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Organic acids as excipients in matrix granules for colon-specific drug delivery

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Abstract

Interest exists in developing site-specific systems for release of a drug in the lower part of the small intestine or in the colon. The aim of this study was to investigate whether drug release rates from enteric matrix granules could be influenced by using organic acids as excipients. Ibuprofen was used as a model drug and Eudragit™ S and Aqoat™ AS-HF as enteric polymers. The dissolution rates of the drug were investigated at different levels of pH (5.8, 6.8 and 7.4). Drug absorption was studied in bioavailability tests in healthy volunteers. In vitro/in vivo correlation was also investigated. It was concluded that although inclusion of an organic acid in a formulation retarded in vitro release of the model drug, no corresponding effect was evident in in vivo studies. Bioavailability tests are therefore important early on during development of new dosage forms or formulations. Although no correlation between in vitro and in vivo results was generally evident correlation could be demonstrated for individual formulations following mathematical transformation of data.

Keywords: Colon-specific drug delivery; Enteric polymer; Ibuprofen; Organic acid; In vitro/in vivo correlation

1. Introduction

Interest in developing site-specific systems for release of a drug substance in the lower part of the small intestine or in the colon has been increasing. Such systems could allow topical treatment of inflammatory bowel diseases such as Crohn's disease or ulcerative colitis. It has also taught that peptides and certain other labile drugs might be administrable orally if they were not released in the upper regions of the gastrointestinal tract (Ashford and Fell, 1993). In some diseases, symptoms are subject to circadian

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rhythms. Colon-specific delivery systems mightalso be useful for chronopharmacotherapy (Nari sawa et al., 1997).

There are several methods for confining drug release to the colon. One of the oldest is employment of enteric polymers as coating materials in relation to tablets, granules or pellets. Such techniques rely on the differences in pH values in the gastrointestinal tract (Friend, 1991). Enteric polymers are soluble at high pH values, for example those in the colon or lower part of the small intestine, but insoluble at the low pH values in the proximal parts of gastrointestinal canal. Acrylic polymers, such as Eudragit™ S, are, for example, suitable for achievement of colonic drug delivery (Rubinstein, 1995). However, the pH in the caecum is usually about one pH unit lower than in the terminal ileum (Evans et al., 1988) and this will have an influence on the value of pH dependent polymers for colonic delivery.

The aim of a previous study (Marvola et al., 1999) was to develop a multiple-unit system resulting in drug release in the colon, using enteric polymers. Film-coated matrix pellets were prepared with enteric polymers as both binders and coating materials. It was found that drug release from the formulations took place in the distal part of the small intestine and the colon if enteric polymers dissolving at pH 7 were used. If the colon is the primary target, the formulation needed development. In the study reported here we investigated whether the pH of the micro-environments of granules of the kinds described above could be decreased by adding organic acids to formulations, and whether dissolution of the enteric material might accordingly be further delayed.

Organic acids have been used to modify drug release from multiple-unit controlled-release formulations with polymers as coating materials. Narisawa et al. (1994, 1996) prepared theophylline and acetaminophen pellets containing, e.g. succinic acid, tartaric acid and citric acid as excipients around nonpareil seeds. The pellets were film-coated with a methacrylate polymer (Eudragit RS™), with the aim of prolonging drug release. The purpose of acid supplementation was to increase drug release rates, because of increases in osmotic pressures inside the coated pellets. Ishibashi et al. (1998) used organic acids as additives in hard gelatin capsules film-coated with an enteric layer, a hydrophilic layer and an acid-soluble layer. Acid solutions were formed inside the capsules, resulting in dissolution of the innermost, acid-soluble, layer of coating.

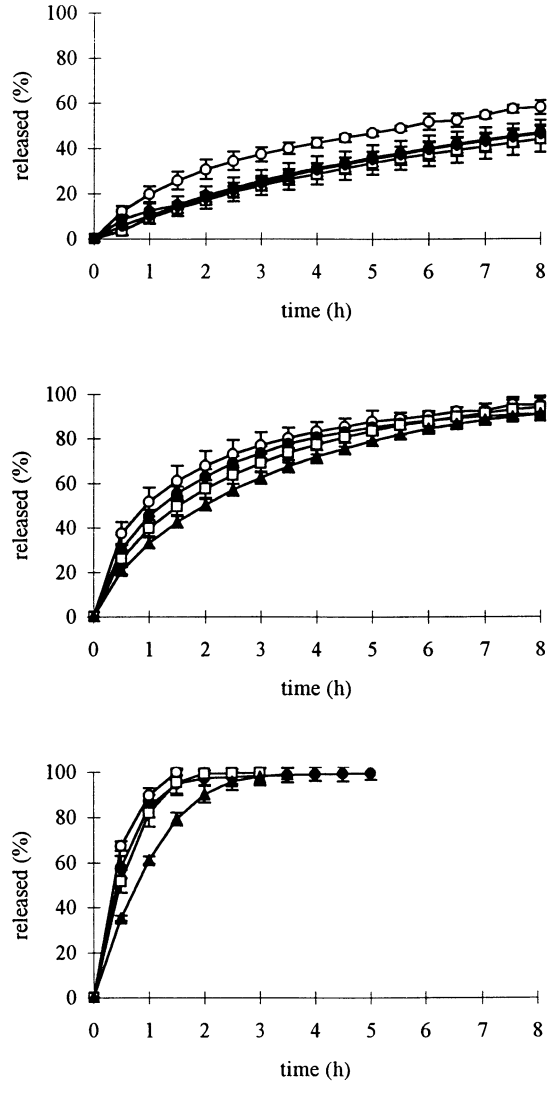

Fig. 1. Effect of amounts of succinic acid on dissolution of ibuprofen from matrix granules. Theoretical amounts of succinic acid: ○ = 0%, ● = 10%, □ = 20%, ▲ = 30%. pH values of dissolution media: 5.8 (top), 6.8 (middle) or 7.4 (bottom), means ± SD, $n = 5$.

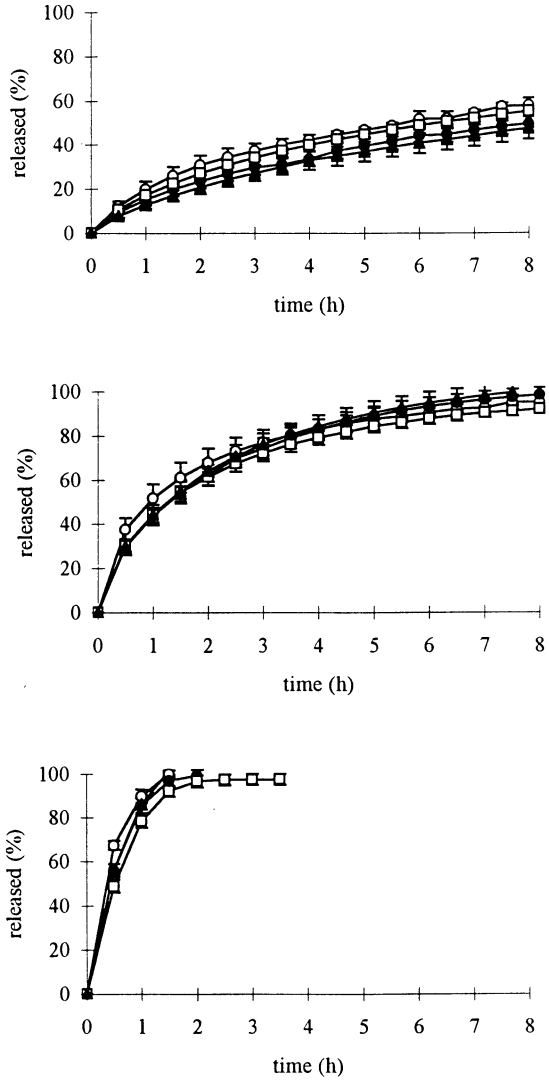

Fig. 2. Effect of amounts of tartaric acid on dissolution of ibuprofen from matrix granules. Theoretical amounts of tartaric acid: ○ = 0%, ● = 10%, □ = 20%, ▲ = 30%. pH values of dissolution media: 5.8 (top), 6.8 (middle) or 7.4 (bottom), means ± SD, $n = 5$.

The formulations we studied differed from those mentioned above in which enteric polymers were used only as coating materials. Our formulations also contained enteric polymer as binders in granule cores, it was thought that acid supplementation in such systems would prolong disintegration of the core system. Ibuprofen was used as a model drug because it is absorbed throughout the gastrointestinal tract (Wilson et al., 1989).

2. Materials and methods

2.1. Materials

The enteric polymers used were the methacrylate copolymer Eudragit S (Röhm Pharma, Germany) and hydroxypropylmethylcellulose acetate succinate Aqoat AS-HF (Shin-Etsu Chemical Co,

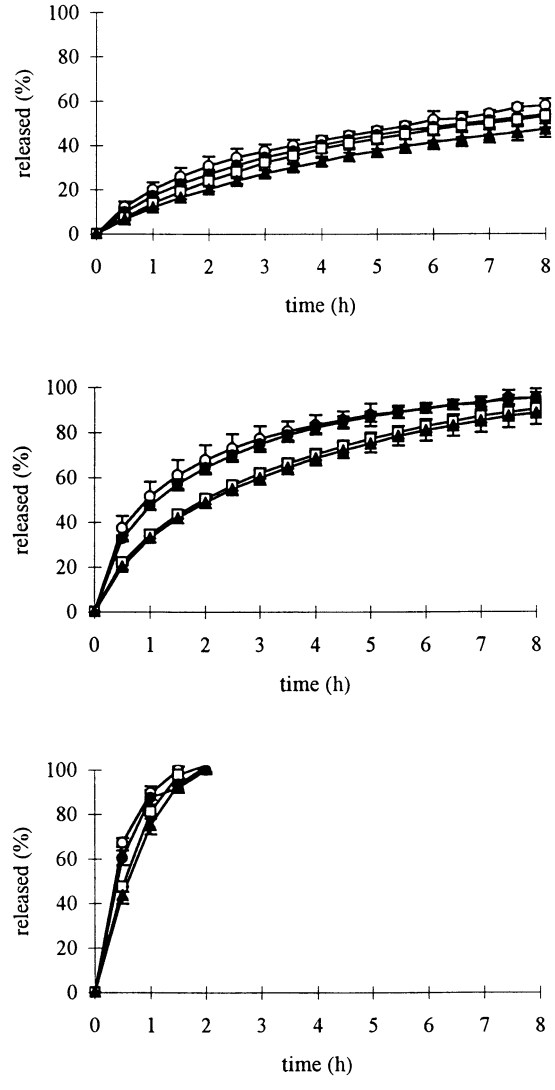

Fig. 3. Effect of amounts of citric acid on dissolution of ibuprofen from matrix granules. Theorectical amounts of citric acid: ○ = 0%, ● = 10%, □ = 20%, ▲ = 30%. PH values of dissolution media: 5.8 (top), 6.8 (middle) or 7.4 (bottom), means ± SD, $n = 5$.

Table 1
Pharmacokinetic parameters of ibuprofen given as matrix granules containing succinic acid as excipient, (mean ± SD, $n = 8$)

Parameter	Theoretical amount of succinic acid in the core			
	0%	10%	20%	30%
t_{lag} (h)	0.4 ± 0.2	0.5 ± 0.2	0.6 ± 0.2	0.5 ± 0.2
t_{max} (h)	5.4 ± 0.5	4.6 ± 0.7[a]	5.5 ± 0.5[a]	5.3 ± 0.7
C_{max} (mg^{-1})	12.3 ± 3.4	14.2 ± 2.9	12.1 ± 3.3	12.2 ± 1.7
$AUC_{0-\infty}$ (mg l^{-1} h)	125.1 ± 47.2	108.1 ± 20.5	108.9 ± 31.0	99.9 ± 18.0
$AUC_{0-12\ h}$ (mg l^{-1} h)	85.0 ± 20.3	87.2 ± 17.0	78.8 ± 19.9	74.4 ± 10.9
MRT (h)	9.1 ± 8.7	5.6 ± 1.1	6.5 ± 2.0	6.2 ± 1.3
$C_{max}/AUC_{0-\infty}$ (h^{-1})	01.0 ± 0.02	0.13 ± 0.02	0.11 ± 0.02	0.13 ± 0.03
$t_{\frac{1}{2}}$ (h)	6.9 ± 7.4	3.9 ± 0.8	4.6 ± 1.4	4.6 ± 1.0

[a] $P < 0.05$ (10% versus 20%, Mann–Whitney U-test).

Japan). The polymers dissolve at pH 7 (Friend, 1991). The additives used in the granules were calcium phosphate ($CaHPO_4$ $2H_2O$) (Ph.Eur.), succinic acid (Ph.Eur.), tartaric acid (Ph.Eur.), citric acid (Ph.Eur.), triethyl citrate (Pfizer & Co, USA) and magnesium stearate (Ph.Eur.). Ibuprofen (Ph.Eur.) was used as the model drug. Ibuprofen is a weak acid, with a pK_a value of 5.3 (Herzfeldt and Kümmel, 1983).

2.2. Preparation of the matrix granules

A 20% solution in ethanol (Oy Primalco Ab, Finland) of the methacrylate polymer (Eudragit S) was prepared. Ibuprofen (60%) was mixed with different diluent combinations (30%). The ratios of calcium phosphate to organic acid in the combinations were 0:3, 1:2, 2:1 or 3:0. Powder masses (200 g) were moistened with binder solution in a mortar and sieved manually through a 2.0 mm sieve. The granules were dried overnight at room temperature. The fraction 1.18–1.68 mm was separated by sieving and used as such or subjected to film coating. The amount of enteric polymer in the dried granules was 10% on the basis of chemical assay of the active ingredient (spectrophotometric measurement at 221 nm).

2.3. Coating procedure

Coating was performed in a fluidized-bed coater (Aeromatic Strea-1, Aeromatic AG, Switzerland). A 200 g of 1.18–1.68 mm granule fraction was coated in each case. The coating solution contained 10% of Aqoat AS-HF, 3.5% of triethyl citrate, 3% of magnesium stearate and 83.5% of demineralized water. The coating solution was prepared according to the instructions of the manufacturer of the polymer and passed through a 0.3 mm sieve before use.

Throughout coating the coating solution was kept in an ice-bath. Granules were preheated for 5 min at 40 ± 5°C outlet temperature. The spraying pressure used during coating was 1 bar, the air flow rate 70 $m^3\ h^{-1}$, and the outlet temperature 40 ± 5°C. The spraying rate was 5 g min^{-1}. Coating was continued until a theoretical weight increase of 20% had been achieved. The granules were dried after coating at the same temperature for 5 min. After the coating, the granules were kept on trays overnight.

2.4. Dissolution tests

Drug release from formulations was studied using the basket method described in USP XXIII (apparatus: Dissolutest 07, Prolabo, France). The dissolution media (500 ml at 37 ± 0.5°C) were pH 5.8, 6.8 and 7.4 phosphate buffers (USP XXIII). The speed of rotation of the basket was 100 min^{-1}. The dissolution apparatus was connected to a flow-through spectrophotometer (Ultrospect II, LKB Biochrom Ltd, UK) via a peristaltic pump. Absorbance at 221 nm was recorded auto-

matically. Absorbances were monitored by a computer running tablet dissolution software (TDS™, LKB Biochrom Ltd, UK).

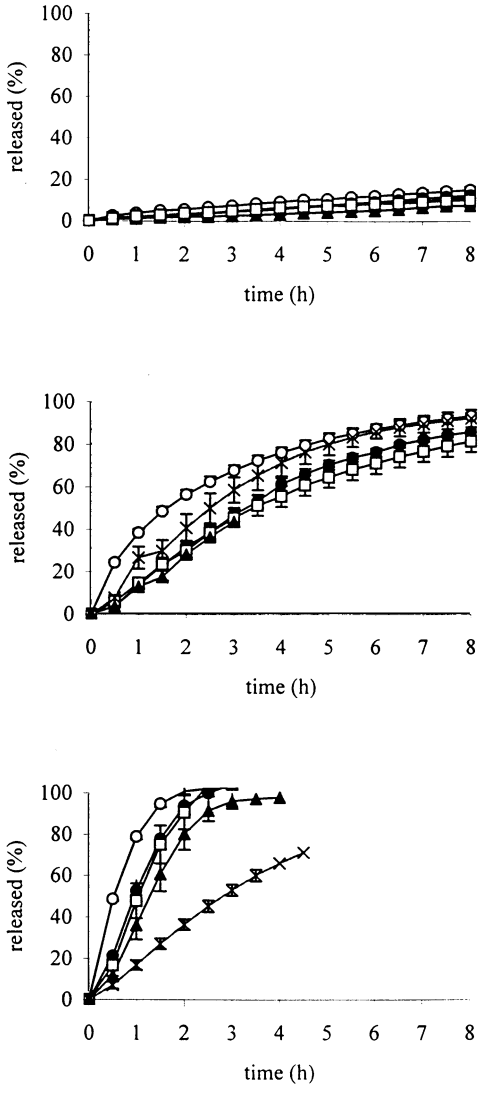

Fig. 4. Dissolution of ibuprofen from Agoat AS-HF coated matrix granules containing citric acid or succinic acid as excipient. Theoretical amounts of acids: ○ = 0%, ● = citric acid 10%, □ = citric acid 20%, ▲ = citric acid 30%, × = succinic acid 30%. pH values of dissolution media:5.8 (top), 6.8 (middle) or 7.4 (bottom), means ± SD, $n = 5$.

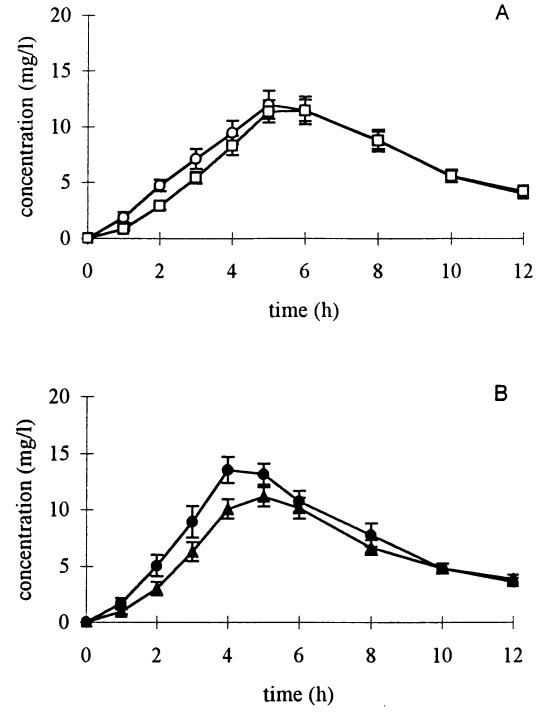

Fig. 5. Bioavailability of ibuprofen from matrix granules containing succinic acid as excipient. Theoretical amounts of succinic acid: ○ = 0%, □ = 20%, ● = 10%, ▲ = 30%. Mean ± SEM, $n = 8$. A and B: different groups of volunteers.

2.5. Bioavailability studies

Four groups (A, B, C, D) of eight healthy volunteers of both sexes participated in randomized cross-over single-dose studies, carried out in accordance with the recommendations of the Declaration of Helsinki (World Medical Assembly 1964) as revised in Tokyo (1975). The ages of the volunteers varied from 20 to 46 years and their weights from 43 to 87 kg. Before the studies the participants underwent physical examination, routine haematological testing (Hb, ESR, S-Alat, S-Asat, S-GT, S-Crea) and ECG examination. The volunteers were informed about possible risks and adverse effects of taking the drug, and written consent was obtained from each. The study protocol had been approved by the ethical committee of the University hospital of Tartu.

The required amounts of each granule formulation studied were dispensed into hard gelatin cap-

sules (size 0, Capsugel AG, Switzerland). The amount of ibuprofen in each capsule was 150 mg. Two ibuprofen capsules were administered to each subject with 200 ml of water following an overnight fast for at least 10 h. Washout periods were at least 1 week. A standard lunch was provided 4 h after drug administration. Blood samples (10 ml) were collected from a forearm vein into heparinized tubes. Plasma was separated after collection and stored at −20° until analysed.

2.6. Plasma assay

Ibuprofen plasma concentrations were determined by means of high-performance liquid chromatography (HPLC) using the method described by Avgerinos and Hutt (1986), with slight modifications. The system was equipped with a Waters Model 501 piston pump, a Waters Model 717 Intelligent Sample Processor, a Waters Model 484 Tunable Absorbance detector operated at 222 nm, and a Waters Model 2.10 Millennium workstation. Sample separation was carried out on a (Bondapak C_{18} reverse-phase silica column (3.9 × 300 mm). The isocratic mobile phase was 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 over the concentration range 0.5–40 mg l^{-1} ($r^2 > 0.9989$). Accuracy, precision, limit of quantitation, specificity and reproducibility were investigated as recommended by Shah et al. (1992).

2.7. Pharmacokinetic parameters

The pharmacokinetic parameters assessed using the Siphar™ pharmacokinetic data analysis program (Simed, France) were lag-time in commencement of drug absorption (t_{lag}), maximum plasma concentration (C_{max}), time to peak concentration (t_{max}), area under the concentration-time curve ($AUC_{0\text{-}12\ h}$ and $AUC_{0\text{-}\infty}$), mean residence time (MRT) and apparent elimination half-life ($t_{\frac{1}{2}}$). The rate of absorption phase was also evaluated by means of the ratio C_{max}/AUC. C_{max} and t_{max} values were used as measured. AUC and MRT values were calculated using the trapezoidal method without logarithmic transformation. The method of Wagner and Nelson was used to calculate the time points at which 10 and 90% had been absorbed ($t_{10\%}$ and $t_{90\%}$). Statistical analyses were carried out using Student's paired t-test, the t-test

Table 2
Pharmacokinetic parameters of ibuprofen given as matrix granules containing succinic acid or citric acid as excipient, (mean ± SD, $n = 8$)

Parameter	Theoretical amount of acid in core			
	0%	Succinic acid 30%	Succinic acid 10%	Citric acid 10%
t_{lag} (h)	0.7 ± 0.4	0.7 ± 0.3	0.7 ± 0.2	0.7 ± 0.2
t_{max} (h)	6.1 ± 1.1	5.1 ± 0.6	4.8 ± 0.5[a]	4.5 ± 0.8[b]
C_{max} (mg l^{-1})	9.5 ± 2.6	10.1 ± 3.1	10.0 ± 4.6	14.0 ± 5.7[c]
$AUC_{0-\infty}$ (mg l^{-1} h)	93.4 ± 30.6	79.9 ± 33.3	100.8 ± 41.5	95.1 ± 43.2
$AUC_{0-12\ h}$ (mg l^{-1} h)	61.7 ± 18.0	61.2 ± 22.6	60.6 ± 29.3	76.2 ± 31.6[c]
MRT (h)	7.5 ± 2.2	5.9 ± 1.3	10.4 ± 8.2	5.4 ± 2.3
$C_{max}/AUC_{0-12\ h}$ (h^{-1})	0.16 ± 0.01	0.17 ± 0.03	0.17 ± 0.04	0.19 ± 0.03[d]
$t_{10\%}$ (h)	1.7 ± 0.9	2.0 ± 0.6	1.8 ± 0.5	2.0 ± 0.7
$t_{90\%}$ (h)	6.4 ± 1.7	5.2 ± 1.0	4.9 ± 1.4	4.3 ± 0.8[e]

[a] $P < 0.05$, succinic acid 10% versus 0%, Mann–Whitney U-test.
[b] $P < 0.01$, citric acid 10% versus 0%, Mann–Whitney U-test.
[c] $P < 0.05$, citric acid 10% versus succinic acid 10% Student's paired t-test.
[d] $P < 0.05$, citric acid 10% versus 0%, Student's t-test for independent groups.
[e] $P < 0.05$, citric acid 10% versus 0%, Student's t-test for independent groups.

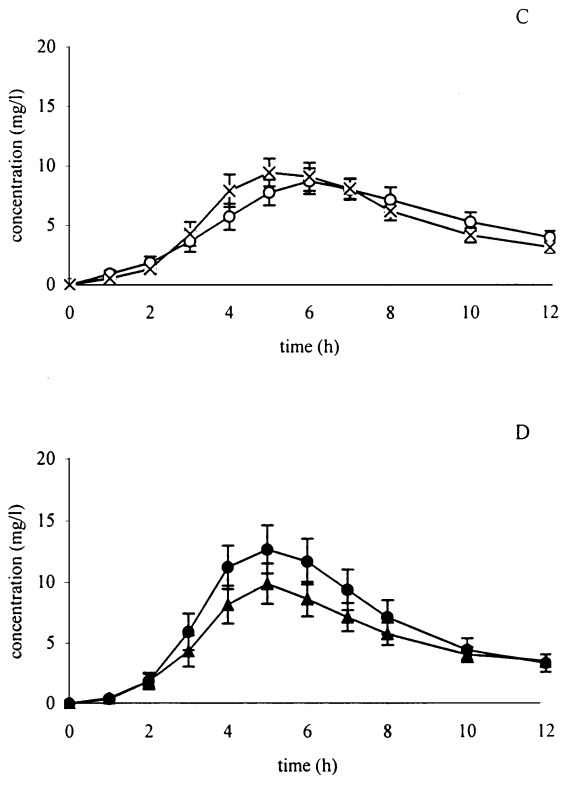

Fig. 6. Bioabailability of ibuprofen from Aqoat AS-HF coated matrix granules containing organic acids as excipients. Theoretical amounts of organic acid: ○ = 0%, × = succinic acid 30%, ▲ = succinic acid 10%, ● = citric acid 10%. Means ± SEM, $n = 8$. C and D: different groups of volunteers.

for independent groups, and the Wilcoxon or Mann–Whitney nonparametric test (for t_{max} values).

2.8. In vitro/in vivo correlation

At the end of the study in vitro/in vivo correlations for each formulation were assessed by positioning the in vitro release curve and the in vivo curve on top of each other. Cumulative dissolution curves were used as the in vitro parameter. The in vivo concentration versus time curve for each volunteer was initially transformed to cumulative amounts absorbed at each time point, using the method of Wagner–Nelson. The correlation between mean values was assessed using the equation of Brockmeier et al. (1983) as modified by Luckow (1994) with slight modifications:

$$t' = a + b \cdot t^c$$

where t' = time in vivo, t = time in vitro, a = lag time in vivo, b = scale factor, and c = shape factor. The scale factor (b) takes account of the fact that in vitro release and in vivo absorption do not follow the same time scales. The shape factor (c) is a measure of the formulation-to-model adaptation.

3. Results and discussion

3.1. In vitro characteristics of uncoated granules

The in vitro characteristics of uncoated granules and the effects of organic acid inclusion on drug release at different pH values were first investigated (Figs. 1–3). As expected, the rate of release of the model drug, ibuprofen, depended markedly on the pH values of the dissolution media, increasing as pH increased. $T_{50\%}$ values for the formulation containing no organic acid were 5.8 h (pH 5.8), 0.8 h (pH 6.8) and 0.3 h (pH 7.4). The release mechanism at pH 5.8 was obviously diffusion of dissolved drug from the matrix. At pH 6.8 and 7.4 the enteric polymer used as binder also dissolved, resulting in fairly rapid drug release. The disintegration pH of an Eudragit S matrix has been shown to be 6.8 (Marvola et al., 1999). Drug release rates at different pH values were, naturally also, affected by the greater solubility of ibuprofen at higher pH-levels.

Replacement of some or all of the calcium phosphate in the matrix with an organic acid retarded drug release. As a rule there was also a correlation between amount of acid and release rate: the greater the amount of acid the greater the retardation (Figs. 1–3). Succinic acid (Fig. 1) and citric acid (Fig. 3) had the greatest effects at pH 6.8, thought to be the most critical in relation to in vivo disintegration of the matrix. With these two acids the $T_{50\%}$ value at pH 6.8 was 2 h. Succinic acid and citric acid were therefore selected for further in vivo studies.

3.2. Bioavailability of uncoated granules

The effect of the amount of succinic acid on bioavailability of the uncoated formulation was studied in two groups of volunteers: Group A received granules containing 0 or 20% of succinic acid, group B with granules containing 10 or 30% of succinic acid. Results are summarized in Table 1 and Fig. 5. Results at the in vitro studies at pH 6.8 suggested that absorption rates would became slower as amounts of succinic acid in the granules increased (Fig. 1, middle). This was seen in vivo results. Absorption from granules containing 20% of succinic acid was somewhat slower than from granules containing no succinic acid (Fig. 5, Group A). Absorption from granules containing 30% of succinic acid was slower than from granules containing 10% (Fig. 5, Group B). However, differences were minimal. Statistical significance existed only in relation to t_{max} values (Table 1). All of the pharmacokinetic parameters in Table 1 for the formulation containing no acid are in good accordance with results from a previous study of this formulation (Marvola et al., 1999). Reproducibility of the manufacturing procedure for the granules is therefore good (minimal batch-to-batch variation).

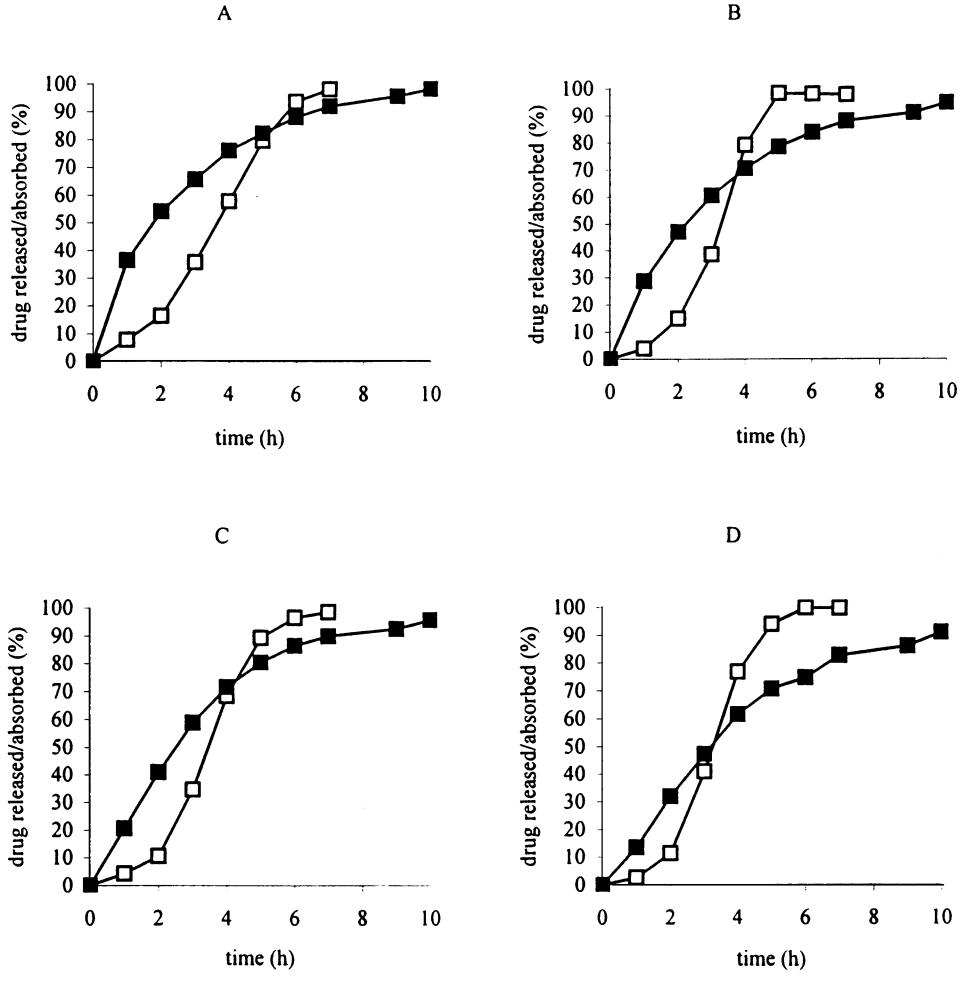

Fig. 7. In vitro/in vivo correlation of enteric coated matrix granules. Each point is a mean value for ibuprofen released in vitro (■) or absorbed in vivo (□). Theoretical amounts of organic acids: A = 0%, B = succinic acid 10%, C = succinic acid 30%, D = citric acid 10%.

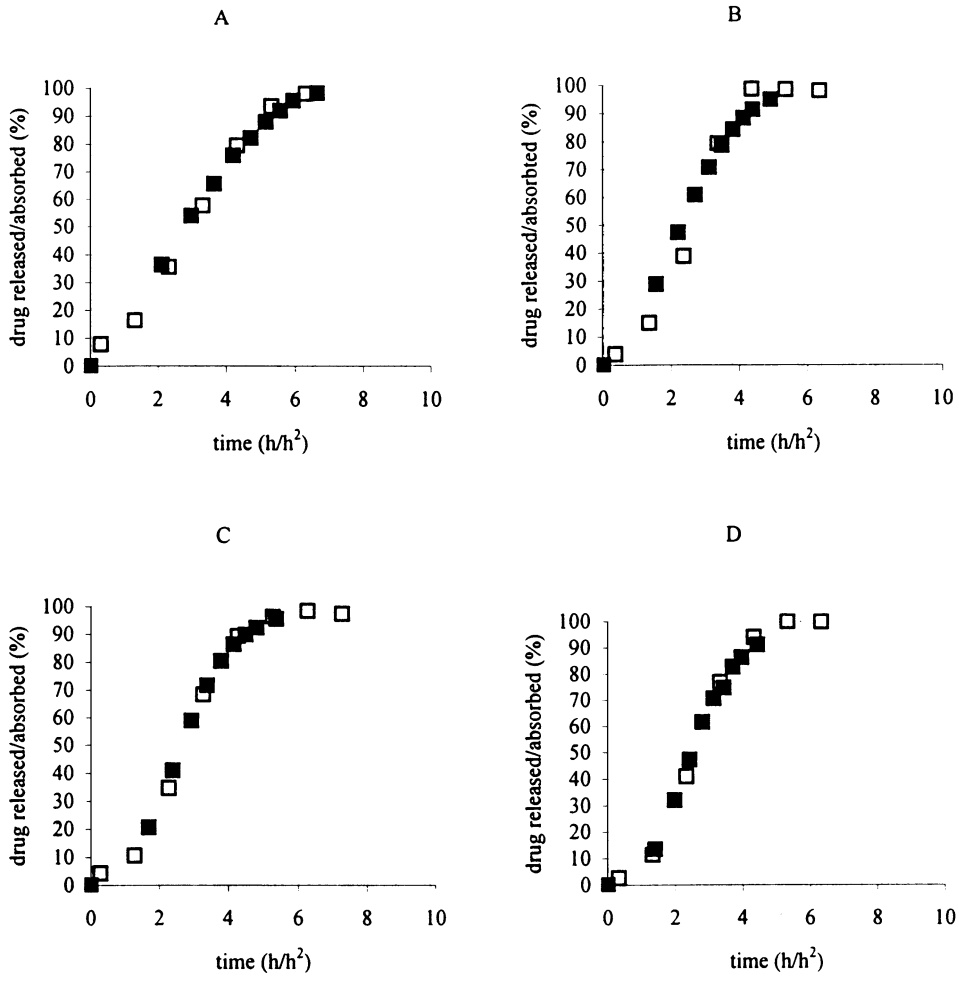

Fig. 8. In vitro/in vivo correlation of enteric coated matrix granules. Each point is a mean value, after transformating, for ibuprofen released in vitro (■) or absorbed in vivo (□). Theoretical amounts of orgainc acids: A = 0%, B = succinic acid 10%, C = succinic acid 30%, D = citric acid 10%.

3.3. In vitro characteristics of film coated granules

Ibuprofen granules containing succinic acid or citric acid were next film-coated. The mean dissolution profiles of ibuprofen are shown in Fig. 4. As expected, release rates of ibuprofen were lower with film-coated granules than with uncoated granules. Release rate depended on the pH value of the dissolution medium. $T_{50\%}$ values for the formulation containing no organic acid were 1.5 h (pH 6.8) and 0.5 h (pH 7.4). At pH 5.8 the release rate of ibuprofen was very low. Only 10% of drug was released in 8 h.

Replacement of calcium phosphate in the granules by citric acid or succinic acid retarded drug release. The rate of drug dissolution from film-coated matrix granules containing 30% succinic acid was slow at pH 7.4. The $T_{50\%}$ value for the formulation was 3 h. There was a correlation between amount of citric acid and drug release rate (Fig. 4). Release rate was as lowest when the most of citric acid was used.

3.4. Bioavailability of film-coated granules

The effect of the amount of succinic acid on bioavailability of film coated granules was studied in the volunteers of Group C. The results are shown in Table 2 and Fig. 6 (top). Absorption from film-coated granules containing 30% succinic acid was slower than from granules containing no succinic acid (Fig. 6, top), but the differences in the pharmacokinetic parameters were not statistically significant (Table 2).

In the in vitro studies 10% of citric acid and 10% of succinic acid had affected drug dissolution rates from film-coated granules. The effect of 10% of succinic acid and citric acid were studied in the volunteers of Group D. There was no difference in absorption rate between these two formulations (Fig. 6, bottom). However, there was a significant difference in $AUC_{0-12\ h}$ values (Table 2). There were also no statistically significant differences in pharmacokinetic parameters relating to the formulations containing 10% or 30% of succinic acid.

3.5. In vitro/in vivo correlation

The highest A level correlation (Leeson, 1995) between in vitro and in vivo profiles was not found with simple positioning of cumulative in vitro release curves on curves of cumulative amounts of drug absorbed. (Fig. 7). Drug release in vitro was faster than in vivo absorption during the early stages. An A-level (point-to-point) correlation was established using the equation of Brockmeier and Luckow (Fig. 8). Optimum values for transformation are shown in Table 3. The shape factor for each formulation was 0.5. Thus the in vitro/in vivo correlation equation can therefore in this particular case be written as; $t' = a + b\sqrt{t}$.

4. Conclusions

The results of the study reported show that replacement of some or all of the calcium phosphate in the matrix with succinic acid or citric acid retarded drug release in vitro from both uncoated and film-coated granules. There was a correlation between amount of organic acid and dissolution rate. Release of ibuprofen was slower from film-coated granules than from uncoated granules.

In vitro retardation of drug release was, however, more marked than retardation of drug in vivo absorption. Mechanical forces obviously were different in vitro and in the gastrointestinal tract. Flows of liquid in the basket of the dissolution apparatus are less forceful than in the stomach and the intestine. A granule core containing water-soluble organic acids becomes weaker than a granule core containing only water-insoluble calcium phosphate as diluent. Weak granules disintegrate more quickly in the gastrointestinal tract than in the dissolution test. Results of dissolution tests cannot therefore be used to predict in vivo fates of the formulations developed. Bioavailability testing at an early stage is therefore important in developing new dosage forms and formulations.

Although no general in vitro/in vivo correlation was evident in the study, formulation-specific, A-level correlations were established using the equation of Brockmeier et al. (1983) and Luckow (1994). This finding supports the idea that in

Table 3
Optimum values for transformation parameters a, b and c in vitro/in vivo correlation tests, using the equation of Brockmeier and Luckow

Acid and its amount in the core	Lag time in vivo (h), a	Scale factor, b	Shape factor, c
None	0.68	2.10	0.5
Succinic acid 30%	0.72	1.70	0.5
Succinic acid 30%	0.65	1.55	0.5
Citric acid 10%	0.67	1.40	0.5

vitro/in vivo correlations tend to be more product-specific than drug-specific or general.

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