Development and Evaluation of Curcumin Loaded *In Situ* Gel Using N-Acyl Chitosan Derivative for Ulcerative Colitis

Sangam Nimkar[1], Amruta Phuse[2], Prasad Deshmukh[3],

[1] Lecturer, Department of Pharmaceutics, Sumatidevi Tompe Institute of Pharmaceutical Science & Research, Chandur Bazar, Dist. Amravati, Maharashtra

[2]Assistant Professor, Department of Pharmaceutics, Dr. Rajendra Gode College of Pharmacy, Amravati, Maharashtra

[3]HOD, Dept. Pharmaceutics, P. R. Pote Patil College of Pharmacy, Amravati, Maharashtra

Corresponding Author Email id: sangamnimkar@gmail.com

ABSTRACT

This study demonstrates the modification of chitosan into N-Butyryl chitosan, N-Octanoyl chitosan, N-Lauryl chitosan, and N-Palmitoyl chitosan. These modified chitosan's were synthesized and characterized using FTIR, XRD, and DSC to confirm the successful attachment of the acyl groups at the amino position. The N-acylated chitosan's were then loaded with the drug curcumin and evaluated for drug loading capacity and swelling index. It was observed that as the carbon chain length increased, solubility also improved, leading to a higher swelling index. The *in situ* gelling system was compressed into tablets and enterically coated. *In vitro* release studies were conducted by exposing the gel to both acidic and basic environments. The enteric coating prevented drug release in the acidic environment, while in phosphate buffer at pH 6.8, drug release occurred. These findings suggest that hydrophobically modified acylated chitosan can be effective for achieving sustained drug release, controlled by the acylation modification.

Keywords: Chitosan**,** N-Acylated chitosan, Curcumin, *In situ* gel, Sustained release.

INTRODUCTION

Chronic inflammatory gastrointestinal disorders, such as Crohn's disease (CD) and ulcerative colitis (UC), are known as inflammatory bowel diseases (IBDs). These disorders are typified by recurring, chronic intestine ulcers [1]. Significant gastrointestinal symptoms brought on by IBD includes abdominal pain. diarrhoea, bleeding, anaemia, and weight loss [2]. Inflammation and ulcers on the large intestine's inner lining are the hallmarks of ulcerative colitis, a chronic inflammatory bowel disease caused by an aberrant immune system reaction. Although ulcerative colitis can strike anybody at any age, those between the ages of 15 and 30 are the most frequently diagnosed with it [3]. Because of the prevalence of a Westernized lifestyle, inflammatory bowel illnesses, such as ulcerative colitis, are most frequent in Northern Europe and North America. Every year, 9 to 20 persons per 100,000 are affected by ulcerative colitis, which has a prevalence of 156 to 291 per 100,000. Ulcerative colitis typically occurs as a main beginning between the ages of 15 and 30, with a minor incidence peak occurring between 50 and 70 years [4].

Although the exact cause of ulcerative colitis (UC) is still unknown, recent research indicates that one of the main pathophysiological pathways is either excessive stimulation or inadequate regulation of the mucosal immune system, and particular emphasis has been given to either the study of mucosal inflammation or immunologic reactions [5]. The severity of ulcerative colitis (UC) and patient-specific factors, such as co-occurring medical illnesses, treatment compliance, and adherence to recommended therapy, all impact the course of UC care. Conventional medications, such as corticosteroids, immune-modulatory drugs, and amino-salicylates, continue to be the mainstay of treatment for ulcerative colitis (UC). Furthermore, curcumin is employed in the treatment of UC [6,7]. Curcumin's clinical application is primarily restricted due to its low water solubility, poor oral absorption, and rapid metabolism [8].

For pharmaceutical technology experts, creating novel treatment systems that strike a good balance between therapeutic efficacy and adverse effects is an exciting task [9]. *In situ* gelling systems intended for local administration have been proposed as an alternative to conventional dosage forms throughout the past decade. These systems, sometimes called "smart materials" since they use stimuli-responsive polymers, don't need

copolymerization agents or organic solvents [10,11,12]. *In situ* gels are the solutions or suspensions that undergo gelation after reaching the particular site due to contact with body fluids or physicochemical changes such as pH, temperature, ionic concentration, UV radiation, presence of specific molecules, or ions, external triggers, etc [13,14]. *In situ* gel exhibits a local action and site specificity, simplicity of administration, high patient compliance, less side effects as compared to other pharmaceutical dosage forms, etc [15,16].

The following are some of the methods and processes that are used to produce or formulate the *in situ* gel formation: physiological stimuli (such as pH and temperature), physical stimulation (such as the diffusion and swelling of a solvent or its exchange), Chemical triggers (such as chemical, photo-, and enzymatic polymerization) [17]. Several polymers, such as chitosan, guar gum, pectin, Carbopol, and HPMC, are used in the development of *in situ* gelling systems [18,19].

Chitosan or deacetylated chitin, is a naturally occurring polycationic linear polysaccharide that is produced by partly removing the acetyl groups from chitin. The primary component of fungi's cell walls and the exoskeletons of insects and crustaceans (such as shrimp and crabs) is chitin [20]. Chitosan having cationic in nature with linear copolymer polysaccharide made up of random distribution of β (1-4) linked 2-amino-2-deoxy-D-glucose (D-glucosamine) and 2- acetamido-2-deoxy-D-glucose (N-acetyl-D-glucosamine). Because chitosan is insoluble in water, its hydrophilicity and high solubility in acidic environments cause it to break down quickly in the stomach, undergo proteolytic breakdown in the gastrointestinal tract, and have poor permeability across the gastrointestinal mucosa, which limits its use in many industries. Therefore, overcoming these obstacles is essential for both successful oral administration and efficient medication transport into the circulation [21].

Chitosan having reactive amino groups at C2 and hydroxyl groups at C3 and C6, it may be modified by acylation, alkylation, quaternization, thiolation, sulfation, phosphorylation, and graft copolymerization to improve its characteristics. In physiological conditions, chitosan may become more hydrophobic by adding hydrophobic alkyl groups. Hydrophobic changes including cholesterol, 5β-cholanic acid, tocopherol, galactosylated O-carboxymethyl, stearic acid, and N-octyl-O-sulfate can result in improvement in swelling as well as sustained release, which facilitates the transport of medications, vitamins, hormones, and proteins [22,23]. Hence the present work was to synthesized and characterized N-acyl derivatives of chitosan with improved swelling property. Synthesized derivatives were used to load curcumin for in situ formulation for the treatment of ulcerative colitis.

Materials & Methods

MATERIALS

Curcumin was gift sample provided by Reliable Nutraceuticals Ltd; Chitosan was gift sample; Butyryl Chloride, Octanoyl Chloride, Lauroyl Chloride, Palmitoyl Chloride; Carbopol, Poly ethylene glycol 6000, Lactose, Magnesium stearate provided by Central Drug House Pvt. Ltd., Eudragit L100 provided by Evonik industries Pvt. Ltd. and All other reagents were of analytical grade and used without further purification.

METHODS

1. Synthesis and Characterization of Chitosan Derivative

1 gm of Chitosan was dissolved in 1% w/v aqueous acetic acid solution (100 mL) and diluted with 85 mL of methanol. Prepared different chitosan solution for the slowly addition of various chlorides of molar equivalent (1:2) of Butyryl chloride (C_4H_7OCl, Mol. Wt.=106.55g), Octanoyl Chloride ($C_8H_{15}OCl$, Mol. Wt.=162.66g), Lauroyl Chloride ($C_{12}H_{23}OCl$, Mol. Wt.=218.77g) and Palmitoyl Chloride ($C_{16}H_{31}OCl$, Mol. Wt.=274.87g) were added separately to the chitosan solution with magnetic stirring for 5 h, respectively. Above mixture was poured into the same volume of methanol and ammonia solution in volume ratio of 7:3. The precipitates were filtered, rinsed with distilled water, methanol, and ether, and then dried [24,25,26]. Characterization of Chitosan and synthesize N-Acyl chitosan derivatives were carried out by Fourier Transform Infrared Spectroscopy (Bruker Alpha II), Differential Scanning Calorimetry (Perkin Elmer DSC-4000) at Institute of Pharmaceutical Education and Research, Wardha and X-Ray Diffraction crystallography (Righaku Miniflex-6000) at Shri. Shivaji Science College, Amravati.

2. Preparation and Characterization of Curcumin loaded Chitosan and its Derivatives

2 mg/ml curcumin solution in absolute ethanol was prepared. chitosan and N-acyl chitosan derivatives (N-Butyryl Chitosan, N-Octanoyl Chitosan, N-Lauroyl Chitosan, N-Palmitoyl Chitosan) were immersed in 10 ml of the curcumin solution to swell to equilibrium, then filtered and dried at room temperature [27].

3. Characterization of Drug loaded Chitosan and its derivatives

3.1. Drug Loading

Dissolve 10 mg of drug-loaded chitosan and its derivatives separately in 10 ml of phosphate buffer (pH 6.8) to determine the amount of drug loading. At 40°C, sonicate the mixture for one hour. Use a spectrophotometer to measure the sample's absorbance at 432 nm after sonication [28].

3.2. Swelling Index

The swelling response of chitosan and its derivatives was determined by immersing a weighed quantity in solutions of pH 6.8. At specific time intervals, the samples were weighed until a constant weight was reached. The equilibrium swelling ratio was then calculated as follows;

$$Swelling\ index = \frac{Ws - Wd}{\text{Wd}}$$

Where; Ws and Wd are the weights of the swollen and dried chitosan's, respectively [29,30].

4. Formulation and evaluation of enteric coated tablet using curcumin loaded chitosan derivatives

The core tablets were prepared using the direct compression method with a Rotary Compression Machine (Rimek-MINI PRESS II DL) [31,32]. The tablet composition is detailed in the Table 1. Enteric coating of core tablets was done by using Eudragit L100, PEG 6000 and coloring agent using the dip coating technique [33,34,35].

Evaluation of tablets were carried out for thickness using vernier caliper, Hardness by Monsanto hardness tester, Friability by Roche Friabilator (Lab India FT1020), Weight variation, Drug content in 100 ml of phosphate buffer (pH 6.8 at 432 nm using a UV-Visible spectrophotometer (Lab India UV3092) [36]. *In situ* gel formation in phosphate buffer at a pH of 6.8 [37], Disintegration by using tablet disintegration tester (Lab India DT1000) and *In vitro* dissolution test using dissolution test apparatus (Lab India DS8000) for 2-hour in 0.1 N HCl solution and then in phosphate buffer pH 6.8 [38,39,40].

Table 1. Formulation of Core tablet

Ingredient	A	B	C	D	E	F
	Chitosan	N-Butyryl Chitosan	N-Octanoyl Chitosan	N-Lauroyl Chitosan	N-Palmitoyl Chitosan	Pure Drug
Drug Loaded Chitosan Equivalent to Weight 3 mg of Drug	85	127	138	135	124	3
Carbopol	2.75	2.75	2.75	2.75	2.75	2.75
Lactose	156.7	114.7	103.7	106.7	117.7	238.7
Sod. Starch Glycolate	3.75	3.75	3.75	3.75	3.75	3.75
Mg. Stearate	1.8	1.8	1.8	1.8	1.8	1.8

**All the ingredients are in mg*

Results and Discussion

1. Synthesis of N-Acyl Chitosan derivative

Chitosan having hydrophilic nature and high solubility in the acidic medium that help into the modification of chitosan to more hydrophobic derivatives because chitosan having reactive amino group and hydroxyl group. The order of reaction C3-OH<C6-OH<C2-NH2 for the acylation process is showing that -NH2 at C2 is more reactive than primary -OH group. The reaction of glucosamine chitosan residue, having amino group at C-2 position to form an amide bond when react with highly reactive acyl chlorides Figure 1. An amide is formed by acylation with C2-NH2 is known as N-acylation [41].

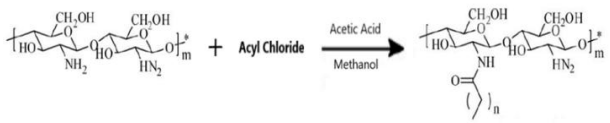

Figure 1. Synthesis Scheme for N-Acyl Chitosan

Figure 2. Prepared N-Acylated Chitosan

2. Characterization of Chitosan and Chitosan Derivatives

2.1. FTIR Spectroscopy of Chitosan and N-Acylated chitosan's

The FTIR spectra of pure chitosan exhibited the characteristic hydroxyl group absorption (3478 cm^{-1}) along with -NH2 bending (1623 cm^{-1}) and the C-O vibration (1026 cm^{-1}), while FTIR spectra of synthesise N-Acyl chitosan shows characteristics absorption peaks at observed at 3000–3800 cm^{-1} for -OH and -NH2, at 2918 cm^{-1} and 2848 cm^{-1} for C-H stretching and at 1068 cm^{-1} for C-O stretching vibration. The absorption of C-H stretching of N-acyl Chitosan at 2918 cm^{-1} and 2848 cm^{-1} increased with elongations of the alkyl side chain. Two major peaks at 1662 cm^{-1} and 1556 cm^{-1} were assigned to C=O stretching (amide I) and N-H bending vibration (amide II). The reaction is highly selective toward N-acylation, as it can be confirmed by the absence of a band present at 1750 cm^{-1} as shown in Figure 3.

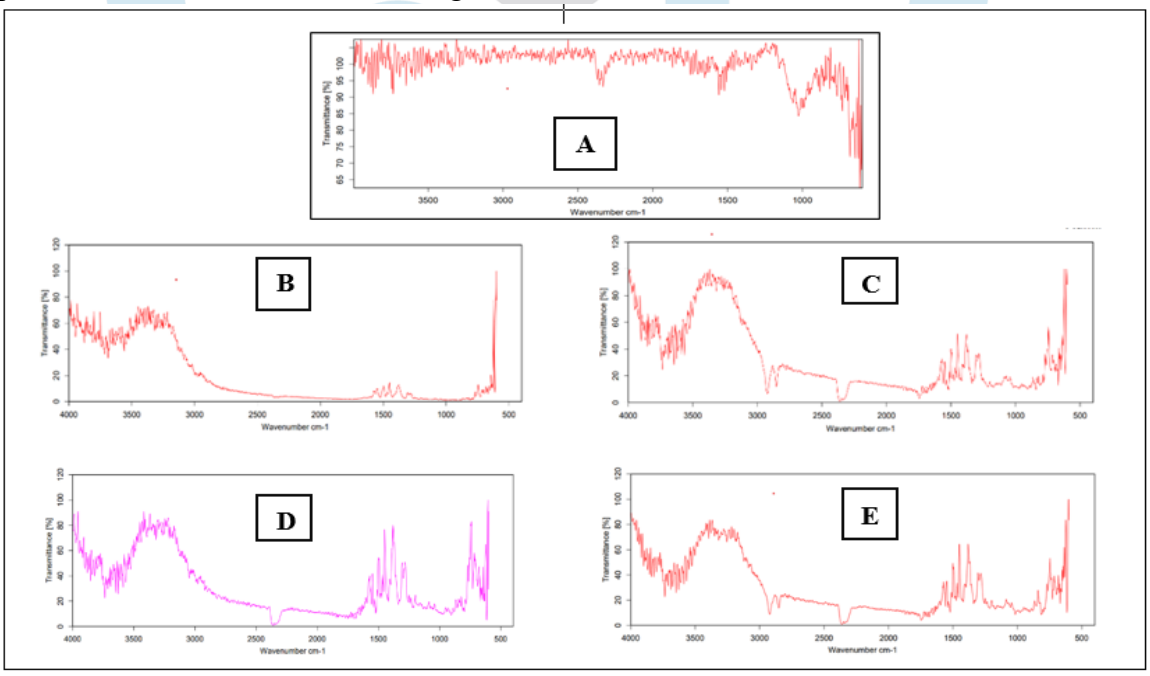

Figure 3. FTIR Spectra Chitosan (A), N-Butyryl Chitosan (B), N-Octanoyl Chitosan (C), N-Lauroyl Chitosan (D), N-Palmitoyl Chitosan (E).

2.2. Differential Scanning Colorimetry

Thermogram produced by Differential Scanning Calorimetry (DSC) offer important insights into the physical transitions of various materials. DSC thermograms show that heat absorption (endothermic process) occurs at these transitions in the case of chitosan and its N-Acyl derivatives. This heat absorption indicates the change from a solid to a liquid state, with the temperature at which this transition occurs referred to as the melting point. The thermograms of the chitosan shows endothermic sharp peak observed at 245.81°C (Onset = 244.67°C) which is melting point of Chitosan as shown in Figure 4. The melting point of chitosan changes after N-Acylation with various chlorides, indicating conformational changes in both pure chitosan and its modified forms. These modified forms of chitosan and their melting points are N-Butyryl chitosan 175.14°C (Onset = 172.17°C), N-Octanoyl chitosan 170.03°C (Onset = 164.66°C), N-Lauroyl chitosan 177.02°C (Onset = 176.03°C) and N-Palmitoyl chitosan 168.12°C (Onset = 165.08°C) as shown in Figure 4.

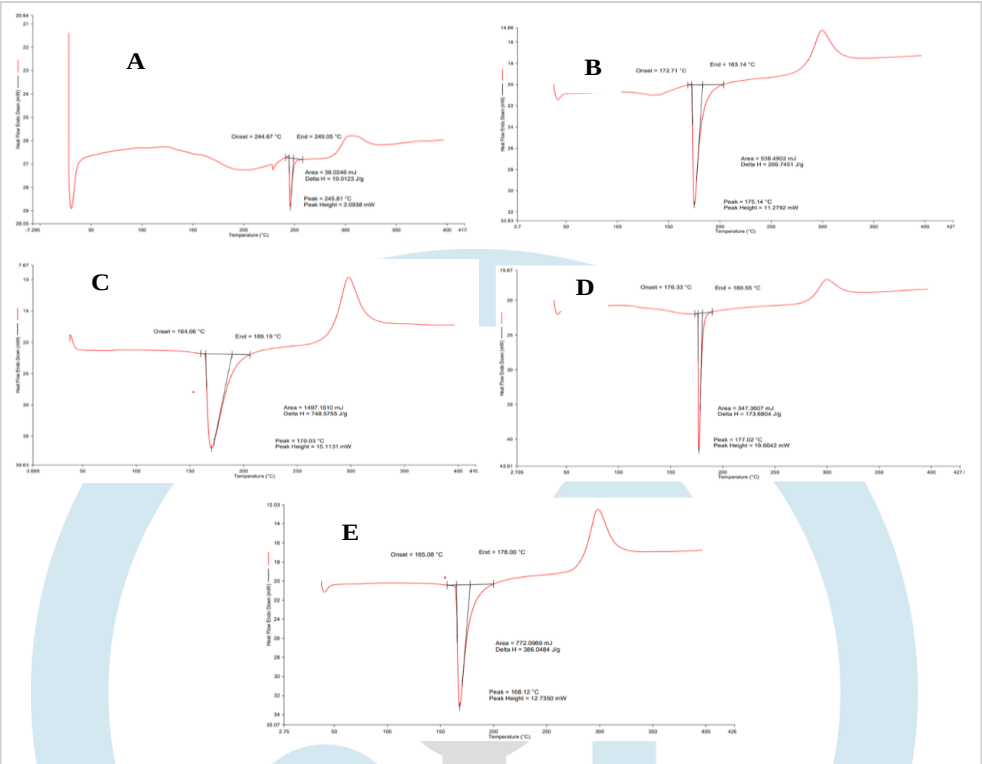

Figure 4. DSC analysis of Chitosan (A), N-Butyryl Chitosan (B), N-Octanoyl Chitosan (C), N-Lauroyl Chitosan (D), N-Palmitoyl Chitosan (E).

2.3. X-Ray Diffraction Crystallography

XRD gives the information about the crystallinity of material. The crystalline nature of chitosan, as evidenced by its diffraction peak at $2\theta = 19.91^0$, is disrupted upon the introduction of acyl substituents. The acyl substitution interferes with the regular arrangement of the chitosan polymer chains, thus reducing crystallinity and increasing amorphous characteristics. This modification, which results in N-acylated chitosan, leads to changes in the 2θ values, indicating a transition from a crystalline to a more amorphous structure. The 2 θ value of various N-Acylated chitosan are N-Butyryl Chitosan 41.9^0, N-Octanoyl Chitosan 42.6^0, N-Lauroyl Chitosan 39.91^0, N-Palmitoyl Chitosan 38.27^0 as shown in Figure 5.

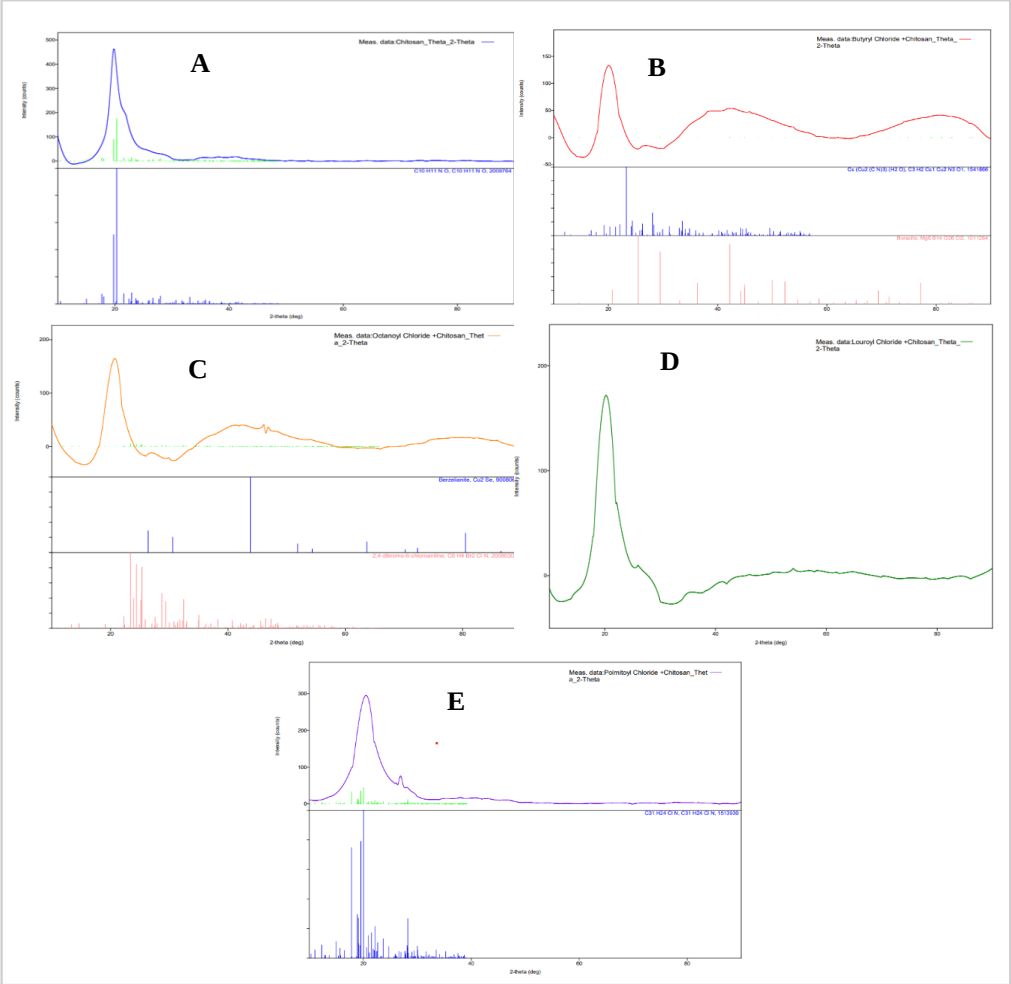

Figure 5. XRD of Chitosan (A), N-Butyryl Chitosan (B), N-Octanoyl Chitosan (C), N-Lauroyl Chitosan (D), N-Palmitoyl Chitosan (E).

3. Preparation and Characterization of curcumin loaded in chitosan and its Derivative

Figure 6 illustrates the drug-loaded chitosan and its derivative that were immersed in the drug solution to acquire the curcumin.

Figure 6. Prepared curcumin loaded chitosan and its derivative

4. Characterization of curcumin loaded Chitosan and its derivatives

4.1. Curcumin Loading in Chitosan and its derivatives

The purpose of drug loading is to investigate the medication's prolonged release from chitosan and its derivatives [42]. The value of drug loading for chitosan and its derivatives is shown in Table 2.

Table 2. Curcumin loaded Chitosan and its derivatives

Sr. No.	Product Name	Drug Loading mg/10mg of product
1	Chitosan	0.3641
2	N-Butyryl Chitosan	0.2435
3	N-Octanoyl Chitosan	0.2282
4	N-Lauroyl Chitosan	0.2179
5	N-Palmitoyl Chitosan	0.2487

4.2. Swelling Index

In order to predict the development of an *in situ* gel, the swelling of chitosan and its derivative is investigated in a phosphate buffer with a pH of 6.8, as shown in Figure 7. The disruption of chitosan's crystalline structure caused by N-Acyl substitution is the reason for the rise in the swelling index. This disruption increases the ability of N-Acylated chitosan to swell by improving the solubility of chitosan and its derivative in water. The swelling index represented in Table 3.

Figure 7. Formation of the Swelling

Table 3. Selling Index of Curcumin loaded Chitosan and Its Derivative

Sr. No.	Product Name	Swelling Index
1	Chitosan	4.9 ± 0.6
2	N-Butyryl Chitosan	5.2 ± 0.7
3	N-Octanoyl Chitosan	5.7 ± 0.8
4	N-Lauroyl Chitosan	5.9 ± 0.5
5	N-Palmitoyl Chitosan	6.4 ± 0.3

5. Evaluation of tablet

5.1. Evaluation of post-compressional parameter

The tablets had a round, biconcave form and were colored a brownish white. Table 4 shows the outcomes of post-compression characteristics for all formulations, including thickness, hardness, friability, and weight variation and drug content.

Table 4. Results of post-compression parameters of core tablet

Formulation	Thickness (mm)	Hardness (kg/cm^2)	Friability (%)	Weight Variation (mg)	Drug Content (%)
A	5.30 ± 0.15	7.8 ± 0.4	0.62 ± 0.03	249.9 ± 2.66	99.29 ± 0.06
B	5.32 ± 0.16	8.3 ± 0.3	0.53 ± 0.04	250.6 ± 2.65	99.3 ± 0.34
C	5.37 ± 0.12	8.5 ± 0.15	0.51 ± 0.06	251.2 ± 2.84	99.46 ± 0.30
D	5.40 ± 0.14	8.7 ± 0.26	0.55 ± 0.08	251.56 ± 3.35	99.53 ± 0.20
E	5.59 ± 0.13	8.6 ± 0.36	0.55 ± 0.08	252.5 ± 3.64	99.5 ± 0.39
F	5.26 ± 0.13	7.6 ± 0.2	0.68 ± 0.03	253.2 ± 3.66	99.96 ± 0.01

5.2. *In Situ* gel formation

The Chitosan is an amine-polysaccharide is also pH dependent, cationic polymer. Neutralization of chitosan aqueous solutions to a pH exceeding 6.2 systematically leads to the formation of a hydrated gel like precipitate also the derivatives of chitosan bearing the good swelling index. Carbopol is a polyacrylic acid polymer, which shows a sol to gel transition in aqueous solution as the pH is raised above its pKa of about 5.5. When these two polymers are combined in a solution, the gel strength in the physiological condition could be significantly enhanced. The transition of the tablet converts into *in situ* gel as shown in Figure 8.

Figure 8. Tablet converted into *in situ* gel formation

5.3. Disintegration time and acid uptake of Enteric coated tablet

The disintegration time and acid uptake of enteric coated tablet is calculated for showing the acid resistance of the coated tablet in 0.1N HCl Figure 9. Which resulting the coating of the polymer is well coated to release the drug at the particular site that is intestine. The coated tablet acid uptake is less than the reference [43]. The result of acid uptake and disintegration time shown in Table 5.

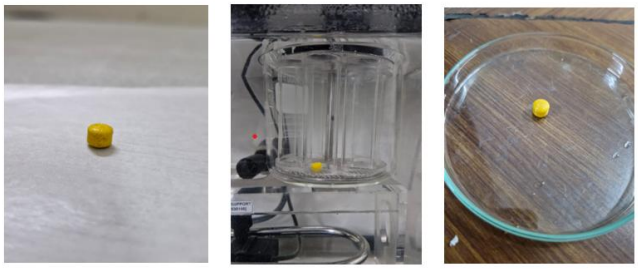

Figure 9. Representing the disintegration of tablet in 0.1 N HCl

Table 5. Acid uptake test for enteric coated tablet in 0.1 N HCl and disintegration time in phosphate buffer pH 6.8

Formulation	Initial weight (mg)	Final weight (mg)	Acid uptake (%)	Disintegration time (min) in 0.1 N HCl	Disintegration time (min) in Phosphate buffer pH 6.8
A	249.3 ± 0.36	260.3 ± 0.65	4.40 ± 0.08	Nil	28 ± 3
B	249.1 ± 0.64	262.5 ± 0.88	5.64 ± 0.05	Nil	37 ± 2
C	249.6 ± 0.26	261.2 ± 0.85	4.64 ± 0.06	Nil	40 ± 4
D	249.4 ± 0.15	263.3 ± 0.55	5.56 ± 0.06	Nil	43 ± 3
E	250.1 ± 0.72	261.4 ± 0.95	4.49 ± 0.14	Nil	45 ± 4
F	248.9 ± 0.32	263.3 ± 0.51	5.76 ± 0.10	Nil	24 ± 3

5.4. *In vitro* dissolution of Enteric coated tablet

The % drug release of formulation A is 96.25 ± 0.08% which contain drug loaded chitosan that release the drug slowly; compared with the other formulation B contain N-Butyryl chitosan drug release is 86.76 ± 0.04%, formulation C contain N-Octanoyl chitosan drug release is 81.95 ± 0.05%, formulation D contain N-Lauroyl chitosan drug release is 76.35 ± 0.10%, formulation E contain N-Palmitoyl chitosan drug release is 72.43 ± 0.11%, and the formulation F contain free form of drug and gelling agent shows rapid releases of drug is 98.92 ± 0.08%. From the release of the drug by N-acylated chitosan resulted in retard the release of the drug gives the sustained release with increasing in the length of acyl group. The drug release at specified time was plotted at cumulative percent drug release verses time (min) curve as shown in Figure 10.

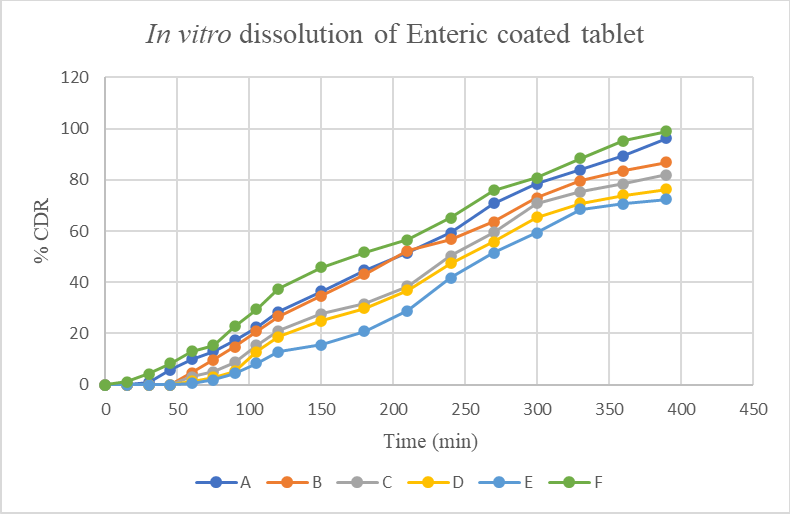

Figure 10. In vitro dissolution of Enteric coated tablet

CONCLUSION

Chitosan had a successful N-acylation modification using Butyryl, Octanoyl, Lauroyl, and Palmitoyl. FTIR, XRD, and DSC were used to assess the acyl group's successful attachment at the amino site. The process of loading drugs into chitosan and its derivatives was completed effectively, and the swelling index and drug loading were assessed. The solubility of chitosan increases with an increase in carbon chain, as seen by the higher solubility index of palmitoyl chitosan when compared to other forms. Compressing the *in situ* gelling system into a tablet form allows for the evaluation of all post compressional parameters for different formulations. The transformation into an *in situ* gel is also successfully examined in phosphate buffer pH 6.8. Using the Eudragit L100, the tablet was enterically coated, and its disintegration time and *in vitro* release research were assessed. The release of the drug in phosphate buffer pH 6.8 resulted in the formulation F shows the rapid release of the drug comparing to other formulation. The more sustained release of drug exhibited by the formulation E. From the present research work it is concluded that N-acylation make significance

improvement in chitosan which help *in situ* gel formation and to retard the release rate of the drug with increase in the chain of the acyl chloride.

ABBREVIATIONS

IBD- Inflammatory Bowel Diseases
UC- Ulcerative Colitis
FTIR- Fourier Transform Infrared Spectroscopy
XRD- X-ray diffraction
DSC- Differential Scanning Calorimetry

REFERENCES

1. Fiocchi C. Inflammatory bowel disease: etiology and pathogenesis. Gastroenterology. 1998;115(1):182-205.
2. Cuzzocrea S, Mazzon E, Dugo L, Caputi AP, Riley DP, Salvemini D. Protective effects of M40403, a superoxide dismutase mimetic, in a rodent model of colitis. Eur J Pharmacol. 2001;432(1):79-89.
3. Tindall WN, Boltri JM, Wilhelm SM. Mild-to-moderate ulcerative colitis: your role in patient compliance and health care costs. J Manag Care Pharm. 2007;13(7 Suppl A):2-15.
4. Danese S, Banerjee R, Cummings JF, Dotan I, Kotze PG, Leong RWL, et al. Consensus recommendations for patient-centered therapy in mild-to-moderate ulcerative colitis: the iSTART approach. Intest Res. 2018;16(4):522.
5. Ardizzone S. Ulcerative colitis. Orphanet Encyclopedia. 2003.
6. Tenci M, Rossi S, Giannino V, Vigani B, Sandri G, Bonferoni MC, et al. An in-situ gelling system for the local treatment of inflammatory bowel disease (IBD): loading of maqui (Aristotelia chilensis) berry extract as an antioxidant and anti-inflammatory agent. Pharmaceutics. 2019;11(11):611.
7. Shehzad A, Rehman G, Lee YS. Curcumin in inflammatory diseases. Biofactors. 2013;39(1):69-77.
8. Dalei G, Das S, Jena SR, Jena D, Nayak J, Samanta L. In situ crosslinked dialdehyde guar gum-chitosan Schiff-base hydrogels for dual drug release in colorectal cancer therapy. Chem Eng Sci. 2023;269:118482.
9. Tenci M, Rossi S, Giannino V, Vigani B, Sandri G, Bonferoni MC, et al. An in-situ gelling system for the local treatment of inflammatory bowel disease (IBD): loading of maqui (Aristotelia chilensis) berry extract as an antioxidant and anti-inflammatory agent. Pharmaceutics. 2019;11(11):611.
10. Yuan Y, Cui Y, Zhang L, Zhu HP, Guo YS, Zhong B, et al. Thermosensitive and mucoadhesive in situ gel based on poloxamer as new carrier for rectal administration of nimesulide. Int J Pharm. 2012;430(1-2):114-119.
11. Deng H, Dong A, Song J, Chen X. Injectable thermosensitive hydrogel systems based on functional PEG/PCL block polymer for local drug delivery. J Control Release. 2019;297:60-70.
12. Ward MA, Georgiou TK. Thermoresponsive polymers for biomedical applications. Polymers. 2011;3(3):1215-1242.
13. Sarada K, Firoz S, Padmini K. In-situ gelling system: a review. Int J Curr Pharma Rev Res. 2014;15(5):76-90.
14. Bhardwaj L, Sharma PK, Malviya R. A short review on gastro retentive formulations for stomach specific drug delivery: special emphasis on floating in situ gel systems. Afr J Basic Appl Sci. 2011;3(6):300-312.
15. Sudhi US, Kumar SS, Nowfiya FN, Mathan S, Dharan SS. Floating oral in-situ gelling system: a review. J Pharm Sci Res. 2020;12(10):1315-1319.
16. Jeong B, Kim SW, Bae YH. Thermosensitive sol-gel reversible hydrogels. Adv Drug Deliv Rev. 2012;64:154-162.
17. Bakliwal SR, Pawar SP. In-situ gel: new trends in controlled and sustained drug delivery system. Int J PharmTech Res. 2010;2:1398-1408.
18. Singh RM, Kumar A, Pathak K. Mucoadhesive in situ nasal gelling drug delivery systems for modulated drug delivery. Expert Opin Drug Deliv. 2013;10(1):115-130.

19. Matanović MR, Kristl J, Grabnar PA. Thermoresponsive polymers: insights into decisive hydrogel characteristics, mechanisms of gelation, and promising biomedical applications. Int J Pharm. 2014;472(1-2):262-275.
20. Chandy T, Sharma CP. Chitosan as a biomaterial. Biomater Artif Cells Artif Organs. 1990;18(1):1-24.
21. Ma D, Zhang LM, Xie X, Liu T, Xie MQ. Tunable supramolecular hydrogel for in situ encapsulation and sustained release of bioactive lysozyme. J Colloid Interface Sci. 2011;359(2):399-406.
22. Prashanth KH, Tharanathan RN. Chitin/chitosan: modifications and their unlimited application potential—an overview. Trends Food Sci Technol. 2007;18(3):117-131.
23. Wang YS, Liu LR, Jiang Q, Zhang QQ. Self-aggregated nanoparticles of cholesterol-modified chitosan conjugate as a novel carrier of epirubicin. Eur Polym J. 2007;43(1):43-51.
24. Xu J, McCarthy SP, Gross RA, Kaplan DL. Chitosan film acylation and effects on biodegradability. Macromolecules. 1996;29(10):3436-3440.
25. Wang X, Du Y, Fan L, Liu H, Hu Y. Chitosan-metal complexes as antimicrobial agent: synthesis, characterization and structure-activity study. Polym Bull. 2005;55:105-113.
26. Cho Y, Kim JT, Park HJ. Preparation, characterization, and protein loading properties of N-acyl chitosan nanoparticles. J Appl Polym Sci. 2012;124:1366-1371.
27. Dalei G, Das S, Jena SR, Jena D, Nayak J, Samanta L. In situ crosslinked dialdehyde guar gum-chitosan Schiff-base hydrogels for dual drug release in colorectal cancer therapy. Chem Eng Sci. 2023;269:118482.
28. Parhi R, Suresh P. Production of solid lipid nanoparticles-drug loading and release mechanism. J Chem Pharm Res. 2010;2(1):211-227.
29. Ata S, Rasool A, Islam A, Bibi I, Rizwan M, Azeem MK, et al. Loading of cefixime to pH sensitive chitosan-based hydrogel and investigation of controlled release kinetics. Int J Biol Macromol. 2020;155:1236-1244.
30. Dalei G, Das S, Jena SR, Jena D, Nayak J, Samanta L. In situ crosslinked dialdehyde guar gum-chitosan Schiff-base hydrogels for dual drug release in colorectal cancer therapy. Chem Eng Sci. 2023;269:118482.
31. Gupta S, Vyas SP. Carbopol/chitosan-based pH triggered in situ gelling system for ocular delivery of timolol maleate. Sci Pharm. 2010;78(4):959-976.
32. Chauhan CS, Naruka PS, Rathore RS, Badadwal V. Formulation and evaluation of prednisolone tablet for colon targeted drug delivery system. J Chem Pharm Res. 2010;2(4):993-998.
33. Khan MJ, Huang WC, Akhlaq M, Raza S, Hamadou AH, Yuning G, et al. Design, preparation, and evaluation of enteric coating formulation of HPMC and Eudragit L100 on carboxylated agarose hydrogel using tartrazine. Biomed Res Int. 2022;2022.
34. Mohan SD, Gupta VRM, Yalamanchili M. Nonaqueous enteric coating application of HPMC and Eudragit L100 on hard gelatin capsules designed to achieve intestinal delivery. J Appl Pharm Sci. 2015;5(1):001-006.
35. Chourey N, Chouksey K. Formulation and evaluation of enteric coated matrix tablets of mesalamine for inflammatory bowel disease. J Drug Deliv Ther. 2022;12(1):56-61.
36. Sivadasan D, Sultan MH, Madkhali O, Javed S, Jabeen A. Formulation and in vitro evaluation of orodispersible tablets of fexofenadine hydrochloride. Trop J Pharm Res. 2020;19(5):919-925.
37. Gupta S, Vyas SP. Carbopol/chitosan-based pH triggered in situ gelling system for ocular delivery of timolol maleate. Sci Pharm. 2010;78(4):959-976.
38. Gupta S, Vyas SP. Carbopol/chitosan-based pH triggered in situ gelling system for ocular delivery of timolol maleate. Sci Pharm. 2010;78(4):959-976.
39. Mohan SD, Gupta VRM, Yalamanchili M. Nonaqueous enteric coating application of HPMC and Eudragit L100 on hard gelatin capsules designed to achieve intestinal delivery. J Appl Pharm Sci. 2015;5(1):001-006.
40. Cole ET, Scott RA, Connor AL, Wilding IR, Petereit HU, Schminke C, et al. Enteric coated HPMC capsules designed to achieve intestinal targeting. Int J Pharm. 2002;231(1):83-95.
41. Harugade A, Sherje AP, Pethe A. Chitosan: a review on properties, biological activities and recent progress in biomedical applications. React Funct Polym. 2023;191:105634.

42. Gupta S, Vyas SP. Carbopol/chitosan-based pH triggered in situ gelling system for ocular delivery of timolol maleate. Sci Pharm. 2010;78(4):959-976.
43. Mohan SD, Gupta VRM, Yalamanchili M. Nonaqueous enteric coating application of HPMC and Eudragit L100 on hard gelatin capsules designed to achieve intestinal delivery. J Appl Pharm Sci. 2015;5(1):001-006.