

Curcumin-encapsulated Polysaccharide Nanocomposite: Formulation Design, Optimization and Characterization

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Abstract

Introduction: Nano systems have gained great attention due to many unique physicochemical characteristics, such as high-capacity for loading of cargo, high stability and ability to entrap both hydrophobic and hydrophilic therapeutics.

Objective: In this research, with the aim of improve the loading efficiency and particle size Chitosan (CS)-Montmorillonite (MMT) nanocomposite were prepared.

Materials and Methods: Cs-MMT nanocomposite were prepared using an ionic gelatinization method for controlled delivery of curcumin and optimized via response surface method. Different formulation and processing variables (Cs concentration, MMT percentage, surfactant concentration, drug amount and sonication time) were used to determine the optimal formulation.

Results: Two parameters of polysaccharide concentration and tween volume were selected to obtain optimal formulation with highest loading efficiency and minimum particle size. Polysaccharide concentration, surfactant concentration and sonication time had higher effect on particle size. MMT addition significantly enhanced the entrapment efficiency of Curcumin. Also, the increase in drug amount (mg/ml) resulted in the increase in entrapment efficiency. Physicochemical characteristics of optimal formulation were determined in terms of entrapment efficiency, release profile, Size, Zeta potential, surface morphology.

Conclusion: Formulation S1 with a particle size of 97 nm, a loading efficiency of 91% and a Zeta potential of 37.2 mV was selected as the optimal formulation. The in vitro release study showed that Curcumin had a slow and sustained release profile at basic pH 7.4, which significantly increased at acidic pH of 4.5. The maximum release was 80% at 37°C, pH 4.5 after 24 hours.

Keyword: Chitosan; Curcumin; Montmorillonite; Ionic gelatinization; Optimal formulation

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1. Introduction

Drug delivery systems (DDSs) can be defined as tools for the effective treatment in patients that enable to transport therapeutic agents more selectively to a target site, to decrease undesirable side-effects on

normal tissues, to protect compounds against enzymatic degradation and to maximize the efficacy of curative drugs [1, 2]. During the past several years, nanotechnology has become one of the most important and exciting majors. Numerous



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nanosystems have emerged to overcome the limitations associated with current methods of therapy and have gained great attention due to many unique physicochemical characteristics, such as high-capacity for loading of cargo, high stability and ability to entrap both hydrophobic and hydrophilic therapeutics [3, 4]. Composites nanoparticles are solid nanoscale particles formed from at least two different materials which can be produced via mechanical or chemical methods [5, 6]. DDSs from natural polymers have become important because of their biocompatibility and nontoxicity [7, 8]. Three components of the controlled delivery systems are a therapeutic agent, the targeting moiety and a carrier matrix. The therapeutic agents may be divided as biologicals, like curcumin, and non-biologicals [9, 10]. Curcumin is the active component of turmeric is an orange-yellow crystalline compound. Over the last century, Curcumin has been found to have anti-neoplastic, antimicrobial, anti-inflammatory and antioxidant activity. However, it has been seldom used in clinical applications. Poor solubility in an aqueous system, fast degradation, and low oral bioavailability have limited the potential of Curcumin for clinical therapeutic application [11]. Entrapment of Curcumin into nanoparticles, as a drug delivery system, is a suitable and helpful way to overcome its limitations [12]. Application of nanoparticles has recently promising results for water-insoluble agents like curcumin [13]. Nanoparticles and nanocomposites have a small size and high surface to volume ratio, which enables them for the crossing of biological barriers and suitable for drug delivery [14, 15]. To this end, the preparation process for the synthesis of curcumin-loaded polysaccharide nanocomposite was optimized through response surface methodology of experimental design. Chitosan has been used to develop a bio-nanocomposite along with Montmorillonite (MMT) nano clay for Curcumin encapsulation. Encapsulation of Curcumin in the Cs-MMT nanocomposite, increased drug solubility in aqueous solutions and stability.

Chitosan (Cs) is a biocompatible and nontoxic polymer have very well structural feasibility for mechanical and chemical modification [16] Chitosan

is biocompatible with living tissues, therefore, does not cause allergic reactions in the body [17] and investigated as matrices for the delivery of drugs. [18] Clay-polymer nanocomposites are getting to play a role in nanoformulations for drug delivery. This is because of their stronger effect on controlled drug release compared to individual clay or polymer [19]. MMT nano clay provides excellent sorption properties because of its rough and porous surface [20]. MMT clay is hydrophilic, nature-friendly nanofiller with very wide specific surface area. It is used as nanofiller in Chitosan-Starch composites. Exfoliation of nanoclay increases polysaccharide–MMT interaction [21]. This study aimed to encapsulate Curcumin into an optimal formulation of Cs-MMT nanocomposite and evaluates the effects of formulation variables and process variables on particle size and entrapment efficiency. Then, the produced nanocomposite was assessed by studying the size and morphology and drug release profile.

2. Materials and Methods

2.1. Materials

Chitosan (low molecular weight), sodium hydroxide, Curcumin, acetic acid, ethyl acetate, hydrochloric acid, absolute ethanol and Tween 20 (surfactant) were obtained from Merck Company. Montmorillonite K₁₀ was purchased from Sigma Aldrich Co., US. The other chemicals were of reagent grade and used without further purification.

2.2. Nanoparticle preparation

Cs nanoparticles were prepared via ionic gelation method. Different Cs solutions (0.25, 0.5, 0.75 and 1 wt.%) were prepared by dissolving Cs powder in 10 ml of 0.5% (v/v) acetic acid solution under stirring (~700 rpm). The Cs solution was regulated to pH 5 with 1M aqueous NaOH solution, then 15 µl of Tween 20 was added to the solutions as the emulsifying agent, to obtain a solution with uniform dispersion and to avoid aggregation at room temperature during stirring. Also, MMT (0.005–0.025 g) (1, 3 and 5% w/w, w.r.t. polymer) was swollen in 10 ml of water for 12 h. It was then stirred vigorously by a mechanical stirrer for 30 h and sonicated for 30 min [22].

2.3. Curcumin entrapment in polysaccharide nanocomposite

At first, different concentrations of Curcumin solution in ethanol, (0.0004–0.004g) (1/100, 1/50, 1/10 and 1/5 w/w, w.r.t. polymer) were added dropwise to MMT suspension (5% w/w, w.r.t. polymer) and was stirred for 15 min. Subsequently, CS nanoparticles solutions were added slowly under probe sonication for 50 min. The resulting suspension was centrifuged (Hermle-Labnet) at 2000 rpm for 2 min. The precipitate was removed, and the supernatant was centrifuged again (Sigma) at 15.000 rpm for 15 min. The supernatant discarded and the precipitate was washed with 1 ml absolute ethanol to remove free Curcumin. Then the particles were re

suspended in deionized water. For freeze-drying, the nanoparticles were stored in liquid nitrogen for 5 min and then transferred to the freeze-dryer.

The physicochemical properties of nanocomposite (e.g., particle size and entrapment efficiency) are affected by the various process and formulation variables. To optimize the formulation of CS-MMT nanocomposite particles, regarding size and drug entrapment, different formulation variables (i.e., Cs concentration, surfactant concentration, MMT percentage, drug amount and sonication time) were evaluated (Table 1).

Table 1. Different formulations of Curcumin-loaded CS-MMT nanocomposite

Formulation code	Concentration Polysaccharide (%)	MMT(^{W/p}) % w.r.t polymer	Tween-20 (μl/ml)	Curcumin (mg/ml)	Sonication Time(min)	Encapsulation efficiency(EE%)
A ₁	1	0	1.5	4	30	51
A ₂	1	5	1.5	0.4	30	9
A ₃	1	3	3	0.4	30	1
A ₄	0.5	5	1.5	4	30	91
A ₅	1	5	3	0.4	60	11
A ₆	1	3	3	4	30	82
A ₇	1	5	1.5	4	60	87
A ₈	0.5	5	3	0.4	60	1
A ₉	0.5	5	3	4	30	79
A ₁₀	0.5	3	3	4	60	79
A ₁₁	1	3	1.5	4	60	88
A ₁₂	0.5	3	1.5	0.4	60	5
A ₁₃	0.5	3	1.5	0.4	30	4
A ₁₄	0.5	5	1.5	4	30	89
A ₁₅	1	5	3	0.4	60	1
A ₁₆	1	5	1.5	4	60	84
A ₁₇	1	5	1.5	0.4	30	9

Table 2. Variants levels for optimization of Curcumin entrapment and particle size of CS-MMT nanocomposite

Symbol	Variant	Code +1	Code 0	Code -1	-1.41	+1.41
A	CS Concentration (mg/ml)	10	7.5	5	3.9	11
B	Surfactant Vol (μl/ml)	3	2.25	1.5	1.19	3.31

2.4. Determination of drug loading and entrapment efficiency

To determine drug loading and entrapment efficiency of curcumin, 1 mg of the lyophilized Cs-MMT nanocomposite particles were dispersed in 1 ml PBS, then 1 ml of ethyl acetate was added. The mixture was shaken and ethyl acetate phase is separated. Free curcumin content in ethyl acetate phase was measured using a UV-Vis spectrophotometer at a wavelength of 419 nm [23]. All tests were conducted in triplicate. The concentration of curcumin was calculated with reference to a regression equation obtained from a constructed calibration curve of curcumin solution. The percentage of curcumin entrapment efficiency (EE) and loading efficiency were computed using equations 1 and 2 [22, 24].

$$\text{Entrapment Efficiency (\%)} = \frac{(\text{Total amount of Curcumin}) - (\text{Free amount of Curcumin})}{(\text{Total amount of Curcumin})} \times 100$$

Eq₁

$$\text{Loading efficiency (\%)} = \frac{(\text{Total amount of Curcumin}) - (\text{Free amount of Curcumin})}{(\text{Total amount of Nanoparticle})} \times 100$$

Eq₂

2.5. Optimization of entrapment efficiency of nanocomposite

In this research, Cs concentration (w/v%, A) and surfactant volume (v/v%, B) were selected as two important factors which affect the entrapment efficiency. To optimize these two factors, response surface method (RSM) was used, and an experiment based on Plackett-Burman design of experiments (DOE) was performed with the two variants (A, B). According to the design of experiments for two variants, 13 samples were synthesized. The range of variant levels in this statistical optimization method was five levels (5 levels including: +1, 0, -1, -1.41, +1.41) (Table 2).

Table 3. Different formulations for optimization of Curcumin entrapment Cs-MMT nanocomposite

Formulation Code	Cs Concentration (mg/ml)	surfactant volume (µl/ml)	Entrapment efficiency (%)	Particle Size (nm)	Zeta Potential (mV)
S ₁	5	1.5	91	97	37.2
S ₂	10	1.5	87	193	32
S ₃	5	3	83	240	30
S ₄	10	3	77	422	33
S ₅	3.9	2.25	87	160	17
S ₆	11	2.25	83	192	39.2
S ₇	7.5	1.19	82	210	30
S ₈	7.5	3.31	78	185	26
S ₉	7.5	2.25	80	229	28.5
S ₁₀	7.5	3	81	198	32.2
S ₁₁	7.5	3	80.5	181	29.5
S ₁₂	7.5	3	80.4	204	31.7
S ₁₃	7.5	3	80	201	34

Table 4. Relevant software parameters for entrapment efficiency of Cs-MMT nanocomposite containing Curcumin

Terms	Sum of Squares	Mean Square	F value	p-value Probe > F
B-B	78.57	78.57	14.71	0.0064
A ²	70.68	70.68	13.23	0.0083

2.6. Nanocomposite characterization

The surface morphology of nanocomposite was observed using Scanning Electron Microscope (SEM) (model KYKY-EM3200) at an accelerated voltage of 26KV. The average size distribution of the nanocomposite particle was determined by DLS analyzer using a Zeta-sizer Zen 3600 (Malvern Instruments, Malvern, UK). Nanoparticle surface charge (zeta potential) was obtained from zeta metric test by zeta sizer of Brookhaven Instruments Corp. The analyses were performed at a scattering angle of 90° after probe sonication of the solution.

2.7. Curcumin release from the nanocomposite

The release of Curcumin from nanocomposite was investigated using dialysis method [25] at two different phosphate buffer mediums (pH 7.4 and 4.5) in a water bath at 37°C. Five ml of the curcumin-loaded nanocomposite solution was transferred into a dialysis bag (Mw cutoff 12000 g/mol) and immersed in 50 ml of phosphate buffer containing ethanol 20% v/v (pH7.4 and pH 4.5) at 37°C. At specified time intervals of 0, 2, 4, 6, 12, 24, 36, 48, 60, 72 and 96 hours, 1.5 ml of samples were elicited and replaced with an equivalent volume of fresh PBS buffer. The released Curcumin content in buffer was determined UV-Vis spectrophotometrically (U.V- T60U; PG Instrument, England) at a wavelength of 419 nm. The amount of released Curcumin was calculated using equation 3:

$$\text{Curcumin released (\%)} = \frac{[\text{Curcumin}]_{\text{rel}}}{[\text{Curcumin}]_{\text{load}}} \times 100$$

Eq₃

Where in, [Curcumin]_{rel} is the amount of Curcumin released from the nanocomposite and [Curcumin]_{load} is the amount of Curcumin encapsulated in the nanocomposite.

3. Results and Discussion

3.1. Nanocomposite characteristics

3.1.1. Morphology observation

Morphology observation of Cs-MMT nanocomposites showed that Curcumin-loaded nanocomposites had a solid dense structure with a spherical shape. The surface of nanocomposite appeared to be homogeneous indicating good compatibility between

Cs and MMT. The average diameter of Cs-MMT nanoparticles in SEM images was within the range of 22.5-31.6 nm (Figure 1). Particle size and poly disparity of Curcumin-loaded Cs-MMT nanocomposite were determined using DLS analysis. According to Figure 2 Particle size distribution of Curcumin-loaded nanocomposite ranged from 97nm to 240 nm. The difference between the particle size obtained via SEM and DLS could be because, DLS measures hydrodynamic radius in suspension solution and the particles were interconnected, but SEM shows particle size in the dry state. Stability of nanoparticles can be confirmed by their zeta potential values. Zeta potential values of the nanoparticles were between +26 to +39.2 mV, which confirmed good stability of the nanoparticles.

3.1.2. Particle size analysis

Particle size (nm) and Zeta Potential (mV) of the Cs-MMT nanocomposite of different formulations (S₁-S₁₃) are shown in Table 3. Maximum size of 422 nm was observed for formulation S₄ with the highest concentration of polysaccharides and surfactant (µl/ml). Under optimum condition, Curcumin loaded Cs-MMT nanocomposite with mean particle size of 97 nm, Zeta potential value of 37.2 mV (Figure 3), and entrapment efficiency of 91% was obtained (Formulation S₁). Cs concentration and sonication time had significant effect on particle size. Addition of MMT to the composition did not have any considerable effect on particle size of the nanocomposite.

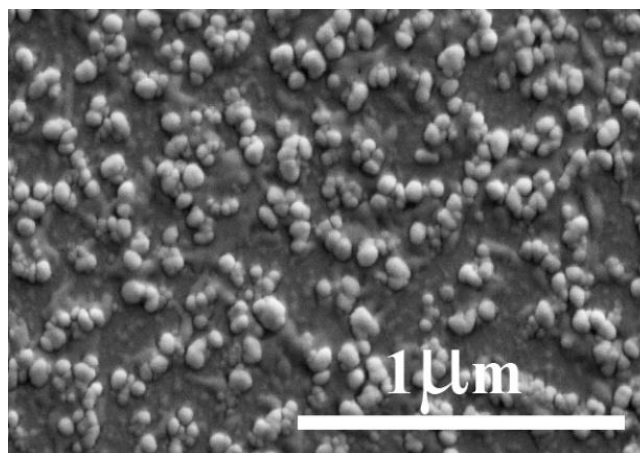


Figure1. SEM photographs of Curcumin-loaded Cs-MMT

3.2. Optimization of entrapment efficiency

Optimization of curcumin entrapment efficiency in different formulations nanocomposite was performed according to Table 3. The results showed that formulation No. S₁ has the highest entrapment efficiency for Cs-MMT nanocomposite (91%). The relevant software parameters for entrapment efficiency of CS-MMT nanocomposite are shown in Table 4. In this software (DOE), p<0.05 was considered as safety level of the index efficiency. According to table 4, the p-value for surfactant volume (variant B) and also Square Cs concentration (variant A²) are less than 0.05, so they are significant in the quadratic equation and, therefore, entrapment efficiency of curcumin is a function of these terms.

Moreover, for a better understanding of the variable model, R₂ (R-Square) is offered. Normally, R₂> 0.7 (R₂ = 0.8056) represents a relatively good correlation

coefficient and, when the value of this index is close to 1, experimental data and model of regression will have greater adjustment and, the model will have higher accuracy. Table 5 presents the selected parameters for entrapment efficiency optimization in curcumin-entrapment Cs-MMT nanocomposite. Therefore, as shown in this table the optimal conditions for entrapment efficiency of curcumin was obtained with Cs concentration of 5 mg/ml, surfactant volume of 1.5 µl/ml, drug amount of 2 mg/ml and MMT amount of 5 w/w%. Figure 4 shows the two-dimensional illustration of parameters effects, Cs concentration and surfactant volume, on encapsulation efficiency rate. According to this figure, with the reducing of Cs concentration (A) and surfactant volume (B) down to an optimal level, entrapment efficiency increases. Statistical models for the relation of entrapment efficiency were obtained by RSM for nanocomposite as reported in Table 5. Where R₁, A², and B are entrapment efficiency, Cs concentration and surfactant volume, respectively. These models are utilized by the statistical software to predict the optimum conditions, which may happen at levels or level combinations.

Results of E.E showed that increase of MMT enhances the entrapment efficiency, and also certain concentration of the drug, increases the entrapment efficiency. Entrapment efficiency increased from 51% to 91% with an increase in MMT concentration from 1w/w% to 5 w/w%. While the further increase in MMT concentration hindered the entrapment efficiency of Curcumin into nanoparticles. This result could be because of the specific physical structure of MMT. MMT has a layered silicate structure; it offers a large surface area in polymer matrix which results in a higher loading of drug molecules [26]. Also entrapment efficiency increased from 9% to 91% when the drug amount increased from 0.4 mg/ml to 4mg/ml, (Table 1). No important increase in entrapment efficiency was observed on further increasing the Sonication time.

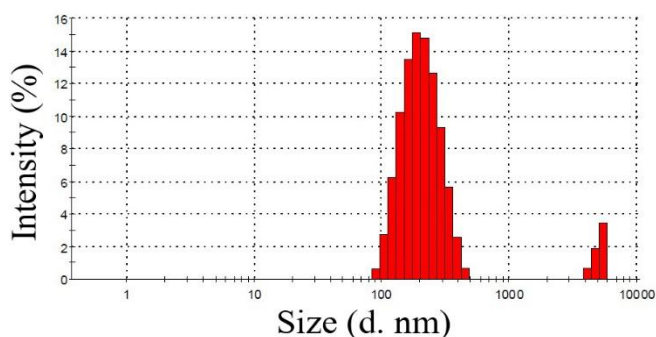


Figure 2. Particle size distribution of Curcumin-loaded nanocomposite

	Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): 37.2	Peak 1: 42.0	100.0	7.31
Zeta Deviation (mV): 41.7	Peak 2: 0.00	0.0	0.00
Conductivity (mS/cm): 0.213	Peak 3: 0.00	0.0	0.00

Result quality **Good**

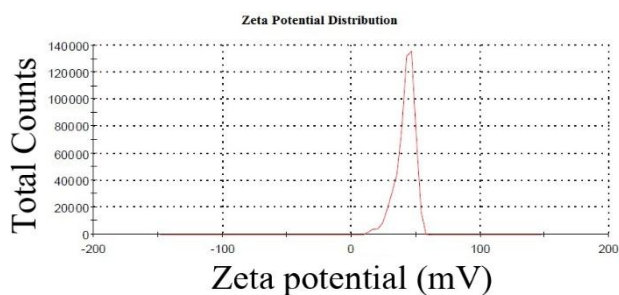


Figure 3. Zeta Potential of Curcumin-loaded nanocomposite

3.3. Release of Curcumin

The release kinetic of Curcumin from nanocomposite was investigated via dialysis method, at two different

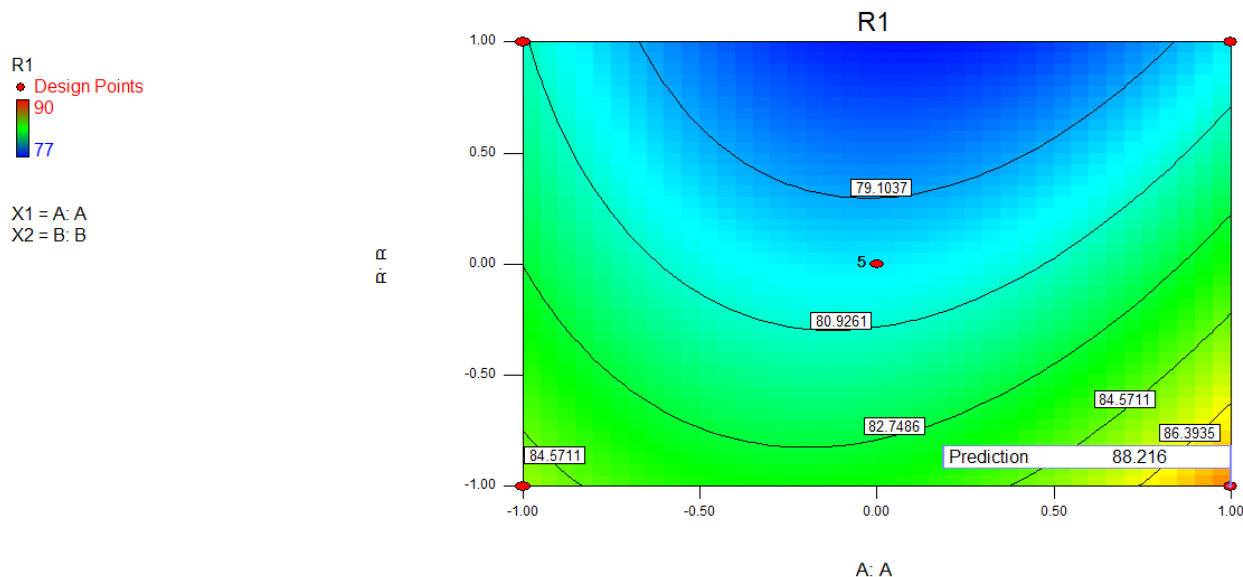


Figure 4. Two-dimensional illustration showing the effect of Cs-CMS concentration and surfactant volume on entrapment efficiency of Cs-MMT nanocomposite

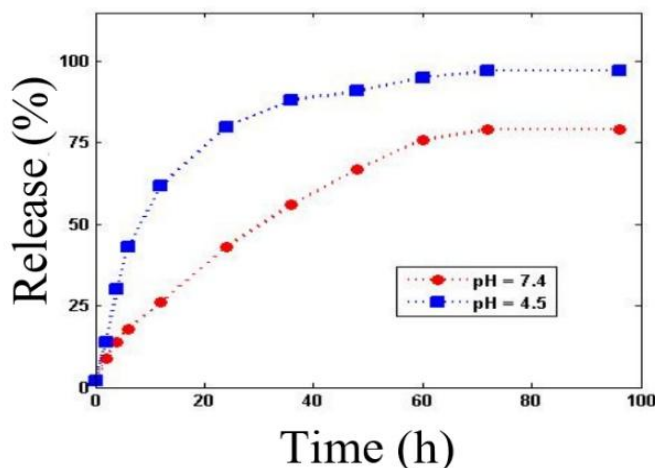


Figure 5. Curcumin release profile from nanocomposite in two different buffers medium (pH 7.4 and 4.5)

buffers medium (pH 7.4 and 4.5) at 37°C, over a period of 96 h (Figure 5). After 24 h, 43% of Curcumin was released from nanocomposite at basic pH 7.4. A primary gradual release, followed by a sustained release of Curcumin was observed at pH 7.4. While at pH 4.5 (gastric conditions), the rate of Curcumin release was higher compared to basic pH 7.4. After 24 h, 80% of Curcumin released from the nanocomposite. This result proved the pH-sensitive behavior of nanocomposite. Curcumin has poor aqueous solubility (>1 µg/ml) and the release of it has made many

challenges. The results indicated that in acid media, by covalent conjugating curcumin on the hydrophilic terminals of pluronic F68 chains via cis-aconitic anhydride linkers, curcumin release rapidly. After 96 h, 60% of curcumin was released at pH 5, while only 40% of curcumin was released at pH 7.4 [25].

4. Conclusion

In the present study, Curcumin loaded Cs-MMT nanocomposite was prepared using ionic gelation method. Different formulations were designed using RSM method to obtain an optimal composition with the highest drug loading and lowest particle size. Entrapment efficiency and particle size were affected by MMT amount, surfactant concentration and polysaccharide concentration. Results showed that the size of the produced Cs-MMT nanocomposite was 31.6nm. Also, the zeta potential was +37.2mV. The maximum amount of curcumin entrapment in nanocomposite was 91%. In the release analysis, at two different buffers medium (pH7.4 and 4.5) showed release at basic pH 7.4, after 72h and at pH 4.5 (gastric conditions), after 96 h, the amount of release was 96% and 77%, respectively.

Table 5. Optimal conditions for curcumin entrapment into nanocomposite

Codes	CS Concentration (mg/ml)	Surfactant volume (µl/ml)	drug amount (mg/ml)	MMT amount (W/w %)	Equation
CS-MMT	5	1.5	2	5	$R_1 = +80.00 - 3.13B + 3.19 A^2$

Conflicts of interest

The authors declare no conflict of interest for this article.

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None declared.

References

[1] Anselmo AC., Mitragotri S., An overview of clinical and commercial impact of drug delivery systems. *J. Control. Releas.*, 2014; 190: 15–28.

[2] Lim E-K., Jang E., Lee K., Haam S., Huh Y-M., Delivery of cancer therapeutics using nanotechnology, *Pharmaceutics.*, 2013; 5: 294–317.

[3] Ficai D., Sandulescu M., Ficai A., Andronescu E., Yetmez M., Agrali O.B., et al., Drug Delivery Systems for Dental Applications, *Current. Org. Chem.*, 2017; 21: 64-73.

[4] Ghorbani F., Zamanian A., Behnamghader A., Daliri-joupari M., A facile method to synthesize mussel-inspired polydopamine nanospheres as an active template for in situ formation of biomimetic hydroxyapatite, *Mat.Sci.Eng:C.*, 2019; 94: 729-739.

[5] Piñón-Segundo E., Mendoza-Muñoz N., Quintanar-Guerrero D., Nanoparticles as Dental Drug-Delivery Systems, *Nano biomat. Clinic. Dentist.*, 2013; 475–495.

[6] Aidun A., Zamanian A., Ghorbani F., Novel bioactive porous starch–siloxane matrix for bone regeneration: Physicochemical, mechanical, and in vitro properties. *Biotech. appl. biochem.*, 2019; 66: 43-52.

[7] Sanko N., Marianne H., Morten R., Gro S., The potential of liposomes as dental drug delivery systems, *E. J. Pharma. Bio pharma.*, 2011; 77: 75–83.

[8] Ghorbani F., Zamanian A., Behnamghader A., Daliri-joupari M., Bone-like hydroxyapatite mineralization on the bio-inspired PDA nanoparticles using microwave irradiation, *Surfac. Interfac.*, 2019; 15: 38-42.

[9] Krausz AE., Adler BL., Cabral V., Navati M., Doerner J., Charafeddine R., et al., Curcumin-encapsulated nanoparticles as innovative antimicrobial and wound

healing agent. *J. Nanomedicine.*, 2015; 11: 195–206.

[10] Song J., Choi B., Jin E.J., Yoon Y., Choi K.H., Curcumin suppresses *Streptococcus mutans* adherence to human tooth surfaces and extracellular matrix proteins. *Eur. J. Clinic. Microbio. Infect.*, 2012; 31: 1347–1352.

[11] Banik BL., Fattahi P., Brown J., Polymeric nanoparticles: the future of nanomedicine, Wiley Interdisciplinary Reviews: Nanomed. Nanobiotech, 2016; 8: 271-299.

[12] Said DE., Elsamad LM., Gohar YM., Validity of silver, chitosan, and curcumin nanoparticles as anti-Giardia agents. *Parasitol Res.*, 2012; 111: 545-554.

[13] Nagpal K., Singh SK., Mishra DN., Chitosan nanoparticles: a promising system in novel drug delivery., *Chem. pharm bull.* 2010; 58: 1423-1430.

[14] Mazzarino L., Travelet Ch., Ortega-Murillo S., Otsuka I., Pignot-Paintrand I. Elaboration of chitosan-coated nanoparticles loaded with curcumin for mucoadhesive applications. *J. Coll. Interf. Sci.*, 2012; 370: 58-66.

[15] Ghorbani F., Zamanian A., Behnamghader A., Daliri-joupari M., A novel pathway for in-situ synthesis of modified gelatin microspheres by silane coupling agents as a bioactive platform., *J. Appl. Polym. Sci.*, 2018; 135: 1-10.

[16] Flora G., Gupta D., Tiwari A. Nano curcumin: a promising therapeutic advancement over native curcumin. *J. Critic. Rev. Therapeu. Drug. Carrier. Sys.*, 2013; 30: 331–368.

[17] Wu TM., Wu CY., Biodegradable poly (lactic acid) / chitosan-modified montmorillonite nanocomposites: Preparation and characterization, *Poly. Degrad. Stabil.*, 2006; 91: 2198-2204.

[18] Hutchinson JM., Montserrat S., Roma F., Corte P., Campos L., Departament de Ma`quines i Motors Te`rmics, ETSEIAT, Universitat Politè`cnica de Catalunya., 2006.

[19] Fathy M., Ahmed A., Use of alginate/montmorillonite nanocomposites as a drug delivery system for curcumin, Thesis, AMERICAN. UNIV. CAIRO., 2015; 1–93.

[20] Dos Santosa BR., Britti Bacalhaub F., Santos Pereira

- Td., Fonseca Souza C., Faez R., Chitosan-Montmorillonite microspheres: A sustainable fertilizer delivery system. *Carbohydrat. Poly.*, 2015; 127: 340–346.
- [21] Müller CMO., Laurindo JB., Yamashita F., Composites of thermoplastic starch and nano clays produced by extrusion and thermo pressing. *Carbohydrat. Poly.*, 2012; 89: 504–510.
- [22] Saikia Ch, Hussain A, Ramteke A, Sharma HK, Maji TK., Carboxymethyl starch-chitosan-coated iron oxide magnetic nanoparticles for controlled delivery of isoniazid. *J. Microencapsul.*, 2015; 32: 29–39.
- [23] Kim TH., Jiang HH., Youn YS., Park Ch W., Tak KK., Lee S., et al., Preparation and characterization of water-soluble albumin-bound curcumin nanoparticles with improved antitumor activity, *Inter. J. Pharm.*, 2011; 403: 285–291.
- [24] Anitha A., Maya S., Deepa N., Chennazhi K.P., Nair S.V., Tamura H., et al., Efficient water soluble O-carboxymethyl chitosan nanocarrier for the delivery of curcumin to cancer cells. *Carbohydrat. Poly.*, 2011; 83: 452–461.
- [25] Shah BR., Li Y., Jin W., An Y., He L., Li Zh., et al., Preparation and optimization of Pickering emulsion stabilized by chitosan-tri polyphosphate nanoparticles for curcumin entrapment. *Food Hydrocol.*, 2016; 52: 369-377.
- [26] Zheng J., Shan J., Fan Z., Yao K., Preparation and properties of gelatin-chitosan/montmorillonite drug-loaded microspheres. *Technol-Mater Sci Ed.*, 2011; 26: 628-633.