

Synthesis of Chitosan Succinate and Chitosan Phthalate and Their Evaluation as Suggested Matrices in Orally Administered, Colon-Specific Drug Delivery Systems

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Summary

The naturally occurring polymer chitosan was reacted separately with succinic and phthalic anhydrides. The resulting semisynthetic polymers were assessed as potential matrices for colon-specific, orally administered drug delivery. Sodium diclofenac was used as the dispersed model drug. The prepared matrices were incorporated into tablets, which were evaluated *in vitro*. The evaluation included dissolution studies conducted under simulated gastrointestinal conditions of pH and transit times. The percentage fluid uptake was used to indicate the ability of the matrix to protect an embedded drug from gastric juices. The prepared matrices resisted dissolution under acidic conditions. On the other hand, improved drug release profiles were observed under basic conditions. Therefore, the results suggest the suitability of the prepared matrices in colon specific, orally administered drug delivery system. However, future *in vivo* testing is planned to fully establish the suitability of the prepared polymers for colon-specific drug delivery.

Introduction

Colon-targeting drug delivery systems have applications in a number of therapeutic areas. These include topical treatment of colon diseases, and oral delivery of medicinal agents that are normally degraded while in the upper gastrointestinal tract. One of the important therapeutic applications of colon targeting delivery systems is in the treatment of disorders of the large intestines. For example, irritable bowel syndrome, colitis, Crohn's disease, colon cancer, and the various infectious diseases of the lower gastrointestinal tract. In such cases, it is necessary to attain high concentrations of the active agent in the large intestines. Oral pharmaceutical preparations, when used for this purpose, are frequently ineffective due to absorption and/or degradation of the active ingredients in the upper part of the gastrointestinal tract. On the other hand, rectal delivery forms, as alternatives, are not always effective due to their erratic local drug distribution profiles^[1]. Suppositories are only effective in the rectal vicinity due to their confined spread^[2], while enema solutions can only offer effective topical treatment to the sigmoid and descending colon^[3]. Rectal dosage forms are less convenient for the patients, particularly when compared to oral dosage forms.

The second important reason for the development of colon-targeting systems, is the fact that some labile systemically acting, orally-administered drugs exhibit poor absorption profiles due to their degradation in the upper part of the

gastrointestinal tract. Drastic pH conditions and the abundance of digestive enzymes in the upper gastrointestinal tract are the major factors behind their poor absorption profiles. Consequently, such agents are usually administered *via* the parenteral route.

The recent explosion in biotechnology, with the concomitant development of many new peptide and protein products, has increased interest in utilizing the colon as a site for drug absorption^[4]. Indeed, the large intestines might be the best site for peptide delivery because of the high residence time and the low activity of the digestive enzymes in this region.

Several potential colon-targeting systems have been developed using a variety of approaches. The most promising are techniques depending on drug coating with pH-sensitive^[5], bacterially-degradable hydrogels^[6] or other matrix systems.

Chitosan, which is partially deacetylated chitin (poly(*N*-acetylglucosamine)) (Figure 1a), and its various synthetic derivatives have recently attracted great interest in connection with the utilization of natural resources in pharmaceutical industry^[7]. Chitosan has been used in various pharmaceutical formulations, both as diluent in direct compression processes^[8] and as vehicle in sustained release systems^[9].

The main properties favoring the use of chitosan in various pharmaceutical preparations include its biological inertness^[10], biodegradability^[11], bioadhesive properties^[12], and its gel-forming properties at low pH ranges^[13]. Further, its chemical features as a polyaminoglycoside allow its physicochemical properties to be modulated by covalent links to different residues. The conjugation of chitosan to various medicinal agents is also facilitated by its nature as aminosugar polymer^[14].

We envisaged the possibility of shifting the optimum pH range for chitosan's gel-forming capacity from acidic to alkaline values. This shift could be carried out through covalently linking chitosan's backbone to acidic residues. Carboxylic acid residues are expected to interact with the neutral or slightly alkaline pH environment of the terminal ileum and the ileocecal junction, thus yielding potentially suitable matrices for orally administered colon specific drug delivery tablets.

In the research discussed here, the amino groups within the chitosan backbone were partially substituted with carboxylic acid residues. This work focuses on the preparation and evaluation of tablets based on chitosan succinate and phthalate conjugates (Figures 1b and 1c). Sodium diclofenac was used as the dispersed medicinal agent. The dissolution rates

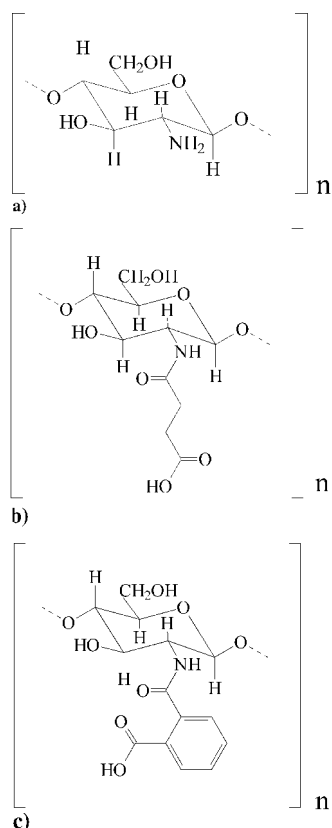


Figure 1: a) Structure of chitosan; b) structure of chitosan succinate conjugate; c) structure of chitosan phthalate conjugate.

of the prepared solid matrices were evaluated under conditions mimicking the gastrointestinal environment, i.e. regarding the different pH ranges and transit times.

However, the generated *in vitro* dissolution results are insufficient to fully establish the suitability of the prepared matrices for colon targeting, nevertheless, they provide good leads for further decisive *in vivo* studies.

Results and Discussion

IR Spectra and UV Measurements of Succinate and Phthalate Conjugates

The UV measurements on the 3N NaOH solutions of the chitosan conjugates indicate the degree of substitution to be 12.0% in the case of chitosan phthalate, and 8.1% in the case of chitosan succinate. The substitution difference is possibly due to solubility differences between phthalic and succinic anhydrides in the reaction media.

The IR spectra of the prepared chitosan conjugates, Figure 2 and Table 1, show amide carbonyl stretching in the range of $1660\text{--}1670\text{ cm}^{-1}$, and carboxylic carbonyl stretching in the range of $1710\text{--}1720\text{ cm}^{-1}$, indicating the formation of amide links with phthalate and succinate moieties. The selective acylation of the amino groups is probably due to their superior nucleophilic character compared to the surrounding hydroxyl groups.

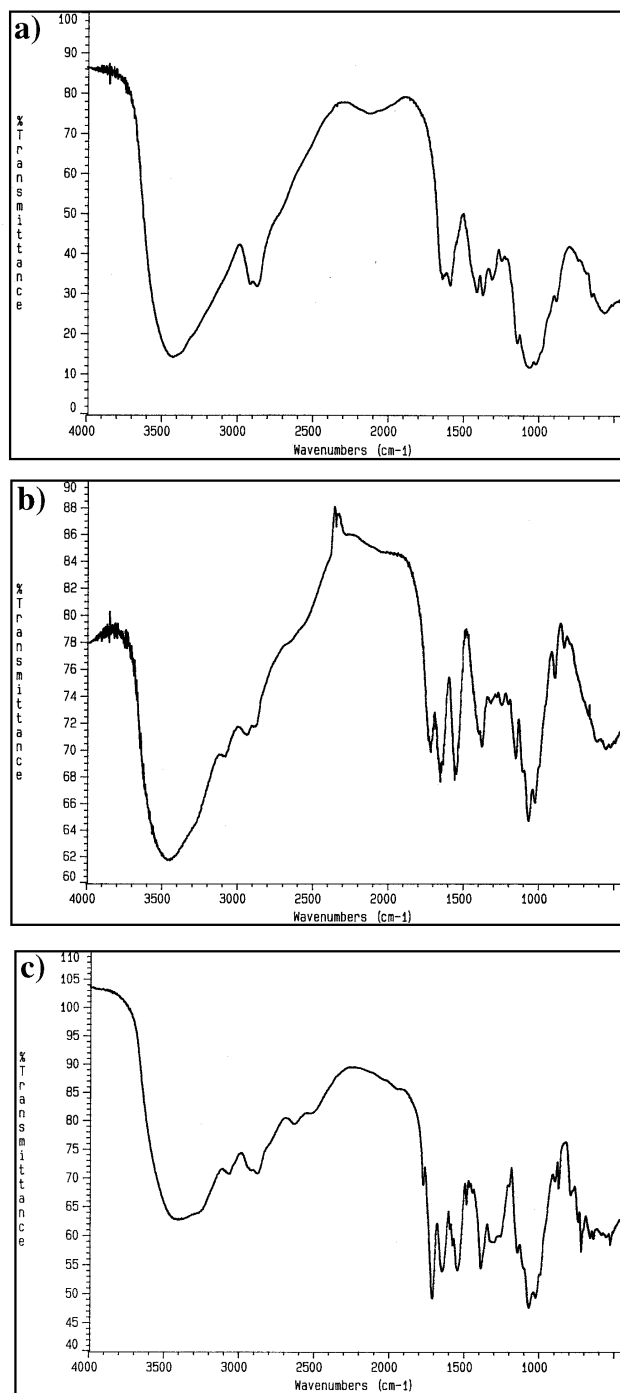


Figure 2: Infrared spectra of : a) chitosan; b) chitosan succinate; c) chitosan phthalate.

Tablet Water Uptake

Water uptake by tablets was used to elucidate the ability of the prepared matrices to protect an embedded drug from gastric juices. Fluid uptake was estimated by measuring the percentage weight increase after incubation in 0.1 M HCl for 2 h. It was observed that the fluid uptake decreases with the increase in the content of the chitosan derivative, as shown in Table 3. This behavior agrees with the explanation that higher polymer content is accompanied with denser and tighter inner

Table 1: Chitosan conjugation products and the corresponding IR stretches and degrees of substitutions.

Product	IR stretching frequencies (KBr disk, cm^{-1})	Degree of substitution (g %)*
Chitosan succinate	Amide carbonyl: 1669 and 1564 Carboxylic carbonyl: 1719	12.0
Chitosan phthalate	Amid carbonyl: 1659 and 1557 Carboxylic carbonyl: 1714 Aromatic ring: 1580	8.1

*The average values of three experiments.

Table 2: The prepared tablet formulas and the corresponding percentages of the different constituents.

Formula	1	2	3
Sodium diclofenac	33%	33%	33%
Lactose	56%	46%	36%
Chitosan derivatives*	10%	20%	30%
Magnesium stearate	1%	1%	1%

* Chitosan phthalate or chitosan succinate.

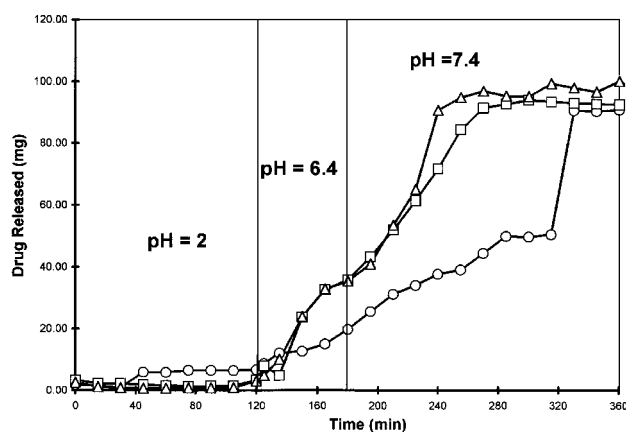
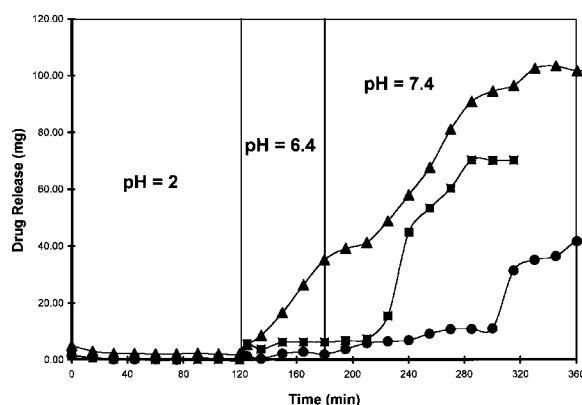
Table 3: Percent fluid uptake for tablets based on chitosan conjugates, expressed as percent weight increase after incubation in HCl (0.1 M) solution.

Chitosan derivative (%)	Weight Increase (%)	
	Chitosan * phthalate	Chitosan succinate*
10	5	7
20	1.4	3.3
30	0.3	1.6

*The average values of three experiments.

network of hydrogen bonds and other dipolar interactions within the polymer matrix. Such interactions are expected to impede water penetration through the matrix, particularly under acidic conditions, since the carboxylic acid residues within the polymer will exist predominantly as the poorly hydrophilic unionized form. It remains to be mentioned that matrices made up of unmodified chitosan dissolved completely in the acidic medium (0.1 M HCl for 2 h), which is not unexpected, as the amino groups within the chitosan structure are predicted to mainly exist as the highly hydrophilic quaternary ammonium cations under acidic conditions. Consequently, allowing unhindered water penetration through the polymer matrix and its eventual dissolution.

From Table 3 it is evident that the fluid uptake was higher in chitosan succinate matrices than in chitosan phthalate. This behavior agrees with the superior hydrophilic character of the succinate moieties. The hydrophobic aromatic rings within phthalate moieties are expected to hinder water penetration.

**Figure 3:** Release profiles of diclofenac sodium from matrices containing different chitosan succinate percent ratios, and at different pH values: Δ 10%, \square 20%, \circ 30%.**Figure 4:** Release profiles of diclofenac sodium from matrices containing different chitosan phthalate percent ratios, and at different pH values: \blacktriangle 10%, \blacksquare 20%, \bullet 30%.

Matrix Hydration and Drug Release

The release of diclofenac sodium from tablets based on chitosan succinate or phthalate was at its highest levels under the alkaline conditions of pH 7.4, as reported in Figures 3 and 4. Moderate drug release was observed under the slightly acidic conditions of pH 6.4. However, almost complete cessation of drug release is observed at pH 2. This behavior agrees with the following explanation. Under alkaline conditions the carboxylic acid moieties are de-protonated yielding hydrophilic carboxylate anions. Hydration of the carboxylate residues promotes the dissolution and the subsequent release of the matrix-dispersed diclofenac sodium. However, under acidic conditions, the carboxy moieties are either partially ionized (at pH 6.4) or predominantly unionized (at pH 2). The uncharged carboxylic acid groups are considerably less hydrophilic compared to their charged conjugate-bases, i.e. the carboxylate anions. Therefore, the modified chitosan matrices acted as expected under acidic conditions, and resisted hydration and the subsequent release of imbedded drugs.

Figures 3 and 4 clearly indicate that the drug release rates are inversely proportional to the amount of the chitosan polymer in each tablet. Maximal drug release profiles are reported for the formulas with the least polymer content and *vice versa*. This behavior is attributed to the thickness of the

polymer depth that has to be hydrated to allow drug release. Higher polymer contents correspond to thicker barriers for hydration and consequently lower drug release rates.

Finally, from Figures 3 and 4, it is evident that under similar provisions of media pH and chitosan content, chitosan succinate matrices released diclofenac sodium at higher rates than chitosan phthalate. The differences can be explained based on the different hydrophilic properties of phthalate and succinate residues. Succinate side chain is undoubtedly more hydrophilic compared to the bulkier aromatic phthalate. Accordingly, succinate residues allow improved polymer hydration with the concomitant improve in drug release rates.

It should be mentioned that matrices made up of chemically unmodified chitosan dissolved completely under acidic conditions (0.1 M HCl for 2 h), as mentioned earlier. Thus, it is not expected that such matrices will stay intact and continue to protect the drug while passing through the gastric juices.

Conclusion

Chitosan succinate and phthalate matrices showed pH dependent release profiles of the entrapped diclofenac sodium. Maximum drug release was observed under pH 7.4, in contrast to pH 2 at which the matrices resisted dissolution. Higher polymer content was found to be associated with reduced drug release rates and fluid uptake. Chitosan phthalate resisted drug release and fluid uptake to a higher extent than chitosan succinate, suggesting the suitability of chitosan phthalate as a supporting matrix for colon-specific drug delivery systems. Further *in vivo* tests are required to fully establish the suitability of the prepared semisynthetic polymers for colon-specific drug delivery, particularly regarding the biodegradability of chitosan phthalate and succinate.

Acknowledgments

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Experimental

Materials and Methods

Materials

Low molecular weight chitosan (molecular mass of 70000) and reagent grade succinic anhydride, phthalic anhydride, and sodium diclofenac were all purchased from Fluka-Aldrich, and were used without further purification. Ultraviolet spectra were recorded on a Cary 1E UV-visible spectrophotometer. Infrared spectra were recorded on a Hitachi 270-50 IR spectrophotometer.

Preparation of Chitosan Succinate and Chitosan Phthalate Conjugates

The reaction was carried out according to the previously reported method^[15]. Briefly, chitosan (1.00 g, corresponding to approximately 6.20 mmols glucosamine) was dissolved in HCl aqueous solution (0.37%, 50 ml) at ambient temperature, and a solution of the anhydride (6.25 mmol; succinic 0.63 g, phthalic 0.92 g) in pyridine (5 ml) was added dropwise with vigorous stirring. The reaction pH was maintained at 7.0 by the dropwise addition of NaOH solution (1.0 M). NaOH addition was continued till the pH was stabilized. After 40 min the reaction was terminated by the addition of NaCl aqueous solution (20%, 200 ml). The resulting precipitate was filtered, washed with acetone and diethyl ether, and desiccated to give chitosan succinate or chitosan phthalate conjugates.

Determination of Degrees of Substitution on Chitosan and UV Analysis

The degrees of phthalate or succinate substitution on chitosan were determined as follows: Chitosan conjugates (0.10 g) were completely hydrolyzed in a NaOH aqueous solution (3.0 M) and over 48 h. The concentrations of phthalic and succinic acids in the hydrolysis solutions were determined by UV measurement, at $\lambda = 232$ nm for phthalic acid and $\lambda = 228$ nm for succinic acid. Non-conjugated chitosan was treated in the same way, and the resulting solution was used as a blank. The degree of substitution (expressed as g%) is defined as the ratio of the measured amount of phthalic or succinic acid (in grams) in the hydrolysis solution, to the amount of the hydrolyzed chitosan conjugates (in grams). Table 1 shows infrared data and degrees of substitution for each product.

Tablet Preparation

A total of six formulations were prepared. In each case, sodium diclofenac was dissolved in a predetermined volume of NaOH aqueous solution (0.1 N). Then, the particular chitosan conjugate (phthalate or succinate) was dispersed in the prepared alkaline drug solution. Subsequently, the resultant dispersion was added to lactose and suitably kneaded in a laboratory mixer. The moist mass was then forced through a 1.7 mm stainless steel sieve. The resulting granules were dried at 50 °C in a Manesty hot air oven over 3 h, during which time a constant dry weight was attained in each case. Each batch of dry granules was passed through a nest of stainless steel sieves suitably arranged on a sieve vibrator (Endecotts). The instrument was switched on to separate the granules into various size fractions. In order to give each batch of granules the same treatment prior to compression, the following procedure was carried out. The dry granule mix was constituted as follows: 0.25 mm undersize, 10%; 0.25 to 0.5 mm, 30%, and 0.5 to 1.00 mm, 60% w/w. The granules of 0.25 mm undersize were mixed, in each case, with the appropriate quantity of magnesium stearate. The coarse granules were then added to the tumbling mixer and the mixing was carried out for 5 min. The granule mix was compressed into tablets of the target weight 300 ± 5 mg in a Manesty F-3 tableting machine fitted with 9.5 mm biconcave punches. The compression pressure level was kept constant for all the batches by adjusting the pressure control knobs to the same setting. The machine speed was set to produce 120 tablet/min. Table 2 illustrates the quantities (expressed as w/w%) of the different constituents in each prepared formula.

Assessment of Tablet-Fluid Uptake

Six tablets of each of the formulations were weighed and incubated in 0.1 M HCl at 37 °C in shaking water bath (100 strokes/min), after 2 h, the tablets were re-weighed and the percentage of fluid taken up by each tablet was calculated.

Table 3 shows the different formulations with the corresponding weight increase due to fluid uptake.

In Vitro Dissolution Tests

The dissolution tests were carried out in a continuous laminar flux apparatus^[16]. Three buffered dissolution media were used over three subsequent stages: pH 2 for 2 h, then pH 6.4 for 1 h, then pH 7.4 for 3 h. The dissolution bath was maintained at 37 °C. In each stage the dissolution medium was pumped (16 ml/min) from a reservoir upwards into the cell containing the tablet, then through a filter to reach the analyzing system containing the spectrophotometric flux cell. The flux cell is adapted for continuous evaluation of the drug absorbance, at $\lambda = 273$ nm for pH 2, and $\lambda = 275$ nm for pH 6.4 and 7.4.

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