicator (Bandelin, Germany). The emulsion (w/o) produced was mixed immediately to allow for complete evaporation of the organic solvent. The nanoparticles were prepared by ultracentrifugation at 15,000 rpm (16000 g) at 25ºC for 20 min using a centrifuge (Fisher Scientific, Germany). The supernatant was then tested for free drug. The pellets were rinsed twice with double distilled water to remove any unentrapped medication and adsorbed Pluronic on the surface of the nanoparticle, and the suspension was freeze-dried for 72 h using a freeze dryer (Labcono, Free Zone 4.5L). Sucrose 5% w/v (Bendosen laboratory chemicals) was used as a cryoprotector or lyoprotector.

Physicochemical analysis

A portion of the formulated nanoparticles were transferred into a plastic cuvette, and the average particle size and polydispersion index (PDI) were determined through dynamic light scattering (DLS) at 25°C and a detection angle of 90°C using photon correlation spectroscopy (PCS). The nanoparticle dispersions were diluted in redistilled water to achieve a signal strong enough for the instrument, depending on the concentration of the drug. The zeta potential of the nanoparticles was determined using a Malvern Zetasizer (Nanoseries) (Malvern Instruments Ltd., Malvern, UK).[14]

Determination of drug entrapment efficiency and drug content

Entrapment efficiency was estimated by the amount of unentrapped drug in the supernatant after centrifugation. Drug content was determined using high performance liquid chromatography (HPLC) with a UV detector at 370 nm according to previously described procedures.[14,15] The drug entrapment efficiency (EE), and drug content (DC) were calculated using the formulae below.

$$\text{EE (\%)} = \frac{AW-EW}{TW}\ x\ 100$$

Where; AW = Actual weight of loaded drug (mg)
EW = Experimental weight of loaded drug (mg)
TW = Theoretical weight of loaded drug (mg)

$$\textbf{DC (\%)} = \frac{Weight\ of\ drug}{Total\ weight\ of\ Nanoparticles}\ x\ 100$$

Scanning electron microscopy

A scanning electron microscope (Quta FEG 650, Hitachi S 3400, Tokyo, Japan) was used to capture images of nanoparticles in order to investigate their structure and surface morphology. To achieve this, a stub of aluminum was cut and then coated with nanoparticles using double-sided adhesive carbon tape. After air drying, gold was sputtered onto them with a sputter coater. The morphology of the coated particles were analysed using a scanning electron microscope.

Drug-excipient interaction and polymorphism studies

Pure quercetin, Eudragit® L-100, a physical mixture of Quercetin and Eudragit® L-100, blank nanoparticles, and Qu-NP formulation were characterized using Fourier transform infrared spectrophotometry (FT-IR). About 2 mg of air-dried samples were mixed with potassium bromide (KBr) and compressed into discs of about 0.1 mm thick using a Mini Hand Press at a pressure of 10 tons per square meter. The IR spectra were obtained by scanning individual sample disc from 4000 to 400 cm^{-1} using an FT-IR spectrophotometer (Nexus FT-IR Spectrometer, Thermo Nicolet).[16]

Powder X-ray diffractometry

The polymorphic characteristics of the blank nanoparticle, the stabiliser Pluronic® F-68, the polymer Eudragit®L-100, their physical mixture, and quercetin was determined using X-ray diffractometer (SEIFERT model JSODEBYEFLEX-2002) with the following operating conditions; temperature range -20°C - 270°C, heating rate 5°C/min, scanning speed 0.5°/min, scanning step 0.02º/min, exposure time 3 secs, and a measuring angle range of 10 - 70º. Each step took exactly one second, and the step size was 0.0482º.

In vitro drug release study

The dialysis bag method was used to determine the in vitro release of quercetin from loaded nanoparticles.[17,18] Qu-NPs equivalent to 0.75 mg quercetin was sealed in a cellulose dialysis membrane (10,000-14,000 MW cutoff) (Spectrum Laboratories Inc., USA), and placed in a dialysis bag. The ends of the bag were tied. The dialysis bag was submerged in 10 mL of simulated gastric fluid (SGF) at pH 1.2 for 2 h, thereafter in phosphate-buffered saline (PBS, pH 6.8; PBS, pH 7.4) for 142 h. Tween 80 (5% v/v) was used in both solutions. The set-up was placed on a magnetic stirrer and heated to 37°C. After 2 h of incubation, the medium was discarded and replaced with a fresh medium containing the same

volume of PBS (pH 6.8 or PBS pH 7.4), while stirring at 100 rpm. To maintain the sink conditions, 1 mL of the released medium was discarded and replaced with 1 mL of a fresh medium. Quantitative drug release analysis was performed using HPLC by comparing the UV absorbance of each medium at 370 nm with a quercetin standard curve. A graph of cumulative amount of drug released versus time was plotted.

In vitro cytotoxicity study (MTT- assay)

The *in vitro* cytotoxic activity of the quercetin nanoparticle formulations and that of free quercetin against non-small cell lung cancer cell line (NCI-H460), and normal human lung fibroblasts cell line (CCD-19Lu) was conducted using the 3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) assay.[19] NCI-H460 cells were cultured in RPMI-1640 (Gibco, Invitrogen, Carlsbad, CA) culture medium containing 10% Fetal Bovine Serum (FBS) (Gibco,Invitrogen, UK), 0.08 mg/mL streptomycin (Gibo, Invitrogen, UK), and incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air in sterile flasks. After a few days, when the cell density has reached 1.0×10^5 cells/cm^2, the cells were transferred to 96-well plates and incubated at 37°C. When the cells became confluent, they were trypsinized and then diluted in the culture medium to achieve a total cell count of 5×10^4 cells/mL. The cell suspension was subsequently transferred to a 96-well plate at a density of 5,000 cells per well, and was left to adhere overnight. Thereafter, the cells were treated with quercetin nanoparticles at different concentrations (0.031, 0.063, 0.125, 0.25, 0.5, and 1.0 mg/mL) in triplicates. The treated cells were incubated at 37ºC for 24 h, and also for 48 h in two sets of experiments, after which 1 mg/mL of MTT solution was added to each well and incubated at 37°C for 2 h in a CO_2 incubator. Wells containing cell-free medium were used as blank, while cells without treatment were used as the control. Finally, the culture medium was removed from each well, and 200 µL of isopropanol was added to each well to solubilise the purple formazan crystals formed. The plates were shaken, and the absorbance was read at 570 nm using an ELISA reader (Bio-Tek Instruments, Winooski,VT) at a reference wavelength of 650 nm using an Ultrospec 1100 Pro UV/Vis Spectrophotometer (Amersham Biosciences, Amersham, UK).

Statistical analysis

Data were expressed as mean ± the standard deviation (SD) of triplicates determination. Statistical analysis was performed using Microsoft Excel, version 2010.

Results and Discussion

Physicochemical properties

The Qu-NPs formulation had a mean particle size of 242.86 ± 20.61 nm, which falls within the particle size range for nanoparticles. The zeta potential was -18.2 ± 2.97 mV and polydispersion index of 0.266 ± 0.008. Negative zeta potential facilitates drug delivery. Nanoparticles have the advantage of being able to deliver the active drugs to cancer cells by selectively using the unique pathophysiology of tumor cells, such as their enhanced permeability and retention (EPR) effect, a passive targeting mechanism that promotes tumour drug accumulation.[20,21] The use of nanoparticles improves *in vivo* drug stability, extend active drug systemic circulation, and allow controlled drug release, resulting in an increase in drug concentration at the tumour site.[22]

Drug entrapment efficiency and drug content

Quercetin entrapment in nanoparticles was calculated indirectly, and it was found that at 0.75 mg/mL, encapsulation efficiency was 71.07 ± 1.16% and quercetin content was 521.89 ± 1.00 mg/mL.

Fourier-transform infrared spectrum

Figure 1 shows the FT-IR spectra of blank NPs, Eudragit® L-100, Qu (pure), physical mixture of Qu and Eudragit® L-100, and Qu-loaded NPs. Qu (pure) exhibited characteristic broad absorption bands at 3570.4 cm^{-1} and 3246.6 cm^{-1}, which is consistent with phenolic O-H stretch vibrations. The aryl ketonic (C=O) stretch vibrations were observed at 1667.4 cm^{-1}. The absorption peaks at 1610.2 cm^{-1}, 1520.6 cm^{-1}, and 1452.5 cm^{-1} were attributed to C-C, C=O, and C=C aromatic stretching vibrations, respectively. The OH bending vibrations of phenols occurred at 1377.2 cm^{-1}. The absorption peak at 1319.9 cm^{-1}, along with peaks between 950 cm^{-1} and 600 cm^{-1}, were attributed to C-H bending vibrations of aromatic hydrocarbons. The C-O stretching vibrations of aryl ethers and phenols were found at 1262 cm^{-1} and 1200 cm^{-1}, respectively. The observation of C-CO-C stretching and bending vibrations of ketones at 1169 cm^{-1} supports the identification of the compound as the flavonoid Qu, in line with the findings of Chourasiya *et al*. (2018).[23] The FT-IR spectrum of Eudragit® L-100 polymer showed bands corresponding to O-H stretching (3400-3700 cm^{-1}). This band is typically broad and indicates the presence of hydroxyl groups (alcohols or phenols) in the polymer. The absorption band at 2800-3100 cm^{-1} is associated with the stretching vibrations of the aliphatic C-H bonds in the polymer's backbone. The vibrations at 1600-1800 cm^{-1} corresponds to the stretching vibration of the carbonyl group (C=O) in the polymer, which is likely from the methacrylic acid units. When Qu and Eudragit® L-100 were physically mixed, the resulting mixture showed absorption bands characteristic of both compounds. The respective frequencies at which these bands were detected were 3405 cm^{-1}, 3326.6 cm^{-1}, 1667 cm^{-1}, 1613.6 cm^{-1}, 1015.3 cm$^{-1,}$ and 826.58 cm^{-1}. The most prominent absorption bands were observed at 3407.4 cm^{-1}, 2932.3 cm^{-1}, 2370.7 cm^{-1}, 1733.8 cm^{-1}, 1644.6 cm^{-1}, 1455.0 cm^{-1}, and 1059.4 cm^{-1}. The spectra of the Qu-loaded NPs showed the disappearance of the phenolic O-H stretch of Qu, and some peaks unique to the Qu-loaded NPs formulation were observed. In the Qu-loaded NPs formation, a decrease in the intensity and broadening of the O-H stretch peak, occurring between 3435 cm^{-1}and 4144 cm^{-1}, was observed. This phenomenon could be attributed to the formation of intermolecular hydrogen bonding interactions between Qu and Eudragit® L-100. Studies have shown that hydrogen bonding can influence the transition from the crystalline state of Qu to the amorphous state.[7,24]

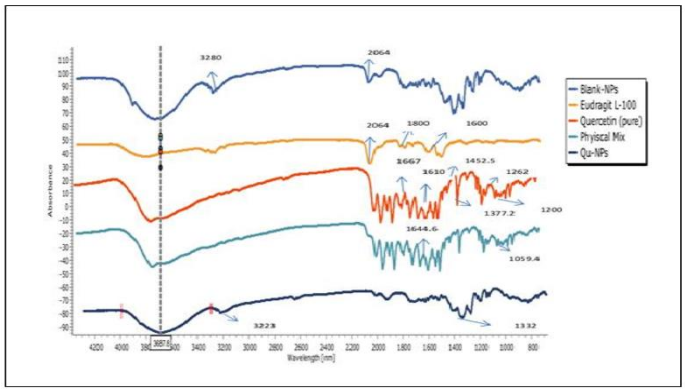

Figure 1: FT-IR spetra of blank NPs, QR(pure) Eudragit® L-100, Physical mixture (QR(pure) / Eudragit® L-100) and Qu loaded NPs

Powder X-ray diffraction

X-ray diffraction analysis of nanoparticles (NPs) provides valuable information about the shape, size, orientation, and molecular arrangement of ordered regions. Figure 2 displays a comparison of the structural properties of blank NPs, Qu (pure), Eudragit® L-100, a physical mixture of Qu and Eudragit® L-100, and Qu-loaded NPs. The diffractogram of blank NPs showed multiple peaks at various angles, with the largest ones at 11.51°, 15.53°, 18.5°, 19.5°, 23.5°, and 28.5°. For pure Qu, five distinct, highly intense peaks were observed at 10.9°, 12.27°, 15.57°, 16.5°, and 27°, indicating its crystalline structure.[34] Eudragit® L-100 exhibited a single peak at 15°–20°, indicating a partial amorphous structure. When Qu and Eudragit® L-100 were physically combined, several spectral peaks of varying intensities were observed at 10.89°, 12.0°, 15.95°, 24.09°, and 27°. The weak peaks resembled

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

those of Qu (pure), while the single, broad-profiled peak can be attributed to Eudragit® L-100. The reduced intensity of Qu peaks in the physical mixture was attributed to the lower amount of Qu and interference with Eudragit® L-100 molecule.[25]

The nanoprecipitation technique was used to prepare Qu-loaded NPs, which produced a diffraction pattern indicating an amorphous state.[26] The DSC analysis confirmed that when Qu was loaded into the NPs, it transformed into an amorphous state. Despite having low-intensity peaks like the blank NPs, Qu-loaded NPs exhibited multiple peaks at 11.76°, 13.52°, 18.38°, 19.78°, 22.38°, and 24.38°, resulting in 81.8% crystallinity. This result can be attributed to the crystallization of Qu-loaded NPs, which occurs when Qu fills empty space in the NPs.[27] Similar observations have been reported by other researchers, using other types of polymers.[28,29] This could explain the increased crystallinity after entrapping the drug. Another possible explanation is that hydrophilic surfactants (stabilisers) dissolve in water rather than an organic solvent, giving the finished product a more crystalline appearance.[30] The nanoprecipitation techniques used to prepare the optimized Qu-loaded NPs allow the amorphous state to easily transition to the crystalline state.[26]

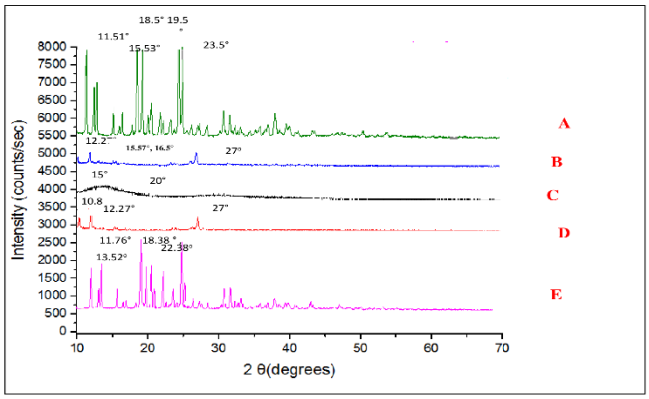

Figure 2: XRD pattern of (A) blank NPs (B) Qu Pure (C)) Eudragit® L-100, (D) Physical mixture (QR(pure) / Eudragit® L-100) (D) Qu loaded NPs

Sample morphology from Scanning Electron Microscopy (SEM)

Figure 3A shows the SEM images of blank NPs, which were smaller in size and had a spherical shape and morphology with a smooth and round surface, whereas Figures 3B, 3C, and 3D show the SEM and UHR-SEM images of Qu-loaded NPs, which have an irregular shape and a rough surface compared to unloaded NPs. The size of all Qu-loaded NPs was less than 260 nm, which was consistent with the information obtained from the mean particle size analysis using dynamic light scattering (DLS).

In vitro release profile of quercetin nanoparticles

In vitro release studies on Quercetin (Qu) were carried out, comparing the release profiles of Qu-loaded nanoparticles (NPs) with plain Qu solution via dialysis. To maintain the sink conditions, 5% (v/v) Tween-80 was added to the release medium. Qu was released from NPs at a slower rate than from a Qu (free) solution, indicating that Qu release from NPs takes longer time. The release took place gradually over a 144-hour period. Approximately 10-20% of the drug was released in the first two hours in simulated gastric fluid (SGF) (pH = 1.2), followed by a very slow release over the next 142 hours in acetate buffer solution (ABS) (pH = 6.8) and phosphate buffer solution (PBS) (pH = 7.4). In the present study, 94.73% of Qu was released from plain Qu (free) solution within 8 hours. The release of Qu from NPs relied on the diffusion of the drug from the polymer's surface and matrix, as well as the polymer's bulk erosion or swelling. In addition, the drug release pattern from NPs showed an initial burst release, followed by a lag phase of relatively gradual release. The high initial burst release can be attributed to the immediate release of Qu that was bound to the NPs' surface and the diffusion of drugs that were close to the NPs' surface.

The release profile curves revealed that Qu from NPs was released at a slower rate than Qu (free) solution because Qu spreads out from the centre of the polymer. The pH-sensitive polymers protected Qu from the acidic environment of the gastrointestinal tract (GIT), hindering release in acetate buffer solution (ABS) (pH = 6.8) and phosphate buffer solution (PBS) (pH=7.4), respectively. Based on these results, Eudragit® L-100 can protect Qu from stomach acid and release it in the small intestine. At low pH, the methacrylic acid moieties in Eudragit® L-100 become protonated, making the polymer insoluble and blocking Qu release.[31] The cumulative release of Qu-loaded NPs in acetate buffer solution (ABS) (pH = 6.8) and phosphate buffer solution (PBS) (pH= 7.4) was 95.87% and 80.7%, respectively, at 142 hours, as shown in Figure 4. Specifically, when Qu diffuses out of the polymer, the formation of hydrogen bonds between Qu and carrier molecules in acetate buffer solution (ABS) (pH=6.8) is more pronounced than in phosphate buffer solution (PBS) (pH= 7.4), which is responsible for the slow release of the Qu in phosphate buffer solution (PBS) (pH=7.4).[32]

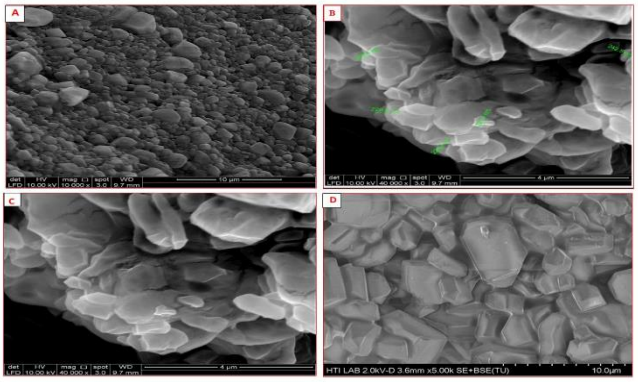

Figure 3: The Scanning Electron Microscope image Bank – NPs **Mag (10.000x), B.C.** The Scanning electron microscope image of optimize Qu loaded NPs. **Mag (40.000x), D** Ultra high resolution scanning electron microscope image of optimized Qu . loaded NPs **Mag (10.000µm)**

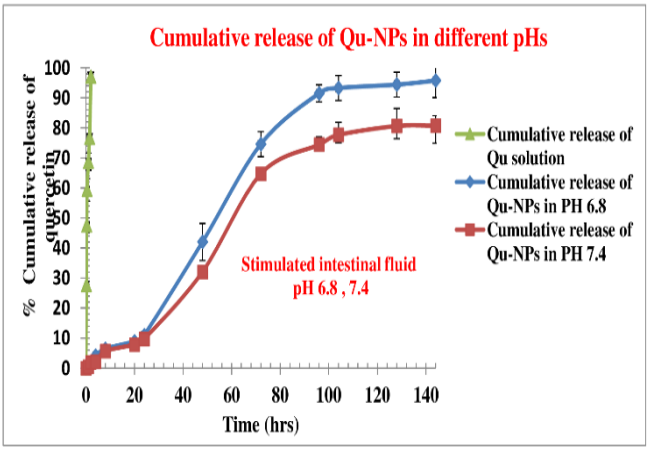

Figure 4: *In vitro* release profile of Qu-loaded Nps. the NPs were initially exposed to stimulated gastric fluid (pH=7.4) for two hrs. phosphate buffer (pH=6.8) and (pH=7.4) for 142 hrs, and Qu solution for 8 hrs Values Means ± SD (n=3)

Cytotoxicity of quercetin nanoparticles

Cell viability studies were conducted to assess the potential cytotoxicity of Qu-loaded NPs on non-small cell lung cancer cell line (NCI-H460) and normal human lung fibroblasts cell line (CCD-19Lu) using the MTT assay. The cells were treated with different concentrations (0.031, 0.063, 0.125, 0.25, 0.5, and 1 mg/mL) of free Qu (pure) and Qu-loaded NPs for 24 and 48 h, depending on time and concentration. Figures 5, 6, 7 and 8 show the viability of (NCI-H460) and (CCD-19Lu) cells,

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

respectively. The results showed that after 24 h of incubation, the plain Qu (pure) demonstrated a significantly more cytotoxic effect than the Qu-loaded NPs due to the controlled release of Qu-loaded NPs. Free Qu (pure) had a stronger effect on non-small cell lung cancer cell lines (NCI-H460) than Qu-loaded NPs, particularly at concentrations of 0.5 and 1 mg/mL. This could be attributed to the sustained release of Qu-loaded NPs, which is consistent with the findings of previous *in vitro* release studies.[17,18,33] Nevertheless, the findings from the present study demonstrated that after 48 h of incubation, the Qu-loaded NPs exhibited a more potent anticancer effect compared to the free Qu (pure) at concentrations of 0.5 and 1 mg/mL. There was no observed cytotoxic effect of free Qu (pure) and Qu-loaded NPs on (CCD-19Lu) cells at any of the concentrations tested after 24 h of incubation. However, the cytotoxic effect of both free Qu (pure) and Qu-loaded NPs increased after 48 h, with higher concentrations, especially at 0.5 and 1 mg/mL. The untreated cells as well as cells treated with lower concentrations of the test samples showed higher viability compared to cells treated with higher concentrations of the samples. This indicates that both free Qu (pure) and Qu-loaded NPs exhibited a concentration-dependent cytotoxic effect.

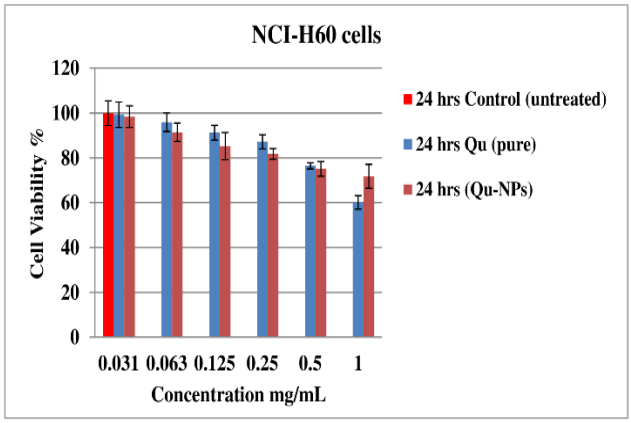

Figure 5: Cytotoxicity effects of Qu (pure) and Qu-loaded NPs in NCF H460 cells after 24hrs of incubation (n=3)

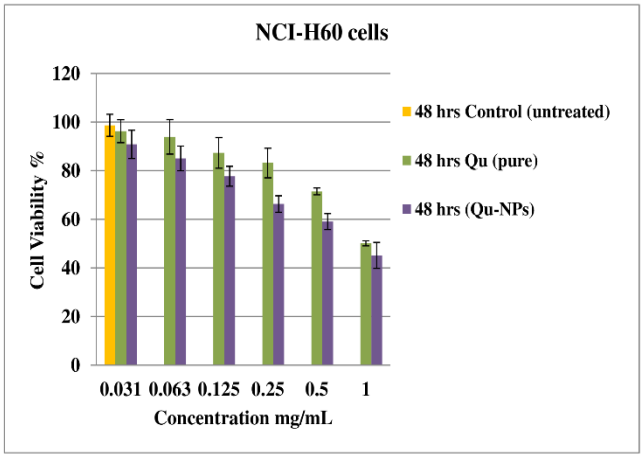

Figure 6: Cytotoxicity effects of Qu (Pure) and Qu-loaded NPs in NCl-H460 cells after 48 hrs of incubation (n=3)

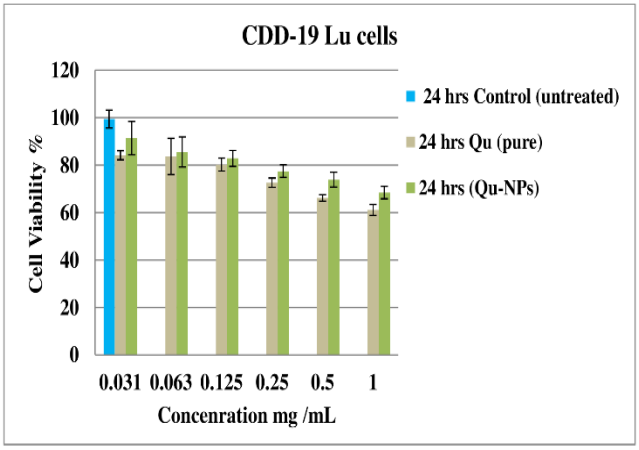

Figure 7: Cytotoxicity effects of Qu (pure) and Qu-loaded NPs in CDD. 19Lu cells after 24hrs of incubation (n=3)

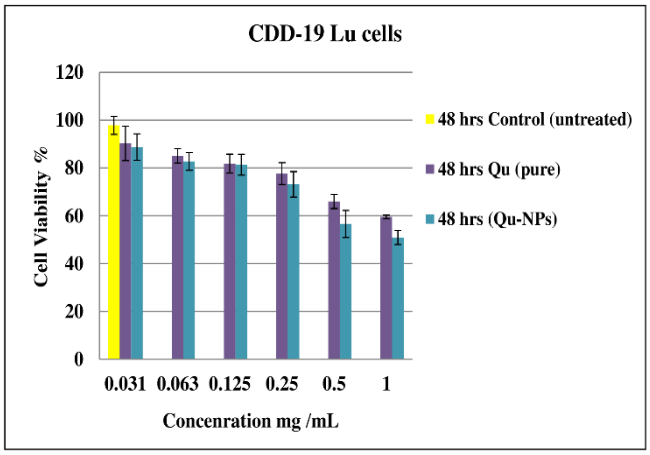

Figure 8: Cytotoxicity effects of Qu (pure) and Qu-loaded NPs in CDD. 19Lu cells after 48hrs of incubation (n=3)

Conclusion

In this study, we present for the first time a nanoparticle formulation of Quercetin based on the pH-sensitive polymer Eudragit® L-100 for Lung-targeted delivery. Excellent drug entrapment efficiency and drug release profile were achieved with a nanoprecipitation-prepared formulation of Qu-loaded Eudragit®L-100 NPs. Intermolecular H-bonding between Qu and the polymer was detected by FT-IR and DSC studies, which demonstrated their compatibility. The NPs were found to contain Qu in an amorphous form, and the ionization of the carboxylate moieties in Eudragit®L100 NPs caused the medication to be released at neutral pH. Qu-loaded NPs showed a high and concentration-dependent toxicity to NCI-H460 cancer cell line. These findings show that conventional pharmaceutical excipients such as Eudragit®L-100 can be used successfully in the production of nanomedicine.. This innovative approach will not only enhance therapeutic efficacy, but also minimizes systemic toxicity associated with conventional chemotherapy and Eudragit® L-100 NPs could serve as a promising platform for optimizing cancer treatment outcomes, thus paving the way for more effective and personalized therapeutic strategies in oncology

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

This research was supported by the Department of Pharmaceutical Technology, School of Pharmaceutical Sciences, Universiti Sains Malaysia.

References

1. World Health Organisation (WHO). Global cancer burden growing, amidst mounting need for services. Available from: https://www.who.int/news/item/01-02-2024-global-cancer-burden-growing--amidst-mounting-need-for-services
2. Lancet T. GLOBOCAN 2018: Counting the toll of cancer. Lancet. 2018; 392(10152):985.
3. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin. 2019; 69(1):7-34.
4. World Health Organisation (WHO). Cancer: Key Facts. Available from: https://www.who.int/news-room/fact-sheets/detail/cancer
5. Zappa C and Mousa SA. Non-small cell lung cancer: current treatment and future advances. Transl Lung Cancer Res 2016; 5(3):288-300.
6. Wu Q, Yang Z, Nie Y, Shi Y, Fan D. Multi-drug resistance in cancer chemotherapeutics: mechanisms and lab approaches. Cancer Lett. 2014; 347(2):159-166.
7. Anwer MK, Al-Mansoor MA, Jamil S, Al-Shdefat R, Ansari MN, Shakeel F. Development and evaluation of PLGA polymer based nanoparticles of quercetin. Int J Biol Macromol. 2016; 92:213-219.
8. Cetin M, Aktas Y, Vural I, Capan Y, Dogan LA, Duman M, Dalkara T. Preparation and *in vitro* evaluation of bFGF-loaded chitosan nanoparticles. Drug Deliv. 2017; 14(8):525-529.
9. Adibkia K, Javadzadeh Y, Dastmalchi S, Mohammadi G, Niri FK, Alaei-Beirami M. Naproxen-eudragit® RS100 nanoparticles: preparation and physicochemical characterization. Colloids Surf B Biointerfaces. 2017; 83(1):155-159.
10. Russo M, Spagnuolo C, Tedesco I, Bilotto S, Russo GL. The flavonoid quercetin in disease prevention and therapy: facts and fancies. Biochem Pharmacol. 2012; 83(1):6-15.
11. Cai X, Fang Z, Dou J, Yu A, Zhai G. Bioavailability of quercetin: problems and promises. Curr Med Chem. 2016; 20(20):2572-2582.
12. Mokale VJ, Naik JB, Verma U, Yadava SK. Preparation and characterization of biodegradable glimepiride loaded PLA nanoparticles by o/w solvent evaporation method using high pressure homogenizer: a factorial design approach. SA J Pharm Pharmacol. 2014; 1(1):1.
13. Adibkia K, Javadzadeh Y, Dastmalchi S, Mohammadi G, Niri FK, Alaei-Beirami M. Naproxen-eudragit® RS100 nanoparticles: preparation and physicochemical characterization. Colloids Surf B Biointerfaces. 2017; 83(1):155-159.
14. Song X, Zhao Y, Hou S, Xu F, Zhao R, He J, Cai Z, Li Y, Chen Q. Dual agents loaded PLGA nanoparticles: systematic study of particle size and drug entrapment efficiency. Eur J Pharm Biopharm. 2018; 69(2):445-453.
15. Xu X, Fu Y, Hu H, Duan Y, Zhang Z. Quantitative determination of insulin entrapment efficiency in triblock copolymeric nanoparticles by high-performance liquid chromatography. J Pharm Biomed Anal. 2016; 41(1):266-273.
16. Tantishaiyakul V, Phadoongsombut N, Kamaung S, Wongwisansri S, Mathurod P. Fourier transform infrared spectrometric determination of paracetamol and ibuprofen in tablets. Pharmazie. 1999; 54(2):111-114.
17. Panwar P, Pandey B, Lakhera PC, Singh KP. Preparation, characterization, and *in vitro* release study of albendazole-encapsulated nanosize liposomes. Int J Nanomed. 2019; 5:101-108.
18. Jain A, Khare P, Agrawal RK, Jain SK. Metronidazole loaded pectin microspheres for colon targeting. J Pharm Sci. 2009; 98(11):4229-4236.
19. van Meerloo J, Kaspers GJL, Cloos J. Cell Sensitivity Assays: The MTT Assay. In: Cree, I. (eds) Cancer Cell Culture. Methods in Molecular Biology, Humana Press. 2011; Vol 731.
20. Maeda H, Sawa T, Konno T. Mechanism of tumor-targeted delivery of macromolecular drugs, including the EPR effect in solid tumor and clinical overview of the prototype polymeric drug SMANCS. J Control Release. 2021; 74(1-3):47-61.
21. Cho K, Wang XU, Nie S, Chen Z, Shin DM. Therapeutic nanoparticles for drug delivery in cancer. Clin Cancer Res. 2018; 14(5):1310-1316.
22. Wicki A, Witzigmann D, Balasubramanian V, Huwyler J. Nanomedicine in cancer therapy: challenges, opportunities, and clinical applications. J Control Release. 2015; 200:138-157.
23. Chourasiya A, Upadhaya A, Shukla RN. Isolation of quercetin from leaves of *Azadirachta indica* and anti-diabetic study of the crude extracts. J Pharm Biomed Sci. 2018; 25:179-181.
24. Abd El-Rahmanand SN, Suhailah S. Quercetin nanoparticles: preparation and characterization. Indian J Drugs. 2016; 2(3):96-103.
25. Mohammadi G, Mirzaeei S, Taghe S, Mohammadi P. Preparation and Evaluation of Eudragit® L100 Nanoparticles Loaded Impregnated with KT Tromethamine Loaded PVA -HEC Insertions for Ophthalmic Drug Delivery. Adv Pharm Bull. 2019; 9(4):593-600.
26. Govender T, Stolnik S, Garnett MC, Illum L, Davis SS. PLGA nanoparticles prepared by nanoprecipitation: drug loading and release studies of a water soluble drug. J Control Release. 1999; 57(2):171-185.
27. Verma A and Stellacci F. Effect of surface properties on nanoparticle–cell interactions. Small. 2010; 6(1):12-21.
28. Kakran M, Sahoo NG, Bao H, Pan Y, Li L. Functionalized graphene oxide as nanocarrier for loading and delivery of ellagic acid. Curr Med Chem. 2021; 18(29):4503-4512. doi:10.2174/092986711797287548.
29. Zhang T, Ma J, Li C, Lin K, Lou F, Jiang H, Gao Y, Yang Y, Ming C, Ruan B. Core-shell lipid polymer nanoparticles for combined chemo and gene therapy of childhood head and neck cancers. Oncol Rep. 2017; 37(3):1653-1661.
30. Trenkenschuh E and Friess W. Freeze-drying of nanoparticles: How to overcome colloidal instability by formulation and process optimization. Eur J Pharm Biopharm. 2021; 165:34.
31. Moustafine RI, Zaharov IM, Kemenova VA. Physicochemical characterization and drug release properties of Eudragit® E PO/Eudragit® L 100-55 interpolyelectrolyte complexes. Eur J Pharm Biopharm. 2006; 63(1):26-36.
32. Pecorini G, Ferraro E, Puppi D. Polymeric Systems for the Controlled Release of Flavonoids. Pharmaceutics. 2023;15(2):628.
33. Derakhshandeh K, Soheili M, Dadashzadeh S, Saghiri R. Preparation and *in vitro* characterization of 9-nitrocamptothecin-loaded long circulating nanoparticles for delivery in cancer patients. Int J Nanomedicine. 2010; 5:463-471.