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PREPARATION AND EVALUATION OF DRUG-CONTAINING CHITOSAN BEADS.

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ABSTRACT

Sulfadiazine beads were prepared by dropping drug-containing solutions of the positively charged polysaccharide, chitosan, into tripolyphosphate (TPP) solutions. The droplets instantaneously formed gelled spheres by ionotropic gelation, entrapping the drug within a three-dimensional network of the ionically linked polymer. To achieve maximum drug content, high payloads, short gelation times, low TPP concentrations, and a low internal to external phase ratio were required. The chitosan beads showed pH-dependent swelling and dissolution behavior. The beads swelled and dissolved in 0.1N HCl, while they stayed intact in simulated intestinal fluid. The release of sulfadiazine in 0.1N HCl decreased with increasing concentration of TPP, but was independent of the TPP concentration in intestinal fluids. The morphology of the beads was investigated by scanning electron microscopy. The porosity of the beads depended on the method of drying.

INTRODUCTION

Chitosan ((1->4)-2-amino-2-deoxy-ß-D-glucan) is a hydrophilic, cationic polyelectrolyte prepared by N-deacetylation of chitin (Figure 1). Chitin is the most plentiful natural polymer next to cellulose and it is obtained from crab and shrimp shells. Chitosan and chitosan derivatives have been used in enzyme and cell immobilization, as flocculants, in the preparation of reverse osmosis membranes, in waste water treatment, and in personal care products (1, 2). In addition, chitosan has a variety of promising medical and pharmaceutical applications. Biomedical

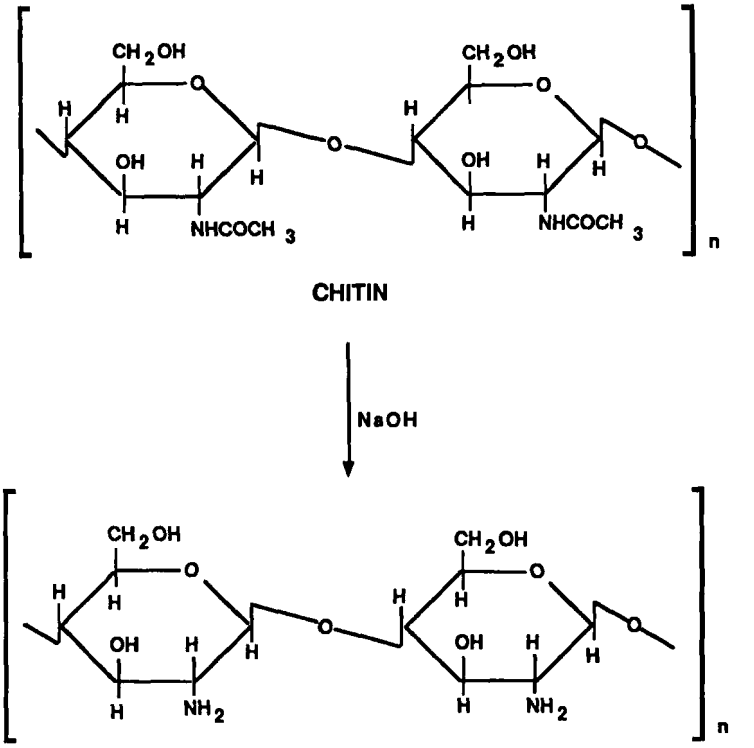


FIGURE 1
Structures of chitin and chitosan.

applications of chitin and chitosan include wound and burn healing, soft and hard contact lenses, and artificial kidney membranes (3). Chitosan has been used as a direct-compression diluent (4), as a vehicle for sustained release of drugs (5-7), as well as for the enhancement of the dissolution rate and bioavailability of water-insoluble drugs (4). Machida et al. studied the enzymatic degradation of chitosan and hydroxypropyl-chitosan *in-vitro* and *in-vivo* and concluded that chitosan might be utilized as a biocompatible and biodegradable carrier for implantable drug delivery systems (8). Heller et al. developed an enzyme-degradable, self-regulated drug delivery system based on partially deacetylated chitin (9).

Sodium alginate beads were prepared by gelling the anionic polysaccharide with $CaCl_2$ solutions to entrap cells (10). In the present investigation, chitosan beads containing sulfadiazine or quinidine were prepared by dropping drug containing chitosan solutions into tripolyphosphate solutions. The interaction of the positively charged chitosan molecules with the anionic counterion, tripolyphosphate, caused the formation of gelled spheres.

The objective of this study was to investigate variables affecting the preparation of, the drug release from, and the morphology of the chitosan beads. Potential pharmaceutical applications could include both sustained and enhanced oral drug delivery systems, depending on the solubility characteristics of the drug.

MATERIALS AND METHODS

Materials

Three different grades of chitosan (Protan Laboratories, Redmond, WA) with viscosities of 50, 750, and 2790 cps (1% w/w in 1% v/v acetic acid) and degrees of deacetylation of 81%, 84%, and 80.4%, respectively, were used. The following chemicals were obtained from commercial suppliers and used as received: caffeine

(MCB Manufacturing Chemist, Inc., Gibbstown, N.J.), quinidine, salicylic acid, sulfadiazine, and tripolyphosphate (Sigma Chemical Co., St.Louis, MO.).

Methods

The drug (1% w/w) was dissolved (caffeine, salicylic acid, or quinidine) or dispersed (sulfadiazine) in a solution of chitosan (1% w/w) in dilute acetic acid (1% v/v). The beads were formed by dropping the bubble-free solution or dispersion (10 ml) through a disposable syringe onto a gently agitated tripolyphosphate solution (100 ml). The chitosan beads were separated after one hour by filtration and briefly rinsed with deionized water. The drying of the gel-like beads was accomplished by either freeze-drying or air-drying for 24 hours followed by oven-drying at 60°C for 6 hours. All batches were prepared in triplicate.

The following preparative variables were investigated in this study: theoretical sulfadiazine loading (10, 20, 30, 40, 50, 75, 90 %), stirring time (0.5, 1.0, 2.0, 3.0, 4.0 hours), TPP concentration (0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 10.0 %), and amount of sulfadiazine added to 100 ml of 2% TPP solution (0, 100, 150, 200 mg).

The sulfadiazine or quinidine content of the beads was determined spectrophotometrically after dissolving the beads in 0.1 N HCl, at λ = 242nm or 251nm, respectively.

The solubilities of sulfadiazine and quinidine were determined by placing an excess of drug in contact with the desired medium. Duplicate samples were shaken for at least 48 hours at 22°C. The samples were filtered and solubilities were obtained by measuring the concentration of the drugs spectrophotometrically after appropriate dilution in 0.1 N HCl.

The release properties of the freeze-dried beads were studied in 0.1N HCl or simulated intestinal fluid (I.F.) (USP XXI) using the USP XXI rotating paddle

apparatus (500 ml, 37°C, 50 rpm). The beads (30 - 100 mg) were added to the dissolution medium and samples of 3 ml were taken and replaced with fresh medium at predetermined time intervals. The maximum concentration of sulfadiazine in the release medium was less than 5% of its solubility. All samples were run in triplicate and assayed spectrophotometrically either directly or after appropriate dilution with 0.1N HCl or simulated intestinal fluid (sulfadiazine: λ = 242nm in both 0.1N HCl and I.F.).

Scanning electron microscopy (SEM) was used to characterize the surface and cross sections of the chitosan beads before and after the dissolution study. Cross sections were obtained by cutting the beads with a razor blade. The dried beads were coated for 70 seconds under an argon atmosphere with gold-palladium (Pelco Model 3 Sputter coater) and observed with a Jeol JSM 35C scanning electron microscope.

RESULTS AND DISCUSSION

The polycationic polysaccharide, chitosan, forms gels with suitable multivalent counterions. The ionic interactions between the positively charged amino groups and the negatively charged counterion, tripolyphosphate, were used in this study to prepare chitosan beads. The protonation of the amino groups enables the dissolution of chitosan by a large number of strong and weak acids (11). Solutions of chitosan in 1% acetic acid were dropped onto TPP solutions and gelled spheres formed instantaneously by ionotropic gelation. The beads were easily manufactured without any sophisticated equipment.

Chitosan is characterized by its degree of deacetylation and its viscosity in 1% (v/v) acetic acid solutions (1% w/w). The shape and preparation of the beads were critically dependent on the viscosity of the chitosan solution. Three chitosan samples with similar degrees of deacetylation but different viscosities were

evaluated for bead formation. Only the high viscosity chitosan sample (2790 cps) resulted in spherical and sufficiently strong beads at a concentration of 1% (w/w) in dilute acetic acid. Beads could not be prepared from samples with viscosities of 50 and 750 cps. In comparison, perfect calcium alginate spheres were formed above a critical solution viscosity of only 30 cps (12). The anionic counterion, TPP, can form either intermolecular or intramolecular linkages with the positively charged amino groups. Kohn reported that stable interchain junction zones formed above a minimum critical sequence length for calcium-alginate gels (13). The interactions were individually weak and long arrays of non-covalent bonds acting together were required for gel stability. The intermolecular linkages, which are responsible for the successful formation of the beads, increase in number with increasing molecular weight. Normal polyelectrolyte or intramolecular binding was probably prevalent with the low viscosity or low molecular weight chitosan samples. This may have prevented strong intermolecular crosslinking, and hence the formation of strong beads.

Most microencapsulation techniques use organic solvents and/or heat (14). In this method, the drugs were entrapped within the chitosan beads in a completely aqueous environment under mild conditions. The pH values of chitosan solutions (1w/w% in 1%v/v acetic acid) and of 4% TPP solutions were 4.1 and 8.9, respectively. The acetic acid within the chitosan droplets was neutralized rapidly by TPP, which diffused into the droplets. In addition, acetic acid diffused into the external phase. The pH-indicators methyl red or bromothymol blue (0.1 ml of a 0.05% solution) were added to the chitosan solution (10 ml) prior to bead formation in order to follow time-dependent pH-changes within the beads during the preparation. The beads were cut at different time periods after bead formation and the color changes of the pH-indicators within the beads were observed visually. The color changes are shown in Table 1. The pH value inside the beads gradually

TABLE 1

Time-dependent pH changes within the wet chitosan beads as visualized by color changes of the pH indicators methyl red and bromothymol blue.

time (min)	color of wet beads (surface / interior)	
	methyl red (pH range 4.2- 6.2) acid (red) - base (yellow)	bromothymol blue (pH range 6.0 - 7.6) acid (yellow) - base (blue)
5	red / red	yellow / yellow
10	yellow / red	blue spots / yellow
15	yellow / red	blue / yellow
20	yellow / yellow	blue / yellow
25		blue / green
35		blue / blue

changed from pH 4.1 (initial pH value of chitosan solution) to neutral pH values within 20 to 35 minutes. One can conclude from these results that this time period was necessary to obtain complete gelling across the beads.

Several drugs with different solubility characteristics were chosen as model compounds for the encapsulation within chitosan beads. This encapsulation procedure works best for water-insoluble drugs. Water-soluble drugs such as caffeine, salicylic acid or quinidine were lost to the TPP phase rapidly (Figure 2). The drugs partitioned completely into the external TPP solution within 15 minutes. To entrap water-soluble drugs, the chitosan spheres have to be separated from the aqueous phase as early as possible.

Alternatively, in the case of ionizable drugs, the pH of the TPP phase could be adjusted to minimize drug solubility. The solubility of quinidine in a 3% TPP solution (pH = 8.9) was only 0.09 g/l. However, the quinidine content in the beads was insignificant (Table 2). Acetic acid diffused into the TPP phase during bead

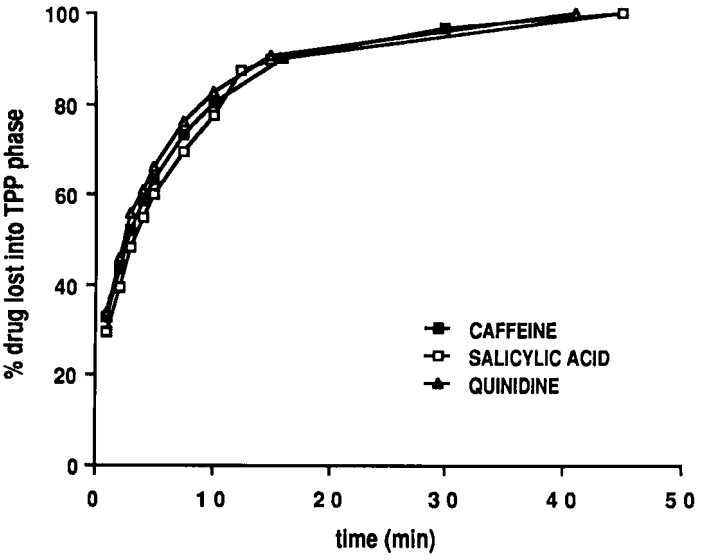


FIGURE 2
Time-dependent loss of water-soluble drugs to the external TPP (4%) phase during the preparation of chitosan beads.

preparation. The pH of the external phase dropped to pH 7.9 and the solubility of quinidine increased to 1.15g/l. Quinidine was lost completely, but it did not precipitate in the external phase. The pH of the TPP phase was then raised to pH 11 by the addition of 1N NaOH. Although the final pH of the TPP phase was 8.97, quinidine could not be entrapped. In contrast to the experiment with the 3% TPP

TABLE 2

Entrapment of quinidine within chitosan beads.

external phase	pH of external phase initial - final	quinidine content, wt% (theoretical content = 50%)
3% TPP	8.94 - 7.89	1.3
3% TPP + 1N NaOH	11.06 - 8.97	1.6
3% TPP + pH 12 buffer	12.07 - 12.04	24.6

solution, quinidine precipitated in the external phase. The pH of the solution close to the bead surface initially dropped because of the loss of acetic acid. This caused quinidine to diffuse out. The pH in the microenvironment increased with time and caused the drug to precipitate. The drug was successfully encapsulated with a pH 12 phosphate buffer, which kept the pH close to the bead surface at higher levels. However, quinidine was still lost, and precipitated. A buffer with a higher capacity may further increase the drug content. The presence of TPP was required for bead formation. A white precipitate, but no droplets were formed when the chitosan-quinidine solution was dropped in the pH 12 buffer. The increase in pH within the beads probably caused quinidine to precipitate in the interior of the beads during the preparation. Although quinidine could be successfully entrapped with this modified technique, the resulting beads were weak. The positively charged amino groups of chitosan were probably converted to the unionized state at the higher pH values. This resulted in reduced ionic interactions or crosslinking with the anionic counterion, TPP.

Sulfadiazine, the water-insoluble model drug, was suspended in the chitosan solution and entrapped successfully. The effect of sulfadiazine payload on the actual sulfadiazine content in the chitosan spheres is shown in Figure 3. The sulfadiazine content increased with increasing payload. High drug loadings up to 80% were achieved. Sulfadiazine was continuously lost to the external tripolyphosphate phase during the preparation of the beads (Figure 4). The pH and the solubility of sulfadiazine within the beads increased because of TPP diffusing into the beads. Sulfadiazine has a solubility of 0.07mg/ml in 1% acetic acid and of 3.5 mg/ml in 3% TPP solution. The sulfadiazine crystals initially dissolved from the surface of the beads, and then with increasing time periods, more drug dissolved from the central regions. The TPP solution to chitosan solution ratio should be kept to a minimum for maximum drug entrapment. More drug was lost with increasing

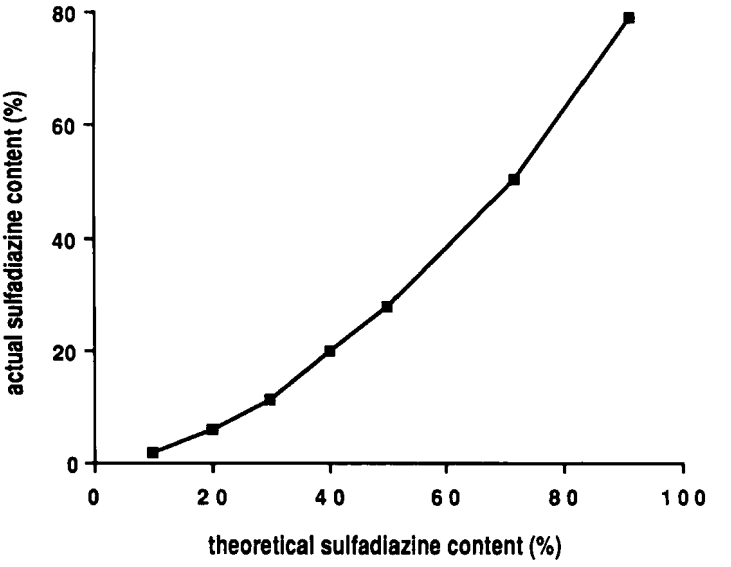


FIGURE 3
Effect of sulfadiazine payload on the actual sulfadiazine content in the chitosan beads (3% TPP).

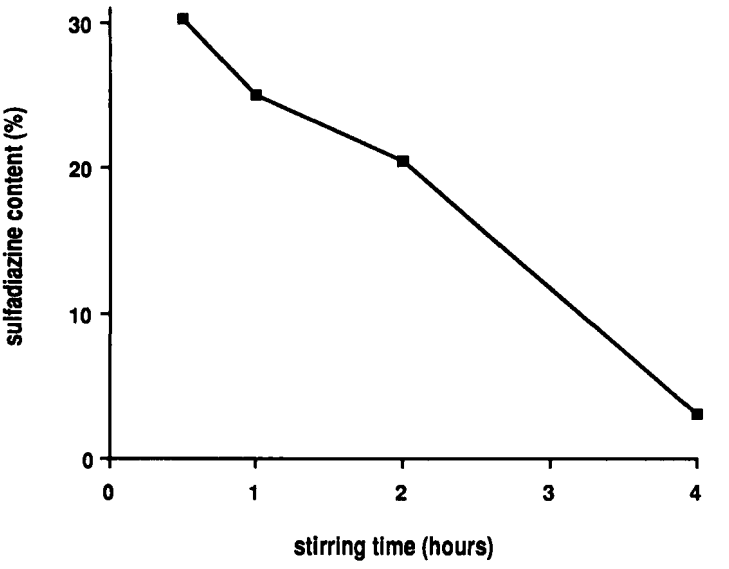


FIGURE 4
Effect of stirring time on sulfadiazine content in the chitosan beads (2% TPP).

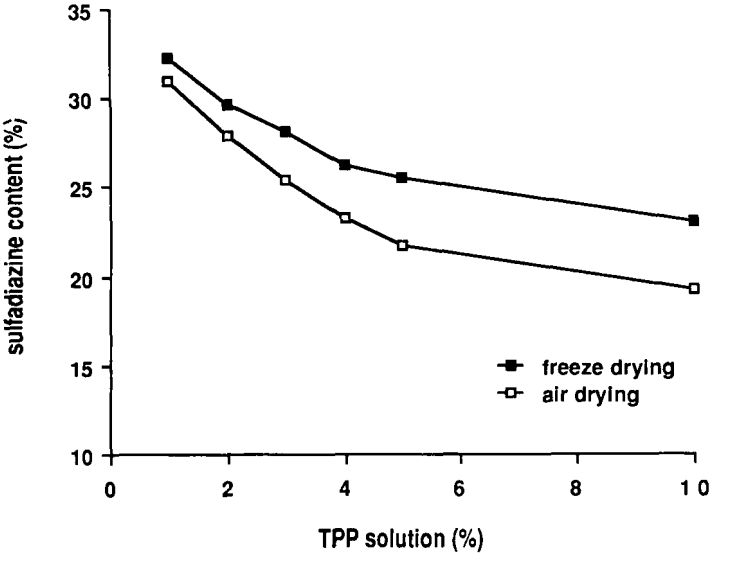


FIGURE 5
Effect of the concentration of tripolyphosphate (TPP) and of the method of drying on the sulfadiazine content in the chitosan beads.

volume of the external phase. The drug content was independent of the total volume used, as long as the phase ratio was kept constant.

The effect of TPP concentration and of the method of drying on drug content is shown in Figure 5. The sulfadiazine content within the beads decreased with increasing TPP concentration. The solubility of sulfadiazine in the external phase and hence the amount of drug lost to the external TPP phase increased with increasing tripolyphosphate concentration (solubility of sulfadiazine in 1% TPP = 2.8 mg/ml, in 3% TPP = 3.5 mg/ml, and in 5% TPP = 3.8 mg/ml). Freeze-drying resulted in higher drug contents within the beads when compared to air-dried beads. The beads shrank significantly during air-drying. Water was exuded and accumulated on the surface of the beads before evaporation. Dissolved sulfadiazine migrated with the water to the outside of the beads. After complete drying, sulfadiazine crystals were visible on the exterior of the beads. During the freeze-

drying process, the beads rapidly solidified. Water transport did not occur and sulfadiazine could not diffuse to the outside of the beads under these conditions. The sulfadiazine content within the beads could be further increased by adding drug to the external TPP phase as shown in Figure 6.

The effect of sulfadiazine loading on drug release in 0.1N HCl and simulated intestinal fluid is shown in Figures 7 and 8. The release rate decreased with increasing drug content in both media. Ground mixtures of chitin or chitosan with drugs have been prepared to improve the dissolution properties and bioavailability of poorly soluble drugs such as griseofulvin, phenytoin, and prednisolone(15-17). A similar enhancing effect was seen in this study. Sulfadiazine is a poorly soluble drug and its solubilities in gastric and intestinal fluids were 0.5 mg/ml and 0.37 mg/ml, respectively. The entrapment of the sulfadiazine crystals within the chitosan matrix rendered the crystal surfaces more hydrophilic. This improved the wettability

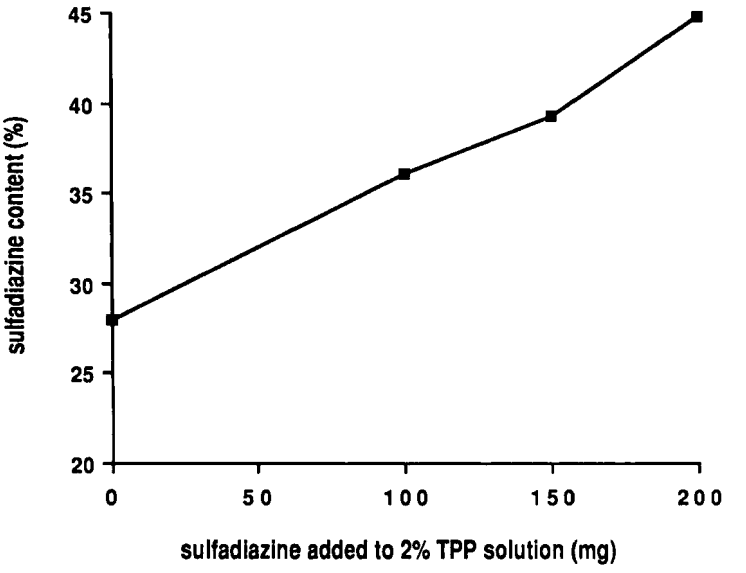


FIGURE 6
Effect of the addition of sulfadiazine to the external tripolyphosphate phase on the sulfadiazine content in the chitosan beads.

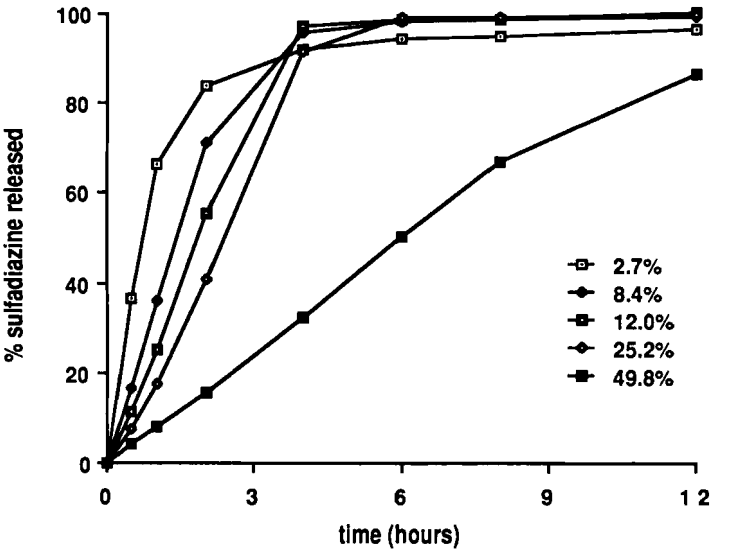


FIGURE 7
Effect of sulfadiazine loading on drug release from chitosan beads in 0.1N HCl.

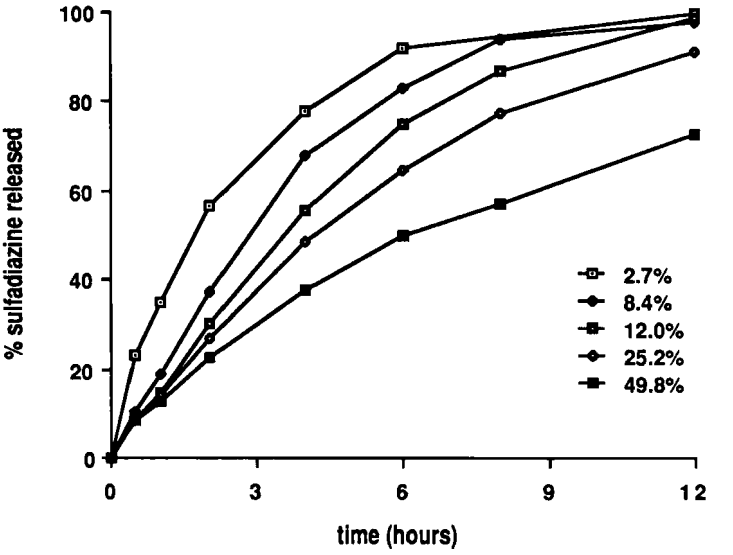


FIGURE 8
Effect of sulfadiazine loading on drug release from chitosan beads in simulated intestinal fluid.

of the crystals by the dissolution media and resulted in the enhancing effect of chitosan on the sulfadiazine release.

The drug release from the chitosan beads depended on the penetration of the dissolution medium into the beads, the eventual swelling and dissolution of the chitosan matrix, and the dissolution and subsequent diffusion of the drug through the swollen or unswollen chitosan matrix. The swelling of the chitosan beads was dependent on the pH of the dissolution medium. The beads when wetted by the acidic dissolution medium swelled extensively, and formed a hydrogel matrix before they dissolved completely. They did not swell or dissolve in simulated intestinal fluid.

Figures 9 and 10 show the effect of the concentration of the gelling agent (TPP) on the sulfadiazine release in 0.1N HCl and intestinal fluid. The anionic counterion, TPP, acts as an ionic crosslinking agent. Drug release from hydrogels

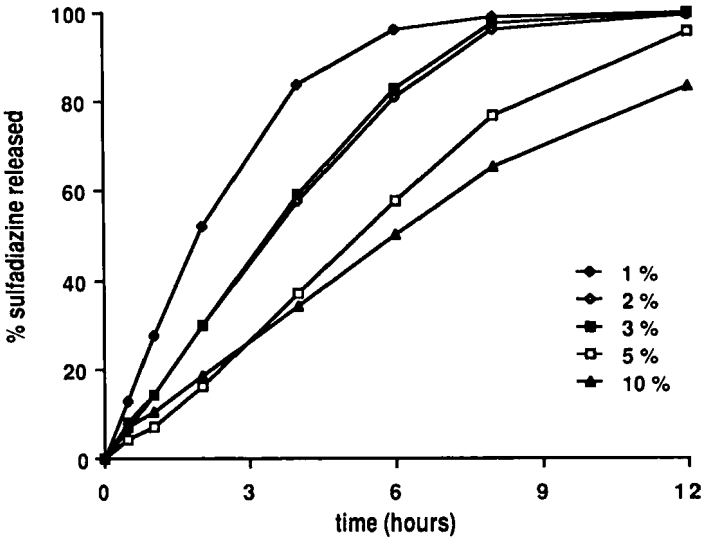


FIGURE 9
Effect of the concentration of tripolyphosphate on the sulfadiazine release from chitosan beads in 0.1N HCl.

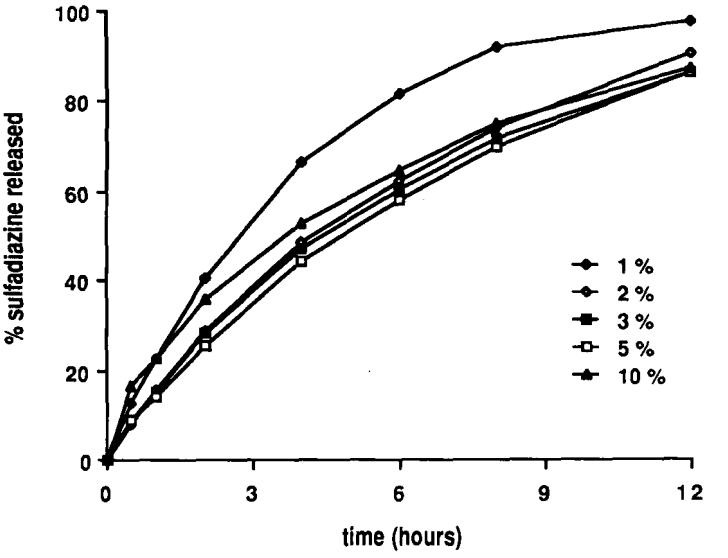


FIGURE 10
Effect of the concentration of tripolyphosphate on the sulfadiazine release from chitosan beads in simulated intestinal fluid.

has been controlled by the degree of crosslinking (18). Increased crosslinking reduced the degree of swelling and the rate of drug release. The same tendency was seen in this study. The sulfadiazine release decreased with increasing concentration of TPP in 0.1N HCl. The dissolution of the drug crystals and subsequent diffusion through the matrix increased as a result of the swelling which increased the free volume of the matrix. Ionization of the free amino groups in 0.1N HCl caused hydration and swelling of the beads prior to the dissolution of chitosan. On the contrary, the beads did not swell or dissolve in simulated intestinal fluid and the effect of the crosslinking agent on the release of sulfadiazine was insignificant. Except for the 1% TPP concentration, the release varied insignificantly with increasing TPP concentrations.

Another interesting property of the beads was the ability to float on the dissolution medium. Sustained release oral delivery has been achieved by using

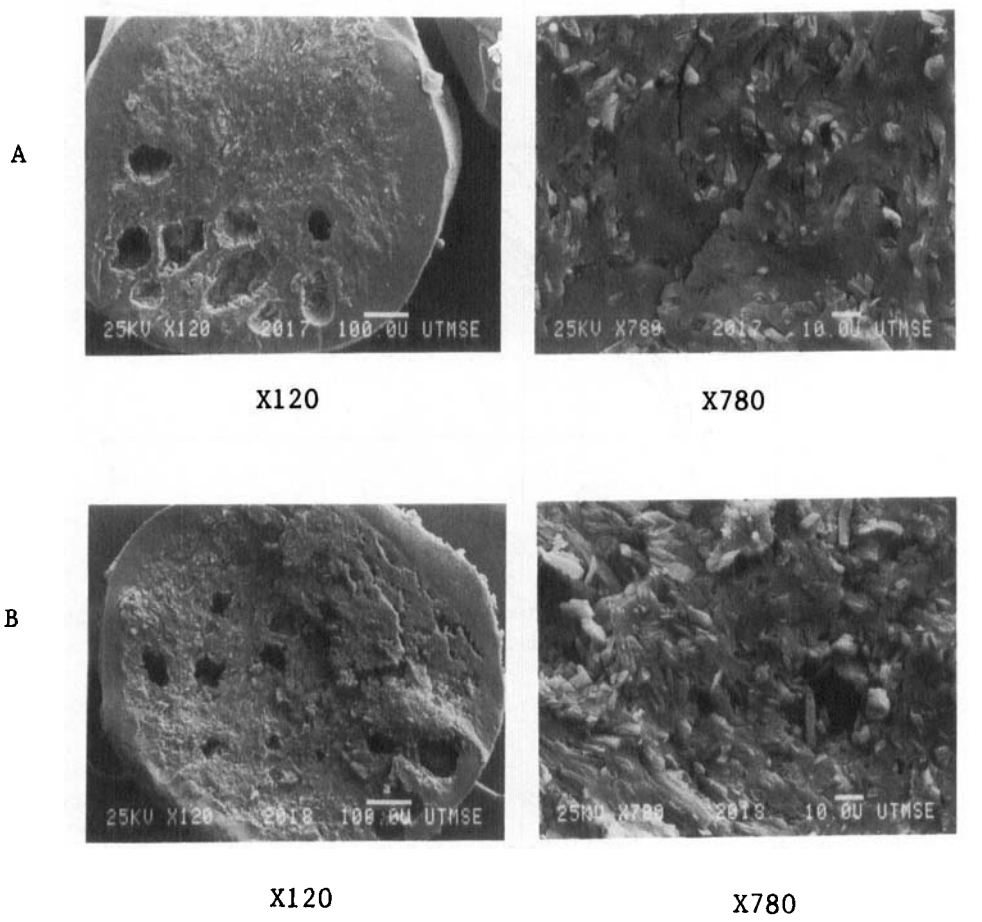
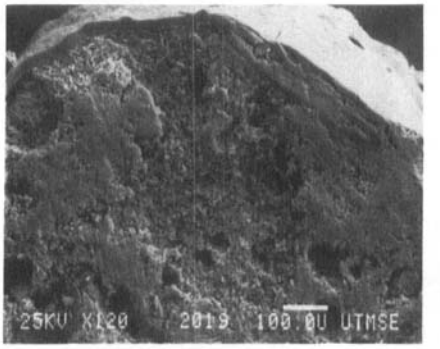
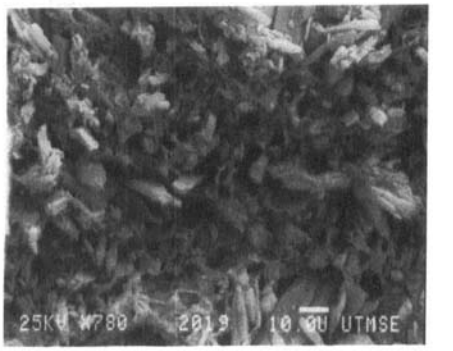


FIGURE 11
Scanning electron micrographs of cross sections of air-dried (11A-D) and freeze-dried (11E) chitosan beads containing different amounts of sulfadiazine: A, 12.2%; B, 25.2%; C, 57.2%; D, 25.2% after dissolution study in intestinal fluid; E, 50.4% (freeze-dried).

C



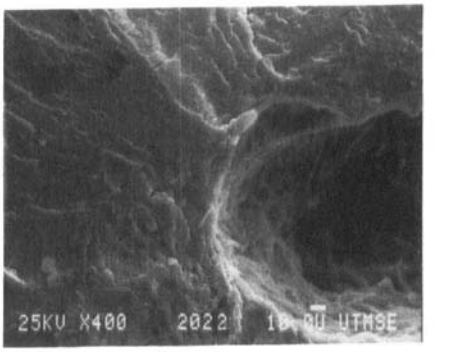
X120



X780

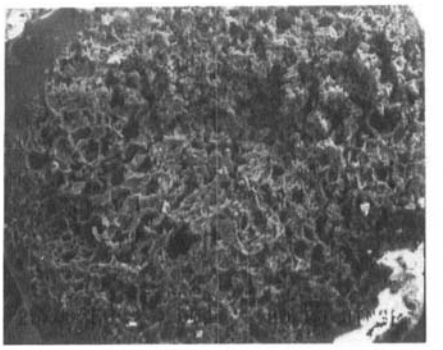
D

X120

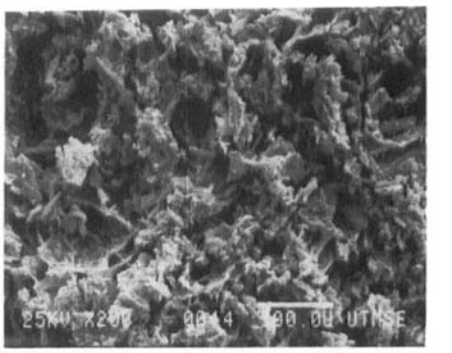


X780

E



X60



X200

FIGURE 11 C-E

formulations which float on the gastric juice (19). Most other ionic gel-forming macromolecules such as sodium alginate and polyacrylic acid are of anionic character and dissolve or swell at higher pH values.

The morphology of the beads was investigated by scanning electron microscopy. The matrix structure depended on the method of drying. SEM-photographs of air-dried chitosan spheres containing different amounts of drugs are shown in Figure 11A-C. The beads shrank during air-drying. The sulfadiazine crystals were embedded in a dense chitosan matrix. The increasing amount of sulfadiazine crystals within the beads was clearly visible. The large openings within the cross sections resulted from entrapped air bubbles incorporated during the preparation of the chitosan-sulfadiazine suspension. Figure 11D shows the crystal-free cross section of a bead following a dissolution study in simulated intestinal fluid. The freeze-dried beads were significantly larger and had a much more porous internal structure when compared to the air-dried beads as shown in Figure 11E. The beads were frozen instantaneously and did not shrink during the freeze-drying process.

In summary, drug containing chitosan beads were successfully prepared by gelling the cationic polysaccharide with the anionic counterion, tripolyphosphate. As with most encapsulation techniques, the drug solubility in the external phase has to be minimized to maximize drug entrapment. To achieve maximum drug content, high payloads, short stirring times, low TPP concentrations, and a low internal to external phase ratio were required. The beads showed pH-dependent swelling and dissolution behavior. They swelled and dissolved in 0.1N HCl, but stayed intact in simulated intestinal fluid. The method of drying determined the porosity of the beads.

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