Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 250 (2003) 227–237
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

In vivo evaluation of matrix granules containing microcrystalline chitosan as a gel-forming excipient

M. Säkkinen [a,*], A. Linna [a], S. Ojala [a], H. Jürjenson [b], P. Veski [b], M. Marvola [a]

[a] *Department of Pharmacy, Division of Biopharmaceutics and Pharmacokinetics, University of Helsinki, P.O. Box 56, FIN-00014 Helsinki, Finland*
[b] *Institute of Pharmacy, University of Tartu, EE-2400 Tartu, Estonia*

Received 1 July 2002; received in revised form 27 September 2002; accepted 30 September 2002

Abstract

Interest in drug delivery to the gastrointestinal tract by means of chitosan has been increasing. In the study reported, the biopharmaceutical properties of granules containing microcrystalline chitosan (MCCh; molecular weight 150 kDa, degree of deacetylation 75%) were evaluated via bioavailability tests in human volunteers. Ibuprofen and furosemide were used as model drugs. With ibuprofen, granules containing 40% of MCCh behaved as a slow-release formulation (t_{max} 2.9 h). With furosemide, the most marked difference between a conventional dosage form and granules containing 40% MCCh was a marked lag time (0.5 h) before absorption from the latter. This difference was reflected in t_{max} values for furosemide. Despite the lag time, AUC values for furosemide were high, indicating that the granules containing MCCh had remained in the stomach and that drug release had taken place in the stomach rather than in the intestine. The results of the bioavailability studies indicate that MCCh matrix granules allow a simple preparation of slow-release and perhaps stomach-specific dosage forms. Use of model drugs differing in relation to sites of absorption in the gastrointestinal tract aided identification of sites of absorption of drugs from the granules. Further studies, including γ-scintigraphic evaluations, will be performed on how the granules behave in the stomach.

Keywords: Microcrystalline chitosan; Slow-release; Stomach-specific; Ibuprofen; Furosemide

1. Introduction

Chitosan is a polysaccharide derived from crustacean chitin with crab and shrimp shell wastes as its principal sources (Shepherd et al., 1997). Gel formation by chitosan at pH values of 1–2, as in the stomach, makes chitosan interesting for study as an excipient for development of slow-release oral dosage forms. The low toxicity and good biocompatibility of chitosan (Muzzarelli et al., 1988; Knapczyk et al., 1989) and the fact that sources of it are abundant, are properties desirable in an excipient.

Results of in vitro studies have shown that chitosan adheres to mucosal tissues, e.g. gastric or intestinal mucosa (Lehr et al., 1992; Gåserød et

* Corresponding author. Tel.: +358-9-1915 9479; fax: +358-9-1915 9138
E-mail address: mia.sakkinen@helsinki.fi (M. Säkkinen).

0378-5173/02/$ - see front matter
PII: S0378-5173(02)00547-1

al., 1998). These adhesive properties have led to increasing interest in the development of slow-release dosage forms with prolonged gastric residence times. Delivery of drugs with sites of action in the stomach has been the main objective (Shah et al., 1999; Remuñan-López et al., 2000). However, little information on the behaviour in man of stomach-specific dosage forms containing chitosan is available. Possible benefits of such dosage forms are under investigation. Dosage forms that release drugs slowly in the upper regions of the gastrointestinal tract could be of value if bioavailabilities of the drugs concerned were low or if toxic metabolites were formed distally in the intestine. If the bioavailability of a drug is low because it is specifically absorbed from sites in the upper regions of the gastrointestinal tract, use of chitosan as an excipient could result in amounts of drug absorbed being increased as a result of prolongation of gastric residence time.

In the study reported here, the biopharmaceutical properties of formulations containing microcrystalline chitosan (MCCh) were studied in human volunteers. The first aim in the study was to determine whether MCCh matrix granules could be useful in preparing slow-release formulations for oral administration. Effects of including enteric polymers in the MCCh formulations were also evaluated. Small amounts of enteric polymers insoluble at gastric pH levels were incorporated in matrices or used as coatings. The second aim was to evaluate whether MCCh granules could be of value for development of dosage forms allowing drug release in the stomach.

Bioavailability studies of the slow-release characteristics of MCCh formulations were initially carried out using ibuprofen as model drug. Ibuprofen was chosen because it is readily absorbed throughout the gastrointestinal tract (Wilson et al., 1989). Its short elimination half life (≈ 2 h) facilitates investigation of the effects of formulation variables on absorption rate. A decrease in drug release rate tends to be reflected in the elimination phase.

In a subsequent step, bioavailability studies to determine the site of drug absorption from MCCh granules in the gastrointestinal tract were carried out using furosemide as the model drug. Furosemide is absorbed to the greatest extent in the upper regions of the gastrointestinal tract, significant amounts of furosemide being absorbed already in the stomach (Staib et al., 1989). Amounts of furosemide absorbed therefore indicate sites of drug release. If substantial amounts of furosemide are released from a dosage form in the stomach and absorbed in the upper regions of the gastrointestinal tract (stomach-specific formulations), amounts of furosemide absorbed are similar to those absorbed from conventional dosage forms. For example, the bioavailability of furosemide is high from floating dosage forms that release the drug slowly in the stomach (Özdemir et al., 2000). If, on the other hand, the drug is released in the intestine, a marked reduction in bioavailability is evident.

2. Materials and methods

2.1. Materials

Microcrystalline chitosan (MCCh) (Novasso Ltd., Finland) of high molecular weight (average 150 kDa) and a low degree of deacetylation ($\approx$ 75%) was used as gel-forming excipient in matrix granules. The MCCh was manufactured from conventional chitosan in accordance with specifications (Struszczyk, 1987) using a continuous method (Finnish Patent, 1991). Microcrystalline grade of chitosan was chosen as excipient because gel formation with formulations containing MCCh is more efficient and consequent drug release more retarded than with formulations containing conventional chitosan (Säkkinen et al., 2002). The effects of incorporation of enteric polymers in granules were studied using hydroxypropylmethylcellulose acetate succinate (Aqoat AS-HF®, Shin-Etsu Chemical Co., Japan) to form coatings and employing methacrylate copolymer (Eudragit S100®, Röhm Pharma, Germany) as a binder. Ibuprofen (Ph. Eur.) and furosemide (Ph. Eur.) were used as model drug substances. Both drugs are weak acids. The pK_a of ibuprofen is 5.3 and that of furosemide 3.9. The Mw of ibuprofen is 206.3 g mol^{-1} and that of furosemide 330.7 g mol^{-1}. Ibuprofen and furosemide are in groups II

and IV, respectively, of the Biopharmaceutics Classification System (Amidon et al., 1995).

2.2. Preparation of the matrix granules

Matrix granules were made from mixtures containing 40% of polymer and 60% of drug. Powder masses incorporating drug and MCCh were granulated using a 2.5% aqueous solution of acetic acid (q.s.). The masses were moistened with the granulating fluid in a mortar and granulated through a 2.0-mm sieve. The granules were dried overnight at room temperature. The size fraction 1.18–1.68 mm was separated by sieving and used as such in the study, or subjected to coating. Granules containing 2.5, 5 or 10% of enteric polymer in the matrices were prepared similarly using a 20% solution of the enteric polymer Eudragit S100® in ethanol (Oy Primalco Ab, Finland) (q.s.) as granulating fluid. Batch size of each formulation was 100 g. Reproducibility of the manufacturing method was checked by preparing at least two batches of each formulation in parallel. Variability in results between the batches was minimal. Presented data are for batch one.

2.3. Coating of matrix granules

The coating solution contained 10% of the enteric polymer Aqoat AS-HF® in demineralized water, 3.5% of triethyl citrate (Fluka Chemie AG, Switzerland) and 3% of magnesium stearate (Ph. Eur.). Solutions were prepared in accordance with the instructions of the polymer manufacturers. Coating was performed using a fluidized-bed coater (Aeromatic Strea-1, Aeromatic AG, Switzerland) equipped with a Würster cylinder. Granules were preheated for 5 min at 40 °C outlet temperature. During coating, the spraying pressure was 1 bar, the airflow rate 70 $m^3\ h^{-1}$ and the outlet temperature 40 °C. The spraying rate was 5 g min^{-1}. Coating was continued until theoretical weight increases of 5, 10 or 20% had been achieved. The granules were dried at 40 °C for 5 min. Batch size of each formulation was 100 g. Reproducibility of the manufacturing method was checked by preparing two batches of each formulation in parallel. Variability in results between the batches was minimal. Presented data are for batch one.

2.4. In vitro release studies

Drug release from granules was studied by means of dissolution tests, using the basket method described in USP 24 (Distek Premiere 5100 Apparatus, Distek, USA). The dissolution medium used was phosphate buffer pH 5.8 (USP 24) (1000 ml, 37 ± 0.5 °C). The speed of rotation was 100 min^{-1}. Results of dissolution tests at pH values of 1–2 would have allowed the in vivo behaviours of chitosan granules in the stomach to be predicted best. However, with ibuprofen and furosemide, the dissolution tests could not be undertaken at very low pH values because of the low solubility of ibuprofen and furosemide at such pH levels.

The required amounts of granules of each formulation were placed into baskets so that the theoretical amount of drug in a dissolution vessel was 12 mg. Amounts of drug released from six parallel samples were determined spectrophotometrically. The dissolution apparatus was connected to a flow-through spectrophotometer (Ultrospec 4000, Pharmacia Biotech, UK) via a peristaltic pump (Icalis PCP490, Icalis Data System, UK). Absorbances at 221 nm (ibuprofen) and 274 nm (furosemide) were recorded automatically, using dissolution software (Icalis Data Systems) and converted to percentages of drug released as a function of time. Drug release from conventional tablet formulations of ibuprofen (Burana® 200 mg, Orion Pharma, Finland) and furosemide (Furesis® 40 mg, Orion Pharma) was also studied using the dissolution system described above.

2.5. In vivo bioavailability studies

Four groups (groups I–IV), each consisting of eight or nine healthy volunteers participated in a series of randomized cross-over single-dose bioavailability studies. The ages of the volunteers ranged from 25 to 42 years, their weights from 56 to 76 kg. Before the studies, each volunteer was examined physically and subjected to routine haematological testing (Hb, ESR, S-Alat, S-Asat,

S-GT, C-Crea) and ECG examination. Each volunteer was informed about possible risks and adverse effects of taking the drugs. Written consent to participation in the studies was obtained. The investigations were carried out in accordance with the recommendations of the Declaration of Helsinki (World Medical Assembly, 1964) as revised in Edinburgh in 2000. The study protocol had been approved by the Ethical Committee of the University Hospital of Tartu.

The required amounts of granules were dispensed into hard gelatin capsules (size 0) so that the amounts of ibuprofen or furosemide per capsule were 100 and 40 mg, respectively. In the bioavailability study of furosemide, a conventional tablet (Furesis® 40 mg, Orion Pharma) was also included for reference. The respective doses of ibuprofen and furosemide in the bioavailability study were 300 and 40 mg. Three ibuprofen capsules or one furosemide capsule or tablet were administered to each subject with 200 ml of water, following an overnight fast for at least 10 h. Lunch was provided 4 h after drug administration. Blood samples were collected from a forearm vein into heparinized tubes. Plasma was separated and stored at −20 °C until analysis. The washout period between formulations was at least 1 week.

2.6. Plasma assay

Drug concentrations in plasma were determined by means of high-performance liquid chromatography (HPLC). In ibuprofen, concentrations of the drug in plasma were determined using the method described by Avgerinos and Hutt (1986), with slight modifications. Determinations were carried out on two samples in parallel. The HPLC system was equipped with a Waters 501 piston pump (Waters, USA), a Waters 717 Autosampler (Waters), a Waters 484 tunable absorbance detector (Waters) operated at 222 nm and a Millennium 32 Chromatography Manager (Waters) workstation. Sample separation was carried out on a 3.9×300-mm column packed with 10-μm reverse-phase silica (μBondapak C18, Waters). The isocratic mobile phase consisted of acetonitrile and 0.1 M sodium acetate (35:65), the pH of which was adjusted to 6.2 with glacial acetic acid. The flow rate was 2 ml min^{-1}. The standard curve was found to be linear ($r^2 > 0.9989$) over the concentration range used (0.5–40 mg l^{-1}). The accuracy and precision of the method were investigated as recommended by Shah et al. (2000). Mean values at the extremes of the concentration range were 0.55 mg l^{-1} (percentage coefficients of variation (CV%) for accuracy and precision were 8.6 and 4.2%, respectively) and 40.5 mg l^{-1} (CV% 1.3 and 1.3%). No interfering peaks were found in the plasma blanks.

Furosemide concentrations in plasma were determined using the method described by Beermann (1982), with slight modifications. Determinations were carried out on three samples in parallel. An internal standard, bumetanide, a phenyl analogue of furosemide, was simultaneously subjected to the plasma procedure. With furosemide, the HPLC system described above was equipped with a Waters 470 fluorescence detector (Waters). The excitation and emission wavelengths were 233 and 389 nm, respectively. The mobile phase was acetonitrile and 0.08 M phosphoric acid (40:60) and the flow rate 1.5 ml min^{-1}. The standard curve was found to be linear ($r^2 > 0.9981$) over the concentration range used (20–2800 μg l^{-1}). The accuracy and precision of the method were investigated as recommended by Shah et al. (2000). Mean values at the extremes of the concentration range were 17.3 μg l^{-1} (CV% for accuracy and precision 13.5 and 8.6%, respectively) and 2790 μg l^{-1} (CV% 0.3 and 3.1%). No interfering peaks were found in the plasma blanks.

2.7. Pharmacokinetic parameters

The pharmacokinetic parameters assessed, using the Siphar® pharmacokinetic data analysis program (Simed, France), were absorption rate constant (k_a), area under the concentration–time curve (AUC), mean residence time (MRT) and apparent elimination half life ($t_{1/2}$). Maximum concentration of drug in plasma (C_{max}) and time to peak concentration (t_{max}) were obtained directly from individual time-versus-plasma–concentration curves. AUC and MRT values were calculated using the trapezoidal method, without logarithmic transformation. The method of

Wagner and Nelson was used to estimate the apparent absorption rate constant (k_a) and to calculate the time at which 90% of drug had been absorbed ($t_{90\%}$). The rate of absorption was also evaluated by means of the ratio C_{max}/AUC. Statistical analyses were carried out using Student's paired t-test or Wilcoxon's non-parametric test (for t_{max} values). When results in two different groups of volunteers were compared, Student's t-test relating to independent groups or the Mann–Whitney non-parametric test (for t_{max} values) was used.

3. Results and discussion

3.1. In vitro drug release

Granules containing MCCh as gel-forming excipient behaved as slow-release formulations of ibuprofen and furosemide (Figs. 1–3). Ibuprofen release was more retarded than furosemide release from corresponding formulations. $T_{50\%}$ values for drug release from formulations containing 40% of MCCh ranged from 1.8 ± 0.3 h for furosemide to 3.1 ± 0.4 h for ibuprofen. The differences in release rates can be explained by differences in the chemical properties of the drugs. At pH 5.8, the

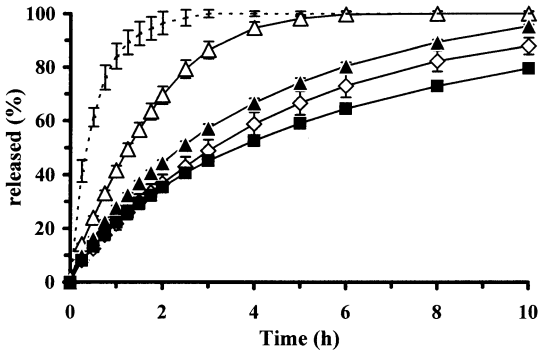

Fig. 1. Release of ibuprofen (at pH 5.8) from matrix granules containing 60% of the drug and microcrystalline chitosan (MCCh) and enteric polymer Eudragit S100® (E) as excipients (mean ± S.D.; $n = 6$). Amounts of MCCh and E in matrices: ◇, MCCh 40%, E 0%; △, MCCh 37.5%, E 2.5%; ▲, MCCh 35%, E 5%; ■, MCCh 30%, E 10%. Reference (conventional tablet): ---.

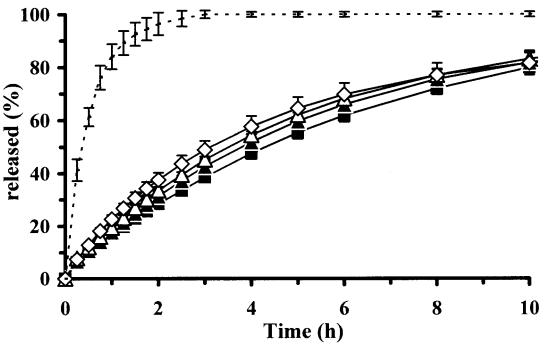

Fig. 2. Release of ibuprofen (at pH 5.8) from coated matrix granules containing 60% of the drug, 40% of microcrystalline chitosan (MCCh) as excipient in the cores and different amounts of the enteric polymer Aqoat AS-HF® as coating (mean ± S.D.; $n = 6$). Amounts of coating in granules: ◇, 0%; △, 5%; ▲, 10%; ■, 20%. Reference (conventional tablet): ---.

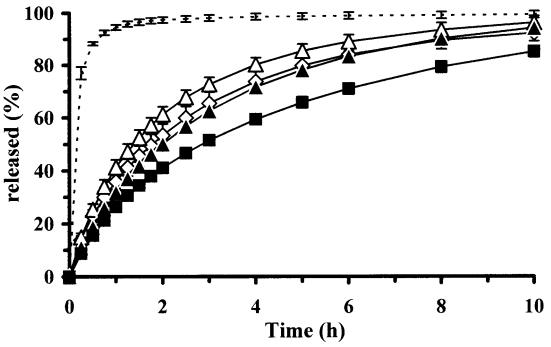

Fig. 3. Release of furosemide (at pH 5.8) from matrix granules containing 60% of the drug and microcrystalline chitosan (MCCh) and enteric polymer Eudragit S100® (E) as excipients (mean ± S.D.; $n = 6$). Amounts of MCCh and E in matrices: ◇, MCCh 40%, E 0%; △, MCCh 37.5%, E 2.5%; ▲, MCCh 35%, E 5%; ■, MCCh 30%, E 10%. Reference (conventional tablet): ---.

stronger acid furosemide (pK_a 3.9) will dissolve more easily than ibuprofen (pK_a 5.3).

In general, drug release from MCCh granules is controlled by gel formation by chitosan in acidic environments, thereafter by both drug diffusion through the gel and erosion of gels as a result of dissolution of chitosan (non-Fickian release) (Säkkinen et al., 2002). Findings relating to release of ibuprofen and furosemide in the study reported here are consistent with previous findings by us that drug solubility substantially affects the slow-

release characteristics of MCCh granules. Slow-release MCCh granules are achievable only with drugs of low solubility (BCS class-II and class-IV drugs). Slightly soluble drugs with low dissolution rates are released more slowly than readily soluble drugs. Release of slightly soluble anionic drug substances may be further retarded by complex formation between positively charged chitosan and negatively charged drug (Rege et al., 1999).

When the enteric polymer Eudragit S100® was incorporated in matrices and replaced 10% of the MCCh, drug release was retarded (Fig. 1). Incorporation of lesser amounts of Eudragit S100® accelerated drug release rates. $T_{50\%}$ values for ibuprofen release at pH 5.8 were 3.1 ± 0.4 h for the formulation containing 40% of MCCh and 3.7 ± 0.2 h for the formulation containing 10% of enteric polymer. Because Eudragit S100 dissolves at pH 6.8 (Marvola et al., 1999), drug release at pH 5.8 was obviously controlled both by gel formation by chitosan and the existence of an insoluble enteric polymer matrix. It is concluded that enteric polymers could be used to reinforce gels formed by MCCh and control drug release at acidic pH values.

Coating of granules containing 40% of MCCh with the enteric polymer Aqoat AS-HF® decreased drug release rates (Fig. 2). $T_{50\%}$ values for ibuprofen release at pH 5.8 were 3.1 ± 0.4 h for uncoated granules and 4.3 ± 0.3 h for granules coated with 20% of the enteric polymer. Because Aqoat AS-HF® has been shown to dissolve at pH 6.7 (Marvola et al., 1999), the high levels of drug release at pH 5.8 indicate that the relatively small amounts of enteric polymer had formed porous coatings. Such porous coatings could be valuable in reinforcing the effects of the gels formed by MCCh at acidic pH values, e.g. in the stomach. Differences between results with amounts of enteric polymer coating ranging from 5 to 20% were moderate.

With furosemide the effects of incorporating enteric polymer in matrices were similar to those with ibuprofen (Fig. 3). Effects of coating were not studied. $T_{50\%}$ values for drug release at pH 5.8 were 1.8 ± 0.3 h for granules containing 40% of MCCh and 2.9 ± 0.2 h for granules containing 10% of enteric polymer. Lesser amounts of enteric polymer had no marked effects.

3.2. In vivo studies

3.2.1. Bioavailability of ibuprofen

Bioavailability studies were initially carried out using ibuprofen as model drug to evaluate whether MCCh granules might be useful in preparing slow-release formulations for oral administration. Results are summarized in Figs. 4 and 5 and Tables 1 and 2.

Granules containing MCCh acted as slow-release dosage forms of ibuprofen. The mean t_{max} for ibuprofen was 2.9 ± 1.1 h for granules containing 40% of MCCh (Table 1). With conventional ibuprofen dosage forms t_{max} occurs usually after about 1 h (Davies, 1998). In our laboratory, t_{max} values of 1.2–1.8 h have been obtained for conventional ibuprofen formulations (Halsas et al., 1999; Honkanen et al., 2001). It is obvious that the efficient formation of gels by MCCh in acidic environments, which has been demonstrated in previous studies in vitro (Säkkinen et al., 2002), retards drug release and drug absorption. Absorption of ibuprofen was not subject to any lag time, indicating that drug absorption from the MCCh granules had already begun in the stomach.

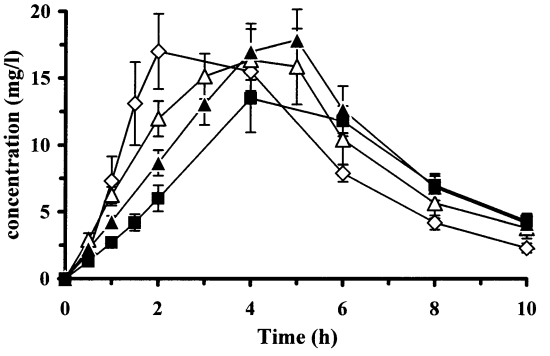

Fig. 4. Concentrations of ibuprofen in plasma after oral administration of matrix granules containing 60% of the drug and microcrystalline chitosan (MCCh) and enteric polymer Eudragit S100® (E) as excipients (mean ± S.E.M.; $n = 8$). Amounts of MCCh and E in matrices: ◇, MCCh 40%, E 0%; △, MCCh 37.5%, E 2.5%; ▲, MCCh 35%, E 5%; ■, MCCh 30%, E 10%.

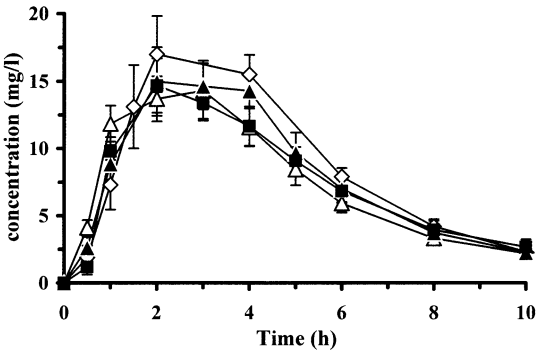

Fig. 5. Concentrations of ibuprofen in plasma after oral administration of coated matrix granules containing 60% of the drug, 40% of microcrystalline chitosan (MCCh) as excipient in cores and different amounts of the enteric polymer Aqoat AS-HF® as coating (mean ± S.E.M.; $n = 8–9$). Amounts of coating in granules: ◇, 0%; △, 5%; ▲, 10%; ■, 20%.

When 2.5, 5 or 10% of enteric polymer was incorporated into matrices, retardation of ibuprofen absorption was apparent (Fig. 4, Table 1). As amounts of insoluble material in matrices increased, i.e. as gel-forming MCCh was replaced with an enteric polymer insoluble at low pH levels, absorption rate constants decreased and t_{max} values increased, to a maximum of 4.7 ± 1.0 h in the case of a formulation containing 10% of enteric polymer. There were statistically significant ($P < 0.05$) differences in relation to the rate parameters k_a, t_{max}, MRT and C_{max}/AUC. There were no marked differences in AUC values.

Coating of granules containing 40% of MCCh with 5, 10 or 20% of an enteric polymer did not decrease rates of absorption (Fig. 5, Table 2). Results of in vitro studies show that granules containing MCCh swell during gel formation, with the swelling being most marked at low pH levels (Säkkinen et al., 2002). Although the results of in vitro dissolution studies at pH 5.8 (Fig. 2) suggested that absorption should become slower as amounts of coating increased, swelling of the granules in the acidic stomach environment could explain why coating had only minor effects on the rate of absorption of ibuprofen in vivo.

Overall, our conclusion is that granules containing MCCh as gel-forming excipient are a simple means of preparing slow-release formulations for oral administration. Absorption could easily be retarded further by incorporating small amounts of enteric polymer in matrices. Results of previous studies by us indicate that increasing the amounts

Table 1
Pharmacokinetic parameters of ibuprofen (300 mg) after oral administration in granules containing microcrystalline chitosan (MCCh) and enteric polymer Eudragit S100® (E) as excipients (mean ± S.D.; $n = 8$)

Parameter	Formulation			
	MCCh 40%[a]	MCCh 37.5%; E 2.5%[b]	MCCh 35%; E 5%[c]	MCCh 30%; E 10%[d]
k_a (h^{-1})	1.4 ± 0.6[b,c,d,*]	0.9 ± 0.2	0.9 ± 0.2	0.8 ± 0.3
t_{max} (h)	2.9 ± 1.1[c,d,**,***]	3.8 ± 1.0	4.5 ± 0.5	4.7 ± 1.0
C_{max} (mg l^{-1})	19.6 ± 6.4[d,**]	19.5 ± 6.5	19.2 ± 6.4	14.2 ± 7.2
$AUC_{0-\infty}$ (mg l^{-1} h)	100.1 ± 22.4	112.2 ± 41.8	126.3 ± 46.7	109.3 ± 34.8
$t_{1/2}$ (h)	2.4 ± 0.5[d,**]	2.8 ± 0.6[d,*]	4.7 ± 4.8	4.9 ± 2.0
MRT (h)	3.4 ± 0.8[d,**]	4.0 ± 0.9[d,*]	5.7 ± 4.3	5.8 ± 1.7
$C_{max}/AUC_{0-\infty}$ (h^{-1})	0.20 ± 0.05[c,d,*,***]	0.18 ± 0.04[d,**]	0.16 ± 0.02[d,*]	0.13 ± 0.03
$t_{90\%}$ (h)	3.0 ± 1.0[d,*]	4.1 ± 1.7	3.7 ± 0.5	4.1 ± 0.8

Student's paired t-test or Wilcoxon's matched-pairs rank test (for t_{max}): column 1 versus 4 and column 2 versus 3. Student's t-test for independent groups or Mann–Whitney U-test (for t_{max}): columns 1 and 4 versus 2 and 3.

[a] Group I.
[b] Group II.
[c] Group II.
[d] Group I.
* Significance level: $P < 0.05$.
** Significance level: $P < 0.01$.
*** Significance level: $P < 0.001$.

Table 2
Pharmacokinetic parameters of ibuprofen (300 mg) after oral administration in chitosan matrix granules containing microcrystalline chitosan (MCCh) as excipient, granules coated with the enteric polymer Aqoat AS-HF® (mean ± S.D.; $n = 8–9$)

Parameter	Formulation			
	Core[a] MCCh 40%	Coat 5%[b]	Coat 10%[c]	Coat 20%[d]
k_a (h^{-1})	1.4 ± 0.6	1.5 ± 0.7	1.4 ± 0.8	1.1 ± 0.6
t_{max} (h)	2.9 ± 1.1	2.1 ± 1.1	2.7 ± 1.0	2.1 ± 0.6
C_{max} (mg l^{-1})	19.6 ± 6.4	16.4 ± 5.7	19.3 ± 8.3	15.2 ± 5.7
$AUC_{0-\infty}$ (mg l^{-1} h)	100.1 ± 22.4	84.8 ± 23.1	90.8 ± 30.2	91.6 ± 30.4
$t_{1/2}$ (h)	2.4 ± 0.5	2.5 ± 0.4	2.4 ± 0.8	2.9 ± 1.3
MRT (h)	3.4 ± 0.8	3.6 ± 0.6	3.5 ± 1.2	4.2 ± 1.9
$C_{max}/AUC_{0-\infty}$ (h^{-1})	0.20 ± 0.05	0.19 ± 0.03	0.21 ± 0.06	0.17 ± 0.05
$t_{90\%}$ (h)	3.0 ± 1.0[b,*]	2.3 ± 0.7[d,*]	2.6 ± 0.9	3.0 ± 0.9

Student's paired t-test or Wilcoxon's matched-pairs rank test (for t_{max}): column 2 versus 3 and 4. Student's t-test for independent groups or Mann–Whitney U-test (for t_{max}): column 1 versus 2–4.
[a] Group I ($n = 8$).
[b] Group III ($n = 9$).
[c] Group III ($n = 9$).
[d] Group III ($n = 9$).
* Significance levels: $P < 0.05$.

of MCCh used, or using MCCh of higher molecular weight, leads to increases in the viscosities of the gels formed by MCCh and decreases in drug release rates (Säkkinen et al., 2002). However, substantial amounts of excipients in formulations containing drug with a high dose, such as ibuprofen, are impractical.

3.2.2. Bioavailability of furosemide

The bioavailability of furosemide was studied from granules containing 40% of MCCh. A conventional tablet and granules containing 10% of enteric polymer in matrices were employed as reference preparations. The bioavailabilities of furosemide from a conventional dosage form and from a formulation containing enteric polymer are representative of furosemide absorption from the stomach and intestine, respectively.

The results of the bioavailability studies of furosemide (Fig. 6) differed markedly from those obtained with ibuprofen. The most marked difference between the conventional dosage form and matrix granules containing 40% of MCCh was that absorption from the latter was subject to a lag time of ≈30 min. This difference was reflected in t_{max} values (Table 3). T_{max} for furosemide was slightly higher for MCCh formulation. However, the effect

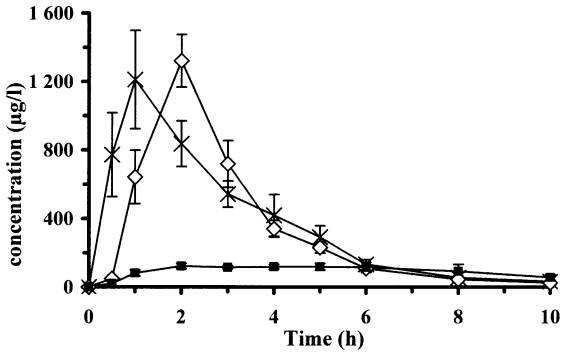

Fig. 6. Concentrations of furosemide in plasma after oral administration of matrix granules containing 60% of the drug and microcrystalline chitosan (MCCh) and enteric polymer Eudragit S100® (E) as excipients (mean ± S.E.M.; $n = 9$). Amounts of MCCh and E in matrices: ◇, MCCh 40%, E 0%; ■, MCCh 30%, E 10%. Reference (conventional tablet): ×.

was not statistically significant. There were no marked differences in AUC or C_{max} values. Furosemide is known to be absorbed to a great extent in the stomach. It is therefore obvious that the lag time was related to gel formation by MCCh. Subsequently, drug diffusion through the gel and gel degradation led to fairly rapid drug release and absorption. Because AUC was not lower with the granule formulation of furosemide

Table 3
Pharmacokinetic parameters of furosemide (40 mg) after oral administration in granules containing microcrystalline chitosan (MCCh) and the enteric polymer Eudragit S100® (E) as excipients

Parameter	Formulation		
	Furesis® tablet[a]	MCCh 40%[b]	MCCh 30% E 10%[c]
k_a (h^{-1})	3.1±1.5	2.1±1.2	1.7±1.2[a,*]
t_{max} (h)	1.5±1.1	1.8±0.4	2.9±2.2
C_{max} (μg l^{-1})	1600±559	1330±446	177±95.6[a,b,***]
$AUC_{0-\infty}$ (μg l^{-1} h)	3800±941	3450±1180	1690±1890[a,b,**]
AUC_{0-10} (μg l^{-1} h)	3720±946	3390±1180	959±519[a,b,***]
$t_{1/2}$ (h)	1.6±0.5	1.4±0.4	5.7±4.2[a,b,*]
MRT (h)	1.9±0.5	1.8±0.4	7.8±5.2[a,b,*,**]
$C_{max}/AUC_{0-\infty}$ (h^{-1})	0.39±0.18	0.39±0.08	0.13±0.04[a,b,*,***]
C_{max}/AUC_{0-10} (h^{-1})	0.40±0.18	0.40±0.08	0.19±0.03[a,b,*,***]

Student's paired t-test or Wilcoxon's matched pairs rank test (for t_{max}).
[a] Group IV.
[b] Group IV.
[c] Group IV.
* Significance levels: $P < 0.05$.
** Significance levels: $P < 0.01$.
*** Significance levels: $P < 0.001$.

than with the conventional tablet, the entire furosemide dose must have been released in the stomach in both cases. The bioavailability of furosemide from the granule formulation was high, despite the clear lag time of absorption, which is indirect evidence that the granules remained in the stomach. If a substantial amount of furosemide dose had been released in the small intestine, the AUC value would have been markedly lower.

Replacement of 10% of the MCCh by enteric polymer strikingly affected the bioavailability of furosemide, as expected. The amount of furosemide absorbed was substantially reduced. AUC_{0-10} values were less than one-third of the corresponding values with the conventional tablet (Table 3). AUC and C_{max} values for the granules studied were, however, similar to those for a commercial slow-release formulation in previous studies (Marvola et al., 1999). It is obvious that incorporation of enteric polymer in matrices hindered disintegration of the granules in the stomach and led to a substantial amount of the furosemide dose being liberated not in the stomach but in the small intestine, resulting in low bioavailability. In many cases it is incomplete absorption of furosemide from the intestine that explains low bioavailability from slow-release dosage forms (Ritschel et al., 1991; Farshi et al., 1995).

In the study reported here, furosemide was chosen as a model drug for evaluation of sites of drug release and absorption from granules containing MCCh. Regional absorption of furosemide from the gastrointestinal tract has been clearly demonstrated by Staib et al. (1989), who used a High-Frequency® capsule to deliver suspensions of furosemide to different regions of the gastrointestinal tract in human volunteers. The results of their study show that furosemide is absorbed to the greatest extent in the upper regions of the gastrointestinal tract, significant amounts of furosemide being absorbed already in the stomach. Amounts of furosemide absorbed decreased significantly in the intestine and the bioavailability of furosemide from the ileum was only 17% of the oral dose. In a recent study by Clear et al. (2001) on conventional tablet formulations of furosemide, findings were similar. When conventional tablets of furosemide were administered using an Intelisite® capsule, markedly lower AUC values were obtained following release of the tablets from the capsule in the duodenum (71% of the oral dose

absorbed) than following oral administration of the tablets (100%). The results of the study by Clear et al. (2001) make it clear that the 'absorption window' of furosemide is narrow. Some of the absorption window is missed if furosemide is released in proximal regions of the small intestine. Because the absorption of furosemide is site-specific, amounts of furosemide absorbed indicate the site of drug release.

Our findings of high bioavailability of furosemide from granules containing 40% of MCCh indicate drug release in the stomach. Taking into account the clear lag time before absorption it can be concluded that MCCh granules remain in the stomach for some time and are perhaps stomach-specific formulations. In general, small granules and pellets (<2 mm) empty from the fasted stomach rapidly, in exponential manners (Khosla and Davis, 1987). If the MCCh granules in our study had passed to the small intestine without remaining in the stomach for a time, bioavailability of furosemide would have been much lower. The findings relating to formulation containing enteric polymer fit well with the idea that granules containing 40% MCCh are stomach-specific. In studies, in vitro chitosan formulations have been shown to adhere to porcine gastric mucosa (Gåserød et al., 1998). The mechanism of adhesion involves adsorption of hydrophilic chitosan on to the mucosal surface and electrostatic attraction between cationic chitosan and anionic mucus glycoprotein. In our study in man, the MCCh granules may have adhered for a time to the gastric mucosa, resulting in prolongation of gastric residence times.

The results of our study show, for the first in human volunteers, that granules containing chitosan could be of value in developing stomach-specific dosage forms. Application has been made for an international patent relating to MCCh granule formulations (PCT/FI01/00322, International Patent Application, 2001). Further studies, including imaging of granules in the gastrointestinal tract by means of γ-scintigraphy, will be performed. The aims of these studies will be to demonstrate whether granules in fact adhere to gastric mucosa and to determine gastric residence times of granules. The physiological factors thought to be most important in limiting gastric residence times of stomach-specific mucoadhesive formulations are mucous turnover and the motility of the gastrointestinal tract (Lee et al., 2000).

4. Conclusions

The results of the bioavailability studies reported here indicate that MCCh matrix granules can be valuable in allowing simple preparation of slow-release dosage forms. Granules in which the content of MCCh was only 40% gave satisfactory slow-release formulation of ibuprofen. However, release rates from MCCh granules depend on the drug concerned. Furosemide absorption from corresponding MCCh formulation was subject to a lag time, followed by fairly rapid drug absorption. Our findings also suggest that MCCh granules remain in the stomach for some time and could therefore be of value in developing stomach-specific dosage forms. Further studies, including γ-scintigraphic investigations, of how such granules behave in the stomach, will be performed.

References

Amidon, G.L., Lennernäs, H., Shah, V.P., Crison, J.R., 1995. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm. Res. 12, 413–420.

Avgerinos, A., Hutt, A.J., 1986. High performance liquid chromatographic determination of ibuprofen in human plasma and urine by direct injection. J. Chromatogr. 380, 468–471.

Beermann, B., 1982. Kinetics and dynamics of furosemide and slow-acting furosemide. Clin. Pharmacol. Ther. 32, 584–591.

Clear, N.J., Milton, A., Humphrey, M., Henry, B.T., Wulff, M., Nichols, D.J., Anziano, R.J., Wilding, I., 2001. Evaluation of the Intelisite capsule to deliver theophylline and frusemide tablets to the small intestine and colon. Eur. J. Pharm. Sci. 13, 375–384.

Davies, N.M., 1998. Clinical pharmacokinetics of ibuprofen. The first 30 years. Clin. Pharmacokinet. 34, 101–154.

Farshi, F.S., Capan, Y., Kes, S., Hincal, A.A., 1995. Formulation and comparative bioavailability of conventional and sustained-release furosemide tablets. STP Pharma. Sci. 5, 361–366.

Finnish Patent FI83426, 1991. Method for continuous manufacture of microcrystalline chitosan. Novasso Ltd.

Gåserød, O., Jolliffe, A.G., Hampson, F.C., Dettmar, P.W., Skjåk-Bræk, G., 1998. The enhancement of the bioadhesive properties of calcium alginate gel beads by coating with chitosan. Int. J. Pharm. 175, 237–246.

Halsas, M., Hietala, J., Veski, P., Jürjenson, H., Marvola, M., 1999. Morning versus evening dosing of ibuprofen using conventional and time-controlled release formulations. Int. J. Pharm. 189, 179–185.

Honkanen, O., Seppä, H., Eerikäinen, S., Tuominen, R., Marvola, M., 2001. Bioavailability of ibuprofen from orally and rectally administered hydroxypropylmethylcellulose capsules compared to corresponding gelatine capsules. STP Pharma. Sci. 11, 181–185.

International Patent Application PCT/FI01/00322, 2001. A peroral pharmaceutical formulation for controlled-release of drug.

Knapczyk, J., Krówczynski, L., Pawlik, B., Liber, Z., 1989. Pharmaceutical dosage forms with chitosan. In: Skajåk-Braek, G., Anthonsen, T., Sandford, P. (Eds.), Chitin and Chitosan: Sources, Chemistry, Biochemistry, Physical Properties and Applications. Elsevier Applied Science, London, pp. 665–670.

Khosla, R., Davis, S.S., 1987. The effect of polycarbophil on the gastric emptying of pellets. J. Pharm. Pharmacol. 39, 47–49.

Lee, J.W., Park, J.H., Robinson, J.R., 2000. Bioadhesive based dosage forms: the next generation. J. Pharm. Sci. 89, 850–866.

Lehr, C.M., Bouwstra, J.A., Schacht, E.H., Junginger, H.E., 1992. In vitro evaluation of mucoadhesive properties of chitosan and some other natural polymers. Int. J. Pharm. 78, 43–48.

Marvola, M., Nykänen, P., Rautio, S., Isonen, N., Autere, A.M., 1999. Enteric polymers as binders and coating materials in multiple-unit site-specific drug delivery systems. Eur. J. Pharm. Sci. 7, 259–267.

Muzzarelli, R., Baldassare, V., Conti, F., Ferrara, P., Biagini, G., Gazzanelli, G., Vasi, V., 1988. Biological activity of chitosan: ultrastructure study. Biomaterials 9, 247–252.

Özdemir, N., Ordu, S., Özkan, Y., 2000. Studies of floating dosage forms of furosemide: in vitro and in vivo evaluations of bilayer tablet formulations. Drug Dev. Ind. Pharm. 26, 857–866.

Rege, P.R., Shukla, D.J., Block, L.H., 1999. Chitinosans as tableting excipients for modified release delivery systems. Int. J. Pharm. 181, 49–60.

Remuñan-López, C., Portero, A., Lemos, M., Vila-Jato, J.L., Nuñez, M.J., Riveiro, P., López, J.M., Piso, M., Alonso, M.J., 2000. Chitosan microspheres for the specific delivery of amoxycillin to the gastric cavity. STP Pharma. Sci. 10, 69–76.

Ritschel, W.A., Menon, A., Sakr, A., 1991. Biopharmaceutic evaluation of furosemide as a potential candidate for a modified release per oral dosage form. Methods Find. Exp. Clin. Pharmacol. 13, 629–636.

Säkkinen, M., Seppälä, U., Heinänen, P., Marvola, M., 2002. In vitro evaluation of microcrystalline chitosan (MCCh) as gel-forming excipient in matrix granules. Eur. J. Pharm. Biopharm. 54, 33–40.

Shah, S., Qaqishi, R., Patel, V., Amiji, M., 1999. Evaluation of the factors influencing stomach-specific delivery of antibacterial agents for *Helicobacter pylori* infection. J. Pharm. Pharmacol. 51, 667–672.

Shah, V.P., Midha, K.K., Findlay, J.W.A., Hill, H.M., Hulse, J.D., McGilveray, I.J., McKay, G., Miller, K.J., Patnaik, R.N., Powell, M.L., Tonelli, A., Viswanathan, C.T., Yacobi, A., 2000. Bioanalytical method validation—a revisit with a decade of progress. Pharm. Res. 17, 1551–1557.

Shepherd, R., Reader, S., Falshaw, A., 1997. Chitosan functional properties. Glycoconj. J. 14, 535–542.

Staib, A.H., Woodcock, B.G., Loew, D., 1989. Remote control of gastrointestinal drug delivery in man. In: Prescott, L.F., Nimmo, W.S. (Eds.), Novel Drug Delivery and Its Therapeutic Application. Wiley, London, pp. 79–88.

Struszczyk, H., 1987. Microcrystalline chitosan. I: preparation and properties of microcrystalline chitosan. J. Appl. Polym. Sci. 33, 177–189.

Wilson, C.G., Washington, N., Greaves, J.L., Kamali, F., Rees, J.A., Sempik, A.K., Lambard, J.F., 1989. Bimodal release of ibuprofen in a sustained-release formulation: a scintigraphic and pharmacokinetic open study in healthy volunteers under different conditions of food intake. Int. J. Pharm. 50, 155–161.