RESEARCH ARTICLE

# Fabrication Of Self Aggregatted Ropinirole Hcl Loaded Nanoparticle Using N-Acyl Chitosan Derivatives

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## Abstract

Self-aggregated drug delivery systems are developed through hydrophobic modification of polymers to impart amphiphilic characteristics, which promote self-assembly into nanosized carriers suitable for efficient drug delivery. N-Acyl derivative include N-Butyryl Chitosan, N-Octanoyl Chitosan, N-Lauroyl Chitosan and N-Palmitoyl Chitosan were synthesized and confirmed by FTIR and XRD. And Evaluated for Self-aggregation property and critical micellar concentration of chemically engineered chitosans and nonsubstituted chitosan was determined by pyrene fluorescence spectroscopy. Critical aggregation concentration was about 0.01 mg/ml for acylated chitosan against 1 mg/ml of chitosan. Ropinirole loaded nanoparticles of acylated chitosans and chitosan were prepared by self-assemble ultrasonication method using probe sonicator in phosphate-buffered saline (PBS) (pH 7.4). Mean ropinirole loading was higher for acylated chitosans as compared to non-substituted chitosans. The particle size of ropinirole-loaded acylated chitosan nanoparticles increased with acyl chain length due to enlargement of the hydrophobic core. Acylation reduced the positive zeta potential by decreasing free amino groups, while the low PDI (<0.5) indicated a fairly monodisperse size distribution. More sustained release of the drug from the bulkier acyl substituted chitosan was observed as compared to chitosan.

**Keywords:** Self-aggregate, N-acyl Chitosan, Critical aggregation concentration, Nanoparticles, Drug delivery.
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## Introduction

The self-assembling (also referred to as self-aggregated) has been defined as the association of certain molecules, macromolecules, or composite materials with themselves to form tri dimensional networks or other structures with new distinguishing properties. The self-assembling process can come off at the molecular or supramolecular level. It can arise by self-association or by an association with other structures through interactions such as hydrogen bond, van der Waals forces, and ionic or hydrophobic interactions. It can also be initiated by an inclusion/complexation mechanism, like the iodine inclusion complex with starch[1,2]. To improve or impart new properties to chitosan, chemical modification of the chitosan chains generally carried out by either grafting of small molecules or polymer chains onto the chitosan backbone or by quaternization of the amino groups. Chitosan chains possess three attractive reactive sites for chemical modification: two hydroxyl groups (primary or secondary) and one primary amine. The site of modification is dictated by the desired application of the final chitosan derivative[3,4,5].

Hydrophilic as well as lipophilic drugs can encapsulated in self-assembled nanoparticles. Introduction of hydrophobic moieties into the chitosan molecules by chemical reaction in order to modify its hydrophobic-hydrophilic balance or make it amphiphiles and produces self-assembly property to chitosan. Monodisperse self-assembled nanoparticles can form by chemically synthesized polymeric amphiphiles in aqueous media or polar medium, and their morphology can be controlled by the chemical structures of hydrophobically modified chitosan such as the molecular weight of chitosan, the types and the degree of substitution (DS) of hydrophobic groups[6]. Critical micelle concentration (CMC) and Critical aggregation concentration (CAC) parameters are very critical pointers of micellization ability and micelle stability, normally CMC values are higher than CAC values. Kinetically stable, slower dissociation permits polymeric micelles to hold their integrity and perhaps drug content while circulating in the blood above or even below CMC/CAC for some time. Advantage of amphiphilic polymers over small molecular surfactants is at the lower value of CMC/CAC can delivers warranty that the micelle will retain its original morphology until reaching the target site, which is a significant[7].

Self-assembled carboxymethyl-hexanoyl chitosan nanocapsules form hollow core–shell structures in water through hydrophobic interactions of hexanoyl groups, while carboxymethyl groups provide stability, enabling sustained doxorubicin release that increases with higher hexanoyl substitution[8]. Self-assembled quaternary ammonium palmitoyl glycol chitosan micellar clusters (10–30 nm) significantly enhance drug delivery, showing improved pharmacodynamic activity, high ocular bioavailability, and drug-loading efficiency up to ten times greater than conventional triblock copolymers[9]. Hydrophobically modified glycol chitosan (HGC) was used to prepare self-assembled

paclitaxel-loaded nanoparticles. The HGC exhibited a low critical aggregation concentration (0.03 mg/mL), indicating high stability. The nanoparticles had a diameter of about 400 nm, high drug-loading capacity, and sustained paclitaxel release, with 80% of the drug released over 8 days in PBS at 37°C. The objective of this research work is to synthesize and characterize fatty acid-grafted chitosan as a self-assembling polymer for nanoparticle formation. Ropinirole will be used as a model drug to develop drug-loaded nanoparticles, which will be evaluated for particle size, zeta potential, entrapment efficiency and in vitro drug release performance.

## Material

Chitosan (55kDa; DDA 81.41%) was purchased from Merck while Butyryl chloride from sigma and Lauroyl Chloride from TCI. Ropinirole HCl was obtained as a gift sample from Glenmark Pharmaceutical Ltd (Mumbai, India). All other reagents were of analytical grade and used without further purification.

## Method

### Synthesis and Characterization of N-Acyl Chitosan

For synthesis of chitosan derivatives, 2.0 g chitosan was dissolved in 100 ml mixing solution of 0.6% (w/v) acetic acid solution and 85 ml of methanol. A molar equivalent (1.2) of Acyl Chloride include Butyryl chloride ($C_4H_7OCl$, MolWt=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 slowly to the chitosan solution in separately with magnetic stirring for 5 h, respectively. The mixture was poured into the same volume of methanol and ammonia solution in volume ratio of 7 to 3. The precipitates were filtered and rinsed with distilled water, methanol, and ether. Then, they were dried in a vacuum at 50°C overnight Characterization of synthesize N-Acyl chitosans were carried out by FTIR and XRD[10,11].

### Evaluation of Self-Aggregation Behavior and Critical Micelle Concentration

The self-aggregation behaviour of N-acyl chitosan in aqueous media and its critical micelle concentration (CMC) were determined by fluorescence spectroscopy, with pyrene as fluorescent compound[12]. Briefly, 10 µL of purified pyrene solution (0.4 mg/mL in ethanol) was added into a test tube; the solvent was driven off and then 10 mL of different concentration of N-acyl chitosan solutions were added with different test tube (the final concentration of pyrene was 2 µM). All the mixtures were sonicated for 30 min in an ultrasonic bath (PCI Instruments Pvt. Ltd, Mumbai). Pyrene emission spectra were recorded using a fluorescence spectrophotometer (Shimadzu RF-5301 PC) of each sample. The sample was excited at 343 nm and emission spectra were

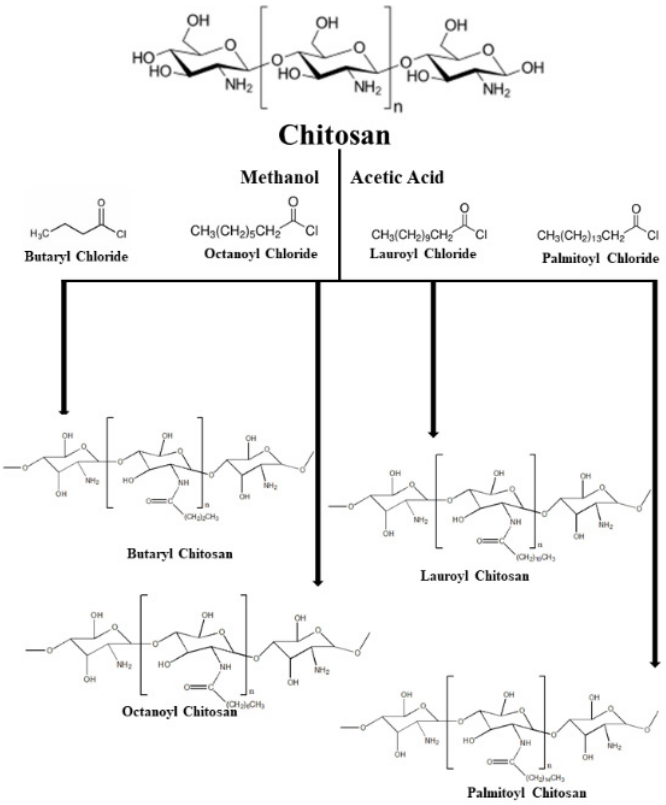


**Figure 1:** Synthesis of acyl chitosans form chitosan and acyl chlorides

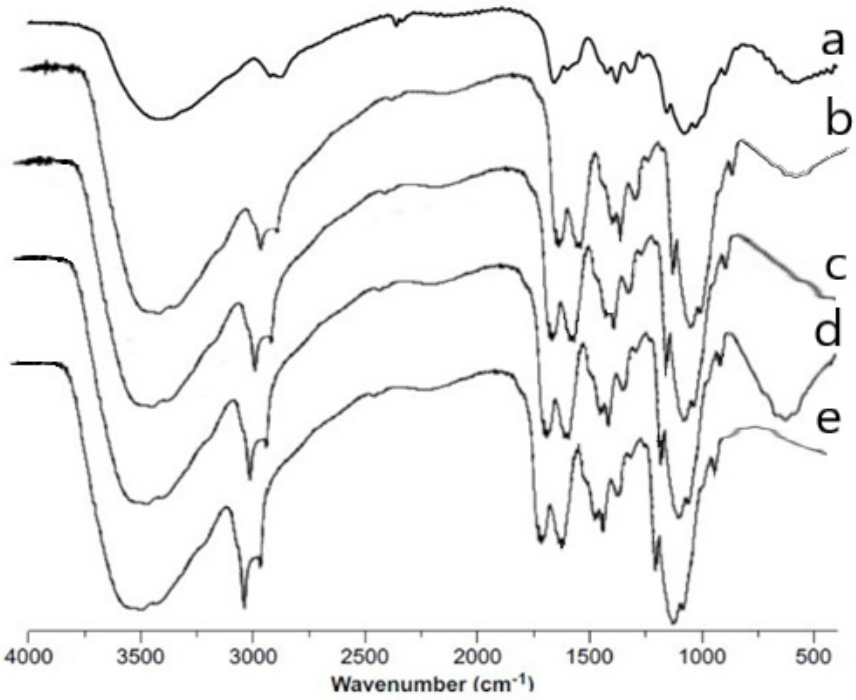


**Figure 2:** FTIR Spectra A (Chitosan), B (N-Butyryl chitosan), C (N-Octanoyl Chitosan), D (N-Lauroyl Chitosan) and E (N-Palmitoyl Chitosan)

obtained in the range of 350–450 nm at an integration time of 1.0 s. The excitation and emission slit opening were 10 and 2.5 nm, respectively[13].

### Preparation and Characterization of Ropinirol HCl loaded N-Acyl Chitosan Nanoparticles

50 mg concentrations of chitosan and modified N-acyl chitosan was suspended in 50 ml of phosphate-buffered saline (PBS) (pH 7.4) and incubated at 40°C for 48 h. Then add aqueous solution of 90 mg ropinirole HCl, then sonicated using a probe type sonifier (BioEra, Ultrasonicator- ULS-01) at 20 W The sonication was repeated three times to get an optically clear solution using a pulse function (pulse on: 10 s and pulse off: 2 s). The resulting nanoparticles suspension was centrifuged (C24BL, Remi Motors Ltd. India) at 20,000 rpm and lyophilized (Penguin Classic Plus, LARK Innovative Fine Tech knowledge, Chennai)[14]. Characterization of Self-aggregation nanoparticle were carried out for % EE, % DL, Mucin binding efficiency[15,16,17].

### *In-vitro* Drug Release Study

*In vitro* drug release study of various formulated ropinirole HCl loaded chitosan and N-acyl chitosan nanoparticles were performed using a dialysis bag (12-14 kDa molecular weight cutoff; Himedia, India) containing 50 ml in pH 7.4 phosphate buffer at 37 ± 0.5°C, 100 rpm using a magnetic stirrer (Remi Motors, India). At regular time intervals, 0.5 ml samples were withdrawn, were analyzed spectrophotometrically at 250 nm by using a UV-Visible spectrophotometer (UV 2401PC, Shimadzu Corporation, Japan) against the blank[18].

## Result And Discussion

### Synthesis and Characterization of N-Acyl Chitosan

C-2 position of chitosan having reactive amino group that form amid bond with acyl chloride (figure 1). Acetic acid used to solvate chitosan while methanol for acyl chloride, product was precipitated with ammonia solution again methanol used to remove unreacted acyl chloride. FTIR spectra confirmed the successful synthesize of N-acyl chitosan, -OH and -$NH_2$ stretching vibrations at 3000–3800$cm^{-1}$, characteristic stretching vibration intensities of –($CH_2$)10– were observed at 2800– 2950 $cm^{-1}$ and at 1068 $cm^{-1}$ for CO stretching vibration. The absorption of C-H stretching of N-acyl CS at 2918 $cm^{-1}$ and 2848 $cm^{-1}$ increased with elongations of the alkyl side chain. The increase of the amide II band in the IR spectra of the product confirms the formation of an amide linkage between amino groups of chitosan and carboxyl groups. The reaction is highly selective toward N-acylation, as it can be confirmed by the absence of a band present at 1750 cm-1, diagnostic of the presence of O-acyl ester groups. These observations are similar with Cho et al. (2012) who studied the acylation of chitosan with propionic acid, hexanoic acid and stearoyl acid[14] (figure 2). while disrupted crystallinity and had been increases by the introduction of acyl substituents by modified

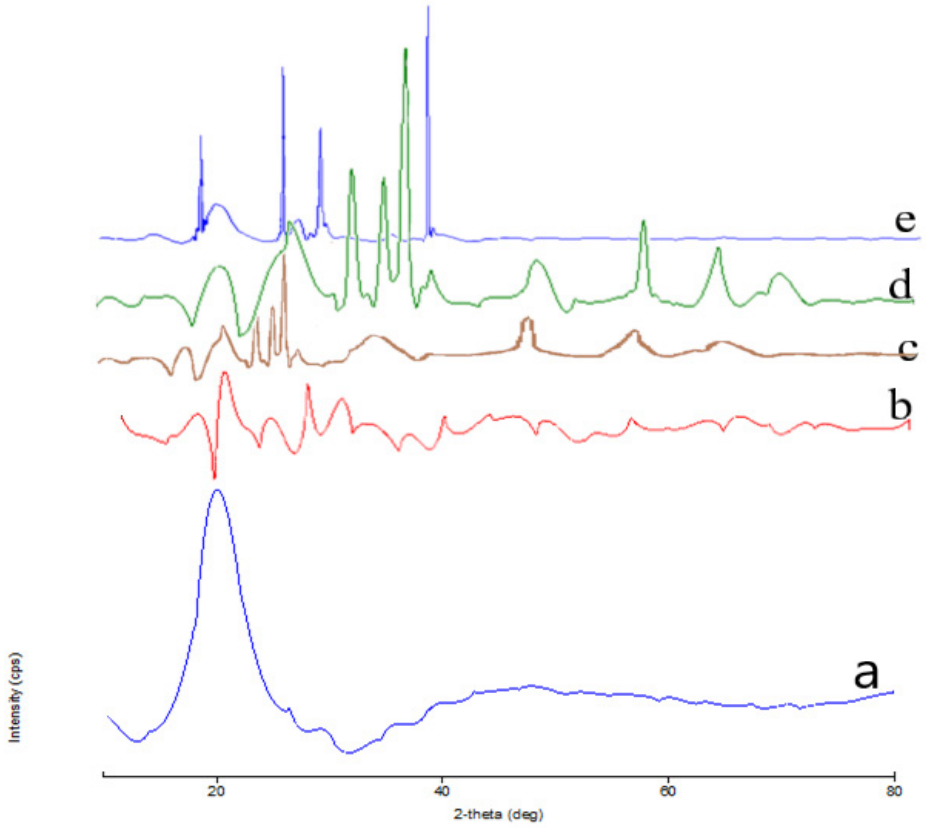


**Figure 3:** XRD of a (Chitosan), b (N-Butyryl chitosan), c (N-Octanoyl Chitosan), d (N-Lauroyl Chitosan) and e (N-Palmitoyl Chitosan)

N-Acyl chitosan (figure 3). Parallel crystallinity behavior was observed by Zong et al. (2000) for hexanoyl, decanoyl and lauroyl chitosan[19].

## Evaluation of Self-Aggregation Behavior and Critical Micelle Concentration

Pyrene was a hydrophobic molecule with low aqueous solubility (=0.3 μM) and its get localize preferentially in the hydrophobic domains of amphipathic molecules[20]. Pyrene monomer emission spectra are associated with vibronic line structures whose intensities show a strong dependence on the polarity of the microenvironment[21]. With increasing concentration, the total emission intensity increased, indicating that the probe transferred from the aqueous medium to the less polar micro-domains such as the interior of the self-aggregated nanoparticle. Figure 4 exhibits the change of $I_{372}/I_{384}$ value as a function of chitosan and N-acyl chitosan with concentration.

The ratio of $I_{372}$ to $I_{384}$ was around 1.13 at the lower concentration and rarely changed until the concentration of N-acyl chitosan was 0.01 mg/mL. But, the ratio of $I_{372}$ to $I_{384}$ steadily decreased with further increase in the concentration of N-acyl chitosans due to the self-association of N-acyl chitosans. CAC was determined by the interception of two straight lines; its value was ≈ 0.01 mg/mL for N-acyl chitosan and 1 mg/mL for chitosan. These observations are similar with Chen et al., 2003 who studied the CAC of linoleic chitosan[22].

## Preparation and Characterization of Ropinirol HCl loaded N-Acyl Chitosan Nanoparticles

Bulky hydrophobic group show a strong tendency of intra- or intermolecular aggregation in polar solvent[23]. The polymeric micelles formed from the molecular assemblage have the ability to take water soluble drugs or low molecular weight organic compounds and disperse them in the aqueous solution. Longer hydrophilic chains and bigger hydrophobic groups help stabilize the micelle structure and protect drug compounds from the environment. N-acylation of chitosan with fatty chain groups can induce a hydrophobic nature to reduce molecular hydrogen bonding and increase stabilization of chitosan by hydrophobic interactions[24]. Particle Size of Ropinirole loaded N-Acyl Chitosan give in Table 1, observing that increasing the particle size with alkyl chain of acyl group due to elongation of hydrophobic side chain, suggesting that the elongation of hydrophobic side chain boosts the hydrophobic core in the N-acyl chitosan nanoparticle.

Zeta potentials of chitosan is positive due to the presence of cationic nitrogen ($NH_3^+$) However N-acyl chitosan nanoparticles were less positive (+) charged in ranges of

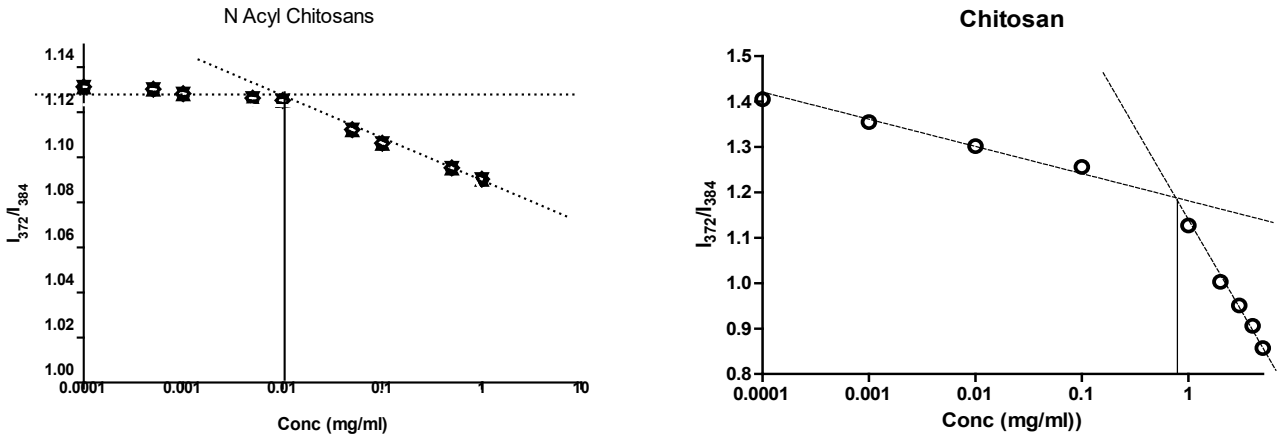


**Figure 4:** Plot of the $I_{372}/I_{384}$ intensity ratio from the fluorescence spectra for N-acyl Chitosans and Chitosan

14.3±1.6 to 16.8±2.9 mV because of a result of decreased in free amino groups due to N-acylation of chitosan. These results of zeta potential are identical with the observations of Lin et al., (2009)[25]. Due to the positive charges of N-acyl chitosan nanoparticles, N-acyl chitosan nanoparticles can be interact to the negative charged cell wall and have a great potential to increase the absorbance and penetration.

PDI for both chitosan and N-acyl chitosan nanoparticles were ranged below 0.3 which signified a fairly monodisperse pattern of size distribution (Figure 5). The slightly increased size of N-acyl chitosan could be due to its longer acyl chain group. The morphology of nanoparticles were observed spherical nature with good structural integrity with the nanosize by Libra 120 transmission electron microscope (Carl Zeiss, Oberkochen, Germany) (Figure 6).

Table 2 present the drug loading and entrapment ability of N-acyl chitosan in compare to chitosan. The Drug loaded in modified N-acyl chitosan nanoparticles is by hydrogen bonding and ionic interaction. For determination of drug loading and entrapment UV-Visible spectroscopic analysis was used. N-acyl chitosan nanoparticles showed appreciably increased DL and EE as compared to chitosan nanoparticles. This could be due to increased hydrogen bonding by the acylation which ensures strong entrapment of ropinirole in nanoparticles. As it was observed that value of DL and EE were increases with increasing length of acyl chain as the increased hydrogen bonding with respect to length of acyl chain. The obtained results suggest that N-acylation with longer alkyl chain results in increased DL in nanoparticles.

Mucoadhesive nanoparticle can adher mucin present in mucous membrane. Acylation modification to chitosan makes hydrophobic nature which was play an important role in mucoadhesion. It is conceivable that, the hydrophilic groups (–OH and –NH2) of chitosan located in the external shell of the micelles and the hydrophobic groups in the cores. Thus, the mucoadhesive property of chitosan should be well maintained upon self-aggregate as these groups are mainly responsible for the mucoadhesive nature of nanoparticle of

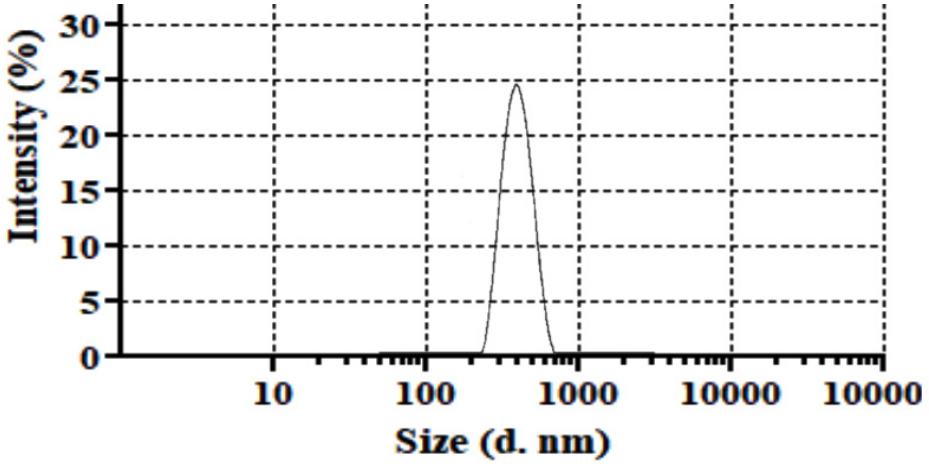


**Figure 5 :** PDI for N-Acyl Chitosan Nanoparticle by Self Assemble Method

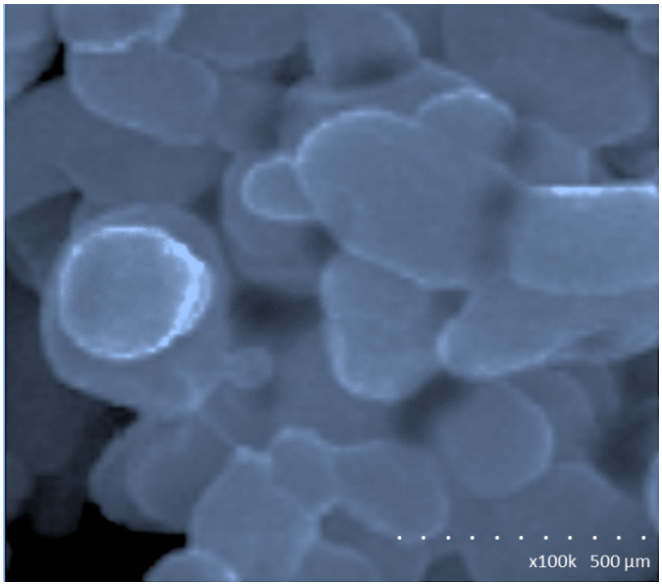


**Figure 6:** SEM for N-Acyl Chitosan Nanoparticle by Self-assemble Method

chitosan and its acylated modified derivatives.

Ropinirole loaded N-acyl chitosan and chitosan evaluated for mucoadhesive property by determination of mucin binding efficiency. Mucoadhesive property was increases with acyl modification along with mucoadhesive were increases with increasing the length of acyl group (Figure 7). Mucoadhesion could be enhanced by increasing the hydrophobicity was investigated by Martin et al. (2002), studies mucoadhesion behaviour of palmitoyl chitosan with different degree of substitution[26].

## *In-vitro* drug release study

The cumulative percentage release of ropinirole loaded chitosan and N Acyl Chitosan nanoparticle was investigated in vitro over a period of 24 h in phosphate buffer pH 7.4 (figure 8). Sustained and steady release was showed by chitosan and N-acyl chitosans nanoparticle over the period of study (24 h) as compared to chitosan nanoparticle, with a biphasic drug release pattern. Initially, for 6 h sustained-release phase was observed, laterally which was showed steady, slower drug release. In the initial phase 56.41±2.16%, 52.66±2.04%, 48.59±1.98%, 47.03±1.94% in phosphate buffer pH 7.4 of ropinirole released by N Butyryl-, N-Octanoyl-, N-Lauroyl- and N-Palmitoyl chitosan nanoparticles respectively because of

**Table 1 :** Zeta Potential, Particle Size and PDI

| | *Zeta Potential* | *Particle Size* | *PDI* |
|---|---|---|---|
| Chitosan | 24.6±2.6 | 271.2±17.5 | 0.365 |
| N-Butyryl Chitosan | 14.4±2.8 | 279.6±16.3 | 0.356 |
| N-Octanoyl Chitosan | 15.4± 2.1 | 286.4±15.8 | 0.380 |
| N-Lauroyl Chitosan | 16.8±2.9 | 293.4±19.3 | 0.410 |
| N-Palmitoyl Chitosan | 14.3±1.6 | 302.4±17.5 | 0.295 |

**Table 2:** % DL and % EE of N-acyl nanoparticle

| | *% DL* | *% EE* |
|---|---|---|
| Chitosan | 55.96±1.1 | 53.64±1.7 |
| N-Butyryl Chitosan | 56.11±1.8 | 58.24±1.4 |
| N-Octanoyl Chitosan | 56.33±1.2 | 61.63±1.9 |
| N-Lauroyl Chitosan | 56.45±0.9 | 66.24±1.1 |
| N-Palmitoyl Chitosan | 56.53±1.6 | 69.35±0.9 |

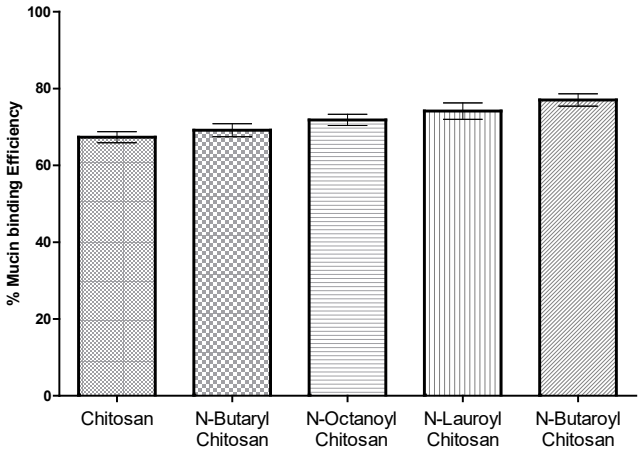


**Figure 7:** Comparative %Mucin Binding Efficiency of Nanoparticle

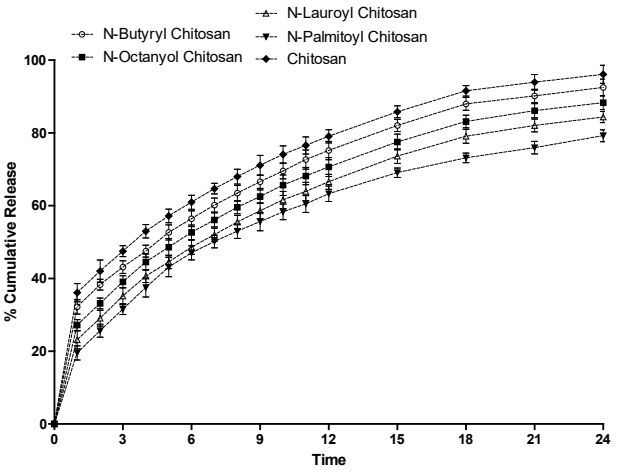


**Figure 8:** % ropinirole release profile with time for chitosan and N-acyl chitosan nanoparticle at 7.4pH

dissolution and passive diffusion of ropinirole presented on or near absorbed onto the surface of nanoparticles or weakly interacted with the hydrophobic cores of the self-assembled nanoparticles. In addition, aqueous medium penetrated into the hydrophobic polymeric matrix leading to slow dissolution and thereby diffusion of the drug from the acyl chitosan nanoparticle matrix initial may also have produced sustained release up to 6 h. After 6 h, a slower release of drug occurred,

leading to release of 92.5±2.27%, 88.28±1.86%, 84.38±1.54%, 79.22±1.64% in phosphate buffer pH 7.4 of ropinirole by N Butyryl-, N-Octanoyl-, N-Lauroyl- and N-Palmitoyl chitosan nanoparticles for 24 h.

The second phase of drug release is probably due to the decrease the concentration of ropinirole in the polymeric matrix, leading to a reduced concentration gradient, causing fewer drugs to be released. Release rate of N-acyl chitosan nanoparticles is also reduced with increasing the length of acyl side chain. This is due to the strengthening of the hydrophobic interaction in N-acyl chitosan nanoparticles with increasing the length of acyl side chain. Once chitosan has been modified with hydrophobic acyl groups, it could be swollen in aqueous condition but not dissolved completely. Hence, the biphasic release pattern of the self-aggregated nanoparticles can be attributed to the localization of ropinirole into and within the immediate layer and core of the prepared nanoparticles. These results agree with the observations described by Klariae et al. 2012[27].

## Conclusion

Chemical modification of chitosan has emerged as an effective approach to enhance its physicochemical and drug delivery properties. Incorporation of bulky hydrophobic fatty acid moieties into the chitosan backbone imparts amphiphilic characteristics, enabling the polymer to self-assemble into nanosized structures in aqueous media. This self-aggregation behaviour contributes to improved drug loading and entrapment efficiency through enhanced polymer–drug interactions. The resulting self-assembled nanoparticles have been extensively investigated for their drug-loading capacity, entrapment efficiency, mucoadhesive properties, and controlled in vitro drug release performance. Owing to these advantageous characteristics, N-acyl-modified chitosan-based self-assembled nanoparticles represent a promising platform for the development of advanced drug delivery systems with broad pharmaceutical applications.

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