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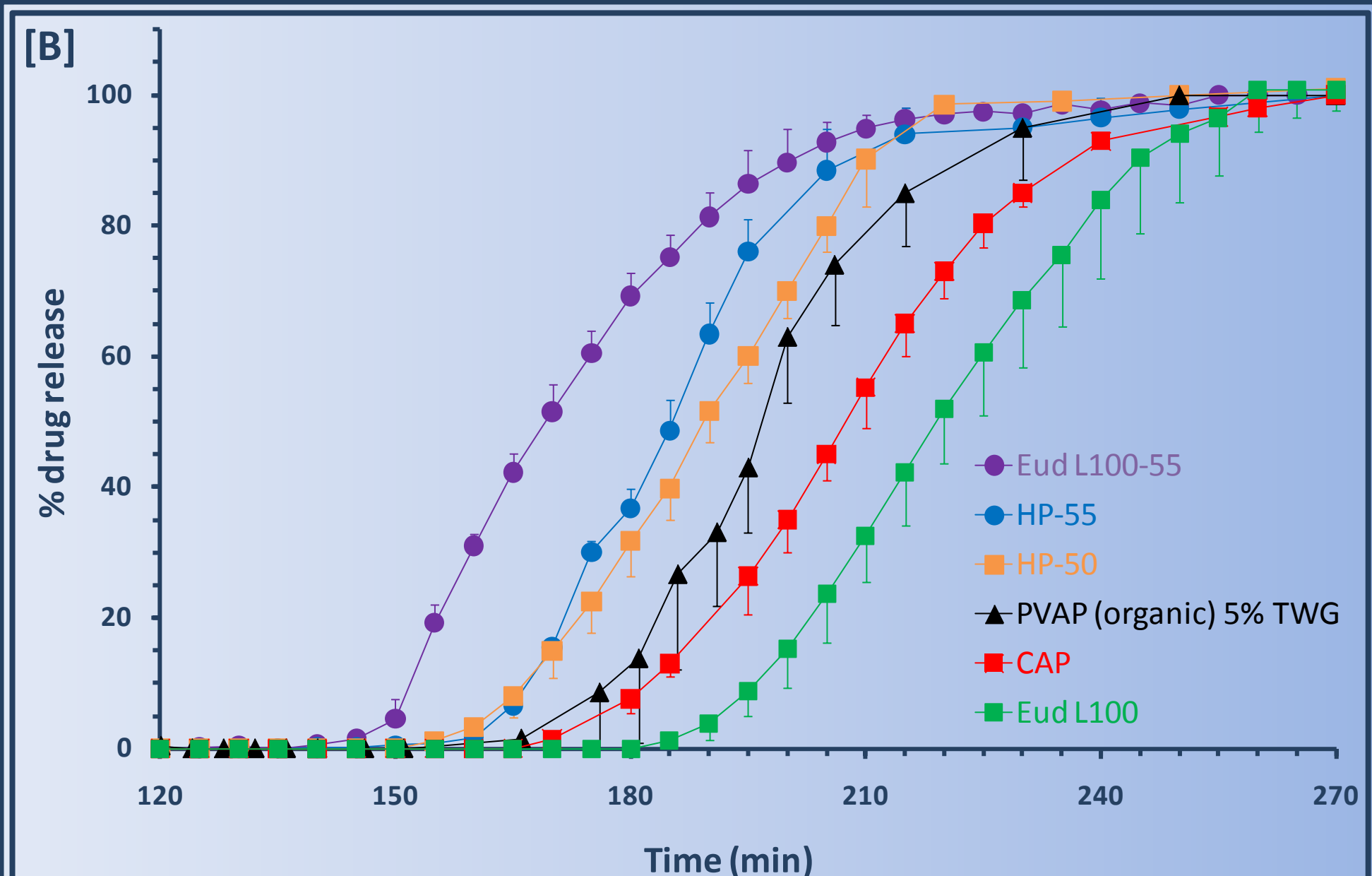
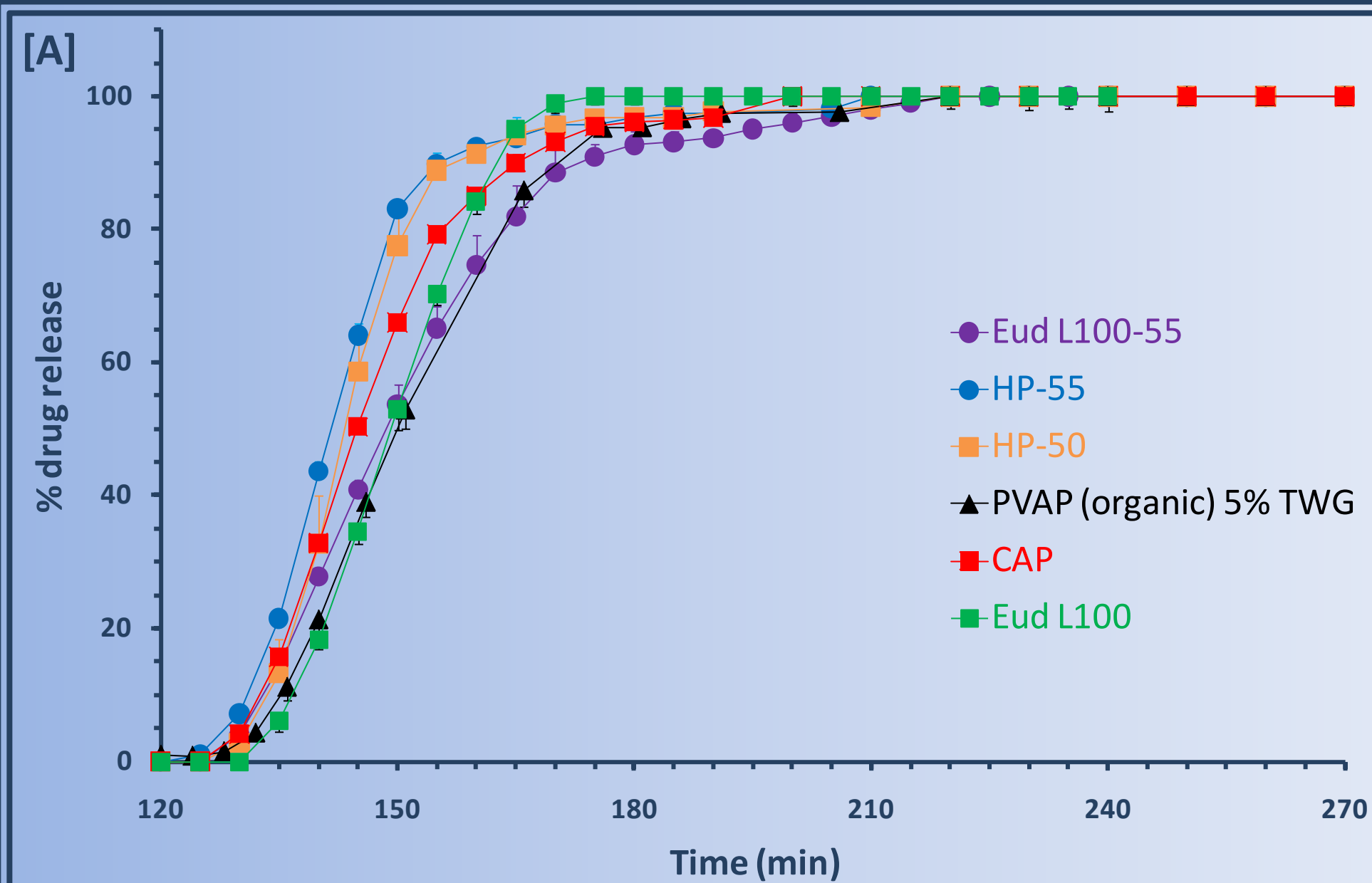
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Realistic and discriminative in vitro release in pH 6.8 [B] physiological bicarbonate buffer compared to [A] compendial phosphate buffer for enteric coated formulations

Evolution of physiological pH 6.8 bicarbonate buffer systems: application to the dissolution testing of enteric products

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Abstract

The use of pH 6.8 compendial phosphate buffer to assess the release of enteric coated products gives rise to poor *in vitro in vivo* correlations because of the inadequacy of the buffer to resemble small intestinal fluids. A more representative and physiological medium, pH 6.8 bicarbonate buffer, was developed here to evaluate the dissolution behaviour of enteric coatings. The bicarbonate system was evolved from pH 7.4 Hanks balanced salt solution to produce a pH 6.8 bicarbonate buffer (modified Hanks buffer, *mHanks*), which resembles the ionic composition and buffer capacity of intestinal fluid. Tablets containing prednisolone were coated with a range of enteric polymers: hypromellose phthalate (HP-50 and HP-55), cellulose acetate phthalate (CAP), hypromellose acetate succinate (HPMCAS-LF and HPMCAS-MF), methacrylic acid copolymers (EUDRAGIT® L100-55, EUDRAGIT® L30D-55 and EUDRAGIT® L100) and polyvinyl acetate phthalate (PVAP). Dissolution of coated tablets was carried out using USP-II apparatus in 0.1 M HCl for 2 h, and subsequently pH 6.8 phosphate buffer or pH 6.8 *mHanks* bicarbonate buffer. In pH 6.8 phosphate buffer, the various enteric polymer coats displayed rapid and comparable dissolution profiles. In pH 6.8 *mHanks* buffer, drug release was slow and marked differences were observed between the various coatings, which is comparable to the reported delayed disintegration times for enteric coated products in the small intestine. In summary, the use of pH 6.8 physiological bicarbonate buffer (*mHanks*) provides more realistic and discriminative *in vitro* release assessment for enteric coated formulations compared to compendial phosphate buffer.

Key words: pH-sensitive polymers; enteric polymers; enteric coatings; modified release; physiological buffers; bicarbonate media; biorelevant dissolution

1. Introduction

The application of an enteric coating to a solid dosage form is a well established approach to prevent drug release in the stomach and allow release in the small intestine. It is used to preclude the degradation of acid-labile actives in the gastric environment or to protect the stomach from irritant compounds [1]. The commonly used enteric coatings employ pH-dependent polymers which contain carboxylic groups. These remain un-ionized in the low pH environment of the stomach, and become ionized in the higher pH conditions of the small intestine, thus allowing the dissolution of the coating and drug release.

The *in vitro* dissolution performance of enteric coatings is usually assessed in compendial pH 6.8 phosphate buffer. In this medium, drug release is typically rapid [3, 6, 9, 19]. However, neither does this reflect the *in vivo* performance of enteric coated products, nor it is sufficient to discriminate the dissolution behaviour between different enteric coatings. *In vivo* gamma scintigraphy studies have shown that there is a substantial time delay (up to 2 h) for such products to disintegrate in the human small intestine post gastric emptying, with different enteric polymer coatings exhibiting varying disintegration times [4, 8, 12, 20, 39]. This *in vitro-in vivo* discrepancy is not surprising considering the inadequacy of the *in vitro* dissolution medium to resemble the luminal fluid of the small intestine in many respects such as ionic composition, buffer capacity, viscosity and volume [2, 11, 18, 25, 26, 33].

The constituent buffer salts, ionic strength and buffer capacity of the dissolution media have been reported to influence drug release from pH-responsive polymer coated dosage forms [7, 15, 22, 26]. Notably, the luminal fluids of the small intestine are buffered by bicarbonate and phosphate levels are very low. Hence, bicarbonate buffers more closely resemble the

environment within the small intestine and would provide a more physiological media for the *in vitro* assessment of enteric products.

We have shown that a pH 7.4 bicarbonate system (Krebs buffer), which simulates the luminal environment of the distal small intestine, provided better *in vitro-in vivo* correlations for a series of enteric coated products for delivery of mesalazine to the ileo-colonic region of the gastrointestinal tract [17]. Conventional bicarbonate buffers are “stable” at a pH of 7.4, however this pH is typically higher than the pH in the proximal small intestine [14]. The objective of this study was to develop a pH 6.8 bicarbonate system, based on Hanks buffer. This physiological medium was then employed to evaluate the dissolution behaviour of a series of enteric polymers from different chemical classes.

2. Materials and Methods

2.1 Materials

The enteric polymers used in this study and their properties are listed in Table 1. Prednisolone was purchased from Aventis Pharma., Antony, France. Lactose (Pharmatose) was obtained from Ellis & Everard, Essex, UK. Cross-linked sodium carboxymethylcellulose was donated by FMC International, Cork, Ireland. Polyvinylpyrrolidone 40,000 was purchased from VWR International Ltd, Poole, UK. Magnesium stearate was purchased from Sigma-Aldrich Co. Ltd., Dorset, UK. Triethyl citrate was obtained from Lancaster Synthesis, Lancashire, UK. Sodium lauryl sulphate and triacetin were sourced from Sigma-Aldrich Co. Ltd., Dorset, UK. Talc (fine powder) was purchased from VWR International Ltd, Poole, UK. Organic solvents used were of analytical grade and were obtained from VWR International Ltd, Poole, UK (ethanol) and Fisher Scientific UK Ltd, Loughborough, UK (acetone and

isopropanol). Salts for preparing buffer solutions were obtained from VWR International Ltd, Poole, UK.

2.2 Preparation of prednisolone tablets

Tablets were prepared containing 5% prednisolone, 88.5% lactose, 5% polyvinylpyrrolidone, 0.5% cross-linked sodium carboxymethylcellulose and 1% magnesium stearate. Tablets were prepared by wet granulation and were produced using a single punch tableting machine (Manesty F3, Liverpool, UK). Cross-linked sodium carboxymethylcellulose (disintegrant) was added both intra- and extra-granularly (50:50). A biconcave 8 mm punch and die set (I Holland, Nottingham, UK) was used to obtain tablets of mass 200 mg (containing 10 mg drug) and crushing strength of 80 N.

2.3 Coating of prednisolone tablets

Enteric coating formulations were prepared either from aqueous polymer dispersions or organic solutions. The compositions of the aqueous and organic coating formulations are listed in Table 2.

Prednisolone tablets were coated using a Strea-1 bottom spray fluidised bed coater (Aeromatic AG, Bubendorf, Switzerland). The coating conditions were optimised for each polymer formulation and are summarized in Table 3. Coating levels of the polymers were determined by the applied amount of polymer per centimetre square of tablet surface (mg/cm^2), except for PVAP where percentage tablet weight gain (TWG %) was used (Table 3). This is because the quantitative composition of the PVAP formulation is not known. After the coating process the tablets were cured in an air-assisted oven at 40°C for 2 hours.

2.4 Dissolution of enteric coated tablets

2.4.1 Acid uptake

All enteric coating formulations at each coating level were evaluated for acid-resistance and uptake. Six coated tablets of each formulation were weighed and subjected to dissolution conditions in 0.1 M HCl. After 2 hours the tablets were removed and excess medium was drained and blotted with filter paper from around the tablets. The tablets were weighed again and the acid uptake by the tablet was calculated according to Equation 1. Formulations were chosen for dissolution testing at the minimum coating level that met the criteria for acid protection, i.e., no more than 10% acid uptake and no visible signs of coat disruption after two hours acid treatment.

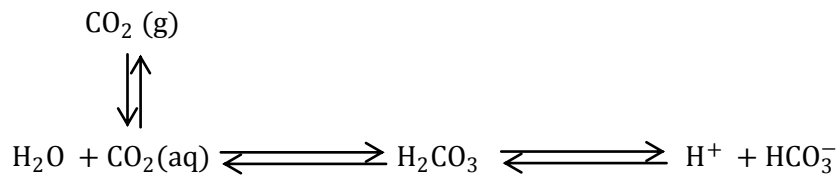
$$\text{Acid uptake (\%)} = \left(\frac{W_f - W_i}{W_i} \right) \times 100$$

Equation 1.

Where W_f is the final tablet weight, W_i is the initial tablet weight.

2.4.2 Development of physiological bicarbonate buffer (mHanks)

Hanks balanced salt solution closely resembles the ionic composition of the small intestinal fluids; however, it has a pH of 7.4, which is too high, and a buffer capacity of 1 mmol/L/ Δ pH, which is too low, compared to human jejunal fluids. Therefore this buffer was modified to achieve a pH of 6.8 and a higher and more relevant buffer capacity (Table 4). Hanks solution is primarily a bicarbonate buffer, in which bicarbonate (HCO_3^-) and carbonic acid (H_2CO_3) co-exist, along with CO_2 (aq) resultant from the dissociation of the latter (Equation 2).



Equation 2

The pH of the buffer system can be altered by adjusting the concentration of the acid (H_2CO_3) and its conjugate base (HCO_3^-) according to the Henderson–Hasselbalch equation (Equation 3). Purging carbon dioxide (g) to Hanks buffer, in excess, increases the concentration of aqueous (CO_2) which promotes the formation of carbonic acid and thus results in a decrease in the pH of the buffer system.

$$\text{pH} = \text{pKa} + \log \frac{[\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]}$$

Equation 3

In this experiment sufficient CO_2 (g) is purged into the system to achieve the desired bicarbonate: carbonic acid ratio, which results in pH 6.8 bicarbonate buffer system (H_2CO_3 pKa, 6.38) while keeping the concentration of bicarbonate unchanged before and after modification. The concentration of bicarbonate in *m*Hanks was determined using a titration method. A known amount of hydrochloric acid was added into *m*Hanks buffer and the excess acid after neutralising bicarbonate is titrated with sodium hydroxide using Autotitrator MPT-2 (Malvern Instruments Ltd., Worcestershire, UK) and the endpoint was determined by a titration curve. The molar concentration of the reacted acid is equal to that of the bicarbonate in the sample; a correction factor is applied for the available phosphate species in the solution. The final ionic composition and buffer capacity of the *m*Hanks buffer are compared with phosphate buffer and human jejunal fluid in Table 4.

The pH in the dissolution media was measured at periodic intervals during the dissolution experiments and was maintained at 6.8 ± 0.05 by continuously sparging CO_2 into the media. Six polyurethane flow tubes (Freshford Ltd., Manchester, UK), one for each dissolution vessel, were used to regulate the CO_2 flow via a manifold assembly.

Buffer capacity (β) of the *m*Hanks was measured by adding aliquots of 0.1M HCl to 100 ml of the buffer system. Buffer capacity was then calculated using Equation 4.

$$\beta = \frac{\Delta AB}{\Delta pH}$$

Equation 4

where ΔAB is the small increment in mol/L of the amount of acid or base added to produce a pH change of ΔpH in the buffer. Buffer capacity was measured at a pH change of 0.5 units on addition of the acid.

2.4.3 *In vitro* drug release

The drug release profiles from the coated prednisolone tablets were carried out using a USP-II apparatus (Model PTWS, Pharmatest, Hainburg, Germany). The tests were conducted in triplicate, in 900 ml dissolution medium maintained at 37 ± 0.5 °C. A paddle speed of 50 rpm was employed. The tests were conducted under sink conditions. Tablets were placed for 2 hours into 0.1 M HCl, and subsequently into pH 6.8 phosphate buffer (Composition: 50 mM KH_2PO_4 and 23.5 mM NaOH; pH adjusted with 1M HCl / NaOH solutions) or pH 6.8 *m*Hanks buffer (Composition: 136.9 mM NaCl, 5.37 mM KCl, 0.812 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.26 mM CaCl_2 , 0.337 mM $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, 0.441 mM KH_2PO_4 , 4.17 mM NaHCO_3 , CO_2 (g) quantity sufficient to reach pH 6.8) (Table 4).

The amount of prednisolone released from tablets coated with HPMCAS-LF, HPMCAS- MF, EUDRAGIT® L30D-55, L100-55 and L100 was determined using an in-line UV spectrophotometer (Cecil 2020, Cecil Instruments Ltd., Cambridge, UK) at the wavelength 247 nm. Data were processed using Icalis software (Icalis Data Systems Ltd, Berkshire, UK). In the case of the tablets coated with CAP, HP-50, HP-55 and PVAP, drug release was determined using HPLC-UV due to the interference of UV absorbance of the polymers at the peak wavelength of prednisolone.

The HPLC-UV system used was a Hewlett Packard 1050 Series HPLC system (Agilent Technologies, UK). Dissolution samples were filtered through 0.22 µm filters (Millipore Ltd, Ireland) and ten microliters were then injected to a reverse phase C8 (5 µm particle size) column (Waters, Massachusetts, USA). The chromatographic conditions were as follows: column temperature of 35 °C, a pressure of 1800 psi, mobile phase consisting of water: tetrahydrofuran: methanol (68.8:25:6.2 v/v), and a flow rate of 1.0 ml/min. Prednisolone was detected at the wavelength of 254 nm.

The *in vitro* drug release data was analysed by two-way ANOVA followed by Tukey post-hoc analysis with 99.8% confidence interval using Univariate General Linear Model tool in PASW Statistics 18 (SPSS Inc., Illinois, USA).

3. Results and discussion

3.1 Acid resistance

The acid uptake results for the various enteric formulations after exposure to 0.1 M HCl for 2 h are shown in Table 5. All organic based enteric coating formulations showed low acid

uptake values at a coating level of 5 mg/cm² (PVAP organic at 5% TWG), indicating good acid resistance. Notably, the aqueous dispersion of the methacrylic acid copolymer, EUDRAGIT® L30D-55, showed comparable acid-resistant properties to its organic version EUDRAGIT® L100-55, at the same coating level of 5 mg/cm². The aforementioned formulations remained intact and showed no physical changes after acid treatment.

The aqueous cellulose based polymer HPMCAS-LF showed poor acid-resistance at 5 mg/cm²; acid uptake values were high and the tablets swelled in acid (Table 5). Therefore, higher coating levels (6, 7 and 8 mg/cm²) were investigated and a coating of 7 mg/cm² was required for sufficient acid protection. This was also the case with HPMCAS-MF, which required a 7 mg/cm² coating level to achieve sufficient acid protection (Table 5). The aqueous coating formulation of PVAP (Sureteric®) also required a higher coating level (7% TWG) to achieve acid-resistance compared to its organic based counterpart (Opadry®) (5% TWG). The film forming mechanisms of the aqueous polymer dispersions are distinct from that of the organic solutions and require complete particle coalescence to obtain film coatings with desired properties [13, 38]; this results in the requirement of a higher coating level for the aqueous formulations to achieve adequate acid-resistance than organic formulations.

The organic formulations with a coating level of 5 mg/cm² and/or 5% TWG were further subjected to dissolution testing. The aqueous EUDRAGIT® L30D-55 formulation was also tested at a coating level of 5 mg/cm². The remaining aqueous formulations were tested at 7 mg/cm² (HPMCAS-LF and HPMCAS-MF) and 7% TWG (PVAP aqueous / Sureteric®).

3.2 Evolution of pH 6.8 bicarbonate buffer (mHanks)

In the present study, pH 6.8 physiological bicarbonate buffer (mHanks buffer) was successfully developed by modification of Hanks buffer (pH 7.4). An attempt has been made

previously to develop pH 6.8 physiological bicarbonate buffers by sparging carbon dioxide to 0.9% sodium chloride solution and the pH was achieved and maintained by titration using 1 N sodium hydroxide [27]. Several issues related with this system were raised including the effect of thermal equilibrium on pH and buffer capacity, loss of carbon dioxide during transfer, cost, long set-up time and more profoundly bubble formation – changing the hydrodynamics of the system, resulting in high variability and poor reproducibility of the dissolution profiles [5]. A modified methodology was proposed later to overcome these issues by keeping sodium hydroxide at a constant concentration and maintaining the pH using carbon dioxide. However, maintaining the pH of this was problematic due to poor resistance of the buffer against pH change and consequently, much higher buffer capacity (30 mmoles/L/ Δ pH) was used for dissolution testing. In the proposed mHanks physiological bicarbonate buffer in the present study, the desired pH was achieved and maintained by sparging carbon dioxide gas into the medium just 2 cm from the liquid surface at a very low flow rate compared to what has been used previously hence avoiding significant changes in hydrodynamics in the dissolution media. In addition, the mHanks closely resembles the ionic composition and buffer capacity of the luminal contents (Table 4), compared to the previously proposed media.

3.3 Dissolution in buffer

Drug release profiles for tablets coated with the organic and aqueous formulations in pH 6.8 phosphate buffer post-exposure to 0.1 M HCl for 2 h are shown in Figures 1A and 2A. All enteric coating formulations, organic and aqueous, showed rapid and similar drug release profiles in pH 6.8 phosphate buffer. This buffer cannot therefore discriminate between the various polymers.

Drug release from prednisolone tablets coated with the organic and aqueous formulations in pH 6.8 *m*Hanks buffer post-exposure to 0.1 M HCl for 2 h are shown in Figures 1B and 2B. Drug release was slower from all enteric coated tablets in pH 6.8 *m*Hanks buffer, compared to that in pH 6.8 phosphate buffer. Moreover, distinctive dissolution profiles were observed for the various enteric coating formulations in this bicarbonate buffer. The dissolution profiles of the organic polymers in *m*Hanks buffer (Figure 1B) are significantly different from each other, except HP-50 and HP-55. The dissolution rank order was EUDRAGIT® L100-55 > HP-55 ≈ HP-50 > PVAP > CAP > EUDRAGIT® L100.

Similar to organic coatings, the enteric formulations based on aqueous polymer dispersions also showed slower drug release and a longer lag time in bicarbonate buffer as compared to compendial phosphate buffer. Since aqueous coatings required a higher weight gain to achieve the sufficient acid resistance, as discussed earlier in section 3.1, therefore this higher weight gain resulted in a thicker coat as compared to their counterpart organic formulations. Consequently, the drug release in the buffer was slower from these aqueous coatings compared to organic coatings (Figure 1 and 2).

In contrast to the aqueous formulations of PVAP and HPMCAS, the methacrylic acid copolymer EUDRAGIT® L30D-55 showed comparable acid resistant properties to its organic form (EUDRAGIT® L100-55) at 5 mg/cm² coating level. Drug release from this aqueous coating system was similar to that from the organic coating system in pH 6.8 *m*Hanks buffer (Figures 1B and 2B). Films formed from this aqueous polymeric dispersion has a minimum film formation temperature lower than room temperature (< 23 °C), and the particles in the latex EUDRAGIT® L30D-55 dispersion have relatively small size (the mean particle size of 0.2 μm) [24]. These properties of the dispersion provide easy particle coalescence during

film formation, and ensured comparable properties of the aqueous film coating as the organic coating.

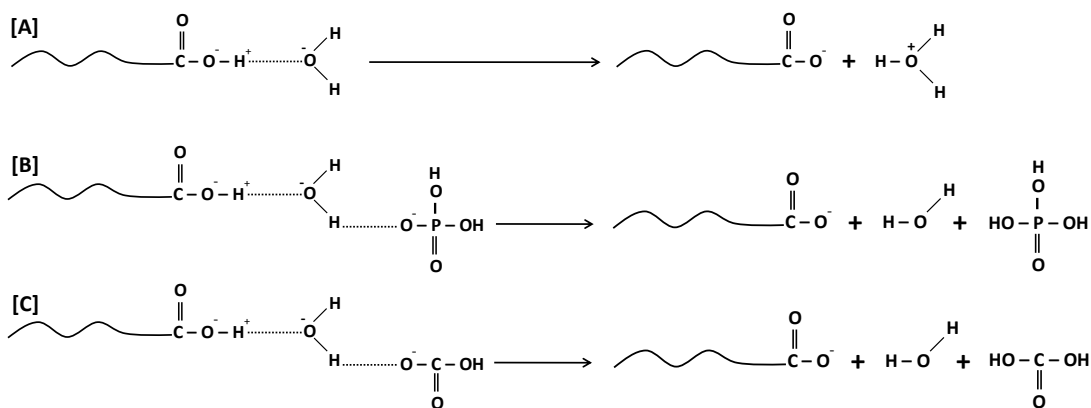
3.4 Distinct behaviour of enteric formulations in physiological bicarbonate media: mechanistic explanation

The mechanism of carboxylic acid polymer dissolution in aqueous solutions is different than that of non-ionic polymers [28] because it involves an additional ionization step that stabilizes the polymer chains. The process of dissolution consists of five steps; (i) diffusion of water and hydroxyl ions into the polymer matrix to form a gel layer, (ii) ionization of polymer chains in the gel layer, (iii) disentanglement of polymer chains out of the gel layer to the polymer-solution interface, (iv) further ionization of polymer chains at the polymer interface, (v) diffusion of disentangled polymer chains away from the interface towards the bulk solution [30]. The rank order in the dissolution of organic polymers observed in *m*Hanks buffer, given the same coating levels were applied, can be explained by the determinant factors for enteric coating dissolution: polymer pKa and chemical structure [31]. Polymers with higher pKa values reflected by higher dissolution pH thresholds, such as EUDRAGIT® L100, showed slower drug release. Apart from pKa, structure of the polymer back bone is also an important factor controlling the dissolution of polyacid polymers. For instance, CAP has a water insoluble back bone and dissolves slower than HPMCP (HP-50/HP-55) and PVAP which have water soluble back bones [10].

Similar to the intestinal fluids, the *m*Hanks buffer is buffered by bicarbonate and also closely resembles the luminal fluids in terms of ionic composition and strength and buffer capacity in contrast to the compendial phosphate buffer. It is notable that drug release in pH 6.8 *m*Hanks buffer was considerably slower than in the compendial phosphate buffer; it not only

discriminated between the different enteric polymer coatings, but is indeed better reflective to the delayed disintegration of enteric coated products in the human intestine *in vivo* [4, 8, 12, 20, 39]. This confirms that most of the enteric coating systems are not interchangeable in terms of drug release as would be suggested by the release data in phosphate buffer, and provides a rank order for these systems in terms of dissolution. To understand this distinct behaviour of enteric polymers in *mHanks*, it is important to understand the interaction of the functional groups at the polymer chain with various ions and buffer species present in the dissolution media.

There have been several reports in the literature, where extremely low rate of dissolution of enteric polymers were reported in normal saline or very weak buffer solutions at pH well above the dissolution thresholds, whereas fast dissolution was observed at same pH at relatively higher strength buffers [15, 31, 36, 37] suggesting that pH is not the only factor controlling the drug release from enteric polymers and other factors also affect the dissociation of the polymer chains. The composition of the dissolution medium, especially the buffer salt, profoundly influences the dissolution rate of enteric polymers. The influence of salts in dissolution of enteric polymers can be explained by general base catalysis. The acid polymers (R-COOH) dissociate through proton transfer to the Brønsted base (H₂O), resulting in the formation of the conjugate base of the polymer and hydronium ions (Equation 5A). In the presence of a basic salt (e.g. HCO₃⁻ or HPO₄⁻), the rate of proton transfer is increased by the higher affinity of the water in accepting proton and consequently the dissolution rate is increased (Equation 5B and 5C). By obeying the Brønsted catalysis law, the dissolution rate was found directly proportional to the pK_a and the concentration of the salts present in the solution [36].



Equation 5. Ionization of carboxylic group in polymer in aqueous media containing [A] only water, [B] phosphate and [C] bicarbonate species

It was also explained that apart from pKa, buffer capacity of the salts also affects acidic polymer dissolution [34]. Hydrogen ions are generated at the polymer-solution interface during polymer dissociation [30] and contribute to a pH drop near the surface of the dissolving carboxylic polymer [21]. Removal of these hydrogen ions at the interface increases the polymer dissolution rate and can be facilitated by reacting with proton acceptors (buffer species), depending on their buffer capacities which directly link to the pKa of the buffer salt. Phosphate buffer has an effective pKa of 7.19 and a resultant higher buffer capacity (23 mmol/L/ Δ pH) thus provides greater driving force for the acidic polymer dissolution than that of bicarbonate with a pKa of 6.31 and a much lower buffer capacity (3.1 mmol/L/ Δ pH). Sheng et al has also suggested that pKa differences in these two buffer species results in different buffer capacity at the solid-liquid interface; where phosphate buffer had about 23 % higher buffer capacity relative to the bicarbonate, at the same pH and buffer concentration [35]. Ionic strength of the dissolution media also have a profound effect on the reaction rate between the polymer film and the basic buffer species; a drastic change in the dissolution rate has been reported from enteric coated formulations with change in ionic strengths of the media [15, 23, 29].

Hence, the pH 6.8 physiological *mHanks* bicarbonate buffer is more appropriate as a dissolution medium to assess the *in vitro* dissolution of enteric polymer coated systems. Further investigations are required to simulate other conditions of the luminal fluids *in vitro* by means of media volume, viscosity and hydrodynamics and a more discriminative effect on drug release can be expected.

4. Conclusions

Drug release from the different enteric coated formulations investigated was rapid and comparable in the commonly used compendial pH 6.8 phosphate buffer, failing to reflect the reported *in vivo* variation and slow release of enteric coated products. A pH 6.8 physiological bicarbonate (*mHanks*) buffer was developed as a dissolution medium to better simulate small intestinal luminal fluid. This buffer was able to discriminate the different enteric polymer coated systems, providing a rank dissolution order, and is likely to improve the *in vitro-in vivo* correlations of these modified release systems. This new knowledge can also be useful in the rational design of enteric coated products designed to target different sites in the small bowel, such as the proximal small intestine or the mid small intestine.

Reference List

1. Agyilirah, G.A. and G.S. Banker, Polymers for enteric coating and applications, in Polymers for controlled drug delivery, P.J. Tarcha, Editor. 1991, CRC press: Boca Raton. p. 39-66.
2. Banwell, J.G., S.L. Gorbach, N.F. Pierce, R. Mitra, and A. Mondal, Acute undifferentiated human diarrhoea in the tropics. . J. Clin. Invest., 1971. 50: p. 890-900.
3. Bianchini, R., M. Resciniti, and C. Vecchio, Technological evaluation of aqueous enteric coating systems with and without insoluble additives. Drug Dev. Ind. Pharm., 1991. 17: p. 1779-1794.
4. Bogentoft, C., M. Alpsten, and G. Ekenved, Absorption of acetylsalicylic acid from enteric-coated tablets in relation to gastric emptying and in-vivo disintegration. J. Pharm. Pharmacol., 1984. 36: p. 350-351.
5. Boni, J.E., R.S. Brickl, and J. Dressman, Is bicarbonate buffer suitable as a dissolution medium? J Pharm Pharmacol, 2007. 59(10): p. 1375-82.
6. Bruce, L.D., H.U. Petereit, T. Beckert, and J.W. McGinity, Properties of enteric coated sodium valproate pellet. Int. J. Pharm., 2003. 264: p. 85-96.
7. Chan, W.A., C.D. Boswell, and Z. Zhang, Comparison of the release profiles of a water soluble drug carried by Eudragit-coated capsules in different in-vitro dissolution liquids. Powder Tech., 2001. 119: p. 26-32.
8. Cole, E.T., R.A. Scott, A.L. Connor, I.R. Wilding, H.U. Petereit, C. Schminke, T. Beckert, and D. Cade, Enteric coated HPMC capsules designed to achieve intestinal targeting. Int. J. Pharm., 2002(231): p. 83-95.
9. Dangel, C., K. Kolter, H.B. Reich, and G. Schepky, Aqueous enteric coatings with methacrylic acid copolymer type C on acidic and basic drugs in tablets and pellets, Part I: acetylsalicylic acid tablets and crystals. Pharm. Tech., 2000: p. 64-70.
10. Davis, M., I. Ichikawa, E.J. Williams, and G.S. Banker, Comparison and evaluation of enteric polymer properties in aqueous solutions. International Journal of Pharmaceutics, 1986. 28(2-3): p. 157-166.
11. Dressman, J.B., R.R. Berardi, L.C. Dermentzoglou, T.L. Russell, S.P. Schmaltz, J.L. Barnett, and K.M. Jarvenpaa, Upper gastrointestinal (GI) pH in young, healthy men and women. Pharm. Res., 1990. 7: p. 756-761.
12. Ebel, J.P., M. Jay, and R.M. Beihn, An in vitro/in vivo correlation for the disintegration and onset of drug release from enteric-coated pellets. Pharm. Res., , 1993. 10: p. 233-238.
13. Eckersley, S.T. and A. Rudin, Mechanism of film formation from polymer latexes. J. Coatings Tech., 1990. 62: p. 89-100.
14. Evans, D.F., G. Pye, R. Bramley, A.G. Clark, T.J. Dyson, and J.D. Hardcastle, Measurement of gastrointestinal pH profiles in normal ambulant human subjects. Gut, 1988. 29: p. 1035-1041.
15. Fadda, H.M. and A.W. Basit, Dissolution of pH responsive formulations in media resembling intestinal fluids: bicarbonate versus phosphate buffers. J. Drug Del. Sci. Technol., 2005. 15: p. 273-279.
16. Fadda, H.M. and A.W. Basit, Drug solubility in luminal fluids from different regions of the small and large intestine of humans. 2009. AAPS2009-003733.
17. Fadda, H.M., H.A. Merchant, B.T. Arafat, and A.W. Basit, Physiological bicarbonate buffers: stabilisation and use as dissolution media for modified release systems. Int J Pharm, 2009. 382(1-2): p. 56-60.
18. Fallingborg, J., Intraluminal pH of the human gastrointestinal tract. Dan. Med. Bull., 1999. 46: p. 183-196.
19. Garcia-Arieta, A., D. Torrado-Santiago, and J.J. Torrado, Comparative study of aqueous and organic enteric coatings of chlorpheniramine maleate tablets. Drug Dev. Ind. Pharm, 1996. 22: p. 579-585.

20. Hardy, J.G., D.F. Evans, I. Zaki, A.G. Clark, H.H. Tonnesen, and O.N. Gamst, Evaluation of an enteric coated naproxen tablet using gamma scintigraphy and pH monitoring. *Int. J. Pharm.*, 1987. 37: p. 245-250.
21. Harianawala, A.I., R.H. Bogner, and M. Bradley, Measurement of pH near dissolving enteric coatings. *Int J Pharm*, 2002. 247(1-2): p. 139-46.
22. Ibekwe, V.C., H.M. Fadda, G.E. Parsons, and A.W. Basit, A comparative in-vitro assessment of the drug release performance of pH responsive polymers for ileo-colonic delivery. *Int. J. Pharm.*, 2006. 308: p. 52-60.
23. Kararli, T.T., C.F. Kirchoff, and J.E. Truelove, Ionic strength dependence of dissolution for Eudragit S-100 coated pellets. *Pharm Res*, 1995. 12(11): p. 1813-6.
24. Lehmann, K., Chemistry and application properties of polymethacrylate coating systems, J.W. McGinity, Editor. 1989, Marcel Dekker, Inc.: New York. p. 153-245.
25. Lindahl, A., A.L. Ungell, L. Knutson, and H. Lennernas, Characterization of fluids from the stomach and proximal jejunum in men and women. *Pharm. Res.*, 1997. 14: p. 497-502.
26. McConnell, E.L., H.M. Fadda, and A.W. Basit, Gut instincts: explorations in intestinal physiology and drug delivery. *Int.J.Pharm.*, 2008. 364: p. 213-226.
27. McNamara, D.P., K.M. Whitney, and S.L. Goss, Use of a physiologic bicarbonate buffer system for dissolution characterization of ionizable drugs. *Pharm Res*, 2003. 20(10): p. 1641-6.
28. Narasimhan, B. and N. Peppas, The physics of polymer dissolution: Modeling approaches and experimental behaviour, in *Polymer Analysis, Polymer Physics*, A. Andrady, Editor. 1997, Springer-Verlag: New York. p. 157-207.
29. Nesbitt, R.U., F.W. Goodhart, and R.H. Gordon, Evaluation of polyvinyl acetate phthalate as an enteric coating material. *International Journal of Pharmaceutics*, 1985. 26(3): p. 215-226.
30. Nguyen, D.A. and H.S. Fogler, Facilitated diffusion in the dissolution of carboxylic polymers. *AIChE Journal*, 2005. 51(2): p. 415-425.
31. Ozturk, S.S., B.O. Palsson, B. Donohoe, and J.B. Dressman, Kinetics of release from enteric-coated tablets. *Pharm. Res.*, 1988. 5: p. 550-564.
32. Phillips, S.F. and J. Giller, The contribution of the colon to electrolyte and water conservation in man. *J Lab Clin Med*, 1973. 81(5): p. 733-746.
33. Schiller, C., C.P. Frohlich, T. Giessmann, W. Siegmund, H. Monnikes, N. Hosten, and W. Weitschies, Intestinal fluid volumes and transit of dosage forms as assessed by magnetic resonance imaging. *Aliment. Pharmacol. Ther.*, 2005. 22: p. 971-979.
34. Shek, E., Buffer capacity, not buffer catalysis, affects the dissolution rate of cellulose acetate phthalate. *Pharm. Ind.*, 1978. 40: p. 981-982.
35. Sheng, J.J., D.P. McNamara, and G.L. Amidon, Toward an in vivo dissolution methodology: a comparison of phosphate and bicarbonate buffers. *Mol Pharm*, 2009. 6(1): p. 29-39.
36. Spitael, J. and R. Kinget, Factors affecting the dissolution rate of enteric coatings. *Pharm. Ind.*, 1977. 39: p. 502-505.
37. Spitael, J., R. Kinget, and K. Naessens, Dissolution rate of cellulose acetate phthalate and the Bronsted catalysis law. *Pharm. Ind.*, 1980. 42: p. 846-849.
38. Vanderhoff, J.W., H.L. Tarkowski, M.C. Jenkins, and E.B. Bradford, Theoretical considerations of the interfacial forces involved in the coalescence of latex particles. *J. Macromol. Chem*, 1966. 1: p. 361.
39. Wilding, I.R., S.S. Davis, R.A. Sparrow, K.J. Smith, K.A. Sinclair, and A.T. Smith, The evaluation of an enteric-coated naproxen tablet formulation using gamma scintigraphy. *Eur. J. Pharm. Biopharm*, 1993. 39: p. 144-147.

Table 1. Enteric polymers used in the study

Polymer	Brand name	Abbreviation	Grade	Soluble at or above pH	Manufacturer/Supplier
Methacrylic acid copolymer	EUDRAGIT®	-	L 100-55	5.5	Evonik Röhm GmbH, Darmstadt, Germany
			L 30D-55	5.5	
			L100	6.0	
Hypromellose acetate succinate	Aqoat®	HPMCAS	LF	5.0	Shin-Etsu Chemical Co., Ltd., Japan
			MF	6.0	
Hypromellose phthalate	-	HPMCP	HP-50	5.0	Shin-Etsu Chemical Co., Ltd., Japan
			HP-55	5.5	
Cellulose acetate phthalate	-	CAP	-	6.0	Eastman Chemical Company, USA
Polyvinyl acetate phthalate	*Opadry® Enteric	PVAP	Organic	5.0	Colorcon Ltd., USA.
	**Sureteric®		Aqueous	5.0	

* Fully formulated coating system containing: PVAP, titanium dioxide, triethyl citrate and stearic acid

** Fully formulated coating system containing: PVAP, talc, polyethylene glycol 3350, sodium bicarbonate, triethyl citrate, purified stearic acid, sodium alginate and colloidal anhydrous silica

Table 2. Composition of the coating formulations**[A]** Aqueous formulations

	EUDRAGIT® L30 D-55	PVAP (aqueous)	HPMCAS-LF	HPMCAS-MF
Polymer weight	20g	20g	20g	20g
Talc	10g (50%*)	-	6g (30%*)	6g (30%*)
Triethyl citrate	2g (10%*)	-	4g (20%*)	4g (20%*)
Sodium lauryl sulphate	-	-	0.6g (3%*)	0.6g (3%*)
Water	128g	113.3g	352g	352g
Solid content of the spray suspension	20%	15%	8%	8%

*Based on polymer weight

[B] Organic formulations

	EUDRAGIT® L100-55	EUDRAGIT® L100	PVAP (organic)	CAP	HP-50	HP-55
Polymer weight	20g	20g	20g	20g	20g	20g
Talc	10g (50%*)	10g (50%*)	-	10g (50%*)	10g (50%*)	10g (50%*)
Triethyl citrate	2g (10%*)	2g (10%*)	-	-	2g (10%*)	2g (10%*)
Triacetin	-	-	-	5g (25%*)	-	-
Isopropanol	279.4g (97%**)	279.4g (97%**)	144g (80%**)	-	-	-
Ethanol	-	-	-	-	230.4g (80%**)	230.4g (80%**)
Acetone	-	-	-	249g (97%**)	-	-
Water	8.6g (3%**)	8.6g (3%**)	36g (20%**)	7.7g (3%**)	57.6g (20%**)	57.6g (20%**)
Solid content of the spray suspension	10%	10%	10%	12%	10%	10%

*Based on polymer weight. ** Based on solvent weight.

Table 3. Coating parameters for the different polymer systems

Formulation	Inlet temp (°C)	Outlet temp (°C)	Fan capacity	Atomizing pressure (bar)	Flow rate (mL/min)	Coating level (mg/cm²)
EUDRAGIT® L30D-55	46	34	17	0.2	4	5
EUDRAGIT® L100-55	40	32	17	0.2	3	5
EUDRAGIT® L100	40	32	17	0.2	3	5
AS-LF	46	43	17	0.2	7	5,6,7,8
AS-MF	56	60	17	0.2	7	5
PVAP(Sureteric®)	58	43	17	0.2	7	5, 6,7,8% TWG
PVAP (Opadry®)	52	40	9	0.2	7	5% TWG
CAP	40	34	17	0.2	5	5
HP-50	46	38	17	0.2	4	5
HP-55	60	43	17	0.2	5	5

Table 4. Comparison of the ionic composition (mM) and buffer capacity of small intestinal fluids [2, 25, 32] and phosphate and *m*Hanks media.

Composition	Human jejunal fluid	Phosphate buffer (0.05M)	<i>m</i>Hanks buffer
Bicarbonate	7.1	Not present	4.17
Phosphate	0.8	50	0.8
Potassium	5.1	50	5.8
Sodium	142	29	142
Chloride	131	Not present	143
Calcium	0.5	Not present	1.3
Magnesium		Not present	0.8
pH	6.8	6.8	6.8
Buffer capacity (mmol/L/ Δ pH)	3.2 [†]	23	3.1

[†] measured from luminal aspirates [16].

Table 5. Acid uptake of enteric coated prednisolone tablets

[A] Organic formulations

	CAP	EUDRAGIT® L 100	EUDRAGIT® L100-55	HP-50	HP-55	PVAP* (Opadry®)
Coating Level (mg/cm ²)	5 [†]	5 [†]	5 [†]	5 [†]	5 [†]	5% [†]
Acid uptake (%)	2.6	4.2	2.7	2.8	2.1	1.2

[B] Aqueous formulations

	EUDRAGIT® L30D-55	HPMCAS-LF				HPMCAS-MF			PVAP* (Sureteric®)			
Coating level (mg/cm ²)	5 [†]	5	6	7 [†]	8	5	6	7 [†]	5%	6%	7% [†]	8%
Acid uptake (%)	3.1	19.4	7.8	7.5	6	9.9	7.8	5.5	30.3	14	8.3	7.2

* coating level based on tablet weight gain (%)

† coating levels selected for dissolution testing

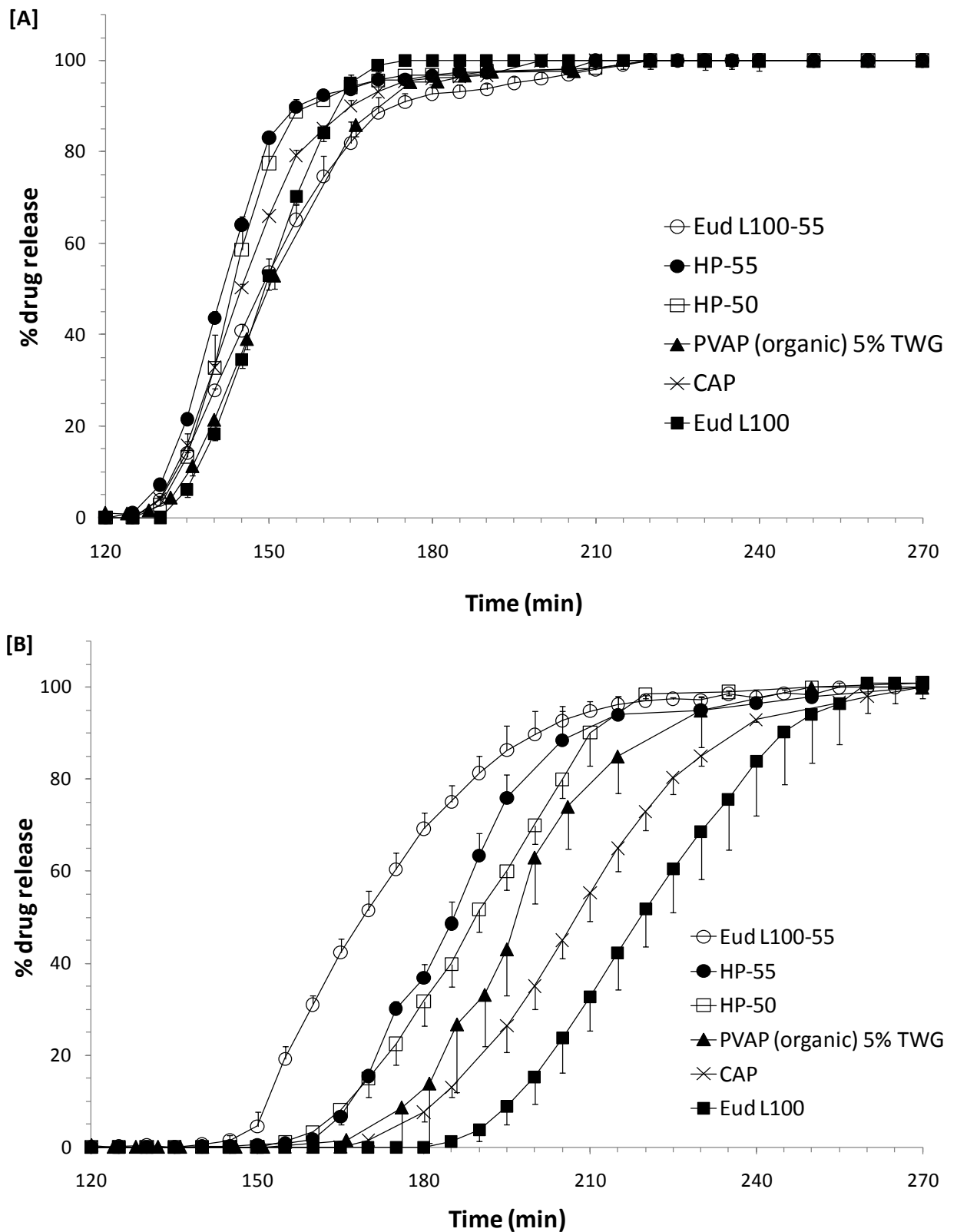


Figure 1. Drug release for organic solution coated (5mg/cm² unless otherwise indicated) prednisolone tablets in 0.1M HCl for 2h (data not shown) followed by pH 6.8 [A] phosphate buffer and [B] bicarbonate buffer

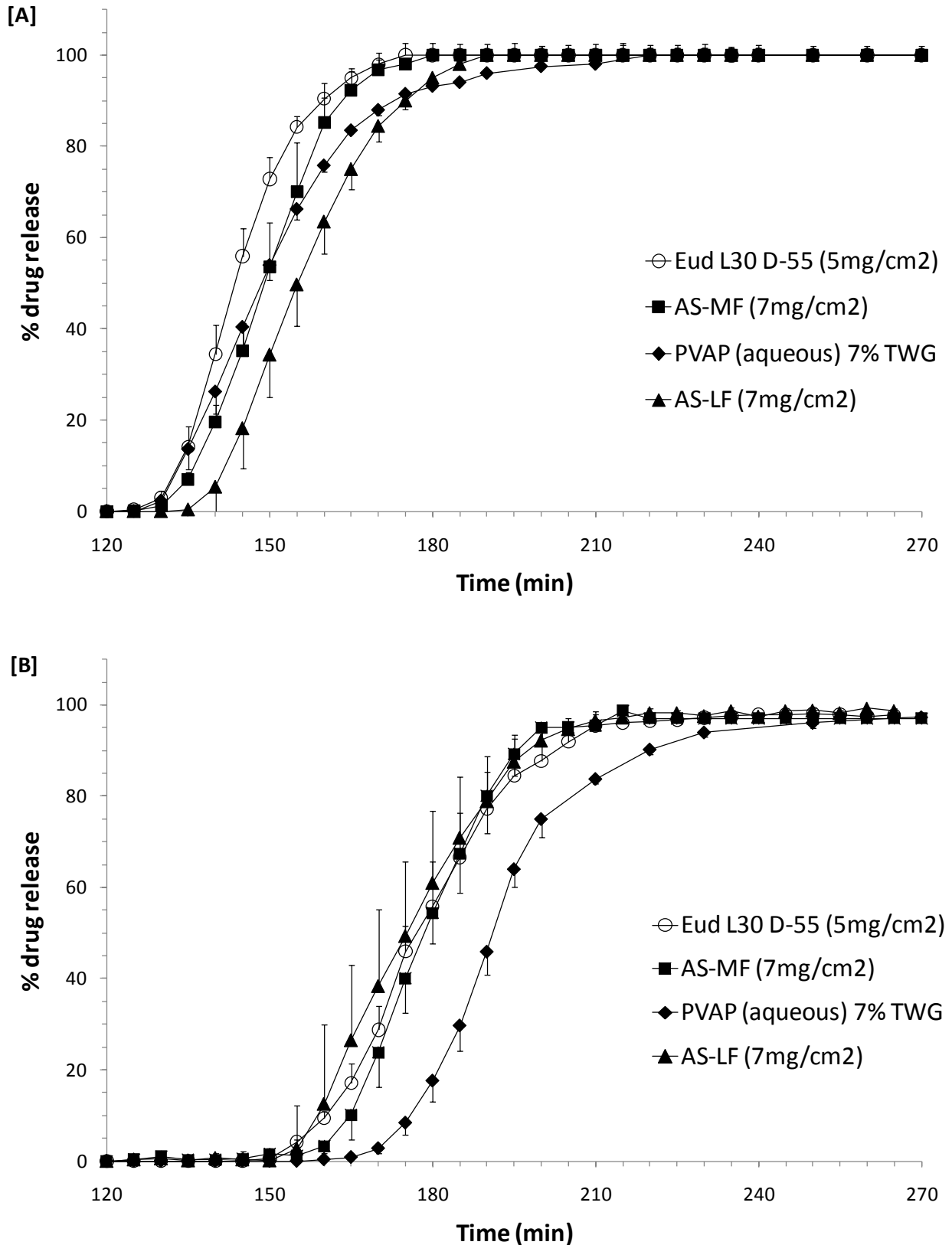


Figure 2. Drug release for aqueous dispersion coated prednisolone tablets in 0.1M HCl for 2h (data not shown) followed by pH 6.8 [A] phosphate buffer and [B] bicarbonate buffer