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Biphasic drug release from film-coated tablets

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Abstract

A study was carried out into the biphasic drug release properties of film-coated paracetamol tablets. The tablet cores were formulated without a disintegrant and film-coated with a coating formulation consisting of pectin, chitosan and hydroxypropylmethylcellulose in a ratio of 6:1:0.37. The tablet cores and the film-coated tablets with coat weight gains (CWGs) of 6, 9 and 13% were evaluated for their water absorption (swelling) and drug release properties. All the tablets absorbed water from pH 6.0 Sorensen's phosphate buffer and the amount of water absorbed increased with an increase in tablet CWG. The addition of 100 μl/50 ml pectinolytic enzymes to the medium resulted in at least a 40% reduction in the amount of water absorption by the tablets, as compared to the medium without enzymes. When the enzyme concentration was increased to 200 μl/50 ml, there was a further reduction (~ 8% w/w) in the amount of water absorbed by the tablets. Drug release was controlled in upper gastrointestinal fluids and decreased with an increase in tablet CWG. Drug release was, however, accelerated in the presence of pectinolytic enzymes, consistent with the entry of the tablets in the colon. An evaluation of the drug release data by the Korsmeyer–Peppas equation showed the involvement of molecular diffusion and other factors such as film/tablet erosion and drug dissolution in drug release.

Keywords: Biphasic drug release; Mixed films; Colon; Pectin; Chitosan

1. Introduction

Natural or modified polysaccharides such as amylose, dextran, chondroitin sulphate, calcium pectinate, pectin, chitosan and crosslinked guar gum have been used as potential carriers for the peroral delivery of drugs to the colon (Ashford et al., 1994) as they are safe, biodegradable and widely available. Of these, the use of pectin and chitosan have shown particular promise as they can form polyelectrolyte complexes when exposed to the various media in the gastrointestinal tract (GIT) (Macleod et al., 1999a). The formation of a complex would be valuable in minimising or controlling drug release in the upper GIT.

Pectin in the form of matrix tablets (Rubinstein et al., 1993; Turkoglu et al., 1999; Ahrabi et al., 2000), compression coatings (Ashford et al., 1993) and film coatings (Wakerly et al., 1996) has been used as a potential carrier for the site specific delivery of drugs to the colon. Mixtures of pectin and chitosan have also been used as compression coatings (Fernandez-Hervas and Fell, 1998) and film coatings for colonic delivery (Macleod et al.,

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1999b,c). The further inclusion of hydroxypropylmethylcellulose (HPMC) into film coats improves the mechanical properties of the film. A modification of these systems can produce biphasic drug delivery where drug release is controlled in the upper GIT while release is accelerated in the colonic region by degradative action of pectinolytic enzymes resident in the colon. This can be valuable in a range of therapeutic areas such as nocturnal asthma (Turner-Warwich, 1988), angina pectoris (Lemmer, 1991; Willich et al., 1992), arthritis or inflammation which are susceptible to diurnal rhythms.

This paper investigates the biphasic release properties of pectin/chitosan/HPMC film-coated tablets, containing paracetamol as a model drug, in simulated GIT conditions. This work extends that reported in an earlier paper on the permeability of pectin/chitosan/HPMC films (Ofori-Kwakye and Fell, 2001).

2. Materials and methods

2.1. Materials

Paracetamol powder and 4-acetaminophen (SigmaUltra® grade) were supplied by Sigma Chemical Company (St. Louis, USA), and were used in the manufacture of paracetamol tablets and as a paracetamol standard, respectively. Lactose (Pharmatose® 450M) was supplied by DMV (Veghel, The Netherlands). Microcrystalline cellulose was obtained in two grades, namely; Emcocel® LM50 (low moisture grade) supplied by Mendell Co. Ltd, (Surrey, UK), and Emcocel® LP200 supplied by Penwest Pharmaceuticals (Surrey, UK), the mixture giving the required tablet properties. Magnesium stearate was supplied by GlaxoSmithKline Ltd, (UK). Povidone USP (PVP) was obtained from GAF Corporation (New York, USA). Pectin USP was a gift from Citrus Colloids (Hereford, UK). High molecular weight chitosan (chitosan HM) was obtained from Sigma-Aldrich (Dorset, UK). HPMC was received as Methocel® E4M Premium grade from Colorcon (Kent, UK). Pectinex® Ultra SP-L enzymes with a standard activity of 26 000 PG/ml at pH 3.5 was obtained from Novo Nordisk Ferment Ltd, (Neumatt, Switzerland). Potassium dihydrogen orthophosphate and disodium hydrogen orthophosphate 2-hydrate were general-purpose reagents from BDH Ltd (Poole, UK). Glycerol and acetic acid (glacial) were supplied by BDH Ltd. Hydrochloric acid with a specific gravity of 1.16 was supplied by Fisher Scientific (Loughborough, UK). Methanol was obtained as Chromasolv® (HPLC grade) from Sigma-Aldrich (GMBH, Germany). Water (HPLC grade) was supplied by Sigma-Aldrich. Distilled water was singly distilled and freshly prepared.

2.2. Tablet manufacture

The wet granulation method was used to prepare granules consisting of two grades of microcrystalline cellulose (Emcocel® LP200, 19.8% w/w; Emcocel® LM50, 19.8% w/w), lactose (Pharmatose® 450M, 19.8% w/w), paracetamol (39.7% w/w), and magnesium stearate (0.8% w/w). The powders, except for the magnesium stearate were massed with 5% w/v aqueous PVP in the bowl of a planetary mixer (Model A120, Hobart Manufacturing Co., UK). The wet mass was passed through a Jackson-Crockatt No. 6 granulator (Allen West Co. Ltd, UK) fitted with a 2.36 mm sieve, and the granules dried overnight at 60 °C. The dried granules were sieved through a 1.00 mm sieve and blended with magnesium stearate. Tablets with a nominal weight of 750 mg (<2 tablets $\pm 5\%$ average weight, none $\pm 10\%$ average weight, $n=20$) were compressed using four stations of a 16 station rotary tablet machine (Model B3B, Manesty Ltd, Liverpool, UK) fitted with a 1.27 cm diameter normal biconcave punch and die sets. The tablets, having a hardness of 9.6 ± 0.7 kp ($n=20$) tested with a Schleuniger hardness tester (Model 2E/205, Schleuniger and Co., Switzerland) and friability of 0.16% (Roche Friabilitor, Erweka, Offenbach, Germany) were stored in a plastic container until use.

2.3. Assay and content uniformity determination

For the assay, 20 tablets were randomly selected, weighed and the mean tablet weight deter-

mined. The tablets were finely powdered in a porcelain mortar and mixed. A quantity of powder equivalent to the mean weight of one tablet was weighed and serially extracted with 0.05-M acetic acid. The final extract was filtered through a 0.45 μm HA membrane filter (Millipore Ltd, UK). Standard solutions of paracetamol in the concentration range 0.5–3.0 mg/100 ml were prepared in 0.05 M acetic acid. The standard solutions and final extract were analysed by reverse-phase HPLC using a mobile phase of 25% methanol in water and flow rate of 1.0 ml/min. For content uniformity determination, 10 tablets were individually weighed and individually analysed using the chromatographic conditions outlined above. The amount of paracetamol in the tablets were calculated using the regression data obtained from calibration plots of the standard solutions.

2.4. Preparation of film coating formulation

Aqueous gels of pectin USP (2% w/w), chitosan HM (1% w/w) and HPMC E4M (1% w/w) were prepared by dispersing the individual powders in 0.1 M HCl and stirring continuously with a mechanical stirrer (Heidolph type RZRI, Germany) until uniform gels were formed. The required amounts of pectin and HPMC gels were weighed and mixed for 30 min, chitosan gel was then added in a slow stream and stirred for a further 30 min. The ratio of pectin, chitosan and HPMC in the coating formulation was 6:1:0.37 (95% pectin/chitosan:5% HPMC). Glycerol was added as plasticiser and mixed for 1 h. To produce a less viscous formulation appropriate for film coating, 0.1 M HCl (2 kg) was added to the formulation and stirred for 1 h. The formulation details are shown in Table 1. The coating formulation having a general composition of pectin USP (0.98% w/w), chitosan HM (0.16% w/w), HPMC E4M (0.06% w/w), glycerol (0.24% w/w) and 0.1 M HCl (98.55% w/w), was left overnight for air bubbles to subside. The coating formulation was prepared on three different days and used to coat the tablets.

Table 1
Details of the film coating formulations

Material	Weight (g)
Pectin USP	70
Chitosan HM	11.7
HPMC E4	4.3
Glycerol	17.1
0.1 M HCl	7010

2.5. Tablet coating process

The film coating of the paracetamol tablet cores was carried out using a Accelacota 10 tablet coater (Manesty Ltd). The coating solution was sprayed onto the tablets (batch size, 1.5 kg) with a Manesty spray gun (Manesty Ltd) in a perforated, 61 cm diameter 316L stainless steel coating drum, modified with a base plate to reduce the working capacity. The coating solution was stirred continuously with a mechanical stirrer to ensure homogeneity. The process parameters used to coat the tablets were: inlet temperature (68–70 °C), outlet temperature (50–52 °C), tablet bed temperature (32–35 °C), spray rate (13–15 g/ml), spray gun distance (18 cm), drum speed (8.7 rpm), inlet air flow (7.5–8.2 m^3/h), atomising air pressure (1.5–2.0 bar), and fan air pressure (0.7–1.0 bar). The tablets were sampled (10 tablets) at regular intervals and weighed to determine the coat weight gain (CWG) of the tablets. When the required CWG was achieved, spraying of the solution was stopped and the tablets dried in the coating drum for a further 10 min before samples were taken. Tablets with CWG of approximately 6, 9 and 13% were produced and stored in plastic bags at room temperature until required.

2.6. Swelling studies

Pectin/chitosan/HPMC film-coated tablets (6, 9 and 13% CWG) were weighed and incubated individually in 50 ml pH 6.0 Sorensen's phosphate buffer in a water bath thermostatically set at 37 °C. The effect of pectinolytic enzymes on the swelling of tablets was investigated by the addition of 100 and 200 μl Pectinex® Ultra SP-L enzymes to the incubation medium. At specified time intervals, the tablets were removed and blotted

dry with filter paper and immediately weighed. Four tablets from each batch were tested. The increase in weight of the tablets (% w/w) was determined using the relationship: $(Wa-Wb/Wb)\times 100$, where Wb and Wa are the weight of the tablets before and after incubation at time t, respectively. Plots were made of the percentage increase in tablet weight (in the presence and absence of pectinolytic enzymes) against time.

2.7. Drug release studies

Drug release studies were carried out on the uncoated and film-coated (6, 9 and 13% CWG) paracetamol tablets with the use of the BP 2000 dissolution apparatus II (paddle method). Tests were carried out with 900 ml dissolution media at 37 ± 0.5 °C, and a paddle speed of 50 rpm. The dissolution media used were 0.1 M HCl (pH 1.5), pH 7.4 Sorensen's phosphate buffer and pH 6.0 Sorensen's phosphate buffer (with and without Pectinex® Ultra SP-L enzymes) to mimic the conditions pertaining in the stomach, small intestine and the colon, respectively. Pectinex® Ultra SP-L enzymes were used to simulate the pectinolytic enzymes in the colon. To mimic the gastrointestinal transit of the tablets, drug release was determined at pH 1.5 (2 h), followed by pH 7.4 (3 h), and finally in pH 6.0 (with and without 4 ml/l pectinolytic enzymes) for 7 h. The effect of pectinolytic enzymes on drug release was investigated using pH 6.0 Sorensen's phosphate buffer containing 0, 2 and 4 ml/l pectinolytic enzymes for 12 h.

At specified time intervals, 5 ml samples of the dissolution media were taken and replaced with fresh media. The collected samples were filtered through 0.45 μm HA membrane filters. The amount of paracetamol in the dissolution media were determined spectrophotometrically (Cecil CE 1021 Spectrophotometer, Cecil Instruments, Cambridge, UK) at a wavelength of 243 nm with the use of regression data from calibration plots of paracetamol in the various media (0.1–3.0 mg/100 ml). From these, plots were made of the percentage paracetamol released from the tablets (mean $\pm$ S.D., $n=3$) versus time.

3. Results and discussion

The tablets cores were formulated in the absence of a disintegrant so as to disintegrate and dissolve slowly once exposed. The mean amount of paracetamol in the tablets was determined by HPLC to be 276 mg (256–298 mg, $n=10$). The tablets thus produced had a uniform distribution of paracetamol in the tablet mass.

Table 2 shows the effect of tablet CWG and pectinolytic enzymes on the water absorption (swelling) of the film-coated tablets. The concentrations of pectinolytic enzymes chosen were similar to those used by other workers (Semde et al., 2000; Macleod et al., 1999b). All the tablets absorbed water in pH 6.0 Sorensen's phosphate buffer. The water absorption of the film-coated tablets in pH 6.0 Sorensen's phosphate buffer increased with an increase in tablet CWG but was reduced in the presence of pectinolytic enzymes. An increase in enzyme concentration caused a further reduction in the water absorption of the tablets. Typical results are shown in Fig. 1. The addition of 100 μl/50 ml pectinolytic enzymes to the medium resulted in at least a 40% reduction in the amount of water absorbed by the tablets, as compared to the medium without enzymes. When the enzyme concentration was increased to 200 μl/50 ml, there was a further reduction ($\sim$ 8% w/w) in the amount of water absorbed by the tablets.

In the absence of enzymes, the absorption of water by the tablets resulted in the formation of a gelatinous layer around the tablets. Tablets with higher CWG formed a much thicker gelatinous

Table 2
Effect of pectinolytic enzymes on water absorption (% w/w) of film-coated tablets (mean $\pm$ S.D., $n=3$) in pH 6.0 Sorensen's phosphate buffer

CWG (%)	Cumulative amount of water absorbed (% w/w)		
	Enzyme concentration		
	0	100 μl/50 ml	200 μl/50 ml
6	101.9 ± 1.1	60.6 ± 1.8	52.6 ± 0.3
9	120.7 ± 2.0	66.6 ± 0.2	57.8 ± 1.7
13	125.3 ± 1.2	67.4 ± 2.6	59.7 ± 0.7

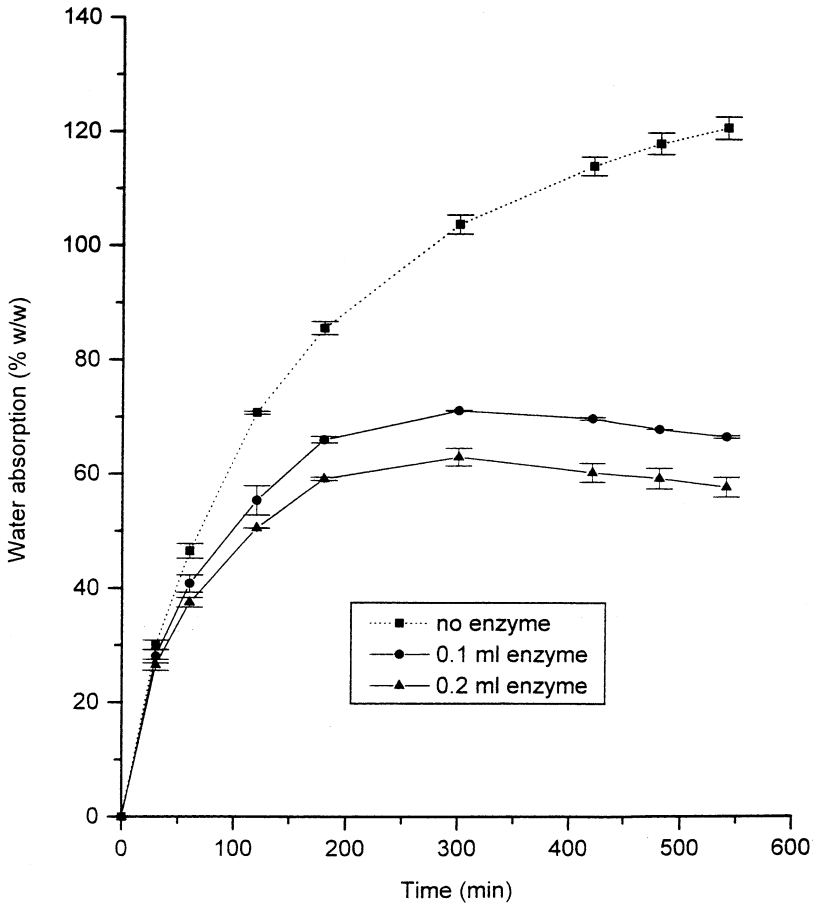

Fig. 1. Water absorption of the coated tablets (9% CWG) as a function of time (mean ± S.D., $n = 4$).

layer around the tablets. These gels will form a diffusion barrier around the tablet cores an increase in which will increase the diffusion path length and hence cause a reduction in drug release. Drug release from the tablets in the absence of pectinolytic enzymes is likely to include drug diffusion through the gel layers and/or erosion of the gel layers formed around the tablets. The stronger and thicker the gels formed the less likely will be their susceptibility to erosion. In the presence of enzymes, the amount of water absorbed by the tablets was minimal and there was no discernible gel formation around the tablets. This means a shorter path length for drug molecules to diffuse. Conversely, pectinolytic enzymes will increase the permeability of the film coatings by increasing the degradation and/or leaching of pectin from the film matrices.

Figs. 2 and 3 show the drug release profiles of the uncoated and film-coated tablets in 0.1 M HCl and pH 7.4 Sorensen's phosphate buffer, respectively. Drug release from the tablets was higher in 0.1 M HCl than in pH 7.4 Sorensen's phosphate buffer. Practically, all the paracetamol (98 ± 2.4%) was released from the uncoated tablets into 0.1 M HCl in 90 min, while 76 ± 5.3% of the drug was released into pH 7.4 Sorensen's phosphate buffer in the same period. The rate of drug release from the uncoated tablets in the two media was low and is indicative of a modified-release paracetamol formulation. The amount of drug released from the film-coated tablets in the two media decreased

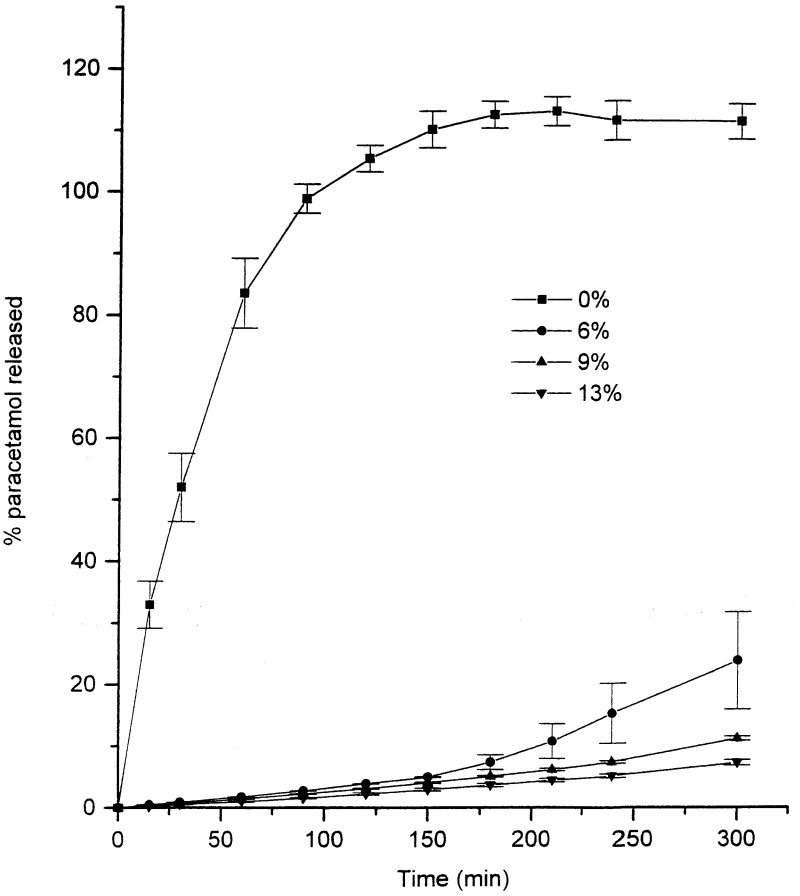

Fig. 2. Release of paracetamol from uncoated and coated tablets in 0.1 M HCl (mean ± S.D., $n = 3$).

with increase in tablet CWG. For instance, after 5 h of dissolution testing in 0.1 M HCl, the film-coated tablets having CWG of 6, 9 and 13% released 24 ± 7.9, 11 ± 0.4 and 7 ± 0.4% of paracetamol, respectively.

Table 3 shows the drug release data from the film-coated tablets in pH 6.0 Sorensen's phosphate buffer in the presence and absence of pectinolytic enzymes. The release of paracetamol from the tablets was higher in the presence of pectinolytic enzymes than in the absence of enzymes. An increase in the concentration of pectinolytic enzymes in the medium resulted in a further increase in the amount of drug released. The amount of drug released, both in the presence and absence of pectinolytic enzymes, was reduced as tablet CWG was increased. Pectinolytic enzymes will attack pectin in the film matrix causing increased degradation and leaching of pectin with the creation of aqueous pores through which drugs can diffuse. The resulting films will have increased permeabilities to drugs (Macleod et al., 1999b; Ofori-Kwakye and Fell, 2001). The drug release profiles of the tablets in the first 150–200 min of the study showed a distinct similarity in the presence and absence of pectinolytic enzymes. This means that a certain amount of hydration and swelling of the film coatings is required for the enzymatic breakdown of the pectin in the mixed film matrix to occur.

Fig. 4 shows the drug release profiles of the film-coated tablets in simulated gastrointestinal condi-

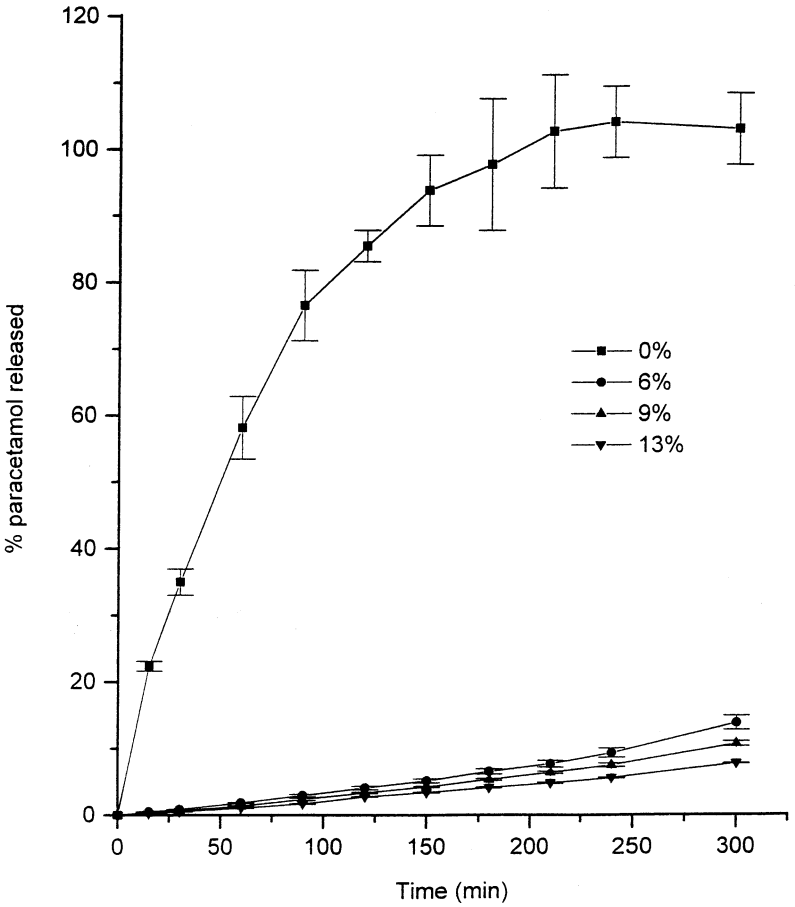

Fig. 3. Release of paracetamol from uncoated and coated tablets in pH 7.4 Sorensen's buffer (mean ± S.D., $n = 3$).

Table 3
The cumulative amount of paracetamol released (mean ± S.D., $n = 3$) after 6 h dissolution testing in pH 6.0 Sorensen's buffer

CWG (%)	Cumulative amount of paracetamol released		
	Enzyme concentration		
	0	2 ml/l	4 ml/l
6	15.8 ± 2.6	22.0 ± 0.3	26.0 ± 1.3
9	9.9 ± 0.5	18.3 ± 1.0	20.9 ± 0.7
13	7.8 ± 0.5	12.2 ± 0.9	14.5 ± 0.2

tions. Drug release was low and controlled by tablet CWG in conditions mimicking the stomach and the small intestine. Minimising the CWG and altering the core formulation would be ways to increase release under upper GI tract conditions. Drug release was, however, accelerated in the presence of pectinolytic enzymes, consistent with the entry of the dosage form in the colon. The acceleration was almost immediate as the tablets were already hydrated. The pectin/chitosan/HPMC film coatings thus demonstrate biphasic release patterns and the influence of CWG suggests a means of optimising the release profile.

The results of the swelling studies were similar to those reported by Semde et al. (2000). These workers determined the water absorption kinetics of isolated films prepared from blends of Eudragit® RS/pectin HM (10:1), Eudragit® NE/pectin HM (10:1), and Eudragit® NE/calcium

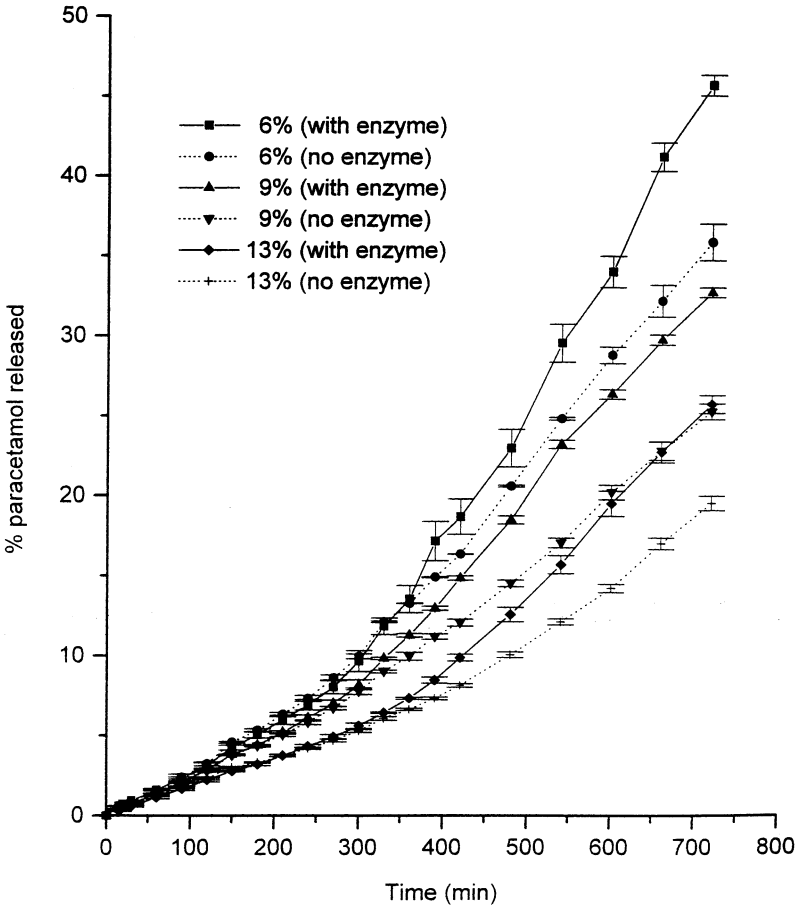

Fig. 4. Release of paracetamol under conditions mimicking gastrointestinal transit (pH 1.5, 2 h; pH 7.4, 3 h; pH 6 ± enzymes, 7 h: mean ± S.D., $n = 3$).

pectinate (10:1) in 0.05 M acetate–phosphate buffer (pH 4.5). They found that the amount of water absorbed by the films in the presence of pectinolytic enzymes was lower than those observed in the absence of the enzymes. These workers have also reported that the presence of pectinolytic enzymes in a dissolution medium of 0.05 M acetate–phosphate buffer (pH 6.0), caused a reduction in the rate of theophylline release from pellets coated with aqueous dispersions of Eudragit® NE30D, Eudragit® RS30D, Aquacoat® ECD30 and Surelease® clear containing pectin HM or calcium pectinate. They attributed the reduction in theophylline release in the presence of pectinolytic enzymes to the rapid degradation and leaching of pectin HM or calcium pectinate from the film coatings, leaving only the insoluble polymers which are less permeable to theophylline. In the current study, however, though the swelling of the pectin/chitosan/HPMC coated paracetamol tablets was reduced in the presence of pectinolytic enzymes, the release of paracetamol from the tablets was increased. Pectin and chitosan are naturally-occurring hydrophilic polymers which combine to form films with enhanced aqueous insolubility than with the use of each polymer alone, restricting leaching in conditions pertaining in the upper GIT. Any leaching of pectin from mixed films containing pectin, chitosan and HPMC on exposure to pectinolytic enzymes would

cause an increase in the porosity of the films making them more permeable to drugs such as paracetamol.

The mechanism of drug release from the uncoated and film-coated tablets was evaluated by means of the Korsmeyer–Peppas equation (Korsmeyer et al., 1983; Peppas, 1985; Ritger and Peppas, 1987; Macleod et al., 1999b; Heng et al., 2001) which is used to describe drug release from swellable dosage forms. The Korsmeyer–Peppas equation is given as:

$$\mathrm{Mt}/\mathrm{M}\mu = kt^n \qquad \text{for } 0 < \mathrm{Mt}/\mathrm{M}\mu < 0.6$$

where Mt and Mμ are the amounts of drug released at time t and the total amount of drug released, respectively, k is a release constant and is related to the structural and geometric properties of the dosage form (Peppas, 1985), and n is the release exponent which gives an indication of the mechanism of drug release. The release data fitted well with the equation with R^2 values of 0.961–0.998. The n values for the coated tablet formulations in the various GIT conditions were close to unity indicating that the coated system provided zero order drug release.

4. Conclusions

The pectin/chitosan/HPMC coated tablets absorbed water in pH 6.0 Sorensen's phosphate buffer and the amount of water absorbed increased with an increase in tablet CWG. Water absorption was, however, reduced in the presence of pectinolytic enzymes. Drug release from the film-coated tablets was controlled by tablet CWG. Manipulation of the tablet CWG will lead to the formation of films capable of controlling the rate and extent of drug release in the GIT. Drug release was accelerated in the presence of pectinolytic enzymes, consistent with the entry of the dosage form in the colon. The coated system produced zero order drug release. The studies have demonstrated the potential of the pectin/chitosan/HPMC film coatings as a carrier for the biphasic delivery of drugs and other bioactive materials.

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References

Ahrabi, S.F., Madsen, G., Dyrstad, K., Sande, S.A., Graffner, C., 2000. Development of pectin matrix tablets for colonic delivery of model drug ropivacaine. Eur. J. Pharm. Sci. 10, 43–52.

Ashford, M., Fell, J.T., Attwood, D., Sharma, H., Woodhead, P., 1993. An evaluation of pectin as a carrier for drug targeting to the colon. J. Control Release 26, 213–220.

Ashford, M., Fell, J.T., Attwood, D., Sharma, H., Woodhead, P., 1994. Studies on pectin formulations for colonic drug delivery. J. Control Release 30, 225–232.

Fernandez-Hervas, M.J., Fell, J.T., 1998. Pectin/chitosan mixtures as coatings for colon-specific drug delivery: an in vitro evaluation. Int. J. Pharm. 169, 115–119.

Heng, P.W.S., Chan, L.W., Easterbrook, M.G., Li, X., 2001. Investigation of the influence of mean HPMC particle size and number of polymer particles on the release of aspirin from swellable hydrophilic matrix tablets. J. Control Release 76, 39–49.

Korsmeyer, R.W., Gurny, R., Doelker, E., Buri, P., Peppas, N.A., 1983. Mechanisms of solute release from porous hydrophilic polymers. Int. J. Pharm. 15, 25–35.

Lemmer, B., 1991. Circadian rhythms and drug delivery. J. Control Release 16, 63–74.

Macleod, G.S., Collett, J.H., Fell, J.T., 1999a. The potential use of mixed films of pectin, chitosan and HPMC for bimodal drug release. J. Control Release 58, 303–310.

Macleod, G.S., Fell, J.T., Collett, J.H., 1999b. An in vitro investigation into the potential for bimodal drug release from pectin/chitosan/HPMC coated tablets. Int. J. Pharm. 188, 11–18.

Macleod, G.S., Fell, J.T., Collett, J.H., Sharma, H.L., Smith, A.-M., 1999c. Selective drug delivery to the colon using pectin:chitosan:hydroxypropylmethylcellulose film-coated tablets. Int. J. Pharm. 187, 251–257.

Ofori-Kwakye, K., Fell, J.T., 2001. Biphasic drug release: the permeability of films containing pectin, chitosan and HPMC. Int. J. Pharm. 226, 139–145.

Rubinstein, A., Radai, R., Ezra, M., Pathak, S., Rokea, J.S., 1993. In vitro evaluation of calcium pectate: a potential colon specific drug delivery carrier. Pharm. Res. 10, 258–263.

Semde, R., Amighi, K., Devleeschouwer, M.J., Moes, A.J., 2000. Effect of pectinolytic enzymes on the theophylline release from pellets coated with water insoluble polymers containing pectin HM or calcium pectinate. Int. J. Pharm. 197, 169–179.

Turkoglu, M., Takka, S., Baran, H., Sakr, A., 1999. Pectin/HPMC drug delivery system for colon targeting—design and in vitro evaluation. Pharm. Ind. 61, 662–665.

Turner-Warwich, M., 1988. Epidemiology of nocturnal asthma. Am. J. Med. 85, 6–8.

Wakerly, Z., Fell, J.T., Attwood, D., Perkins, D., 1996. Pectin/ethyl cellulose film coating formulations for colonic drug delivery. Pharm. Res. 13, 1210–1212.

Willich, S.W., Goldberg, R.J., Maclure, M., 1992. Increased onset of sudden cardiac death in the first three hours after awakening. Am. J. Cardiol. 70, 65–68.

Peppas, N.A., 1985. Analysis of Fickian and non-Fickian drug release from polymers. Pharm. Acta Helv. 60, 110–111.

Ritger, P.L., Peppas, N.A., 1987. A simple equation for description of solute release. II. Fickian and anomalous release from swellable devices. J. Control Release 5, 37–42.