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Synthesis, Characterization and Evaluation of Some Chitosan Conjugates

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Natural polymers are widely used in pharmaceutical field as excipients. In the present study, degree of deacetylation of chitosan was determined and conjugated with amino acids, mono and dicarboxylic acid. Conjugates were characterized by infrared spectroscopy and differential scanning calorimetry. The synthesized conjugates were studied for the degree of substitution. Paracetamol tablets were prepared with chitosan and chitosan conjugates and evaluated for various quality control parameters. Release study at pH 2 and pH 6.8 was performed. The present study shows that the chitosan conjugates show very slow release at pH 2 and faster release at pH 6.8, data suggests that chitosan conjugates delay/sustain the drug release.

Keywords: Chitosan, Chitosan conjugates, Paracetamol, Dissolution.

INTRODUCTION

The basic goal of drug delivery system is to deliver the therapeutically active agent to the site of action. Various problems that are encountered by a drug to reach the site of action include poor aqueous solubility, lack of site specificity, low bioavailability *etc*. Therefore a lot of efforts are being made to develop the delivery systems that are site specific [1-3]. Polymers are widely used for targeting the drug to a specific target. Chitosan is a linear binary hetropolysaccaharide consisting of two units of N-acetyl D-glucosamine and D-glucosamine, obtained by partial deacetylation of chitin. Chitosan is biodegradable, biocompatible, antimicrobial, anticholesterolemic, antioxidant and anti-inflammatory. Chitosan is a weak amphiphilic base poorly soluble in water at neutral and alkaline pH, but soluble in dilute acidic solutions below its pKa (6.3) in which it converts glucosamine units into the soluble protonated forms [4-6].

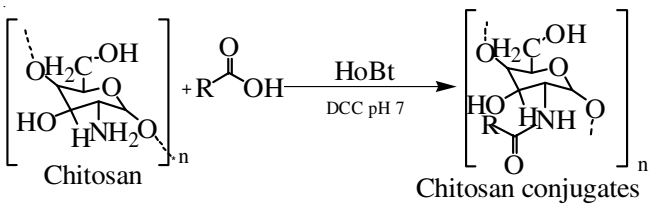
Chitosan is a weak base and an acidic environment is needed to convert its glucosamine units into the uncoiled state, positively charged and water soluble form. In a dosage form at neutral and basic pH, chitosan loses its charge and precipitates from solution, thus increasing the absorption of drug. The tablet forms a gel matrix that inhibits the dissolution of the chitosan because chitosan is soluble in acidic pH.

Chitosan has gel forming ability from acidic to basic values. This shift can be done by means of covalently binding chitosan backbone to acidic residues. The residues of carboxylic acid are known to have interaction at basic pH environment of the terminal ileum and the ileocaecal junction, thereby giving more appropriate matrices for orally administered colon specific drug delivery dosage forms [7].

EXPERIMENTAL

Chitosan was obtained from Mahatani Chiosan Pvt. Ltd., Verabal, Gujarat, India. Paracetamol was obtained from Macleods Pharmaceutical Ltd., Baddi, H.P. DCC (dicyclohexyl carbodimide) and HoBt (hydroxy benzo triazole) were purchased from Loba chemie India.

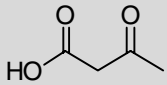
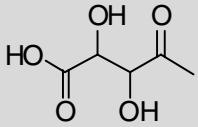
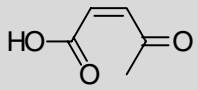
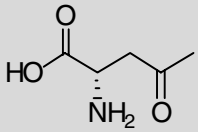
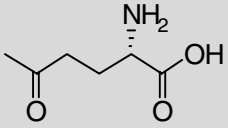
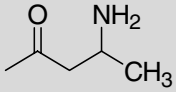
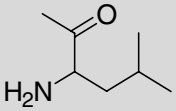
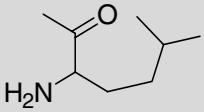
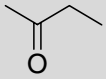
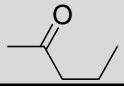
Synthesis of chitosan conjugates: Chitosan (1 %) solution was prepared in hydrochloric acid (1 %), pH was adjusted to 4 using 1 M sodium hydroxide. Then amino acids/mono or dicarboxylic acid and DCC (dicyclohexyl carbodimide) were taken in a conical flask. After 10 min of hydroxy benzotriazole (HoBt) was added to above solution. This reaction mixture was stirred at room temperature for 6 h. The product was precipitated by adjusting the pH of the reaction mixture to 7.5 using 1 M NaOH and precipitate was washed with water several times [8-15] (**Scheme-I**).

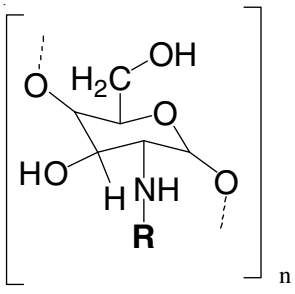


Scheme-I: Synthesis of chitosan conjugates

For synthesis of A1, A2, A3, A4, A5, A6, A7, A8, A9, A10, 0.005 mol of acids, malonic acid (0.52 g), tartaric acid (0.73 g), maleic acid (0.58 g), aspartic acid (0.66 g), glutamic acid (0.735 g), L-alanine (0.44 g), L-valine (0.67 g), L-leucine (0.65 g), propionic acid (0.37 g) and butyric acid (0.67 g), respectively, were added to the chitosan solution along with 0.005 mol of DCC (1.03 g). After 10 min 0.005 mol of HoBt (0.67 g) was added and the product was precipitated as mentioned in Table-1.

TABLE-1
STRUCTURE OF CHITOSAN CONJUGATES

Name of conjugate	Compound code	R
Chitosan malonate	A1	
Chitosan tartarate	A2	
Chitosan maleate	A3	
Chitosan aspartate	A4	
Chitosan glutamate	A5	
Chitosan L alanine	A6	
Chitosan L-valine	A7	
Chitosan L- leucine	A8	
Chitosan propionate	A9	
Chitosan butyrate	A10	



General structure of chitosan conjugates

Degree of deacetylation of chitosan and chitosan conjugates: Degree of deacetylation (DD) of chitosan and chitosan conjugates was determined by titrimetric analysis [16], 0.13 g chitosan was dissolved in 25 mL of 0.1 N hydrochloric acid and stirred for 30 min until completely dissolved [17,18]. The solution was titrated with 0.1 N sodium hydroxide using phenolphthalein as indicator. The degree of deacetylation was calculated as follows:

$$NH_2\ (\%) = \frac{(C_1V_1 - C_2V_2) - 0.016}{G\,(100 - W)} \times 100$$

where, C_1 is concentration of NaOH (N),C_2 is concentration of HCl (N), V_1 is volume of NaOH solution (mL) and V_2 is volume of HCl solution (mL), G is sample, weight (G) and W is the water percentage.

$$DD\ (\%) = \frac{NH_2}{9.94} \times 100$$

where 9.94 % is the theoretical NH_2 percentage.

Tablet formulation of chitosan conjugates: Tablets were prepared by direct compression method. The composition of tablet formulation is given in Table-2. Different batches of paracetamol tablets with chitosan and chitosan conjugates were prepared by direct compression. The granules were passed through sieve no. 40. Avicel and lactose were used as diluents. The fraction was blended with magnesium stearate and talc and compressed into tablets (multi station tablet compression) using 9 mm flat-faced punches [19-23].

TABLE-2
COMPOSITION OF TABLET FORMULATION

Ingredients	Quantity (%)
Paracetamol	65
Chitosan/chitosan conjugates	10
Microcrystalline cellulose	8
Lactose monohydrate	15
Talc	1
Magnesium stearate	1

Evaluation of tablets: The thickness and diameter of the tablets was determined using vernier caliper. The hardness of the tablet was determined using Monsanto hardness tester. The friability of the tablets was determined using Roche fribilator. Weight variation test of the tablets was carried out as per the official method [24].

Dissolution studies: Dissolution study was carried out using dissolution apparatus type 2 (USP). The study was carried out in 900 mL of phosphate buffer pH 2 for 2 h and then 900 mL of phosphate buffer pH 6.8 from 3 to 24 h. The dissolution medium was maintained at 37 ± 0.5 °C. The paddle was lowered so that the lower end of the stirrer was 25 mm above from the base of the beaker. The pre weighed tablet was then introduced into the dissolution flask and paddle was rotated at 50 rpm. At different time intervals, 10 mL of sample was withdrawn and analyzed spectrophotometrically at 243 nm for the drug release and replaced by 10 mL of fresh medium [12,25].

RESULTS AND DISCUSSION

Characterization of chitosan and chitosan conjugates: Degree of substitution of chitosan and synthesized conjugates

was determined by titrimetric method. Degree of substitution indicates the percentage of free amino group and is reported in Table-3, conjugate with highest degree of substitution was chitosan aspartate (A4) while lowest substitution was observed with chitosan leucine (A8) conjugate. Degree of deacetylation of chitosan was found to be 83.30 %.

TABLE-3
DEGREE OF DEACETYLATION AND DEGREE OF SUBSTITUTION OF CHITOSAN CONJUGATES

Chitosan conjugate	Degree of deacetylation (%)	Degree of substitution (%)
A1	44.49	38.81
A2	51.70	31.60
A3	50.94	32.36
A4	45.27	38.03
A5	53.01	30.29
A6	58.19	25.11
A7	72.19	11.11
A8	68.52	14.78
A9	58.84	24.46
A10	56.71	26.59

Structure confirmation of chitosan conjugates was done using FTIR ALPHA spectrophotometer. Carbonyl (-CONH) stretching in range of 1680-1625 cm^{-1} due to amide bond confirms the formation of conjugates [26,27]. The DSC thermogram of chitosan conjugates, have shown extra endothermic peak at 210-230 °C, which was not present in the DSC thermogram of chitosan, confirmed the conjugation of chitosan with amino, mono and dicarboxylic acid [28].

Evaluation of prepared tablets: The tablets of different batches showed uniform hardness (4.5 ± 0.8 to 5.5 ± 0.12). The friability (0.39 ± 0.06 to 0.55 ± 0.04) and weight variation (498 ± 1.64 to503 ± 3.2) of different batches of tablets was found within the prescribed limits.

Dissolution studies were performed to study the drug release from the formulation. *In vitro* dissolution study was carried out using USP type 2 dissolution apparatus. Drug release was performed at pH 2.0 and pH 6.8. At pH 2, dissolution was carried out for 2 h and a highest release was observed with chitosan leucine (A8) while lowest release was observed with chitosan maleate (A3). At pH 6.8 dissolution was carried out for 3 to 24 h and maximum release was shown by chitosan butyrate (A10) while lowest release was shown by chitosan maleate (A3) which suggests that among all the derivatives chitosan maleate shows highest sustained release. This was followed by chitosan alanine (A6), chitosan aspartate (A4) and chitosan malonate (A1) (Figs. 1 and 2).

Conclusion

The present study demonstrated the successful application of the combination of chitosan conjugates to sustain the paracetamol release from paracetamol tablets upto 24 h at different pH conditions. Chitosan conjugates of different amino acids, mono and dicarboxylic acid have shown slow release at pH 2. At pH 6.8 amount of drug released increases, but none of the conjugates showed complete release. Thus it can be concluded that for targeted intestinal delivery, chitosan conjugates can be developed. The present data suggests that chitosan conjugates with drug could also be use for colon-specific drug delivery system.

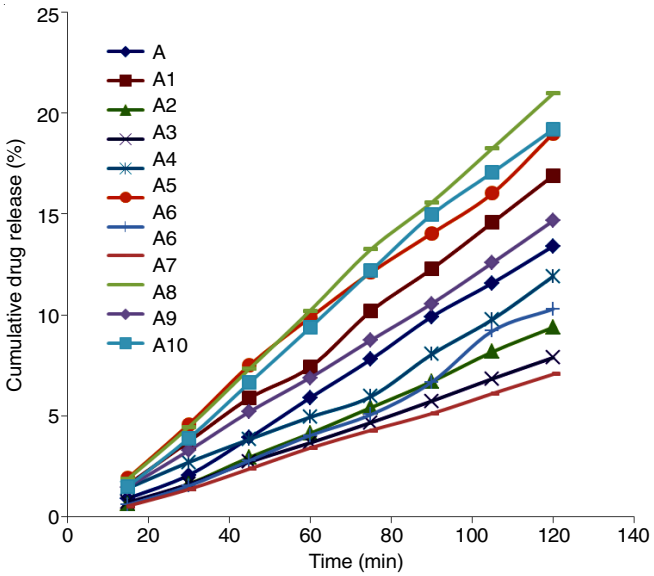


Fig. 1. *In vitro* release of paracetamol tablets in phosphate buffer pH 2

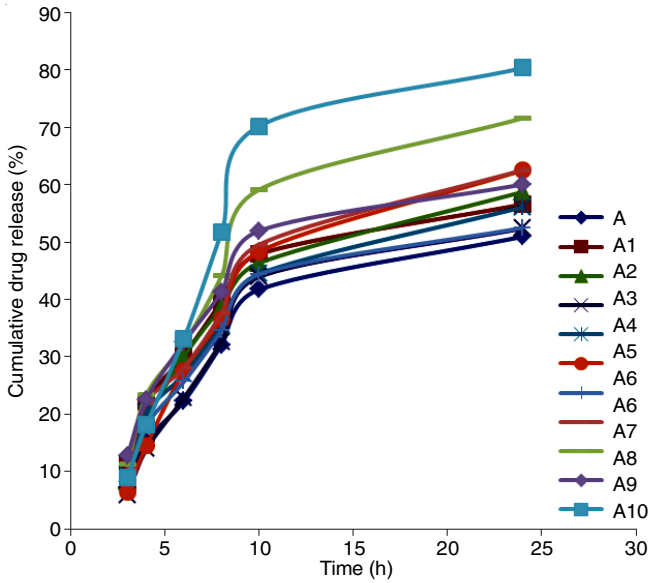


Fig. 2. *In vitro* release of paracetamol tablets in phosphate buffer pH 6.8

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