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# Hepatic Targeting of Conconavalin-A Appended Myristoyl Chitosan Nanoparticles Containing Epirubicin

**Pooja Sharma[1],Rayasa Ramachandra Murthy*[1]**

*1.Nanomedicine Research Centre, I.S.F College of Pharmacy, Moga, Punjab- 142001*

## ABSTRACT

Hepatic targeting of Concanavalin A appended myristoyl chitosan nanoparticles containing Epirubicin. Myristoyl chitosan was synthesised by reacting native chitosan with myristoyl chloride and degree of acylation was determined by Ninhydrin assay. Nanoparticles of chitosan and myristoyl chitosan were prepared by ionic gelation and the method was optimised for processing parameters based on particle size, zeta potential and entrapment efficiency (EE). The nanoparticles of chitosan and myristoyl chitosan were conjugated with concanavalin A by incubation and the conjugation was confirmed by zeta potential measurement. The surface morphology of the optimized formulation was checked with the help of SEM and was further studied for organ distribution studies in Wistar rat model. Myristoyl chitosan synthesised was confirmed by FT-IR studies. Degree of acylation was found out to be 42.2 ± 2.7%. The optimized Con A conjugated nanoparticles prepared by chitosan (Ch17) and myristoyl chitosan (MCh17) was found to be spherical in shape with particle size 244.4nm and 275.8nm, zeta potential of 0.307mV and 0.133mV, entrapment efficiency 45.01±1.32% and 40.10±1.23% respectively. *In vitro* drug release (PBS 7.4) from Ch17 was 93.02±1.66% and followed Higuchi model, while release from MCh17 was 68.53±2.27% and followed Peppas model. Both the formulation were stable for 1 month at the temperature of 2-8$^{o}$C. *In vivo* liver uptake of MCh17 nanoparticles was 93.6±10.11% while it was 87.0±7.55% with Ch17. Epirubicin loaded MCH17 nanoparticle showed high uptake by liver with concomitant reduction in blood level of Epirubicin in comparison to Ch17 nanoparticles.

**Keywords:** Chitosan, Concanavalin A, Epirubicin, Liver targeting, Myristoyl chitosan, Nanoparticles.

*Corresponding Author Email: poojapjo87@gmail.com

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## INTRODUCTION

Liver acts as a target for drug delivery due to occurrence of several fatal conditions which include chronic hepatitis, enzyme deficiency, and hepatoma. For liver-targeting of systems, passive trapping of particles by reticuloendothelium or active targeting using hepatic receptor and ligand bearing particulates recognition is required[1]

Potent drugs might not have much efficacy in-vivo or might pose adverse effects. So, various types of drug carriers such as nanoparticles (NPs), liposome, micelles, microemulsion, nanoemulsion are used for liver drug targeting[2]

Epirubicin (Epi) is the most preferred anthracycline[3,4] because it has a more favourable therapeutic index[5] reduces cardiac toxicity and gives Epiglucuronide and epirubicinol as metabolism product along with subsequent biliary excretion of the glucuronide conjugate[6] shows a favourable pharmacokinetic profile and has fewer toxicological effects[5,7] and has higher cumulative dose than doxorubicin before cardiotoxicity can limit further therapy[8]

Conventional dosage forms for this drug has several limitations that can be solved by controlled drug delivery systems such as improved efficacy, reduced side effects, improved patient compliance, and can be utilized in the form of nanocarriers in drug delivery[9.] I/V administration of drug solution distributes the drug throughout the body. That is why, novel formulation of Epi (NPs) were formulated for targeting drug at the site of action.

Chitosan (Ch) is a natural polymer consisting of randomly arranged β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl- D-glucosamine (acetylated unit) and is prepared by the partial N-deacetylation of chitin, a natural biopolymer derived from crustacean shells such as crabs, shrimps and lobsters. Ch is non-toxic, biodegradable and biocompatible, safe, inexpensive polymer with very attractive biological properties, such as permeation-enhanceer ,mucoadhesive, anticoagulant and antimicrobial activity and so on. These properties render Ch a very suitable material as a drug delivery carrier[10] The ability to modify the chemical structure of Ch's makes it an appropriate biomaterial for therapeutic applications. Ch has both reactive amino and hydroxyl group. This strong functionality of Ch (two hydroxyl groups (C3, C6) and one primary amine group (C2) per-repeat unit) gives it a considerable opportunity of chemical modification[11]. In addition, Ch amino groups (at C2 position) are nucleophilic and reactive at higher pH values. They are a suitable site for chemical modifications and for enzyme immobilization[12,13] In the present study, N-acylation of Ch with myristoyl chloride has been done for introducing the hydrophobicity for drug delivery in the form of matrix. It was postulated that

derivatization would reduce the hydrophilicity of the matrix and produce a formulation, which is controlled release in comparison to Ch[14]

Nanoparticles (NPs) are solid colloidal particles with diameters ranging from 1- 1000nm[15]and delivers a higher concentration of pharmaceutical agent to a desired location, controls drug delivery, high intracellular uptake of NPs than microparticles, effective permeation through cell membranes, stability in the blood stream and suited for intravenous (i/v) delivery.

*Asialoglycoprotein receptors (ASGP-R)* are the main receptors present in hepatocytes (major cells present in liver), so it is desirable to label the carriers with *ASGP-R* specific ligands as galactose, lactose, acetylgalactosamine, asialofetuin and concanavalin A.

Conconavalin A (ConA) is a lectin (carbohydrate-binding protein), originally extracted from the jack-bean, *Canavaliaensiformis*. It has a specific binding capacity to certain structures specifically in various sugars, glycoproteins, and glycolipids, mainly internal and non reducing terminal α-D-mannosyl and α-D-glucosyl groups. It is widely used in biology and biochemistry for the characterization of glycoproteins and other sugar-containing entities present on the surface of various cells. A recent report by Lei and Chang (2009) demonstrated potent therapeutic effect of Con A against experimental hepatoma (liver cancer) and was found to be sequestered more by hepatic tumor cells, in preference to surrounding normal hepatocytes[16] Hence in this study an attempt has been made to target epirubicin loaded chitosan and myristoyl chitosan nanoparticles to liver cancer cells using Con A as ligand.

## MATERIALS AND METHOD :

Epirubicin (Drug) was obtained as a gift sample from M/s Sun Pharmaceuticals Ltd, Vadodara, India. Chitosan (lntermolecular weight), tri-polyphosphate (TPP), tween 80 and myristoyl chloride were procured from Sigma-Aldrich, Bangalore, India. Conconovalin A (Con A), D-glucosamine (100% free amino groups) and ninhydrin reagent were supplied by Hi Media Laboratories Pvt. Ltd, Mumbai, India. All other materials used were of analytical or pharmaceutical grade.

### ACYLATION OF CHITOSAN :

**Synthesis of myristoyl chitosan (MCh):**

2.5 gm of Ch was dissolved in 300 mL of 0.12 M acetic acid and was stirred on magnetic stirrer for 24 hours to ensure total solubility. The pH was adjusted to 7.2 by slow addition of 0.1M NaOH with strong agitation, yielding a gel slurry. Myristoyl chloride (10 mL) was then added drop wise to the above gel slurry. It was again stirred for 6 hrs and preparation was neutralized

up to pH 6.8–7.0 and then precipitated with acetone. The precipitate, collected by filtration, was washed with hot methanol (50–60$^{o}$C) and decanted. The washing was repeated three times to eliminate free fatty acids. Finally, the products were dried with pure acetone to obtain MCh[17]

**Structural analysis of MCh by Fourier-transform infrared (FT-IR) analysis**

FT-IR spectra was recorded using spectrophotometer (Perkin Elmer, USA) foranalysis in the spectral region (4000–400 $cm^{-1}$) with 64 scans recorded at a 4 $cm^{-1}$ resolution.

**Measurement of degree of acylation by ninhydrin assay**

The unchanged amino groups remaining after acylation were determined by the method described by Curotto and Aros[18]. 0.1 mg/ mL solutions of myristoyl chitosan were prepared in $CH_3COOH$ (0.5M ) and HCl (0.274M ) by continuous stirring at 20$^{o}$C for 24 h. $CH_3COOH$ and HCl were added in 1:1 ratio. 0.5 mL of acetate buffer (4 M, pH 5.5) was added to the above solution and 2 mL ninhydrin reagent (Sigma, USA) was added. The tube containing solution of MCh was placed in a boiling water bath for 20 min. The solutions were cooled and their absorbances at 570 nm were read. D-glucosamine (Sigma) solutions (100% free amino groups) were used to generate a standard curve.

**Preparation of drug loaded NPs of Ch and MCh**

NPs were prepared by the method reported by Calvo et al. with appropriate modifications. 2.5 mg Chwas dissolved in 1% v/v acetic acid solution (10mL) and 10 mg drug was mixed with chitosan solution. 0.35% w/v tween 80 was added and mixed. Tri poly phosphate (TPP) (5 mg) was dissolved in 10 mL distilled water. TPP solution was added drop wise to chitosan solution containing drug, using high speed homogenizer i.e. 5000 rpm (with constant stirring). When all the TPP solution was added into Ch solution, the homogenization was continued for 5 mins[19]. The same procedure was repeated for myristoyl chitosan nanoparticles except that the myristoyl was dissolved in 2% v/v acetic acid solution and stirred overnight on magnetic stirrer for complete solubilization. Formulations were evaluated on the basis of size, PDI and entrapment efficiency

**Ligand conjugation onto the Ch and MCh based NPs**

Ch and MCh based NPs were modified by using a model ligand i.e. Con-A. 1 mg Con-A was dissolved in 2 mL of 0.1M phthalate buffer of pH 5.2, mixed with functionally activated 10 mg/mL NPs (5mL), and incubated in a rotator at 25°C for 2 hrs. The zeta potential measurement was used to optimize the time of incubation. After the conjugation, particles were removed and centrifuged. Pellet was collected and washed several times with PBS pH 7.4[20]

**CHARACTERISATION OF OPTIMIZED FORMULATION**

Optimized formulation was characterized for various parameters like morphology, particle size determination, zeta potential, entrapment efficiency by the method described below.

**Morphology**

Scanning electron microscopy (SEM) was employed for visualization of shape, size and surface morphology of the prepared NPs. The NPs samples were taken in a piece of black tape and fixed to the sample holder and visualized under low vacuum and three viewing fields were selected for different magnifications. The magnification giving best resolution has been depicted in results[21.]

**Particle size distribution and zeta potential analysis**

Particle size, size distribution (PDI) and zeta potential of optimized formulation was determined by laser diffractometery using Beckman coulter Delsa TM Nano C Particle size analyzer.

**Determination of Entrapment efficiency (EE)**

Nanoparticle suspension prepared using known quantity of epirubicin was centrifuged at 25000 rpm for 30 min at controlled temperature of $4^0$C. The obtained pellet was re-dispersed into 10 mL of deionised water and vortexed for 5 min. The prepared dispersion was centrifuged at 14000 rpm for 10 min and supernatant was separated. The supernatant was analyzed at 484 nm by using spectrophotometer (Perkin Elmer, Japan). EE was calculated using following formula[22]

$$\% \text{ EE} = \frac{\text{Total Amount of drug added - Amount of drug in supernatant}}{\text{Total amount of drug added}} \times 100$$

**In-vitro drug release**

The pellet (weight equivalent to 10 mg epirubicin) obtained after centrifugation was re-suspended in 1 mL PBS (pH 7.4) and the suspension was put in a dialysis membrane. 100 mL PBS (pH 7.4) was put in 3 different flasks of 250 mL each. Dialysis membranes containing suspension of free drug, Ch NP's, MCh NP's were dipped into different flasks containing PBS (pH 7.4). The flasks were incubated at $37\pm1^0$ C in shaker incubator. Aliquots of 3mL were withdrawn from the release media at pre-determined time intervals and an equivalent amount of fresh PBS pH 7.4 was added to the release medium after each collection of aliquot[23,24] The withdrawn aliquots were analyzed for the drug content by UV spectrophotometer at 484 nm. Dissolution results were submitted to analysis of variance (one-way ANOVA) employing Daniel Soper statistics calculator version 3.0 BETA to determine differences between the means obtained. F values calculated between each formulations were compared with the tabled F values at $p < 0.05$.

**Stability Studies**

Stability of NPs of Ch and MCh containing Epi was investigated at 2–8$^0$C (storage temperature of Epi) for 1 month. Suspension of NPs were placed in USP type 1 glass vial closed with rubber stopper, sealed with aluminium seal. At predetermined time interval, samples were withdrawn from the vials and studied for particle size distribution and drug content. After 5, 10, 15 and 30 days particles size, zeta potential and drug content was observed.

**In-vivo studies**

Organ distribution studies were done on healthy Wistar rats (200-230gm) of either sex by injecting a single bolus intravenous injection of EPI at 7 mg of Epi⁄ kg (n = 3 in total). The animals were fasted overnight. Liver drug concentration was obtained after intravenous (i/v) administration of Epi solution, Epi in Ch and Epi in MCh NPs. Three groups of rats (three per group) were taken for organ distribution studies. Rats were sacrificed by cervical dislocation and the liver was removed. The liver samples were rinsed with normal saline. These samples were then homogenized and immediately centrifuged at 6000 rpm for 30 minutes at 4$^0$C temperature. Acetontrile was added to the supernatant for de-proteinization. Using these aliquots of the homogenate, the total Epi (that released from NPs plus the remainder in NPs), concentration was determined by UV spectrophotometry.

Statistical method: In-vivo liver uptake results were submitted to analysis of variance (one-way ANOVA) employing Daniel Soper statistics calculator version 3.0 BETA to determine differences between the means obtained. F values calculated between each formulations were compared with the tabled F values at p=0.05.

## RESULTS AND DISCUSSIONS:

**Synthesis and characterization of myristoyl chitosan (MCh) :**

MCh was synthesized and acylation of Ch was confirmed by FTIR (Figure 1). The peaks in FTIR spectrum of MCh at 2850 cm$^{-1}$ and 2919 cm$^{-1}$ were ascribed to –$CH_2$ stretching. The intensity of band is strong in MCh in comparison to Ch and was proportional to the acyl chain length. While the bands at 1640 cm$^{-1}$ and 1552 cm$^{-1}$ correspond to amide bond C=O stretching and N-H bending vibrations, respectively. A broad band in the region of 3200–3500 cm$^{-1}$ in all the cases was assigned to the N-H and O-H stretching vibrations.

The peak at about 1740 cm$^{-1}$ indicated the presence of ester group formed by O-acylation. But, the absence of this peak in the synthesized product confirmed that only N-acylation occurred during synthesis. These results clearly confirmed that the chitosan was substituted[25,26,27] .

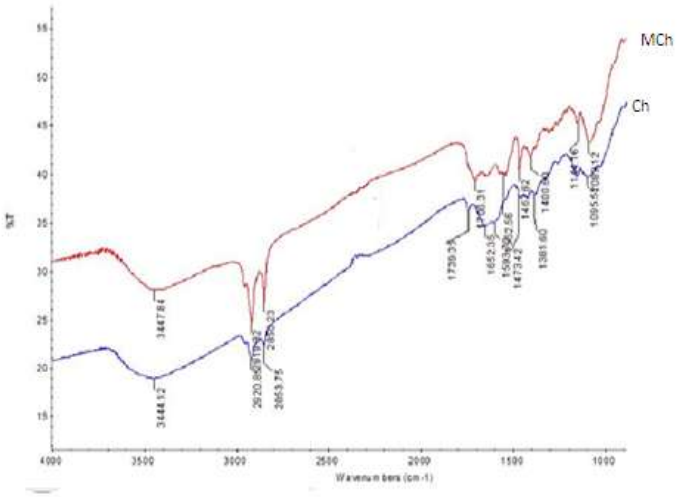



**Figure 1: Comparison of IR spectra of Chitosan and myristoyl chitosan**

**Measurement of degree of acylation by ninhydrin assay :**

The degree of acylation as determined by Ninhydrin assay method was found out to be 42.2 ± 2.7%.

**Preparation of drug loaded NPs of Ch and MCh**

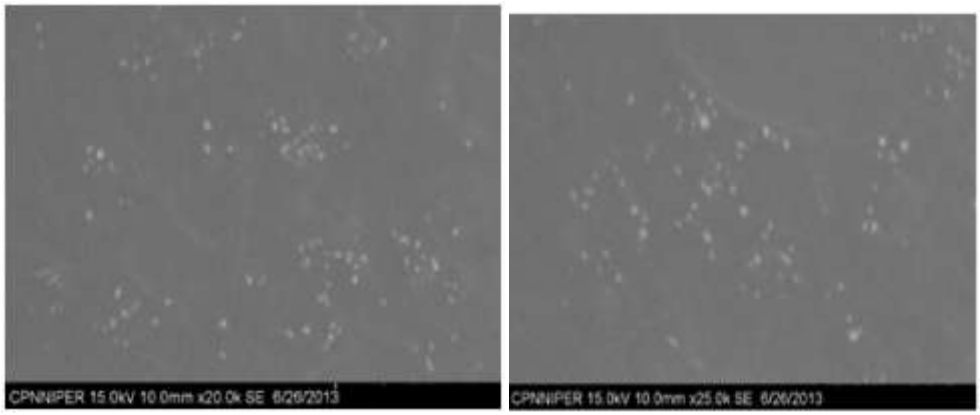
Nanoparticles of Ch and MCh were prepared by ionic gelation method. Amongst the drug loaded formulations, the average particle size, PDI and zeta potential of drug loaded nanoparticles of chitosan (Ch15) was found out to be 240.9 nm, 0.225, 49.67 mV respectively. In case of nanoparticles of myristoyl chitosan (MCh15), the average particle size, PDI and zeta potential was found out to be 271.9 nm, 0.278, 39.99 mV respectively. The zeta potential of MCh15 was less positive as compared to Ch15.

**Ligand conjugation onto the Ch and MCh based NPs and characterization of optimized formulation :**

Con A was selected as ligand moiety as it has affinity to cell surface receptors specific to polysaccharides. Covalent linkage was used as method for ligand attachment. Zeta potential was measured to confirm the conjugation of appended NPs.

**Morphology :**

Optimized formulation was visualized under SEM. Figure 2 shows SEM images of optimized NPs of appended Ch (A) and MCh (B). SEM images revealed that NPs formed were small in size and were almost spherical in shape.



A B

**Figure 2: SEM images of appended (A) Ch NPs and (B) MCh NPs**

**Particle size distribution analysis:**

Size and size distribution of optimized Con A appended Ch NPs and MCh NPs were determined by photon correlation spectroscopy method using zeta sizer (Beckman coulter) Delsa$^{TM}$ Nano C. The size, of appended Ch17 and MCh17 was found out to be 244.4±9.56 nm and 275.8±11.35 nm. The PDI values were 29.01±1.86 and 27.11±2.16 respectively. The entrapment efficiency (EE) of epirubicin in Ch17 (45.01±1.32%) was high as compared to MCh17 (40.10±1.23%). (n=6).

**In-vitro drug release:**

*In vitro* drug release from Ch17 and MCh 17 performed using dialysis bag method is shown in figure 3. The percentage release was 95.81±2.22% from drug solution in about 4hrs. Drug release from Ch17, MCh17 showed an initial burst release followed by 93.02±1.66% and 68.53±2.27% release from Ch17 and MCh17 in 20hrs.(n=6).

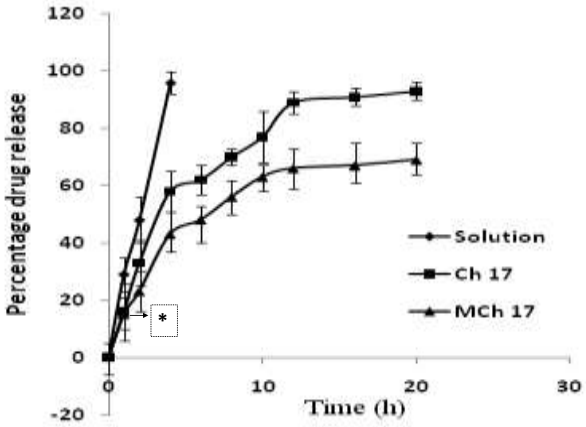



**Figure 3: % Release Vs time curve of drug solution, Ch17, MCh17 in PBS pH 7.4 (n=6; P= 0.05).**

***** Insignificant betweenCh17 and MCh17 at t=1 h. Significant between soln & Ch17; Soln & MCh 17 ; Ch 17 & MCh 17 at all other time points.

**Study of drug release kinetics and mechanism of drug release**

For studying the release behaviour of drug release, different kinetic models were used including zero order, first order, Peppa's model and Higuchi model. Regression values ($R^2$) for release profiles obtained by plotting % drug release vs time as per the kinetic equations for various models are tabulated in table 1. The regression value ($R^2$) represents the extent up to which a particular model is applicable. Drug release profile from batch Ch17 in PBS pH 7.4, showed initial burst release followed by release corresponding to Higuchi model ($R^2$ = 0.930). These results are due to adsorbed drug particles at the surface of NPs, showing initial burst release followed by the drug embedded in the matrix showing Higuchi release behaviour.

In case of MCh17, the drug release in PBS pH 7.4, $R^2$ value of 0.975 was observed for the Peppa's model. The exponent value (n) obtained in this model (0.574) indicated that the drug release was by anomalous diffusion or non Fickian diffusion and erosion controlled rate release. Based on the water solubility of MCh at physiological pH, it can be concluded that erosion of the polymer chain plays an important role in the drug release from MCh nanoparticles.

**Table 1: Comparative Regression values ($R^2$) for release profiles obtained Batch Ch 17 and MCh 17 fitted to various kinetic models**

| Kinetic model | Equation, (Plot) | Plot | Batch Ch17 | Batch MCh17 |
|---|---|---|---|---|
| zero order | Mt = Kt | Mt vs t | 0.807 | 0.854 |
| first order | log Mt = Mog Mo – Kt/2.303 | lot Mt vs t | 0.78 | 0.827 |
| Peppas model | $Mt/M\alpha = K.t^n$ | log Mt/Mα vs log t | 0.898 | 0.975 |
| Higuchi model | $Mt/A = [D(2Co-Cs)Cs.t]^{½}$ | Mt vs √t | 0.930 | 0.908 |

Mt : % drug release at time t; Mα: % drug release at infinite time; K: Constant; Co: Solubility; Cs Saturated solubility; D Diffurional co-efficient: n: peppas exponent.

One-way (ANOVA) was applied to compare mean percentage drug release from each formulation at different time points and the F values were calculated at 0.05 level of significance. On comparison with the tabled F values at p=0.05, it was concluded that the percentage dissolution between epirubicin solution and Ch 17 or MCh 17 is significant (p=0.05) at all time points. However, the percentage dissolution between Ch 17 and MCh 17 is insignificant (F < 4.97) at only one point of time (at t=1 h) and at all other time point it is significant (F>4.97).

**Stability Studies :**

Stability studies (table 2 and 3) were conducted for 30 days. Ch17 and MCh17 were found to be stable upto a period of 30 days at a temp of 5±3$^0$C, by calculating the size and % residual drug content of NPs. Size of Ch17 and MCh17 was found to be 262.4nm and 289.4nm respectively and % residual drug content was found to be 89.6% and 90.6% respectively.

**Table 2. Particle size determination of NPs for a period of 30 days at 5±3$^0$C**

| Formulations | Average particle size (nm) | | | | |
|---|---|---|---|---|---|
| | At 5±3$^0$C | | | | |
| | 0 days | 5 days | 10 days | 20 days | 30 days |
| Ch17 | 244.4±9.56 | 246.5±8.94 | 249.0±10.38 | 256.5±12.14 | 262.2±9.36 |
| MCh17 | 275.8±11.35 | 276.6±12.56 | 279.6±11.63 | 283.1±13.42 | 289.4±14.72 |

N=6

**Table 3 . % residual drug content of NPs for a period of 30 days at 5±3$^0$C**

| Formulations | % Residual drug content | | | | |
|---|---|---|---|---|---|
| | At 5±3$^0$C | | | | |
| | 0 days | 5 days | 10 days | 20 days | 30 days |
| Ch17 | 100 | 98.1±1.24 | 95.4±0.96 | 91.9 ±0.84 | 89.6±1.02 |
| MCh17 | 100 | 99.1±1.42 | 96.7 ±1.08 | 93.3 ±1.52 | 90.6±1.18 |

N=6

**In-vivo studies** :

Tissue distribution studies performed to compare the liver uptake of Ch17 and MCh17 are given in figure 4. Tissue distribution studies shows that 93.6±10.11% MCh17 was targeted to liver in 30min, and 80.0±6.55% was observed in liver after 4 hrs. In case of Ch17, 87.0±7.55% was targeted to liver in 30min and 65.0±5.00% of drug was retained in liver after 4hrs.

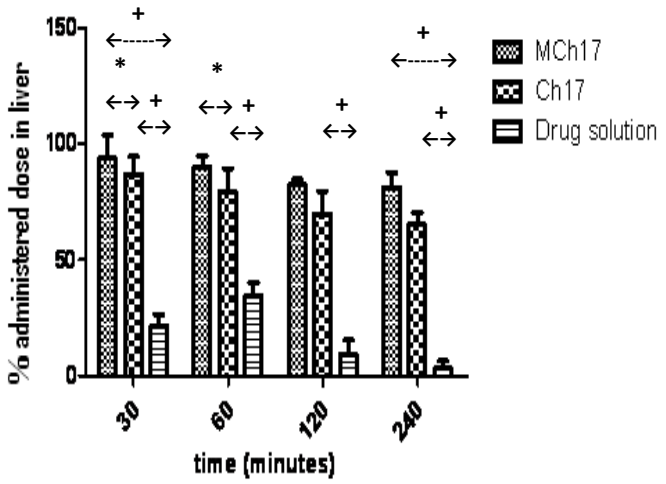



**Figure 4**: **Percentage of i.v. administered dose of epirubicin in liver with time in Wister rats (n=3; p=0.05). Note: * Insignificant & $^+$ Highly Significant between arrows represented.**

One-way (ANOVA) was applied to compare liver uptake of epirubicin at different time points after IV administration of different formulations (Soln, Ch 17 and MCh 17) in Wistar rat model (n=3) and the F values were calculated at 0.05 level of significance. On comparison with the tabled F values at p=0.05, it was concluded that the extent of liver uptake of the drug between epirubicin solution and Ch 17 or MCh 17 is significant (p=0.05) at all time points. However, the comparative liver uptake between Ch 17 and MCh 17 was insignificant up to 2 hours post administration ($F < 7.71$) while after 2 hours the difference was found significant ($F > 7.71$).

Both N and O acylation is observed using lower chain fatty acids (acetyl, propionyl and butyryl chitosan), while only N-acylation occurs while using higher chain fatty acids such as lauroyl, myristoyl, palmitoyl etc[26]. Hence, a long chain fatty acid, myristoyl chloride was selected for hydrophobic modification of chitosan. For the synthesis of MCh, a highly reactive myristoyl chloride (an acid chloride) was interacted with amino group of Ch[28] The amino groups at C2 position, being nucleophilic and highly reactive and hence reacts with myristoyl chloride. This makes them a suitable position for chemical modifications[22,29,30]

pH 7.2 was selected due to the reactivity of C-2 position of Ch at this pH only. The degree of acylation was restricted upto 42.2± 2.7% because, some free amino groups are required for reaction with TPP for cross linking during the preparation of nanoparticles. 100% acylation is not advisable as it hinders the formation of NPs due to non-availability of free amino groups.

Ionic gelation method uses ionic interaction for the transition of materials from liquid to gel. The ionically cross-linked NPs are formed by the interaction of amino groups of the chitosan backbone with salts like TPP, thus exploiting the cationic nature of Ch and its derivatives[31,32,33,34]. As per reports, this method has advantage over chemical cross-linking that possible toxicity of the reagents and other undesirable effects may be avoided. In this method, aqueous medium is used for the formulation of NPs in which the fatty acid chains reorganise themselves to form micelles by intra- or intermolecular association between hydrophobic moieties in order to reduce the interfacial tension. The hydrophobic masses are removed from the aqueous environment resulting in the formation of polymeric micelles with core/shell structure[35]. TPP is used for the stabilization of micelles because the unmodified amino groups of MCh interact with TPP resulting in the formation of NPs.

Homogenization results in the reduction of the size of particles to an extent, beyond which there is aggregation of particles due to increase in surface free energy caused by size reduction leading to agglomeration[36]. Hence optimization of critical sonication time was performed by preparing trial batches at different RPM for varied intervals of time with particle size and PDI values as

response parameter. Optimized batch was prepared by homogenization at 5000 RPM for 5 minutes.

The average particle size of MCh NPs was found out to be slightly higher as compared to Ch NPs, which is due to longer fatty acid chain. The corresponding zeta potential values of Ch NPs and MCh NPs were 49.67mV and 39.99mV respectively. This might be due to the presence of $NH_3^+$ density on the surface of NPs[37]. Due to the reduction in the number of free groups due to N-acylation of Ch, there is a reduction in the zeta potential of MCh formulations

The molecular weight (Mw) of Ch affects the shape of particles and hence, Ch of intermediate Mw was selected. Low or intermediate Mw Ch produces spherical NPs. but high Mw Ch failed to yield NPs which might be due to the high viscosity of the polymer.

Con-A was covalently conjugated onto the NPs through a coupling reaction between free amine groups of Con-A and the hydroxyl groups of polymers. This process was selected because in this process, physical adsorption is relatively random and chaotic along with weak association which might result in breakage of bond in comparison to covalent linkage[39] The hydroxyl groups of carbohydrates interact for the binding interactions with lectins. As the number of CH bond and the size of carbohydrate structure increases, the probability of bond formation increases. There is a high density of hydroxyl groups in carbohydrates that might act as an acceptor for two hydrogen bonds and as a donor for a single hydrogen bond simultaneously[39].

After conjugation, the zeta potential of both Ch17 and MCh17 were found near about same. This shows that lectins had been attached onto both the NPs.

Drug release is mainly controlled by erosion, diffusion and swelling. In first 4 hours, 57.55±6.77% and 43.35±2.78% of drug was released from Ch17 and MCh17. This initial, fast release was given by the drug molecules localized on the surface of the NPs[40].. However, the drug release from MCh17 showed monophasic character fitting into the Peppas equation with exponent value, n=0.574 confirming the anomalous release with probability of polymer erosion.

It was observed that maximum drug release in 20hr for Ch17 was 93.02±1.66% while only 68.53±2.27% was released in 20hrs from MCh17. This could be due to slow but constant swelling and erosion of MCh17 due to relative hydrophobicity of the polymer chain[41]..

In the preparation of nanoparticles, the drug loading may be done either during the preparation of NPs or after its preparation. In the present work, Epi was loaded during preparation of NPs. As the MCh forms hydrophobic core surrounded by a hydrophilic outer shell; it can serve as reservoir for various hydrophobic drugs[35]. Epirubicin, being hydrophilic in nature, has less

entrapment in MCh17 as compared to Ch17 as in Ch17, there is no such hydrophobic core and hence more entrapment of hydrophilic drug.

When the hydrophobic moiety is conjugated to a Ch molecule, the resulting amphiphile may form self-assembled NPs that can encapsulate a quantity of drugs and deliver them to a specific site of action[44]..

Stability studies of NPs show both physical and chemical stability. NPs are usually prone to change in size either due to aggregation of vesicles or due to leakage of the drug encapsulated.Results show that both the formulations were stable at $5\pm3^0$C for 1 month.

In general, liver-targeting systems employ passive trapping of particles by RES uptake or active targeting based on recognition between hepatic receptor and ligand-bearing particulates[1.]. Particles greater than 100 nm in diameter are rapidly taken up by the RES in the liver, spleen, lung and bone marrow, while smaller-sized particles tend to have a prolonged circulation time. When the NPs of particle size well above 100 nm but below 200 nm are administered i/v, they are easily recognized and captured by the phagocytic cells of the body's immune system[42,43]. These cells are generally dumped in RES organs particularly liver. Hence, this process of particle recognition and capture which is normally extremely efficient can be utilized strategically to target liver by modulating particle size, and to an even extent, the surface properties of the particles. The particles that have a more hydrophobic surface are efficiently removed from circulation, but hydrophilic particles can resist opsonin coating and consequently are cleared more slowly.

## CONCLUSION :

Acylated derivative of chitosan (Ch) such as myristoyl chitosan (MCh) was synthesised and characterised. MCh was prepared by reacting native Ch with myristoyl chloride and attachment of myristoyl group to the backbone of Ch was confirmed by FTIR analysis. MCh can improve solubility and provide sustained release of drug. Hence, the prepared MCh was considered as a useful tool for further development work in the present project.

Nanoparticles (NPs) of Ch and MCh were prepared by ionic gelation method. The NPs were optimised for processing parameters based on particle size, zeta potential and entrapment efficiency (EE). EE was found high in case of Ch NPs as compared to MCh NPs, probably due to the amphiphilic nature of MCh and the hydrophobic moiety interfering with encapsulation of water soluble drug. The NPs of Ch and MCh were conjugated with concanavalin A (Con A) by incubation. Conjugation was confirmed by measuring zeta potential of optimised formulations.

Zeta potential was decreased as compared to previous results because of the conjugation of ligand. After optimization, the surface morphology was checked with the help of SEM. The particles were found out to be nearly spherical. Drug release from Ch NPs was higher in comparison to MCh NPs over a period of 20 hrs. NPs of Ch and MCh provide satisfactory stability under refrigeration (2-8$^0$C) for 30 days. From the *in vivo* studies, it was concluded that a surface-modified NPs reduced blood Epirubicin level after i/v administration. The Con A appended NPs showed high Epirubicin uptake by liver, due to its relative high affinity for polysaccharide receptors over expressed in liver. However, NPs of MCh were easily and rapidly recognized by the RES uptake system of liver. Hence it is concluded that MCh NPs are better carrier for drug delivery to liver as compared to Ch NPs.

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