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Original Article

Preparation and Characterization of Theophylline-Chitosan Beads as an Aapproach to Colon Delivery

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Abstract

Chitosan with excellent biodegradable and biocompatible characteristics has received attention as an oral drug delivery vehicle for controlled-release formulations. In this study an enteric-coated capsule containing theophylline-chitosan beads based on 2^3 factorial designs was prepared as a colon drug delivery system. The theophylline-chitosan gel beads were formulated by adding the drug-containing solution of chitosan into tripolyphosphate solutions, dropwise. The obtained beads were washed with water and freeze-dried before filling into the capsules. Eudragit® S100 was then used to enteric-coat the prepared capsules. Drug entrapment efficiency and the effects of different variables including: bead morphology, swelling behavior of the beads and the release behavior of the system on these parameters were investigated. Results showed that the highest and lowest swelling ratio is obtained at pH 4.5 and 7.2, respectively. These studies have shown that chitosan concentration and drug polymer weight ratio significantly affect the drug entrapment. Decreasing the drug solubility in external phase caused a significant increase in drug loading. External phase saturation with theophylline and tripolyphosphate, as well as decreasing temperature, have increased drug loading. Furthermore, the lowering of temperature had a significant effect on bead's hardness. The release of theophylline from freeze-dried beads filled in enteric-coated capsules was also investigated. Release of theophylline was prolonged with saturation of both drug and tripolyphosphate in the external phase. Results showed that the release of theophylline from chitosan beads is possibly due to more than one mechanism, possibly dissolution, diffusion and relaxation of the polymer chains.

Keywords: Chitosan; Bead; Drug delivery; Colon; Theophylline.

Introduction

Colonic drug delivery (CDD) for either local or systemic effects has been the subject of much research over the last decade. This method of drug delivery has several advantages including protection of drug from harsh environment of stomach and small intestine, avoiding drug absorption from upper GIT and increased bioavailability of some drugs. CDD is performed using different polymers through various drug delivery systems such as chitosan beads, which are the subject of the present investigation.

Chitosan, a cationic polysaccharide obtained from chitin, is a suitable polymer for drug delivery. Chitin is one of the most important natural polysaccharides and is found in crustaceous shells or in cell walls of fungi. This polysaccharide has been used in food industry, medicine and drug delivery systems. However, due to its low solubility in many common

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solvents, chitin is not widely used for industrial applications (1, 2). Chitosan, obtained by deacetylation of chitin, is soluble in aqueous acidic media due to the presence of amino groups. Bioadhesive properties, biocompatibility and biodegradability of chitosan, have made this polymer a potential and suitable carrier for biomedical and drug delivery applications (3). This polymer has also been used in oral sustained release formulations and implantable drug delivery systems (3). In addition, it has been shown that chitosan can interfere with dietary fat absorption (4).

Nagai et al. (5) used chitosan with other excipients for preparation of controlled release tablets and found that the drug release rate was directly proportional to the amount of chitosan within formulations. Nigalaye et al. (6) prepared theophylline sustained release tablets using a hydrocolloidal matrix system of chitosan, carbomer-934P and citric acid and showed that at concentrations above 50% (w/w), chitosan formed an insoluble non-erosive type matrix; whereas, at lower concentrations (less than 33%) a fast-releasing matrix system was obtained. Miyazaki et al. (7) found that the addition of sodium alginate to chitosan-containing tablets could improve their extended release property. Similar results have also been published by Kawashima et al. (8), who suggested that citric acid could form chitosan gel and, thereby, improve the sustained release properties of the system.

Beads with spherical shape prepared by complexation between positively charged macromolecules, such as chitosan and negatively charged molecules, like tripolyphosphate (TPP) has received attention as a controlled release drug delivery system (9). Tripolyphosphate with negative charge is able to interact with cationic chitosan through electrostatic forces (10). Thus, not only reversible physical crosslinking is substituted for chemical crosslinking, the possible toxicity of reagents and other undesirable effects could also be prevented.

Preparation of TPP/chitosan complex through the addition of chitosan droplets into a tripolyphosphate solution has been reported by Bodmeier et al. (9). Aral and Akbuga (11) have produced strong and durable TPP/chitosan beads by coating the bead's surface with sodium alginate to form a polyelectrolyte complex film. Shu and Zhu (12) reported a novel approach to prepare TPP/chitosan beads for controlled release drug delivery. Their studies showed that the prepared TPP/chitosan beads had a more homogeneous structure and beads were strengthened greatly. Sezar and Akbuga (13) studied the effect of different variables such as drug concentration, type and concentration of chitosan, pH value of TPP solution, volume of internal and external phase, gelation time and drying condition on various properties of chitosan beads. They showed that concentration of both chitosan and TPP have an effects the drug loading. The structure and strength of the beads might be dependent on the gelation time and drying condition.

Certain polymers, with a charged moiety, are pH-sensitive and can be used as coating agents to protect contents of tablets, capsules or pellets from gastric fluid and can, therefore, be used for colon targeting. A number of commercially available methacrylic resins, popularly known as Eudragit, are being used for colon-targeted formulations. Methacrylic acid- methyl metacrylate copolymers, (Eudragit® S), contains 30% methacrylic acid units and dissolves at pH values higher than 7.0. This polymer is a suitable coating agent for colon drug delivery system.

The main aim of the present investigation was to prepare a suitable enteric-coated capsule containing theophylline-chitosan beads for colon delivery. Furthermore, the effect of some factors, such as the concentration of chitosan and TPP, as well as the drug: polymer ratio on drug loading of theophylline-chitosan beads was investigated by utilizing the 2^3 factorial designs.

Experimental

Materials

Chitosan (98% deacetylated, viscosity of 1% *w/v* solution, 264 mPa.s) was a gift from Primex (Iceland). Tripolyphosphate was purchased from Sigma (Vienna, Austria). Eudragit® S100 was a gift from FMC. Other chemicals and solvents were of pharmaceutical or analytical grades, and used as received.

Table 1. Factors used in the factorial design experiment.

Factor	Low level	High level
Chitosan concentration (%) (X1)	1.5	2
Tripolyphosphate solution (%) (X2)	5	10
Drug:polymer weight ratio (X3)	0.5:1	1:1

Equipment

Spectrophotometer (Shimadzu 1201, Japan), pH-meter (Corning 120, UK), freeze-drier (Rewart Edwards High Vacuum 30P.2.T.S N114, UK).

Methods

Characterization of chitosan

Two different methods were used to determine the degree of deacetylation (DD). According to a modified acid-base titration method (14), chitosan (0.50 g) was dissolved in 20.0 mL 0.10N HCl and titrated pH-metrically with a standardized solution of a 0.10 N NaOH solution. The curve constructed has two equivalent points related to the excess of HCl and the protonated amino groups. DD was calculated based on equation 1.

$$DD = 16.1\ (Y-X)\ f/w \qquad \text{(Eq.1)}$$

Where Y and X are the consumed NaOH volume at the equivalent points (mL), f is molarity of the NaOH solution and w is the initial weight of chitosan (g).

Infrared spectroscopy was also used for determining DD according to a previously reported method (15).

Molecular weight determination

For determination of the chitosan average molecular weight (MW), five various concentrations of chitosan solution in acetic acid-sodium acetate buffers were prepared. The relative viscosity was obtained with a capillary viscometer at 30 ± 0.05ºC. Next, the intrinsic viscosity was determined and the molecular weight of chitosan was calculated based on the Mark-Houwink equation (16)

$$[\eta]=k.MW^{a}, \qquad \text{(Eq.2)}$$

Where $k= 1.64\ 10^{-30}\ DD^{14}$
and $a= -1.02\ 10^{-2}\ DD +1.82$.

Table 2. Types of freeze-dried bead formulations based on the 2^3 factorial design experiment. Drug loading (run in triplicate) was selected as the dependent variable (Y).

Formulation Code	X1	X2	X3	Y % (mean±sd)
F1	1.5	5	0.5	4.7±0.32
F2	2	5	0.5	16.93±0.85
F3	1.5	10	0.5	11.52±0.45
F4	1.5	5	1	22.66±0.22
F5	2	10	0.5	13.75±0.54
F6	2	5	1	28.39±0.43
F7	1.5	10	1	12.12±0.64
F8	2	10	1	40.15±0.64

Factorial design experiments

Chitosan beads were obtained based on the 2^3 factorial design. Chitosan concentration (X1), tripolyphosphate concentration (X2) and drug:polymer weight ratio (X3) were selected as independent variables (Table 1). The drug entrapment efficiency of the beads (Y) is the response parameter or the dependent variable (Table 2). Furthermore, release studies as well as the saturation effect of external phase with tripolyphosphate or theophylline on drug loading were investigated.

Preparation of chitosan beads

Initially, 200 mg chitosan was dissolved in 10 ml of a 1% acetic acid solution under stirring for 20 min at room temperature. Then, theophylline was dispersed in this solution and finally theophylline-chitosan mixture was added dropwise the into tripolyphosphate aqueous solution at room temperature, using a syringe. The formed beads were allowed to stand in the tripolyphosphate solution for 15 min to be cured. The beads were separated with paper filter, then washed twice with water and dried by freeze- drying.

Coating process

Dried beads (500 mg) were placed in hard capsules (size 1) and coated using the pan coating procedure. First, Eudragit® S100 was dissolved in acetone, then 1% triethyl citrate (as plastisizer) was added and stirred to obtain a homogeneous solution. Spray coating was carried out using a coater made of stainless steel with an atomizing nozzle of 0.5 mm in diameter. Compressed air with a pressure of 2 bars was used to atomize the coating solution with a spray rate of 0.8 g/min. The inlet temperature and coating time were 50ºC and 30 min, respectively. Coating weight and thickness were

determined to be 45±3 mg and 0.32±0.01 mm, respectively (data are Mean±SD, n=3).

Particle size determination

For each formulation, diameter of 100 beads were measured with a micrometer and the mean particle size was determined.

Drug loading

Beads were initially broken in pH 1.2 HCl and, after filtration; their theophylline-contents assayed spectrophotometrically at 272nm.

Drug release studies

The release of theophylline from freeze-dried beads filled in enteric-coated capsules was studied using the USP basket method (Apparatus I) at 50 rpm and in 900 mL of dissolution fluid at 37 ± 0.5ºC. Six coated capsules were tested in pH 1.2 simulated gastric fluid (SGF) for the first 1.5 h, pH 6.0 phosphate buffer for the second 1.5 h and pH 7.2 phosphate buffer for the remaining period of time (4.5 h). At set intervals, 5 ml samples were removed and replaced with equal volumes of the buffer solution. The amount of drug released was measured spectrophotometrically at 272 nm. The amount of theophylline released was plotted against time in different media.

Results and discussion

The average molecular weight and intrinsic viscosity of the starting chitosan calculated from a DD-dependent Mark-Houwink relationship (16), was determined to be 10.26 10^5 g/mol and 1050 cm^3/g, respectively.

Two different methods, pH-metric titration and infrared spectroscopy were used to determine the percentage of chitosan deacetylation. The value resulted from the pH-metric titration (DD 0.94) was inagreement with the FTIR (DD 0.91) spectroscopic method, both matching the value reported by the manufacturer (0.98). In the IR spectrum (Figure 1), the amide bond at 1655 cm^{-1}, representing the N-acetyl group content, and the hydroxyl bond at 3450 cm^{-1}, as an internal standard, were used to determine the percentage of acetylated amine groups. The percentage of acetylated amine groups were calculated by equations 3 and 4, as follows: (15)

$$\%N\text{-acetyl}=(A_{1655}/A_{3450})(100/1.33) \quad (3)$$

$$DD= 100-\%N\text{-acetyl} \quad (4)$$

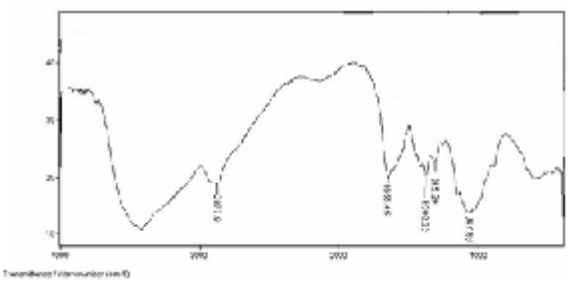

Figure 1. FTIR spectrum of chitosan.

After freeze-drying, all of the beads were found to be spherical. The mean particle size of eight different formulations were between 0.642±0.019 to 0.825±0.011 mm. The particle size of different formulations is depicted in Figure 2. Scanning electron microscopy (SEM) was used to investigate the morphology of chitosan beads (for mulation F8). The surface of dried beads seemed to be smooth and did not shrink during the freeze-drying process (Figure 3).

Chitosan, with a polycationic characteristic, forms gel beads with the negatively charged tripolyphosphate counterion. These studies have shown that the shape and preparation of the beads were critically dependent on the viscosity of the chitosan, as well as the concentration of tripolyphosphate solution. When a 1% chitosan solution was used, no beads were formed. However, smooth beads were obtained upon dropping 1.5 and 2% chitosan solution into different concentrations of tripolyphosphate solutions.

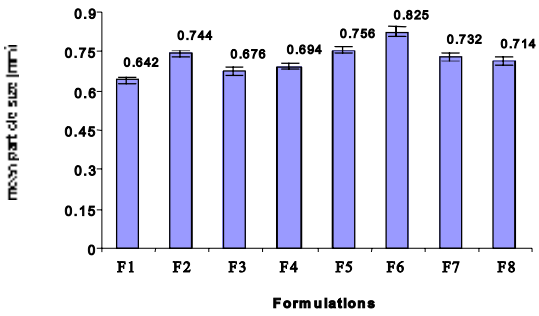

Figure 2. Mean particle size of different formulations of beads.

Table 3. Results of the analysis of variance for 2^3 factorial experiments (run in triplicate).

Source of variation	Exp. 1	Exp. 2	Exp. 3	d.f.	Mean square	F ratio	Significance
F1	4.8	4.6	4.9	-	-	-	-
F2	14.1	13.9	13.6	1	1969.101	1542.976	0.000
F3	11.4	11.3	11.7	1	876.163	686.556	0.000
F4	12.9	10.6	11.2	1	686.833	538.198	0.000
F5	33.2	35.8	36.7	1	60.153	47.121	0.000
F6	32.5	34.5	30.1	1	17.019	13.336	0.002
F7	22.9	22.8	22.4	1	22.835	17.893	0.001
F8	42.1	40.8	41.6	1	104.125	81.592	0.000
Experimental. error				16	1.276		

Bodmeier et al. (9) examined the effect of pH on drug loading of some model drugs. They showed that pH changes the drug entrapment efficiency due to the increase in drug solubility within the external phase. However, theophylline with a pKa of 8.77 did not show significant changes by changing the pH of the external phase between 4 and 10.

To study the swelling ratio of the formulations prepared, beads were dispersed in various solutions with different pH and their swelling property was investigated visually. The highest swelling ratio was obtained at pH 4.5, whereas at pH 7.2, beads swelled slowly but they were broken after 2-3 h (Figure 4).

Various formulations (F1-F8) were prepared using the 2^3 factorial design procedure. An optimum drug loading of 40.15% ± 0.64 (n=6) was obtained with formulation 8 (Table 2). Three more formulations (F9-F11) were also prepared to increase the drug entrapment efficiency by different methods. In formulation 9, the external phase was saturated with tripolyphosphate and as result the loading increased up to 51.37%±0.45. Surprisingly, saturation of theophylline in the external phase caused a tremendous shift in drug loading, up to 91.71%±1.2 in formulation 10. Furthermore, decreasing the external phase temperature increased the drug entrapment to 78.61%±0.78 (formulation 11). Table 3 shows the results of ANOVA for the 2^3 factorial design experiments. All factors and interactions have significant effect on drug loading ($P<0.005$). Increasing the chitosan concentration caused an increase in drug loading, possibly due to the higher ability of gel formation. Tripolyphosphate concentration also showed a significant effect on drug content at drug/ polymer weight ratio of 0.5:1. This might explain why more tripolyphosphate was needed to obtain the required gel strength when the drug:polymer ratio was increased. Furthermore, saturation of the external phase with tripolyphosphate or theophylline caused a significant increasing in drug loading. This could be due to reduced concentration gradient and, therefore, reduced diffusion of drug toward beads surface and thereby dissolution in the external phase. Reduction of external phase temperature also caused an increase in drug entrapment in formation F11, possibly due to the reduction in of theophylline solubility within the external phase. Nevertheless, lowering the temperature could also increase the stiffness of the beads. Freeze-dried beads showed a higher drug loading in comparison to the air-dried beads. This may be due to the migration of theophylline in the air-dried method. Theophylline could migrate with water to the surface of the beads in the air-dried method and the beads could shrink after water evaporation. In contrary, in the freeze-drying process, since just the frozen water molecules sublimate, the drug could not migrate to the surface and beads are intactly solidified.

Since formulations F8-F11 had the highest drug entrapment efficiencies, they were selected for release studies. Release profiles of theophylline from the prepared chitosan beads

Table 4. Kinetic constants (k), diffusional exponents (n) and determination coefficient (r^2) determined by the linear regression of ln (M_t/M_∞) against ln t.

Formulation	n(X± SD,n=3)	k(X± SD,n=3)	r^2
F8	0.5776±0.0271	0.0118±0.0060	0.980
F9	0.7780±0.0327	0.0138±0.0033	0.965
F10	0.5889±0.0334	0.0105±0.0077	0.989
F11	0.4908±0.0802	0.0152±0.0056	0.965

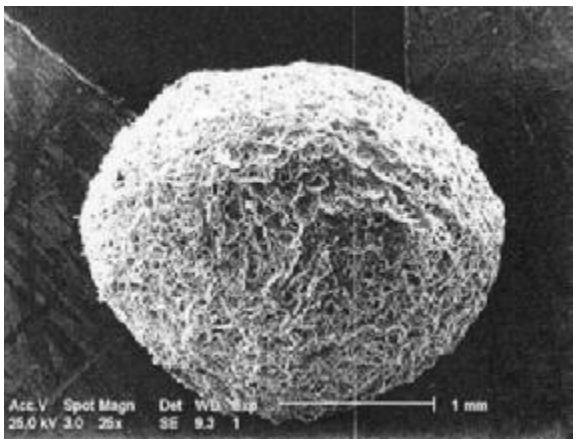

Figure 3. Scanning electron micrographs (SEM) of chitosan beads prepared after the freeze-drying process (Formulation 8), using a magnification of x 25.

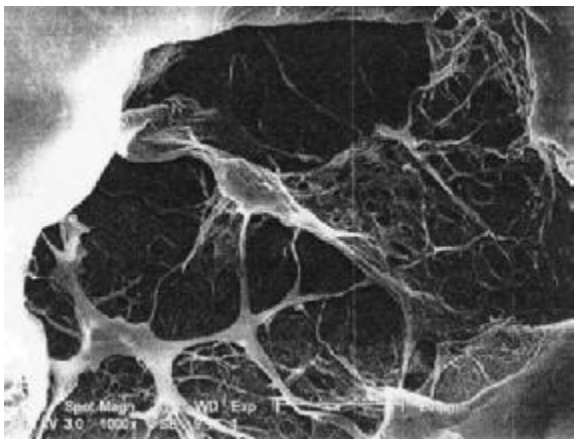

Figure 4. Scanning electron micrographs (SEM) of chitosan beads prepared in aph 7.2 phosphate buffer solution (Formulation 8), under a magnification of x 1000.

(formulations 8, 9, 10 and 11) are depicted in Figure 5. Since Eudragit® S100 is a pH-dependent polymer and is not sensitive to low pH, the release of theophylline from the enteric-coated capsules in simulated gastric medium and pH 6.0 phosphate buffer solution was not significant. However, coated capsules were degraded and the beads began to release their theophylline after a short lag time and with a fast rate, when exposed to pH 7.2. phosphate buffer solution. The release profile (Figure 5) shows a lag-phase and two different release phases. The lag-phase reveals a delay in release, as was expected from the pH-dependent system used. The other two phases are related to the release of drug from the matrix. During the first release phase, nearly 70% of the entrapped drug was released over a period of less than 40 min, which might suggest a fast phenomena like diffusion in porous system, dissolution of drug or

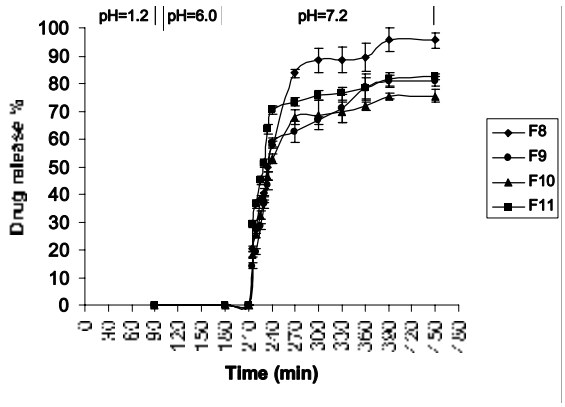

Figure 5. Release of theophylline from chitosan beads filled in enteric-coated capsules (formulations 8, 9, 10 and 11, n=6, mean ± s.p).

dissociation of drug-polymer molecular complexes. As analysis of data shows that $0.5<n<1.0$, combination of the above-mentioned mechanisms are expected, which all are highly possible in our system before swelling. After this first step, release rate decreased several times. This might be due to swelling of the polymers, which is expected to reduce the release rate. The swelling observed with the system (data not shown) supports this suggestion. As the system shows a constant release rate, the decreased rate is surely not due to the decreased thermodynamic activity or concentration gradient. After swelling, either diffusion through throws a swollen matrix or the process of swelling itself could be the rate limiting step. Our analysed data for this part shows that n is nearly 1.0 (data not shown), revealing that most of the release is being controlled by swelling, which is expected for the polymer. Gupta et al. Investigated the drug release behavior of chitosan beads (17). Their studies showed that the release of diclofenac sodium depends greatly on the swelling of the beads. Furthermore, at pH 7.2-7.4, there is a very limited swelling; thus the drug entrapped within the beads can not be released easily.

As shown in Figure 5, saturation of the external phase with tripolyphosphate in formulation No. 9 decreases drug release in comparison to formulation F8; which could be due to an increased degree of crosslinking. Furthermore, an increment of bead hardness in formulation 11 caused a decrease in dissolution

rate, possibly due to the slower penetration of the medium in to this formulation.

Fickian and non-Fickian (anamolous) behaviors have been used for determining the mechanism of drug release from polymeric systems. It is difficult to determine the exact release kinetic in such complex systems. However, we tried to analyze the drug release data, using a general equation as follows:

$$M_t/M_\infty = kt^n \qquad (5)$$

where M_t is the amount of drug released in a given time, M_∞ is the total amount of theophylline within the beads, k and n are equation constants and t is the time. Also a logarithmic form of this equation could be used in these systems (18). The initial section of the release curves (Figure 5) ($M_t/M_\infty<0.6$) was analyzed by this equation and the equation constants were determined (data are presented in Table 4). For all formulations, the values of the exponent n were between 0.5 and 1, indicating a non-Fickian transport. This suggests that transport is possibly controlled by diffusion and/or relaxation of the polymer chains.

Similar results have also been reported by takka et al. (19), who investigated the release mechanism of nicardipine-alginate gel beads. Their investigation showed that values of the exponent n lies between 0.5 and 1.0, which indicate a non-Fickian transport controlled by diffusion and relaxation of the polymer.

Conclusion

Chitosan, a natural biocompatible and biodegradable polymer, was used as a vehicle for the preparation of theophylline beads and the effect of different formulation variables on properties of system prepared was investigated. Results have indicated that the saturation of the external phase with theophylline, tripolyphosphate and a decrease in temperature of external phase could affect the properties of the system. It was also shown that the release of theophylline from chitosan beads was governed with more than one mechanism (possibly dissolution, diffusion and relaxation of the polymer chains). Data obtained shows that all variables should be taken into account during the formulation of such a system.

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