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# Citric acid as a pH-regulating additive in granules and the tablet matrix in enteric-coated formulations for colon-specific drug delivery

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Colon-specific drug-delivery systems have been extensively investigated over the last decade. The aim of the study reported here was to investigate whether times of commencement of drug liberation and absorption could be controlled by varying the amount of citric acid in granule cores or in the tablet matrix in enteric-coated multiple-unit tablets. One of the most important aims was to determine the optimal amounts and locations of citric acid in formulations intended as drugs targeted at the colon. Ibuprofen was used as the model drug. Drug release rates were studied in phosphate buffer at pH 6.8 and 7.4. A gradient dissolution study at pH 1.2, 6.8 and 7.4 was undertaken with two formulations. Drug absorption was studied by means of bioavailability tests. We concluded that the drug release rate could be controlled *in vitro* by changing the amount of citric acid in granule cores or the tablet matrix. *In vivo* tests confirmed that between 10 and 15% citric acid in the tablet matrix delayed the commencement of drug absorption most. This kind of formulations could be suitable for preparation of colon-specific dosage forms. It is probably unnecessary to include citric acid in granule cores. No logical correlation between *in vitro* and *in vivo* results was obtained.

## 1. Introduction

Colon-specific drug delivery systems have been the subject of extensive investigation and development over the last decade. Colon-specific systems can increase the effectiveness of local treatment of colonic disease. Lowering the necessary dose would reduce systemic side effects. The colon could also be the site of absorption following oral administration of protein and peptide drugs, which are inactivated in the upper parts of the gastrointestinal tract. Colon-specific systems could be used in diseases in which symptoms display circadian variation, and when treatment during the early hours of the morning would be most effective.

There are several means by which drug release can be targeted at the colon. Our research group has been trying to develop a colon-specific oral dosage form using enteric polymers (Marvola et al. 1999; Nykänen et al. 1999; Marvola and Nykänen 2001; Nykänen et al. 2001). We have prepared enteric-coated tablets using enteric-coated granules in which the binder is also an enteric polymer. The final stage in the development procedure has been to study whether drug release can be further delayed by adding a small amount of an organic acid to the tablet matrix and/or granules.

It has already been shown (Nykänen et al. 2001) that commencement of drug absorption can be markedly delayed by inclusion of small amounts of an organic acid (e.g. citric acid) in the tablet matrix and/or granules. The effect was found to be greatest when the acid was added to the tablet matrix, and weakest when the acid was added to granule cores. The greatest delay in drug release *in vivo* was achieved with a formulation in which citric acid was incorporated in both tablet matrix and granules. However, it was concluded that such a formulation might delay drug release too much. Further studies are therefore needed to determine the optimal amount of citric acid and its optimal distribution between the tablet matrix and granule cores.

The aim of this study was to arrive at an optimal colon-specific formulation. The effect of the site of citric acid in the formulation on drug release and absorption, especially on lag times, was investigated. We first studied whether commencement of drug liberation and absorption could be controlled by varying the amount of citric acid in granule cores from none to 10%, keeping the amount of citric acid in the tablet matrix constant at 10%. We then investigated the properties of formulations in which the percentage of citric acid in the tablet matrix was 10, 11.7, 13.3 or 15 and no citric acid was incorporated in the granule cores. Nine formulations were subjected to *in vitro* dissolution tests. Four of these were selected for bioavailability studies in healthy volunteers. Ibuprofen (a weak acid with a $pK_a$ value of 5.3) was used as model drug because it is absorbed throughout the gastrointestinal tract (Wilson et al. 1989). Its permeability is high and it falls in group II of the Biopharmaceutical Classification System (Amidon et al. 1995). Its elimination half-life is short (about 2 h) and $t_{max}$ can be achieved in 1.25 h with conventional formulations (Ritschel 1992). Changes in

drug release and absorption are therefore easily identified via changes in pharmacokinetic parameters in bioavailability studies.

## 2. Investigations, results and discussion

### *2.1. Tablet formulations*

Nine enteric-coated tablet formulations were prepared from enteric-coated matrix granules (Table 1, Table 2). The amount of model drug, ibuprofen, in each case was 100 mg. Citric acid was used as a pH-regulating additive in granules and in the tablet matrix. The percentage of citric acid in the tablet matrix was 0, 10, 11.7, 13.3 or 15 when there was no citric acid in the granules. The percentage of citric acid in the tablet matrix was 10 when there was 2.5, 5, 7.5 or 10% citric acid in the granules. Eudragit S was used as matrix former in granules and Aqoat AS-HF as film former in granules and in the tablets. Aqoat AS-HF was selected as film former because it is water soluble. The enteric polymers used dissolve about at pH 7 (Peeters and Kinget 1993; Bauer and Kesselhut 1995).

### *2.2. In vitro characteristics of enteric-coated tablets containing different amounts of citric acid in granules*

The drug release rate from enteric-coated formulations containing 10% citric acid in the tablet matrix and 0 to 10% citric acid in the granules was studied at pH 6.8 and 7.4 (Fig. 1). The drug release rate was increasingly retarded as the percentage of citric acid in the granules changed from zero to 10, at both pH levels. The effect of citric acid was so great that the $t_{50\%}$ value increased from 3.4 h to 5 h as the percentage of citric acid in the granules increased from zero to 2.5%, at pH 6.8. With 10% citric acid in the granules $t_{50\%}$ was over 8 h at pH 6.8. At pH 7.4 $t_{50\%}$ was 1.3 h when there was no citric acid in the granules. The value increased to 2.4 h when the granules contained 5% citric acid, and to 2.7 h when the granules contained 10% citric acid.

The pH-regulating effect of citric acid in the granules lowers the dissolution rate of enteric polymer situated in the granule coating in multiple-unit tablets. In previous studies on plain enteric-coated granules, we noted that the drug release rate from enteric-coated granules was lower when granules contained 10% citric acid than when the granules contained no citric acid, but that the release rate did not decline further if the percentage of citric acid was increased to 20 or 30 (Nykänen et al. 1999). In the study reported here it was found that the drug release rate from enteric-coated tablets could be controlled by adding up to 10% citric acid just to the granules.

**Table 1: Percentage compositions of uncoated granules**

| | Granule 0 | Granule 2.5 | Granule 5 | Granule 7.5 | Granule 10 |
|---|---|---|---|---|---|
| Ibuprofen | 60 | 60 | 60 | 60 | 60 |
| Calcium phosphate | 30 | 27.5 | 25 | 22.5 | 20 |
| Citric acid | 0 | 2.5 | 5 | 7.5 | 10 |
| Eudragit S 100 | 10 | 10 | 10 | 10 | 10 |

**Table 2: Percentage compositions of uncoated tablets**

| | A | B | C | D | E | F | G | H | I |
|---|---|---|---|---|---|---|---|---|---|
| Granule 0 | 48.5 | – | – | – | – | 48.5 | 48.5 | 48.5 | 48.5 |
| Granule 2.5 | – | 48.5 | – | – | – | – | – | – | – |
| Granule 5 | – | – | 48.5 | – | – | – | – | – | – |
| Granule 7.5 | – | – | – | 48.5 | – | – | – | – | – |
| Granule 10 | – | – | – | – | 48.5 | – | – | – | – |
| Emcocel LP 200 | 39.5 | 39.5 | 39.5 | 39.5 | 39.5 | 49.5 | 37.8 | 36.2 | 34.5 |
| Citric acid | 10 | 10 | 10 | 10 | 10 | 0 | 11.7 | 13.3 | 15 |
| Magnesium stearate | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Talc | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

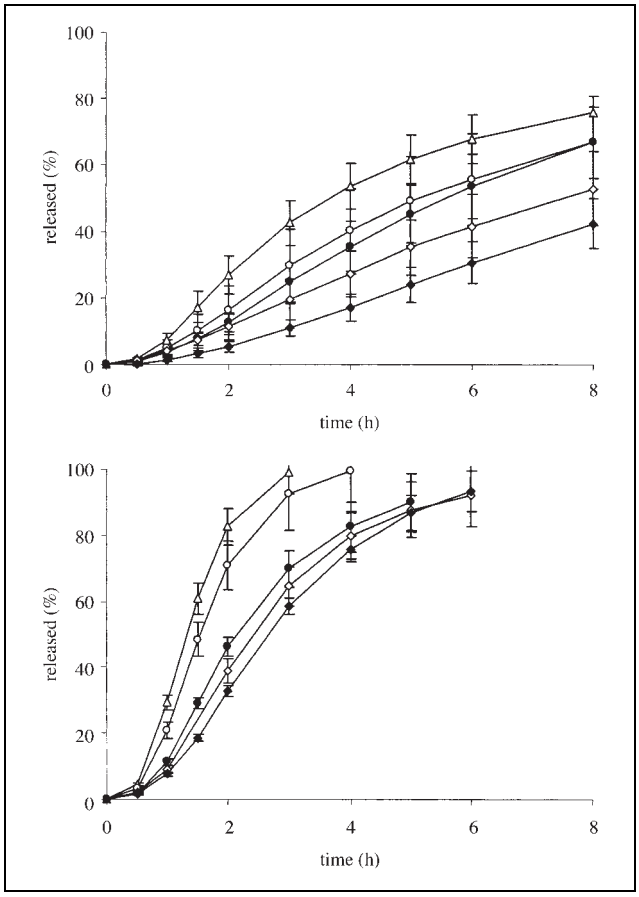


Fig. 1: Dissolution of ibuprofen from enteric-coated multiple-unit tablets containing 10% citric acid in the tablet matrix and no citric acid (△) or 2.5% (○), 5% (●), 7.5% (◇), 10% (◆) citric acid in granules. Dissolution-media pH values: 6.8 (above), 7.4 (below), means ± SD, n = 6

### *2.3. In vitro characteristics of enteric-coated tablets containing different amounts of citric acid in the tablet matrix*

The drug release rate from enteric-coated tablets was determined when the amount of citric acid in the tablet matrix was 0, 10, 11.7, 13.3 and 15%. The results are shown in Fig. 2. The rate of release of the model drug decreased as the amount of citric acid in the tablet matrix increased up to 11.7% (Fig. 2). Increasing the percentage of citric acid in the tablet matrix beyond 11.7 did not further reduce the release rate. It was concluded that when the tablet matrix contained 11.7% or less citric acid the rate of dissolution of enteric coating declined as the amount of citric acid present increased. The effect was particularly evident at pH 7.4. Enteric polymers dissolved faster at pH 7.4 than at pH 6.8. It is thus obvious that, when present in large amounts, water-soluble citric acid can accelerate the dissolution of enteric polymers more strongly at pH 7.4 than at 6.8. When the percentage of citric acid in

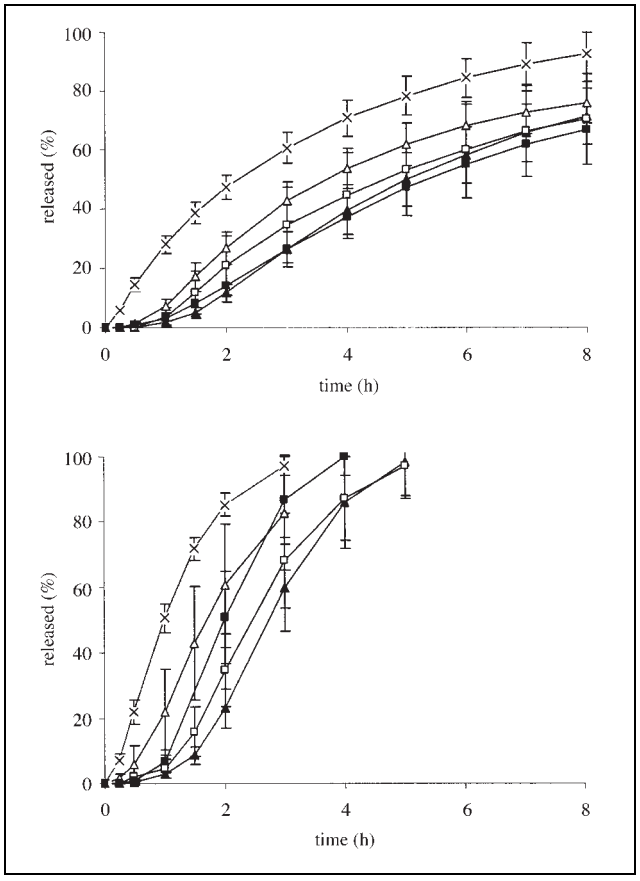


Fig. 2: Dissolution of ibuprofen from enteric-coated multiple-unit tablets containing no citric acid (x), 10% (△), 11.7% (▲), 13.3% (□) or 15% (■) citric acid in the tablet matrix and no citric acid in granules. Dissolution-media pH values: 6.8 (above), 7.4 (below), means ± SD, n = 6

the tablet matrix exceeds 11.7, degradation of the tablet matrix is greater than at lower amounts of citric acid.
Lag-times of about 1 h were seen at pH 7.4 when the amount of citric acid in the tablet matrix ranged between 11.7 and 15%. Dissolution of enteric polymers in such formulations in the terminal small intestine, where the pH value exceeds 7.4, could be delayed.

### *2.4. Gradient dissolution study*

A gradient dissolution study was performed using two enteric-coated tablet formulations containing 2.5% citric acid in the granules and 10% citric acid in the tablet matrix, or no citric acid in the granules and 15% citric acid in the tablet matrix.

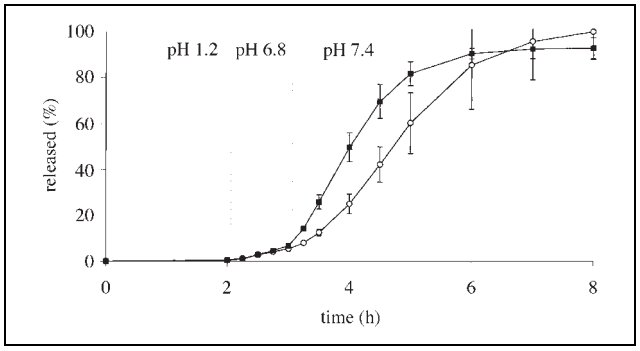


Fig. 3: Dissolution of ibuprofen form enteric-coated multiple-unit tablets containing 10% citric acid in the tablet matrix and 2.5% citric acid in granules (○), or 15% citric acid in the tablet matrix and no citric acid in granules (■). means ± SD, n = 3

Dissolution profiles of the formulations studied are shown in Fig. 3. No drug was released from either formulation for 2 h at pH 1.2. At pH 6.8 only 5% of ibuprofen was released during a one-hour exposure. There were no marked differences between the formulations at pH 6.8. Drug release at 7.4 was faster from the formulation containing no citric acid in the granules than from the formulation containing 2.5% citric acid in granules. This result is in accordance with results of other dissolution studies, in which we noted that incorporation of citric acid in granules in a multiple-unit tablet retarded drug release longer than when there was no acid in the granules. The results of studies of the effect of the amount of citric acid in the tablet matrix showed that citric acid accelerated disintegration of the tablet matrix and increased the drug dissolution rate, especially at high pH (7.4). In the dissolution study reported here the result was the same. Drug dissolution was faster from tablets containing 15% citric acid in the tablet matrix than from tablets containing 10% citric acid in the tablet matrix when the formulations were displaced to pH 7.4 from pH 6.8. From these results it is obvious that formulations of the kind studied could remain intact in the stomach, and that drug could be released after a lag-time of at least 3 h in the terminal small intestine and caecum.

### *2.5. Bioavailability from enteric-coated tablets*

In our study we first investigated the bioavailability of ibuprofen from two enteric-coated formulations in eight volunteers. In one formulation the percentage of citric acid in the granules was 2.5, which is less than in our previous study, and the percentage of citric acid in the tablet matrix was 10. In the other formulation there was no citric acid in the granules and 15% citric acid in the tablet matrix.
Absorption from both formulations was similar (Fig. 4). For both formulations the amounts absorbed increased after a lag-time of 2–8 h. In some volunteers absorption from the formulation with 2.5% citric acid in the granules was measurable only after 8 h (Fig. 5). After our previous *in vivo* study we reported that it might be possible to prepare colon-specific formulations by incorporating citric acid in the tablet matrix of multiple-unit enteric-coated tablets (Nykänen et al. 2001). In the same study, it was also noted that inclusion of citric acid in the granules could determine the site of drug liberation. A citric acid

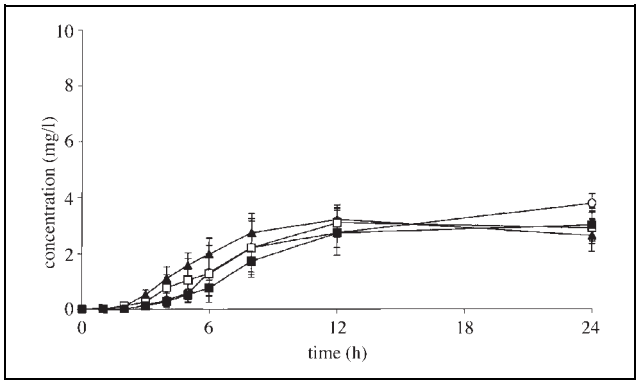


Fig. 4: Mean plasma concentrations of ibuprofen (300 mg) after administration of enteric-coated multiple-unit tablets containing 10% citric acid in the tablet matrix and 2.5% citric acid in granules (○) or 11.7% (▲), 13.3% (□) or 15% (■) citric acid in the tablet matrix and no citric acid in granules. means ± SEM, n = 8

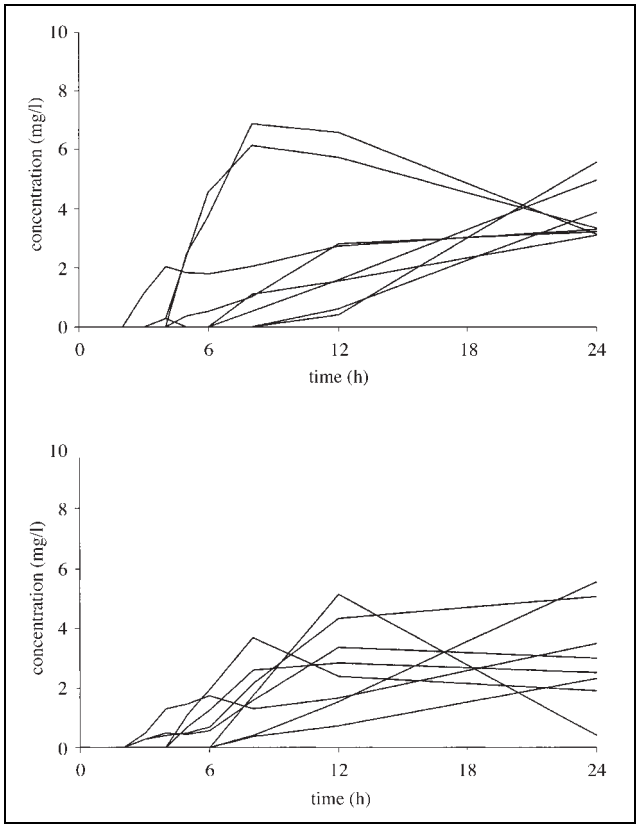


Fig. 5: Individual plasma concentration curves of ibuprofen (300 mg) after administration of enteric-coated multiple-unit tablets containing 10% citric acid in the tablet matrix and 2.5% citric acid in the granules (above) or 15% citric acid in the tablet matrix and no citric acid in granules (below)

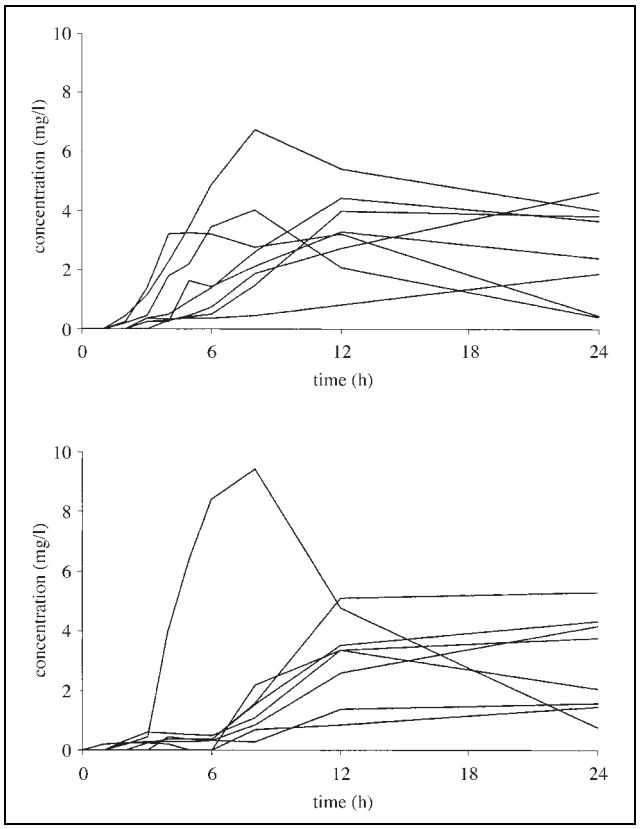


Fig. 6: Individual plasma concentration curves of ibuprofen (300 mg) after administration of enteric-coated multiple-unit tablets containing 11.7% citric acid (above) or 13.3% citric acid (below) in the tablet matrix and no citric acid in granules

content of 10% in the granules was found to be excessive. The results of the first bioavailability study reported now lead us to conclude that addition of citric acid to granules may be unnecessary. There was a lag time of 5.1 h in relation to absorption from the formulation containing 2.5% citric acid in the granules. This is appropriate for a colon-specific formulation, but absorption after the lag time was fairly slow. Inclusion of citric acid in the granules retarded absorption too much.

We therefore carried out a second bioavailability study to determine the effect of including citric acid in the tablet matrix alone. Eight healthy volunteers took enteric-coated tablets containing 11.7 and 13.3% citric acid in the tablet matrix. The mean concentration vs. time curves show that absorption from the tablets was increasingly retarded, after a lag time, as the percentage of citric acid increased (Fig. 4). This finding, and results of previous bioavailability tests with tablets containing 0 (Nykänen et al. 2001), 10 (Nykänen et al. 2001) or 15% citric acid in the tablet matrix and no citric acid in the granules, allow further conclusions to be drawn. It is obvious that lag times before commencement of drug absorption gradually lengthened as the amount of citric acid in the tablet matrix increased. For example, lag time in relation to commencement of drug absorption lengthened from 0.5 h to 1.75 h when the amount of citric acid in the tablet matrix increased from 0 to 11.7%. It is therefore important to incorporate citric acid in the tablet matrix.

Individual concentration vs. time curves also show that lag times can be lengthy if the amount of citric acid in the tablet matrix is high (Fig. 6). There were statistically significant differences in lag times when the amount of citric acid in the tablet matrix was increased to 15% from either 11.7% or 13.3% (Table 3).

It was impossible to calculate all of the pharmacokinetic parameters because in most volunteers $C_{max}$ was not achieved during the 24 h of bioavailability testing (Table 3). Multiple-unit formulations can remain in the colon for as long as several days. Accordingly, bioavailability testing for at least 48 h is necessary if $AUC_{0-\infty}$ values are needed. In the study reported here $AUC_{0-24\,h}$ values were about 50 mg $l^{-1}$ h. With a conventional ibuprofen capsule (300 mg) they were about 120 mg $l^{-1}$ h (Halsas et al. 1999).

**Table 3: Time at which 50% of the ibuprofen (100 mg) was dissolved *in vitro* ($t_{50\%}$) and pharmacokinetic parameters of ibuprofen (300 mg) from *in vivo* bioavailability study**

| Citric acid in granules (%) | 2.5 | 0 | 0 | 0 |
|---|---|---|---|---|
| Citric acid in the tablet matrix (%) | 10 | 11.7 | 13.3 | 15 |
| Parameter | | | | |
| $t_{lag}$ (h)[d] | 5.13 ± 2.32 | 1.75 ± 0.71[ab] | 2.00 ± 1.85[ac] | 4.00 ± 1.85 |
| $t_{lag}$ (h) median | 5 | 2 | 1.5 | 4 |
| $t_{lag}$ (h) range | 2–8 | 1–3 | 0–6 | 2–6 |
| $AUC_{0-24\,h}$ (mg $l^{-1}$ h)[d] | 54.8 ± 26.8 | 56.7 ± 23.5 | 53.7 ± 25.5 | 47.4 ± 14.4 |
| $t_{50\%}$ pH 7.4[e] | 1.32 | 2.78 | 2.47 | 2.01 |
| $t_{50\%}$ pH 6.8[e] | 5.08 | 5.27 | 4.02 | 5.23 |

Enteric-coated tablets containing 10% citric acid in the tablet matrix and 2.5% citric acid in granules, or 11.7%, 13.3% or 15% citric acid in tablet matrix and no citric acid in granules

[a] $p < 0.01$, comparison with result in first column
[b] $p < 0.01$, comparison with result in last column
[c] $p < 0.05$, comparison with result in last column
[d] means ± SDs, n = 8
[e] means, n = 6

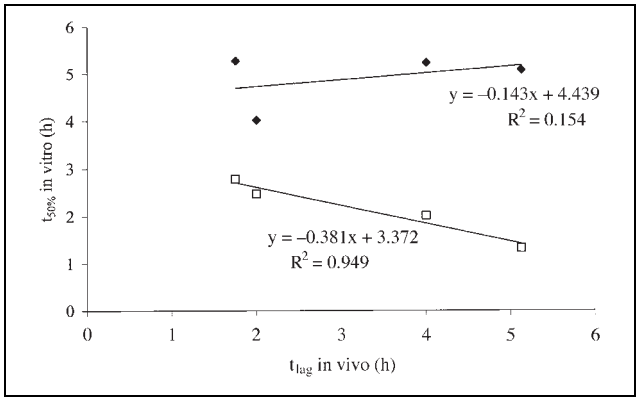


Fig. 7: Level C in vitro/in vivo correlation for enteric-coated multiple-unit ibuprofen formulations containing 10% citric acid in the tablet matrix and 2.5% citric acid in granules, or 11.7%, 13.3% or 15% citric acid in the tablet matrix and no citric acid in granules. Dissolution-media pH values; 7.4 (□) and 6.8 (◆)

In a previous study we concluded that multiple-unit enteric-coated tablets containing citric acid as a pH-regulating excipient might be used to achieve colon-specific drug delivery. In the present study we tried to determine the optimal place and amount of citric acid when colon-specific formulations are prepared. The results of bioavailability tests confirmed our earlier assumptions regarding the optimal location of citric acid in formulations. Between 10 and 15% citric acid in the tablet matrix may be appropriate for preparation of colon-specific formulations. Such formulations might result in fairly rapid absorption after a lengthy lag time. A formulation containing citric acid in the tablet matrix might remain intact longer in the small intestine than one without acid in the tablet matrix. The inclusion of citric acid in the tablet matrix improved site specificity. When citric acid is also present in the granules, drug liberation and absorption may be too slow after disintegration of the tablet matrix. Inclusion of citric acid in the granules is thus probably unnecessary.

### *2.6. In vitro/in vivo correlations*

Single-point level-C correlations for formulations at both *in vitro* pH levels studied are shown in Fig. 7. There was no significant linear *in vitro*/*in vivo* correlation in relation to the dissolution studies at pH 6.8. At pH 7.4 the slope of the curve was negative. As discussed above, the presence of citric acid, which is water-soluble, in a tablet matrix can promote degradation of the tablet matrix *in vitro* when the citric acid content exceeds 11.7%. However, the results of bioavailability studies show that increasing the amount of citric acid in the tablet matrix can lengthen the lag time. This leads us to conclude that it is impossible to predict the results of bioavailability studies from the results of *in vitro* dissolution studies. It is therefore important to carry out bioavailability tests in volunteers during development of the formulations.

## 3. Experimental

#### *3.1. Preparation of matrix granules*

Five granule formulations were prepared (Table 1). A 20% solution in ethanol (Oy Primalco Ab, Finland) of the methacrylate polymer Eudragit S (Röhm Pharma, Germany) was prepared. Ibuprofen (60%, Ph. Eur.) was mixed with various combinations of diluents (30%), calcium phosphate ($CaHPO_4$ $2H_2O$, Ph. Eur.) and citric acid (Ph. Eur.).The percentages of citric acid in the granules were 0, 2.5, 5.0, 7.5 and 10. 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 1.18 to 1.68 mm fraction was separated by sieving and subjected to film coating. The amount of enteric polymer in the dried uncoated granules was 10% based on chemical assay of the active ingredient (spectrophotometric measurement at 221 nm).

#### *3.2. Coating procedure*

Coating was performed in a fluidized-bed coater (Aeromatic Strea-1, Aeromatic AG, Switzerland). One hundred grams of granules were coated in each case. The coating solution contained 10% of hydroxypropylmethylcellulose acetate succinate Aqoat AS-HF (Shin-Etsu Chemical Co., Japan), 3.5% of triethyl citrate (Pfizer Co., USA), 3% of magnesium stearate (Ph. Eur.) and 83.5% of demineralised water. The coating solution was prepared according to the instructions of the polymer manufacturer, and was passed through a 0.3 mm sieve before use.
The coating solution was kept in an ice bath while coating was in progress. Granules were preheated for 5 min. at an outlet temperature of $40 \pm 5$ °C. The spraying pressure used during coating was 1 bar, the air flow rate 70 $m^3$/h, and the outlet temperature $40 \pm 5$ °C. The spraying rate was 5 g/min. 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.

#### *3.3. Preparation of tablets*

Tablet masses were prepared just before tableting. Coated granules, microcrystalline cellulose (Emcocel LP 200, Penwest Pharmaceuticals Co., U.K.) and citric acid (if used) were mixed in a Turbula mixer (W. A. Bachofen, Switzerland) for 5 min. Magnesium stearate and talc (Ph. Eur.) were added and mixing for a further 2 min. Tablets were compressed using concave 11 mm punches in a Korch EK-O single-punch press (Erweka Apparatebau GmbH, Germany). The weight of each tablet was 412 mg. Compression forces were chosen to give a tablet hardness, as measured using a Schleuniger 2-E/205 tablet hardness tester (Dr. K. Schleuniger and Co., Switzerland), of 60–70 N. The tablets were enteric-coated with Aqoat AS-HF, using the method mentioned above, except that the air flow rate was 100 $m^3$/h. One hundred grams of tablets were coated in each case. Coating was continued until a theoretical weight increase of 12% has been achieved.

#### *3.4. In vitro dissolution tests*

Drug release from uncoated and enteric-coated tablets was studied using the basket method described in USP 24 (apparatus: Dissolutest 07, Prolabo, France). The dissolution medium (900 ml at $37 \pm 0.5$ °C) was phosphate buffer of either pH 6.8 or pH 7.4 (USP 24). Dissolution studies were carried out using all uncoated and enteric-coated tablet formulations in both phosphate buffers. 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 automatically. Absorbances were monitored by means of a computer running tablet dissolution software (TDS™, LKB Biochrom Ltd, UK).
A gradient dissolution study was conducted with two formulations. The tablets were kept in 4 ml of 0.1 N hydrochloric acid (pH 1.2) for 2 h. A dissolution study was then carried out in phosphate buffer at pH 6.8 for 1 h. This was followed by testing in phosphate buffer at pH 7.4. The dissolution apparatus and method were otherwise as described above.

#### *3.5. In vivo studies*

##### *3.5.1. Bioavailability studies*

Two groups of eight healthy volunteers of both sexes participated in randomised cross-over single-dose studies carried out in accordance with the recommendations of the Declaration of Helsinki (World Medical Assembly 1964) as amended in Edinburgh (2000). The ages of the volunteers varied from 18 to 42 years and their weights from 56 to 75 kg. Before the studies the participants underwent a physical examination, routine haematological testing and an 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 Tartu University Hospital.
The amount of ibuprofen in each tablet was 100 mg. Three ibuprofen tablets were administered to each subject with 200 ml of water, following an overnight fast for at least 10 h. Washout periods were at least one 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 °C until analysed.

##### *3.5.2. Plasma assay*

Ibuprofen plasma concentrations were determined by means of HPLC, using the method described by Avgerinos and Hutt (1986) with slight modifications. The HPLC system used has been described previously (Nykänen

et al. 2001). The standard curves were found to be linear over the concentration range 0.3 to 30 mg $l^{-1}$ ($r^2 > 0.9980$) or 0.2 to 40 mg $l^{-1}$ ($r^2 > 0.9998$). Accuracy, precision, limit of quantitation, specificity and reproducibility were investigated as recommended by Shah et al. (2000).

#### *3.5.3. Pharmacokinetic parameters*

The pharmacokinetic parameters assessed using the Siphar pharmacokinetic data analysis program (Simed, France) were maximum plasma concentration ($C_{max}$), time to peak concentration ($t_{max}$) and area under the concentration-time curve ($AUC_{0-24\,h}$). $C_{max}$ and $t_{max}$ values were used as measured. The lag time for absorption ($t_{lag}$) was calculated from raw data. AUC values were calculated using the trapezoidal method, without logarithmic transformation. Statistical analyses were carried out using Student's paired t-test or Student's t-test for independent groups and the Wilcoxon nonparametric test or Mann-Whitney non-parametric test for $t_{max}$ and $t_{lag}$ values.

### *3.6. In vitro/in vivo correlation*

Level-C *in vitro/in vivo* correlation was investigated using the time at which 50% of the drug had dissolved ($t_{50\%}$) as the *in vitro* parameter and lag time in relation to commencement of absorption ($t_{lag}$) as the *in vivo* parameter. The reason for selecting $t_{50\%}$ as the *in vitro* parameter was because it reveals retardation of drug release by citric acid. The pharmacokinetic parameters $t_{max}$ and AUC were not used in investigating *in vitro/in vivo* correlation because they could not be calculated for all volunteers after 24 h of bioavailability testing. Selection of $t_{lag}$ as the *in vivo* parameter was because the retardant effect of citric acid on commencement of drug absorption was being particularly investigated. The *in vitro* parameter was plotted against the *in vivo* parameter, and linear regression calculations were undertaken. Correlation was investigated at both pH levels.

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