

Article

pH-Responsive Colloidal Guar Gum Nanoparticles for Rosmarinic Acid Delivery: Role of the Degree of Carboxymethylation

Ayşe Selen Yildirim ¹ and Özlem Erdal Altıntaş ^{2,*} 

¹ Institute of Postgraduate Education, Department of Nutrition and Dietetics, Afyonkarahisar Health Sciences University, 03200 Afyonkarahisar, Turkey

² Faculty of Health Sciences, Department of Nutrition and Dietetics, Afyonkarahisar Health Sciences University, 03200 Afyonkarahisar, Turkey

* Correspondence: ozlem.altintas@afsu.edu.tr

Abstract

The oral delivery of polyphenolic compounds such as rosmarinic acid (Ros) is limited by poor gastrointestinal stability and early release, resulting in low bioaccessibility. Herein, carboxymethylated guar gum (cmGG)-based nanoparticles were developed as a pH-responsive colloidal delivery system to enhance Ros stability, prevent early release, and improve intestinal bioaccessibility. In this context, pH-responsiveness refers to pH-dependent modulation of degradation, and stabilization along the gastrointestinal tract, rather than an abrupt pH-triggered burst release. Guar gum was chemically modified to different degrees of carboxymethylation to enhance its colloidal stability under gastrointestinal conditions, reduce polymer degradation, and enable a more controlled release of the phenolic compound Ros. Comparative evaluation of cmGG systems with varying degrees of carboxymethylation revealed that nanoparticles prepared from highly substituted cmGG exhibited superior colloidal stability and acid resistance, contributing to effective protection of Ros under gastric conditions. Ros-loaded guar gum nanoparticles effectively suppressed release at acidic pH while enabling controlled and sustained release at intestinal pH. Simulated gastrointestinal digestion studies demonstrated that Ros-loaded carboxymethylated guar gum nanoparticles significantly enhanced the gastrointestinal stability and bioaccessibility of Ros compared with non-carboxymethylated guar gum nanoparticles. Overall, these findings indicate that the degree of carboxymethylation is a critical design parameter for tuning colloidal behavior and release performance under the varying pH conditions encountered throughout the gastrointestinal tract in guar gum-based nanoparticle systems.

Keywords: bioaccessibility; carboxymethylated guar gum; colon-targeted nanoparticles; stability; simulated gastrointestinal model; rosmarinic acid



Academic Editor: Anna Trybala

Received: 31 December 2025

Revised: 29 January 2026

Accepted: 6 February 2026

Published: 10 February 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and

conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction

Oral administration of bioactive compounds is one of the most preferred routes in pharmaceutical treatments due to patient compliance, ease of administration, and low invasiveness. However, the physiological conditions of the gastrointestinal (GI) system particularly enzymatic degradation, low pH, the mucosal barrier, and rapid intestinal transit encountered before reaching the target site adversely affect the bioaccessibility of bioactive compounds, defined as the fraction released from the carrier matrix during gastrointestinal digestion and rendered available for absorption. Consequently, the bioavailability of the

compound, referring to the fraction that is absorbed, reaches systemic circulation, and exerts a biological effect is limited, leading to reduced therapeutic efficacy [1–4]. Due to its comparatively low enzymatic degradation, the colon is recognized as an optimal site for the absorption of proteins and bioactive compounds. To achieve targeted delivery, systems designed for the colon should limit the release of their payload in the upper gastrointestinal tract and ensure that the compound is released only upon reaching the colon, thereby eliciting the desired physiological response. Various approaches applied for this type of carrier system include encapsulating the bioactive compound with pH-sensitive polymers, developing pro-bioactive compounds, time-dependent and pH-dependent release systems, applying colon-specific biodegradable polymers, enzyme-controlled delivery systems, osmotic and pressure-controlled delivery systems, and delivery systems incorporating nanotechnology [5–7]. Colon-targeted bioactive compound delivery systems aim to avoid early release of the bioactive compound in the upper GI tract, protecting it until it reaches the colon, thereby achieving release only in the target region. This reduces systemic exposure while increasing local therapeutic effect and minimising side effects [8]. Consequently, colon-targeted nanoparticle systems have been at the centre of controlled release and targeted treatment strategies in recent years. Various kinds of polymers are used to achieve an optimal formulation of colon-targeted delivery systems, with biodegradable biopolymers being preferred, particularly due to their ability to accommodate various delivery regimens and colon bacteria. Among these, guar gum, a natural polysaccharide, has great potential for use in colon-specific delivery systems due to its various advantages, like low toxicity, good stability, and a favourable biodegradability profile [9]. Guar gum (GG) is a naturally emergent galactomannan polysaccharide obtained from the dried seeds of *Cyamopsis tetragonoloba*, a member of the Leguminosae family. GG consists of a linear backbone composed of chemically β -1,4-linked d-mannose units and dissolves due to the occurrence of randomly linked α -1,6-linked galactose units as side chains. This structure gives it water retention, gel-forming, and microflora and biodegradation capabilities [10,11]. Its high sensitivity to microbial degradation in the colon, its pH-dependent swelling that enables colon-specific release of the bioactive compound, and its sustained bioactive compound release property have demonstrated its potential as a candidate for a colon-targeted delivery system [12,13]. At the same time, GG acts as an indigestible food component with prebiotic properties by specifically promoting the growth and/or activity of certain bacterial species in the colon, resulting in positive effects on the host and contributing to overall well-being. However, properties such as irregular hydration rate, decreased viscosity when stored, and low thermal stability limit the use of GG in some applications. Furthermore, although natural GG demonstrates colon-focused delivery potential, the limitations described above can prevent the dosage form from achieving optimal performance. Rapid hydration at colonic pH causes sudden release of the bioactive compound, resulting in a significant portion of the bioactive compound remaining unabsorbed with lower bioavailability. Furthermore, the sudden increase in the concentration of the bioactive compound can also lead to local irritation and systemic side effects. The presence of colonic microflora also makes GG more susceptible to microbial attack, which can disrupt the structural integrity of the GG backbone, leading to sudden release of the bioactive compound and subsequent disadvantages in the delivery profile [9,14]. To overcome these challenges, the chemical modification of GG has proven to be an effective strategy, enabling the physicochemical properties of the resulting polymer to be adjusted according to the intended application. Within this framework, carboxymethylated guar gum (cmGG) has emerged as a potentially effective functional material for diverse applications. The carboxymethylation process is carried out by attaching carboxymethyl ($-\text{CH}_2\text{-COO}^-$) groups to the hydroxyl groups of the polymer, thereby obtaining carboxymethylated guar gum (cmGG) [14,15]. This modifi-

cation enhances the polymer's ionic nature and hydrophilicity, improving its solubility in aqueous systems. Compared to its natural form, cmGG exhibits more regular water uptake, controlled swelling behaviour, and better gel-forming capacity. Furthermore, the negative charge imparted by the carboxymethyl groups provides electrostatic stability on the nanoparticle surface, preventing particle agglomeration and increasing the long-term stability of the system [16–18]. From a colloidal science perspective, the enhanced nanoparticle stability can be qualitatively interpreted within the Derjaguin–Landau–Verwey–Overbeek (DLVO) framework, where surface charges contribute to electrostatic repulsion, while additional steric effects arising from the polymer chains further support colloidal stabilization beyond classical DLVO considerations [19]. Carboxymethylation also improves the polymer's pH sensitivity: while exhibiting limited swelling in the low-pH conditions of the top GI tract, it provides greater ionisation and swelling at colonic pH (approximately 6.8–7.4). Thus, it becomes possible to protect the cargo from the upper GI tract and transport it to the colon, where controlled release occurs [20]. Furthermore, the interactions between Ros and the polymer matrix are strengthened by interfacial affinity, contributing to retention of the bioactive compound and preventing premature release. Therefore, cmGG has emerged as an important polymer in the development of pH- and microbiota-responsive systems. Carboxymethylation also does not reduce the biodegradability of the polymer by preserving its degradability by the microbiota; on the contrary, it may facilitate access by bacterial enzymes in the colon. The literature reports that cmGG has been successfully used in colon-specific systems with microbiota-triggered release mechanisms [9,20,21]. Thus, cmGG enables a dual-controlled colon-targeting mechanism that is both pH-sensitive and microbiota-triggered. cmGG-based biomaterials have uplifted the quality level in colon-targeted delivery systems for bioactive compounds. Countless formulations, for instance films, capsules, nanoparticles, tablets, microspheres, etc., are designed to reach the target in colon-targeted delivery systems. Among these, polymeric nano- or microparticles rank among the most common classes and supply many advantages over other systems. Nano- or microparticles are endowed with the remarkable capability to encapsulate bioactive compounds. In addition to promoting the cellular association of the bioactive compound, nano- or microparticles always provide a slower and more controlled release profile. From a colloidal science perspective, the stability, controlled release, and performance of these nanoparticles are influenced by interfacial affinity between the polymer matrix and the encapsulated compound, as well as steric and electrostatic stabilization effects [9,20,22]. In recent years, secondary metabolites derived from plants have emerged as valuable bioactive agents for promoting human health. Rosmarinic acid (Ros), a phenolic compound with notable biological activity, is predominantly found in members of the Lamiaceae and Boraginaceae families. Its biosynthesis occurs through enzyme-catalyzed reactions involving the amino acids tyrosine and phenylalanine. In contrast, chemical synthesis of Ros requires an esterification reaction between caffeic acid and 3,4-dihydroxyphenyl lactic acid, which introduces two additional phenolic rings into the molecule. Extensive research has highlighted Ros's broad therapeutic potential, demonstrating efficacy against various conditions such as cancer, diabetes, inflammatory and neurodegenerative disorders, as well as liver diseases [23,24]. Ros, among the foremost polyphenol-based antioxidants, is gaining increasing attention due to its bioactive properties, including anti-inflammatory, anti-diabetic, anti-hypertensive, anti-cancer, and antibacterial activities. Furthermore, due to its high antioxidant capacity, this natural polyphenol has recently attracted attention for its use as a nutraceutical compound in the food industry [25]. Despite Ros's high therapeutic potential, its intrinsic properties, such as poor water solubility and limited bioavailability, have inferiorated its translation into clinical applications. Ros has been extensively studied for its therapeutic functions and nutraceutical features in improving human health, de-

pending on its noteworthy pharmacological and medicinal properties [26–28]. Recently, the establishment of valuable nutraceuticals and effective medicinal products from natural origins has gained attention. Nevertheless, the efficacy of these bioactive compounds varies depending on their absorption, stability, and bioavailability. Some improvements in these adverse characteristics can be realized by encapsulating the compounds in liposomes or polymeric microparticles or nanoparticles [24]. The success of colon-targeted systems depends not only on formulation development but also on the accurate assessment of their behaviour throughout gastrointestinal transit. To this end, *in vitro* digestion models are increasingly utilized. These models mimic the oral, gastric, small intestinal, and colonic stages by simulating pH changes, enzymatic hydrolysis, bile salts, and microbiota effects. Through these simulations, the stability, payload retention capacity, and controlled release potential of numerous bioactive compound-loaded nanoparticles can be evaluated in gastrointestinal (GI) environments [29,30]. Therefore, these models are of critical importance for understanding the response of pH- and microbiota-sensitive polymers such as cmGG to the digestive environment.

This study aims to effectively deliver rosmarinic acid (Ros), which stands out for its superior therapeutic effects and nutraceutical properties, to the colon by preventing its early absorption in the upper gastrointestinal tract and enabling its controlled release in the colon. To this end, polymeric nanoparticles were developed from guar gum derivatives with different degrees of carboxymethylation. The stability of the resulting carboxymethylated nanoparticles was systematically evaluated under *in vitro* simulated gastric and simulated intestinal fluid conditions, and the nanoparticle formulation exhibiting the highest stability in both environments was selected. Ros was loaded into the most stable formulation, and the resulting Ros-loaded nanoparticles were thoroughly characterized. In addition, Ros-loaded carboxymethylated guar gum-based nanoparticles (Ros-cmGG@Np) were comparatively evaluated against Ros-loaded unmodified guar gum nanoparticles (Ros-GG@Np) in terms of controlled release, stability, and bioaccessibility under Ros-specific *in vitro* gastrointestinal system conditions. The novelty of this study lies in the systematic investigation of carboxymethylated guar gum-based nanoparticles for the oral delivery of Ros and in elucidating the effects of polymer modification (carboxymethylation) on nanoparticle behavior under varying pH conditions of the gastrointestinal tract. This comparative and integrated evaluation conducted within a single *in vitro* model provides a meaningful contribution to the limited literature on colon-targeted delivery systems for Ros.

2. Material and Methods

2.1. Chemicals and Reagents

Rosmarinic acid (Mw = 360.31; CAS 2028-92-5), research-grade guar gum (Mw = 200 kDa; CAS 9000-30-0), and 25% glutaraldehyde (Mw = 100.12 g/mol; CAS 111-30-8) used in this study were obtained from Sigma-Aldrich (Darmstadt, Germany), whereas monochloroacetic acid was supplied by Merck (Darmstadt, Germany). All reagents required for the INFOGEST digestive system (including inorganic salts, enzymes (pepsin P7000, pancreatin P7545, lipase L3126, Type II) and bile salts) were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Caco-2 (ATCC HTB-37) and HT-29 (ATCC HTB-38) cells were obtained from the American Type Culture Collection (ATCC) (Manassas, VA, USA). EMEM, DEMEM and supplements were purchased from CEFO Ltd., Seoul, Republic of Korea, and the MTT Cell Proliferation Assay Kit was purchased from Bio Vision, BioVision Inc., Milpitas, CA, USA. All other solvents and reagents used in the study were of analytical grade.

2.2. Carboxymethyl Guar Gum Synthesis (cmGG)

The synthesis of cmGG was performed by adapting the aqueous medium method previously described by Bachra et al. (2022) [31]. Briefly, 10 g of GG was dispersed in 50 mL of deionized water within a round-bottom flask equipped with a mechanical stirrer and stirred overnight. The solution was then sonicated for 1–2 h to eliminate entrapped air bubbles until a clear colloidal solution was obtained. Subsequently, 10 mL of NaOH solution (10% *w/v*) was added dropwise at a rate of 1 mL/min and the mixture was stirred at room temperature for 15 min. Monochloroacetic acid (MCA) in varying amounts, as specified in Table 1, was dissolved in 5 mL of deionized water and gradually added to the reaction mixture, resulting in the formation of three distinct formulations. Each formulation was continuously stirred for 30 min, followed by heating the reaction mixture to 60 °C for 4 h. The reaction products were extracted with ethanol and centrifuged three times (10,000 rpm). The pH of the extract was adjusted to 7.0 using glacial acetic acid. Cold ethanol (50 mL) was then introduced as a non-solvent to precipitate the product, which was subsequently collected, washed multiple times with methanol, and dried under vacuum [31]. Finally, the obtained cmGG products were ground, sieved, and stored at +4 °C in powder form for further use.

Table 1. Formulation for cmGG synthesis and degree of substitution of cmGGs.

GG (g)	MCA (g)	Formulations	DS
10	3	cmGG-1	0.32
10	6	cmGG-2	0.57
10	9	cmGG-3	0.88

2.3. Determination of the Degree of Substitution (DS)

The evaluation of the capacity of the existing carboxylic acids in the cmGG formulations (degree of substitution) presented in Table 1 was carried out using the acid-base titration technique reported by Yadav et al. [32], with minor alterations. Initially, the cmGG formulations (1.5 g) were treated with 50 mL of 2 M NaCl solution at room temperature for 48 h and stirred in a magnetic stirrer to change them to the hydrogen form prior to titration. The cmGG dispersions converted to the hydrogen form were extracted and dried at 60 °C for 2 h. Dry H-cmGG (0.5 g) was dissolved in 50 mL of 0.1 M NaOH solution and then back-titrated with excess NaOH using phenolphthalein as an indicator with standardised 0.1 M HCl solutions [32]. The degree of substitution (DS) of the functionalised polymer functional groups was calculated using Equation (1):

$$w_a = \frac{C_{NaOH}V_{NaOH} - C_{HCl}V_{HCl}}{m}$$

$$DS = 162w_a / (5900 - 58w_a) \quad (1)$$

Here, the mass fraction of the single bond CH₂COOH (acetyl group); the molar concentrations of the standard NaOH and HCl solutions, *C*_{NaOH} and *C*_{HCl}; 59 g/mol, the molar mass of the single bond CH₂COOH; *V*, the volume of NaOH (50.00 mL), and *V*_{HCl}, volume of HCl used for the titration of excess NaOH; *m* (g), the mass of polymer obtained; and 62 g/mol, the molar mass of anhydroglucose unit, are applied.

2.4. Characterisation of cmGG Derivatives (FT-IR)

The molecular bond characterisation of the cmGG formulations listed in Table 1 was analysed using FT-IR spectroscopy at a wavelength of 500–4000 cm^{−1}. For cmGG polymers

in dry powder form, the samples were pressed into KBr pellets and placed between KBr salt plates for analysis at the specified wavelength.

2.5. Synthesis of cmGG Polymeric Nanoparticles (cmGG@Np)

Carboxymethyl guar gum nanoparticles (cmGG@Np) were synthesized by oil emulsification in water and in situ polymer crosslinking [33]. First, 1 mL of Span 80 (2% *v/v*) was introduced to 10 mL of DCM (dichloromethane) to form the oil phase. 0.5% (*w/v*) solution of the cmGG formulations (Table 1) was prepared in 30 mL of dH₂O and added dropwise to the oil phase, and the resulting mixture was stored in a continuous magnetic stirrer. Once mutual saturation occurs of the oil and continuous phases, the mixture was sonicated for 10 min (30 s on/10 s off, 40% amplitude) and then 10 mL of glycerol was added dropwise. The resulting nanosuspension was crosslinked by adding 1 mL of glutaraldehyde (25% *v/v*) and left overnight at 37 °C for nanoparticle formation. After washing twice with dH₂O, the nanoparticles were obtained by cold centrifugation at 20,000 rpm for 30 min. The obtained nanoparticles were lyophilised and stored at +4 °C. Nanoparticles obtained from cmGGs containing different amounts of carboxylic acid were labelled as cmGG-1@Np, cmGG-2@Np, and cmGG-3@Np.

2.6. Stability of cmGG@Np in Simulated Gastrointestinal Fluids

The stability of cmGG@Np was assessed based on a method previously depicted by Brodkorb et al. (2019) in the INFOGEST digestion model [34]. The composition and enzyme activity of simulated gastrointestinal fluids containing pepsin for each phase (mouth, stomach, and small intestine) were synthesized and measured following a method previously described by Zhou et al. (2020) [35]. They were evaluated in simulated gastric fluid (SGF, pH 3.0) and simulated intestinal fluid (SIF, pH 7.4) containing pancreatin and lipase.

Oral phase: A total of 5 g of each sample (cmGG-1@Np, cmGG-2@Np, and cmGG-3@Np) was prepared according to established protocols and mixed with 5 mL of simulated salivary fluid. The mixture was gently agitated for 2 min to mimic mastication and saliva interaction, with the fluid containing essential inorganic salts.

Gastric phase: A total of 5 mL of the oral mixture was combined with 10 mL of simulated gastric fluid and acidified to pH 3.0 using HCl. Pepsin (1 mL, ~2000 U/mL) was added, and the solution was stirred for 2 h to simulate gastric digestion under acidic and enzymatic conditions.

Intestinal phase: The complete gastric digest was transferred to simulated intestinal fluid containing double-distilled water, bile salts, and CaCl₂. The pH was adjusted to 7.4 using NaOH. Pancreatin and lipase (~2000 U/mL) were incorporated, and the mixture was stirred for 2 h to replicate small intestinal conditions. Ionic strength was maintained with inorganic salts, and bile salts were adjusted to a final concentration of 10 mmol/L. After digestion, the mixture was centrifuged at 12,000 rpm and 4 °C for 50 min and the supernatant was collected for subsequent analysis.

Free cmGG was incubated in the presence of SGF and SIF and used as a control. Samples of 1 mL were collected 15, 30, 45, 60, 90 and 120 min after the addition of SGF and 15, 30, 45, 60, 90 and 120 min after the addition of SIF. Equal volumes were added to maintain sink conditions. The degradation reactions of pepsin and trypsin were stopped by adding 100 µL of ice-cold acetonitrile solution containing 0.1% (*v/v*) trifluoroacetic acid, respectively. The samples were extracted by incubation with pH 7.4 PBS, and the absorbance values of the solubilized cmGG in the upper phase were measured using a spectrophotometer (cmGG λ_{max} = 315 nm). The cmGG concentration in the gastrointestinal environment was calculated using a calibration curve. Gastrointestinal stability was calculated using

Equation (2). Ros cmGG-1, cmGG-2 and cmGG-3 calibration curve. A calibration curve for cmGG derivatives was obtained using aqueous solutions prepared in the concentration range of 10–100 mg/mL. Absorbance measurements were performed at 315 nm ($\lambda_{\max}$) using a UV–Vis spectrophotometer, and the calibration curve was constructed by plotting absorbance versus concentration (Supplementary Material Figure S1: cmGG-1 calibration curve, Figure S2: cmGG-2 calibration curve and Figure S3: cmGG-3 calibration curve):

$$\text{stability}(\%) = \frac{C_{\text{digesta}} \times 8}{C_{\text{initial}}} \times 100 \quad (2)$$

where C_{initial} and C_{digesta} represent the cmGG concentrations in the initial and digestion phases, respectively. The coefficient “8” in Equation (2) accounts for the cumulative dilution that emerges as the samples pass through the three phases of in vitro digestion, and this is a coefficient obtained by following the INFOGEST protocol [34].

2.7. Degradation Studies of cmGG@Np Under Different pH Conditions

Degradation studies were conducted to evaluate the pH-dependent structural degradation behavior of cmGG@Np formulations with different degrees of carboxymethylation (cmGG-1@Np, cmGG-2@Np, and cmGG-3@Np). The experiments were performed based on the method reported by Nzilu et al. (2023), with minor modifications [36]. Each cmGG@Np formulation (5 mg) was separately dispersed in phosphate-buffered saline (PBS) adjusted to different pH values (pH 3.0, pH 5.5, and pH 7.4) and incubated at 37 °C under gentle and continuous agitation. At predetermined time intervals (0, 2, 4, 8, 12, and 24 h), 1 mL aliquots were withdrawn from each suspension, and an equal volume of fresh PBS at the corresponding pH was added to the system to maintain sink conditions throughout the experiment. To terminate the degradation process, the samples were centrifuged, and the resulting supernatants were collected for further analysis. The degree of nanoparticle degradation was quantitatively evaluated by monitoring time-dependent changes in cmGG concentration in the cmGG@Np suspensions using a UV–Vis spectrophotometer ($\lambda_{\max} = 315$ nm). The degradation percentage was calculated using the equation provided below (Equation (3)), and cmGG concentrations under different pH conditions were determined based on calibration curve (Supplementary Material Figure S1: cmGG-1 calibration curve, Figure S2: cmGG-2 calibration curve and Figure S3: cmGG-3 calibration curve).

$$\text{Degradation} (\%) = \frac{(A_0 - A_t)}{A_0} \times 100 \quad (3)$$

where A_0 represents the initial cmGG concentration and A_t corresponds to the cmGG concentration determined at time t .

2.8. Ros-Loaded cmGG@NP Synthesis (Ros-cmGG@NP)

Nanoparticles containing rosmarinic acid (Ros-cmGG@NP) were fabricated by utilizing the cmGG-3 polymer, which demonstrated superior stability in both simulated gastric (SGF) and intestinal (SIF) fluids. The Ros-cmGG-3@NP formulation was synthesized via an oil-in-water emulsification method combined with in situ polymer crosslinking [33]. In brief, 10 mg of Ros was solubilized in 10 mL of dichloromethane (DCM), followed by the addition of 1 mL of Span 80 (2% v/v) to generate the oil phase. A 0.5% (w/v) aqueous solution of cmGG-3 (30 mL) was then gradually introduced into the oil phase under continuous magnetic stirring. Upon reaching equilibrium between the oil and aqueous phases, the system was sonicated for 10 min at 40% amplitude. Subsequently, 10 mL of glycerol was incorporated dropwise. Crosslinking was achieved by adding 1 mL of 25% glutaraldehyde, and the nanosuspension was incubated overnight at 37 °C to facilitate nanoparticle for-

mation. The nanoparticles were collected by cold centrifugation at 20,000 rpm for 30 min, followed by lyophilization, and stored at +4 °C for further applications.

2.9. Physicochemical Characterization

Field emission scanning electron microscope (FE-SEM)

The shape and surface morphology of Ros-cmGG-3@Np were observed using a FE-SEM (Sigma300, Carl Zeiss Co., Ltd., Oberkochen, Germany) at an acceleration voltage of 10.00 kV. After adhering the samples onto a copper plate with conductive adhesive, freeze-dried samples on the surface were coated with gold, and the morphological characteristics of the nanoparticles were evaluated.

Mean Particle Size, Polydispersity Index (PDI), and Zeta Potential (ζ)

The particle size of Ros-cmGG-3@Np was determined in terms of the average particle diameter and polydispersity index (PDI) using a Malvern Zeta Nano S 90 (Malvern Instruments, Malvern, UK), which operates on the principle of dynamic light scattering (DLS) by analyzing the Brownian motion of colloidal particles. DLS measurements were performed with a 1 mg/mL particle suspension in Millipore water. The zeta potential was quantified at 25 °C using a Malvern zeta potential instrument (Nano Sight NS500, NanoSight Ltd., Malvern, UK), based on electrophoretic mobility under an applied electric field.

2.10. Adsorption Experiments

Entrapment efficiency and loading capacity

The entrapment efficiency and loading capacity of Ros were evaluated using a modified approach developed by Ullah et al. (2022) [37]. In detail, 10 mg of the nanoparticle formulation was accurately weighed and dispersed in 10 mL of distilled water, followed by continuous magnetic stirring at 200 rpm for 2 h to ensure complete hydration. The resulting suspension was then centrifuged at 5000 rpm for 30 min to separate the unencapsulated fraction [37]. The supernatant obtained after centrifugation was collected, and the concentration of free Ros was evaluated spectrophotometrically at its characteristic absorption peak ($\lambda_{\max} = 255$ nm). The amount of unencapsulated Ros in the supernatant was quantified to indirectly assess both the loading capacity and the entrapment efficiency (%EE) of Ros within the nanoparticles. Following centrifugation, the nanoparticle pellet was resuspended in phosphate-buffered saline (PBS) and appropriate aliquots were taken and passed through a filter. The entrapment efficiency of Ros was subsequently determined by measuring the absorbance of the filtrate at the compound's characteristic maximum wavelength using UV-visible spectroscopy, based on the calibration curve provided in Supplementary Figure S4 and calculated according to Equation (4). A calibration curve for Ros was obtained using aqueous solutions prepared in the concentration range of 0–100 $\mu\text{g/mL}$. Absorbance measurements were performed at 255 nm ($\lambda_{\max}$) using a UV-Vis spectrophotometer, and the calibration curve was constructed by plotting the absorbance versus concentration:

$$EE (\%) = \frac{Ros_{total} - Ros_{free}}{Ros_{total}} \times 100 \quad (4)$$

2.11. Evaluation of Bioaccessibility in a Simulated Gastrointestinal Model

The digestive performances of the Ros-GG@Np and Ros-cmGG-3@Np formulations were assessed using the standardized INFOGEST in vitro digestion protocol [34]. The preparation of simulated gastrointestinal media as well as the enzymatic activities employed for the oral, gastric, and intestinal steps followed the procedure reported by Zhou et al. (2020) [35]. A concise overview of the individual digestion stages is Section 2.6.

2.12. Gastrointestinal Stability and Bioaccessibility of Ros After Digestions

Gastrointestinal stability was determined using Equation (2), and bioaccessibility was determined using Equation (5). The concentrations of Ros in the initial and micellar phases were determined using the Ros calibration curve shown in Supplementary Figure S4.

$$\text{bioaccessibility (\%)} = \frac{C_{\text{micelle}}}{C_{\text{initial}}} \times 100 \quad (5)$$

Here, C_{initial} and C_{micelle} represent the Ros concentrations in the initial and micelle phases, respectively.

2.13. In Vitro Release Studies

The Ros release properties of Ros-GG@Np and Ros-cmGG-3@Np in a simulated in vitro gastrointestinal environment were evaluated under pH 3.0 SGF, pH 6.8 SIF, and pH 7.4 SIF conditions using the dialysis diffusion technique. Suspensions of Ros-GG@Np and Ros-cmGG-3@Np at a concentration of 10 mg/mL in PBS were placed in a dialysis bag (MW cut-off 12 kDa) and then immersed separately into the respective dissolution media. The samples were incubated at 37 °C under stirring at 50 rpm, and aliquots (1 mL) were collected at defined time points, with equal volumes of SGF or SIF added to maintain sink conditions. The degradation reactions of pepsin and trypsin were stopped by adding 100 µL of ice-cold acetonitrile solution containing 0.1% (v/v) trifluoroacetic acid [34]. Samples were incubated with pH 7.4 PBS for extraction, and the absorbance of dissolved Ros in the supernatant was assessed through a spectrophotometer at Ros $\lambda_{\text{max}} = 255$ nm. The concentration of Ros in the gastrointestinal environment was assessed by employing a calibration curve (Supplementary Figure S4) and Equation (6):

$$\text{cumulative release(\%)} = \frac{\text{The cumulative amount of Ros released from cmGG-3@Np}}{\text{total amount of loaded Ros}} \times 100 \quad (6)$$

2.14. Evaluation of In Vitro Release Kinetics

The in vitro gastrointestinal release kinetics of Ros from Ros-cmGG-3@Np were evaluated using four different mathematical models (Zero-order, First-order, Higuchi, and Hixson–Crowell). The R^2 values obtained from each model were compared to select the best-fitting model for Ros release. The equations used for the release kinetics are defined as follows [38]:

$$\text{Zero – order kinetic model : } Q_t = Q_0 + K_0t$$

$$\text{First – order kinetic model : } \log Q_t = \log Q_0 + K_1t$$

$$\text{Higuchi model : } Q_t = K\sqrt{t}$$

$$\text{Hixson – Crowell model : } Q_t = Kxt^n$$

Here, Q_t represents the amount of Ros released at time t , and Q_0 is the amount of Ros released initially. K is the kinetic constant, and n is the release exponent representing the Ros release mechanism.

2.15. In Vitro Cytocompatibility (Caco-2 and HT-29)

The cytotoxic effects of Ros-GG@Np and Ros-cmGG-3@Np nanoparticles on human colon-derived cell lines (Caco-2 and HT-29) were evaluated using an indirect cytotoxicity method in accordance with ISO 10993-12 [39] and ISO 10993-5 [40].

Preparation of extracts:

To obtain the extracts of Ros-GG@Np and Ros-cmGG-3@Np, nanoparticles at a concentration of 0.2 g/mL were incubated in the respective culture media (EMEM for Caco-2, DMEM for HT-29) at 37 °C for 72 h.

Cell culture conditions:

Caco-2 cells were cultured in EMEM and HT-29 cells in DMEM, both fortified with 10% Fetal Bovine Serum (FBS) and 1% penicillin-streptomycin. Cells were sustained under humidified conditions at 37 °C in a 5% CO₂ incubator.

Experimental procedure:

Cell suspensions at a density of 4×10^3 cells/mL were seeded into 96-well plates at a volume of 200 µL per well. After overnight incubation, the culture medium was removed and replaced with 200 µL of nanoparticle extracts at concentrations of 0.2, 0.1, 0.05, and 0.025 g/mL. The plates were incubated for 4 and 24 h at 37 °C.

MTT assay:

Upon the completion of the incubation period, cellular viability was evaluated through the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. The generated formazan crystals were solubilized in DMSO, and the absorbance was subsequently recorded at 570 nm using a spectrophotometric method. The half-maximal inhibitory concentration (IC₅₀) values for both cell lines were calculated using a variable slope (three-parameter) nonlinear regression model of inhibitor-response in GraphPad Prism 9.0. Analyses were performed in eight replicates (n = 8) for each condition.

2.16. Statistical Analysis

All experiments were performed in at least three replicates, and the data are expressed as mean ± S.D. The statistical significance of cytocompatibility results was analyzed using two-way ANOVA with multiple comparisons. Differences were considered statistically significant at $p < 0.05$.

3. Results and Discussion

3.1. Controlled Carboxymethylation of Guar Gum: Effect of Degree of Substitution on Colloidal Behavior

Carboxymethylated guar gum (cmGG) was successfully synthesized via the etherification of guar gum with MCA in an aqueous medium. Increasing the amount of MCA resulted in a progressive increase in the degree of substitution (DS), yielding cmGG-1 (DS = 0.32), cmGG-2 (DS = 0.57), and cmGG-3 (DS = 0.88) (Table 1). The DS represents the average number of carboxymethyl groups introduced per anhydroglucose unit of the guar gum backbone, which theoretically contains three hydroxyl groups. DS value of 0.88 therefore indicates that nearly one hydroxyl group per glucose unit was substituted by a carboxymethyl moiety. Importantly, the introduction of carboxymethyl groups increases both the hydrophilicity and the density of ionizable functional groups within the polymer structure. This modification is expected to play a crucial role in subsequent colloidal behavior by enhancing polymer hydration, chain expansion, and the availability of negatively charged carboxylate groups (–COO[−]) under physiological pH conditions. From a colloidal science perspective, these features are anticipated to contribute to improved electrostatic repulsion and steric stabilization in nanoparticle systems prepared from highly substituted cmGG [41]. Consistent with previous reports (Ahuja et al., 2013 [42]; Yadav et al., 2024 [32]; Bachra et al., 2022 [31]), the controlled modulation of DS enables the rational tuning of physicochemical and colloidal properties, including solubility, ionic character, and interparticle interactions [31,32,42]. This demonstrates that GG derivatives with varying degrees of carboxymethylation can be controlled to confer intended functionalities such as solubility, ion-exchange capacity, or biocompatibility.

3.2. Characterization of cmGG Formulations

The FT-IR spectra of cmGG derivatives (cmGG-1, cmGG-2, and cmGG-3), synthesized using GG with varying ratios of MCA, were analyzed to confirm the successful occurrence of the carboxymethylation process. The FT-IR data shown in Figure 1 validate the chemical modification of GG and its cmGG derivatives. In the GG spectrum, a broad signal at 3440 cm^{-1} corresponds to the stretching vibration of the hydroxyl (-OH) group. The peak near 1650 cm^{-1} is attributed to water molecules associated with the polymer. The region around 1400 cm^{-1} arises from $-\text{CH}_2$ deformation vibrations. The highly linked C-CO, C-OH, and C-O-C stretches of the polymer backbone are represented as peaks in the 870 – 1260 cm^{-1} range. These characteristic peaks of GG have also been observed in a number of studies [43–45]. In the cmGG formulations obtained by modifying GG, an absorption band appears around 3350 , 3290 and 3245 cm^{-1} due to -OH stretching vibrations. The intensity of this peak decreases as the carboxylic acid content increases. In the cmGG-3 formulation, which has the highest carboxylic acid content, the -OH stretching vibration is observed at 3245 cm^{-1} . Peaks at 2915 cm^{-1} correspond to C-H stretching vibrations of $-\text{CH}$ and $-\text{CH}_2$ groups. In the cmGG formulations, two distinct peaks at 1615 cm^{-1} and 1450 cm^{-1} , resulting from the symmetric and asymmetric stretching vibrations of COO^- groups, indicate the presence of $-\text{COOH}$ groups. Peaks at 1070 cm^{-1} and 1020 cm^{-1} are attributed to the bending vibrations of $\text{CH}_2\text{-O-CH}_2$ in the polymer structure [32,46–48].

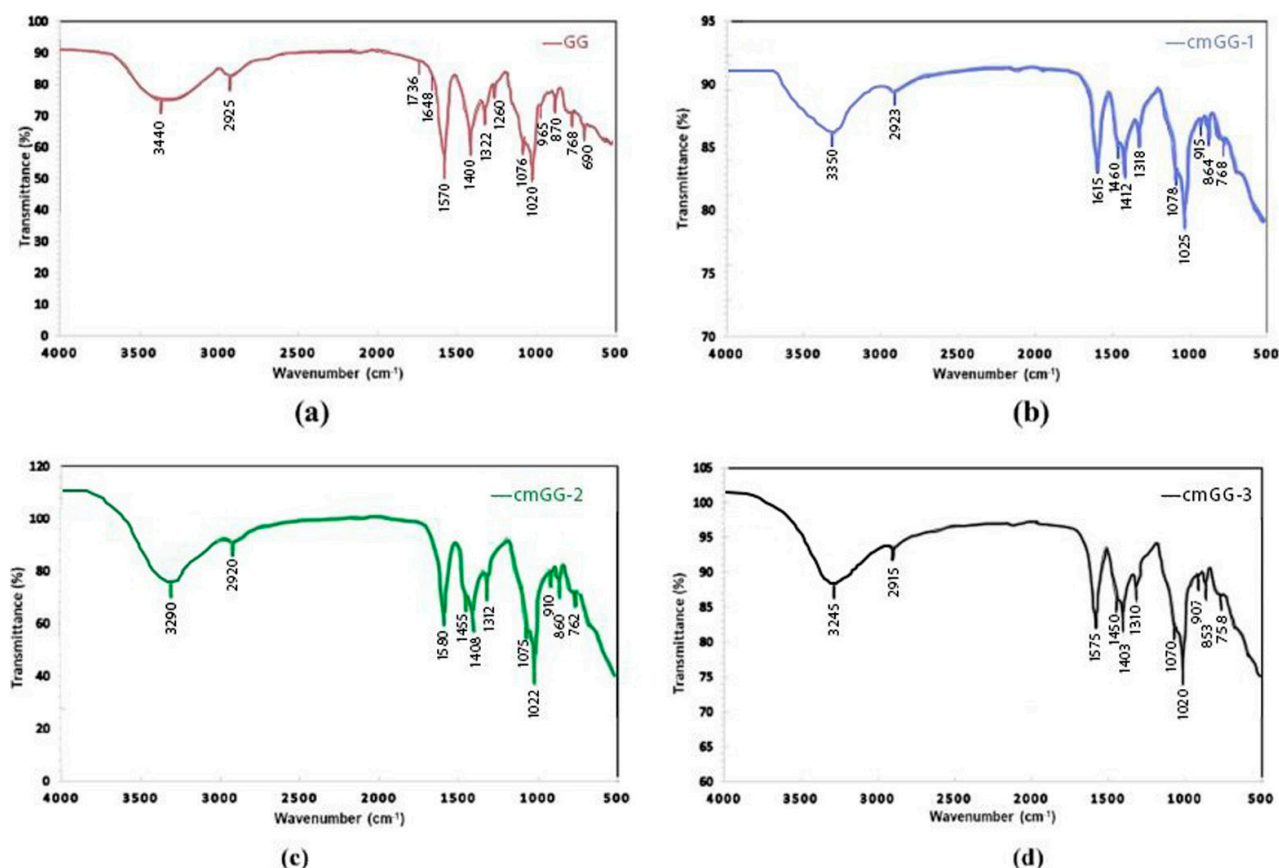


Figure 1. FT-IR spectra of GG and cmGG formulations: (a) GG, (b) cmGG-1, (c) cmGG-2 and (d) cmGG-3.

3.3. pH-Dependent Colloidal Stability of cmGG@Np in Simulated Gastrointestinal Fluids

The stability profiles of carboxymethylated guar gum nanoparticles (cmGG-1@Np, cmGG-2@Np, and cmGG-3@Np) in simulated gastric fluid (SGF, pH 3.0) and simulated

intestinal fluid (SIF, pH 7.4) are presented in Figure 2. In SGF, all formulations exhibited a gradual decrease in stability over the incubation period (0–120 min), which can be attributed to hydrolytic degradation under acidic conditions. However, the extent of stability loss was strongly dependent on the degree of substitution (DS). Among the tested formulations, cmGG-3@Np, possessing the highest carboxymethylation degree, maintained the highest stability throughout the incubation, retaining 80.9% stability at 120 min. In contrast, cmGG-1@Np with the lowest DS showed the most pronounced degradation, with stability decreasing below 55.2% at the same time point, while cmGG-2@Np exhibited an intermediate stability of 72.5%. This trend clearly demonstrates that increased carboxymethylation enhances the structural integrity of cmGG nanoparticles under acidic conditions. The introduction of carboxymethyl groups increases polymer hydrophilicity and promotes inter-chain electrostatic repulsion, which mitigates acid-catalyzed glycosidic bond cleavage [49]. In addition, under low pH conditions, partial protonation of carboxylate groups facilitates intramolecular hydrogen bonding, contributing to the maintenance of a cohesive polymer network [50]. From a colloidal science perspective, these effects can be interpreted as a combination of electrostatic and steric stabilization mechanisms, which suppress aggregation and structural collapse in acidic environments. Similar stabilization effects resulting from hydroxyl-to-carboxymethyl substitution have been previously reported, highlighting the role of steric hindrance and electrostatic effects in enhancing resistance to acidic degradation [17,18]. The superior stability of cmGG-3@Np therefore indicates its strong potential as a carrier system for gastric-resistant and controlled-release applications. Following SGF incubation for 120 min, the nanoparticles were transferred to simulated intestinal fluid (SIF, pH 7.4). In SIF, all formulations exhibited a relatively smaller reduction in stability compared to SGF, which can be attributed to the lower hydrolytic susceptibility of guar gum chains under neutral or mildly basic conditions. At pH 7.4, carboxymethyl groups are predominantly ionized ($-\text{COO}^-$), resulting in increased surface charge density and strong electrostatic repulsion between polymer chains. This repulsive force contributes to the preservation of nanoparticle structural integrity by preventing polymer chain aggregation or collapse. In the context of this study, the pH-dependent behavior of cmGG@Np should be interpreted not as an abrupt, pH-triggered burst release, but rather as changes in the degradation and stabilization mechanisms of the polymer matrix along the gastrointestinal pH gradient. This release behavior is primarily governed by the pH-dependent structural integrity and stability of the polymer matrix. Within the DLVO framework, the enhanced stability observed for higher-DS formulations can be qualitatively attributed to the increased dominance of electrostatic repulsion over attractive forces. However, this interpretation should be regarded as descriptive rather than fully mechanistic, as polymer chain steric hindrance and matrix swelling may also contribute to colloidal stabilization. Consequently, cmGG-3@Np, characterized by the highest charge density, exhibited the greatest stability in SIF. These findings are consistent with literature reports demonstrating that carboxymethylated polysaccharides maintain colloidal stability at neutral pH through ionic swelling and electrostatic balance [51]. Baghel et al. (2023) similarly reported that guar gum and its derivatives exhibit high structural integrity in intestinal environments, supporting their suitability for controlled-release applications [15]. Overall, increased carboxymethylation significantly enhances the stability of cmGG nanoparticles in both SGF and SIF; however, the dominant stabilization mechanisms are pH-dependent. Hydrogen bonding and steric protection prevail under acidic conditions, whereas electrostatic repulsion and charge-mediated stabilization govern colloidal behavior in intestinal environments [52].

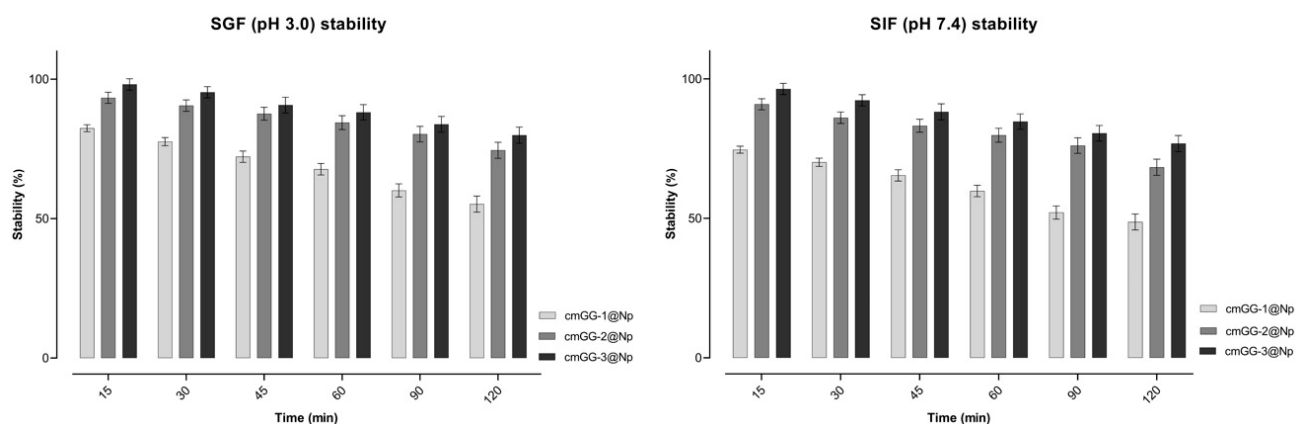


Figure 2. Stability of cmGG@Np in SGF (pH 3.0) and SIF (pH 7.4) (mean $\pm$ SD, $n = 3$).

3.4. Degradation Behavior of cmGG@Np Under Different pH Conditions

Different pH-dependent degradation (%) profiles of carboxymethylated guar gum nanoparticles with varying degrees of carboxymethylation (cmGG-1@Np, cmGG-2@Np, and cmGG-3@Np) are presented in Figure 3. The obtained results indicate that the degradation percentage varies depending on both the environmental pH and the degree of carboxymethylation of the polymer matrix. Across all investigated pH conditions, cmGG-3@Np exhibited the lowest degradation (%) values (in PBS, 24 h: pH 3.0, $36.8 \pm 2.3\%$; pH 5.5, $19.4 \pm 1.6\%$; pH 7.4, $15.6 \pm 1.7\%$) whereas cmGG-1@Np showed higher degradation (%) levels (in PBS, 24 h: pH 3.0, $62.7 \pm 3.4\%$; pH 5.5, $34.5 \pm 2.4\%$; pH 7.4, $31.2 \pm 2.2\%$). cmGG-2@Np displayed an intermediate behavior between these two formulations. This trend suggests that increasing the degree of carboxymethylation enhances the resistance of cmGG nanoparticles to different pH conditions. Under acidic pH conditions, a time-dependent increase in degradation (%) was observed for all formulations. However, higher degrees of carboxymethylation were associated with lower degradation (%) values. This observation indicates that cmGG@Np formulations with higher carboxymethylation degrees better preserve their structural integrity in acidic environments. In neutral and mildly basic pH conditions, the degradation (%) values of all cmGG@Np formulations were lower than those observed under acidic conditions. In particular, cmGG-3@Np maintained the lowest degradation (%) values within this pH range, indicating that the nanoparticle structure remained more stable. This behavior reflects the enhanced structural stability of the nanoparticle matrix. Overall, the degradation (%) results obtained under different pH conditions demonstrate that the structural degradation behavior of cmGG nanoparticles is dependent on the degree of carboxymethylation, with highly carboxymethylated formulations exhibiting lower degradation (%) values. The pH-dependent degradation (%) results obtained in this study are consistent with previously reported findings on polysaccharide-based nanoparticles containing carboxyl groups. Gao et al. (2014) evaluated the pH responsiveness of carboxymethyl chitosan-based nanoparticles by monitoring changes in particle size at different pH values and reported that the nanoparticle structure was more markedly affected under acidic conditions, whereas enhanced structural stability was observed in neutral and basic pH ranges [53]. This behavior was attributed to pH-dependent variations in the ionization degree of carboxyl groups. Similarly, in the present study, cmGG nanoparticles exhibited higher degradation (%) under acidic pH conditions, while maintaining greater structural stability under neutral and mildly basic environments. Moreover, a decrease in degradation (%) was observed with increasing degrees of carboxymethylation, indicating that cmGG-based formulations with higher carboxymethylation levels possess improved resistance to pH-induced structural deterioration.

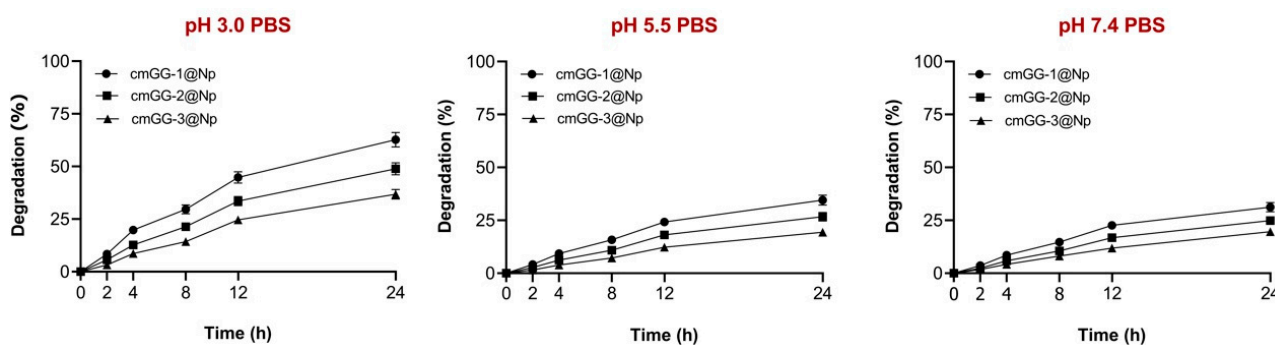


Figure 3. Degradation of cmGG@Np under different pH conditions (in PBS pH 3.0, pH 5.5 and pH 7.4) (mean $\pm$ SD, $n = 3$).

3.5. Physicochemical and Colloidal Characterization

Figure 4 presents the FE-SEM images, the average particle size, the polydispersity index (PDI), and the zeta potential of Ros-cmGG-3@Np nanoparticles synthesized via oil-in-water emulsification followed by in situ polymer crosslinking. The FE-SEM images reveal that Ros-cmGG-3@Np nanoparticles possess a smooth surface morphology, a spherical shape, and a monodisperse appearance. This uniform morphology indicates a high degree of structural homogeneity, which is a critical prerequisite for reproducible colloidal behavior and consistent performance in controlled release applications [54]. The average particle size of Ros-cmGG-3@Np was determined to be 155 nm, with a PDI value of 0.21. This PDI value indicates a narrow particle size distribution and confirms the monodisperse nature of the nanoparticle system. According to the literature, colloidal systems exhibiting PDI values in the range of 0.1–0.25 are generally considered homogeneous and physically stable, with a reduced tendency toward aggregation [55]. The combination of nanoscale particle size and low PDI suggests that the formulation process enabled effective control over particle nucleation and growth, resulting in a well-dispersed colloidal system. The zeta potential of Ros-cmGG-3@Np was measured as -13.3 mV, reflecting the presence of ionized carboxyl groups on the nanoparticle surface derived from the carboxymethylated guar gum backbone. Compared to reported anthocyanin-loaded CA-CMC (casein and carboxymethyl cellulose) nanocomplexes with average size of ~ 210 nm and PDI 0.33 [56], Ros-cmGG-3@Np nanoparticles were smaller (155 nm) and more monodisperse (PDI 0.21), suggesting enhanced colloidal stability and homogeneity. This moderately negative surface charge contributes to electrostatic repulsion between nanoparticles, thereby limiting particle interactions. Although it is widely reported that absolute zeta potential values exceeding ± 30 mV are typically associated with strong electrostatic stabilization and highly stable aqueous suspensions [57], the relatively low PDI and absence of aggregation observed in the present system indicate that electrostatic repulsion alone does not govern colloidal stability. Instead, the observed colloidal stability of Ros-cmGG-3@Np can be rationalized by a combined stabilization mechanism. In addition to electrostatic interactions, the hydrated cmGG polymer chains extending into the surrounding aqueous medium provide steric stabilization, which creates an entropic barrier against particle aggregation. This mixed electrostatic–steric stabilization behaviour is qualitatively consistent with extended DLVO concepts commonly invoked for polymer-based colloidal systems. In such systems, steric contributions are often considered dominant; however, extended DLVO should be regarded as a descriptive framework, as factors such as polymer chain conformation, hydration, and matrix dynamics may also influence dispersion stability beyond classical surface charge considerations [58]. Overall, the physicochemical characteristics of Ros-cmGG-3@Np demonstrate the formation of a stable and homogeneous colloidal nanoparticle system suitable for gastrointestinal delivery applications.

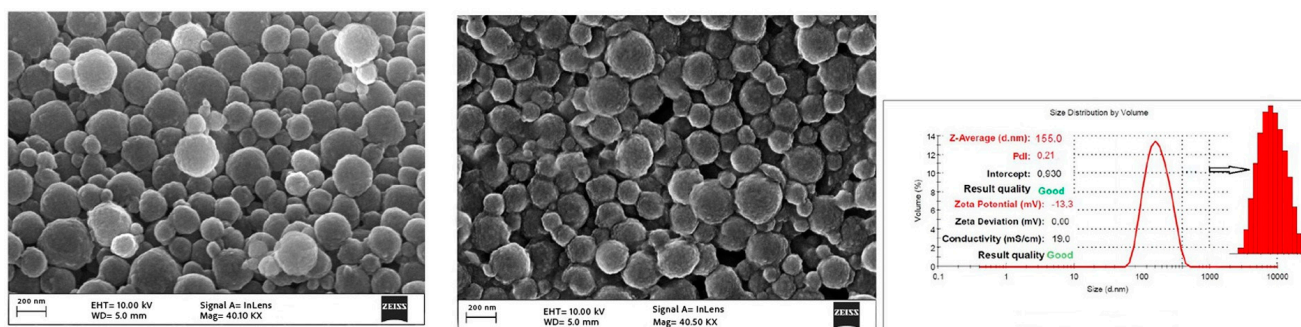


Figure 4. FE-SEM images, particle size, and zeta potential of Ros-cmGG-3@Np.

3.6. Adsorption Analyses

Entrapment efficiency and loading capacity

The cmGG-3 polymer, which possesses the highest degree of carboxymethylation, contains a greater number of ionized carboxylate ($-\text{COO}^-$) and hydroxyl ($-\text{OH}$) groups, providing a high density of surface functional sites capable of interacting with Ros molecules. These functional groups enable strong hydrogen bonding and, to some extent, electrostatic interactions with the carboxylic and phenolic hydroxyl groups of Ros, resulting in a high interfacial affinity between the polymer matrix and the bioactive compound [46,59]. Such surface-driven interactions facilitate effective adsorption and molecular anchoring of Ros within the cmGG-3 network, accounting for the high entrapment efficiency (65.48%). Ros, a polyphenolic compound bearing aromatic rings with phenolic $-\text{OH}$ and carboxylic $-\text{COOH}$ groups [60], further benefits from this affinity through multiple hydrogen-bonding interactions and partial electrostatic attraction, which directly contribute to the high loading capacity (56.62%). Moreover, the increased degree of carboxymethylation enhances the hydrophilic character and controlled swelling capacity of the polymer in aqueous media, promoting Ros diffusion into the matrix while maintaining effective retention and preventing premature leakage, a balance that is particularly favorable for moderately polar molecules such as Ros [61,62].

3.7. pH-Dependent Release Behavior and Diffusion-Controlled Kinetics of Ros-Loaded cmGG Nanoparticles

The *in vitro* release behavior of rosmarinic acid (Ros) from non-carboxymethylated guar gum nanoparticles (Ros-GG@Np) and carboxymethylated guar gum nanoparticles (Ros-cmGG-3@Np) was evaluated at 37 °C under simulated gastrointestinal conditions, including simulated gastric fluid (SGF, pH 3.0) and simulated intestinal fluid (SIF, pH 6.8 and pH 7.4) (Figure 5). Distinct release profiles were observed depending on both the environmental pH and the degree of carboxymethylation of the polymer matrix.

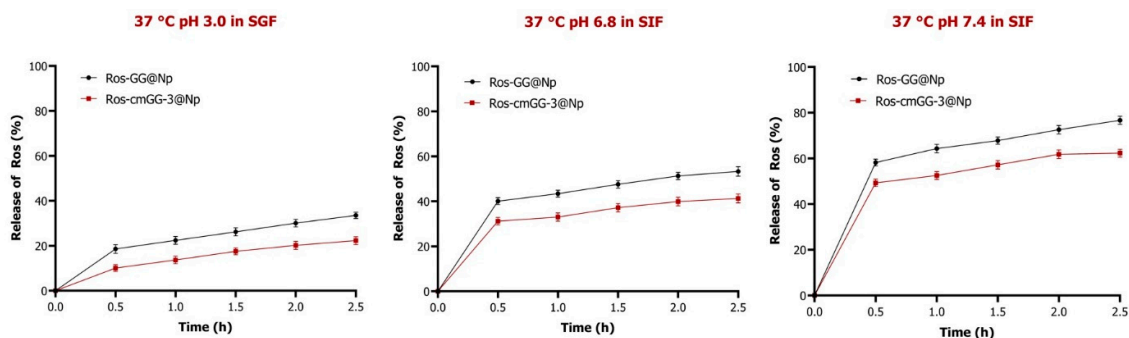


Figure 5. *In vitro* release profiles of Ros-GG@Np and Ros-cmGG-3@Np simulating gastrointestinal conditions (SGF, pH 3.0; SIF, pH 6.8; and SIF, pH 7.4) (mean $\pm$ SD, n = 8).

Under acidic SGF conditions (pH 3.0), Ros-GG@Np exhibited a cumulative release of 33.5% within 2.5 h, whereas Ros-cmGG-3@Np showed a significantly lower release of 22.3%. This reduced initial release can be attributed to the high degree of carboxymethylation in cmGG-3, where abundant carboxylate (-COO^-) and hydroxyl (-OH) groups promote strong hydrogen bonding and partial electrostatic interactions with the phenolic and carboxylic functional groups of Ros, thereby restricting its rapid diffusion from the polymer matrix [63,64]. Moreover, the more densely substituted cmGG-3 network introduces steric constraints that further limit molecular mobility under acidic conditions. A comparable pH-dependent suppression of release under acidic conditions has been reported for other CMGG-based delivery systems. In a previous study, bovine serum albumin (BSA) microencapsulated within $\text{Ca}^{2+}/\text{Ba}^{2+}$ -crosslinked CMGG beads exhibited markedly limited release in simulated gastric environments, while nearly complete release occurred upon exposure to pH 7.4 [65]. Although this system differs in carrier geometry and crosslinking strategy, the findings support the ability of carboxymethylated guar gum matrices to suppress premature release in acidic media and to promote enhanced payload release under intestinal conditions. At pH 6.8 (SIF), Ros-GG@Np demonstrated a faster release profile, reaching 53.3%, whereas Ros-cmGG-3@Np displayed a more controlled release of 41.2%. This behavior can be explained by the preservation of the structural organization and stability of the polymer matrix resulting from the ionization of carboxymethyl groups at near-neutral pH values. The balance between polymer hydration and electrostatic repulsion among ionized -COO^- groups results in a diffusion-dominated release process, in which steric resistance within the swollen polymer matrix moderates Ros diffusion [66]. Such electrostatic repulsion-driven stabilization aligns with established colloidal principles, where repulsive interactions prevent polymer chain aggregation and maintain structural homogeneity. Under pH 7.4 SIF conditions, Ros-GG@Np released 76.7% of its encapsulated Ros within 2.5 h, whereas Ros-cmGG-3@Np showed a comparatively lower release of 62.3%. Although extensive ionization occurs at higher pH in both systems, the higher charge density and increased availability of functional groups in cmGG-3 favor stronger polymer–phenolic compound interactions through hydrogen bonding and residual electrostatic attraction. Concurrently, the increased steric crowding within the highly substituted polymer network creates a denser diffusion barrier, collectively contributing to the sustained release behavior observed for Ros-cmGG-3@Np. From a colloidal interaction perspective, the controlled release behavior of Ros-cmGG-3@Np under the varying pH conditions throughout the gastrointestinal system is governed by the combined effects of electrostatic stabilization and steric hindrance, which together regulate molecular diffusion from the nanoparticle matrix. Overall, these findings demonstrate that a high degree of carboxymethylation in guar gum not only enhances nanoparticle stability and Ros loading efficiency but also enables pH-dependent, diffusion-controlled release throughout gastrointestinal environments. The synergistic contributions of electrostatic repulsion and steric stabilization establish cmGG-3 nanoparticles as an effective colloidal carrier system for the controlled oral delivery of polyphenolic compounds such as Ros [66–69].

The release kinetics of Ros from Ros-cmGG-3@Np are presented in Figure 6. Carboxymethylation of the GG polymer introduces hydrophilic carboxymethyl groups into the polymer backbone, which enhances the polymer's resistance to pH-dependent degradation and promotes structural stabilization across the varying pH conditions of the gastrointestinal tract, thereby directly influencing the release behavior of Ros. The higher density of ionized carboxylate (-COO^-) groups enhances electrostatic repulsion between polymer chains, inhibiting aggregation and maintaining nanoparticle integrity, while steric stabilization from the highly substituted polymer network regulates Ros diffusion from the matrix. Consequently, the carboxymethylation renders the polymer more hydrophilic and forms

a more homogeneous matrix, allowing Ros to diffuse in a controlled manner through the polymer network [15,70–72]. The release kinetics of Ros-cmGG-3@Np increased proportionally with the square root of time and showed a good fit to the Higuchi model, indicating that diffusion plays a major role in the release process. However, given the pH-responsive and swellable nature of the cmGG matrix, the Higuchi model should be regarded as an empirical description of the release behaviour rather than a definitive mechanistic interpretation, as polymer swelling and matrix relaxation may also contribute to the overall release profile [73]. In contrast, the release kinetics of Ros-GG@Np could not be clearly described by a single classical model due to the structural and chemical heterogeneity of the non-carboxymethylated polymer. In this system, the release mechanism likely involves a combination of diffusion, polymer erosion, and surface-associated release processes.

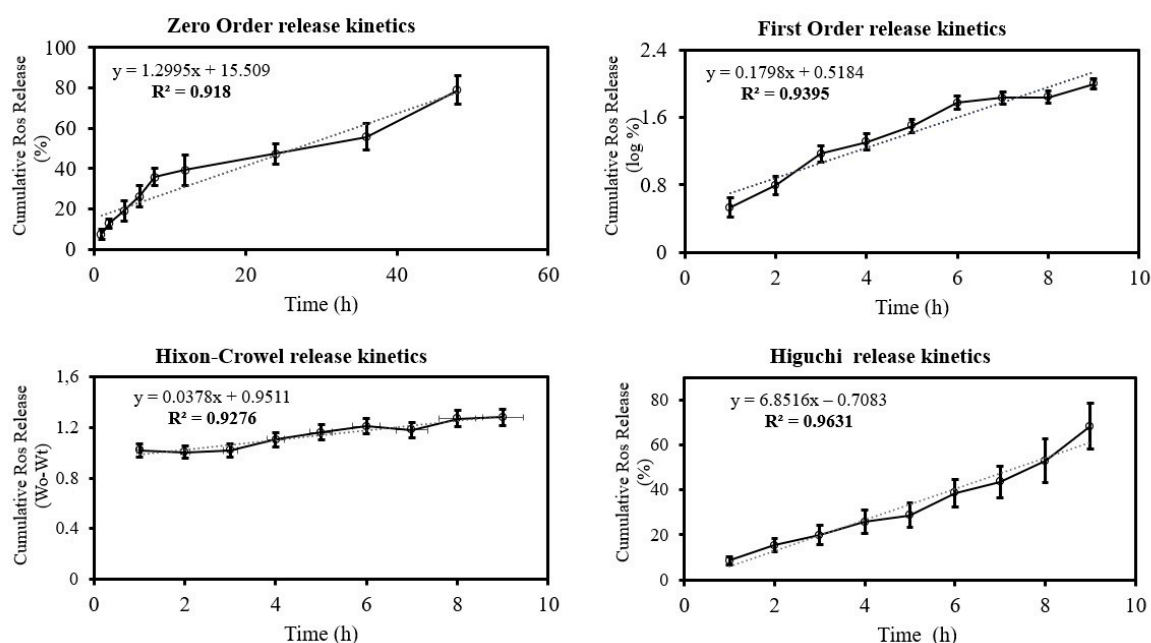


Figure 6. Release kinetics of Ros from Ros-cmGG-3@Np (mean $\pm$ SD, $n = 3$).

3.8. Gastrointestinal Stability and Bioaccessibility of Ros-Loaded cmGG Nanoparticles

The gastrointestinal stability and bioaccessibility of Ros-GG@Np and Ros-cmGG-3@Np were evaluated using the standardized INFOGEST simulated digestion model (Figure 7). The results demonstrated that Ros-GG@Np exhibited 48.3% gastrointestinal stability and 40.2% bioaccessibility, whereas Ros-cmGG-3@Np showed significantly higher values of 75.4% and 70.1%, respectively. Here, gastrointestinal stability refers to the fraction of Ros retained intact within the nanoparticle matrix throughout digestion, while bioaccessibility represents the proportion of Ros transferred into the micellar phase and rendered available for intestinal absorption after digestion. These findings clearly indicate that carboxymethylation markedly enhances both the gastrointestinal stability and bioaccessibility of Ros-loaded nanoparticles. Carboxymethylation of guar gum enhances polymer–water interactions, improving the stability of nanoparticles under varying pH conditions throughout the gastrointestinal tract and limiting premature degradation of Ros. The presence of ionized carboxylate ($-\text{COO}^-$) groups on the nanoparticle surface imparts a negative surface charge, generating electrostatic repulsion between particles and thus preventing aggregation under dynamic gastrointestinal conditions. The combined effects of steric protection and electrostatic stabilization preserve the colloidal integrity of Ros-cmGG-3@Np during digestion. Importantly, enhanced colloidal stability directly contributes to improved bioaccessibility. Well-dispersed and structurally intact nanoparticles facilitate

a more efficient transfer of Ros into the mixed micellar phase formed during intestinal digestion, increasing the fraction of Ros available for absorption. Notably, compared to other polysaccharide-based nanocarrier systems, the gastrointestinal bioaccessibility of Ros-cmGG-3@Np is quantitatively superior. Compared with recent polysaccharide-based nanoparticle systems, the bioaccessibility of Ros-cmGG-3@Np is superior. In CA-CMC nanoparticles loaded with anthocyanins [56], the encapsulated anthocyanins exhibited enhanced stability and bioaccessibility under simulated gastrointestinal conditions, but the retention rates after intestinal digestion were lower than that of Ros-cmGG-3@Np. Similarly, in gliadin/sodium carboxymethyl cellulose (G/CMC) nanoparticles encapsulating phloretin, the bioaccessibility of free phloretin was low, about 23%, whereas encapsulation increased it to approximately 55% [74]. The enhanced bioaccessibility in these systems was attributed to improved solubility and protection by the nanoparticle matrix. In contrast, Ros-cmGG-3@Np achieved a bioaccessibility of 70.1%, surpassing both CA-CMC-anthocyanin and G/CMC-phloretin systems. This improvement is due to the high colloidal stability, steric hindrance, polymer hydration, and electrostatic stabilization provided by the ionized carboxylate groups, which collectively protect Ros from premature degradation and enhance its transfer into the intestinal micellar phase [75–79]. These results demonstrate that Ros-cmGG-3@Np represents a highly effective colon-targeted oral delivery system, outperforming previously reported polysaccharide-based nanocarriers in terms of both gastrointestinal protection and bioaccessibility.

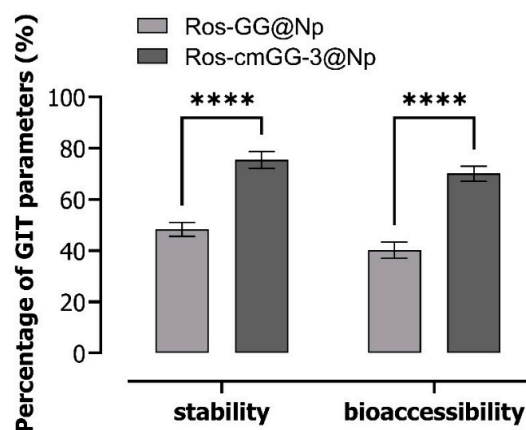


Figure 7. Gastrointestinal stability and bioaccessibility of Ros-GG@Np and Ros-cmGG-3@Np (mean $\pm$ SD, $n = 6$, **** indicates $p < 0.0001$).

3.9. Effect of Ros-Loaded Nanoparticles on the Viability of Caco-2 and HT-29 Cells

The cytotoxic effects of Ros-GG@Np and Ros-cmGG-3@Np systems were evaluated on two human colon-derived cell lines, Caco-2 and HT-29 (Figures 8 and 9). These cell lines were selected for complementary purposes: Caco-2 cells serve as a well-established intestinal epithelial barrier model for evaluating nanoparticle biocompatibility and epithelial interactions, whereas HT-29 cells, derived from colorectal adenocarcinoma, provide a relevant model to assess antiproliferative and cytotoxic effects on malignant cells [80–82]. MTT assay results demonstrated that both nanoparticle systems reduced cell viability in a concentration-dependent manner in Caco-2 cells; however, Ros-cmGG-3@Np exhibited a lower IC_{50} value (0.0949 g/mL) compared to Ros-GG@Np ($IC_{50} = 0.1558$ g/mL), indicating enhanced biological activity. This behavior can be attributed to carboxymethylation-induced modifications in surface chemistry, which increase hydrophilicity and impart a higher negative surface charge to the nanoparticles, thereby enhancing their interaction with cellular membranes and facilitating cellular uptake [83,84]. In HT-29 cells, a similar but more pronounced trend was observed, with lower IC_{50} values for Ros-cmGG-3@Np

(0.042 g/mL) compared to Ros-GG@Np (0.051 g/mL), suggesting increased sensitivity of cancer cells to the carboxymethylated system. This enhanced cytotoxicity may be explained by the higher endocytic activity of malignant cells, combined with the controlled intracellular release of Ros, leading to prolonged cellular exposure and amplified antiproliferative effects. Overall, the differential response between Caco-2 and HT-29 cells indicates that carboxymethylation not only improves nanoparticle stability and delivery efficiency but also promotes selective cytotoxicity toward cancer cells, while maintaining acceptable biocompatibility in intestinal epithelial-like cells.

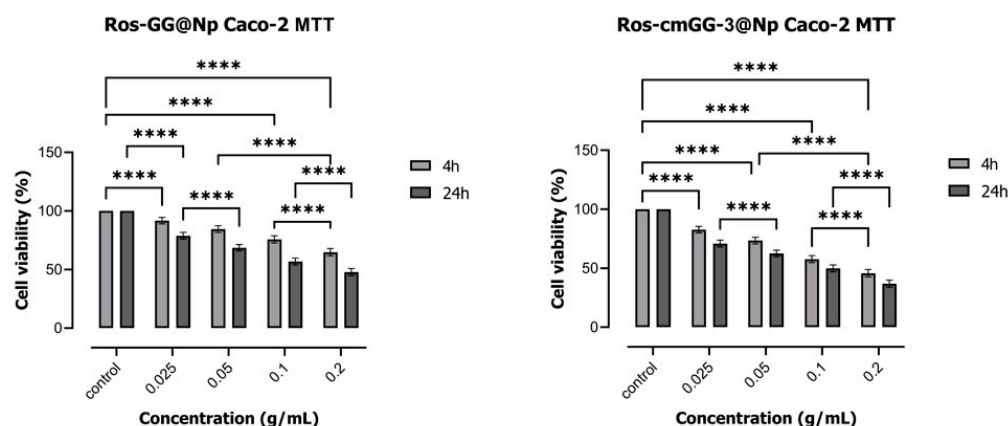


Figure 8. Cell viability of Caco-2 cells treated with Ros-GG@Np and Ros-cmGG-3@Np (mean $\pm$ SD, $n = 8$, **** indicates $p < 0.0001$).

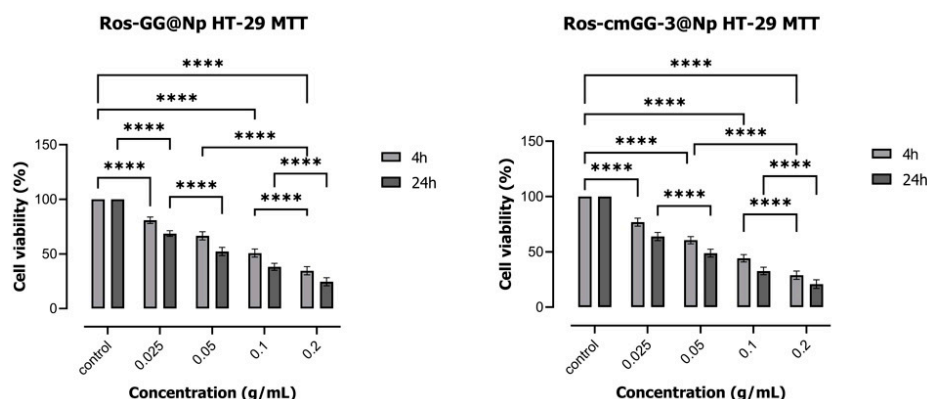


Figure 9. Cell viability of HT-29 cells treated with Ros-GG@Np and Ros-cmGG-3@Np (mean $\pm$ SD, $n = 8$, **** indicates $p < 0.0001$).

4. Conclusions

In this study, carboxymethylated guar gum (cmGG) derivatives with different degrees of substitution (DS) were evaluated as functional carriers for the colon-targeted oral delivery of rosmarinic acid (Ros). An increase in the degree of carboxymethylation markedly enhanced the hydrophilic and ionic character of the polymer, which directly influenced the stability and performance of the nanoparticles under gastrointestinal conditions. Results from the in vitro simulated gastrointestinal digestion model demonstrated that nanoparticles derived from cmGG exhibited DS-dependent stability profiles, with the highest structural integrity observed for the cmGG-3@Np formulation. cmGG-3@Np maintained its structural integrity throughout both gastric and intestinal phases, providing suitable robustness for colon-targeted delivery systems. Physicochemical characterization revealed that Ros-cmGG-3@Np nanoparticles exhibited a nanometric particle size, low polydispersity, and a negative surface charge, indicating a stable and homogeneous

structure. The high degree of substitution promoted strong interactions between Ros and the polymer matrix, contributing to high encapsulation and loading efficiencies. Release studies showed that Ros-cmGG-3@Np effectively limited premature release in acidic gastric environments while exhibiting a controlled, diffusion-dominated release profile under intestinal conditions. This behavior facilitated more efficient transport of Ros to the colon.

Post-digestion analyses demonstrated that Ros-cmGG-3@Np outperformed unmodified guar gum nanoparticles in terms of gastrointestinal stability and bioaccessibility. These findings indicate that the cmGG-3@Np system protects rosmarinic acid against gastrointestinal degradation, enhances its biological accessibility, and provides an effective platform for colon-targeted oral delivery.

Overall, the results demonstrate that cmGG-3@Np nanoparticles constitute a highly effective carrier platform for increasing the oral bioaccessibility of phenolic compounds such as rosmarinic acid, reducing gastrointestinal degradation, and enabling colon-targeted, controlled release. Their behavior in response to pH changes throughout the gastrointestinal tract positions cmGG-3 nanoparticles as promising candidates for functional foods, nutraceuticals, and colon-targeted pharmaceutical applications. Nevertheless, to further expand the potential of this system, future studies should include in vivo pharmacokinetic validation, detailed investigations of microbiota–polymer interactions, and scale-up studies aimed at clinical translation.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/colloids10010021/s1>. Figure S1: Rosmarinic acid calibration curve; Figure S2: cmGG-1 calibration curve; Figure S3: cmGG-2 calibration curve; Figure S4: cmGG-3 calibration curve.

Author Contributions: Conceptualization, Ö.E.A.; Methodology, A.S.Y. and Ö.E.A.; Investigation, A.S.Y. and Ö.E.A.; Data Curation, A.S.Y. and Ö.E.A.; Formal Analysis, Ö.E.A.; Resources, A.S.Y. and Ö.E.A.; Validation, A.S.Y. and Ö.E.A.; Visualization, Ö.E.A.; Supervision, A.S.Y. and Ö.E.A.; Project Administration, Ö.E.A.; Writing—Original Draft Preparation, Ö.E.A.; Writing—Review and Editing, Ö.E.A. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by Afyonkarahisar Health Sciences University Scientific Research Projects Coordination Unit (Grant No: 25.LİS.017).

Data Availability Statement: All the data provided in the manuscript.

Acknowledgments: Additionally, this work is based on the master's thesis of one of the co-authors, Ayşe Selen YILDIRIM. During the preparation of the manuscript, artificial intelligence-based tools were used solely for language and grammatical editing.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Adeyanju, A.A. Absorption, bioavailability, bioaccessibility, metabolism, and excretion of bioactive food phytochemicals. In *Plant Food Phytochemicals and Bioactive Compounds in Nutrition and Health*, 1st ed.; CRC Press: Boca Raton, FL, USA, 2024; p. 25. [CrossRef]
2. Cai, D.; Han, C.; Liu, C.; Ma, X.; Qian, J.; Zhou, J.; Li, Y.; Sun, Y.; Zhang, C.; Zhu, W. Chitosan-capped enzyme-responsive hollow mesoporous silica nanoplatforms for colon-specific drug delivery. *Nanoscale Res. Lett.* **2020**, *15*, 123. [CrossRef]
3. Tie, S.; Tan, M. Current advances in multifunctional nanocarriers based on marine polysaccharides for colon delivery of food polyphenols. *J. Agric. Food Chem.* **2022**, *70*, 903–915. [CrossRef] [PubMed]
4. Martínez, E.; Gamboa, J.; Finkielstein, C.V.; Cañas, A.I.; Osorio, M.A.; Vélez, Y.; Llinas, N.; Castro, C.I. Oral dosage forms for drug delivery to the colon: An existing gap between research and commercial applications. *J. Mater. Sci. Mater. Med.* **2025**, *36*, 24. [CrossRef] [PubMed]

5. Awad, A.; Madla, C.M.; McCoubrey, L.E.; Ferraro, F.; Gavins, F.K.; Buanz, A.; Gaisford, S.; Orlu, M.; Siepmann, F.; Siepmann, J.; et al. Clinical translation of advanced colonic drug delivery technologies. *Adv. Drug Deliv. Rev.* **2022**, *181*, 114076. [[CrossRef](#)] [[PubMed](#)]
6. Batool, R.; Mudassir, J.; Khan, M.A.; Zafar, S.; Rana, S.J.; Abbas, N.; Muhammad, S.; Arshad, M.S.; Muhammad, S. Fabrication and characterization of celecoxib-loaded chitosan/guar gum-based hydrogel beads. *Pharmaceuticals* **2023**, *16*, 554. [[CrossRef](#)]
7. Dalei, G.; Das, S. Carboxymethyl guar gum: A review of synthesis, properties and versatile applications. *Eur. Polym. J.* **2022**, *176*, 111433. [[CrossRef](#)]
8. Amidon, S.; Brown, J.E.; Dave, V.S. Colon-targeted oral drug delivery systems: Design trends and approaches. *AAPS PharmSciTech* **2015**, *16*, 731–741. [[CrossRef](#)]
9. Manna, S.; Karmakar, S.; Sen, O.; Sinha, P.; Jana, S.; Jana, S. Recent updates on guar gum derivatives in colon-specific drug delivery. *Carbohydr. Polym.* **2024**, *334*, 122009. [[CrossRef](#)]
10. George, A.; Shah, P.A.; Shrivastav, P.S. Guar gum: Versatile natural polymer for drug delivery applications. *Eur. Polym. J.* **2019**, *112*, 722–735. [[CrossRef](#)]
11. Verma, V.; Meena, J.; Saini, T.; Saini, S.; Malik, R. Carboxymethyl guar gum: Bridging the gap between biopolymers and advanced functional materials. *Polym.-Plast. Technol. Mater.* **2025**, *64*, 1715–1744. [[CrossRef](#)]
12. Soltani, M.D.; Meftahizadeh, H.; Barani, M.; Rahdar, A.; Hosseinihah, S.M.; Hatami, M.; Ghorbanpour, M. Guar (*Cyamopsis tetragonoloba* L.) plant gum: From biological applications to advanced nanomedicine. *Int. J. Biol. Macromol.* **2021**, *193*, 1972–1985. [[CrossRef](#)] [[PubMed](#)]
13. Verma, D.; Sharma, S.K. Recent advances in guar gum-based drug delivery systems and their administrative routes. *Int. J. Biol. Macromol.* **2021**, *181*, 653–671. [[CrossRef](#)]
14. Sharahi, M.; Bahrami, S.H.; Karimi, A. A comprehensive review on guar gum and its modified biopolymers: Their potential applications in tissue engineering. *Carbohydr. Polym.* **2025**, *347*, 122739. [[CrossRef](#)] [[PubMed](#)]
15. Baghel, M.; Sakure, K.; Giri, T.K.; Maiti, S.; Nakhate, K.T.; Ojha, S.; Sharma, C.; Agrawal, Y.; Goyal, S.; Badwaik, H. Carboxymethylated gums and derivatization: Strategies and significance in drug delivery and tissue engineering. *Pharmaceuticals* **2023**, *16*, 776. [[CrossRef](#)] [[PubMed](#)]
16. Nooy, A.E.J.; Rori, V.; Masci, G.; Dentini, M.; Crescenzi, V. Synthesis and preliminary characterisation of charged derivatives and hydrogels from scleroglucan. *Carbohydr. Res.* **2000**, *324*, 116–126. [[CrossRef](#)]
17. Abhishek, R.; Ahuja, M. Evaluation of carboxymethyl moringa gum as nanometric carrier. *Carbohydr. Polym.* **2017**, *174*, 896–903. [[CrossRef](#)]
18. Badwaik, H.R.; Al Hoque, A.; Kumari, L.; Sakure, K.; Baghel, M.; Giri, T.K. Moringa gum and its modified form as a potential green polymer used in biomedical field. *Carbohydr. Polym.* **2020**, *249*, 116893. [[CrossRef](#)]
19. Scott, D.M.; Prud'homme, R.K.; Priestley, R.D. Effects of the polymer glass transition on the stability of nanoparticle dispersions. *Soft Matter* **2023**, *19*, 1212–1218. [[CrossRef](#)]
20. De Anda-Flores, Y.; Carvajal-Millan, E.; Campa-Mada, A.; Lizardi-Mendoza, J.; Rascon-Chu, A.; Tanori-Cordova, J.; Martínez-López, A.L. Polysaccharide-based nanoparticles for colon-targeted drug delivery systems. *Polysaccharides* **2021**, *2*, 626–647. [[CrossRef](#)]
21. Moutaharrik, S.; Palugan, L.; Cerea, M.; Meroni, G.; Casagni, E.; Roda, G.; Martino, P.A.; Gazzaniga, A.; Maroni, A.; Foppoli, A. Colon drug delivery systems based on swellable and microbially degradable high-methoxyl pectin: Coating process and in vitro performance. *Pharmaceutics* **2024**, *16*, 508. [[CrossRef](#)]
22. Renukadevi, J.; Ranjani, M.; Sneha, P.; Hussain, J.S.; Shakthi, H. Gut-targeted nutraceutical delivery: Engineering microbiome-responsive nutraceutical interfaces. *Food Bioeng.* **2025**, *4*, 248–261. [[CrossRef](#)]
23. Waqar, M.A.; Mubarak, N.; Khan, A.M.; Khan, R.; Shaheen, F.; Shabbir, A. Advanced polymers and recent advancements on gastroretentive drug delivery system: A comprehensive review. *J. Drug Target.* **2024**, *32*, 655–671. [[CrossRef](#)] [[PubMed](#)]
24. Noor, S.; Mohammad, T.; Rub, M.A.; Raza, A.; Azum, N.; Kumar Yadav, D.; Hassan, I.; Asiri, A.M. Biomedical features and therapeutic potential of rosmarinic acid. *Arch. Pharmacol. Res.* **2022**, *45*, 205–228. [[CrossRef](#)] [[PubMed](#)]
25. Guan, H.; Luo, W.; Bao, B.; Cao, Y.; Cheng, F.; Yu, S.; Fan, Q.; Zhang, L.; Wu, Q.; Shan, M. A comprehensive review of rosmarinic acid: From phytochemistry to pharmacology and its new insight. *Molecules* **2022**, *27*, 3292. [[CrossRef](#)]
26. Choi, S.J.; Oh, S.S.; Kim, C.R.; Kwon, Y.K.; Suh, S.H.; Kim, J.K.; Park, G.G.; Son, S.Y.; Shin, D.H. Perilla frutescens extract ameliorates acetylcholinesterase- and trimethyltin chloride-induced neurotoxicity. *J. Med. Food* **2016**, *19*, 281–289. [[CrossRef](#)]
27. Değer, U.; Çavuş, Y. Investigation of the role of rosmarinic acid treatment in regulating inflammation, cell damage, and angiogenesis in rat ovarian torsion and detorsion models. *Acta Cir. Bras.* **2020**, *35*, e202000304. [[CrossRef](#)]
28. Kim, J.S.; Lee, J.H.; Hong, S.M.; Cho, K.; Kim, S.Y. Salvia miltiorrhiza prevents methylglyoxal-induced glucotoxicity via the regulation of apoptosis-related pathways and the glyoxalase system in human umbilical vein endothelial cells. *Biol. Pharm. Bull.* **2022**, *45*, 51–62. [[CrossRef](#)]

29. Ośko, J.; Nasierowska, K.; Grembecka, M. Application of in vitro digestion models in the evaluation of dietary supplements. *Foods* **2024**, *13*, 2135. [\[CrossRef\]](#)
30. Wang, Y.; Li, Z.; Bao, Y.; Cui, H.; Li, J.; Song, B.; Wang, M.; Li, H.; Cui, X.; Chen, Y.; et al. Colon-targeted delivery of polyphenols: Construction principles, targeting mechanisms and evaluation methods. *Crit. Rev. Food Sci. Nutr.* **2023**, *65*, 64–86. [\[CrossRef\]](#)
31. Bachra, Y.; Grouli, A.; Damiri, F.; Zhu, X.X.; Talbi, M.; Berrada, M. Synthesis, Characterization, and Swelling Properties of a New Highly Absorbent Hydrogel Based on Carboxymethyl Guar Gum Reinforced with Bentonite and Silica Particles for Disposable Hygiene Products. *ACS Omega* **2022**, *7*, 39002–39018. [\[CrossRef\]](#)
32. Yadav, H.; Maji, B.; Maiti, S. Novel succinoylated carboxymethyl guar gum nanocarriers of glimepiride for controlling type-2 diabetes. *Med. Nov. Technol. Devices* **2024**, *22*, 100309. [\[CrossRef\]](#)
33. Deepika, C.; Shivani, U.; Kumar, M.S.; Geeta, S. Preparation and Characterization of Celecoxib entrapped guar gum nanoparticles targeted for oral drug delivery against colon cancer: An in vitro study. *J. Drug Deliv. Ther.* **2020**, *10*, 14–21. [\[CrossRef\]](#)
34. Brodtkorb, A.; Egger, L.; Alminger, M.; Alvito, P.; Assunção, R.; Ballance, S.; Bohn, T.; Bourlieu-Lacanal, C.; Boutrou, R.; Carrière, F.; et al. INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nat. Protoc.* **2019**, *14*, 991–1014. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Zhou, H.; Tan, Y.; Lv, S.; Liu, J.; Mundo, J.L.M.; Bai, L.; Rojas, O.J.; McClements, D.J. Nanochitin-stabilized pickering emulsions: Influence of nanochitin on lipid digestibility and vitamin bioaccessibility. *Food Hydrocoll.* **2020**, *106*, 105878. [\[CrossRef\]](#)
36. Nzilu, D.M.; Madivoli, E.S.; Makhanu, D.S.; Wanakai, S.I.; Kiprono, G.K.; Kareru, P.G. Green synthesis of copper oxide nanoparticles and its efficiency in degradation of rifampicin antibiotic. *Sci. Rep.* **2023**, *13*, 14030. [\[CrossRef\]](#)
37. Ullah, S.; Azad, A.K.; Nawaz, A.; Shah, K.U.; Iqbal, M.; Albadrani, G.M.; Al-Joufi, F.A.; Sayed, A.A.; Abdel-Daim, M.M. 5-fluorouracil-loaded folic-acid-fabricated chitosan nanoparticles for site-targeted drug delivery cargo. *Polymers* **2022**, *14*, 2010. [\[CrossRef\]](#)
38. Zaman, M.; Butt, M.H.; Siddique, W.; Iqbal, M.O.; Nisar, N.; Mumtaz, A.; Nazeer, H.Y.; Alshammari, A.; Riaz, M.S. Fabrication of pegylated chitosan nanoparticles containing tenofovir alafenamide: Synthesis and characterization. *Molecules* **2022**, *27*, 8401. [\[CrossRef\]](#)
39. Nguyen, A.K.; Goering, P.L.; Reipa, V.; Narayan, R.J. Toxicity and photosensitizing assessment of gelatin methacryloyl-based hydrogels photoinitiated with lithium phenyl-2,4,6-trimethylbenzoylphosphinate in human primary renal proximal tubule epithelial cells. *Biointerphases* **2019**, *14*, 021007. [\[CrossRef\]](#)
40. Miller, F.; Hinze, U.; Chichkov, B.; Leibold, W.; Lenarz, T.; Paasche, G. Validation of eGFP fluorescence intensity for testing in vitro cytotoxicity according to ISO 10993-5. *J. Biomed. Mater. Res. Part B Appl. Biomater.* **2017**, *105*, 287–296. [\[CrossRef\]](#)
41. La Mesa, C.; Risuleo, G. Stabilization of Food Colloids: The Role of Electrostatic and Steric Forces. In *Some New Aspects of Colloidal Systems in Foods*; IntechOpen: London, UK, 2018. [\[CrossRef\]](#)
42. Ahuja, M.; Singh, S.; Kumar, A. Evaluation of carboxymethyl gellan gum as a mucoadhesive polymer. *Int. J. Biol. Macromol.* **2013**, *53*, 114–121. [\[CrossRef\]](#)
43. Iqbal, D.; Nazir, A.; Iqbal, M.; Yameen, M. Green synthesis and characterization of carboxymethyl guar gum: Application in textile printing technology. *Green Process. Synth.* **2020**, *9*, 212–218. [\[CrossRef\]](#)
44. Elsaedi, S.M.; Zaki, E.G.; Omar, W.A.E.; Soliman, A.A.; Attia, A.M. Guar gum-based hydrogels as potent green polymers for enhanced oil recovery in high-salinity reservoirs. *ACS Omega* **2021**, *6*, 23421–23431. [\[CrossRef\]](#)
45. Kumar, A.; Kumari, S.; Parmanand; Sharma, S.K. Constructing the nanomixture of guar gum and Fe₃O₄ for photocatalytic degradation of dyes and heavy metal. *J. Mater. Sci. Mater. Electron.* **2022**, *33*, 2643–2653. [\[CrossRef\]](#)
46. Gong, H.; Liu, M.; Chen, J.; Han, F.; Gao, C.; Zhang, B. Synthesis and characterization of carboxymethyl guar gum and rheological properties of its solutions. *Carbohydr. Polym.* **2012**, *88*, 1015–1022. [\[CrossRef\]](#)
47. Dodi, G.; Pala, A.; Barbu, E.; Peptanariu, D.; Hritcu, D.; Popa, M.I.; Tamba, B.I. Carboxymethyl guar gum nanoparticles for drug delivery applications: Preparation and preliminary in-vitro investigations. *Mater. Sci. Eng. C* **2016**, *63*, 628–636. [\[CrossRef\]](#) [\[PubMed\]](#)
48. Jana, P.; Mitra, T.; Selvaraj, T.K.R.; Gnanamani, A.; Kundu, P.P. Preparation of guar gum scaffold film grafted with ethylenediamine and fish scale collagen, cross-linked with ceftazidime for wound healing application. *Carbohydr. Polym.* **2016**, *153*, 573–581. [\[CrossRef\]](#)
49. Javanbakht, S.; Shaabani, A. Carboxymethyl cellulose-based oral delivery systems. *Int. J. Biol. Macromol.* **2019**, *133*, 21–29. [\[CrossRef\]](#)
50. Ferraz, J.; Pollini, M.F.O.; Cardoso, V.M.B.; Nunes, S.; Chorilli, M.; Roque-Borda, C.A.; Meneguim, A.B. Evaluating the Impact of High-Esterified Pectin on Retrogradation and Film-Forming Properties of High-Amylose Starch. *Polysaccharides* **2025**, *6*, 78. [\[CrossRef\]](#)
51. Zhao, H.; Li, J.; Cong, S.; Hou, H.; Zhang, G.; Bi, J. Fabrication and Anti-Swelling Properties of Gelatin/Sodium Alginate–Carboxymethyl Chitosan-Based Cationic Coordination Hydrogels. *Foods* **2025**, *14*, 3149. [\[CrossRef\]](#)
52. Thakur, V.; Dhiman, S.; Singh, T.G.; Bhatia, R.; Awasthi, A. The Cutting Edge Quest: Epic Saga of Carboxymethyl Guar Gum in Drug Delivery and Roads Ahead. *Polym. Adv. Technol.* **2025**, *36*, e70119. [\[CrossRef\]](#)

53. Gao, C.; Liu, T.; Dang, Y.; Yu, Z.; Wang, W.; Guo, J.; Zhang, X.; He, G.; Zheng, H.; Yin, Y.; et al. pH/redox responsive core cross-linked nanoparticles from thiolated carboxymethyl chitosan for in vitro release study of methotrexate. *Carbohydr. Polym.* **2014**, *111*, 964–970. [[CrossRef](#)] [[PubMed](#)]
54. Chudasama, M.; Goyary, J. Nanostructured materials in food science: Current progress and future prospects. *Next Mater.* **2024**, *5*, 100206. [[CrossRef](#)]
55. Gürsoy, N.; Yilmaz Öztürk, B.; Dağ, İ. Synthesis of intracellular and extracellular gold nanoparticles with a green machine and its antifungal activity. *Turk. J. Biol.* **2021**, *45*, 196–213. [[CrossRef](#)] [[PubMed](#)]
56. Cui, H.; Si, X.; Tian, J.; Lang, Y.; Gao, N.; Tan, H.; Bian, Y.; Zang, Z.; Jiang, Q.; Bao, Y.; et al. Anthocyanins-loaded nanocomplexes comprising casein and carboxymethyl cellulose: Stability, antioxidant capacity, and bioaccessibility. *Food Hydrocoll.* **2022**, *122*, 107073. [[CrossRef](#)]
57. Clogston, J.D.; Patri, A.K. Zeta potential measurement. In *Characterization of Nanoparticles Intended for Drug Delivery*; McNeil, S., Ed.; Humana Press: New York, NY, USA, 2011; Volume 697, pp. 63–70. [[CrossRef](#)]
58. Österberg, M.; Henn, K.A.; Farooq, M.; Valle-Delgado, J.J. Biobased nanomaterials—The role of interfacial interactions for advanced materials. *Chem. Rev.* **2023**, *123*, 2200–2241. [[CrossRef](#)]
59. Espíndola, C. Some nanocarriers' properties and chemical interaction mechanisms with flavones. *Molecules* **2023**, *28*, 2864. [[CrossRef](#)]
60. Lešnik, S.; Bren, U. Mechanistic insights into biological activities of polyphenolic compounds from rosemary obtained by inverse molecular docking. *Foods* **2022**, *11*, 67. [[CrossRef](#)]
61. Obireddy, S.R.; Lai, W.F. ROS-generating amine-functionalized magnetic nanoparticles coupled with carboxymethyl chitosan for pH-responsive release of doxorubicin. *Int. J. Nanomed.* **2022**, *17*, 589–601. [[CrossRef](#)]
62. Kocaaga, B. Injectable PVP/CMC Hydrogels with Tissue Adhesion, Hemostatic Function, and Sustained Drug Release. *J. Appl. Polym. Sci.* **2026**, e70363. [[CrossRef](#)]
63. Aiping, Z.; Jianhong, L.; Wenhui, Y. Effective loading and controlled release of camptothecin by O-carboxymethylchitosan aggregates. *Carbohydr. Polym.* **2006**, *63*, 89–96. [[CrossRef](#)]
64. Hu, G.; Lan, X.; Peng, B.; Liao, J.; Xiong, Y. Water resistant, biodegradable and flexible corn starch/carboxymethyl cellulose composite film for slow-release fertilizer coating materials. *Int. J. Biol. Macromol.* **2024**, *260*, 129476. [[CrossRef](#)] [[PubMed](#)]
65. Thimma, R.T.; Tammishetti, S. Barium chloride crosslinked carboxymethyl guar gum beads for gastrointestinal drug delivery. *J. Appl. Polym. Sci.* **2001**, *82*, 3084–3090. [[CrossRef](#)]
66. Wang, L.; Gang, X.; Xiao, Y.; Ren, Y.; Wang, J.; Niu, B.; Li, W. Sodium Alginate/carboxymethyl chitosan-CuO hydrogel beads as a pH-sensitive carrier for the controlled release of curcumin. *Eur. Polym. J.* **2023**, *192*, 112069. [[CrossRef](#)]
67. Daneshmoghannlou, E.; Miralinaghi, M.; Moniri, E.; Sadjady, S.K. Fabrication of a pH-responsive magnetic nanocarrier based on carboxymethyl cellulose-aminated graphene oxide for loading and in-vitro release of curcumin. *J. Polym. Environ.* **2022**, *30*, 3718–3736. [[CrossRef](#)]
68. Singh, J.; Nayak, P. pH-responsive Polymers for Drug Delivery: Trends and Opportunities. *J. Polym. Sci.* **2023**, *61*, 2828–2850. [[CrossRef](#)]
69. Najafi, M.; Pourmadadi, M.; Abdous, M.; Rahdar, A.; Pandey, S. Formulation of double nanoemulsions based on pH-sensitive carboxymethyl cellulose/Starch copper doped carbon quantum dots for quercetin controlled release. *J. Mol. Liq.* **2024**, *400*, 124543. [[CrossRef](#)]
70. Rahman, M.S.; Hasan, M.S.; Nitai, A.S.; Nam, S.; Karmakar, A.K.; Ahsan, M.S.; Shiddiky, M.J.A.; Ahmed, M.B. Recent developments of carboxymethyl cellulose. *Polymers* **2021**, *13*, 1345. [[CrossRef](#)]
71. Kurhade, R.R.; Shaikh, M.S.; Nagulwar, V.; Kale, M.A. Advancements in carboxymethyl cellulose (CMC) modifications and their diverse biomedical applications: A comprehensive review. *Int. J. Polym. Mater. Polym. Biomater.* **2024**, *74*, 1043–1067. [[CrossRef](#)]
72. Zarrintaj, P.; Ganjali, M.R.; Salmankhani, A.; Mashhadzadeh, A.H.; Munir, M.T.; Salehnia, F.; Rezapour, M.; Habibzadeh, S.; Saeb, M.R. Carboxymethylated polysaccharides in drug delivery. In *Tailor-Made Polysaccharides in Drug Delivery*; Academic Press: Cambridge, MA, USA, 2023; pp. 63–81. [[CrossRef](#)]
73. Askarizadeh, M.; Esfandiari, N.; Honarvar, B.; Sajadian, S.A.; Azdarpour, A. Kinetic modeling to explain the release of medicine from drug delivery systems. *ChemBioEng Rev.* **2023**, *10*, 1006–1049. [[CrossRef](#)]
74. He, J.-R.; Zhu, J.-J.; Yin, S.-W.; Yang, X.-Q. Bioaccessibility and intracellular antioxidant activity of phloretin embodied by gliadin/sodium carboxymethyl cellulose nanoparticles. *Food Hydrocoll.* **2022**, *122*, 107076. [[CrossRef](#)]
75. Yu, N.; Wang, J.; Jiang, C.; Nie, X.; Hu, Z.; Ye, Q.; Meng, X.; Xiong, H. Development of composite nanoparticles from gum Arabic and carboxymethylcellulose-modified Stauntonia brachyanthera seed albumin for lutein delivery. *Food Chem.* **2022**, *372*, 131269. [[CrossRef](#)] [[PubMed](#)]
76. Zhang, J.; Zhang, X.; Wang, Q.; Wu, C. Changes of physicochemical properties and bioactivities of resveratrol-loaded core-shell biopolymer nanoparticles during in vitro gastrointestinal digestion. *Food Chem.* **2023**, *424*, 136444. [[CrossRef](#)] [[PubMed](#)]

77. Rahmadiawan, D.; Shi, S.C. Enhanced stability, superior anti-corrosive, and tribological performance of Al₂O₃ water-based nanofluid lubricants with tannic acid and carboxymethyl cellulose over SDBS as surfactant. *Sci. Rep.* **2024**, *14*, 9217. [[CrossRef](#)] [[PubMed](#)]
78. Huang, J.; Wang, F.; Zhang, Y.; You, C.; Li, X. Development of self-assembled (+)-Nootkatone delivery system using Gliadin-Carboxymethyl chitosan composite nanoparticles: Fabrication, characterization, and pharmaceutical application. *Int. J. Biol. Macromol.* **2025**, *318*, 145086. [[CrossRef](#)]
79. Zhang, J.; Gan, C.; Xu, K.; Wang, H.; Li, H.; Yang, L.; Sun, S. Fabrication and characterization of zein/gelatin/carboxymethyl starch nanoparticles as an efficient delivery vehicle for quercetin with improved stability and bioaccessibility. *Int. J. Biol. Macromol.* **2025**, *308*, 142409. [[CrossRef](#)]
80. Song, H.; He, A.; Guan, X.; Chen, Z.; Bao, Y.; Huang, K. Fabrication of chitosan-coated epigallocatechin-3-gallate (EGCG)-hordein nanoparticles and their transcellular permeability in Caco-2/HT29 cocultures. *Int. J. Biol. Macromol.* **2022**, *196*, 144–150. [[CrossRef](#)]
81. Ejazi, S.A.; Louisthelmy, R.; Maisel, K. Mechanisms of nanoparticle transport across intestinal tissue: An oral delivery perspective. *ACS Nano* **2023**, *17*, 13044–13061. [[CrossRef](#)]
82. Pang, H.; Yu, W.; Wu, Y.; Nie, X.; Huang, G.; Xu, Z.P.; Chen, C.; Han, F.Y. Enhanced Epithelial Cell Uptake of Glycol Chitosan-Coated PLGA Nanoparticles for Oral Drug Delivery. *Adv. Ther.* **2025**, *8*, 2400547. [[CrossRef](#)]
83. Li, L.; Zhang, P.; Li, C.; Guo, Y.; Sun, K. In vitro/vivo antitumor study of modified-chitosan/carboxymethyl chitosan “boosted” charge-reversal nanoformulation. *Carbohydr. Polym.* **2021**, *269*, 118268. [[CrossRef](#)]
84. Patrojanasophon, P.; Singpanna, K.; Rojanarata, T.; Opanasopit, P.; Ngawhirunpat, T.; Pengnam, S.; Pornpitchanarong, C. Folate receptor-targeted thiol-maleimide clicked chitosan/carboxymethyl cellulose nanoparticles for cisplatin delivery in oral carcinoma. *Int. J. Biol. Macromol.* **2025**, *290*, 138976. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.