Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 290 (2005) 145–154
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

The influence of excipients on the diffusion of ibuprofen and paracetamol in gastric mucus

Lance R. Shaw[a], William J. Irwin[a], Tim J. Grattan[b], Barbara R. Conway[a,*]

[a] *Medicines Research Unit, School of Life and Health Sciences, Aston University, Aston Triangle, Birmingham B4 7ET, UK*
[b] *GlaxoSmithkline Consumer Healthcare, St Georges Avenue, Weybridge, UK*

Received 23 September 2004; received in revised form 22 November 2004; accepted 30 November 2004

Abstract

The aim of this study was to examine the diffusion of commonly administered analgesics, ibuprofen and paracetamol, through gastric mucus. As ibuprofen and paracetamol are often formulated with alkalising excipients, or are commonly co-administered with antacids that have been demonstrated to alter their absorption, diffusion was also studied in the presence of a range of soluble and insoluble antacids or buffering agents. The effect of pH, which has been demonstrated to modify the properties of mucus, was also studied. Mucus was a significant barrier to diffusion for both drugs, compared to an unstirred aqueous layer with diffusion rates significantly lower in the presence of a mucus barrier for both drugs; ibuprofen diffusion also demonstrated a significant increase in the lag time. Paracetamol diffusion was not significantly affected by addition of any antacid, whereas ibuprofen rates were affected and the diffusion lag time for ibuprofen was significantly reduced in all cases. Isolated increases in pH increased the rate and reduced the lag time for ibuprofen diffusion. It was shown that mucus acts as a passive barrier in the case of paracetamol diffusion, and an interactive barrier to ibuprofen diffusion. Changes in mucus viscosity at different pH values may be responsible for the observed changes in ibuprofen diffusion rate.

Keywords: Mucus; Diffusion; Ibuprofen; Paracetamol; Antacids

1. Introduction

Although paracetamol and ibuprofen are well-established non-prescription analgesics, future developments are likely to include new formulations to achieve rapid absorption for a fast onset of action and prolonged absorption to extend the duration of action (Prescott, 2003). Alkalising agents in the form of co-administered antacids or formulation excipients have been found to have an effect on the absorption of the analgesic drugs ibuprofen and paracetamol. For example, Albin et al. (1985) found that the co-administration

* Corresponding author. Tel.: +44 204 3918; fax: +44 121 359 0562.
E-mail address: b.r.conway@aston.ac.uk (B.R. Conway).

0378-5173/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2004.11.028

of an aluminium hydroxide/magnesium hydroxide antacid delayed the time to peak plasma concentration for paracetamol. Grattan et al. (2000) demonstrated different absorption rates for paracetamol formulations containing sodium bicarbonate and calcium carbonate, the former being more quickly absorbed and the second being more slowly absorbed compared to the commercial formulation, demonstrated by differences in C_{max} and t_{max}. Co-administration of magnesium hydroxide has been shown to increase the absorption rate of ibuprofen, although not significantly (Neuvonen and Kivisto, 1994; Maenpaa et al., 2004). The $AUC_{0-1\,h}$ increased by 65% and the C_{max} increased by 31% although t_{max} was not significantly different (Neuvonen and Kivisto, 1994). Also, Hannula et al. (1985) studied the absorption of ibuprofen from capsules containing aluminium hydroxide, calcium carbonate or sodium bicarbonate as the primary excipient. Their results demonstrated significant differences in absorption rates, with formulations containing calcium carbonate or sodium bicarbonate being rapidly absorbed and the aluminium hydroxide-containing formulation being very poorly absorbed.

Explanations for these changes in absorption have been proposed, e.g. the in vitro dissolution behaviour has a rank-order correlation with the in vivo absorption rate (Hannula et al., 1985). The increased absorption rate of paracetamol in a formulation containing sodium bicarbonate as the primary excipient (Grattan et al., 2000) was attributed to an increase in gastric-emptying rate produced by sodium bicarbonate (O'Mahony et al., 2000). It is more likely that a combination of factors, including gastric emptying, contribute to the enhanced absorption rate (Kelly et al., 2003).

However, the inclusion of alkalising excipients in a formulation or coadministration of such excipients could also significantly alter the barrier properties of gastric mucus and this may contribute to the observed alterations in bioavailability.

This study investigates the role of mucus as a barrier to the absorption of paracetamol and ibuprofen. Diffusion of the drugs through gastric mucus was measured and the effects of a range of soluble and insoluble additives on diffusion investigated. The effect of pH on drug diffusion through the mucus was also studied. Native pig gastric mucus (PGM) was selected as a mucus model and the experiments were conducted using a three-compartment diffusion cell.

2. Materials and methods

2.1. Materials

Paracetamol, ibuprofen and sodium bicarbonate (extra fine grade) were obtained from GlaxoSmithKline (Weybridge, UK). [^{14}C]Ibuprofen (50.3 mCi mmol^{-1}, 0.1 μCi ml^{-1}) was obtained from ICN (Irvine, CA, USA). Hydrochloric acid, aluminium hydroxide, calcium carbonate, magnesium hydroxide, sodium hydroxide, sodium chloride, potassium chloride, boric acid, di-sodium phosphate, citric acid, mono-basic potassium phosphate, magnesium oxide and Optiphase 'Hi-safe' 3 were obtained from Aldrich (Poole, UK). All materials were pharmaceutical or analytical grade as appropriate. Double-distilled water was generated in-house using a Fison's Fi-Stream Still.

2.2. Preparation of mucus

The stomachs of a minimum of three freshly slaughtered fasted pigs were obtained from a local abattoir. Each stomach was opened along the greater curvature, inverted, any food content removed mechanically and finally washed with double-distilled water. The mucus was collected using a smooth-faced spatula, ensuring no underlying mucosa was removed, from all regions of the stomach, i.e. cardia, fundus, body and pylorus regions, as it has been shown that differences in some mucus characteristics (e.g. glycosylation, sulphation and buoyant density) are apparent in mucus obtained from the different regions (Nordman et al., 1998). The mucus was mechanically mixed with the spatula until visually homogeneous and then stored overnight at 4 °C before use. The pH range of the mucus samples was pH 4.8–6.2 with a mean and standard deviation of 5.7 ± 0.4 (n = 10) measured using a combination electrode.

2.3. Drug diffusion across mucus

In-house manufactured side-on three-compartment diffusion cells were used to perform the diffusion experiments (Holbrook, 1991). Each cell consisted of a donor and receiver compartment, 15 ml volume per compartment, and a central compartment designed to hold a 1 mm thick mucus layer with diameter of 1 cm. 0.45 μm polycarbonate filter membranes and metal gauze filters were used to physically stabilize the mu-

cus layer and prevent direct contact between the mucus and medium. Stirring rates were set to the highest value where consistent stirring could still be obtained, to ensure the donor cell-aqueous/first membrane barrier drug transfer rate was maximised, and the effects on hydrodynamic conditions on drug diffusion were minimised. Each cell was connected serially to a Churchill circulating water pump set at a temperature of 37 °C.

An equal volume of donor solution, as detailed below, was added to the donor cell for each experiment. Aliquots of 0.1 ml of the receiver solution (detailed below) were sampled every 20 min for a minimum of 200 min, the sample volume being replaced with an equal volume of receiver solution.

For studying the effect of mucus on the drug diffusion rate, i.e. mucus control, aqueous control and filter experiments, the receiver solutions consisted of 0.05 M HCl. The donor solutions were 5 mg ml^{-1} paracetamol in 0.05 M HCl, or saturated ibuprofen solution (≈0.05 mg ml^{-1}) (Shaw, 2001) in 0.05 M HCl, spiked with an aliquot of radio-labelled ibuprofen to give an activity of ≈150,000 DPM ml^{-1}.

2.4. The effect of antacids on drug diffusion through mucus

For additives exhibiting incomplete dissolution, i.e. aluminium hydroxide, magnesium hydroxide, magnesium oxide and calcium carbonate studies, the receiver solution compositions were saturated solutions of the antacid in 0.05 M HCl. The donor solutions consisted of either 5 mg ml^{-1} paracetamol in 0.05 M HCl, or 0.05 M HCl pre-saturated with ibuprofen and filtered before saturation with the antacid under test. The solutions were maintained at 37 °C and filtered through a 0.45 μm membrane filter immediately prior to use. The ibuprofen donor solutions were then spiked with the radio-labelled drug as before. This method ensured that, for all experiments, the drug concentration in the donor cells was unchanged.

For the soluble excipient/antacid, i.e. sodium bicarbonate, the receiver solution consisted of 15 mmol/200 ml sodium bicarbonate in 0.05 M HCl and the donor cell solution was 15 mmol/200 ml sodium bicarbonate in 0.05 M HCl containing 5 mg ml^{-1} paracetamol or pre-saturated ibuprofen (≈0.05 mg ml^{-1}). In each case the sodium bicarbonate was added, slowly, in situ.

2.5. The effect of pH on ibuprofen diffusion through mucus

For studying the effects of pH-modification on the diffusion rate, the receiver solutions consisted of phosphate buffer (USP, 2000), adjusted to 0.15 M with sodium chloride, at the pH under investigation. The donor solutions were 0.05 mg ml^{-1} ibuprofen in the USP buffer, filtered through a 0.45 μm membrane filter before being spiked with an aliquot of radio-labelled ibuprofen to give an activity of ≈150,000 DPM ml^{-1}.

2.6. Calculations

The steady-state flux of the drug through the membrane was determined from the slope of M_t versus time plot and the permeability constant calculated from Eq. (1)

$$J = k_p C_V \quad (1)$$

where J represents the flux, k_p the permeability coefficient and, C_v the initial drug concentration. The permeability coefficient was calculated by dividing the steady-state flux by the initial drug concentration.

Comparison of two groups of data was conducted using Student's unpaired *t*-test. Comparison of more than two groups of data was conducted using single-factor ANOVA analysis. If the ANOVA analysis highlighted differences in the mean values between the groups, a post-analysis Dunnett's test was performed, comparing the test groups to the control group.

2.7. Analytical procedures

Paracetamol was analysed by HPLC according to an adaptation of the method described by Krieger (1987). The HPLC system was composed of a Wisp 712 autoinjector, Waters 484 variable wavelength UV detector and Waters 600E system controller (Massachusetts, USA) using a Techsphere ODS-2 5 μM 150 mm × 4.6 mm column (Welwyn Garden City, UK). For paracetamol, the mobile phase was methanol:0.75% acetic acid (1:3), injection volume was 20 μl, wavelength used 280 nm and the flow rate was 1.0 ml min^{-1}. Caffeine was used as an internal standard. A blank solution was checked for interferences and sample dilution was undertaken if required to produce drug concentrations within a

linear working range. The radio-labelled drug, ibuprofen, was analysed using a 1900TR scintillation counter (Packard, IL, USA), with the sample aliquots (0.2 ml) mixed with 10 ml of Optiphase Hi-safe 3.

3. Results and discussion

3.1. Selection of mucus model and diffusion method

Pig gastric mucus (PGM) consists mainly of water, between 80 and 95%, electrolytes and glycoproteins, commonly referred to as mucins (MacAdam, 1993; Bansil et al., 1995). Mucins are defined structurally as large ($M_w = 10^6$ to 10^7 Da), viscous proteins composed of approximately 75% carbohydrate and 25% amino acids linked via *O*-glycosidic bonds between *N*-acetylgalactosamine and serine or threonine residues (Bansil et al., 1995). The dry weight composition of pig intestinal mucus has been reported as being approximately: 5% mucin, 37% lipids, 39% proteins, 6% DNA and 13% other by Larhed et al. (1998).

Generally, three mucus models are used in diffusion experiments. The first of these is crude mucus, obtained and used directly (Livingston and Engel, 1995). The second model uses mucus, purified and prepared to separate the high molecular weight mucin fraction (Kearney and Marriott, 1986). The third method involves the use of dried crude or semi-purified mucus, which is reconstituted before use. It is generally accepted that the mucin component of mucus is the fraction responsible for the viscous and gel-forming nature of mucus (Allen and Snary, 1972; Meyer and Silberberg, 1978), and the purified fraction is often used.

Bhaskar et al. (1991) demonstrated that the viscosity of mucus can be varied with pH, ionic strength and purification process. Of particular interest, they showed that for purified PGM, the viscosity decreased by a factor of approximately 100 over a pH range of 2–7. They also showed that this profound change was dramatically reduced when the ionic strength was increased using NaCl (range 0–2.0). Further to this, it has been shown that the degree and rate of swelling is dependent on the pH and ionic strength (Bansil et al., 1995). Preliminary studies showed that reconstituted, dried crude PGM demonstrated a decrease in viscosity with increasing pH. For example, a 10% (w/w) solution of PGM had a viscosity of 32.7 $mm^2\,s^{-1}$ at pH 3.3 decreasing to 25.4 $mm^2\,s^{-1}$ at pH 6.2. For 5 and 10% (w/w) solutions a decrease in viscosity of approximately 30% and 25% over the pH range 2–7 was found.

Although it has been reported that a rehydrated PGM model could not produce rheological properties equivalent to those of natural mucus (Nared-Kocevar et al., 1997), viscosity is only one of the factors affecting diffusion. It has also been reported that crude gastric mucus is a more rigorous absorption barrier when studying ionisable drugs (Legen and Kristl, 2001).

As the mucus layer in the stomach is likely to be a more considerable barrier to drug absorption than mucus in the intestine; it is much thicker, reported as being between 576 ± 81 μm in the human stomach (Bickel and Kauffman, 1981) whereas the mucus in the intestine and colon has been reported as being between 50 and 450 μm, summarised by MacAdam (1993), and as approximately 40 μm in dogs and humans, summarised by Macheras et al. (1995), it was decided to use gastric mucus for these experiments. Also, as the investigation involves the alkalisation of the drug environment by pH-modifying excipients/antacids, it is apparent that the greatest changes will occur in the unbuffered, and highly acidic, environment of the stomach rather than the neutral/buffered environment of the small intestine. Thus gastric mucus rather than intestinal mucus was selected (Larhed et al., 1997) and crude PGM from the stomach was used in this study.

Stability of the mucus was confirmed by measuring the diffusion of paracetamol through one batch daily for three days ($n = 4$ per individual experiment) with single factor ANOVA statistical analysis ($P = 0.43$). The inter-batch variation of mucus was verified by measuring the diffusion of paracetamol through four separate batches ($n = 4$ per individual experiment) with single factor ANOVA statistical analysis ($P = 0.29$) (Shaw, 2001).

3.2. Retardation of diffusion rate of model drugs by mucus

Fluxes across the membrane filter, unstirred water layer and 1 mm mucus layer are shown in Table 1 for both paracetamol and ibuprofen. The mucus was found to significantly retard the diffusion rate to $62.2 \pm 7.3\%$ of the diffusion rate through the unstirred layer for paracetamol (*t*-test, $P = 0.002$). For Ibuprofen, the mu-

Table 1
Paracetamol and ibuprofen flux through mucus and controls ($n = 3$; mean ± S.D.)

Flux (mg cm^{-2} s^{-1})	Paracetamol	Ibuprofen
Filter	$1.85 \times 10^{-3} \pm 1.0 \times 10^{-4}$	$1.41 \times 10^{-5} \pm 7.0 \times 10^{-7}$
Mucus	$1.39 \times 10^{-4} \pm 4.8 \times 10^{-6}$	$5.95 \times 10^{-7} \pm 3.6 \times 10^{-8}$
Unstirred aqueous layer	$2.27 \times 10^{-4} \pm 2.8 \times 10^{-5}$	$1.83 \times 10^{-6} \pm 7.3 \times 10^{-8}$

cus was found to significantly retard the diffusion rate to 32.7 ± 2.1% of the diffusion rate through the unstirred layer (*t*-test, $P = 0.00001$). The permeability coefficient, K_p, through mucus was determined to be $2.78 \times 10^{-5} \pm 9.7 \times 10^{-7}$ cm s^{-1} for paracetamol and $1.19 \times 10^{-5} \pm 7.3 \times 10^{-7}$ cm s^{-1} for ibuprofen.

3.3. The effect of dissolved antacids on the diffusion rate of ibuprofen and paracetamol

The results from the dissolved excipients/antacids paracetamol study are summarised in Table 2. For paracetamol, single-factor ANOVA statistical analysis of the lag times, revealed no significant change to the lag time by any of the antacids/excipients ($P = 0.38$) nor the diffusion rates ($P = 0.15$). The results from the dissolved excipients/antacids and ibuprofen study are summarised in Table 3. The addition of aluminium hydroxide effectively eliminated the diffusion of ibuprofen. In all other cases, the lag times were significantly reduced by addition of the antacids/excipients. The diffusion rate of ibuprofen was significantly reduced by the addition of either sodium bicarbonate or magnesium oxide, and significantly increased in the presence of magnesium hydroxide or calcium carbonate.

3.4. The effect of pH on the diffusion rate of ibuprofen

The final series of experiments was designed to isolate the effect of pH on diffusion from other factors by the use of a wide range of pH buffers, adjusted to have the same ionic strength. Pre-dissolved drug was included at concentrations equivalent to those estimated in vivo. The results from the pH modification study, for ibuprofen, are summarised in Table 4. It can be observed that there is a trend of reduction in lag time with increasing solution pH. In addition, there is clear evidence that the diffusion rate of ibuprofen increases with increasing solution pH. The diffusion profiles for ibuprofen at the different pH values are illustrated in Fig. 1.

4. Discussion

The layer of mucus which covers the gastrointestinal tract is associated with protection and lubrication. Mucus has been demonstrated to be a barrier to both diffusion and absorption of drugs (MacAdam, 1993; Larhed et al., 1998; Khanvilkar et al., 2001), with the mucus layer reducing the diffusion rate of drugs substantially, when compared to an aqueous unstirred layer, in several studies (Holbrook, 1991; Matthes et al., 1992; Bhat et al., 1995). The diffusion coefficient of a drug through mucus depends on the viscoelasticity of the mucus gel, the relative size of the drug molecule and any interactions it may have with the mucus. Interactions between the mucus components and the drug are likely to be more important in determining the barrier properties than the gel structure itself (Khanvilkar et al.,

Table 2
The effect of dissolved antacid/excipient additive on the diffusion rate of paracetamol (5 mg ml^{-1}) through mucus

Experiment	Flux ($\times 10^4$ mg cm^{-2} s^{-1}) ± S.D.	Lag time (min) ± S.D.	k_p ($\times 10^5$ cm s^{-1}) ± S.D.	Mean data R^2
Mucus control	1.39 ± 0.05	18.3 ± 3.9	2.78 ± 0.097	0.9984
Sodium bicarbonate	1.18 ± 0.19	18.7 ± 4.0	2.37 ± 0.37	0.9966
Magnesium hydroxide	1.49 ± 0.30	10.3 ± 2.8	3.00 ± 6.00	0.9975
Magnesium oxide	1.40 ± 0.12	15.2 ± 10.7	2.80 ± 0.25	0.9937
Calcium carbonate	1.32 ± 0.13	16.5 ± 5.8	2.63 ± 0.27	0.9987
Aluminium hydroxide	1.21 ± 0.11	16.6 ± 2.0	2.42 ± 0.22	0.9964

Flux and lag time values are the mean of three data-points ± S.D. R^2 was determined by linear regression analysis of the mean of the replicates.

Table 3
The effect of dissolved antacid/excipient additive on the diffusion rate of ibuprofen (0.05 mg ml^{-1}) through mucus

Experiment	Flux ($\times 10^8$ mg cm^{-2} s^{-1}) ± S.D.	Lag time (min) ± S.D.	k_p ($\times 10^6$ cm s^{-1}) ± S.D.	Mean data R^2
Mucus control	59.5 ± 3.7	118.4 ± 6.7	11.9 ± 0.7	0.9917
Sodium bicarbonate	24.8 ± 9.1	11.9 ± 8.3**	5.0 ± 1.9**	0.9423
Magnesium hydroxide	161.5 ± 15.0	76.0 ± 7.0**	32.3 ± 2.7**	0.9888
Magnesium oxide	30.2 ± 8.8	−2.0 ± 14**	6.0 ± 1.7*	0.9643
Calcium carbonate	88.8 ± 18.8	29.2 ± 1.6**	17.8 ± 3.8*	0.9938
Aluminium hydroxide	0.43 ± 0.46	N/A	0.09 ± 0.09**	n.d.

Flux and lag time values are the mean of three data-points ± S.D. R^2 was determined by linear regression analysis of the mean of the replicates. n.d.: not determined.

* Significantly different from control (Dunnett's test $P < 0.05$).

** Significantly different from control (Dunnett's test $P < 0.01$).

2001). It is also known that pH influences the physicochemical properties of mucus. Using a purified mucus model, Bhaskar et al. (1991) observed that a large decrease in mucus viscosity occurred as the pH was raised. It has been observed that the diffusion rate of clarithromycin was reduced to approximately 20–30% of the control rate when aluminium- and magnesium-containing antacids were added to semi-purified mucus (Grübel and Cave, 1998). This was in contrast to the increased penetration rate reported with amoxicillin (Grübel and Cave, 1997).

The conditions and concentrations used in the excipient/antacid studies were designed to mimic co-administration of the ingredient with the analgesic (either as separate doses or combined within the same preparation) within the stomach.

Three potential interaction mechanisms were considered: pH modification, i.e. the role of drug and mucus ionisation on mucus/drug binding and drug diffusion rate, the role of hydrophobic interactions and, thirdly, other known specific interactions for each antacid.

Mucus has predominantly ionisable groups within the glycoprotein, *N*-acetylneuraminic (sialic) acid and esterified sulphate, which both have a pK_a of 2.6 (Kearney and Marriott, 1986). Paracetamol has a pK_a variously reported as between 9.0 and 9.5 and even as high as 10.14 (Fairbrother, 1974); 9.5 was used here as the mean of reference values. For ibuprofen, from the reported data, a mean pK_a of 5.2 was calculated (Moffat, 1986). After each experiment, the pH of the donor solution was measured and utilised to calculate the percentage ionised for drug and mucus (Table 5).

Although glycoprotein is a highly soluble and extensively hydrated macromolecule, it also contains globular protein regions (Kearney and Marriott, 1987). Depending on the amino acid sequence, these regions may be sufficiently hydrophobic for drug–protein interactions (Khanvilkar et al., 2001). Also, gastric mucus glycoproteins have been shown to be extensively esterified with long-chain fatty acids (Slomiany et al., 1983). With such lipophilic regions in the glycoprotein, a hydrophobic interaction with the drug is a realistic possibility. Bhat et al. (1996) confirmed that a non-specific hydrophobic interaction was a major factor involved in binding of a non-homologous series of drugs. Tetracycline has been shown to bind to purified, reconstituted PGM (Kearney and Marriott, 1987). For tetracycline, at low pH, all ionisable groups are protonated and a net charge of +1 arises from the dimethyl-

Table 4
The effect of pH on the diffusion rate of ibuprofen (0.05 mg ml^{-1}) through mucus

Experiment	Flux ($\times 10^8$ mg cm^{-2} s^{-1}) ± S.D.	Lag time (min) ± S.D.	k_p ($\times 10^6$ cm s^{-1}) ± S.D.	Mean data R^2
pH 2	46.8 ± 18.9	143.2 ± 10.6	9.36 ± 3.8	0.9901
pH 4	61.0 ± 31.5	152.3 ± 11.2	12.2 ± 6.3	0.9937
pH 6	123.0 ± 7.0	49.3 ± 4.2	24.6 ± 1.4	0.9982
pH 8	133.0 ± 2.6	24.2 ± 4.9	26.6 ± 1.0	0.9987

Flux and lag time values are the mean of three data-points ± S.D. R^2 was determined by linear regression analysis of the mean of the replicates.

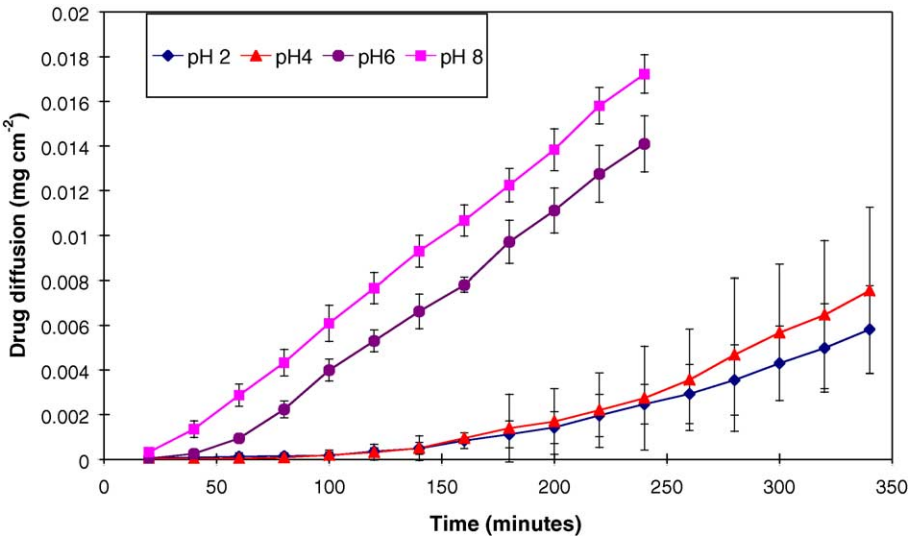

Fig. 1. The effect of pH on the diffusion profile of ibuprofen through mucus. Results are the mean of at least three data series ± S.D.

amino group ($pK_a = 9.7$). A change to a net charge of 0 occurs above pH 3 due to the ionisation of the acid group of the tricarbonyl methane system ($pK_a = 3.3$), at which point the molecule is zwitterionic. Above pH 7, the net charge changes to −1 as the acidic β-diketone system ($pK_a = 7.7$) ionises. It was postulated that taking the two pH/charge profiles, between pH 2 and 3, the drug and purified PGM have opposite charges and hence would be able to interact electrostatically. Above pH 3, the drug becomes effectively neutral and although electrostatic binding is reduced, a hydrophobic interaction is now possible, leading to enhanced binding. A fall in the binding level above pH 7 would be coincident with a reduction in the amount of the neutral species. Levels of binding, calculated theoretically, matched the observed results. As the lag time is intrinsically linked to the degree of binding, this approach was applied to the current data in terms of lag time analysis.

Considering paracetamol, from Table 2, it was deduced that for all the diffusion experiments, the lag times were not significantly different, being in the range of 10–19 min. From a knowledge of the solubility of paracetamol and its structure, i.e. phenolic and acetamido functional groups, it can be concluded that it is unlikely to interact hydrophobically with mucus and further, when it is becomes negatively charged at high pH, it will be electrostatically repulsed by the mucus. Over the pH range of the experiment, any changes in the interaction of paracetamol with mucus have no effect on its diffusion through the mucus.

For paracetamol, in terms of steady-state flux and, therefore, permeability constant, no significant differences were observed following addition of any of the antacid/excipients. This implies, firstly, that any reduction in the viscosity of the mucus with increasing pH is not sufficient to produce a measurable effect using

Table 5
Calculated values of ionised species at experimental pH, for mucus, ibuprofen and paracetamol

Antacid/excipient	Measured pH post-experiment	Mucus % ionised	Ibuprofen % ionised	Paracetamol % ionised
Mucus control	1.3	4.8	0.0	0.0
Sodium bicarbonate	7.4	100.0	92.6	0.8
Calcium carbonate	6.7	100.0	92.6	0.2
Aluminium hydroxide	3.8	94.0	2.0	0.0
Magnesium hydroxide	9.1	100.0	100.0	28.5
Magnesium oxide	9.2	100.0	100.0	33.4

this method, and secondly, that the vehicle-membrane partition coefficient, *P*, is not changed by pH. One reason for this could be that paracetamol is predominantly unionised over pH range within the experiments as shown in Table 5 and therefore limited electrostatic repulsion would occur, therefore not altering *P* by this mechanism. Changes in the size of the diffusing molecule, e.g. through complexation with metal ions, would be unlikely to be detected with this technique.

For ibuprofen, increasing pH decreased the measured lag time substantially and the drug diffusion rate was greatly increased (Table 3). These observations may be explained as follows. At low pH values (i.e. 2–4) ibuprofen exists primarily in the unionised state and mucus is also predominately unionised. Ibuprofen has extensive alkyl group substitution on the *para*-position of the aromatic ring and is therefore highly lipophilic. Accordingly, there is a strong possibility that hydrophobic interactions can occur between the lipophilic groups of the glycoprotein macromolecule and the unionised ibuprofen. This would lead to extensive binding of ibuprofen and mucus and, depending on the concentration of ibuprofen, a significant lag time. When the pH was raised to between pH 6–8, where mucus is 100% ionised and ibuprofen is >90% ionised in all cases, the hydrophobic interaction would be eliminated, to be replaced by an electrostatic repulsion interaction. This explains the very large reduction in the lag time. In terms of the increased diffusion rate with increasing pH, for ibuprofen, this may be explained by the observations of Bhaskar et al. (1991), who noted a large decrease in mucus viscosity as the pH was raised. In addition, at the pH in the mucus control experiment, the lag time was large as expected, due to the potential mechanism as explained above.

For magnesium oxide, magnesium hydroxide, sodium bicarbonate, and calcium carbonate experiments, the pHs were raised to between pH 6–8 and the lag times were reduced in line with expectations, with the potential mechanism as explained above. Sodium bicarbonate and magnesium oxide significantly reduced the diffusion rate, to between 40–50% of the mucus control. These results cannot be explained using the hypothesis detailed above alone.

Calcium carbonate and magnesium hydroxide showed an increase in the diffusion rate of ibuprofen to 149% and 271% of the mucus control, respectively. As saturated solutions of the drug in antacid were filtered before use, the increased rates are not simply due to an increased solubility. It has been shown that calcium can bind to mucus at high pH (Forstner and Forstner, 1975). It has also been suggested that Ca^{2+} combines with negative charges on mucin, especially those of sialic acid groups. This mechanism may be a factor in the increased diffusion rate observed with the calcium carbonate additive. Magnesium hydroxide has been shown to increase the absorption of ibuprofen (Neuvonen, 1991) and it has also been shown to reduce the gastric tolerability of ibuprofen (Maenpaa et al., 2004). It has been proposed that the presence of magnesium ions may interfere with mucin aggregation (Grübel et al., 1997) although the mechanism has not yet been identified. It has also been suggested that increased solubility, leading to more potent inhibition of COX-1 or transient rebound effects, may be responsible for the reduced tolerability. These results suggest that increased penetration of ibuprofen through gastric mucus may also be a significant factor.

For aluminium hydroxide, the antacid effectively eliminated the diffusion of ibuprofen through the mucus layer and the K_p was not significantly different from zero. As paracetamol diffusion was not similarly affected by aluminium hydroxide, the specific precipitation of mucin by aluminium can be discounted (Exley, 1998). Theoretically, there are four ways that this could occur, the size of the diffusing molecule could become so large that it could not penetrate the gel structure, the vehicle membrane partition coefficient, *P*, could be altered such that no partitioning into the mucus occurred, the viscosity of the mucus could increase dramatically thus eliminating diffusion and finally, the aluminium hydroxide could form an insoluble complex with ibuprofen, eliminating the diffusion gradient. This final possibility is unlikely as no evidence of complexation was detected in the donor solution.

5. Conclusions

The native PGM was found to act as a significant barrier to the diffusion of ibuprofen and paracetamol compared to an unstirred aqueous layer. The addition of antacids with changes in pH does not measurably change the barrier properties of PGM to paracetamol. However, ibuprofen diffusion through PGM is affected over the experimental pH range, changing the barrier

properties in terms of reduced lag time and modified diffusion rate. In addition, changing the pH in isolation produced a decrease in lag time and increase in diffusion rate as the pH was increased. For ibuprofen, the mucus and drug properties were compared. It was hypothesised that the lag times observed correlated with a drug/mucus hydrophobic interaction that was eliminated at higher pHs. In addition, observed increases in diffusion rate at higher pHs correlated with cited work where PGM viscosity was shown to decrease as pH is raised. The model differentiated between two model drugs, with different pK_a values and lipophilicities, and highlighted different barrier mechanisms that may be involved, depending on the drug under study.

Acknowledgments

The authors would like to thank the EPSRC and GlaxoSmithKline Consumer Health for financial support for L.R. Shaw.

References

Albin, H., Demotes-Mainard, F., Vincon, G., Bedjaoui, A., Begaud, N., 1985. Effect of two antacids on the bioavailability of paracetamol. Eur. J. Clin. Pharmacol., 251–253.

Allen, A., Snary, D., 1972. The structure and function of gastric mucus. Gut 13, 666–672.

Bansil, R., Stanley, E., Lamont, J.T., 1995. Mucin biophysics. Annu. Rev. Physiol. 57, 635–657.

Bhaskar, R.K., Gong, D., Bansil, R., Pajevic, S., Hamilton, J.A., Turner, B.S., Lamont, J.T., 1991. Profound increase in viscosity and aggregation of pig gastric mucin at low pH. Am. J. Physiol. 261, 827–832.

Bhat, P.G., Flanagan, D.R., Donovan, M.D., 1995. The limiting role of mucus in drug absorption: drug permeation through mucus solution. Int. J. Pharm. 126, 179–187.

Bhat, P.G., Flanagan, D.R., Donovan, M.D., 1996. Drug binding to gastric mucus glycoproteins. Int. J. Pharm. 134, 15–25.

Bickel, M., Kauffman, G.L., 1981. Gastric gel mucus thickness: 16,16-dimethyl prostaglandin E2 and carboxolone. Gastroenterology 80, 770–775.

Exley, C., 1998. The precipitation of mucin by aluminium. J. Inorg. Biochem. 70, 195–206.

Fairbrother, J.E., 1974. Acetaminophen. In: Florey, K. (Ed.), Analytical Profiles of Drug Substances, vol. 3. Academic Press, USA, pp. 1–109.

Forstner, J.F., Forstner, G.G., 1975. Calcium binding to intestinal goblet cell mucin. Biochim. Biophys. Acta 386, 283–292.

Grattan, T.J., Hickman, R.A., Darby-Dowman, A., Hayward, M.A., Boyce, M.J., Warrington, S., 2000. A pilot biostudy to investigate the pharmacokinetics of acetaminophen (paracetamol) from two commercially available acetaminophen products and three development formulations containing acetaminophen in combination with sodium bicarbonate or calcium carbonate. Eur. J. Pharm. Biopharm. 43, 225–229.

Grübel, P., Cave, D.R., 1997. Penetration of amoxicillin and clarithromycin through gastric mucus is affected by the aluminium–magnesium containing antacid Riopan. Gastroenterology 112, A135.

Grübel, P., Bhaskar, K.R., Cave, D.R., Stanley, H.E., Lamont, J.T., 1997. Interaction of an aluminium–magnesium containing antacid and gastric mucus: possible contribution to the cytoprotective function of antacids. Aliment. Pharmacol. Ther. 11, 139–145.

Grübel, P., Cave, D.R., 1998. Factors affecting solubility and penetration of clarithromycin through gastric mucus. Aliment. Pharmacol. Ther. 12, 569–576.

Hannula, A.M., Marvola, M., Rajamaek, M., Ojantakanen, S., 1985. Effects of pH regulators on the bioavailability of ibuprofen from hard gelatin capsules. Acta Pharm. Fenn. 7, 221–227.

Holbrook, P.A., 1991. Biological barriers to the nasal delivery of peptide drugs. PhD Thesis. Aston University.

Kearney, P., Marriott, C., 1986. The effects of mucus glycoproteins on the bioavailability of tetracycline. I. Dissolution rate. Int. J. Pharm. 28, 33–40.

Kearney, P., Marriott, C., 1987. The effects of mucus glycoproteins on the bioavailability of tetracycline. II. Binding. Int. J. Pharm. 35, 211–217.

Kelly, K., O'Mahony, B., Lindsay, B., Jones, T., Grattan, T.J., Rostami-Hodjegan, A., Stevens, H.N.E., Wilson, C.G., 2003. Comparison of the rates of disintegration, gastric emptying, and drug absorption following administration of a new and a conventional paracetamol formulation, using gamma scintigraphy. Pharm. Res. 20, 1668–1673.

Khanvilkar, K., Donovan, M.D., Flanagan, D.R., 2001. Drug transfer through mucus. Adv. Drug Deliv. Rev. 48, 173–193.

Krieger, D.J., 1987. Liquid chromatographic determination of acetaminophen in tablets—collaborative study. J. Assoc. Off. Anal. Chem. 70, 212–214.

Larhed, A.W., Artursson, P., Grasio, J., Bjork, E., 1997. Diffusion of drugs in native and purified gastrointestinal mucus. J. Pharm. Sci. 86, 660–665.

Larhed, A.W., Artursson, P., Bjork, E., 1998. The influence of intestinal mucus components on the diffusion of drugs. Pharm. Res. 15, 66–71.

Legen, I., Kristl, A., 2001. Comparative permeability of some acyclovir derivatives through native mucus and crude mucin dispersions. Drug Dev. Ind. Pharm. 27, 669–674.

Livingston, E.H., Engel, E., 1995. Modelling of the gastric gel mucus layer: application to the measured pH gradient. J. Clin. Gastroenterol. 21, S120–S124.

MacAdam, A., 1993. The effect of gastrointestinal mucus on drug absorption. Adv. Drug Deliv. Rev. 11, 221–251.

Macheras, P., Reppas, C., Dressman, J.B., 1995. Biopharmaceutics of Orally Administered Drugs. Ellis Horwood, London, 127 pp.

Maenpaa, J., Tarpila, A., Jouhikainen, T., Ikavalko, H., Loyttyniemi, E., Perttunen, K., Neuvonen, P.J., Tarpila, S., 2004. Magnesium hydroxide in ibuprofen tablet reduces the gastric mucosal tolerability of ibuprofen. J. Clin. Gastroenterol. 38, 41–45.

Matthes, I., Nimmerfall, F., Sucker, H., 1992. Mucusmodelle zur untersuchung von intestinalabsorptionmechanismen II. Pharmazie 54, 203–211.

Meyer, F.A., Silberberg, A., 1978. A model for mucus glycoprotein structure. Comparison with sunmaxillary mucins. Biorheology 25, 799–801.

Moffat, A.C., 1986. Clarke's Isolation and Identification of Drugs. Pharmaceutical Press, London.

Nared-Kocevar, J., Kristl, J., Korbar-Smid, J., 1997. Comparative rheological investigation of crude gastric mucin and natural gastric mucus. Biomaterials 18, 677–681.

Neuvonen, P.J., 1991. The effect of magnesium-hydroxide on the oral absorption of ibuprofen, ketoprofen and diclofenac. Br. J. Clin. Pharmacol. 31, 263–266.

Neuvonen, P., Kivisto, K.T., 1994. Enhancement of drug absorption by antacids. Clin. Pharmacokinet. 27, 120–128.

Nordman, H., Davis, J.R., Carlstedt, I., 1998. Mucus glycoprotein from pig gastric mucosa: different mucins are produced by the surface epithelium and glands. Biochem. J. 331, 687–694.

O'Mahony, B., Foster, J., Soutar, S., Wilson, C.G., McMillan, N., 2000. An investigation into the prokinetic action of sodium bicarbonate in man using magnetic resonance imaging. J. Pharm. Pharmacol. 52, 6.

Prescott, L.F., 2003. Future perspectives with paracetamol. Drugs 63, 51–56.

Shaw, L.R., 2001. The development of a novel in vitro method model suitable for the prediction of bioavailability. PhD Thesis. Aston University.

Slomiany, A., Slomiany, B., Witas, H., Aono, M., Newman, L.J., 1983. Isolation of fatty acids covalently bound to the gastric mucus glycoproteins of normal and cystic fibrosis patients. Biochem. Biophys. Res. Commun. 113, 286–293.

The United States Pharmacopoeia (USP 24), 2000. United States Pharmacopoeial Convention, Inc., Rockville, USA.