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Formulation of Controlled Release Matrix Tablet using synthesized N-acyl Thiolated Chitosan derivative

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Abstract: Chitosan derivative was successively synthesized by initial preparation of acylthiourea reagent using ammonium thiocyanate and making it to react with at primary amino groups of chitosan and then reduced to yield thiolated chitosan. Due to the formation of disulfide bonds with mucus glycoproteins, mucoadhesiveness is augmented. The thiol groups were then quantified using Ellman's reagent. The derivatives inherit good swelling property in neutral and alkaline media. The different derivatives containing thiol groups were formulated into tablets using reference drug for evaluation. The thiolated chitosan display in situ gelling features due to the pH-dependent (alkaline pH) formation of inter-molecular disulfide bonds which makes the application of thiolated chitosan on intestinal mucosa and can guarantee prolonged controlled release of embedded therapeutic ingredients.
Keywords: Thiolated Chitosan, acyl isothiocyanate, Isopropanolol, Aluminium isopropoxide, Mucoadhesion, Matrix tablet.

1. Introduction:

Chitosan is devoid of pharmaceutical use because of poor or no water solubility and alkaline solubility is far impossible. Literature states that acylation of chitosan at amino position results in increase in its water solubility. Further, some chitosan conjugates with thiol containing moieties have shown swelling property in alkaline pH. The article deals with synthesis of some novel derivatives which contains thiol group obtained in stepwise reaction. The derivatives were prepared by applying a simple thiouride synthetic pathway which is then further reduced to form a Thiolated Chitosan derivative. The derivative shows a good mucoadhesion, water solubility, swelling in alkaline pH which can be used for controlled release for intestinal release. The derivatives retain the acid solubility inherited from the precursor. The derivatives thus have good pharmaceutical properties to be used as a polymer in intestinal delivery of drug.

2. Materials and Physico-chemical characterization:

2.1 Materials:

Chitosan (medium molecular mass: 400 KDa; degree of deacetylation: 83–85%) was purchased from Research Laboratories, Acylchloride, ammonium thiocyanate, Elman's Reagent were obtained from Research Lab and aluminium isopropoxide was procured from SIGMA. All chemicals were of analytical grade. NMR analysis was performed at IIT (Powai).

2.2. Preparation of acylthiocyanate reagent:

The solution of ammonium thiocyanate (0.011 mol) in dry acetone (25 ml) was prepared. Benzoyl chloride (0.01 mol) was added slowly in above solution with stirring. The reaction mixture was subjected to microwave irradiation for 3mins at 560 watts power to yield an acylthiocyanate reagent. [1]

2.3. Synthesis of acylthiouride of chitosan:

A solution of chitosan in 2%AcOH/MeOH (50 ml) was added slowly to the above solution so as to maintain reflux condition. After the addition was complete, the mixture was stirred for 90 min at room temperature, which is separated as solid precipitate on pouring in NaOH solution (pH 10). [2, 3]

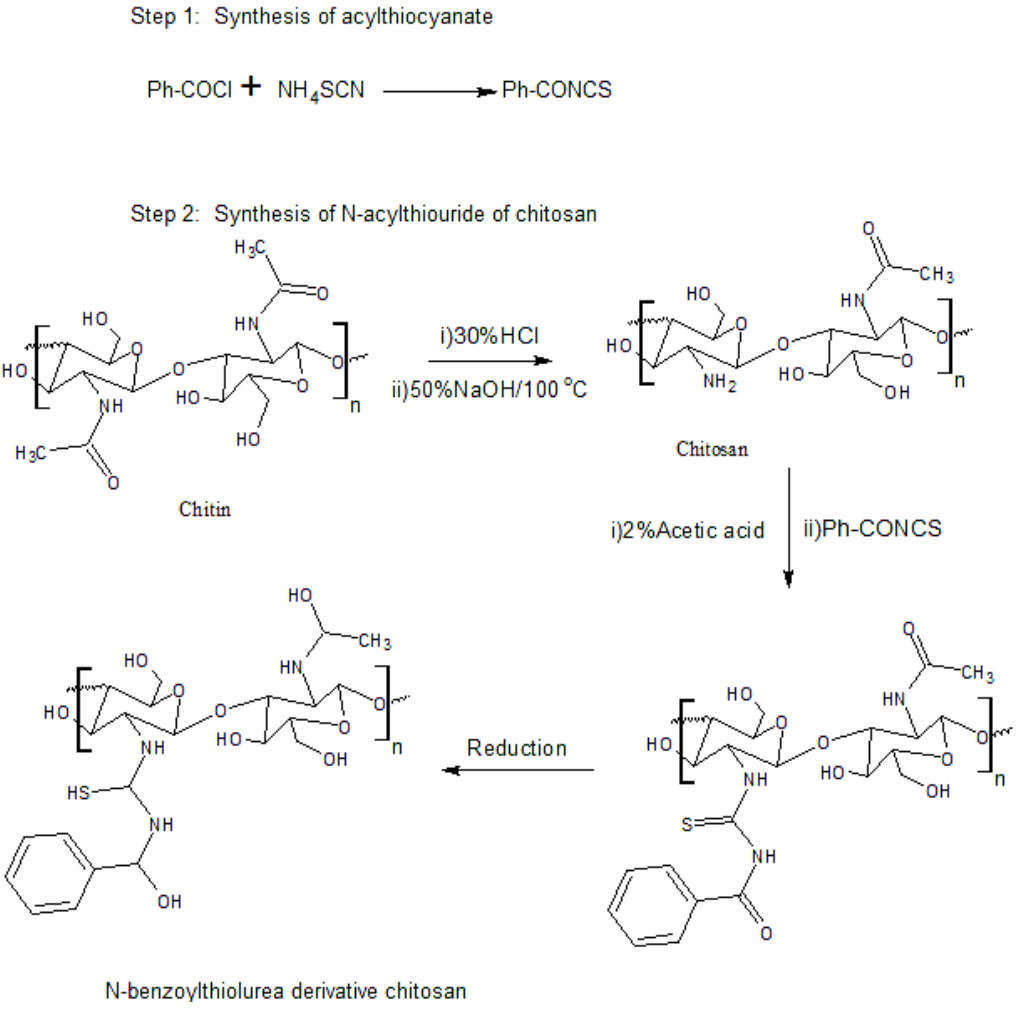
2.4. Synthesis of Thiolated Chitosan derivative:
The above precipitate was washed with acetone thoroughly to remove the traces of acylthiocyanate. The product was then treated with aluminium isopropoxide in isopropanaol which reduces the thioketone group from acylthiouride to form the thiol resulting in the formation of Thiolated Chitosan. The aluminium isopropoxide in isopropanaol solution is as specific reducing agent for ketones [4] (*Scheme 1*).

2.5.Procedure for Quantification of Sulfhydryl Groups:
Prepare the dilution buffer (0.1 M Sodium phosphate, 1 mM EDTA, pH 8.0) and DTNB [(5,5'-dithio-*bis*-(2-nitrobenzoic acid)] working solution. Store at +4°C. Dissolve 4mg of DTNB in 1 ml dilution buffer. Prepare a set of Sulfhydryl standard (Cysteine HCl) with sample dilution buffer (or distilled water). Dissolve Cysteine HCl (26.34 mg) to prepare 1.5 mM solution, then serial dilutions 1.25 mM, 1.0 mM, 0.75 mM, 0.5 mM and 0.25 mM and was used immediately. Add the following components to test tubes:

250 µl Sample/Standard
2.5 ml dilution buffer
50 µl DTNB reagent

The resulting solution was incubated for 15 minutes and absorbance was measured at the wavelength of 412 nm and concentration of Sulfhydryl group was determined using Cysteine as standard solution. Molar extinction coefficient at 412 nm of DTNB is 14,150 $M^{-1}cm^{-1}$. [5, 6]

2.6. Swelling Index:
Swelling index of the synthesized derivative was determined by soaking 150mg flat faced 8 mm tablet in 6ml of water in a Petri-dish. Initial and final weights of the tablets were recorded .
Swelling index was calculated by using following formula:
Swelling index= Final weight – initial weight/ Final weight x 100



Scheme 1: N-benzoyl Thiolated Chitosan derivative synthesis

Table 1: Quantification of thiol group by Elman's reagent:

Sr. no.	Sample*	Absorbance (412 nm)	Sulfhydryl content (moles)
1	Chitosan	-	-
2	TC1	0.3333	0.6608 x 10-7

*TC1: N-benzoyl thiolated Chitosan derivative

Table 2: Swelling Index of N-acyl thiolated chitosan derivatives:

Sr. no.	Sample	Initial weight (W1)	Final weight (W2)	Swelling Index (%)
1	Chitosan	0.1022	0.1142	1.2
2	TC1	0.1508	0.5112	70.5

2.7. Procedure for Quantification of Sulfhydryl Groups:

Prepare the dilution buffer (0.1 M Sodium phosphate, 1 mM EDTA, pH 8.0) and DTNB [(5,5'-dithio-*bis*-(2-nitrobenzoic acid)] working solution. Store at +4°C. Dissolve 4mg of DTNB in 1 ml dilution buffer. Prepare a set of Sulfhydryl standard (Cysteine HCl) with sample dilution buffer (or distilled water). Dissolve Cysteine HCl (26.34 mg) to prepare 1.5 mM solution, then serial dilutions 1.25 mM, 1.0 mM, 0.75 mM, 0.5 mM and 0.25 mM and was used immediately. Add the following components to test tubes:

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2.8. Swelling Index:

Swelling index of the synthesized derivative was determined by soaking 150mg flat faced 8 mm tablet in 6ml of water in a Petri-dish. Initial and final weights of the tablets were recorded

Swelling index was calculated by using following formula:

Swelling index= Final weight – initial weight/ Final weight x 100

2.9. Solubility test:

The solubility of the copolymers was tested in several organic solvents, distilled water as well as in 0.1 M phosphate buffer (pH 4.0), 0.1 M phosphate buffer (pH 7.0) and DMSO. The samples were soaked in each solvent at the concentration of 5 mg/ml [7].

2.10. Mucoadhesivity assessment:

Recently, it has been shown that polymers with thiol groups provide much higher adhesive properties than polymers generally considered to be mucoadhesive. The enhancement of mucoadhesion can be explained by the formation of covalent bonds between the polymer and the mucus layer which are stronger than non-covalent bonds. This theory was supported by the results of assessment of Mucoadhesive strength demonstrated a positive correlation between the degree of modification with thiol bearing moieties and the adhesive properties of the polymer. [8, 9]

The measurement of the Mucoadhesive strength, the pure polymer was taken and the disc of 150 mg. was prepared by using 8mm die on the rotary compression machine. After that the tablet was removed and used for the measurement of the mucoadhesive strength .

Table 3: Solubility assessment of thiolated Chitosans.

SAMPLE	AQ.ACETIC ACID (2%)	PBS^{+} (pH 4.8)	WATER	PBS (pH 7.4)	$DMSO^{++}$
Chitosan	++++	++	-	-	-
TC1	++++	++++	++++	++ (SWELLS)	++ (SWELLS)
TC2	++++	++++	++++	++++ (SWELLS)	+++ (SWELLS)
TC3	++++	++++	++++	++ (SWELLS)	++ (SWELLS)
TC4	++++	++++	++++	+++ (SWELLS)	+++ (SWELLS)
TC5	++++	++++	++++	++++ (SWELLS)	+++ (SWELLS)

+ Phosphate buffer solution, ++ Dimethylsulfoxide

Table 4: Mucoadhesivity assessment of thiolated Chitosans:

Sr. no.	Sample	Weight required (gm)	Mucoadhesive Strength (Strain) dyne cm/s^2
1	Chitosan	1.168	1800
2	TC1	6.111	9416.98

Table 5: % Release (Average with %Dissolution Efficiency & Mean Dissolution Time)

Sr.No.	Time (Hrs)	Avg. %R	Amt. (mg)	% DE*	MDT**
1	0	0.000	0.00	0.00	0.00
2	1	2.135	1.28	1.07	0.50
3	2	2.390	1.43	1.66	1.11
4	3	53.570	32.14	10.44	2.22
5	4	45.681	27.41	20.23	2.40
6	5	56.758	34.05	26.43	3.06
7	6	0.000	0.00	30.98	3.08

*dissolution efficiency, **Mean dissolution time.

2.11. Thiol derivative as Controlled Release matrix:

Thiolated Chitosan represents, primarily due to its Mucoadhesive properties, a valuable tool for non-invasive drug delivery. The longer residence time of formulations based on mucoadhesive polymers at the absorption site is believed to contribute to an increased absorption rate of the incorporated drug. However, such an enhanced bioavailability can be achieved only if a controlled release of the active agent out of the formulation is provided. Thiolated Chitosans also display, besides their strong mucoadhesive and permeation enhancing properties, excellent cohesive properties. The cohesion and stability of a drug delivery system over the intended duration of drug liberation is often a substantial requirement for a controlled release. The usefulness of thiolated Chitosans as carrier matrices for controlled drug release was demonstrated with model drug as Amodiaquine. [8, 9]

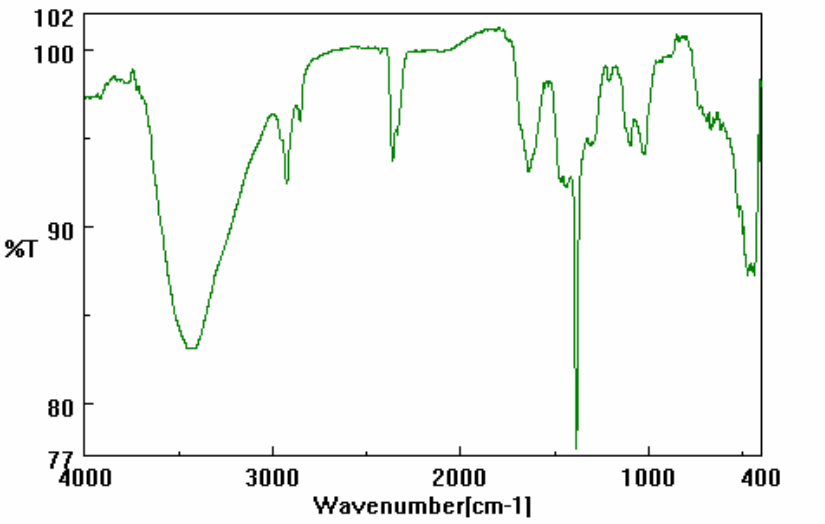

Figure 1: IR spectra for N-benzoyl thiolated chitosan derivative

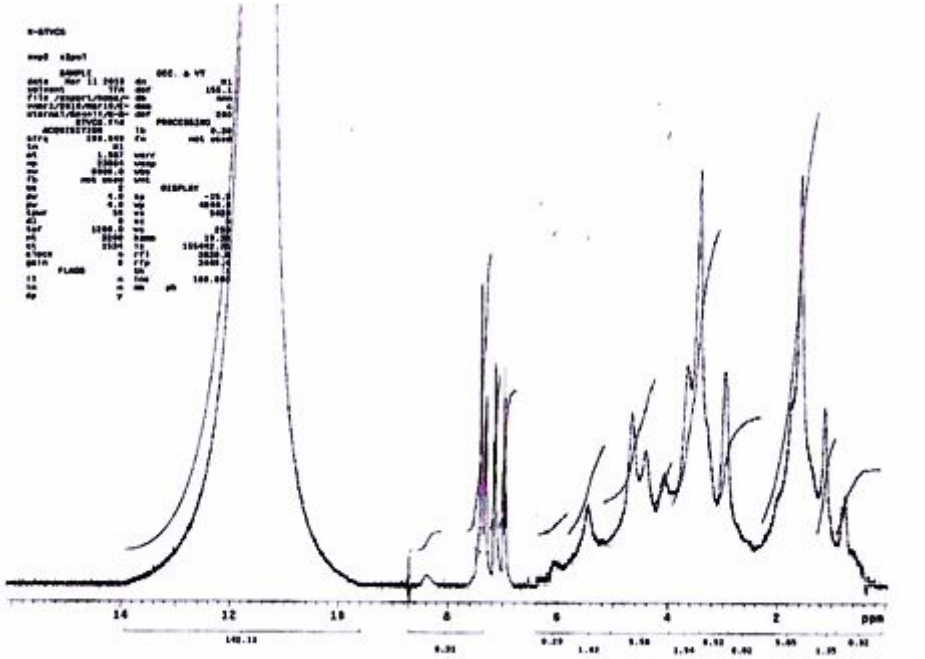

Figure 2: NMR spectra for N-benzoyl thiolated chitosan derivative

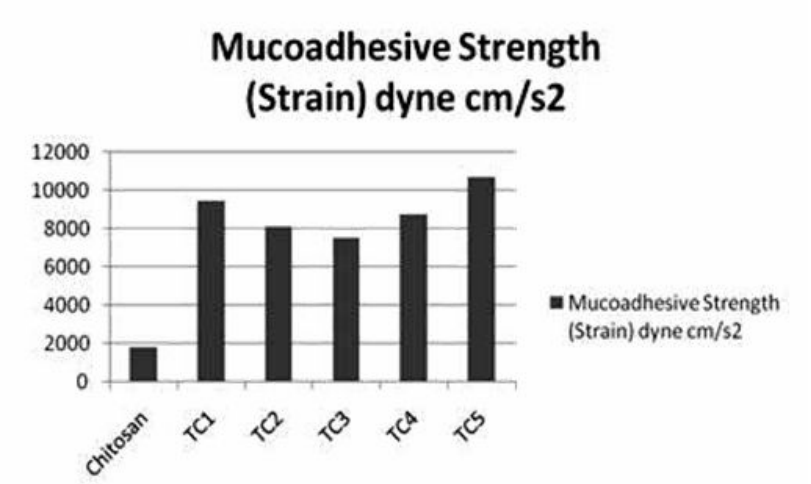

Figure 3: Mucoadhesive strength for N-benzoyl Thiolated Chitosan derivatives

Figure 4: Release pattern of N-benzoylthiolurea derivative for a Model drug (Amodiaquine)

3. Results & Discussion:

Thiolated Chitosan derivative synthesized is N-Benzoyl thiolated chitosan derivative. The IR spectra (*figure 1*) and NMR spectra (*figure 2*) were assessed for structural confirmation.

Significant IR peaks:

3435.56, O-H Stretch (H-bonded alcohols); 2064.42, C-O stretch overtone (aryl-alkyl ether); 1383.68, S-H stretch (aliphatic); 658.571, C-S Stretch.

Significant NMR peaks:

5.067, tetrahydropyran methane; 4.414, 2 amine, 1 thiol of N-C(SH)-N ;4.161, tetrahydropyran methine C-O-, -N-C=O, -O from methane; 1.58, Thiol.

The resultant thiolated polymer derivative have shown good swelling property and enhanced mucoadhesive strength due to the intermolecular disulfide linkage between the polymer and mucosa (*figure 3*). Also the derivative has shown a promising sustained release property (*figure 4*).

Because of its mucoadhesive properties the resultant thiolated polymer derivative have shown prolong residence time of the dosage form in the small intestine, the permeation enhancing effect, Sustained drug release and good oral bioavailability of various poorly absorbed drugs improved.

4. Conclusion:

Thus, the given research work proves from the result obtained that:

The Spectral data corresponds to the anticipated structures and referring to literature it is confirmed that Sulfhydryl functional group showed a dramatic change in the polymer's properties. Also Mucoadhesiveness strongly improved.

5. Ackowledgement:

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