

Article

Modified Cellulose Nanocrystals Encapsulating Cannabigerol: A Step Forward in Controlling Intestinal Inflammatory Disorders

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Abstract: Cannabigerol (CBG) from *Cannabis sativa* L. is known for its anti-inflammatory, antibacterial, and antioxidant properties, showing potential against intestinal inflammation. However, its lipophilic nature limits its absorption and stability. Researchers have explored cellulose nanocrystals (CNCs) to deliver lipophilic compounds and enhance their biological outcomes. This study investigated the capability of modified CNC with cetyltrimethylammonium bromide (CTAB) to effectively deliver CBG. The encapsulation process's impact on cytotoxicity, biological activity, and controlled release during digestion was assessed. Results indicated that CNC-CTAB encapsulation significantly reduced CBG's cytotoxicity on intestinal cells, allowing safer administration of higher doses. The antioxidant and anti-inflammatory properties of the encapsulated CBG were retained, resulting in a decrease in reactive oxygen species and cytokine levels in intestinal cells. Additionally, the system inhibited the growth of the intestinal pathogen *Campylobacter jejuni*. The study supports using CNC-CTAB as an efficient delivery system to enhance CBG's potential against intestinal inflammation. Incorporating this system into food matrices could lead to novel functional foods for managing intestinal inflammation.

Keywords: CBG; delivery system; CNC-CTAB; cytotoxicity; biological potential; intestinal inflammatory disorders



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1. Introduction

Cannabigerol (CBG) is a terpenophenolic phytocannabinoid present in the plant *Cannabis sativa* L. CBG is not psychotomimetic (i.e., it does not produce effects on the mind similar to a psychotic state) and is the precursor molecule, in the *C. sativa* plant, of the most abundant phytocannabinoids, such as tetrahydrocannabinol (THC) and cannabidiol (CBD) [1]. CBG exhibits affinity and activity characteristics between D9-THC and CBD at the cannabinoid receptors (CB₁ and CB₂) but appears to be unique in its interactions with α -2 adrenoceptors and serotonin 1A receptors (5-HT_{1A}) [2]. Studies indicate that CBG possesses interesting biological activities, namely, anti-inflammatory, antibacterial, and antifungal activities, regulation of the redox balance, and neuromodulatory effects [3,4]. This molecule has been reported to have therapeutic potential in treating diseases such as neurologic disorders and inflammatory bowel diseases [2]. However, the lipophilic nature of CBG and other cannabinoids is a significant challenge for developing an effective formulation for optimal biological effect. Accordingly, cannabinoids present very low aqueous solubility, resulting in poor bioavailability when administered through numerous oral formulations. Additionally, possible cytotoxic effects associated with high concentrations restrict the quantity of biomolecules that can be administered to the body, which in turn further impacts their biological potential.

These constraints could potentially be surpassed through the utilization of enabling delivery systems. These systems may have the capacity to maintain and improve the functionality of bioactive molecules, enhancing their bioaccessibility and bioavailability, while also enabling a controlled and sustained release [5]. In this context, a range of formulations aimed at oral delivery of cannabinoids has been reported. Self-nano-emulsifying drug delivery systems (SNEDDS) have demonstrated that lipid type—either medium- or long-chain triglycerides—influences the bioavailability and lymphatic uptake of CBD, with long-chain triglycerides improving systemic availability and reducing first-pass metabolism [6]. Similarly, controlled-release and gastro-retentive formulations help extend cannabinoids' narrow absorption window in the upper gastrointestinal tract, which is essential for conditions requiring steady therapeutic levels, such as chronic pain and epilepsy. These gastro-retentive tablets, based on egg albumin and surfactants, have shown improved bioavailability and extended release when compared to conventional CBD solutions [7]. Cyclodextrins such as α -CD, β -CD, and γ -CD improve CBD's therapeutic potential by allowing encapsulation within their hydrophobic cavities, enhancing solubility and cytotoxic efficacy against cancer cells [8]. Moreover, innovative formulation techniques involving cyclodextrins and mesoporous silica have proven effective in enhancing the solubility of cannabidiol (CBD), thereby contributing to improved therapeutic outcomes [9]. However, cetyltrimethylammonium bromide (CTAB)-modified cellulose nanocrystals (CNCs) have never been tested for encapsulation of CBD. The CTAB-modified surface creates a hydrophobic interface on CNCs, enhancing compatibility with lipophilic compounds like cannabinoids, being a potentially superior alternative for its delivery. These systems have demonstrated high encapsulation efficiencies, often exceeding 90%, and exhibited sustained release properties, beneficial for chronic cannabinoid therapies [10–13]. Unlike cyclodextrins or lipid-based SNEDDS, CTAB-modified CNCs offer a more straightforward, scalable approach using renewable cellulose, making them a highly sustainable option. Additionally, its positive surface charge is expected to improve intestinal absorption by interacting favorably with cellular membranes, promoting better uptake.

This work pioneers the use of CNC-CTAB structures as vehicles for the delivery of CBG, as a means to offer an efficient and sustained release of this molecule and promote its intestinal biological functions. The compatibility of the designed delivery system was evaluated via *in vitro* cytotoxicity studies toward intestinal cells to confirm a safe application. The biological potential of the delivery system was assessed via *in vitro* experiments, investigating antioxidant, antimicrobial, and anti-inflammatory activities. Moreover, an *in vitro* model mimicking gastrointestinal (GI) digestion was employed to evaluate how the digestion conditions influenced the release profile of CBG. As per our knowledge, this is the first study investigating CNC-CTAB structures for the delivery of CBG, providing for the first time comprehensive evaluations of encapsulation and release properties, intestinal cytotoxicity, and biological potential of CNC-CTAB-encapsulated CBG. These findings offer novel insights into the applicability and therapeutic potential of CNC-CTAB as a promising platform for CBG delivery.

2. Materials and Methods

2.1. Reagents and Materials

The chemicals employed in the experiments were of analytical grade or superior. Commercial CNC, characterized by a needle-like morphology with dimensions of 10–20 nm in width and 50–400 nm in length, was generously provided by Cellulose Lab (Fredericton, NB, Canada). CTAB (cetyltrimethylammonium bromide) with a purity of over 99.0% was sourced from Sigma-Aldrich (St. Louis, MO, USA). The CNC modification using CTAB followed the method described by Casanova et al. (2023) [13]. CBG isolate (purity >98%) was obtained via fermentation from Amyris (Lot: 146754; Emeryville, CA, USA). Phytocannabinoid Mixture 10 comprising cannabidiolic acid, cannabigerolic acid, cannabigerol, cannabidiol, tetrahydrocannabivarin, cannabinol, tetrahydrocannabinolic acid A, Δ^9 -tetrahydrocannabinol, Δ^8 -tetrahydrocannabinol, and ($\pm$)-cannabichromene

was sourced from Cayman Chemical (Ann Arbor, MI, USA). Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) and ascorbic acid (>99%) were obtained from Sigma-Aldrich.

2.2. CBG Encapsulation by Spray Drying

Predetermined quantities of CBG were dissolved in ethanol, and an aqueous solution of CNC-CTAB was introduced into the ethanolic solution to achieve a final CNC-CTAB concentration of 2% (*w/v*) and a bioactive to carrier ratio of 1:3 in 70% ethanol (*v/v*). The resulting mixture was stirred for 30 min and the loaded particles were separated by centrifugation. Unbound CBG in the supernatant was quantified, and the pellet underwent two washes with distilled water through centrifugation. Drying was performed using a BÜCHI B-290 Mini Spray Dryer (Flawil, Switzerland) with a 0.5 mm nozzle. The mixture at 0.75% (*w/v*) was fed at 4 mL/min with an inlet temperature of 120 °C. The spray gas flow was set to 670 L/h and aspiration rate to 75% (30 m³/h).

2.3. Cannabigerol Quantification

CBG was quantified by gas chromatography–mass spectrometry (GC-MS). Samples were dissolved in dichloromethane, derivatized by the reagent N,O-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (Merck, Rahway, NJ, USA) and analyzed on GC-QqQ model EVOQ (Bruker, Karlsruhe, Germany) mass spectrometer, with a Rxi-5Sil MS column (30 m × 250 µm × 0.25 µm). Helium served as the carrier gas at a consistent flow rate of 1 mL/min, and the conditions followed the outlined by Luz-Veiga et al. [14]. The injector was set at 340 °C with a split of 10, and the oven temperature program consisted of initial conditions at 60 °C with a 1 min hold, followed by a ramp to 200 °C at 10 °C/min with a 1 min hold, then a further ramp to 315 °C at 3 °C/min with another 1 min hold. Finally, there was an increase of 5 °C/min until reaching 340 °C, and a 15 min hold at this temperature. The transfer line was maintained at 300 °C. The quadrupole operated with an electron ionization energy of 70 eV in positive mode, the source temperature set at 280 °C, and a scan range from 30 to 1000 Da. Compound identification was performed by comparing the obtained mass spectra with a phytocannabinoid standard mixture, as well as through comparison with the NIST library and the free online spectral repository SpectraBase™ (<https://spectrabase.com/>).

2.4. Characterization of Encapsulated CBG Particles

The encapsulation *yield* (%) was calculated according to Equation (1). Encapsulation efficiency (*EE*%) and loading capacity (*LC*%) were computed using Equations (2) and (3).

$$\text{yield (\%)} = \frac{m_{\text{final}}}{\text{Cur}_{\text{added}} + \text{carrier}_{\text{added}}} \times 100 \quad (1)$$

$$\text{EE (\%)} = \frac{\text{CBG}_{\text{added}} - \text{CBG}_{\text{unbound}}}{\text{CBG}_{\text{added}}} \times 100 \quad (2)$$

$$\text{LC (\%)} = \frac{\text{CBG}_{\text{added}} - \text{CBG}_{\text{unbound}}}{\text{final mass}} \times 100 \quad (3)$$

Zeta potential (0.1%, *w/v*, in aqueous suspensions) was measured at 25 °C using dynamic light scattering (DLS) with a Malvern Zetasizer Nano ZSP (Malvern, UK). A 10 mW He-Ne laser (633 nm) and a folded capillary cell were used for measurements at a 173° detection angle. Particle size was determined by laser diffraction using a Malvern Mastersizer 3000 (Malvern, UK). Cellulose delivery systems (refractive index: 1.468, absorption index: 0.01) were analyzed in distilled water at 3500 rpm, with obscuration between 5 and 15%, applying the Mie theoretical model to determine size distribution by volume.

Morphological assessments were conducted using a Thermo Scientific™ Pro Scanning Electron Microscope, with observations carried out under high vacuum conditions and an acceleration voltage ranging from 5 to 10 kV. Before analysis, the samples were mounted on observation stubs with double-sided adhesive carbon tape (NEM tape, Nisshin, Japan)

and coated with Au/Pd (target SC510-314B from ANAME, S.L., Madrid, Spain) using a Sputter Coater (Polaron, Bad Schwalbach, Germany).

The spectra for Attenuated Total Reflection Fourier-Transform Infrared Spectroscopy (ATR-FT-IR) were obtained using a PerkinElmer Frontier™ MIR/FIR spectrometer. Scans were conducted across a range of 550–4000 cm^{-1} , with 16 scans per sample at a spectral resolution of 4 cm^{-1} . Each analysis was carried out in duplicate.

2.5. Cell Lines and Culture Conditions

Human colon carcinoma Caco-2 cells were obtained from the European Collection of Authenticated Cell Cultures (ECACC, UK) and cultured at 37 °C in 95% air and 5% CO_2 . The cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) containing 4.5 g/L glucose, L-glutamine, and no pyruvate, supplemented with 10% Fetal Bovine Serum, 1% Penicillin–Streptomycin–Fungizone, and 1% Non-Essential Amino Acids Solution (MEM NEAA) (ThermoScientific, Waltham, MA, USA). Experiments were conducted using Caco-2 cells at passages 28 to 32.

2.6. Cytotoxicity Evaluation

Cytotoxicity assessment was conducted following the ISO 10993-5:2009 standard, utilizing Caco-2 cells [15]. Caco-2 cells were cultured to approximately 80% confluence, then detached using TrypLE Express and seeded at 1×10^4 cells/well in a 96-well microplate. After 24 h, the culture media was replaced with media containing CNC-CTAB encapsulating CBG at concentrations of 0.01 to 0.32 g/L, along with free CBG at corresponding concentrations (0.0025–0.08 g/L). A death control was included with 10% DMSO, and plain media served as growth control. After another 24 h, Presto Blue reagent was added, and after a 2 h incubation, fluorescence intensity was measured using a microplate reader (excitation: 560 nm; emission: 590 nm). Results were expressed as percentages of cell metabolism inhibition, and the highest biocompatible concentration of each sample was selected for further biological activity assessments.

2.7. Antioxidant Potential

2.7.1. 2,2-Diphenyl-1-picrylhydrazyl-Free-Radical (DPPH) Assay

The established 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay was used to evaluate free-radical scavenging activity, based on the method by Schaich et al. (2015) [16]. This method was selected due to its common use in antioxidant research, allowing for reliable comparisons with previous studies and suitability for investigating new targets. A stock DPPH solution (600 μM) was prepared in methanol and stored at -20°C . A fresh solution was prepared by diluting this stock to 0.600 ± 0.100 at 515 nm. For the assay, 25 μL of the sample (0.12 g/L for the delivery system and 0.01 g/L [30 μM] for CBG), Trolox (7.5–240 μM), ascorbic acid (0.05 g/L, 280 μM), or solvent was mixed with 175 μL of the DPPH solution. After a 30 min incubation at 25 °C, absorbance was measured at 515 nm. Scavenging activity was expressed as a percentage reduction in absorbance relative to the control, with results reported as μmol Trolox equivalent (TE) per gram of sample.

2.7.2. Oxygen Radical Absorbance Capacity (ORAC) Assay

The Oxygen Radical Absorbance Capacity (ORAC) assay was conducted following the procedure described by Coscueta et al. (2019) with minor adjustments [17]. This method is widely used for assessing antioxidant capacity, providing a reliable framework for comparing results across studies. In black 96-well microplates, samples at specified biocompatible concentrations (0.12 g/L for the delivery system, 0.01 g/L [30 μM] for CBG, and 0.09 g/L for CNC-CTAB), Trolox standards (10–80 μM), and ascorbic acid (0.05 g/L, 280 μM) as an antioxidant control were mixed with fluorescein (70 nM). The plate was pre-incubated for 10 min at 37 °C, then 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH) solution (18 mM) was added. Fluorescence (excitation: 485 nm; emission: 528 nm) was recorded every minute for 80 min. Results were calculated by determining

the difference in the areas under the fluorescein decay curves for samples and blanks, and expressed as μmol of Trolox equivalent per gram of sample using a calibration curve.

2.7.3. Production of Reactive Oxygen Species

The production of reactive oxygen species in Caco-2 cells was evaluated according to the method described by Casanova et al. (2013) [13]. Briefly, cells were seeded at 1×10^4 cells/well in 96-well black polystyrene microplates. After 24 h, the medium was replaced with phenol red-free media containing samples at biocompatible concentrations (0.12 g/L for the delivery system, 0.01 g/L [30 μM] for CBG, and 0.09 g/L for CNC-CTAB), with and without an oxidative stimulus (H_2O_2 at 100 μM). The cells were incubated for another 24 h. Positive controls included Trolox (15 μM) and ascorbic acid (500 μM), while plain media served as the control. Following incubation, a DCFDA probe (25 μM) was added, and fluorescence intensity (excitation: 485 nm; emission: 520 nm) was measured at 1 h intervals for 4 to 6 h until saturation was reached.

2.8. Immunomodulation

The monolayer immunomodulatory assays in Caco-2 cells were conducted following the protocol outlined by Costa et al. (2023) [18]. Briefly, cells were seeded at a density of 2.5×10^5 cells/well in a 24-well microplate and incubated for 24 h at 37 °C. The culture medium was then replaced with media containing samples at biocompatible concentrations (0.12 g/L for the delivery system, 0.01 g/L [30 μM] for CBG, and 0.09 g/L for CNC-CTAB), with or without an inflammatory stimulus (interleukin-1 β , 0.01 $\mu\text{g/mL}$). Betamethasone (40 μM) served as a positive anti-inflammatory control, while plain media with and without IL-1 β served as the basal activity control. After incubation, supernatants were collected and centrifuged. Interleukins 6 (IL-6) and 8 (IL-8) and Tumor Necrosis Factor- α (TNF- α) were measured using enzyme-linked immunosorbent assay (ELISA) kits (Abcam Human IL-6 Elisa Kit and Bio-Legend Max Human Elisa Kit IL-8 and TNF- α). Protein was extracted from the cells and quantified using the Pierce™ BCA Protein Assay Kit for normalization of ELISA results. Cytokine levels were expressed in pg/mL, with results presented as fold changes relative to the cytokine levels in the basal stimulated control to minimize variability in the assays.

2.9. Antimicrobial Activity

The antimicrobial efficacy of CNC-CTAB-encapsulated CBG, free CBG, and the carrier was examined against *Escherichia coli* ATCC 25922, *Salmonella enteritidis* ATCC 13076, *Listeria innocua* ATCC 33091, and *Campylobacter jejuni* ATCC 33560 following the Clinical and Laboratory Standards Institute (CLSI-M07-A9) guidelines. Mueller–Hinton agar was prepared with biocompatible concentrations of 0.12 g/L for the delivery system, 0.01 g/L (30 μM) for CBG, and 0.09 g/L for CNC-CTAB. The agar was inoculated with the microorganisms at 10^4 CFU/drop and incubated at 37 °C for 48 h, with *Campylobacter jejuni* cultured in 5% O_2 conditions. After incubation, microbial growth inhibition was assessed visually by comparing the plates against a dark matte surface, where no microbial growth indicates antimicrobial activity against the tested microorganisms.

2.10. Release Profile Under Simulated Digestion

CNC-CTAB encapsulating CBG, free CBG, and the carrier were subjected to in vitro gastrointestinal (GI) digestion based on the standardized European IN-FOGEST protocol for static simulation of GI food digestion [19,20]. Water suspensions were prepared at concentrations of 4.8 g/L for the delivery systems, 0.2 g/L for CBG, and 3.6 g/L for CNC-CTAB, which were combined with simulated gastric fluid (SGF) in a 1:1 (v/v) ratio. The gastric phase was initiated by adjusting the pH to 3.0 with HCl and adding $\text{CaCl}_2(\text{H}_2\text{O})_2$ to a final concentration of 0.15 mM. Pepsin was introduced to achieve an activity of 2000 U/mL, and the solution was incubated for 120 min at 37 °C with stirring. To simulate the intestinal phase, simulated intestinal fluid (SIF) was added to the digesta in a 1:1 (v/v)

ratio, with the pH adjusted to 7.0 using NaOH. Pancreatin (with a trypsin activity of 100 U/mL) and bile salts (10 mM) were incorporated, along with $\text{CaCl}_2(\text{H}_2\text{O})_2$ to a final concentration of 0.6 mM. This mixture was also incubated for 120 min at 37 °C. Samples (300 μL) were withdrawn hourly, combined with 700 μL of ethanol, and centrifuged for CBG quantification. The enzymatic activity was halted by heating the samples to 80 °C for 10 min after digestion.

2.11. Statistical Analysis

The analyses were performed in triplicate, with results presented as mean values and their standard deviations. Assuming a normal distribution of the data, verified by the Shapiro–Wilk Test, a one-way analysis of variance (ANOVA) was conducted. To identify significant differences between mean values, post-hoc Tukey’s test ($p < 0.05$) was applied. Statistical analyses were conducted using STATISTICA 10.0 Software (TIBCO Software Inc., Palo Alto, CA, USA).

3. Results

3.1. Characterization of Encapsulated CBG Particles

CBG was encapsulated into CTAB-modified CNC using spray-drying, a widely utilized encapsulation method in industrial applications [21]. The resulting encapsulated CBG particles were evaluated for yield, encapsulation efficiency (EE), loading capacity (LC), zeta potential (ZP), size, and morphology. The results are shown in Table 1 and Figure 1.

Table 1. Yield, encapsulation efficiency (EE), loading capacity (LC), zeta potential (ZP), and size of CNC-CTAB particles encapsulating CBG.

Delivery System	Yield (%)	EE (%)	LC (%)	Zeta Potential (mV)	Particle Size	
					Dv 50 (μm)	D 4:3 (μm)
CNC-CTAB_CBG	69.50	78.56 ± 1.89	28.26 ± 0.68	-21.80 ± 0.17	7.20	9.15

CNC: cellulose nanocrystals; CTAB: modified with cetyltrimethylammonium bromide.

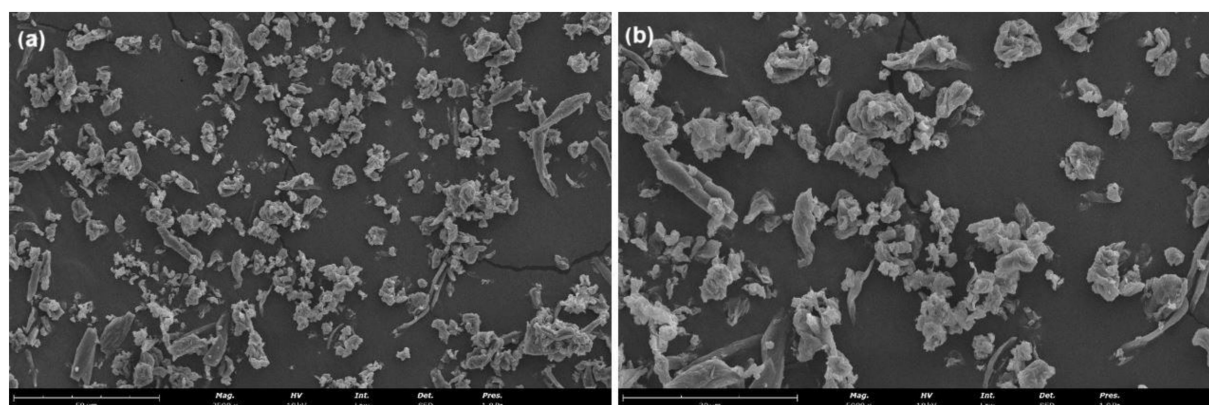


Figure 1. Scanning electron microscope images of spray-dried CNC-CTAB encapsulating CBG (magnifications of (a) 2500 $\times$ and (b) 5000 $\times$, with scale bars representing 50 μm and 30 μm , respectively).

The incorporation of CBG into the CNC-CTAB delivery system demonstrated a significant encapsulation efficiency (EE) of $78.56\% \pm 1.89$. This aligns with previously reported findings for CNC-CTAB carriers encapsulating lipophilic biomolecules such as curcumin, docetaxel, and paclitaxel, which showed EE values ranging from 80% to 90% [10,13]. The binding observed is probably due to the interaction between the lipophilic side chain of CBG and the hydrophobic domain of CNC-CTAB (C-H groups), supported by both electrostatic and hydrophobic forces. Additionally, the phenolic hydroxyl moieties in CBG may engage in hydrogen bonding with the polar head of CTAB [22]. Furthermore, the

surfactant monomers adsorbed onto the system can undergo rearrangement, promoting hydrophobic interactions among surfactant alkyl chains and leading to the formation of surfactant clusters that contribute to an enhanced binding of CBG [10,13].

The system exhibited a loading capacity of ca. 28% and a mass yield of about 70%, both promising outcomes for laboratory-scale spray-drying processes. The encapsulation of CBG within CNC-CTAB maintains moderate stability, as evidenced by a significantly negative zeta potential value of approximately -22 mV. According to laser diffraction-based particle size analysis, the mean diameter ($D[4.3]$) is $9.15\text{ }\mu\text{m}$, with half of the sample volume ($Dv(50)$) consisting of particles smaller than $7.20\text{ }\mu\text{m}$. It is noteworthy that, while nanomaterials were used to construct the delivery system, the spray drying process predominantly increased the particle size from the nanoscale to the microscale. This transition holds commercial appeal, as materials at the microscale may encounter fewer regulatory obstacles associated with the use of nanomaterials, potentially simplifying market access. SEM images (Figure 1) depict irregular, primarily spheroid particles ranging in diameters from 2 to $10\text{ }\mu\text{m}$, aligning with the findings from the particle size analysis.

The majority of these microstructures exhibited textured surfaces with indentations, likely stemming from shrinkage during the drying and cooling phases, subsequently causing surface deformations [23]. This phenomenon closely corresponds to morphologies documented in existing literature regarding spray-dried nanocellulose delivery systems [23,24]. Some CBG appear to exist as free crystals characterized by smoother, non-spherical particles that dried independently. This could be a result of precipitation occurring prior to the spraying process, when the biomolecule solution was introduced to the CNC suspension, or it may be attributed to differing drying kinetics between the biomolecule and CNC [24].

Figure 2 presents the ATR-FT-IR spectra for CNC-CTAB encapsulating CBG, alongside spectra for pure CBG and CNC-CTAB alone. The CBG spectrum exhibits characteristic peaks at 2960 cm^{-1} , indicating C-H stretching; 1620 cm^{-1} and 1580 cm^{-1} , associated with C=C stretching, and 1450 cm^{-1} , related to CH_2 bending. These vibrational bands are also evident in the encapsulation system even though at lower intensities, suggesting the successful incorporation of CBG into the CNC-CTAB matrix [13]. There were no significant shifts in the characteristic peaks of CBG within the loaded system, suggesting that the chemical structure of CBG remained intact after encapsulation and indicating a non-covalent interaction between CBG and the CNC-CTAB matrix. The CTAB modification of CNC introduces hydrophobic domains that can interact with the lipophilic regions of CBG molecules. Additionally, the positively charged quaternary ammonium groups of CTAB may interact with any slightly negatively charged regions of CBG (e.g., phenolic hydroxyl group, ether oxygen, and pi-electron), while the hydroxyl groups present in both CNC and CBG can potentially form hydrogen bonds, further stabilizing the encapsulation. Van der Waals interactions also likely contribute to the overall stability of CBG within the CNC-CTAB matrix, while the surfactant monomers on CNC-CTAB can reorganize and induce hydrophobic interactions between its alkyl chains to form surfactant clusters, further favoring CBG binding. These non-covalent interactions collectively facilitate the encapsulation of CBG without altering its chemical structure, as evidenced by the preservation of its characteristic FTIR peaks. This analysis aligns with previous research on encapsulating lipophilic compounds and demonstrates the effectiveness of CNC-CTAB as a carrier for CBG, potentially enhancing its stability and bioavailability for various applications.

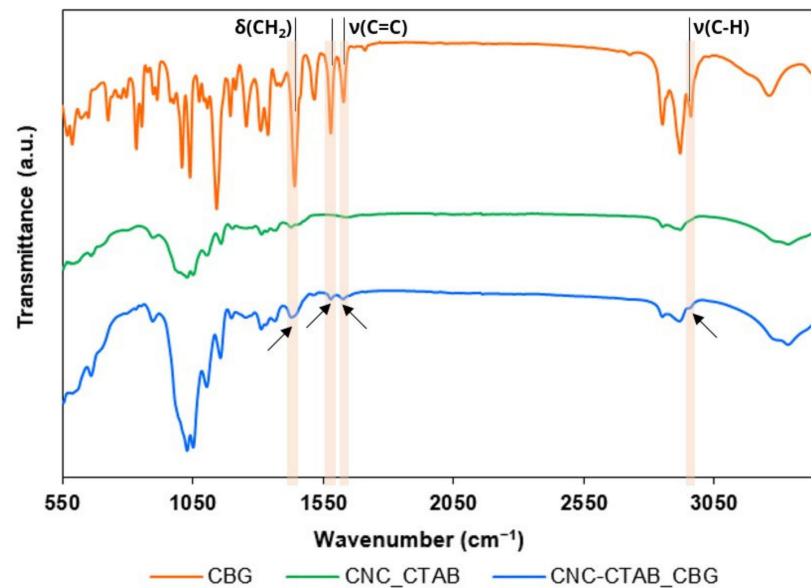


Figure 2. ATR-FT-IR spectra for CNC-CTAB encapsulating CBG, CNC-CTAB, and free CBG. Significant absorption bands are highlighted in orange and indicated by arrows, corresponding to specific functional groups or vibrational modes discussed in the text.

3.2. Cytotoxicity

Considering the potential gastrointestinal effects of the developed CBG delivery systems, the cytotoxicity of the systems was evaluated using the intestinal cell line Caco-2 to identify the highest non-cytotoxic concentration. The results are presented in Figure 3 for CNC-CTAB encapsulating CBG (0.01–0.32 g/L), alongside the free biomolecule at equivalent concentrations to those present in the encapsulated systems, considering a 3:1 carrier-to-biomolecule active ratio (0.0025–0.08 g/L).

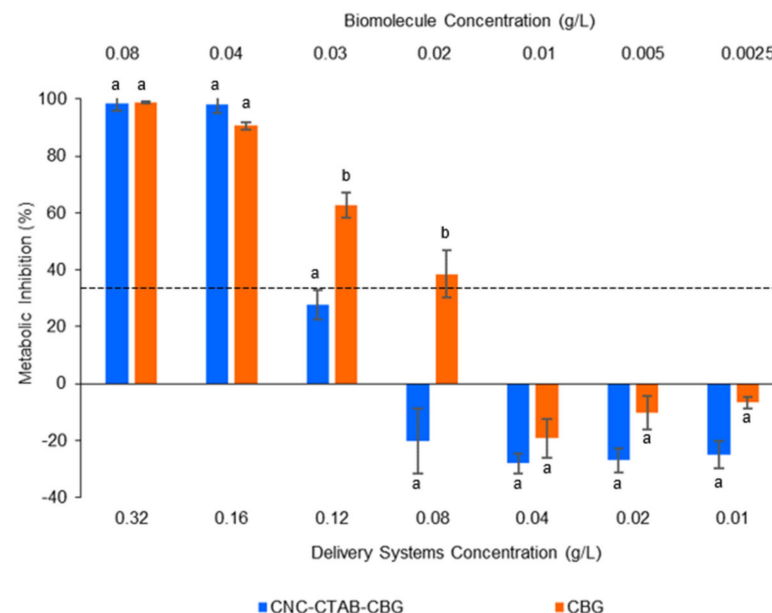


Figure 3. Impact of CNC-CTAB systems encapsulating CBG and free CBG upon Caco-2 cells metabolic activity. The dotted line indicates the 30% cytotoxicity threshold as specified by ISO 10993-5:2:2009. Different letters denote statistically significant differences ($p < 0.05$) observed among the samples tested at each concentration.

Considering a cell cytotoxicity threshold of <30% metabolic inhibition (as per ISO 10993-5:2009), CBG demonstrated statistically significant reductions ($p < 0.05$) in cell metabolism at concentrations of 0.02 g/L (60 μ M) and higher, while concentrations up to 0.01 g/L (30 μ M) were found to be safe for Caco-2 cells (Figure 3). Existing literature supports a concentration-related effect of CBG on Caco-2 cells, with a decrease in cell viability observed at the highest concentration tested (30 μ M) [25]. CNC-CTAB delivery systems encapsulating CBG exhibited no cytotoxic effects on Caco-2 cells up to 0.12 g/L (<30% metabolic inhibition). Consequently, these systems were able to mitigate CBG cytotoxicity toward Caco-2 cells ($p < 0.05$), as they maintained safe concentrations of 0.12 g/L, containing approximately 0.03 g/L of the biomolecule. This suggests a potential threefold increase in CBG dosage (from 0.01 g/L to 0.03 g/L). Utilizing encapsulation techniques to alleviate ingredient toxicity proves to be a valuable strategy for enhancing the applicability and biological functions of active biomolecules [26,27]. The bio-compatible concentrations of each sample (0.12 g/L for the delivery system and 0.01 g/L or 30 μ M for CBG) were employed for subsequent biological activity assessments.

3.3. Biological Potential

3.3.1. Antioxidant Potential

CBG's recognized biological effects include antioxidant, anti-inflammatory, and antibacterial properties [3,28]. However, understanding the potential impact of encapsulation on these bioactivities is crucial to determining the viability of the delivery system. In this context, the present study examines the antioxidant capacity of the CNC-CTAB delivery system encapsulating CBG, alongside the unencapsulated biomolecule and the carrier itself. This evaluation employs distinct yet complementary methodologies. Initially, the samples underwent assessments to determine their inherent scavenging and reducing abilities via radical scavenging assays (DPPH and ORAC) with ascorbic acid serving as the antioxidant reference. The results are presented in Figure 4.

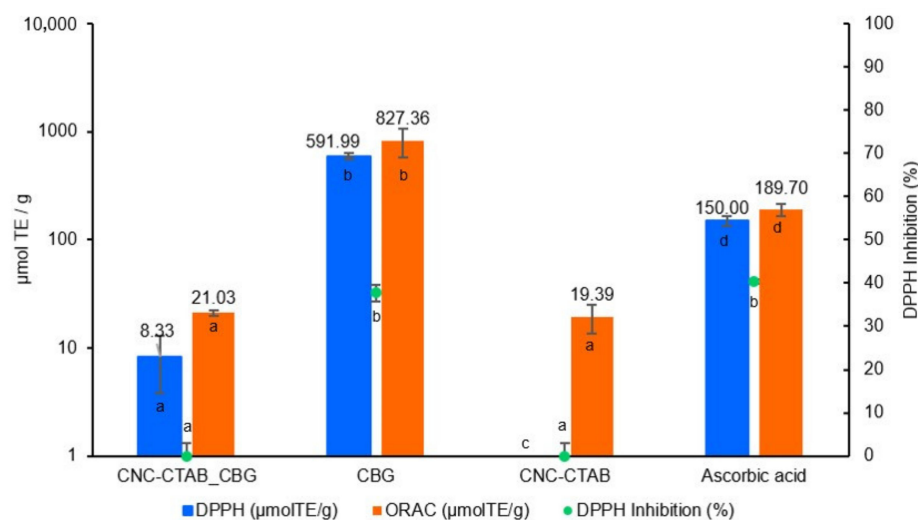


Figure 4. Antioxidant activity of CNC-CTAB encapsulating CBG, free CBG, and the carrier (CNC-CTAB) assessed using DPPH and ORAC assays compared to ascorbic acid. The results for the ORAC and DPPH assays expressed in μ mol TE/g are represented by the columns (left y-axis). The green dot indicates the DPPH assay results expressed as a percentage of inhibition (right y-axis). Statistically significant differences ($p < 0.05$) between the various samples for each assay are represented by different letters.

CBG exhibited promising antioxidant activity in both the DPPH assay (591.99 μ mol TE/g) and the ORAC assay (827.36 μ mol TE/g), with Trolox equivalent values superior to those of ascorbic acid ($p < 0.05$), and DPPH inhibition close to 40%, similar to the antioxidant control ($p > 0.05$). Literature studies have also shown that the antioxidant activities of cannabinoids, in-

cluding CBG, are stronger than Trolox in DPPH and ORAC methods, which has been attributed to the presence of phenolic groups, OH groups, and double bonds in the CBG molecule [29,30]. In fact, the authors observed a decrease in the antioxidant activity among cannabinoids in the order CBG, CBD, and THC, suggesting that it results from the decrease in the number of OH groups and double bonds in individual molecules [30]. While CBG demonstrates notable antioxidant activity across DPPH and ORAC assays, its radical-scavenging effectiveness measured lower than that of some polyphenols, particularly curcumin (4675.64 $\mu\text{mol TE/g}$ through DPPH and 76.69% DPPH inhibition) [31]. Curcumin's higher performance in these assays can be attributed to its polyphenolic structure, which provides additional phenolic groups that actively neutralize free radicals, lending it superior chemical antioxidant capacity in comparison to CBG. CBG antioxidant activity decreased significantly ($p < 0.05$) after encapsulation, with the CNC-CTAB delivery system encapsulating CBG showing relatively low antioxidant activity in the DPPH assay (8.33 $\mu\text{mol TE/g}$, 0.10% DPPH inhibition) and the ORAC assay (21.03 $\mu\text{mol TE/g}$) (Figure 4). The free carrier material, CNC-CTAB, did not exhibit relevant antioxidant activity in these assays.

3.3.2. Production of Reactive Oxygen Species (ROS)

While radical scavenging techniques offer insights into the chemical antioxidant efficacy of samples, it is essential to acknowledge that the biological antioxidant capacity may be determined by factors not exclusively captured through these measurements alone [16]. Therefore, we evaluated the antioxidative potential of CNC-CTAB encapsulating CBG, as well as free CBG and the carrier, in mitigating H_2O_2 -induced oxidative stress in intestinal Caco-2 cells by assessing reactive oxygen species (ROS) production. The findings are depicted in Figure 5.

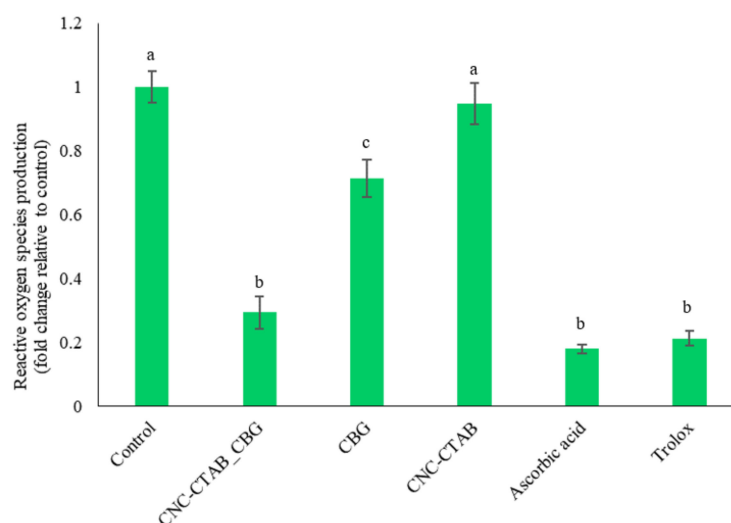


Figure 5. Antioxidant activity of CNC-CTAB encapsulating CBG, free CBG, and CNC-CTAB, as well as ascorbic acid and Trolox, by their influence on ROS production in Caco-2 cells subjected to the oxidative stress of H_2O_2 , relative to the control group. Statistical differences between the samples are indicated by different letters ($p < 0.05$).

CBG, at the highest non-cytotoxic concentration tested (30 μM), was able to reduce the production of ROS in Caco-2 cells stimulated by H_2O_2 by ca. 30% ($p < 0.05$). In the literature, several studies have demonstrated the efficacy of CBG in protecting cells from oxidative damage exerted by exposure to H_2O_2 , namely, in CTX-TNA2 astrocyte cells [29] and RAW264.7 macrophages [32]. Studies have shown that CBG is able to counteract oxidative stress and regulate redox balance by directly affecting components of the redox system or by acting indirectly through the activation of CB2 cannabinoid receptors. Specifically, CBG was reported to inhibit oxidative stress by (i) down-regulation of main oxidative markers (iNOS, nitrotyrosine and PARP-1); (ii) activation of the membrane

receptor PARP- γ ; (iii) modulation of the expression of superoxide dismutase (SOD-1); (iv) preventing I κ B- α phosphorylation and translocation of the nuclear factor- κ B (NF- κ B); and (iv) modulation of MAP kinases pathway [1,3,32,33]. Borrelli et al. (2013) have shown CBG effectiveness in ameliorating experimental colitis through a reduction in nitric oxide formation by macrophages via CB2 receptor activation and by leading to a diminution of ROS in the intestinal epithelial cells [34]. Other authors have confirmed that cannabinoids inhibit oxidative and nitrosative stress through the modulation of iNOS expression and reduction in ROS, and that CBG exerts antioxidant activity comparable to that of vitamin E [30,35,36]. Interestingly, despite the differences in the radical-scavenging capacities of CBG and the polyphenol curcumin, this disparity significantly diminishes when examining cellular antioxidant activity, particularly in reducing ROS production by Caco-2 cells under oxidative stress. In this biological context, both curcumin and CBG have demonstrated comparable efficacy, each achieving nearly equivalent reductions in cellular ROS levels [31]. This finding suggests that while the polyphenol's chemical structure offers superior radical scavenging in vitro, CBG's antioxidant activity may be equally effective within cellular environments, where factors such as cellular uptake, metabolism, and interaction with cellular signaling pathways play essential roles in modulating ROS. The free carrier material CNC-CTAB did not show any significant reduction in the production of ROS by Caco-2 cells when subjected to an oxidizing agent (H₂O₂).

The encapsulated CBG demonstrated a significant ability ($p < 0.05$) to reduce ROS production by Caco-2 cells exposed to the oxidizing agent, resulting in an approximate 70% reduction. This effectiveness was comparable to the positive control compounds, Trolox and ascorbic acid ($p > 0.05$) (Figure 5). Remarkably, the encapsulated CBG surpassed the unbound biomolecule at its non-cytotoxic concentration in terms of diminishing ROS production within intestinal cells. This heightened antioxidant efficacy may be ascribed to the increased level of CBG within the encapsulated system, gradually released from the carrier over time. By upholding non-toxic levels for cells, the encapsulated system facilitated an enhanced antioxidant response. While the antioxidant capacity evaluated through radical scavenging methods showed a reduction after encapsulation, the assessment of ROS production in cells revealed an increasing trend. This difference could likely be attributed to the varying exposure durations of the methods, as the extended timeframe of the cellular assay allows for the gradual release of the bioactive from the carrier, promoting sustained antioxidant effects while retaining safe levels of the biomolecule.

3.3.3. Immunomodulation

Disturbances in the process of intestinal inflammation can precipitate severe intestinal conditions, such as inflammatory bowel diseases (IBDs). The development of IBDs is closely linked to the involvement of inflammatory mediators, with IL-1 β playing a significant role [18,37,38]. This investigation assessed how CNC-CTAB delivery systems containing CBG, as well as free CBG and the carrier itself, influenced the inflammatory response in Caco-2 cells triggered by IL-1 β . The research concentrated on the levels of cytokines IL-6 and IL-8, using betamethasone as a standard reference for anti-inflammatory efficacy. The results are presented in Figure 6.

From Figure 6, significant ($p < 0.05$) reductions in cytokine secretion were observed for CBG-treated cells, indicating a relevant anti-inflammatory effect. CBG reduced the secretion of IL-6 and IL-8 cytokines by ca. 60% and 40%, respectively, compared to the stimulated control cells. CBG has been found to modulate inflammatory processes by significantly reducing I κ B- α phosphorylation, thus reducing the transcriptional activity of NF- κ B, which is responsible for the transcription of pro-inflammatory cytokines such as TNF α and IL-1 β [3,32]. Furthermore, CBG has been reported to reduce the activity of diacylglycerol lipase, the enzyme responsible for the biosynthesis of 2-arachidonoylglycerol, the most abundant ligand for CB1 and CB2 cannabinoid receptors, as well as the activities of COX-1 and COX-2, which are involved in the metabolism of arachidonic acid and production of prostaglandins, lipid mediators associated with inflammation [3,39]. In addition,

CBG exhibits a significant activity against several receptors from the transient receptor potential (TRP) family, acts as a potent agonist of $\alpha 2$ adrenergic receptors, and moderately blocks 5-HT1A receptors, which, combined with the effects mentioned above, potentiates its biological activity [2,40]. Although CNC-CTAB in its free form demonstrated a slight pro-inflammatory effect, leading to an approximate 20% increase in the production of IL-6 and IL-8, encapsulation did not significantly impact CBG's inflammatory response in terms of IL-6 and IL-8 secretion levels in IL-1 β stimulated Caco-2 cells ($p > 0.05$) (Figure 6). CBG-encapsulated systems were capable of lowering cytokine production to levels comparable to those achieved with the anti-inflammatory agent betamethasone, indicating the potential anti-inflammatory activity of these systems.

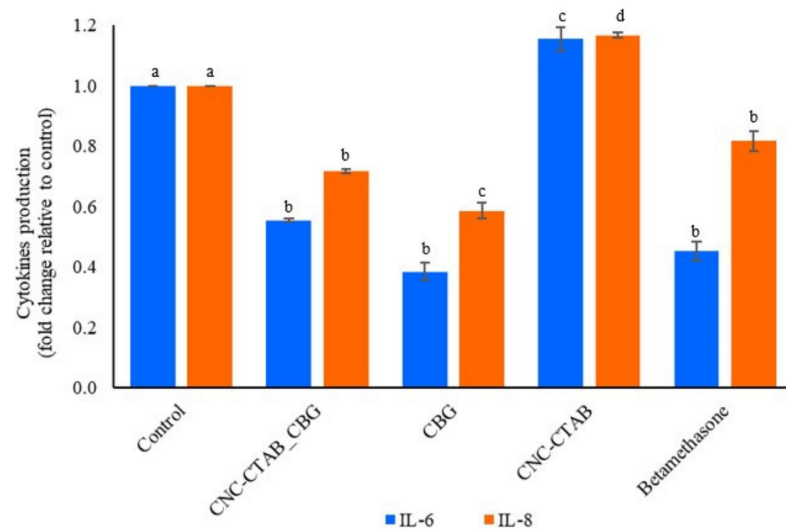


Figure 6. Immunomodulatory properties of CNC-CTAB encapsulated CBG, free CBG, the carrier, and betamethasone (positive control) on the Caco-2 cell line, employing IL-1 β as a pro-inflammatory stimulus. The findings were assessed against the basal control level, with varying letters indicating significant statistical differences ($p < 0.05$) among the cytokine responses for each sample.

3.3.4. Antimicrobial Activity

The antimicrobial effectiveness of CNC-CTAB encapsulating CBG, as well as free CBG and the carrier, was assessed against common microorganisms linked to gastrointestinal conditions. Due to the aqueous insolubility of the delivery system, the previously established biocompatible concentrations were evaluated using the incorporation method. The study focused on four reference strains: *Escherichia coli*, *Salmonella enteritidis*, *Listeria innocua*, and *Campylobacter jejuni*, which include both Gram-positive and Gram-negative bacteria. The findings are outlined in Table 2.

Table 2. Antimicrobial properties of CNC-CTAB loaded with CBG (0.12 g/L), CBG (30 μ M), and CNC-CTAB (0.09 g/L) against four reference microorganisms linked to the gastrointestinal tract.

Sample	<i>E. coli</i>	<i>S. enteritidis</i>	<i>L. innocua</i>	<i>C. jejuni</i>
CNC-CTAB_CBG	—	—	—	+
CBG	—	—	—	—
CNC-CTAB	—	—	—	+

(—) Absence of antimicrobial activity (microorganism growth present); (+) Antimicrobial activity (inhibition of microorganism growth). Tested strains were *Escherichia coli*, *Salmonella enteritidis*, *Listeria innocua*, and *Campylobacter jejuni*.

According to Table 2, using the employed method, none of the samples were capable of inhibiting the growth of *Escherichia coli*, *Salmonella enteritidis*, and *Listeria innocua* at the concentrations tested. CBG has demonstrated antimicrobial activity against various strains, including methicillin-resistant *Staphylococcus aureus* (MRSA) [41], *Streptococcus mutans* [42],

Staphylococcus epidermidis, *Streptococcus pyogenes*, and *Propioniferax innocua* [14]. CBG has shown antimicrobial activity by altering bacterial membrane properties, such as membrane hyperpolarization, reduction in fluidity, and increased permeability [42]. However, CBG MIC values of 500 μM have been observed for *E. coli* [14], which may explain the inability to inhibit microbial growth at the concentration tested (30 μM). While free CBG had no impact on the growth of *Campylobacter jejuni* at the tested concentration, free CNC-CTAB and CNC-CTAB loaded with CBG successfully inhibited its growth. Despite the high CBG concentration present in the delivery system, its hydrophobic nature poses challenges to its release within the agar medium. As a result, the antimicrobial outcomes obtained in this study for encapsulated CBG are likely attributed to CNC-CTAB, a view supported by the antimicrobial effect demonstrated by the free carrier material. While CNC itself has not been associated with significant antimicrobial activity [43], the CTAB surfactant has exhibited antimicrobial traits linked to its amphipathic structure's positive charge [44]. The observed antimicrobial effects of CNC-CTAB encapsulating CBG against *C. jejuni* hold great promise, particularly in the context of gastrointestinal delivery. *Campylobacter jejuni* stands out as a leading cause of bacterial enteritis and colonic inflammation, and it has been recognized as one of the most common risk factors for IBDs and irritable bowel syndrome (IBS). Evidence suggests that *C. jejuni* disrupts intestinal epithelial structure and function and thereby permits the translocation of luminal material, including resident intestinal bacteria, into the subepithelial compartment, which may prime the intestine for subsequent inflammation [45].

3.4. Release Profile Under Digestion Conditions

To replicate the digestive conditions and analyze how CBG is released from CNC-CTAB in the gastrointestinal tract, an in vitro digestion model was used. This model followed the standardized European INFOGEST procedure for simulating static in vitro digestion [19,20]. The experiment was conducted for both the encapsulated CBG and free CBG, with the release patterns displayed in Figure 7.

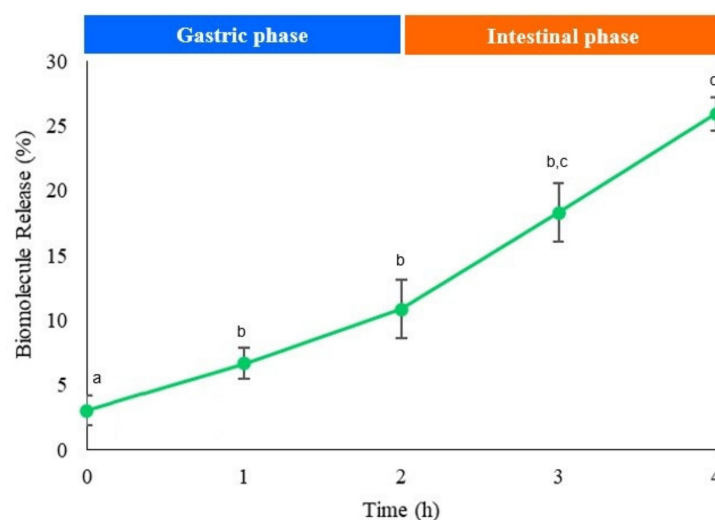


Figure 7. Release profile of CBG during the in vitro simulation of gastrointestinal digestion using the CNC-CTAB delivery system. Variations in letters denote statistically significant differences ($p < 0.05$) observed between the time points.

CBG exhibited a biphasic release pattern, characterized by an initial slow release in the gastric phase followed by a sustained release in the intestinal phase. Approximately 10% of CBG was released within the first 2 h in the gastric environment, while an additional 25% was released over the subsequent 4 h in the intestinal phase (Figure 7). This release profile is particularly interesting for intestinal delivery of CBG, as it allows for prolonged exposure of the bioactive compound to intestinal cells, promoting its biological activities,

such as antioxidant and anti-inflammatory effects. Additionally, the sustained release of CBG in the intestinal phase may enhance its antimicrobial activity against pathogenic gut microbiota, including *Campylobacter* spp. The observed release pattern suggests that the Korsmeyer–Peppas model could be appropriate for describing CBG's release kinetics. This model is particularly appropriate for polymeric systems and can account for both Fickian diffusion and non-Fickian transport mechanisms. The Korsmeyer–Peppas model is especially useful for interpreting complex and time-dependent release patterns, such as the biphasic release observed with CBG in the gastric and intestinal phases [46]. The results highlight the potential of encapsulated CBG, due to its unique interactions with cannabinoid and serotonin receptors, for applications aimed at intestinal inflammation and microbial infections.

4. Conclusions

CBG (cannabigerol), a lipophilic cannabinoid, faces challenges such as degradation and low aqueous solubility, which hinder its oral bioavailability. However, its diverse biological activities present significant commercial potential. This study investigates CNC-CTAB structures as carriers for the intestinal delivery of CBG, aiming for effective and prolonged release to enhance its biological effects. A key finding is that CNC-CTAB encapsulating CBG shows no cytotoxicity toward intestinal cells at concentrations up to 0.12 g/L, effectively reducing CBG's inherent cytotoxicity. This highlights the potential of encapsulation for safer CBG delivery. Experiments with Caco-2 cells demonstrated that encapsulated CBG significantly reduced reactive oxygen species (ROS) production induced by hydrogen peroxide, illustrating its capacity to counteract oxidative stress. Importantly, the encapsulated system exhibited enhanced ROS-reducing efficacy compared to free CBG, all while maintaining safe levels for intestinal cells. The unique receptor-mediated interactions of CBG contribute to its anti-inflammatory effects, which were not significantly altered by encapsulation. The encapsulated system effectively lowered cytokine production to levels comparable to the anti-inflammatory drug betamethasone. Furthermore, the CNC-CTAB delivery system exhibited antimicrobial properties against *Campylobacter jejuni*, underscoring its potential in addressing this pathogen and alleviating associated gastrointestinal inflammation. The slow-release profile of the encapsulated CBG under gastrointestinal conditions ensures prolonged exposure of the bioactive compound to intestinal cells and pathogens, enhancing its biological functions. Given the critical role of inflammation, redox imbalance, and the involvement of *Campylobacter jejuni* in intestinal inflammatory diseases, our findings emphasize the potential of this encapsulation strategy to improve CBG's efficacy in combating such conditions while addressing cytotoxicity concerns. This study is the first to explore the biocompatibility, biological capabilities, and gastrointestinal release profile of CNC-CTAB delivery systems encapsulating CBG. The results point to the promise of encapsulated CBG, particularly its receptor-mediated effects, for applications targeting intestinal inflammation and microbial infections, enabling safer administration at higher doses.

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