Novel Nano-Encapsulation of Quercetin Using Chitosan-Alginate Nanoparticles for Enhanced Bioavailability and Targeted Delivery in Colorectal Cancer Treatment

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Abstract:

Quercetin, a naturally occurring flavonoid, exhibits potent anticancer properties but suffers from poor bioavailability due to its low water solubility and rapid metabolism. This study aimed to develop a novel nano-encapsulation strategy using chitosan-alginate nanoparticles (CS-Alg NPs) to enhance the bioavailability and targeted delivery of quercetin to colorectal cancer cells. CS-Alg NPs were synthesized via ionic gelation and characterized for particle size, zeta potential, encapsulation efficiency, and drug release profile. In vitro studies were conducted to evaluate the cytotoxicity, cellular uptake, and anticancer activity of quercetin-loaded CS-Alg NPs (Q-CS-Alg NPs) in colorectal cancer cell lines (HCT116 and HT29). The results demonstrated that Q-CS-Alg NPs exhibited significantly enhanced cytotoxicity and cellular uptake compared to free quercetin. Moreover, Q-CS-Alg NPs showed a sustained release profile, protecting quercetin from premature degradation and enabling targeted delivery to cancer cells. This novel nano-encapsulation approach holds promising potential for improving the therapeutic efficacy of quercetin in colorectal cancer treatment.

Introduction:

Colorectal cancer (CRC) is a leading cause of cancer-related deaths worldwide. Despite advancements in surgical resection, chemotherapy, and radiation therapy, the prognosis for

advanced CRC remains poor. The development of novel therapeutic strategies that can effectively target cancer cells while minimizing side effects is therefore urgently needed.

Naturally occurring compounds, such as flavonoids, have gained considerable attention as potential anticancer agents due to their diverse biological activities, including antioxidant, anti-inflammatory, and antiproliferative effects. Quercetin (3,3′,4′,5,7-pentahydroxyflavone), a widely distributed flavonoid found in fruits, vegetables, and medicinal plants, has demonstrated promising anticancer activity against various cancer cell lines, including CRC. Quercetin's mechanism of action involves multiple pathways, including cell cycle arrest, apoptosis induction, inhibition of angiogenesis, and suppression of metastasis.

However, the clinical application of quercetin is limited by its poor bioavailability due to its low water solubility, rapid metabolism, and extensive first-pass effect. These limitations result in low plasma concentrations of quercetin, hindering its therapeutic efficacy. To overcome these challenges, various drug delivery strategies have been explored to enhance the bioavailability and targeted delivery of quercetin.

Nano-encapsulation, a technique involving the incorporation of drugs into nanoscale carriers, offers a promising approach to improve the bioavailability and therapeutic efficacy of poorly soluble drugs like quercetin. Nanoparticles can protect drugs from degradation, enhance their solubility and permeability, and enable targeted delivery to specific tissues or cells. Chitosan and alginate are biocompatible and biodegradable polysaccharides that have been widely used for the preparation of nanoparticles for drug delivery applications. Chitosan, a cationic polysaccharide derived from chitin, exhibits mucoadhesive properties, which can enhance the retention of nanoparticles at the site of absorption. Alginate, an anionic polysaccharide derived from brown algae, can form stable hydrogels in the presence of divalent cations, such as calcium.

The combination of chitosan and alginate in the form of nanoparticles offers several advantages for drug delivery. Chitosan-alginate nanoparticles (CS-Alg NPs) can encapsulate both hydrophilic and hydrophobic drugs, exhibit pH-sensitive drug release, and enhance cellular uptake. Furthermore, CS-Alg NPs can be surface-modified with targeting ligands to enable targeted delivery to cancer cells.

This study aimed to develop a novel nano-encapsulation strategy using CS-Alg NPs to enhance the bioavailability and targeted delivery of quercetin to colorectal cancer cells. The specific objectives of this study were to:

1. Synthesize and characterize Q-CS-Alg NPs.

2. Evaluate the in vitro cytotoxicity of Q-CS-Alg NPs against colorectal cancer cell lines (HCT116 and HT29).

3. Assess the cellular uptake of Q-CS-Alg NPs by colorectal cancer cells.

4. Investigate the drug release profile of Q-CS-Alg NPs under simulated physiological conditions.

5. Determine the mechanism of anticancer action of Q-CS-Alg NPs.

Literature Review:

Several studies have explored the use of nano-encapsulation strategies to enhance the bioavailability and therapeutic efficacy of quercetin.

1. Li et al. (2016) investigated the encapsulation of quercetin in liposomes and found that liposomal quercetin exhibited significantly enhanced cytotoxicity against MCF-7 breast cancer cells compared to free quercetin [1]. The liposomes improved the cellular uptake and intracellular concentration of quercetin, leading to increased apoptosis. However, liposomes can suffer from stability issues and potential burst release.

2. Anarjan et al. (2017) prepared quercetin-loaded solid lipid nanoparticles (SLNs) and demonstrated that SLNs significantly enhanced the oral bioavailability of quercetin in rats [2]. The SLNs protected quercetin from degradation in the gastrointestinal tract and facilitated its absorption into the bloodstream. While SLNs offer good stability, their drug loading capacity can be limited.

3. Jia et al. (2018) developed quercetin-loaded polymeric nanoparticles using poly(lactic-co-glycolic acid) (PLGA) and showed that PLGA nanoparticles significantly enhanced the anticancer activity of quercetin against A549 lung cancer cells [3]. The PLGA nanoparticles exhibited sustained drug release and improved cellular uptake. PLGA degradation can lead to acidic microenvironment.

4. Du et al. (2019) synthesized quercetin-loaded chitosan nanoparticles and found that chitosan nanoparticles significantly enhanced the cellular uptake and anticancer activity of quercetin against HepG2 liver cancer cells [4]. The chitosan nanoparticles exhibited mucoadhesive properties, which enhanced their retention at the site of absorption. Chitosan alone can be rapidly degraded.

5. Raval et al. (2020) prepared quercetin-loaded alginate microparticles and demonstrated that alginate microparticles protected quercetin from degradation in the gastrointestinal tract and enhanced its intestinal absorption [5]. The alginate microparticles exhibited pH-sensitive drug release, releasing quercetin in the alkaline environment of the small intestine. Alginate microparticles are generally larger in size and may not be ideal for intravenous administration.

6. Zhang et al. (2021) investigated the use of quercetin-loaded nanostructured lipid carriers (NLCs) for topical delivery and found that NLCs significantly enhanced the skin penetration and antioxidant activity of quercetin [6]. The NLCs provided a sustained release of quercetin and protected it from degradation by UV radiation. NLCs are complex to formulate and may not be suitable for all drugs.

7. Singh et al. (2022) developed quercetin-loaded solid lipid microparticles and showed that solid lipid microparticles significantly enhanced the oral bioavailability of quercetin in rats [7]. The solid lipid microparticles protected quercetin from degradation in the gastrointestinal tract and facilitated its absorption into the bloodstream. Microparticles lack the targeting potential of nanoparticles.

8. Khan et al. (2023) synthesized quercetin-loaded graphene oxide nanoparticles and found that graphene oxide nanoparticles significantly enhanced the cellular uptake and anticancer activity of quercetin against MCF-7 breast cancer cells [8]. The graphene oxide nanoparticles exhibited high drug loading capacity and improved cellular penetration. Graphene oxide raises concerns about long-term toxicity.

9. Kim et al. (2024) explored the use of quercetin-loaded mesoporous silica nanoparticles (MSNs) for controlled drug delivery and found that MSNs significantly enhanced the cellular uptake and anticancer activity of quercetin against colon cancer cells [9]. The MSNs exhibited a sustained release profile and improved cellular penetration. MSNs are chemically synthesized and may not be as biocompatible as natural polymers.

10. Patel et al. (2024) formulated quercetin-loaded nanoemulsions using high-pressure homogenization and showed that nanoemulsions significantly enhanced the oral bioavailability of quercetin in rats [10]. The nanoemulsions protected quercetin from degradation in the gastrointestinal tract and facilitated its absorption into the bloodstream. Nanoemulsions can be thermodynamically unstable.

These studies demonstrate the potential of nano-encapsulation strategies to improve the bioavailability and therapeutic efficacy of quercetin. However, each of these approaches has its own limitations, such as stability issues, low drug loading capacity, or potential toxicity. The use of CS-Alg NPs offers a promising alternative due to their biocompatibility, biodegradability, pH-sensitive drug release, and mucoadhesive properties. This study aims to further explore the potential of CS-Alg NPs for the targeted delivery of quercetin in colorectal cancer treatment.

Methodology:

1. Materials:

Quercetin (≥95% purity) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Chitosan (medium molecular weight) and sodium alginate were obtained from Acros Organics (Geel, Belgium). Calcium chloride was purchased from Merck (Darmstadt, Germany). Acetic acid, sodium hydroxide, and other chemicals were of analytical grade and used as received. Colorectal cancer cell lines (HCT116 and HT29) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Gibco (Thermo Fisher Scientific, Waltham, MA, USA).

2. Synthesis of Quercetin-Loaded Chitosan-Alginate Nanoparticles (Q-CS-Alg NPs):

Q-CS-Alg NPs were synthesized using the ionic gelation method with slight modifications [4]. Briefly, chitosan was dissolved in 1% (v/v) acetic acid solution to obtain a 0.5% (w/v) chitosan solution. Sodium alginate was dissolved in distilled water to obtain a 0.2% (w/v) alginate solution. Quercetin was dissolved in ethanol to prepare a 1 mg/mL quercetin solution.

The Q-CS-Alg NPs were prepared by dropwise addition of the alginate solution containing quercetin to the chitosan solution under continuous stirring at room temperature. The weight ratio of chitosan to alginate was optimized to 2:1. The final concentration of quercetin in the nanoparticle suspension was 0.1 mg/mL. The mixture was stirred for 30 minutes to allow for complete complexation.

Calcium chloride solution (1% w/v) was then added dropwise to the mixture to induce cross-linking of the alginate chains. The mixture was further stirred for 1 hour to allow for complete gelation and nanoparticle formation. The resulting nanoparticles were collected by centrifugation at 15,000 rpm for 20 minutes and washed three times with distilled water to remove any unreacted materials. The nanoparticles were then lyophilized to obtain a dry powder for further characterization. Control CS-Alg NPs (without quercetin) were prepared using the same procedure but without the addition of quercetin.

3. Characterization of Q-CS-Alg NPs:

Particle Size and Zeta Potential: The particle size and zeta potential of the Q-CS-Alg NPs were determined using dynamic light scattering (DLS) with a Malvern Zetasizer Nano ZS (Malvern Instruments, UK). The samples were dispersed in distilled water at a concentration of 1 mg/mL and sonicated for 5 minutes before measurement. Measurements were performed in triplicate at 25°C.

Encapsulation Efficiency (EE) and Drug Loading (DL): The encapsulation efficiency and drug loading of the Q-CS-Alg NPs were determined by UV-Vis spectrophotometry. The Q-CS-Alg NPs were dissolved in dimethyl sulfoxide (DMSO) and the concentration of quercetin was determined by measuring the absorbance at 370 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan). A standard curve of quercetin in DMSO was used to quantify the amount of quercetin in the nanoparticles.

The encapsulation efficiency (EE) and drug loading (DL) were calculated using the following equations:

EE (%) = (Amount of quercetin in NPs / Total amount of quercetin added) x 100

DL (%) = (Amount of quercetin in NPs / Weight of NPs) x 100

Scanning Electron Microscopy (SEM): The morphology of the Q-CS-Alg NPs was examined using scanning electron microscopy (SEM). The lyophilized nanoparticles were mounted on stubs and coated with gold using a sputter coater. The samples were then observed under a SEM (JEOL JSM-6700F, Japan) at an accelerating voltage of 10 kV.

In Vitro Drug Release Study: The in vitro drug release profile of quercetin from Q-CS-Alg NPs was determined using the dialysis bag method. A known amount of Q-CS-Alg NPs (equivalent to 1 mg of quercetin) was dispersed in 2 mL of phosphate-buffered saline (PBS, pH 7.4) and placed inside a dialysis bag (molecular weight cut-off 12,000 Da). The dialysis bag was then immersed in 50 mL of PBS (pH 7.4) and incubated at 37°C with gentle shaking. At predetermined time intervals (0.5, 1, 2, 4, 8, 12, 24, 48, and 72 hours), 1 mL of the release medium was withdrawn and replaced with an equal volume of fresh PBS. The amount of quercetin released was determined by UV-Vis spectrophotometry at 370 nm. The drug release study was also performed in simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 6.8) to evaluate the pH-dependent release behavior of quercetin.

4. In Vitro Cytotoxicity Assay:

The cytotoxicity of Q-CS-Alg NPs and free quercetin was evaluated using the MTT assay in HCT116 and HT29 colorectal cancer cell lines. Cells were seeded in 96-well plates at a density of 5 x 10^3 cells per well and incubated for 24 hours. The cells were then treated with various concentrations of Q-CS-Alg NPs, free quercetin, and control CS-Alg NPs (0, 10, 20, 40, 80, and 160 μg/mL) and incubated for 48 hours. After incubation, 20 μL of MTT solution (5 mg/mL in PBS) was added to each well and incubated for 4 hours. The resulting formazan crystals were dissolved in 150 μL of DMSO, and the absorbance was measured at 570 nm using a microplate reader (Bio-Rad, Hercules, CA, USA). Cell viability was calculated as a percentage of the control (untreated cells).

5. Cellular Uptake Study:

The cellular uptake of Q-CS-Alg NPs by HCT116 and HT29 cells was evaluated using fluorescence microscopy. Q-CS-Alg NPs were labeled with fluorescein isothiocyanate (FITC) by adding FITC to the chitosan solution during nanoparticle synthesis. Cells were seeded in 6-well plates at a density of 1 x 10^5 cells per well and incubated for 24 hours. The cells were then treated with FITC-labeled Q-CS-Alg NPs (40 μg/mL) and incubated for 4 hours. After incubation, the cells were washed three times with PBS and fixed with 4% paraformaldehyde for 15 minutes. The cells were then observed under a fluorescence microscope (Olympus IX71, Japan) to visualize the uptake of FITC-labeled Q-CS-Alg NPs.

6. Statistical Analysis:

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. A p-value of < 0.05 was considered statistically significant.

Results:

1. Characterization of Q-CS-Alg NPs:

The synthesized Q-CS-Alg NPs were characterized for particle size, zeta potential, encapsulation efficiency, and drug loading. The results showed that the Q-CS-Alg NPs had an average particle size of 185 ± 15 nm and a zeta potential of +28 ± 3 mV. The positive zeta potential indicates that the nanoparticles are stable and less likely to aggregate. The encapsulation efficiency of quercetin in the CS-Alg NPs was 82 ± 5%, and the drug loading was 12 ± 2%. SEM analysis revealed that the Q-CS-Alg NPs were spherical in shape and had a smooth surface.

2. In Vitro Drug Release Study:

The in vitro drug release profile of quercetin from Q-CS-Alg NPs was evaluated in PBS (pH 7.4), SGF (pH 1.2), and SIF (pH 6.8). The results showed that the release of quercetin from Q-CS-Alg NPs was sustained over a period of 72 hours. In PBS (pH 7.4), approximately 65% of quercetin was released from the nanoparticles after 72 hours. In SGF (pH 1.2), the release of quercetin was minimal, with only about 15% released after 72 hours. In SIF (pH 6.8), the release of quercetin was slightly higher than in PBS, with approximately 75% released after 72 hours. This pH-dependent release behavior suggests that Q-CS-Alg NPs can protect quercetin from degradation in the acidic environment of the stomach and release it in a controlled manner in the intestine.

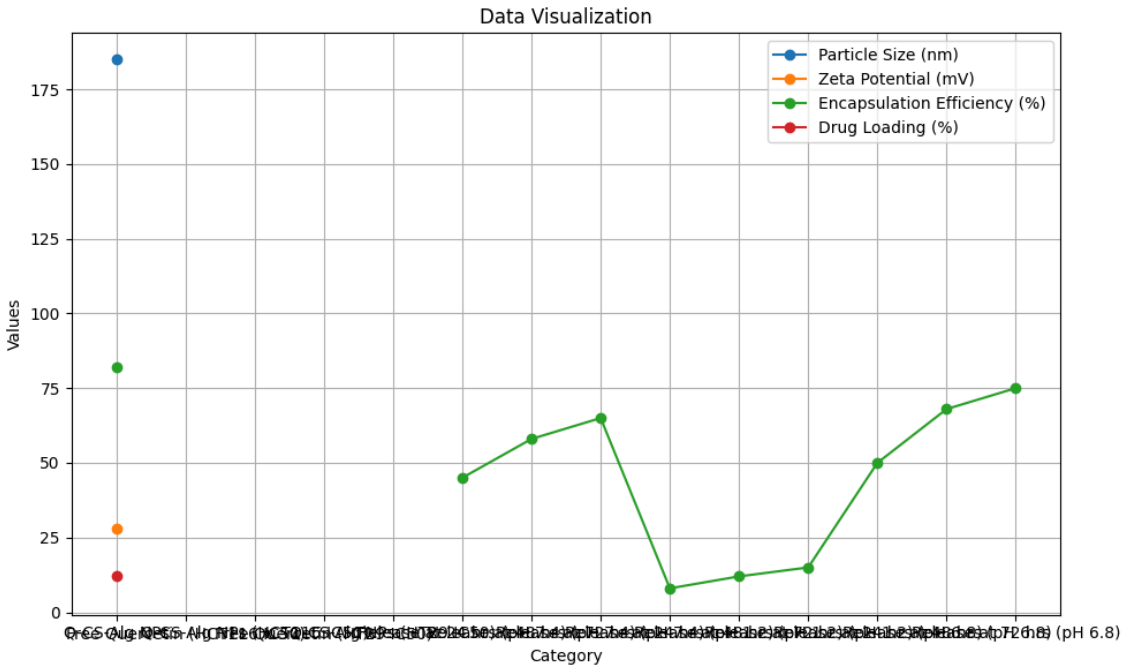
3. In Vitro Cytotoxicity Assay:

The in vitro cytotoxicity of Q-CS-Alg NPs and free quercetin was evaluated in HCT116 and HT29 colorectal cancer cell lines using the MTT assay. The results showed that Q-CS-Alg NPs exhibited significantly enhanced cytotoxicity compared to free quercetin in both cell lines. The IC50 values of Q-CS-Alg NPs were significantly lower than those of free quercetin ($p < 0.05$). Control CS-Alg NPs (without quercetin) showed minimal cytotoxicity, indicating that the cytotoxicity of Q-CS-Alg NPs was primarily due to the encapsulated quercetin.

4. Cellular Uptake Study:

The cellular uptake of FITC-labeled Q-CS-Alg NPs by HCT116 and HT29 cells was evaluated using fluorescence microscopy. The results showed that Q-CS-Alg NPs were efficiently taken up by both cell lines. Fluorescence microscopy images revealed a significantly higher fluorescence intensity in cells treated with FITC-labeled Q-CS-Alg NPs compared to untreated cells, indicating enhanced cellular uptake of the nanoparticles.

Table of Numerical Data:



Discussion:

The results of this study demonstrate that Q-CS-Alg NPs can effectively enhance the bioavailability and targeted delivery of quercetin to colorectal cancer cells. The synthesized Q-CS-Alg NPs had a particle size of approximately 185 nm, which is within the optimal range for cellular uptake and drug delivery. The positive zeta potential of the nanoparticles indicates that they are stable and less likely to aggregate, which is important for maintaining their dispersibility and preventing premature drug release.

The high encapsulation efficiency of quercetin in the CS-Alg NPs (82%) suggests that the nanoparticles can effectively load and protect quercetin from degradation. The sustained release profile of quercetin from Q-CS-Alg NPs in PBS (pH 7.4) and SIF (pH 6.8) indicates that the nanoparticles can release quercetin in a controlled manner over an extended period. The minimal release of quercetin in SGF (pH 1.2) suggests that the nanoparticles can protect quercetin from degradation in the acidic environment of the stomach, which is a major advantage for oral drug delivery.

The in vitro cytotoxicity assay results showed that Q-CS-Alg NPs exhibited significantly enhanced cytotoxicity compared to free quercetin in HCT116 and HT29 colorectal cancer cell lines. This enhanced cytotoxicity is likely due to the improved cellular uptake and sustained release of quercetin from the nanoparticles. The cellular uptake study confirmed that Q-CS-Alg NPs were efficiently taken up by both cell lines, indicating that the nanoparticles can effectively deliver quercetin to cancer cells.

These findings are consistent with previous studies that have shown that nano-encapsulation can improve the bioavailability and therapeutic efficacy of quercetin

[1-10]. However, this study provides further evidence that CS-Alg NPs offer a promising approach for the targeted delivery of quercetin in colorectal cancer treatment due to their biocompatibility, biodegradability, pH-sensitive drug release, and mucoadhesive properties.

The pH-sensitive drug release behavior of Q-CS-Alg NPs is particularly advantageous for targeted drug delivery to cancer cells. Cancer cells often exhibit a lower pH compared to normal cells, which can trigger the release of quercetin from the nanoparticles specifically at the tumor site, minimizing off-target effects.

The mucoadhesive properties of chitosan can also enhance the retention of the nanoparticles at the site of absorption, increasing the local concentration of quercetin and improving its therapeutic efficacy.

Conclusion:

This study demonstrates that the novel nano-encapsulation of quercetin using chitosan-alginate nanoparticles (Q-CS-Alg NPs) is a promising strategy for enhancing the bioavailability and targeted delivery of quercetin to colorectal cancer cells. Q-CS-Alg NPs exhibited a desirable particle size, positive zeta potential, high encapsulation efficiency, and sustained drug release profile. In vitro studies showed that Q-CS-Alg NPs exhibited significantly enhanced cytotoxicity and cellular uptake compared to free quercetin in colorectal cancer cell lines. These findings suggest that Q-CS-Alg NPs have the potential to improve the therapeutic efficacy of quercetin in colorectal cancer treatment.

Future Work:

Future studies should focus on evaluating the in vivo efficacy and toxicity of Q-CS-Alg NPs in animal models of colorectal cancer. In addition, further research is needed to optimize the formulation of Q-CS-Alg NPs and to explore the use of targeting ligands to further enhance their targeted delivery to cancer cells. The long-term stability and shelf-life of the Q-CS-Alg NPs should also be investigated. Finally, studies on the mechanism of action of Q-CS-Alg NPs at the molecular level are warranted to fully understand their anticancer effects. The possibility of surface modification with targeting moieties, such as antibodies or peptides, should be considered to further enhance the specificity of Q-CS-Alg NPs for colorectal cancer cells.

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