In vitro dissolution of proton-pump inhibitor products intended for paediatric and geriatric use in physiological bicarbonate buffer

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Abstract

Proton-pump inhibitor (PPI) products based on enteric coated multiparticulates are design to meet the needs of patients who cannot swallow tablets such as children and older adults. Enteric coated PPI preparations exhibit delays in *in vivo* absorption and onset of antisecretory effects, which is not reflected by the rapid *in vitro* dissolution in compendial pH 6.8 phosphate buffer commonly used for assessment of these products. A more representative and physiological medium, pH 6.8 *m*Hanks bicarbonate buffer, was used in this study to evaluate the *in vitro* dissolution of enteric coated multiparticulate-based PPI products. Commercially available omeprazole, lansoprazole and esomeprazole products were subject to dissolution tests using USP-II apparatus in pH 4.5 phosphate buffer saline for 45 minutes (acid stage) followed by pH 6.8 phosphate buffer or pH 6.8 *m*Hanks bicarbonate buffer. In pH 6.8 phosphate buffer, all nine tested products displayed rapid and comparable dissolution profiles meeting the pharmacopeia requirements for delayed release preparations. In pH 6.8 *m*Hanks buffer, drug release was delayed and failed the pharmacopeia requirements from most enteric coated preparations. Despite that the same enteric polymer, methacrylic acid – ethyl acrylate copolymer (1:1), was applied to all commercial multiparticulate-based products, marked differences were observed between dissolution profiles of these preparations. The use of pH 6.8 physiological bicarbonate (*m*Hanks) buffer can serve as a useful tool to provide realistic and discriminative *in*

vitro release assessment of enteric coated PPI preparations and to assist rational formulation development of these products.

Key words: pellets, dysphagia, modified release, physiological buffers, bicarbonate media, biorelevant dissolution

1. Introduction

Proton pump inhibitors (PPIs) are highly effective in gastric acid suppression and are increasingly used in the treatment of acid-related disorders such as gastroesophageal reflux disease and peptic ulcer disease (L. S. Welage, 2003). PPIs are acid-labile compounds; they rapidly degrade at pH levels below 4. Consequently, most PPI products are available as enteric coated (delayed release) dosage forms to protect the active drug in the stomach and release the drug in the small intestine. For patients who cannot swallow conventional tablets such as children and older patients, alternative PPI formulations have been developed including granules in sachets, pellet-enclosed capsules, orally dispersible tablets and MUPS (Multiple-Unit Pellet System) tablets. These formulations are based on the encapsulation of the active compound in enteric coated multiparticulates (granules, pellets, micropellets or microcapsules) of varying sizes.

Enteric coatings applied to solid dosage forms employ polymers which contain carboxylic acid groups and exhibit pH-dependent dissolution. The dissociation of the enteric polymer and the resultant drug release from coated products in aqueous media

are affected not only by the pH of the media but also by their composition and other characteristics, such as the type of buffer species, ionic strength and buffer capacity (W. A. Chan et al., 2001; H. M. Fadda and A. W. Basit, 2005; V. C. Ibekwe et al., 2006). Commonly, the *in vitro* dissolution of enteric coated preparations is assessed in pH 6.8 phosphate buffer, albeit, this compendial buffer solution does not reflect the constitution of the luminal fluids of the small intestine and consequently gives poor prediction of the *in vivo* performance of these products. Phosphate content in the intestinal fluids is relatively neglectable and the principal buffer specie is bicarbonate. Efforts have been made to develop and utilise physiological solutions buffered by bicarbonate for dissolution testing of solid dosage forms coated with pH-responsive polymer systems (H. M. Fadda and A. W. Basit, 2005; H. M. Fadda et al., 2009; F. Liu et al., 2010). These media resemble more closely the physiological environment within the intestine and have been proven to provide better *in vitro-in vivo* correlations than conventional phosphate buffers.

The enteric coatings applied to PPI products tend to hinder their absorption and delay the onset of antisecretory effect. It can take up to 4 hours for delayed-release PPIs to achieve maximum plasma concentration after oral ingestion (J. R. Horn and C. W. Howden, 2005). It was reported that immediate release omeprazole preparations stabilised using bicarbonate buffers provide faster absorption and onset of gastric acid suppression compared to delayed release omeprazole formulations (B. Hepburn and B. Goldlust, 2003). In addition, rates of absorption are highly variable for different PPI preparations (J. R. Horn and C. W. Howden, 2005). *In vivo* performances of enteric coated dosage forms are affected by their gastrointestinal transits especially gastric emptying times. Since enteric coated multiparticulates do not show typical *in*

vivo disintegration, neither conventional pharmacokinetic studies nor scintigraphies can fully evaluate their *in vivo* performances taking into account variations in gastric emptying. Pharmacoscintigraphy studies, a combination of scintigraphy with pharmacokinetic studies, are required to gain an understanding of the *in vivo* dissolution behaviour of these enteric coated products post-gastric emptying (I. R. Wilding et al., 2001). A predictive *in vitro* dissolution testing can serve as a useful tool during formulation development by providing discriminative *in vitro* data to guide the rational selection of desired formulation features. The aim of this study was to evaluate the *in vitro* dissolution of enteric coated multiparticulate PPI products in a pH 6.8 physiological bicarbonate (*m*Hanks) buffer. This assessment was conducted on various commercially available delayed release PPI products intended for use in children and individuals with swallowing difficulties such as older patients.

2. Materials and Methods

2.1 Materials

Omeprazole, lansoprazole and esomeprazole commercial products available in the UK that are based on enteric-coated multiparticulates were included in the study (Table 1). Mepradec (omeprazole) was included as a tablet–enclosed capsule (10 mm oblong tablet) and as a comparison to multiparticulate-based products. These were obtained from respective producers (Table 1). Omeprazole, lansoprazole and esomeprazole standards were purchased from Sigma-Aldrich Co. Ltd., Dorset, UK. Salts for preparing buffer solutions were purchased from Sigma-Aldrich Co. Ltd., Dorset, UK.

2.2 Particle size analysis

All multiparticulate-based products contain enteric-coated pellets or granules. The particle size of these multiparticulates was measured using either a laser diffraction particle size analyser or an analytical sieve shaker. Table 2 lists products analysed using each method. For pellet-enclosed capsules, contents of 10 capsules were emptied and weighted before the subsequent analysis. For sachets, oro-dispersible tablets and MUPS tablets, 10 tablets or contents of 10 sachets were dispersed in 0.1M HCl. The dispersed pellets were collected using filter papers and placed in an oven (40 °C) to dry for 6 hours. Dried pellets were weighed before subject to subsequent analysis.

Laser diffraction particle size analysis was applied to products with a particle size smaller than 875μm. For each product tested under the laser diffraction particle size analysis, dry pellets (2g) were filled in the sample vials and were fed into a laser diffraction particle size analyser (Sympatec). Results were displayed as the median particle size (X_{50}). Using the sieve method, dry pellets were put through a series of analytical test sieves mounted on an analytical sieve shaker (Copley Scientific, AS200). The opening diameters of the sieves were 2000, 1400, 1000, 710, 500, 355, 250, 180, 125 and 90μm. The sieves were shaken for 10 minutes. Pellets remained on each sieve were collected and weighted.

2.3 In *vitro* drug release

Drug release from the commercial PPI products was evaluated using a USP II apparatus (Model PTWS, Pharmatest, Hainburg, Germany). The tests were conducted in six repetitions, in 900 ml dissolution medium maintained at 37 $\pm$ 0.5 °C. A paddle

speed of 50 rpm was employed. The tests were conducted under sink conditions. Each product was placed for 45 minutes into pH 4.5 phosphate buffer (0.05 M KH_2PO_4) as the acid stage of the test according to British Pharmacopeia for testing gastric-resistant PPI products (British Pharmacopoeia Commission, 2014). Patients taking PPI products can have an elevated gastric pH ranging from 3 to 6 with substantial time period having gastric pH higher than 4.0 (S. Hata et al., 2013; D. C. Metz et al., 2006). Therefore, a higher pH level was used to test gastric-resistance of enteric coated PPI products than commonly used 0.1 M HCl. After the acid stage, the products were subsequently transferred into pH 6.8 phosphate buffer (composed of 50 mM KH_2PO_4 and 23.5 mM NaOH; pH adjusted with 1M HCl / NaOH solutions) or a pH 6.8 *m*Hanks buffer (F. Liu et al., 2010). The *m*Hanks buffer was adapted from Hanks' balanced salt solution (J. H. Hanks, 1975) composed of 136.9 mM NaCl, 5.37 mM KCl, 0.812 mM $MgSO_4.7H_2O$, 1.26 mM $CaCl_2$, 0.337 mM $Na_2HPO_4.2H_2O$, 0.441 mM KH_2PO_4, 4.17 mM $NaHCO_3$. A sufficient quantity of CO_2(g) was purged into the media to reach pH 6.8 (F. Liu et al., 2010). The pH of the *m*Hanks buffer was maintained by sparging CO_2 into the media during dissolution studies. For products containing gastro-resistant multiparticulates (pellets and microgranules), after the acid stage test the pellets or granules were carefully collected using a 125 μm sieve to avoid any product loss and transferred to the buffer stage test.

The quantity of active ingredients released from the commercial enteric coated products was determined using an in-line UV spectrophotometer (Cecil 2020 model, UK) at the wavelengths of 299, 283 and 299 nm for omeprazole, lansoprazole and esomeprazole respectively. Data were processed using Icalis software (Icalis Data Systems Ltd, Berkshire, UK). Drug release lag times (t_{lag}), t_{80} and release rate were

calculated for all formulations in pH 6.8 phosphate and *m*Hanks buffers. The t_{lag} is defined as the first time point when the percentage cumulative drug release is greater than 5%. The t_{80} is the first time point when the percentage cumulative drug release has reached above 80%. The drug release rate is the slop of the linear plot of percentage drug release against time.

Buffer capacity (β) of the buffers used in the dissolution test was measured by adding aliquots of 0.1M HCl to 100 ml of the buffer until a pH change of 0.5 units. The measurements were conducted in triplicates. Buffer capacity was then calculated using Eq.(1).

$$\beta = \frac{\Delta AB}{\Delta pH} \quad (1)$$

Where ΔAB is the small increment in mol/L of the amount of acid added to produce a pH change of ΔpH in the buffer.

The *in vitro* drug release data was analysed by two-way ANOVA followed by post-hoc analysis by Tukey with 99.8% confidence interval using Univariate General Linear Model tool in PASW Statistics 21 (SPSS Inc., Illinois, USA).

182
183 Table 1. Commercial PPI products included in the study

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Brand name	Strength	Formulation	Enteric coating	Manufacturer
Esomeprozole				
Nexium	10 mg	Gastro-resistant granules for oral suspension, sachet	Methacrylic acid – ethyl acrylate copolymer (1:1) 30% dispersion	AstraZeneca
Emozul	20 mg	Gastro-resistant pellet-enclosed capsule	Methacrylic acid – ethyl acrylate copolymer (1:1) 30% dispersion	Consilient Health Ltd
Actavis	20 mg	Dispersible tablet containing gastro-resistant pellets (MUPS tablet*)	Methacrylic acid – ethyl acrylate copolymer (1:1) 30% dispersion	Actavis Group PTC
Lansoprazole				
Actavis	15 mg	Gastro-resistant pellet-enclosed capsule	Methacrylic acid – ethyl acrylate copolymer (1:1) 30% dispersion	Actavis Group PTC
Almus	15 mg	Gastro-resistant pellet-enclosed capsule	Methacrylic acid – ethyl acrylate copolymer (1:1) 30% dispersion	Zentiva
Zoton	15 mg	Oro-dispersible tablet containing gastro-resistant microgranules (MUPS tablet*)	Methacrylic acid – ethyl acrylate copolymer (1:1) 30% dispersion; polyacrylate dispersion 30%	Pfiz er
Omeprazole				
Actavis	10 mg	Gastro-resistant pellet-enclosed capsule	Methacrylic acid – ethyl acrylate copolymer (1:1) 30% dispersion	Actavis Group PTC

Mepradec	10 mg	Tablet enclosed capsule	Hypromellose acetate succinate	Dexcel Pharma Ltd
Almus	10 mg	Gastro-resistant pellet-enclosed capsule	Methacrylic acid – ethyl acrylate copolymer (1:1) 30% dispersion	Sandoz Ltd
Losec caps	10 mg	Gastro-resistant pellet-enclosed capsule	Methacrylic acid – ethyl acrylate copolymer (1:1) 30% dispersion	AstraZeneca
Losec MUPS	10 mg	Dispersible tablet containing gastro-resistant pellets (MUPS tablet*)	Methacrylic acid – ethyl acrylate copolymer (1:1) 30% dispersion	AstraZeneca
Mezzopram	10 mg	Dispersible tablet containing gastro-resistant pellets (MUPS tablet*)	Methacrylic acid – ethyl acrylate copolymer (1:1) 30% dispersion	Sandoz Ltd

*MUPS: Multiple-Unit Pellet System

3. Results

Particle sizes of the multiparticulate-based products vary substantially among the tested preparations (Table 2). The omeprazole product Losec MUPS had smallest particle size with over 70% particles in the range of 180-250 μm. All pellet-enclosed capsule formulations had larger particle sizes compared to the tablet forms or sachet formulations. The majority of pellet-enclosed capsules contained pellets with a particle size larger than 1mm.

Table 2. Particle size of multiparticulate products

Brand name	Formulation	Particle size	
		Sieve method, μm (% weight)	Laser diffraction (X_{50}), μm
Esomeprozole			
Emozul	Pellet-enclosed capsule	1400 - 2000 (100%)	n/a
Nexium	Granules (sachet)	n/a	648
Actavis	MUPS tablet	500 - 710 (21%); 355 - 500 (75.23%); 250 - 355 (3.77%)	494
Lansoprazole			
Actavis	Pellet-enclosed capsule	1000 -1400 (83.63%); 710 - 1000 (16.37%)	n/a
Almus	Pellet-enclosed capsule	1000 -1400 (7.65%) 710 -1000 (92.35%)	n/a
Zoton	Oro-dispersible tablet (MUPS tablet)	n/a	352
Omeprazole			

Almus	Pellet-enclosed capsule	1400 - 2000 (52.56%); 1000 - 1400 (45.67%); 710 - 1000 (1.77%)	n/a
Actavis	Pellet-enclosed capsule	1400 - 2000 (13.73%); 1000 - 1400 (86.27%)	n/a
Losec caps	Pellet-enclosed capsule	1400 - 2000 (8.65%); 1000 - 1400 (91.35%)	n/a
Mezzopram	MUPS tablet	500 - 710 (95.21%); 335 - 500 (4.79%)	n/a
Losec MUPS	MUPS tablet	335 - 500 (5.45%); 250 - 335 (26.96%); 180 - 250 (73.04%)	n/a

n/a: not applied

The buffer capacities of the pH 4.5 phosphate buffer (acid stage), pH 6.8 phosphate buffer and pH 6.8 *m*Hanks buffer were 0.88 ± 0.10, 23.21 ± 0.07 and 3.38 ± 0.15 mmol/L/ΔpH respectively. Drug release profiles from commercial products containing esomeprazole, lansoprazole and omeprazole are presented in Figures 1-3. For all products tested, a significant difference ($p<0.005$) was found between the dissolution profiles of each product in pH 6.8 phosphate buffer and in pH 6.8 *m*Hanks buffer (Figures 1-3). The dissolution profiles between different brands of the same active ingredient (esomeprazole, lansoprazole or omeprazole) in pH 6.8 phosphate buffer did not show significant differences (Figures 1a, 2a and 3a). In pH 6.8 *m*Hanks buffer, the dissolution profiles of the three esomeprazole products were found to be significantly different from each other ($p<0.005$) (Figure 1b). For lansoprazole products tested in pH 6.8 *m*Hanks buffer, the dissolution profile of the brand Zoton is significantly different from the other two brands (Almus and Actavis) ($p<0.005$); however, the dissolution profiles of the brands Almus and Actavis did not show a significant difference (Figure 2b). The dissolution profile of brand Almus containing

omeprazole in pH 6.8 *m*Hanks buffer did not show a significant difference from the brands Losec and Mepradec containing the same drug (Figure 3b). All other dissolution profiles of omeprazole products in pH 6.8 *m*Hanks buffer as shown in Figure 3b were significantly different from each other ($p<0.005$).

The drug release lag times, t_{80} and release rates (k) are shown in Tables 3 and 4. Drug release from all tested products was immediate (lag times between 5 to 10 minutes) in pH 6.8 phosphate buffer with t_{80} ranging from 5.8 ± 2.0 to 37.5 ± 6.1 minutes, complying pharmacopoeia requirements (e.g. 80% drug release in 45 minutes as specified in British Pharmacopoeia (British Pharmacopoeia Commission, 2014)). A substantial delay in the onset time of drug release (t_{lag} ranging from 20.8 ± 2.0 to 53.3 ± 2.6 minutes) was observed in all tested products in pH 6.8 *m*Hanks buffer. Furthermore, only one product, omeprazole Losec caps, had more than 80% drug release within 45 minutes (t_{80} 44.2 ± 2.0 minutes). All other enteric coated products had less than 80% drug release within 45 minutes (t_{80} ranging from 45.8 ± 2.0 to 92.5 ± 5.2 minutes) in pH 6.8 *m*Hanks buffer and therefore failed the pharmacopeia requirement.

Table 3. The t_{lag} (minute), t_{80} (minute) and release rate (k, % release/minute) from esomeprazole and lansoprazole products in pH 6.8 phosphate and *m*Hanks buffers. The t_{lag} and t_{80} are presented as post-acid exposure times (excluding the time period in acid stage).

Buffer solution (pH 6.8)	Esomeprazole									Lansoprazole								
	Actavis			Emozul			Nexium			Actavis			Almus			Zoton		
	t_{lag}	t_{80}	k	t_{lag}	t_{80}	k	t_{lag}	t_{80}	k	t_{lag}	t_{80}	k	t_{lag}	t_{80}	k	t_{lag}	t_{80}	k
Phosphate	5.0 ± 0.0	25.0 ± 0.0	4.0 ± 0.2	10.0 ± 0.0	25.0 ± 0.0	4.9 ± 0.1	10.0 ± 0.0	43.3 ± 6.1	2.8 ± 0.2	5.0 ± 0.0	20.0 ± 0.0	3.2 ± 0.1	5.0 ± 0.0	16.7 ± 2.6	6.9 ± 0.2	5.0 ± 0.0	5.8 ± 2.0	17.3 ± 1.0
*m*Hanks	21.7 ± 2.6	60.8 ± 2.0	2.5 ± 0.4	53.3 ± 2.6	75.8 ± 2.0	3.3 ± 0.4	46.7 ± 5.2	75.0 ± 5.5	3.3 ± 0.5	20.8 ± 2.0	51.7 ± 5.2	3.0 ± 0.4	30.0 ± 0.0	47.5 ± 2.7	5.4 ± 0.4	31.7 ± 2.6	56.7 ± 6.1	2.9 ± 0.6

Table 4. The t_{lag} (minute), t_{80} (minute) and release rate (% release/minute) from omeprazole products in pH 6.8 phosphate and *m*Hanks buffers. The t_{lag} and t_{80} are presented as post-acid exposure times (excluding the time period in acid stage).

Buffer solution (pH 6.8)	Actavis			Mepradec			Almus			Losec caps			Losec MUPS			Mezzopram		
	t_{lag}	t_{80}	k	t_{lag}	t_{80}	k	t_{lag}	t_{80}	k	t_{lag}	t_{80}	k	t_{lag}	t_{80}	k	t_{lag}	t_{80}	k
Phosphate	5.0 ± 0.0	25.0 ± 0.0	4.1 ± 0.2	5.0 ± 0.0	20.0 ± 0.0	3.6 ± 0.7	10.0 ± 0.0	20.0 ± 0.0	7.2 ± 0.8	7.5 ± 2.7	12.5 ± 2.7	14.7 ± 0.8	5.0 ± 0.0	31.7 ± 4.1	3.0 ± 0.4	5.0 ± 0.0	37.5 ± 6.1	3.3 ± 0.3
*m*Hanks	41.7 ± 2.6	64.2 ± 2.0	4.3 ± 0.2	30.8 ± 2.0	54.2 ± 6.6	3.6 ± 0.7	30.8 ± 2.0	45.8 ± 2.0	4.0 ± 0.6	27.5 ± 2.7	44.2 ± 2.0	5.0 ± 0.5	35.8 ± 2.0	67.5 ± 5.2	2.7 ± 0.7	21.7 ± 14.4	92.5 ± 5.2	1.9 ± 0.1

4. Discussion

Physiological bicarbonate buffers have been previously proven to be more realistic dissolution media compared to compendial phosphate buffers and provide better discrimination between enteric coated drug delivery systems (V. C. Ibekwe et al., 2006; F. Liu et al., 2010). The current study is the first to apply bicarbonate buffer to compare *in vitro* dissolution performances of commercially available enteric coated multiparticulate products targeting to the small intestine. In agreement with previous reports, compendial pH 6.8 phosphate buffer failed to distinguish dissolution profiles between different enteric coated multiparticulate PPI products. However, these products showed significantly different dissolution profiles in the pH 6.8 *m*Hanks bicarbonate media in most cases. In addition, drug release from the enteric coated products was immediate and rapid in pH 6.8 phosphate buffer. However, marked delay in drug release onset time was noted in the more realistic pH 6.8 *m*Hanks buffer which simulates closely the human jejunal fluid. For most products tested, except Nexium (esomeprazole), Actavis (omeprazole) and Mepradec (omeprazole), drug release rates (k) were slower in the bicarbonate buffer compared to the phosphate buffer.

Drug release from enteric coated products is determined by the dissolution of the carboxylic acid polymers used for the coating which, in turn, is determined by the ionization of the polymers in aqueous solutions. The presence of various ions and buffer species in the dissolution media profoundly influences the ionization rate of the polymer (J. Spitael and R. Kinget, 1977). According to Bronsted catalysis law, the dissolution rate of a carboxylic acid polymer is directly proportional to the p*K*a of the buffer specie in the solution (J. Spitael et al., 1980). Buffer capacity of the salt which

links to its p*K*a also plays a role in the acidic polymer dissolution (E. Shek, 1978). In addition, the composition and strength of other ions present in the solution have an effect on the polymer dissociation (F. Liu et al., 2010). The p*K*a and buffer capacity are distinct between pH 6.8 phosphate buffer (p*K*a 7.19, buffer capacity 23.1 ± 0.3 mmol/L/ΔpH) and pH 6.8 bicarbonate buffer (p*K*a 6.31, buffer capacity 3.1 ± 0.2 mmol/L/ΔpH) (F. Liu et al., 2010). The ionic composition and strength of the two dissolution media are also remarkably different. All these factors explain the differences in dissolution profiles of enteric coated systems in the phosphate buffer and the bicarbonate buffer.

Drug release from a core surrounded by an enteric coating is governed by two dynamic and simultaneous processes, the dissolution of the enteric polymer and the diffusion of the drug through the dissolving polymer layer. Ozturk et al (1988) described that as the enteric polymer dissolves, the thickness of the polymer layer reduces and the release rate of the drug increases over time. For enteric coated tablets, tablet disintegration is presumed to coincide with 95% completion of the polymer dissolution and the subsequent drug release is dependent on the dissolution of the drug from the disintegrated tablet core (S. S. Ozturk et al., 1988). The enteric coated multiparticulates used in this study are based on microcrystalline cellulose or sugar spheres as core materials which do not show typical disintegration as shown in tablets. It can be speculated that the onset time of drug release from coated pellets is mostly affected by the dissolution rate of the enteric polymer in the aqueous medium. The drug release rate (k) at the linear plot of the dissolution graph and the complete release of the drug can be affected by both the dissolution of the polymer and the release of the drug from the core.

Previous reports have compared the dissolution of enteric coated systems applying different enteric polymers in bicarbonate buffer (F. Liu et al., 2010). Their distinct dissolution profiles were explained by differences in the polymer p*K*a and chemical structure, such as the aqueous solubility of the polymer backbones and the degree of substitution (M. Davis et al., 1986; S. S. Ozturk et al., 1988). However, the commercial enteric coated products used in this study are all (except Mepradec) based on the same enteric polymer, methacrylic acid – ethyl acrylate copolymer (1:1) 30% aqueous dispersion (commercially available as Eudragit® L 30D-55), according to their Summary of Product Characteristics (SPC) (Datapharm Communications Limited, 2013). The enteric polymer used in Mepradec (omeprazole) is hypromellose acetate succinate (HPMCAS) which is also commonly used in aqueous based coating systems. Previous study showed that when HPMCAS was applied at a coating level providing sufficient acid-resistance, drug release from coated tablets was comparable to that from tablets coated with Eudragit® L 30D-55 in bicarbonate buffer (F. Liu et al., 2010). As such, the difference in drug release between the products investigated in this study indicates that formulation factors other than the enteric polymer could play a role in determining the dissolution of the coating and the resultant drug release.

Particle size of the multiparticulate-based products ranges from around 250 μm to 2000 μm, which might affect the dissolution of the polymer and drug release due to available surface area in contact with aqueous media. The rank order of drug release for esomeprazole products (Actavis < Nexium < Emozul) in pH 6.8 *m*Hanks buffer correlated well with their particle sizes (Actavis < Nexium < Emozul); the product with the smallest particle size showed fastest drug release. The rank order was

obtained by comparing the drug release onset time between the products. If two products have the same onset time, the release rate was then compared. This relationship between drug release rank order and particle size was not observed for lansoprazole and omeprazole products. The rank order of drug release for omeprazole products is Mezzopram < Losec Caps < Almus < Mepradec (tablet) < Losec MUPS < Actavis, which shows no relationship with the rank order of particle size, Losec MUPS < Mezzopram < Actavis ≈ Losec Caps ≈ Almus < Mepradec (tablet). For lansoprazole products, the brand Zoton showed slowest drug release profile in pH 6.8 *m*Hanks buffer despite that it has the smallest particle size. Zoton (lansoprazole) is an oro-dispersible tablet containing gastro-resistant microgranules. The SPC of this product suggests that other than methacrylic acid copolymer a second polymer "polyacrylate dispersion 30 percent" was used for the coating (Datapharm Communications Limited, 2013). Although the grade of the polyacrylate polymer was not specified, it has been reported that ethyl acrylate-methyl methacrylate copolymer 30% dispersion (Eudragit® NE 30D) was used in addition to Eudragit® L 30D-55 in developing gastric-resistant PPI formulations (T. Shimizu et al., 2003; R. N. Tirpude and P. K. Puranik, 2011). Eudragit® NE 30D is insoluble in water independent of pH and is used for sustained release film coatings. This polymer has a very low glass transition temperature of 13°C and can be used in polymer blends with other acrylic polymers to decrease glass transition temperature and thus increase film flexibility (Y. El-Malah and S. Nazzal, 2008; S. Kucera et al., 2009). This is beneficial during the compression of gastric-resistant microgranules into oro-dispersible tablets to prevent film cracking. However, the presence of the insoluble Eudragit® NE 30D in the enteric coating could decrease drug release rate and may be the reason for the slower release as observed in Zoton compared to other lansoprazole products.

It is interesting to note that the omeprazole product Losec Caps showed significantly faster drug release in pH 6.8 *m*Hanks buffer than Losec MUPS (omeprazole) despite having larger particle size and being produced by the same manufacturer. Losec MUPS tablet comprise omeprazole enteric coated microgranules which are compressed into orally disintegrating tablets. To ensure that the coated microgranules are able to withstand the compression into tablets, it is likely that thicker coatings or extra coating layers are required to maintain sufficient gastric-resistance. It has been reported that the enteric-coated microgranules in lansoprazole orally disintegrating tablets comprise seven layers, a core, an under-coating layer, three layers of enteric-coating and an over-coating layer to improve stability, reduce damage during compression and neutralize the taste of the microgranules (F. Baldi and P. Malfertheiner, 2003). This may explain the longer onset time for drug release and slower drug release rate in *m*Hanks buffer from Losec MUPS compared to Losec Caps.

Coating thickness could be a factor affecting drug release rate from different enteric coated products. When methacrylic acid copolymer was applied onto prednisolone-loaded pellets (1mm in diameter), dissolution was significantly faster from pellets obtained 15% weight gain from the coating compared to those with 20% weight gain (data not shown). The inclusion of other formulation additives could also influence drug release. For example, the type and amount of plasticizers used in the coating formulation affect the mechanical properties of the coating during dissolution and the drug release rate (H. M. Fadda et al., 2008). It is likely that drug release from the final coated product is determined by the interplay of the different factors. An investigation

of the individual contribution to drug release by these factors would be of interest for future research.

Significant lag times in drug release from enteric coated tablets in physiological bicarbonate buffers were reported previously, which correlates to the reported delay in disintegration times of enteric coated tablets in the human intestine (C. Bogentoft et al., 1984; J. P. Ebel et al., 1993; F. Liu et al., 2010). Results in this study show that a similar delay in drug release occurred in bicarbonate buffer from enteric coated multiparticulate PPI products, intended for use in patients who are unable to swallow intact tablets especially children, older patients and patients with swallowing difficulties. The *in vivo* dissolution of enteric coatings and the resultant drug release from coated products are determined by physiological factors of the gastrointestinal tract such as gastric emptying and the pH, volume, ionic composition, and buffer capacity of the intestinal fluids. Currently there is a lack of knowledge in these physiological factors in children especially in the very young age groups such as neonates and infants (H. K. Batchelor et al., 2014). For example, small intestinal pH was reported to be comparable to adults in older children (8-14 years old); however, no data available for younger age groups (J. L. Kaye, 2011). It is therefore unclear how these physiological factors affect the dissolution of enteric coated products in children. However, the available fluid volume for dissolution is significantly lower in the intestine of young children than in that of adults (H. K. Batchelor et al., 2013). It can be speculated that the observed delay in drug release from these products in *m*Hanks buffer is likely to result in delayed dissolution *in vivo* in paediatric patients. There are indeed reports of unabsorbed enteric coated omeprazole pellets in the gastric contents or stool of infants (C. Tuleu et al., 2008). A study reported that half of

395 the critically ill pediatric patients who received nasogastric administration of
396 omeprazole suspensions either did not respond to the treatment or required significant
397 dose titration to achieve gastric acid suppression (J. A. Haizlip et al., 2005).
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399 The potential delay in *in vivo* drug release from enteric-coated PPI products reflects
400 literature reports on the slow exertion of their maximum antisecretory effects. Suzuki
401 et al. reported that it took significantly longer time to reach a gastric pH of 3
402 following lansoprazole administration (3.75 ± 0.48 hours) compared to that following
403 famotidine administration (2.24 ± 0.51 hours) (T. Suzuki et al., 2008). In another
404 study, the mean gastric pH increased to above 4 within 15 minutes after the
405 administration of immediate release omeprazole (containing non-enteric coated
406 omeprazole stabilised using sodium bicarbonate) (P. O. Katz et al., 2007). In contrast,
407 mean gastric pH did not reach 4 until 3 hours after the administration of enteric coated
408 esomeprazole and more than 5 hours after the dosing of enteric coated lansoprazole.
409 This is in agreement with reported delays in *in vivo* drug absorption from enteric
410 coated PPI products. Boussery et al. showed that the time to reach maximum plasma
411 drug concentration (t_{max}) was 0.57 ± 0.16 and 2.36 ± 1.74 hours for immediate release
412 omeprazole suspensions and enteric coated omeprazole MUPS tablets respectively in
413 patients with severe neurodevelopmental problems (K. Boussery et al., 2011). In
414 addition, the MUPS tablets showed high inter-individual variation in reaching t_{max}
415 (ranging from 1 to 6 hours). Similar results were shown in another study conducted in
416 healthy volunteers in both fasted and fed conditions (Z. Liu et al., 2013). This is better
417 reflected by the *in vitro* drug release results in pH 6.8 mHanks buffer than phosphate
418 buffer, as the former reveals the difference in drug release profiles between the tested
419 PPI products as well as the variation in drug release in the six dissolution test

repetitions of the same product (as shown by the higher standard deviation in t_{lag}, t_{80} and release rate).

To gain an understanding of the correlation between the *in vitro* dissolution results and *in vivo* absorption parameters of enteric coated products, one must take into account of gastric emptying time of these preparations. Unlike tablets, pellets empty from the stomach in consecutive portions over a period of time (J. M. Newton, 2010). Marked intra- and inter-individual variability in the gastric emptying kinetics of pellets has been reported even under fasting conditions, with emptying time varying from 15 minutes to more than 3 hours (I. Locatelli et al., 2009). Locatelli et al have attempted to develop a mathematical model to described gastric emptying of pellets under fasting conditions and have suggested an overall mean value of approximately 40 minutes to guide the development of *in vitro* dissolution methods (I. Locatelli et al., 2009). Since previously reported pharmacokinetic profiles of enteric coated PPI multiparticulate products do not provide gastric emptying values, an attempt is made to add the suggested average gastric emptying time to the t_{80} of drug release in pH 6.8 phosphate and *m*Hanks buffer and compared to reported *in vivo* t_{max} values under fasting conditions. The reported *in vivo* t_{max} values for enteric coated esomeprazole multiparticulate preparations range from 1.3 to 2.0 hours (N. Bladh et al., 2007; M. B. Sostek et al., 2003). The *in vitro* drug release t_{80} values (including a mean gastric emptying time of 40 minutes) of esomeprazole products are 1.1-1.4 and 1.7-1.9 hours in pH 6.8 phosphate buffer and *m*Hanks buffer respectively. For lansoprazole products, the reported *in vivo* t_{max} values are in the range of 1.6 to1.9 hours (J. W. Freston et al., 2003; K. Iwasaki et al., 2004) and the *in vitro* t_{80} values are 0.8-1.0 and 1.5-1.6 hours in pH 6.8 phosphate buffer and *m*Hanks buffer respectively. The

445 reported *in vivo* t_{max} values for omeprazole products range from 1.9 to 4.0 hours (K.
446 Boussery et al., 2011; S. Karim et al., 2014; Z. Liu et al., 2013) and the *in vitro* t_{80}
447 values are 0.9-1.3 and 1.2-2.2 hours in pH 6.8 phosphate buffer and *m*Hanks buffer
448 respectively. A closer relationship between the t_{80} values in *m*Hanks buffer and the *in*
449 *vivo* t_{max} can be observed compared to phosphate buffer, indicating a potential for
450 improved *in vitro-in vivo* correlation. It needs to be noted that using an average gastric
451 emptying time overlooks the intra- and inter- individual variations in gastric emptying
452 of pellets and oversimplifies the complex nature of the process. A better evaluation in
453 *in vitro- in vivo* correlation of these enteric-coated multiparticulate formulations can
454 be achieved using pharmacoscintigraphy studies taking into account of gastric
455 emptying times of individual pharmacokinetic profiles.
456
457 Dynamic dissolution media based on bicarbonate buffers were repo rted recently
458 which resemble the aboral pH changes in the intestine (G. Garbacz et al., 2014; H. A.
459 Merchant et al., 2014). An average increase in drug release lag time of about 10
460 minutes was observed from enteric coated formulations in the dynamic dissolution
461 system compared to the static bicarbonate buffer used in this study. Although it is
462 apparent that the dissolution testing under dynamic pH change mode would reflect
463 better the pH gradients in the human intestine, *in vivo* such real-time pH profile varies
464 significantly inter- and intra-individually. It is impractical to echo this variation even
465 using the dynamic dissolution system. Furthermore, there is not sufficient data
466 available in the pH values relevant to intestinal transit time in children or older people
467 to support the design of a meaningful dynamic pH change for testing these products.
468 Therefore, the static pH 6.8 bicarbonate buffer is used in this study and it was able to

discriminate between different enteric coated commercial multiparticulate PPI products and revealed their inherent shortcomings of delayed drug release.

4. Conclusions

Significant delay in drug release was identified from commercial enteric coated PPI products intended for paediatric and geriatric use in pH 6.8 physiological bicarbonate (*m*Hanks) buffer. This buffer was able to discriminate between the different enteric coated multiparticulate preparations, providing a rank dissolution order. This knowledge reflects literature reports on the delay in absorption and onset of antisecretory effects of these products and is likely to improve *in vitro-in vivo* correlations. The *vitro* dissolution using the bicarbonate buffer can be a useful tool in the rational design of enteric coated PPI products to meet the needs of different patient populations.

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(a) (b)

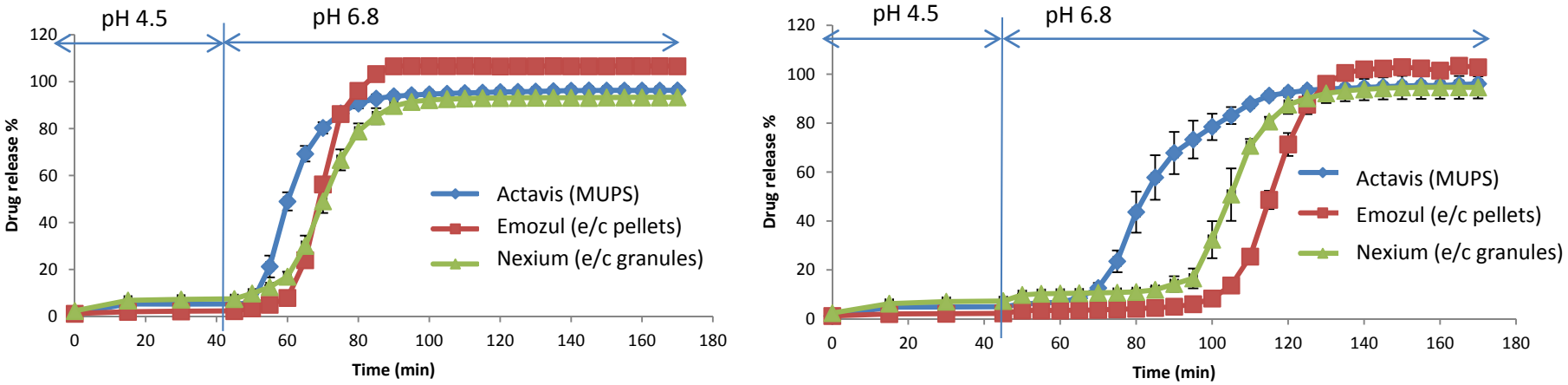
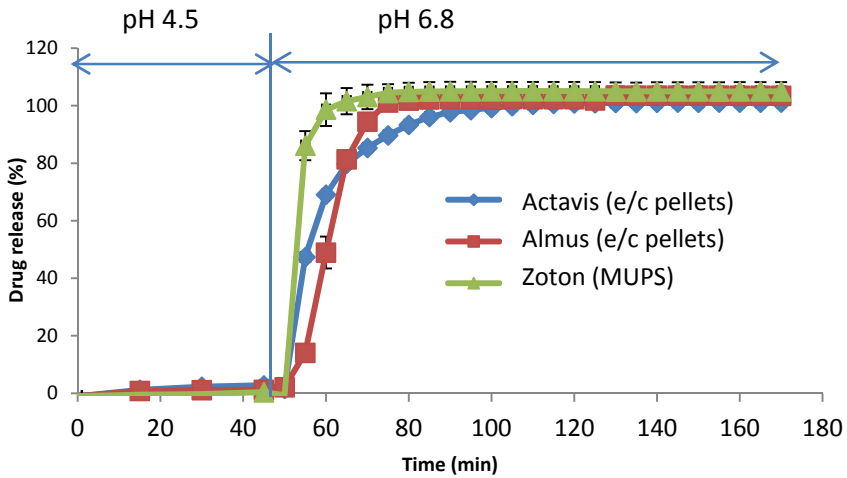


Figure 1. Drug release from esomeprazole enteric-coated products in pH 4.5 PBS (45 minutes), and subsequent pH 6.8 phosphate buffer (a) and *m*Hanks buffer (b)

a)



(b)

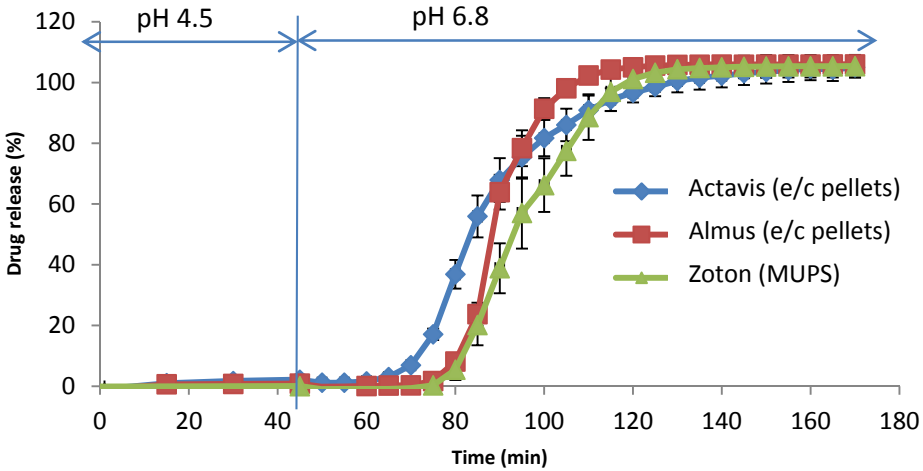


Figure 2. Drug release from lansoprazole enteric-coated products in pH 4.5 PBS (45 minutes), and subsequent pH 6.8 phosphate buffer (a) and *m*Hanks buffer (b)

(a) (b)

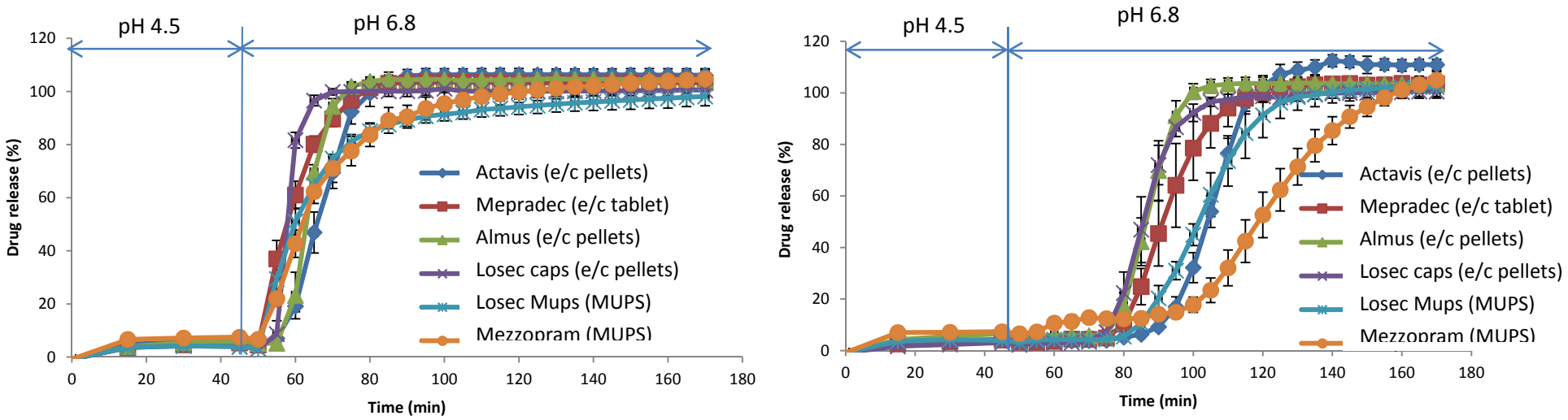


Figure 3. Drug release from omeprazole enteric-coated products in pH 4.5 PBS (45 minutes), and subsequent pH 6.8 phosphate buffer (a) and *m*Hanks buffer (b)

513 References
514
515 Baldi, F., Malfertheiner, P., 2003. Lansoprazole fast disintegrating tablet: a new
516 formulation for an established proton pump inhibitor. Digestion 67, 1-5.
517 Batchelor, H. K., Fotaki, N., Klein, S., 2014. Paediatric oral biopharmaceutics: key
518 considerations and current challenges. Advanced drug delivery reviews 73, 102-126.
519 Batchelor, H. K., Kendall, R., Desset-Brethes, S., Alex, R., Ernest, T. B., 2013.
520 Application of in vitro biopharmaceutical methods in development of immediate
521 release oral dosage forms intended for paediatric patients. Eur. J. Pharm. Biopharm. 85,
522 833-842.
523 Bladh, N., Blychert, E., Johansson, K., Backlund, A., Lundin, C., Niazi, M., Pettersson,
524 G., Fjellman, M., 2007. A new esomeprazole packet (sachet) formulation for
525 suspension: in vitro characteristics and comparative pharmacokinetics versus intact
526 capsules/tablets in healthy volunteers. Clin. Ther. 29, 640-649.
527 Bogentoft, C., Alpsten, M., Ekenved, G., 1984. Absorption of acetylsalicylic acid from
528 enteric-coated tablets in relation to gastric emptying and in-vivo disintegration. J.
529 Pharm. Pharmacol. 36, 350-351.
530 Boussery, K., De Smet, J., De Cock, P., Vande Velde, S., Mehuys, E., De Paepe, P.,
531 Remon, J. P., Van Bocxlaer, J. F., Van Winckel, M., 2011. Pharmacokinetics of two
532 formulations of omeprazole administered through a gastrostomy tube in patients with
533 severe neurodevelopmental problems. Br. J. Clin. Pharmacol. 72, 990-996.
534 British Pharmacopoeia Commission, 2014. British Pharmacopoeia Volume V
535 Appendices. Appendix XII B. Dissolution, London.
536 Chan, W. A., Boswell, C. D., Zhang, Z., 2001. Comparison of the release profiles of a
537 water soluble drug carried by Eudragit-coated capsules in different in-vitro dissolution
538 liquids. . Powder Tech 119, 26-32.
539 Datapharm Communications Limited, 2013. The electronic Medicines Compendium
540 (eMC).
541 Davis, M., Ichikawa, I., Williams, E., Banker, G., 1986. Comparison and evaluation of
542 enteric polymer properties in aqueous solutions. Int. J. Pharm. 28, 157-166.
543 Ebel, J. P., Jay, M., Beihn, R. M., 1993. An in vitro/in vivo correlation for the
544 disintegration and onset of drug release from enteric-coated pellets. Pharm. Res. 10,
545 233-238.
546 El-Malah, Y., Nazzal, S., 2008. Novel use of Eudragit NE 30D/Eudragit L 30D-55
547 blends as functional coating materials in time-delayed drug release applications. Int. J.
548 Pharm. 357, 219-227.
549 Fadda, H. M., Basit, A. W., 2005. Dissolution of pH responsive formulations in media
550 resembling intestinal fluids: bicarbonate versus phosphate buffers. J Drug Deliv. Sci.
551 Tec. 15, 273-279.
552 Fadda, H. M., Hernandez, M. C., Margetson, D. N., McAllister, S. M., Basit, A. W.,
553 Brocchini, S., Suarez, N., 2008. The molecular interactions that influence the plasticizer
554 dependent dissolution of acrylic polymer films. J. Pharm. Sci. 97, 3957-3971.
555 Fadda, H. M., Merchant, H. A., Arafat, B. T., Basit, A. W., 2009. Physiological
556 bicarbonate buffers: stabilisation and use as dissolution media for modified release
557 systems. Int. J. Pharm. 382, 56-60.
558 Freston, J. W., Chiu, Y. L., Mulford, D. J., Ballard, E. D., 2nd, 2003. Comparative
559 pharmacokinetics and safety of lansoprazole oral capsules and orally disintegrating
560 tablets in healthy subjects. Aliment. Pharmacol. Ther. 17, 361-367.

561 Garbacz, G., Kolodziej, B., Koziolek, M., Weitschies, W., Klein, S., 2014. A dynamic
562 system for the simulation of fasting luminal pH-gradients using hydrogen carbonate
563 buffers for dissolution testing of ionisable compounds. Eur. J. Pharm. Sci. 51, 224-231.
564 Haizlip, J. A., Lugo, R. A., Cash, J. J., Vernon, D. D., 2005. Failure of nasogastric
565 omeprazole suspension in pediatric intensive care patients. Pediatr. Crit. Care Med. 6,
566 182-187.
567 Hanks, J. H., 1975. Hanks' balanced salt solution and pH control. TCA manual/Tissue
568 culture Association 1, 3-4.
569 Hata, S., Arai, M., Maruoka, D., Tanaka, T., Matsumura, T., Suzuki, T., Nakagawa, T.,
570 Katsuno, T., Imazeki, F., Yokosuka, O., 2013. Intragastric acidity during the first day
571 following administration of low-dose proton pump inhibitors: a randomized crossover
572 study. Clinics and research in hepatology and gastroenterology 37, 296-301.
573 Hepburn, B., Goldlust, B., 2003. Comparative effects of an omeprazole antacid
574 complex-immediate release (OACIR) and omeprazole delayed-release (OME-DR) on
575 omeprazole pharmacolinetics and gastric pH in healthy subjects. Gastroenterology 124,
576 A228 (Abstract).
577 Horn, J. R., Howden, C. W., 2005. Review article: similarities and differences among
578 delayed-release proton-pump inhibitor formulations. Aliment. Pharmacol. Ther. 22
579 Suppl 3, 20-24.
580 Ibekwe, V. C., Fadda, H. M., Parsons, G. E., Basit, A. W., 2006. A comparative in vitro
581 assessment of the drug release performance of pH-responsive polymers for ileo-colonic
582 delivery. Int J Pharm 308, 52-60.
583 Iwasaki, K., Yoshikawa, Y., Shibata, N., Takada, K., Sakurai, Y., Takagi, N., Irie, S.,
584 Nakamura, K., 2004. Evaluation of fast disintegrating lansoprazole tablet in human
585 subjects. Drug Metab. Pharmacokinet. 19, 227-235.
586 Karim, S., Hay, Y. K., Baie, S. H., Bukhari, N. I., Murtaza, G., 2014. Study of
587 comparative bioavailability of omeprazole pellets. Acta Pol. Pharm. 71, 463-468.
588 Katz, P. O., Koch, F. K., Ballard, E. D., Bagin, R. G., Gautille, T. C., Checani, G. C.,
589 Hogan, D. L., Pratha, V. S., 2007. Comparison of the effects of immediate-release
590 omeprazole oral suspension, delayed-release lansoprazole capsules and delayed-release
591 esomeprazole capsules on nocturnal gastric acidity after bedtime dosing in patients with
592 night-time GERD symptoms. Aliment. Pharmacol. Ther. 25, 197-205.
593 Kaye, J. L., 2011. Review of paediatric gastrointestinal physiology data relevant to oral
594 drug delivery. Int. J. Clin. Pharm. 33, 20-24.
595 Kucera, S., Shah, N. H., Malick, A. W., Infeld, M. H., McGinity, J. W., 2009. Influence
596 of an acrylic polymer blend on the physical stability of film-coated theophylline pellets.
597 AAPS PharmSciTech 10, 864-871.
598 Liu, F., Merchant, H. A., Kulkarnia, R. P., Alkademia, M., Basit, A. W., 2010.
599 Evolution of physiological pH 6.8 bicarbonate buffer systems: application to the
600 dissolution testing of enteric products. Eur. J. Pharm. Biopharm. 78, 151-157.
601 Liu, Z., Ding, L., Zhong, S., Cao, X., Jiang, L., Duan, H., 2013. Pharmacokinetics of a
602 new immediate-release compound omeprazole capsule and its comparison with the
603 enteric-coated formulation under fasting and fed conditions. Drug research 63, 370-
604 375.
605 Locatelli, I., Mrhar, A., Bogataj, M., 2009. Gastric emptying of pellets under fasting
606 conditions: a mathematical model. Pharm. Res. 26, 1607-1617.
607 Merchant, H. A., Goyanes, A., Parashar, N., Basit, A. W., 2014. Predicting the
608 gastrointestinal behaviour of modified-release products: Utility of a novel dynamic
609 dissolution test apparatus involving the use of bicarbonate buffers. Int. J. Pharm. 475,
610 585-591.

611 Metz, D. C., Amer, F., Hunt, B., Vakily, M., Kukulka, M. J., Samra, N., 2006.
612 Lansoprazole regimens that sustain intragastric pH > 6.0: an evaluation of intermittent
613 oral and continuous intravenous infusion dosages. Aliment. Pharmacol. Ther. 23, 985-
614 995.
615 Newton, J. M., 2010. Gastric emptying of multi-particulate dosage forms. Int. J. Pharm.
616 395, 2-8.
617 Ozturk, S. S., Palsson, B. O., Donohoe, B., Dressman, J. B., 1988. Kinetics of release
618 from enteric-coated tablets. Pharm. Res. 5, 550-565.
619 Shek, E., 1978. Buffer capacity, not buffer catalysis, affects the dissolution rate of
620 cellulose acetate phthalate. Pharm. Ind. 40, 981-982.
621 Shimizu, T., Kameoka, N., Iki, H., Tabata, T., Hamaguchi, N., Igari, Y., 2003.
622 Formulation study for lansoprazole fast-disintegrating tablet. II. Effect of triethyl citrate
623 on the quality of the products. Chem. Pharm. Bull. (Tokyo) 51, 1029-1035.
624 Sostek, M. B., Chen, Y., Skammer, W., Winter, H., Zhao, J., Andersson, T., 2003.
625 Esomeprazole administered through a nasogastric tube provides bioavailability similar
626 to oral dosing. Aliment. Pharmacol. Ther. 18, 581-586.
627 Spitael, J., Kinget, R., 1977. Factors affecting the dissolution rate of enteric coatings.
628 Pharm. Ind. 39, 502-505.
629 Spitael, J., Kinget, R., Naessens, K., 1980. Dissolution rate of cellulose acetate
630 phthalate and the Bronsted catalysis law. Pharm. Ind. 42, 846.
631 Suzuki, T., Yamaguchi, T., Odaka, T., Kobayashi, M., Seza, A., Kouzu, T., Yokosuka,
632 O., 2008. Four-day continuous gastric pH monitoring following anti-acid secretory
633 drug administration: cross-over test to assess the early effects. Aliment. Pharmacol.
634 Ther. 27, 66-71.
635 Tirpude, R. N., Puranik, P. K., 2011. Rabeprazole sodium delayed-release
636 multiparticulates: Effect of enteric coating layers on product performance. J. Adv.
637 Pharm. Technol. Res. 2, 184-191.
638 Tuleu, C., Arenas-Lopez, S., Robinson, C., McCarthy, D., Paget, R. I., Tibby, S.,
639 Taylor, K. M., 2008. 'Poppy seeds' in stomach aspirates: is oral omeprazole
640 extemporaneous dispersion bioavailable? Eur. J. Pediatr. 167, 823-825.
641 Welage, L. S., 2003. Pharmacologic properties of proton pump inhibitors.
642 Pharmacotherapy 23, 74S-80S.
643 Wilding, I. R., Coupe, A. J., Davis, S. S., 2001. The role of gamma-scintigraphy in oral
644 drug delivery. Advanced drug delivery reviews 46, 103-124.
645

646