Pharmaceutical Research Laboratory: Department of Pharmaceutical Sciences, Dr. H. S. Gour University, Sagar, India

Chitosan hydrochloride based microspheres of albendazole for colonic drug delivery

G. Rai, S. K. Jain, S. Agrawal, S. Bhadra, S. S. Pancholi, G. P. Agrawal

Received August 25, 2003, accepted April 27, 2004

Prof. Dr. G. P. Agrawal, Department of Pharmaceutical Sciences, Dr. H. S. Gour University, Sagar, (M.P.) India.
suniljain25in@yahoo.com

Pharmazie 60: 131–134 (2005)

Microspheres of chitosan hydrochloride (CH) were prepared in order to deliver albendazole specifically into the colon. Microspheres were prepared by an emulsion method using different ratios of drug and CH (1 : 1 to 1 : 5), agitation speeds (500 to 1500 rpm) and concentrations of glutaraldehyde in toluene as the cross-linking agent (0.25 to 1.0% w/v). The effect of polymer concentration, stirring rate and concentration of cross-linking agent on the particle size and drug loading was studied. With an increase in CH concentration, the average particle size was increased. Increased agitation speed reduced the size of the microspheres but higher agitation speed resulted in irregularly shaped microspheres. Increasing the concentration of cross-linking agent produced more regularly shaped microspheres of smaller size. The drug loading was highest at a drug: CH ratio of 1 : 3, stirring speed 1000 rpm and 0.75% w/v concentration of cross-linking agent. The effect of CH concentration on *in vitro* drug release from the microspheres was evaluated in simulated g.i.t fluids. A comparative *in vitro* drug release study of the optimized formulation was carried out in simulated colonic fluid, with and without 2% rat caecal content. The drug release in 24 h was 48.9% in colonic fluid without rat caecal content, and 76.5% in colonic fluid with rat caecal contents.

1. Introduction

Chitosan, or β 2-amino-2-deoxy-D-glucose, is a hydrophilic biopolymer obtained industrially by hydrolysing the aminoacetyl groups of chitin, the main component of the shells of crabs, shrimp and krill, by alkaline treatment. The major drawback to its use in colon-specific release is its insolubility at the alkaline pH of the colon thus hindering enzyme activity (Paul and Sharma 2000; Ravi Kumar 2000).

As a part of our research programme for investigating the controlled and targeted delivery of drugs to the colon, microspheres of chitosan salt containing albendazole were prepared. Albendazole was selected for colonic delivery because the colon is the major site of residence of several types of worms and furthermore very little albendazole reaches the colon after oral administration (Krishanaiah et al. 2003). With this system, our aim was to avoid drug delivery in the upper GIT and target the drug to the terminal ileum and colonic region.

2. Investigations, results and discussion

The CH microspheres were prepared by an emulsion method. Glutaraldehyde saturated toluene was used as the cross linker because of the solubility of toluene in an oily phase. It produced uniform and surface crosslinked quite small and almost spherical microparticles as can be seen in the scanning electron photomicrograph (Fig. 1).

The effect of polymer concentration, stirring rate and concentration of cross-linking agent on the particle size of the microspheres was studied (Table). The mean diameter of the CH microspheres was found to increase from 206.8 μm to 236.8 μm with increasing CH concentration viz. drug : CH ratio from 1 : 1 to 1 : 5. The increase in size of the microspheres may be attributed to an increase in viscosity of the polymer with increasing concentration, which resulted in larger emulsion droplets and finally greater microsphere size.

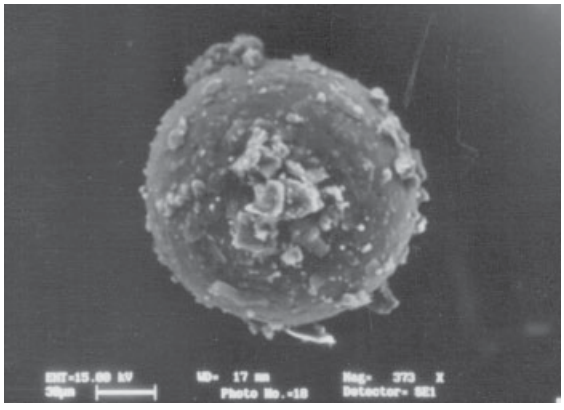

Fig. 1: Scanning electron photomicrograph of chitosan microsphere

Table: Effect of drug: CH ratio, stirring rate and concentration of cross linking agent on average diameter and drug content of CH microspheres

Parameter	Process variable	Average diameter (μm)	Drug content (%)
Albendazole:	1 : 1	206 ± 5	70 ± 1
CH ratio	1 : 2	214 ± 3^{ns}	71 ± 6^{ns}
	1 : 3	$220 \pm 3^{**}$	74 ± 3^{ns}
	1 : 4	$229 \pm 3^{**}$	68 ± 3^{ns}
	1 : 5	$237 \pm 3^{***}$	68 ± 4^{ns}
Stirring rate	500	230 ± 3	71 ± 4
(rpm)	1000	$221 \pm 3^{**}$	76 ± 4^{ns}
	1500	212 ± 4^{ns}	73 ± 5^{ns}
Cross-linking	0.25	231 ± 3	64 ± 1
agent concentration	0.50	$224 \pm 3^{*}$	68 ± 3^{ns}
(% w/v)	0.75	$219 \pm 3^{**}$	76 ± 5^{ns}
	1.00	$210 \pm 1^{**}$	70 ± 6^{ns}

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns – not significant, One way ANOVA test (Dunnett's t test), Data shown are the mean of three readings ± standard deviation

The mean diameters of the microspheres prepared using various agitation speeds i.e. 500, 1000 and 1500 rpm were found to be 230.3, 220.7 and 211.7 μm respectively. The dispersion of the acetic acid solution of the drug and CH in the form of droplets in the oil phase depends on the agitation speed of the system, and hence with an increase in agitation speed, the size of the microspheres reduced, although higher agitation speed resulted in microspheres of irregular shape. The mean diameters of the microspheres prepared using various concentrations of cross linking agent (glutaraldehyde) i.e. 0.25%, 0.5%, 0.75% and 1.0%, were found to be 231.1, 224.2, 218.6 and 209.6 μm respectively. The more cross-linking agent was added, the more regular were the microspheres formed, and the size of the microspheres was reduced from 231.1 μm to 209.6 μm. This may be explained by a while tightening of the polymeric network leading to shrinkage of the microsphere as the concentration of cross-linking agent increased.

The drug loading of the microspheres was investigated with respect to different processing variables i.e. drug : CH ratio, stirring rate and concentration of cross linking agent (Table). Maximum drug loading was observed at a drug : CH ratio of 1 : 3 (73.6%), stirring rate 1000 rpm (75.8%) and 0.75% w/v cross-linking agent concentration

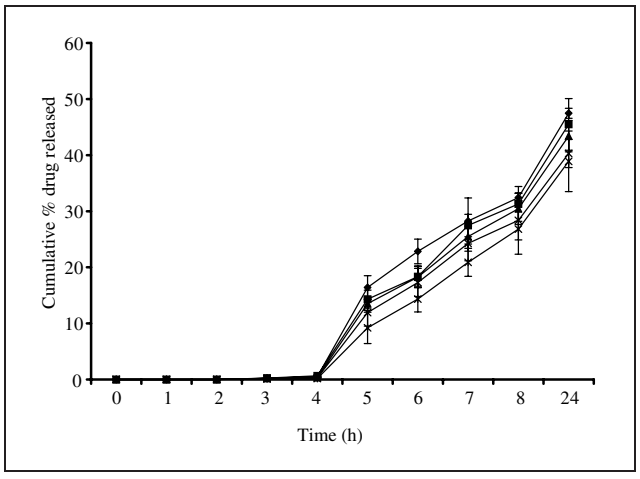

Fig. 2: Percent cumulative *in vitro* drug release profile of albendazole from CH microspheres containing different drug: CH ratio i.e. 1 : 1 (–◆–), 1 : 2 (–■–), 1 : 3 (–▲–), 1 : 4 (–X–) and 1 : 5 (–∗–) in simulated GIT fluids

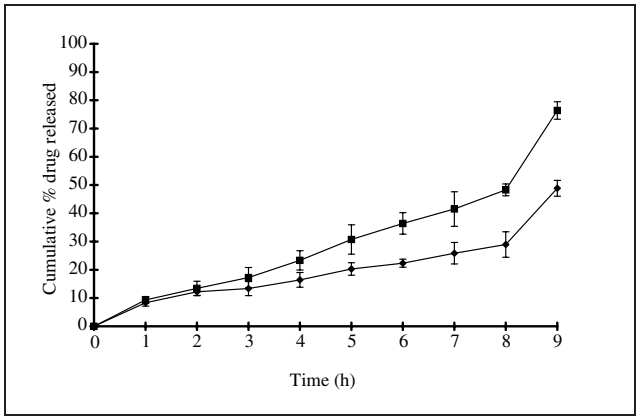

Fig. 3: Effect of caecal content on percent albendazole release from CH microspheres in colonic fluid (PBS, pH 7.4) without caecal content (–◆–) and with 2% caecal content (–■–)

(76.5%). The range of percentage drug loading from 73.6 to 76.5% indicates that approx. 25% of the drug appears to have been leached out to the external phase during the emulsification process. The higher stirring rate prevented the coacervate droplets from coalescing and producing smaller microspheres but it did not have any significant effect on drug loading.

The cumulative % drug release curve from the CH microspheres showed the desired rate as there was no measurable drug release up to 2 h in simulated gastric fluid (pH 1.2) while at pH 4.5 the drug release was quite insignificant (< 1%) up to 4 h. The release of albendazole from CH microspheres in simulated intestinal fluid (pH 7.0) up to 24 h was decreased with increased CH concentration. Thus drug release could be controlled by varying the concentration of CH (Fig. 2). Albendazole, a very hydrophobic drug might have dissolved in the CH polymer matrix and hence its release from the microspheres with a higher proportion of CH was slow.

On the basis of the above results, CH microspheres were prepared under optimized conditions viz. drug : CH ratio 1 : 3, stirring speed 1000 rpm and cross-linking agent concentration 0.75% w/v.

These microspheres were found to be spherical in shape, distributed in the size range 200 to 250 μm with average size of 220 μm. The particles exhibited a smooth surface as indicated in the scanning electron photomicrograph (Fig. 1). Drug entrapment into the optimized microspheres was found to be 70 to 75%.

In the presence of 2% w/v caecal content *ex vivo* drug release study was also performed in PBS (pH 7.0) using a USP XXI dissolution test apparatus. The presence of rat caecal contents in the dissolution medium resulted in improved drug release from CH microspheres as compared to the control. *In vitro* drug release in 24 h in simulated colonic fluid without rat caecal content was 48.9%, but drug release in simulated colonic fluid with 2% rat caecal contents was 76.5%. The rat caecal content in the dissolution medium increased drug release from CH microspheres, which may be attributed to various anaerobic bacteria present in the caecum responsible for the degradation of CH (Fig. 3). Hence drug release in the simulated colonic fluid with caecal content may be due to the combined effect of diffusion and erosion.

The CH microspheres in the form of a lyophilized powder were stored in glass bottles at 4 ± 1 °C, 25 ± 1 °C and 50 ± 1 °C for one month and evaluated for any change in shape and structural integrity by microscopic

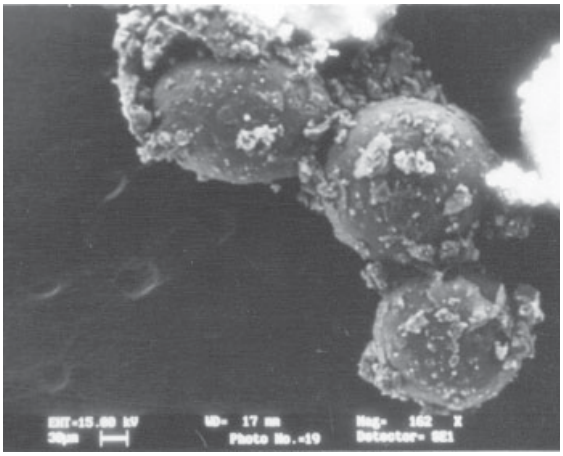

Fig. 4: Scanning electron photomicrograph of chitosan microspheres of albendazole showing structural integrity after storage at 50 °C for one month

examination and residual drug content. At 50 ± 1 °C, the microspheres lost their spherical shape (Fig. 4) indicating their instability at higher temperature. Agglomerates of microspheres were formed after one month storage at 50 °C, which may be attributed to softening and fusion of the polymer. The percentage residual drug content of the microspheres was found to be 98.2%, 98.7% and 98.2% at 4°, 25° and 50 °C respectively after 30 days of storage.

3. Experimental

3.1. Materials

Albendazole was procured as a gift sample from S. A. Pharma, Sagar, India. High-molecular-weight chitosan (MW 60,0000, viscosity 400 mPa and degree of deacetylation 80%), was purchased from Fluka, Buchs, Switzerland. Glutaraldehyde, liquid paraffin, toluene and other solvents were purchased from Himedia Chemical, India. All other chemicals used in the dissolution studies were of analytical reagent grade and were used as received.

3.2. Preparation of chitosan salts

Chitosan (0.25g) was dissolved at room temperature in 50 ml water containing hydrochloric acid in the molar ratio 1 : 1 (moles monomer : moles acid). The solution was spray-dried (Buchi Mini Spray Dryer, B-191, Switzerland) with an inlet temperature of 90–100 °C and the product was collected (Orienti et al. 2002).

3.3. Infrared spectrometry

Infrared (IR) spectra of chitosan and its hydrochloride salt were recorded with a Jasco FT-IR-410 spectrophotometer. The samples were prepared in the form of compressed KBr disks.

3.4 Preparation of microspheres

The microspheres were prepared by an emulsion method with modifications (Benedetti et al. 1990; Abd El-Hameed and Kellaway 1997). A polymeric solution with the desired drug : polymer ratio was prepared in 5% aqueous acetic acid. This solution was dispersed in 37.5 ml of liquid paraffin (1 : 1 of light and heavy liquid paraffin) containing 0.15 g of Span 80 in a 100 ml beaker. This dispersion was stirred using a stainless steel half moon paddle at speed 500 rpm to 1500 rpm for 2 min and the glutaraldehyde solution in toluene (0.25–1.0 ml) was added dropwise and stirred for 1–4 h. The paddle contained three blades with a length of 1 cm each. After stirring, the microspheres were centrifuged and then washed with hexane, methanol and finally with acetone. The microspheres were collected and dried in a hot air oven at 50 °C. The effect of the process variables – drug: polymer ratio, stirring rate and concentration of cross-linking agent (glutaraldehyde in toluene) – on the particle size and size distribution of the microspheres, drug entrapment efficiency and drug release was studied. CH microspheres were prepared using different drug : polymer ratios i.e. 1 : 1 to 1 : 5, while keeping the other two variables constant i.e. stirring speed 1000 rpm and cross-linking agent concentration 0.75% w/v. Similarly, microspheres were also prepared at different stirring rates viz. 500, 1000 and 1500 rpm, while keeping the drug: polymer ratio 1 : 3 and concentration of cross-linking agent 0.75% w/v. In the same way microspheres were prepared using different concentrations of cross-linking agent i.e. 0.25 to 1.0% w/v while keeping the drug : polymer ratio 1 : 3 and stirring speed 1000 rpm.

3.5. Characterization of microspheres

3.5.1. Morphology and particle size

Morphological study of the microparticles was performed by scanning electron microscopy (SEM). The prepared microspheres were freeze dried at −30 °C for 48 h and coated with gold palladium under an argon atmosphere for 150 s to achieve a 20 nm film (Sputter coater, SCD 004, BAL-TEC, Balzers, Furstentum, Liechtenstein). The coated samples were then examined with a scanning electron microscope (Jeol JSM-1600, Tokyo, Japan). The particle size of the prepared microspheres was determined by an optical microscope using a calibrated ocular micrometer (Leica, Germany).

3.5.2. Drug content

An accurately weighed quantity of CH microspheres (equivalent to 100 mg of albendazole) was digested in 10 ml of methanol and allowed to stand for 24 h for complete extraction of albendazole. The solution was filtered and albendazole was assayed spectrophotometrically (GBC Cintra-10 Spectrophotometer) at 291 nm (Mandal et al. 1992). Each determination was made in triplicate.

3.5.3. Release studies

An accurately weighed amount of CH microspheres, equivalent to 100 mg of albendazole, was added to 500 ml of dissolution medium and the release of albendazole from the CH microspheres was investigated using a USP XXI rotating paddle dissolution apparatus (Model DT-06, Erweka, Germany) at 100 rpm and 37 °C. The simulation of gastrointestinal transit conditions was achieved by altering the pH of dissolution medium at different time intervals. The pH of the dissolution medium was kept at 1.2 for 2 h with 0.1 N HCl. Then 1.7 g of KH_2PO_4 and 2.225 g of $Na_2HPO_4 \cdot 2\,H_2O$ were added to the dissolution medium, adjusting to pH 4.5 with 1.0 M NaOH and the release rate study was continued for a further 2 h. After 4 h, the pH of the dissolution medium was adjusted to 7.0 and maintained up to 24 h. The final volume in all cases was 500 ml. Samples were withdrawn from the dissolution medium at various time intervals using a pipette fitted with a microfilter and analyzed spectrophotometrically at 291 nm. All dissolution studies were performed in triplicate.

In vitro drug release study in the presence of colonic fluid containing 2% rat caecal content. The drug release studies were also carried out in simulated colonic fluid using a USP XXI dissolution rate test apparatus (100 rpm, 37 ± 1 °C). A weighed amount of microspheres was placed in the 200 ml of dissolution media (PBS pH 7.0) containing 2% w/v rat caecal content. The experiment was carried out with continuous CO_2 supply into the dissolution medium. At different time intervals, the samples were withdrawn and replaced with fresh PBS. The experiment was continued up to 24 h. The withdrawn samples were pipetted into a series of 10 ml volumetric flasks and volumes were made up to the mark with PBS and centrifuged. The supernatant was filtered through Whatman filter paper and the filtrate analyzed for albendazole content at 291 nm using GBC Cintra spectrophotometer.

3.6. Stability

The selected formulation of microspheres was stored in amber colored glass bottles at 4 ± 1 °C (FT), 25 ± 1 °C (RT) and 50 ± 1 °C (HT) for a period of 30 days and observed for change in morphology and percentage residual drug content, if any. The samples were analysed periodically for residual drug content at time intervals of 10 days for one month.

Acknowledgements: One of the authors, G. Rai, expresses his sincere thanks to U.G.C. for awarding the Junior Research Fellowship during his M. Pharm. course. The authors gratefully acknowledge S. A. Pharma, Sagar for the supply of albendazole as a gift sample. The authors wish to thank the Director, IMMS, New Delhi for providing SEM facilities.

References

Abd El-Hameed MD, Kellaway IW (1997) Preparation and *in vitro* characterization of mucoadhesive polymeric microspheres as intra-nasal delivery systems. Eur J Pharm Biopharm 44: 53–60.

Benedetti LM, Topp EM, Stella VJ (1990) Microspheres of hyaluronic acid estersfabrication methods and *in vitro* hydrocortisone release. J Control Rel 13: 33–41.

Hirano S, Seino H, Akiyama I, Nonaka I (1990) Chitosan: a bio-compatible material for oral and intravenous administration. In: Geblein CG, Dunn RL (ed.) Progress in Biomedical Polymers, Plenum, New York, p. 283–289.

Knapczyk J, Krowczynski L, Krzek J, Brzeski M, Nurnberg E, Schnek O, Struszcyk H (1989) Requirements of chitosan for pharmaceutical and biomedical applications. In: Skak-Braek G, Anthonsen T, Sandford P (ed.) Chitin and Chitosan: Sources, Chemistry, biochemistry, Physical Properties and Applications, Elsevier, London, p. 657–663.

Krishanaiah YSR, Latha K, Nageswara R, Karthikeyan RS, Bhaskar P and Satyanarayana V (2003) Development of colon targeted oral guar gum matrix tablets of albendazole for the treatment of Helminthiasis. Ind. J Pharm Sci 65: 378–85.

Mandal SC, Bhattacharya M, Maity AK (1992) Determination of albendazole in tablet formulation by u. v. spectrophotometric method. Indian Drugs 29: 323–325.

Orienti T, Cerchiara B, Luppi F, Bigucci G, Zuccari V (2002) Influence of different chitosan salts on the release of sodium diclofenac in colon-specific delivery. Int J Pharm 238: 51–59.

Paul W and Sharma CP (2000) Chitosan, a drug carrier for the 21st century: a review. STP Pharma Sci 101: 5–22.

Ravi Kumar MNV (2000) A review of chitin and chitosan applications. React Funct Pol 46: 1–27.