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Formulation and In Vitro Characterisation of Chitosan-Alginate Nanoparticles Loaded with Curcumin for Targeted Colon Cancer Drug Delivery: Effect of Polymer Ratio on Encapsulation Efficiency, Release Kinetics, and Cytotoxicity

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Abstract

Curcumin (diferuloylmethane), the principal bioactive polyphenol of Curcuma longa, exhibits documented antiproliferative, anti-inflammatory, and pro-apoptotic activity across multiple cancer cell lines, yet its clinical translation is severely hampered by pharmacokinetic limitations including aqueous solubility below 11 ng/mL at physiological pH, extensive first-pass metabolism, and rapid systemic clearance yielding oral bioavailability typically below 1% in humans. Polymeric nanoparticle encapsulation using mucoadhesive polysaccharides that resist gastric and small intestinal degradation while releasing cargo in the colonic environment — exploiting colonic pH (6.5–7.4) and microbial enzyme-mediated polymer degradation — represents a promising strategy for colon-targeted curcumin delivery. This study reports the preparation, optimisation, and in vitro characterisation of chitosan-sodium alginate nanoparticles (CS-SA NPs) loaded with curcumin via ionic gelation method, investigating the effect of four chitosan:alginate polymer ratios (1:1, 1:2, 2:1, and 1:3 w/w) on encapsulation efficiency (EE%), drug loading capacity (DLC), particle size, zeta potential, polydispersity index (PDI), and in vitro release profile. Optimal formulation (CS:SA = 1:2, designated F3) achieves EE of 84.3 ± 2.1%, particle size 186 ± 12 nm, PDI 0.183, zeta potential -28.4 ± 1.8 mV, and cumulative curcumin release of 78.6% at 24 hours under simulated colonic fluid (pH 6.8, rat caecal content 2% w/v). In vitro cytotoxicity against HCT-116 colon cancer cell line confirms IC50 of 8.4 µg/mL for F3 nanoparticles versus 42.6 µg/mL for free curcumin solution — a 5.1-fold enhancement attributed to nanoparticle-mediated cellular uptake enhancement and sustained intracellular release. FTIR and DSC analysis confirm curcumin encapsulation and polymer-drug compatibility. These results support further in vivo evaluation of CS-SA nanoparticles as a colon-targeted curcumin delivery platform.

Keywords: *curcumin, chitosan, alginate, nanoparticles, colon cancer, drug delivery, ionic gelation, HCT-116, encapsulation efficiency, release kinetics, zeta potential*

1. Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed malignancy globally, with an estimated 1.93 million new cases and 935,000 deaths in 2020 (GLOBOCAN 2020). In India, CRC incidence has been increasing at approximately 5% annually in urban populations over the past two decades, driven by dietary westernisation, rising obesity prevalence, physical inactivity, and declining dietary fibre consumption. First-line chemotherapeutic regimens for metastatic CRC — FOLFOX (fluorouracil, leucovorin, oxaliplatin) and FOLFIRI (fluorouracil, leucovorin, irinotecan) — achieve objective response rates of 40–60% but are associated with significant systemic toxicity including myelosuppression, peripheral neuropathy, and severe gastrointestinal adverse effects that limit dose intensity and treatment duration.

The ethnopharmacological record of Curcuma longa root extract in Ayurvedic medicine for gastrointestinal conditions, combined with an extensive preclinical evidence base demonstrating curcumin's inhibition of NF-κB, COX-2, and Wnt/β-catenin signalling pathways centrally implicated in CRC pathogenesis, has generated sustained research interest in curcumin as an adjunctive or standalone CRC therapeutic. Phase I and II clinical trials have established curcumin's excellent safety profile at oral doses up to 12 g/day, confirming its clinical tolerability while simultaneously documenting the pharmacokinetic limitations (peak plasma concentrations of 0.5–1.8 µM after 12 g oral dose) that preclude achievement of the in vitro active concentrations (10–50 µM range for antiproliferative effects in cell culture models) in tumour tissue.

Chitosan, a deacetylated derivative of chitin produced from crustacean exoskeletons, possesses mucoadhesive properties (electrostatic interaction of its positively charged amino groups with negatively charged mucin glycoproteins), inherent antibacterial activity, and established biocompatibility and biodegradability. Sodium alginate, an anionic polysaccharide derived from brown seaweed, forms hydrogels upon ionic crosslinking with divalent cations (calcium) or electrostatic interaction with cationic polymers such as chitosan. The CS-SA polyelectrolyte complex exploits the opposite charges of these polymers (chitosan pKa ~6.5; alginate pKa ~3.5) to form stable nanoparticles by spontaneous ionic gelation without organic solvents or toxic crosslinkers — a significant process safety advantage over PLGA or lipid-based nanoparticle systems. The colonic microbiota's abundant production of alginate lyase and chitosanase enzymes provides additional specificity for colonic drug release from CS-SA matrices beyond the pH-responsive swelling mechanism.

While individual chitosan nanoparticle and alginate microsphere systems for curcumin delivery have been extensively reported, the systematic investigation of polymer ratio effects on nanoparticle physicochemical properties and in vitro performance of the binary CS-SA system under rigorous simulated gastrointestinal conditions remains incomplete. This study addresses that gap with a methodical four-ratio investigation under identical preparation and characterisation conditions, with the explicit goal of identifying an optimal formulation for further preclinical development.

2. Materials and Methods

2.1 Materials

Curcumin (purity ≥ 98%, HPLC grade) was obtained from Sigma-Aldrich (St. Louis, USA). Chitosan (deacetylation degree ≥ 85%, medium molecular weight 190–310 kDa) and sodium alginate (viscosity 2% solution: 25–35 cP, Mw ~80,000 Da) were procured from HiMedia Laboratories (Mumbai, India). Calcium chloride dihydrate, acetic acid (glacial), phosphate buffer salts, and all HPLC-grade solvents were purchased from Merck (India). Rat caecal content for simulated colonic fluid preparation was obtained following ethical approval from the Institutional Animal Ethics Committee (IAEC Approval No. KPH/IAEC/2023-24/07). HCT-116 human colon carcinoma cells were obtained from National Centre for Cell Science (NCCS), Pune.

2.2 Preparation of Curcumin-Loaded CS-SA Nanoparticles

Curcumin-loaded nanoparticles were prepared by the ionic gelation/polyelectrolyte complexation method. Curcumin (5 mg) was dissolved in 1 mL ethanol and added dropwise to 10 mL aqueous chitosan solution (0.1% w/v in 1% acetic acid) under magnetic stirring at 500 rpm. The curcumin-chitosan dispersion was then added dropwise to sodium alginate solution (varying concentrations per the four formulations F1-F4 corresponding to CS:SA ratios of 1:1, 2:1, 1:2, and 1:3 respectively) under continued stirring at 1000 rpm for 30 minutes at room temperature. CaCl2 solution (0.1 M, 1 mL) was added as the crosslinking agent and stirring continued for 30 additional minutes. The nanoparticle suspension was centrifuged (15,000 rpm, 30 min, 4°C; Beckman Coulter Optima L-90K), supernatant collected for encapsulation efficiency determination, and the pellet was resuspended in Milli-Q water and lyophilised with mannitol (3% w/v) as cryoprotectant.

2.3 Physicochemical Characterisation

Particle size, PDI, and zeta potential were determined by dynamic light scattering (DLS) using a Malvern Zetasizer Nano ZS (Malvern Instruments, UK) at 25°C in triplicate. Encapsulation efficiency was calculated as: EE% = [(Total curcumin added - Free curcumin in supernatant) / Total curcumin added] × 100. Curcumin concentration in supernatant was determined by UV-Vis spectrophotometry at 425 nm using a standard curve (r^2 = 0.9994, range 1-50 µg/mL in ethanol:water 50:50). Surface morphology was examined by Field Emission Scanning Electron Microscopy (FESEM, ZEISS GeminiSEM 300) on gold-sputter-coated lyophilised samples.

2.4 In Vitro Drug Release

In vitro release was conducted using dialysis bag method (MW cutoff 12,000 Da) in three sequential pH media simulating gastrointestinal transit: simulated gastric fluid (SGF, pH 1.2, 2 hours), simulated intestinal fluid (SIF, pH 6.8 with pancreatin, 3 hours), and simulated colonic fluid (SCF, pH 6.8 with 2% w/v rat caecal content, 19 hours). Release medium samples (3 mL) were withdrawn at specified time points and replaced with fresh medium. Curcumin concentration was determined by HPLC (reverse-phase C18 column, mobile phase acetonitrile:water:acetic acid 65:34:1, detection at 425 nm, flow rate 1 mL/min). Release kinetic modelling was performed using DDSolver Excel add-in for zero-order, first-order, Higuchi, Korsmeyer-Peppas, and Hixson-Crowell models.

Table 1. Formulation Compositions and Physicochemical Characteristics of CS-SA Curcumin Nanoparticles (n=3, mean ± SD)

Parameter	F1 (1:1)	F2 (2:1)	F3 (1:2)	F4 (1:3)
Particle Size (nm)	248 ± 18	312 ± 24	186 ± 12	214 ± 16
PDI	0.241	0.308	0.183	0.196
Zeta Potential (mV)	-19.3 ± 1.2	+12.4 ± 0.9	-28.4 ± 1.8	-31.2 ± 2.1
EE (%)	71.2 ± 3.4	64.8 ± 2.9	84.3 ± 2.1	79.6 ± 3.2
DLC (%)	8.4 ± 0.6	7.6 ± 0.5	9.9 ± 0.4	9.4 ± 0.7
Yield (%)	78.3 ± 4.1	72.1 ± 3.8	88.6 ± 2.9	84.2 ± 3.5

3. Results and Discussion

3.1 Effect of Polymer Ratio on Physicochemical Properties

Table 1 summarises the physicochemical characterisation results for all four formulations. Particle size varied significantly with polymer ratio: F2 (CS:SA = 2:1) showed the largest particles (312 ± 24 nm), attributable to the excess positive charge on chitosan promoting inter-particle aggregation during ionic gelation before the electrostatic equilibrium is established. F3 (CS:SA = 1:2) produced the smallest particles (186 ± 12 nm) with the lowest PDI (0.183), indicating a well-defined nanoparticle size distribution attributable to the excess alginate coating stabilising particle surfaces through steric and electrostatic repulsion. The negative zeta potential of F3 (-28.4 ± 1.8 mV) and F4 (-31.2 ± 2.1 mV) reflects alginate dominance at the nanoparticle surface, while F2's positive zeta potential (+12.4 ± 0.9 mV) confirms chitosan surface dominance. The absolute zeta potential values for F3 and F4 exceed the commonly cited ±25 mV threshold for electrostatically stabilised colloidal systems, predicting adequate storage stability.

Encapsulation efficiency followed the order F3 (84.3%) > F4 (79.6%) > F1 (71.2%) > F2 (64.8%). The higher EE of F3 compared to F1 confirms that excess alginate relative to chitosan improves curcumin entrapment — attributed to alginate's hydrophobic domain interaction with curcumin's extended conjugated system providing additional non-electrostatic binding capacity within the nanoparticle matrix. F4's slightly lower EE than F3 despite higher alginate content may reflect the excess unbound alginate in solution at the highest alginate ratio (1:3) partitioning some drug into the aqueous phase before complete nanoparticle formation. These trends are consistent with Sarmento et al. (2007) and Azevedo et al. (2014) who reported similar optimal polymer ratio effects in CS-SA insulin nanoparticle systems.

3.2 FTIR and DSC Characterisation

FTIR spectra of curcumin, blank CS-SA nanoparticles, and curcumin-loaded F3 nanoparticles were compared. Curcumin showed characteristic absorption bands at 3510 cm^{-1} (O-H stretch), 1628 cm^{-1} (C=O stretch of β-diketone enol form), 1601 cm^{-1} (C=C aromatic stretch), and 1027 cm^{-1} (C-O-C stretch). The chitosan NH_2 band at 1596 cm^{-1} and alginate COO^- asymmetric stretch at 1620 cm^{-1} are present in blank nanoparticles. In the curcumin-loaded F3 spectrum, the shift and broadening of the 1628 cm^{-1} band to 1614 cm^{-1} and reduction in the 3510 cm^{-1} O-H intensity confirm physical encapsulation with hydrogen bonding interaction between curcumin's OH groups and the polymer matrix. Absence of new peaks confirms absence of covalent chemical interaction — consistent with physical entrapment rather than chemical conjugation.

DSC thermograms reveal curcumin's characteristic melting endotherm at 183.4°C in the pure drug. This endotherm is absent in the F3 curcumin-loaded nanoparticle thermogram, confirming that curcumin is molecularly dispersed or amorphised within the polymer matrix — a state associated with enhanced dissolution rate relative to crystalline curcumin and consistent with the enhanced in vitro release and cytotoxicity performance observed in subsequent experiments.

3.3 In Vitro Release and Kinetic Modelling

The cumulative in vitro release profiles (Figure 4) demonstrate the pH-responsive and enzyme-triggered colonic release behaviour of F3 nanoparticles. In SGF (pH 1.2, 0–2 h), curcumin release from F3 was limited to 8.2 ± 1.4% — substantially lower than free curcumin suspension (26.4 ± 2.1% in SGF) — confirming gastric protection by the alginate coating, which remains intact at low pH due to protonation of carboxylic groups. In SIF (pH 6.8, 2–5 h), release increased to 24.6 ± 2.8% (cumulative), reflecting partial ionisation of alginate and chitosan swelling. The most significant release occurred in SCF (pH

6.8 with caecal content, 5–24 h), reaching 78.6 ± 3.2% cumulative release at 24 h — attributed to alginate lyase and chitosanase activity in the rat caecal content degrading the polymer matrix.

Release kinetic modelling of the SCF phase data for F3 formulation showed best fit to the Korsmeyer-Peppas model (R^2 = 0.9841, n = 0.58), with the release exponent n between 0.5 and 1.0 indicating anomalous (non-Fickian) transport — a combined mechanism of diffusion through the swollen polymer matrix and polymer erosion by enzymatic degradation. This finding is mechanistically consistent with the dual release mechanism proposed for CS-SA matrices in colonic environments.

Table 2. Release Kinetic Model Parameters for F3 Formulation in Simulated Colonic Fluid Phase

Kinetic Model	R^2	k (rate constant)	n (if applicable)
Zero-order	0.9124	3.42 %/h	—
First-order	0.9312	0.0814 h^{-1}	—
Higuchi	0.9687	18.43 %/$h^{0.5}$	—
Korsmeyer-Peppas	0.9841	12.61	0.58
Hixson-Crowell	0.9241	0.0342 h^{-1}	—

3.4 In Vitro Cytotoxicity Against HCT-116 Cells

MTT assay results show concentration-dependent cytotoxicity of F3 nanoparticles and free curcumin against HCT-116 cells at 48 hours. The IC50 of F3 nanoparticles (8.4 ± 0.6 µg/mL) was 5.1-fold lower than free curcumin solution (42.6 ± 3.2 µg/mL) and 3.2-fold lower than blank CS-SA nanoparticles (IC50 > 100 µg/mL, confirming polymer biocompatibility). The markedly enhanced cytotoxicity of the encapsulated formulation is attributed to three mechanistic factors: (i) nanoparticle endocytosis bypassing the P-glycoprotein efflux pump that limits free curcumin intracellular accumulation in HCT-116 cells (which overexpress P-gp); (ii) sustained intracellular curcumin release from endosome-escaped nanoparticles maintaining therapeutic intracellular concentrations over 48 hours; and (iii) the amorphous state of encapsulated curcumin providing faster intracellular dissolution compared to crystalline free curcumin. These cytotoxicity findings are consistent with the enhanced cellular uptake studies required for future mechanistic characterisation.

4. Conclusion

Chitosan-alginate nanoparticles prepared by ionic gelation at a 1:2 polymer ratio (F3) represent the optimal curcumin delivery formulation in this systematic study, achieving 84.3% encapsulation efficiency, 186 nm particle size with PDI 0.183, adequate colloidal stability (-28.4 mV zeta potential), pH-responsive gastric protection (8.2% SGF release), and colonic enzyme-triggered release (78.6% at 24h in SCF). The 5.1-fold improvement in IC50 against HCT-116 colon cancer cells compared to free curcumin confirms the delivery system's therapeutic advantage. FTIR and DSC analyses confirm physical encapsulation and curcumin amorphisation — a key determinant of enhanced dissolution and bioactivity. The F3 formulation warrants in vivo pharmacokinetic and pharmacodynamic evaluation in CRC rodent models to translate the in vitro findings toward preclinical proof-of-concept.

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