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Development of Biopolymer Based Matrix Type Multiple Unit Systems for Sustained Release of Diclofenac Sodium: *In vitro* and *In vivo* Evaluation

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The objective of the present study was to investigate the applicability of matrix type chitosan treated alginate multiple unit systems (MUS) for sustained release of diclofenac sodium. The multiple unit systems (MUS) were prepared by the ionotropic gelation method. Spherical MUS with 1.852 ± 0.041–2.173 ± 0.265 mm diameter range and 66.66 ± 3.21 to 78.55 ± 6.49% entrapment efficiency were produced. The addition of chitosan increased the swelling of MUS in acidic conditions and reduced the drug release from MUS. The fluoroscopic study reveals that the MUS retained in gastrointestinal tract (GIT) for more than 12 h and distributed throughout the GIT. The *in vivo* evaluation in healthy human volunteers of the MUS and that of Voveran SR tablets each containing 100 mg drug revealed that the MUS was bioequivalent to Voveran SR producing a non-significantly different ($p > 0.05$) AUC. This study demonstrates that the matrix type chitosan treated alginate MUS can be a good alternative to sustained release tablets to deliver diclofenac sodium and expected to be less of an irritant to gastric and intestinal mucosa.

Keywords: diclofenac sodium; multiple unit systems; chitosan; sodium alginate; sustained release; bioavailability

1 Introduction

Use of hydrophilic polymer matrix is one of the most popular approaches in formulating an extended-release dosage form (1–3). In recent years, there has been great interest in the development of MUS. The use of natural biopolymers in dosage form design received considerable attention, especially from the viewpoint of safety with decreased side effects and use of polymer blends represents a potential way of achieving required release properties. Among these, chitosan and sodium alginate are promising biopolymers for multiple unit oral drug delivery systems (4–8). MUS have several advantages over conventional single unit dosage forms for controlled release, in terms of bioavailability i.e., more consistent blood levels, predictable gastrointestinal transit, less localized gastrointestinal disturbances and greater product safety (9).

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Chitosan, the N-acetylated product of the polysaccharide chitin, is gaining increasing importance in drug delivery to the gastrointestinal tract (GIT) owing to its good biocompatibility, non-toxicity and biodegradability. In the early 1980s, chitosan was proposed as a useful excipient for either sustaining the release of water-soluble drugs (10) or enhancing the bioavailability of poorly water-soluble compounds (11). It has been shown that chitosan is mucoadhesive and enhances the penetration of macromolecules across the intestinal and nasal barriers (12–14). Furthermore, chitosan has been presented as a useful polymer for colon-specific drug delivery due to its specific biodegradation by the colonic bacteria (15). But its use in oral administration is restricted by its fast dissolution in the stomach and thus the limited capacity for controlling the release of drugs. Also, chitosan has intrinsic anti-ulcer effect by adhesive action on gastric epithelial cells and/or by neutralizing the hydronium ions in the gastric fluid (16, 17).

Sodium alginate, an anionic polysaccharide, forms a cured gel matrix in the presence of calcium. Calcium alginate is able to incorporate drug within its gel matrix, and thus acts as a vehicle for the sustained release of orally administered drugs (18–20). Several attempts have been made to control

the drug release by modifying the structure of the gel matrix in calcium alginate (21, 22). Calcium alginate containing another polysaccharide additive such as chitosan and calcium alginate beads coated with chitosan has been reported previously for controlled drug delivery and enhancement of bioadhesive properties (23–25). Also, alginate has shown anti-ulcer and mucoprotective properties (26, 27).

Diclofenac sodium is a potent non-steroidal anti-inflammatory drug (NSAID) which has anti-inflammatory, analgesic and antipyretic properties. Diclofenac sodium is a phenyl acetic acid derivative with pKa value of 4.0. As a result, diclofenac sodium is practically insoluble in acidic solution, but dissolves in intestinal fluid and water. In order to diminish diclofenac sodium gastrointestinal (GI) irritation (28), effective enteric coated or sustained release dosage forms have been developed.

Side effects, mainly at the gastric level, are well known, following oral administration of an NSAID. Therefore, the efforts of many researchers have been concerned to solve these problems through a variety of techniques of protection of the gastric mucosa or alternatively to prevent the NSAID release in this district. The objective of the present study was to evaluate the potential utility of natural biopolymers such as sodium alginate and chitosan in sustaining the release of diclofenac sodium in GIT. We also investigated the possible applicability of chitosan treated matrix type alginate systems as sustained release system. We prepared MUS of alginate with dispersed chitosan using aqueous solvents and evaluated *in vitro* and *in vivo*.

2 Experimental

2.1 Materials

Chitosan was gifted by the Central Institute of Fisheries Technology and India Sea Foods (degree of deacetylation, 80.80%; Kochi, India). Sodium alginate (Keltone HVCR) was kindly supplied by International Specialty Products (ISP) (Hyderabad, India). Diclofenac sodium was obtained from Rachana Labs (Hyderabad, India). The internal standard naproxen was gifted by Divis Laboratories (Hyderabad, India). All other reagents and solvents used were of analytical grade procured from Merck (Mumbai, India). The reference product Voveran SR® 100 mg tablets (Novartis, India) were procured from local market.

2.2 Preparation of Multiple Unit Systems

Diclofenac sodium (1 gm) and varying quantities of chitosan (0.5 to 3 gm) were added to sodium alginate (2 gm), previously dissolved in distilled water and stirred until homogenous. The resultant solutions contain 2% w/w sodium alginate, 1% w/w diclofenac sodium and varying concentrations (0.5 to 3% w/w) of chitosan. These solutions (20 ml each) were dropped using a hypodermic syringe into 100 ml of 1.5% w/v calcium chloride solution under mild agitation and the mixture was then stirred slowly for an additional 10 min. Then the formed MUS were filtered, washed and dried at room temperature. Table 1 lists the formulations prepared.

2.3 Determination of MUS Size

The size distribution of the MUS was determined by sieve analysis. The percent frequency was the proportion of MUS obtained in the different sieves relative to the total amount of particles used for the analysis. Using the frequency data, the log-normal distribution on a probability scale was plotted and the geometric mean diameter and the geometric standard deviation were calculated.

2.4 Entrapment Efficiency

The MUS (50 mg) were digested in 500 ml of phosphate buffer (pH 7.4). The solution was filtered and absorbance of diclofenac sodium was measured using UV spectrophotometer (SL 164, Elico, Hyderabad, India) at 276 nm. The entrapment efficiency was determined by the following formula:

$$\text{Entrapment efficiency (\%)} = \frac{\text{Actual quantity of drug present in MUS}}{\text{Theoretical quantity of drug present in MUS}} \times 100$$

Table 1. Composition of multiple unit systems showing size and entrapment efficiency

Formulation	Drug (gm)	Sodium alginate (gm)	Chitosan (gm)	Particle size (mm) (Mean ± SD) (n = 3)	Entrapment efficiency (%) (Mean ± SD) (n = 3)
MUS1	1	2	0	1.852 ± 0.041	78.55 ± 6.49
MUS2	1	2	0.5	2.043 ± 0.158	66.66 ± 3.21
MUS3	1	2	1	2.066 ± 0.164	72.04 ± 1.69
MUS4	1	2	2	2.152 ± 0.098	73.88 ± 3.13
MUS5	1	2	3	2.173 ± 0.265	72.98 ± 4.78

2.5 Swelling Studies

Known quantities (100 mg) of various MUS were placed in 10 ml volumetric graduated cylinders containing 0.1 N HCl (pH 1.2) and phosphate buffer (pH 6.8). The length of MUS column (graduated cylinder) and thereby swelling was measured at appropriate time intervals. The studies were performed in triplicate and average volume of MUS was calculated. Relative swelling of MUS was calculated as percentage of the volume at time 0.

2.6 In vitro Release Studies

In vitro drug release from MUS was studied by means of a USP type II dissolution test apparatus (Disso 2000, LabIndia, India) equipped with an auto sampler and fraction collector for the collection and replenishment of sample and dissolution medium respectively. The study was conducted with an agitation speed of 50 rpm in 0.1 N HCl (pH 1.2) for 2 h followed by phosphate buffer (pH 6.8) for 10 h at $37 \pm 0.5^{\circ}C$. Before the dissolution test, required amounts of MUS of each formulation were placed in the dissolution vessels to give a theoretical amount of drug in each vessel of 100 mg. At appropriate time intervals, 5 ml samples were withdrawn and assayed spectrophotometrically at 276 nm. All dissolution runs were performed in six replicates.

2.7 GI Transit Behavior

The gastrointestinal transit behavior of the formulation was visualized using fluoroscopy (low energy X-rays, Siemens Fluorovision, Germany) under the supervision of a radiologist. The study protocol was approved by the institutional ethical committee (Kakatiya University, Warangal, India). Three healthy human volunteers participated in the study. The ages of the volunteers ranged from 23 to 29 years, their weights from 62 to 70 kg. MUS containing radio-opaque marker (barium sulphate) were prepared in a similar manner to formulation MUS4 by replacing the drug. Gelatin capsules containing 50 MUS each were administered to each subject with 200 ml of water, after the subject had fasted overnight. Lunch was provided 5 h after administration of radio-opaque formulation.

2.8 In vivo Bioavailability Study

Two groups, each of eight healthy male volunteers, participated in a randomized crossover single dose study. The ages of the volunteers ranged from 24 to 31 years, their weights from 62 to 69 kg. Written consent for participation in the study had been obtained. The bioavailability protocol was approved by the institutional ethical committee (Kakatiya University, Warangal, India). The amount of diclofenac sodium administered in the study was 100 mg. The required amount of formulation MUS4 was dispensed into hard-gelatin capsules. A light breakfast was provided after overnight fasting. After 30 min, capsules and reference product Voveran SR® 100 mg tablets (VSR) (Novartis, India) were administered to each subject with 200 ml of water. Lunch was provided 4 h after drug administration. Blood samples of 5 ml were collected at 0, 1, 2, 3, 3.5, 4, 4.5, 6, 8, 12, and 24 h. The samples were allowed to clot and centrifuged at 3000 rpm for 10 min. Serum was separated and stored at $-20^{\circ}C$ until analysis.

2.9 Estimation of Diclofenac in Human Serum

Diclofenac concentrations in serum were determined by means of high-performance liquid chromatography (HPLC) using the method described by El-Sayed et al. (29), with slight modifications. The method involved the addition of 100 μl of 1% w/v internal standard (naproxen) to 0.5 ml of serum samples and mixed on a vortex mixer for 2 min. To the above samples, 500 μl of 500 mM HCl was added, vortexed for 3 min and extraction was accomplished by addition of 5 ml dichloromethane. After vortexing for 5 min and centrifuging for 10 min at 3500 rpm, the supernatant was transferred to a centrifuge tube and evaporated to dryness. The residue was reconstituted in 100 μl of acetonitrile and injected into the loop injector.

The HPLC system was equipped with a pump (LC-10AT, Shimadzu, Japan), an injection port (Rheodyne, USA), a reversed phase C18 column (250 × 4.6 mm, 5 μ, Phenomenex, USA) and a UV detector (SPD 10A, Shimadzu, Japan). HPLC mobile phase was composed of 55% (v/v) acetonitrile, 15% (v/v) methanol and 30% (v/v) water (pH adjusted to 3.2 with orthophosphoric acid) at a flow rate of 1 ml/min with the detector wavelength set at 278 nm.

2.10 Pharmacokinetics of MUS

Peak plasma concentrations (C_{max}), the corresponding times at which these are reached (T_{max}) and the area under the serum concentration time curve (AUC) for individual subject were calculated using KINETICA™ software (Inna Phase Corp., 2000). All the data was statistically analyzed using Sigmastat software package (Jandel Corp., California). Paired *t*-test was used for comparison of pharmacokinetic parameters and the difference was considered significant when $p < 0.05$.

3 Results and Discussion

3.1 Characterization of Multiple Unit Systems

Using this technique (ionotropic gelatin method) spherical MUS with a high drug content could be prepared. The composition, size and drug loading capacity of MUS were given in Table 1. The MUS were in the size range of 1.852 ± 0.041 to 2.173 ± 0.265 mm and roughly spherical in shape. Chitosan concentration could affect the MUS size. Chitosan treated alginate MUS were larger than alginate

MUS. This might be due to an increase in total quantity of the formulation ingredients. The entrapment efficiency of the MUS was varied from 66.66 ± 3.21% to 78.55 ± 6.49%. Diclofenac sodium showed high entrapment efficiency in alginate and chitosan treated alginate MUS irrespective of the concentration of chitosan. When comparison is made between alginate MUS and chitosan treated alginate MUS, the entrapment efficiency of this drug was lower in chitosan treated alginate MUS. The presence of chitosan in dispersed form throughout the matrix decreased the drug loading efficiency of MUS.

The results of swelling study are given in Figures 1 and 2. Swelling of MUS was influenced by the environmental pH. MUS prepared with chitosan showed the highest swelling in 0.1 N HCl. Gradual increase in swelling was observed up to 1.5 h and remained almost constant thereafter. A significant ($p < 0.05$) difference in swelling was observed after addition of 0.5% w/w of chitosan. A further increase in chitosan concentration has shown a gradual but little increase in swelling. This may be due to gelling of chitosan in strong acidic conditions. In contrast to these results, MUS prepared with alginate alone showed highest swelling in phosphate buffer (pH 6.8). An increase in chitosan concentration resulted in decreased swelling in phosphate buffer. The properties of the polymers were generally affected by their swelling behavior, water uptake and hydration state (30–32). Therefore, slow swelling is a requisite to avoid the formation of an over hydrated form that loses its integrity before the drug release at the target. No cracks were observed on the surface of the MUS and they remained intact in both the media studied. The intact nature of the MUS is required to maintain a slow drug release during their transit through GIT.

3.2 Drug Release Studies

The release profiles of MUS were shown in Figure 3. From the release profiles it was clear that most of the drug was released in phosphate buffer (pH 6.8). All the formulations released less than 4% of diclofenac sodium in 0.1 N HCl during the first 2 h due to very poor solubility of diclofenac

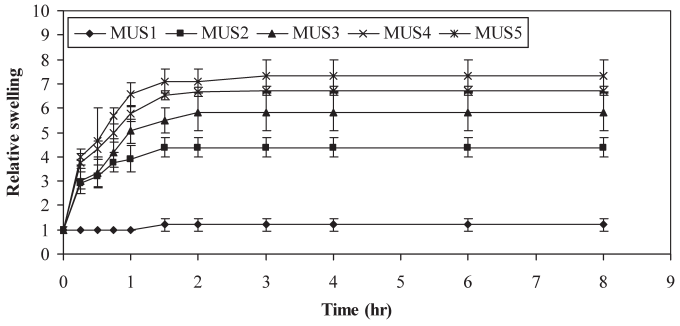

Fig. 1. Relative swelling of diclofenac sodium multiple unit systems in 0.1 N HCl. Each point represents mean value ± standard deviation (n = 3).

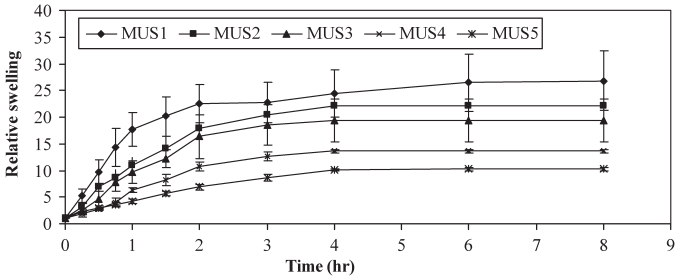

Fig. 2. Relative swelling of diclofenac sodium multiple unit systems in phosphate buffer (pH 6.8). Each point represents mean value ± standard deviation (n = 3).

sodium in gastric fluids (33). An increase in the chitosan concentration from 0.5% w/w to 3% w/w reduced diclofenac sodium release from MUS in phosphate buffer (pH 6.8). Release of drug from formulations MUS4 and MUS5 was slow when compared to formulations MUS1, MUS2, and MUS3. About 50% of the drug was released within 2.75, 2.85, 2.90, 3.80, and 4.20 h from the formulations MUS1, MUS2, MUS3, MUS4, and MUS5, respectively. Similarly, 90% of drug release was observed in 2.65, 5.70, 5.95, 8.4 and 9.4 h. There was no significant ($p > 0.05$) difference in drug release between MUS4 and MUS5. When a comparison is made between the release properties of MUS4 in 0.1 N HCl followed by phosphate buffer (pH 6.8) and directly in phosphate buffer (pH 6.8), a significant reduction in drug release from MUS4 was observed in 0.1 N HCl followed by phosphate buffer (pH 6.8) (Figure 4). Thus the addition of chitosan to the gel structure in acidic conditions reduced the drug release from MUS (34).

When a comparison is made between the release properties of MUS4 and reference product (Voveran SR® tablet), the release was almost similar in acidic environment where the drug is very poorly soluble (33). Drug release from MUS4 was faster compared to reference product in phosphate buffer (pH 6.8), where the drug is soluble. This could be due to the change in formulation ingredients and multiple unit system possessing larger surface area.

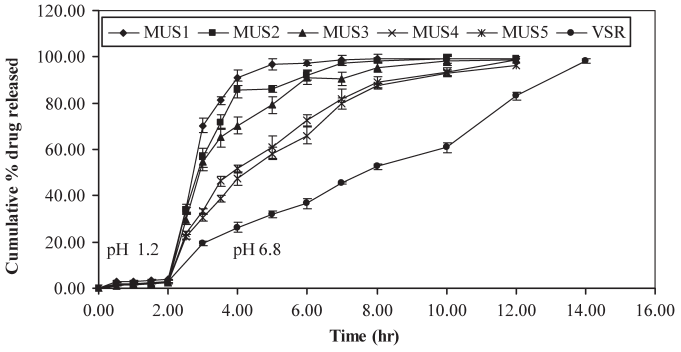

Fig. 3. Release profiles of diclofenac sodium from multiple unit systems and Voveran SR. Each point represents mean value ± standard deviation (n = 6).

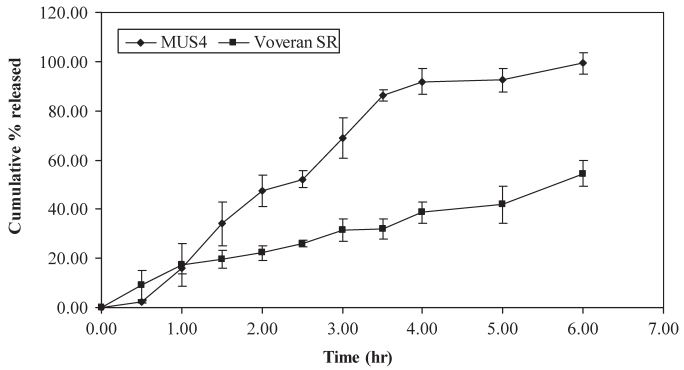

Fig. 4. Release profiles of diclofenac sodium from formulation MUS4 and Voveran SR in phosphate buffer (pH 6.8). Each point represents mean value ± standard deviation (n = 6).

The description of dissolution profiles has been attempted using different release models. The data were evaluated according to the following equations:

$$\text{First-order: } \ln M_t = \ln M_0 + K_1 t$$

$$\text{Zero-order: } M_t = M_0 + K_0 t$$

$$\text{Higuchi model: } M_t = K_H \sqrt{t}$$

$$\text{Korsmeyer–Peppas model: } M_t/M_0 = K_K t^n$$

where M_t is the amount of drug dissolved in time t, M_0 the initial amount of drug, K_1 the first-order release constant, K_0 the zero-order release constant, K_H the Higuchi rate constant, K_K the release constant and n is the diffusional release exponent indicative of the operating release mechanism.

The correlation coefficient (R^2) was used as an indicator of the best fitting, for each of the models considered. The correlation coefficient (R^2) ranges from 0.3549 to 0.8024, 0.4355 to 0.9067, 0.5239 to 0.9570 and 0.5218 to 0.9398 for first-order, zero-order, Higuchi and Korsmeyer–Peppas release models respectively. For the formulation (MUS4) selected for *in vivo* bioavailability study, the best fit was achieved with the application of Higuchi ($R^2 = 0.9456$) and Korsmeyer-Peppas ($R^2 = 0.9157$) models. On the other hand, n values (0.5393 to 0.9390) indicated that the drug release from all the formulations is by non-Fickian diffusion.

3.3 GI Transit Behavior

The fluoroscopic study reveals that the MUS remained in the stomach for about 30–60 min, and then passed into the upper intestinal tract where they stayed up to 3 h followed by the small intestine up to 6 h and retained for longer time in the colon (more than 6 h). The administered formulation was not detected by radiography after 24 h, which could be due to the degradation of polymers in the colon and/or evacuation during the passage of the bowel. X-ray photograph obtained at 12 h shows a maximum number of MUS in the colon (Figure 5). The tested formulation could be useful for the sustained delivery of diclofenac sodium as it stayed at different sites and spread over a larger area of the GIT.

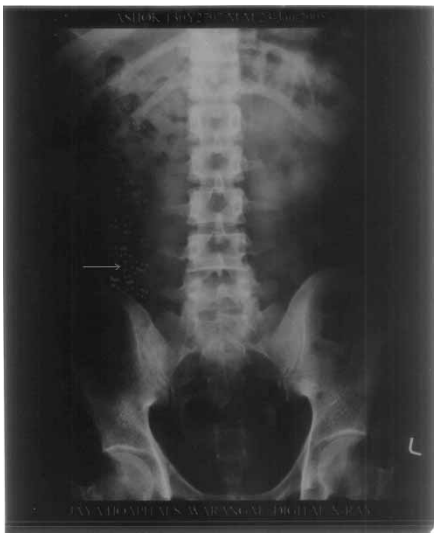

Fig. 5. Photograph of X-ray study recorded at 12 h after oral administration of blank formulation of MUS4 in human volunteer.

3.4 In vivo Bioavailability Study

The mean (±SD) plasma concentrations of diclofenac at each time point following administration of MUS4 and Voveran SR are shown in Figure 6 and the pharmacokinetic parameters were listed in Table 2. Rapid release and absorption of

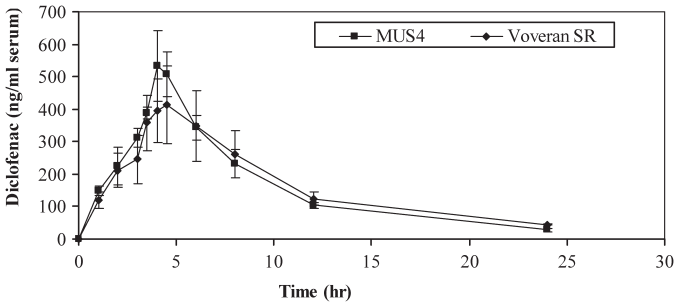

Fig. 6. Mean serum levels of diclofenac after oral administration of formulation MUS4 and Voveran SR 100 mg tablets. Each point represents mean value ± standard deviation (n = 8).

Table 2. Pharmacokinetics of diclofenac following oral administration of formulation MUS4 and Voveran SR 100 mg tablets

Pharmacokinetic parameter	MUS4 (Mean ± SD)	Voveran SR (Mean ± SD)
C_{max} (ng/ml)	565.43 ± 94.27	516.27 ± 58.25
T_{max} (hr)	3.89 ± 0.22	4.13 ± 0.44
AUC_{0-24} (ng · hr/ml)	3718.73 ± 191.58	3506.14 ± 617.37

diclofenac sodium occur from MUS4 producing C_{max} of 565.43 ± 94.27 ng/ml with a respective T_{max} of 3.89 ± 0.22 h, while Voveran SR showed slightly lower C_{max} of 516.27 ± 58.25 ng/ml with a respective T_{max} of 4.13 ± 0.44 h. The AUC_{0-24} values for the formulated MUS4 and Voveran SR were 3718.73 ± 191.58 and 3506.14 ± 617.37 ng hr ml^{-1}, respectively. On comparing the formulated MUS4 and Voveran SR as shown in Table 2, it was found that they are non-significantly different ($p > 0.05$) with respect to their C_{max}, T_{max}, and AUC_{0-24}. The results of *in vivo* study clearly indicate that matrix type chitosan treated alginate MUS were bioequivalent to the sustained release Voveran SR 100 mg tablets.

4 Conclusions

As a conclusion, matrix type multiple unit systems containing chitosan and alginate can be a good alternative to the single unit and sustained release dosage forms as it produced similar bioavailability. Side effects, mainly at the gastric level, are well known, following oral administration of an NSAID. Hence, safety becomes a primary requisite in the treatment. MUS containing chitosan and alginate are expected to be less of an irritant to gastric and intestinal mucosa, since alginate and chitosan were proved to possess anti-ulcer and mucoprotective properties and shows additive and/or synergistic effects.

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6 References

1. Rao, Y.M., Veni, J.K. and Jayasagar, G. (2001) *Drug Dev. Ind. Pharm.*, **27(8)**, 759–766.
2. Samani, S.M., Montaseri, H. and Kazemi, A. (2003) *Eur. J. Pharm. Biopharm.*, **55(3)**, 351–355.
3. Velasco, M.V., Ford, J.L., Rowe, P. and Rajabi-Siahboomi, A.R. (1999) *J. Control. Rel.*, **57(1)**, 75–85.
4. Remunan-Lopez, C., Lorenzo-Lamosa, M.L., Vila-Jato, J.L. and Alonso, M.J. (1998) *Eur. J. Pharm. Biopharm.*, **45(1)**, 49–56.
5. Tapia, C., Escobar, Z., Costa, E., Sapag-Hagar, J., Valenzuela, F., Basualto, C., Gai, M.N. and Yazdani-Pedram, M. (2004) *Eur. J. Pharm. Biopharm.*, **57(1)**, 65–75.
6. Orienti, I., Cerchiara, T., Luppi, B., Bigucci, F., Zuccari, G. and Zecchi, V. (2002) *Int. J. Pharm.*, **238(1–2)**, 51–59.
7. Sugimoto, K., Yoshida, M., Yata, T., Higaki, K. and Kimura, T. (1998) *Biol. Pharm. Bull.*, **21(11)**, 1202–1206.
8. Chatchawalsaisin, J., Podczeck, F. and Newton, J.M. (2004) *Int. J. Pharm.*, **275(1–2)**, 41–60.
9. O'Reilly, S., Wilson, C.G. and Hardy, J.G. (1987) *Int. J. Pharm.*, **34(3)**, 213–216.
10. Hou, W.M., Miyazaki, S., Takada, M. and Komai, T. (1985) *Chem. Pharm. Bull.*, **33(9)**, 3986–3992.
11. Sawayanagi, Y., Nambu, N. and Nagai, T. (1982) *Chem. Pharm. Bull.*, **30(12)**, 4464–4467.
12. Lehr, C.M., Bouwstra, J.A., Schacht, E.H. and Junginger, H.E. (1992) *Int. J. Pharm.*, **78(1)**, 43–48.
13. Illum, L., Farraj, N.F. and Davis, S.S. (1994) *Pharm. Res.*, **11(8)**, 1186–1189.
14. Borchard, G., Lueszen, H.L., de Boer, G.A., Coos Verhoef, J., Lehr, C.M. and Junginer, H.E. (1996) *J. Control. Rel.*, **39(2–3)**, 131–138.
15. Tozaki, H., Fujita, T., Yamamoto, A., Muranishi, S., Sugiyama, T., Terabe, A., Matsumoto, T. and Suzuki, T. (1996) *Proc. Int. Symp. Control. Release Bioact. Mater.*, **23**, 551–552.
16. Radi, H. and Mansoor, A. (2004) *Int. J. Pharm.*, **272(1–2)**, 999–1008.
17. Hillyard, I.W., Doczi, J. and Kiernan, P.B. (1964) *Proc. Soc. Exp. Biol. Med.*, **115(4)**, 1108–1112.
18. Nokhodchi, A. and Tailor, A. (2004) *Il Farmaco*, **59(12)**, 999–1004.
19. Tonnesen, H.H. and Karlsen, J. (2002) *Drug Dev. Ind. Pharm.*, **28(6)**, 621–630.
20. Murata, Y., Kofuji, K. and Kawashima, S. (2003) *J. Biomater. Sci, Polymer Edn.*, **14(6)**, 581–588.
21. Sezer, A.D. and Akbuga, J. (1999) *J. Microencapsul.*, **16(2)**, 195–203.
22. Iskakov, R.M., Kikuchi, A. and Okano, T. (2002) *J. Control. Rel.*, **80(1–3)**, 57–68.
23. Gaserod, O., Jolliffe, I.G., Hampson, F.C., Dettmar, P.W. and Skjak-Braek, G. (1998) *Int. J. Pharm.*, **175(2)**, 237–246.
24. Murata, Y., Miyamoto, E. and Kawashima, S. (1996) *J. Control. Rel.*, **38(2)**, 101–108.
25. Murata, Y., Tsumoto, K., Kofuji, K. and Kawashima, S. (2003) *Chem. Pharm. Bull.*, **51(2)**, 218–220.
26. Nagaoka, M., Shibata, H., Kimura-Takagi, I., Hashimoto, S., Aiyama, R., Ueyama, S. and Yokokura, T. (2000) *BioFactors*, **12(1–4)**, 267–274.
27. Katayama, S., Ohshita, J., Sugaya, K., Hirano, M., Momose, Y. and Yamamura, S. (1998) *Int. J. Mol. Med.*, **2(6)**, 675–679.
28. Todd, P.A. and Sorkin, E.M. (1998) *Drugs*, **35(3)**, 244–285.
29. El-Sayed, Y.M., Abdel-Hameed, M.E., Suleiman, M.S. and Najib, N.M. (1988) *J. Pharm. Pharmacol*, **40(10)**, 727–729.
30. Mortazavi, S.A. and Smart, J.D. (1993) *J. Control. Rel.*, **25(3)**, 197–203.
31. Duchene, D. and Ponchel, G. (1992) *Biomaterials*, **13(10)**, 704–714.
32. Lehr, C.M. (1996) *Eur. J. Drug Met. Pharmacokinet.*, **21(2)**, 139–148.
33. Nokhodchi, A., Farid, D., Najafi, M. and Adrangui, M. (1997) *Drug Dev. Ind. Pharm.*, **23(11)**, 1019–1023.
34. Sabnis, S., Rege, P. and Block, L.H. (1997) *Pharm. Dev. Technol.*, **2(3)**, 243–255.