Title: Regulating the pH of bicarbonate solutions without purging gases: Application to dissolution testing of enteric coated tablets, pellets and microparticles

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Abstract

Dissolution media based on bicarbonate buffers closely mimic the environment of intestinal fluids and thus improve *in vitro in vivo* correlation compared to phosphate buffers. Purging gases into the medium is used as a method to stabilise bicarbonate buffers; however, this causes issues due to the disturbance of the hydrodynamics in the dissolution vessel. The aim of this study was to develop a novel system to regulate and stabilise the pH of bicarbonate buffers without purging gases for the application of dissolution testing of enteric coated products. A novel enclosure system was applied to the USP II dissolution vessel to supply N_2 and CO_2 gases above the dissolution medium without purging into the solution. Drug release from enteric coated predinisolone microparticles (216.9 µm), pellets (1.25 mm) and commercially available tablets was determined in 0.1M HCl and subsequently in pH 6.8 phosphate buffer or pH 6.2-6.8 bicarbonate buffers generated by titration of the acidic medium *in situ* using USP II apparatus. Supplying N_2 at 3-4 bar and CO_2 at 0.1 bar were able to increase the pH of the bicarbonate buffer from pH 6.2 to 6.8 within 45 min and subsequently stabilise the medium pH at 6.8 ± 0.05 pH units. Enteric coated microparticles showed much faster drug release in the physiological bicarbonate buffers than tablets and pellets. The novel bicarbonate-based dissolution system moves forward the application of the physiological bicarbonate buffers for testing pharmaceutical products to meet compendial requirements.

Key words: enteric coating, biorelevant, dissolution, in vitro testing, bicarbonate

1 Introduction

The purposes of *in vitro* dissolution testing are to discriminate between formulations and to predict how a dosage form will behave *in vivo*. Phosphate and other compendial buffers are generally employed due to their high buffer stability during dissolution testing; however it is not the main physiological buffer species in the intestine where most oral drugs are absorbed. The predominant buffer in this region is bicarbonate. The use of bicarbonate buffer-based media for dissolution testing was proven to provide better prediction to *in vivo* performance and discrimination of oral products designed to target different segments of the gastrointestinal tract (Fadda and Basit, 2005; Liu et al., 2011; Liu and Shokrollahi, 2015). The challenge of applying bicarbonate buffers is the loss of carbon dioxide (CO_2) from the solution causing pH to rise and leading to poor reproducibility of the dissolution test.

Various methods have been investigated to stabilise bicarbonate buffers including continuous purging of CO_2 into the medium solution, application of a layer of liquid paraffin and complete sealing of the dissolution vessel to prevent gas escape (Fadda et al., 2009; Liu et al., 2011). A pHysio-stat® system was developed which uses a pH electrode to monitor the pH of the dissolution media and a gas diffuser to bubble in CO_2 or nitrogen (N_2) gases to maintain the pH of the medium throughout the dissolution test (Garbacz et al., 2013). A similar Auto pH System™ incorporated pH monitoring to control the release of helium (pH increasing) and CO_2 (pH decreasing) gases to develop a dynamic bicarbonate buffer-based dissolution system capable of simulating the real-life pH-gradients in the intestinal lumen (Merchant et al., 2014a; Merchant et al., 2014b).

Bubbling gases into the dissolution medium causes disruption to the hydrodynamics of the medium which could affect dissolution rate of certain drugs and thus not meeting compendial requirements (Garbacz et al., 2013; Merchant et al., 2014b). Gas bubbling could generate

foaming when biorelevant media are used such as those containing surfactants and Fasted State Simulated Intestinal Fluid (FASSIF) and Fed State Simulated Intestinal Fluid (FESSIF) (Boni et al., 2007). When a two-stage dissolution testing is required, e.g. transferring from pH 1 (stomach) to pH 6.8 (intestine) for testing delayed release (enteric coated) products in USP II paddle apparatus, the test product (such as enteric coated tablets) needs to be picked up and transferred from acidic to bicarbonate-based media for a complete media change (Liu and Shokrollahi, 2015). This is not appropriate for multiparticulates such as pellets and microparticles which can be lost during the media transfer.

The aim of this study was to develop a novel method of stabilising and regulating the pH of the bicarbonate buffer without the need of bubbling gases. We also aim to develop a single-vessel method for media transfer from acidic conditions to bicarbonate buffer eliminating the need of complete media change for testing enteric-coated products including tablets, pellets and microparticles.

2 Materials and methods

2.1 Materials

Prednisolone (micronized) was purchased from Sanofi (France). Inert spherical particles of microcrystalline cellulose (MCC; Cellets® 100 and 1000) were purchased from Pharmatrans Sanaq AG (Switzerland). Hypromellose (Methocel™ E5) was donated by Colorcon (UK). Talc (Pharm M) was purchased from Imerys Talc (Italy). Methacrylate polymer Eudragit® L30 D-55 was supplied as free samples by Evonik AG (Germany). Triethyl citrate (TEC) and magnesium stearate were purchased from Acros Organics (UK) and glycerol monostearate (GMS) Imwitor® 900K from IOI Oleo GmbH (Germany). Gastric-resistant (enteric polymer polyvinyl acetate phthalate in the coating) prednisolone 5 mg-dose tablets (batch # PW242; Actavis Group PTC, UK) were used as commercially available product for comparative

investigation. FaSSIF/FeSSIF/FaSSGF poweder was purchased from Biorelevant.com. All reagents for dissolution testing were purchased from Fisher Scientific (UK).

2.2 Regulating the pH of bicarbonate solutions using a novel enclosure system

To overcome the issues associated to bubbling gases to regulate or stabilise the pH of bicarbonate buffers, we used an enclosure device fitted onto the dissolution vessel (USP I and II) to facilitate gas diffusion into the dissolution medium containing hydrogen carbonate (Liu et al., 2019). Gases, N_2 (pH increasing) and CO_2 (pH decreasing), were supplied via inlets into the enclosure system, distributed through a ring-shaped diffuser and released via multiple outlets pointing vertically (90°) or angled (45°) towards the surface of the dissolution medium **(Fig. 1).** The enclosure device comprised a plate attached to a ring-shaped chamber. The plate contained two apertures which were connected to the gas supply at one end (facing upwards) and the hollow cavity of the ring-shaped chamber at the other end. At the bottom of the chamber (opposite the plate), a number of orifices were made to evenly distribute the gases on the surface of the dissolution medium and to facilitate gas diffusion.

2.3 A single-vessel media change and pH-regulation method

For testing enteric-coated products, a media change is required from acidic medium of 0.1 M HCl (gastric condition) to pH 6.8 buffer (intestinal condition). When bicarbonate buffer is used, the acidic medium is usually discharged from the vessel and replaced with the buffer. This is labour intensive and unsuitable for testing multiparticulate-based products. In this study, we used a single-vessel media transfer method, whereby the enteric-coated product was first tested in 700 mL 0.1 M HCl. Once the test is completed, 100 mL of 0.65 M NaOH solution was added to the HCl solution by pouring directly into the vessel and the pH was adjusted to 2.0 - 3.0 using 1 M HCl or 1 M NaOH. A salt solution (100 mL) containing hydrogen carbonate was then added to the above medium solution by pouring directly into the vessel to reach the final

composition of the medium equivalent to a modified Hanks buffer (composition: 63.57 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 $NaHCO_3$) (Liu et al., 2011). The pH was adjusted to the desired level (e.g. pH 6.2 or 6.8) using 1 M HCl or 1 M NaOH.

The pH of the dissolution medium was monitored and regulated during dissolution using a pH-monitoring/controlling system (NICO 2000, UK) which regulated the supply of the pH-increasing (N_2) and decreasing (CO_2) gases through electric valves via the enclosure system described above. The pH values of the bicarbonate-based media (pH 6.8) were measured at different locations in the dissolution vessel (bottom, middle and top) to evaluate homogeneity of gas distribution and uniformity of pH throughout the vessel.

Buffer capacity (β) of the bicarbonate buffer was measured by adding aliquots of 0.1 M HCl to 100 mL of the buffer solution and was calculated using Equation 1.

$$\beta = \frac{\Delta AB}{\Delta pH} \quad \text{Eq. 1}$$

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. All buffer capacity measurements were conducted in triplicate.

2.4 Compatibility with bio-relevant media

FaSSIF/FeSSIF/FaSSGF powder was added to 900 ml pH 6.8 bicarbonate buffer prepared using methods described above to obtain Fasted State Simulated Intestinal Fluid (FaSSIF, 2.016 g powder added) or Fed State Simulated Intestinal Fluid (FeSSIF, 10.08 g powder added). The pH of the bicarbonate buffer was maintained at pH 6.8 ± 0.5 using two methods by 1) bubbling CO_2 and N_2 directly into the solution at 0.1 bar and 2) supplying CO_2 (0.1 bar) and N_2 (4 bar) through the enclosure device.

2.5 Preparation of prednisolone-loaded microparticles and pellets

Prednisolone was layered onto microcrystalline cellulose (MCC) cores (Cellets® 100 and Cellets® 1000) using a fluid bed coater (Mini-Glatt; Glatt GmbH, Germany). The drug-loading suspension contained prednisolone, hypromellose, talc and deionised water (9.79, 1.04, 1.83 and 87.34 % w/w, respectively). Hypromellose was dissolved in deionised water. Prednisolone and talc were added to the solution and dispersed for 5 min using a propeller mixer (RZR 2051 control, Heidolph Instruments, Germany) at 750 rpm. The resultant suspension was filtered through a 250 µm mesh sieve and kept under continuous stirring with a magnetic stirrer during the drug loading process. The suspension was sprayed through a 0.5 mm nozzle at 2.0 – 2.5 g/min maintaining a 30 ºC product temperature with 46 ºC inlet air temperature. The inlet air flow rate was 18 ± 0.5 m^3/h with 2 bar atomisation pressure. The spray process was completed once 10 % drug loading was achieved.

2.6 Coating of prednisolone microparticles and pellets

Prednisolone-loaded MCC particles (Cellets® 100 and 1000) were coated with a Eudragit® L30 D-55 dispersion containing triethyl citrate (10 % w/w), glycerol monostearate (GMS, 5 % w/w), polysorbate 80 (2 % w/w), and deionised water (all percentages based on dry polymer). Half of the required deionised water were heated to 75-80 °C and GMS was added to the heated water under continuous stirring with a magnetic stirrer. Triethyl citrate and polysorbate 80 were added to the GMS emulsion which was stirred continuously for a further 10 min followed by homogenisation using a rotor-stator homogeniser (Ultra-Turrax T25, IKA-Werke GmbH, Germany) at 10,000 rpm and 75-80 °C for 10 min. The remaining half of the deionised water was added to the hot dispersion under continuous stirring using a magnetic stirrer and allowed to cool to 30 °C. The resultant dispersion was added to the Eudragit® L 30 D-55 dispersion

under continuous stirring using a magnetic stirrer and were filtered through a 250 µm mesh sieve before coating.

The polymer dispersion was sprayed through a 0.5 mm nozzle at 1.0–1.5 g/min maintaining a 25-28 ºC product temperature with 32-40 ºC inlet air temperature. The inlet air flow rate was set to 19 ± 0.5 m^3/hr with 1.5 bar atomisation pressure. Continuous vibration was applied during the polymer coating processes using a pneumatic linear vibrator (NTS 180 NFL, Netter Vibration, Germany). During coating process, magnesium stearate was periodically added (every 15 min, at 0.1 % based on starting cores for each addition) to the coating chamber through an external feeding port (Mohylyuk et al., 2019). At the end of the coating process the coated particles were dried for 20 min at 25 °C in the processing chamber. After 10 min of drying, 1 g of silicon dioxide was added to the coating column through the external feeding port to separate the free-flowing particles and particles stuck in the Wurster column (Mohylyuk et al., 2019). The coating weight gains for the pellets and microparticles achieved were 18 % and 73 % respectively. All polymer-coated particles were cured at 40 °C for 24 hours in an oven (Heratherm OMS60; Thermo Electron LED GmbH, Germany).

The free flowing particles discharged from the coater were analysed using an analytical sieve shaker (AS200, Retsch GmbH, Germany) fitted with sieves of mesh sizes 90, 125, 180, 250, 355 and 710 µm. Light microscopy (GXL3230, GT Vision Ltd, England) was used to identify the size ranges of coated particles that were not agglomerated. The percentage yield of the coating process was calculated using Equation 2 (Mohylyuk et al., 2019).

$$\%\, Yield = \frac{weight\ of\ non{-}agglomerated\ free\ flowing\ particles}{Total\ weight\ of\ coated\ particles}\ x\ 100 \qquad \text{Eq. 2}$$

Coating thickness was determined for the pellets and microparticles using light microscopy (GXL3230, GT Vision Ltd, England). The average particle diameter of 100 uncoated and coated pellets/particles was measured and the coating thickness was calculated using Equation

3. Scanning electron microscopy (SEM) images were obtained for the coated microparticles and pellets using a Phenom ProX (Lambda Photometrics, UK).

182

183 $$Coating\ thickness\ (\mu m) = \frac{Average\ diameter\ of\ coated\ particles\ (\mu m) - Average\ diameter\ of\ uncoated\ particles (\mu m)}{2}$$ Eq. 3

184

The specific surface area (SSA) of pellets and microparticles for 5 mg prednisolone dose was calculated using Equation 4. To calculate the mass of a single pellet/particle (*w*), approximately 0.02 g pellets/microparticles were weighed using a 6-point balance and the mass was divided by the number of particles in the weighed sample (manually counted under light microscopy). The test was conducted in triplicate.

$$SSA(mm^2) = \left(\frac{Total\ weight\ of\ particles\ of\ 5mg\ dose,mg}{Weight\ of\ one\ particle,mg}\right) \times \pi \times Diameter^2 \qquad \text{Eq. 4}$$

2.7 Drug release tests of enteric-coated microparticles, pellets and tablets

Prednisolone release from enteric-coated microparticles, pellets and tablets was evaluated using a USP-II apparatus (DIS 6000, Copley Scientific, UK) at 37 ± 0.5 °C with a paddle speed of 100 rpm. Drug release was tested for 2 h in 700 mL (for media change into bicarbonate buffer) or 750 mL (for media change into phosphate buffer) of 0.1 M HCl solution and subsequently in one of the three media: 1) 1000 mL of pH 6.8 phosphate buffer (by adding 250 mL of a 0.2 M solution of trisodium phosphate dodecahydrate in the vessel), 2) 900 mL of pH 6.8 bicarbonate buffer prepared as described in Section 2.3, and 3) pH 6.2-6.8 bicarbonate buffer where the bicarbonate buffer was prepared as described in Section 2.3 to reach a pH level of 6.2 and the pH value was gradually increased to pH 6.8 in 45 min by supplying N_2 through the enclosure and pH-regulating system. For the preparation of both the phosphate and bicarbonate buffers, the operations of adding the buffer and adjusting the pH were completed within 5 min. The bicarbonate-based media were stabilised at pH 6.8 for the required duration by supplying the pH-regulating gases through the enclosure and pH-regulating system. All buffer stage tests were performed for a total of 2 h.

The quantity of prednisolone released from the products was determined using a closed loop pumping system and in-line UV-quantification (T70+, PG Instruments, UK) at a wavelength of 247 nm. All tests were conducted in triplicate. Drug release lag time (t_{lag}) in the buffer stage

testing was determined as the x-intercept of steady state phase of drug release in the buffer tests. Complete drug release (t_{85}) was calculated for drug release in the buffer media by using the first time point where 85 % drug release was observed.

The dissolution data were analysed by using a two-way ANOVA with 95 % confidence interval using Microsoft Excel (Microsoft Corporation, Washington, USA).

3 Results

3.1 pH-regulation and media change

The use of the enclosure system allowed the pH of the bicarbonate buffer to be regulated without substantial disruption to the surface of the media. The design of the enclosure system allowed gases to be supplied at 90° or 45° to the surface of the media under different pressures. To simulate the gradual pH increase in the upper small intestine, N_2 gas was supplied through the enclosure system to increase the pH of the bicarbonate-based medium from pH 6.2 to 6.8. **Fig. 2a** shows that increasing the pressure of the N_2 gas (from 3 to 4 bar) increased the rate of pH rise and N_2 gas supplied vertically (at 90°) was more efficient in increasing the pH than at 45°. Once the pH value has reached pH 6.8, it was stabilised at pH 6.8 ± 0.05 using CO_2 (0.1 bar) and N_2 (3-4 bar) through the enclosure system (**Fig. 2b**).

The single vessel media change was reproducibly achieved from 700 mL 0.1 M HCl to pH 6.18 ± 0.10 (n=8) and pH 6.80 ± 0.04 (n=8) bicarbonate buffer using the titration method (**Tab. 1**). The final pH of the media was adjusted to pH 6.2 ± 0.05 or pH 6.8 ± 0.05. **Tab. 2** shows the pH values measured at different locations in the dissolution vessel using the pH 6.8 bicarbonate buffer. The buffer capacities for the pH 6.2 and pH 6.8 bicarbonate buffer after pH adjustment were 5.04 ± 0.29 mmoles/L/ΔpH and 3.31 ± 0.24 mmoles/L/ΔpH respectively.

231 Bubbling CO_2 and N_2 gases at 0.1 bar into the pH 6.8 bicarbonate buffer containing FaSSIF
232 and FeSSIF powder generated foaming in the media. When CO_2 (0.1 bar) and N_2 (4 bar) gases
233 were supplied through the enclosure device, no foaming was observed for 1 h.

234 **3.2 Drug release from enteric coated prednisolone microparticles, pellets and tablets**

235 Polymer coating of Eudragit® L30 D-55 was successfully achieved using prednisolone-loaded
236 microparticles (Cellets® 100, diameter D_{50} = 160.33 ± 2.09 µm for uncoated cores) and pellets
237 (Cellets® 1000, diameter D_{50} = 1140 ± 50 µm for uncoated cores), obtaining yield of 87 % and
238 100 % respectively. The particle size of polymer coated microparticles and pellets were 216.94
239 ± 0.48 µm and 1199 ± 23 µm respectively. The measured coating thicknesses of microparticles
240 and pellets were 23.5 and 29.5 µm respectively with no statistical difference between the two
241 (n=100, p>0.05). The specific surface areas for a 5 mg prednisolone dose were 1453 and 225
242 mm^2 for the microparticles and pellets respectively. **Fig. 3** shows the SEM images of coated
243 microparticles and pellets.

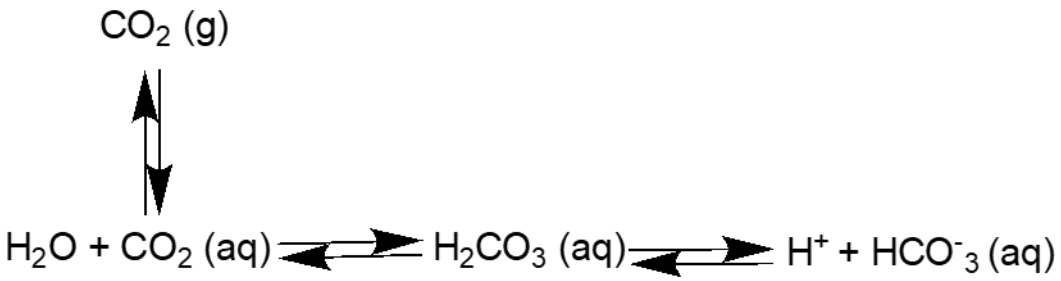
244 There was very low drug release in 0.1 M HCl for 2 h for the enteric-coated microparticles (0.6
245 ± 0.02 %) and pellets (0.7 ± 0.6 %). For the commercially available prednisolone enteric-coated
246 tables, 10 ± 1.2 % drug release was detected at the end of the 2 h acid stage test.

247 In pH 6.8 phosphate buffer, drug release was immediate after 2 h acid treatment for the
248 microparticles, pellets and tablets (**Fig. 4**) with no significant differences between all three
249 formulations ($p > 0.05$). All formulations reached 85 % drug release within 20 min (**Tab. 3**).
250 In pH 6.8 bicarbonate buffer, the drug release lag time and t_{85} were much longer for pellets and
251 tablets compared to that in phosphate buffer (significantly different for both $p < 0.05$) (**Fig. 5**,
252 **Tab. 3**). In comparison, much shorter lag time was shown for the microparticles than pellets
253 and tablets (significantly different to both $p < 0.05$) (**Fig. 5**, **Tab. 3**). For pellets and tablets, the
254 drug release lag time and t_{85} were longer in bicarbonate buffer pH 6.2-6.8 than that in

255 bicarbonate buffer pH 6.8 (significantly different for both $p < 0.05$) (**Fig. 6**, **Tab. 3**) and the
256 microparticles again showed shorter lag time and t_{85} than pellets and tablets (significantly
257 different to both $p < 0.05$) (**Fig. 6**, **Tab. 3**).

4 Discussion

Physiological bicarbonate buffers can provide superior prediction of *in vivo* behaviour of certain pharmaceutical products in comparison to compendial phosphate buffers. Several studies have reported better *in vivo in vitro* correlations of enteric coated products using bicarbonate buffers than phosphate buffers (Liu and Shokrollahi, 2015; Merchant et al., 2014b; Varum et al., 2014). Jede et al showed that using biorelevant bicarbonate buffers improved prediction of *in vivo* supersaturation and precipitation of poorly soluble weakly basic drugs than phosphate buffers (Jede et al., 2019). However, the instability of bicarbonate-based buffers caused by the evaporation of CO_2 gas during the dissolution testing presents a barrier for its use as an *in vitro* tool. A progressive increase in media pH was noted during *in vitro* dissolution testing using bicarbonate buffers (Garbacz et al., 2014), as explained by Equation 5.



Eq. 5

Bubbling CO_2 (g) under the surface of the media compensates this loss and decreases the media pH, whereas, purging an inert gas e.g. N_2, helium or a mixture of these gases with air can remove the dissolved CO_2 in the solution and thus increase media pH (Garbacz et al., 2014). Applying this concept, automated systems (e.g. the pHysio-stat®, pHysio-grad® and the Auto pH system™) have been made available to provide a practical solution to regulate and stabilise the pH bicarbonate buffers and to simulate the pH gradients in the human intestinal lumen (Garbacz et al., 2014; Merchant et al., 2014b). Purging gases using these devices have been utilised by recent studies applying bicarbonate buffer-based dissolution methods (Jede et al., 2019; Karkossa and Klein, 2017; Shibata et al., 2016).

There are issues associated with bubbling gases into the bicarbonate-based dissolution media. Firstly, it causes disturbance to the hydrodynamics of the dissolution media, potentially affecting the drug release rate. It was suggested that the gas diffuser should be placed in the upper part of the dissolution medium during purging to minimise the effect on drug release, as the test samples such as tablets are usually located at the bottom of the dissolution vessel (Garbacz et al., 2014). However, the extent of the impact of gas sparging on drug release remains unclear. Boni et al observed greater movements of multiparticulate formulations (pellets) in the dissolution medium caused by bubbling CO_2 into bicarbonate buffers than that caused by the use of phosphate buffers (Boni et al., 2007), which could potentially increase drug release rate. Secondly, gas bubbling into bicarbonate buffers can cause foaming when biorelevant media, e.g. FaSSIF and FeSSIF, were used which contain bile salts and lecithin (Amaral Silva et al., 2019; Boni et al., 2007). Recently Jede et al reported the use of "biorelevant bicarbonate buffer" by adding FaSSIF powder into 22.5 mM bicarbonate buffer with pH regulated using the pHysio-grad® (Jede et al., 2019). The authors did not discuss whether any foaming was observed.

Boni et al reported a method of supplying CO_2 above the bicarbonate solution to maintain the pH value of the medium during dissolution testing (Boni et al., 2007). This approach was not effective because of the open design of the conventional dissolution vessel lids for USP I and USP II apparatus which cannot prevent escape of the supplied gas. Fadda et al. investigated methods providing complete sealing of the bicarbonate media solution including application of a layer of liquid paraffin and a complete sealed lid of the dissolution vessel to prevent gas escape (Fadda et al., 2009). These static methods were effective in stabilising the media pH but cannot provide dynamic pH regulation. In this study we used a specially designed partial enclosure system that was fitted onto the dissolution vessel (USP I and USP II) to prevent gas escape and improve the efficiency of gas supply. Gases (CO_2 or N_2) were supplied into the gas

304 inlet of the device and released via multiple outlets to generate even distribution of the gas
305 above the dissolution medium and maximise the contact of the gas with the solution. This
306 effectively created a micro-environment with increased partial pressure of CO_2 or N_2 to
307 facilitate gas diffusion into the medium and achieve pH regulation.

308 The partial pressure of a gas was considered as the amount of gas which can diffuse into a
309 solution from the surface interface above the liquid (Kotz JC et al., 2012). According to Henry's
310 law, the concentration of a gas dissolved in a solvent is proportional to the partial pressure of
311 the gas (Equation 6) (Henry, 1803).

312 $$P_i = H_{ij} x_i \qquad \text{Eq. 6}$$

313 Where P_i is the partial pressure of component *i* in the gas; H_{ij} is the Henry's law constant for
314 solute *i* in solvent *j* and X_i is the mole fraction of component *i* in the liquid. As part of Henry's
315 law, Dalton's law describes the partial pressure of a gas in a gas mixture (Equation 7) (Smith
316 and Missen, 2005):

317 $$P_i = P_{total} x_i \qquad \text{Eq. 7}$$

318 Where P_i is the partial pressure, P_{total} is the sum of pressures for the mixture of gases and x_i is
319 the mole fraction of the gas of interest in the total mixture of gases. In this study, the enclosure
320 system used was not completely sealed and therefore the P_{total} above the dissolution medium
321 was considered to be constant and equivalent to the atmosphere pressure. The partial pressure
322 of a given gas above the dissolution medium, e.g. CO_2 or N_2, was therefore proportional to its
323 mole fraction in the gas mixture. During dissolution testing, the head space of the dissolution
324 vessel was initially filled with air, a mixture of the ideal gases N_2, oxygen, argon and CO_2 plus
325 water vapour and various trace components. When CO_2 or N_2 was supplied through the
326 enclosure system to the head space, the partial pressure of the respective gas increases and thus
327 increasing its concentration dissolved in the medium.

328 Supplying CO_2 through the enclosure system was much more efficient in decreasing the
329 medium pH (0.1 bar of the gas was used) than supplying N_2 in increasing the pH (3-4 bar was
330 required). The two gases, CO_2 and N_2, have the same solubility at 37 ºC (0.0014 g/kg) (Smith
331 and Missen, 2005). However, the dissolved CO_2 (aq) interacted with water directly generating
332 carbonic acid which dissociated and released hydrogen ion resulting in pH decrease (Eq.5). In
333 comparison, the effect of N_2 supply was indirect which functioned by reducing the partial
334 pressure of CO_2 in the gas mixture above the dissolution medium. This in turn decreased the
335 dissolved CO_2 concentration in the medium, moving Equation 5 to the left and thus increasing
336 the pH. This process was much slower than that related to CO_2 supply. Suppling N_2 gas at
337 higher pressure into the enclosure system resulted in faster medium pH increase, which can be
338 explained by Henry's law and Dalton's law. The angle of gas outlet to the surface of the
339 solution affected the rate of pH change. It is likely that distributing the gas vertically towards
340 the surface of the medium solution promoted better contact of the gas to the solution than
341 supplying the gas in an angle. Once diffused through the surface of the media, gases were
342 homogeneously distributed in the dissolution vessel and the pH values were uniform
343 throughout the vessel. Preliminary observations showed that bubbling gases into the
344 bicarbonate buffer incorporating FaSSIF and FeSSIF powder caused foaming which was not
345 the case when the gases were supplied through the enclosure device, showing compatibility
346 with biorelevant media.

347 Compendial dissolution method for testing delayed release (enteric coated) formulations
348 includes a single-vessel media change from acidic condition (0.1 M HCL) to pH 6.8 phosphate
349 buffer. No similar method was reported using bicarbonate buffer solutions. In this study we
350 applied a reproducible method to achieve single-vessel media change for two-stage dissolution
351 testing in bicarbonate buffers. This method is particularly useful for testing multiparticulate-
352 based pharmaceutical products, including liquids with suspended microparticles, Multi Unit

353 Pellet Systems (MUPs), powder for reconstitution and granules in capsules which are
354 considered suitable for use in paediatric and geriatric patients (Liu et al., 2014). When
355 bicarbonate buffer is used for testing delayed release formulations, a complete media change
356 (discarding the acidic medium and replacing with bicarbonate buffer) is usually carried out,
357 which faced challenges in full recovery of the multiparticulate units during media transfer
358 especially when USP II (paddle) method is required (Liu and Shokrollahi, 2015). The current
359 titration method reproducibly increased the medium pH and changed the ionic composition of
360 the medium from stomach to intestinal conditions with comparable buffer capacities to
361 previous reports at pH 6.8 and pH 6.2 (Merchant et al., 2014b; Varum et al., 2014).

362 During gastrointestinal transit, the acidic content arriving from the stomach is neutralized by
363 bicarbonate secreted into the duodenum by the pancreas, resulting in a drastic increase in the
364 pH value from the stomach to the duodenum. The luminal pH of the proximal small intestine
365 usually lies within the range of 5.5 to 7.0, gradually increasing to 6.5-7.5 in the distal ileum
366 (Evans et al., 1988). The pH gradient in the small intestine determines the time and site of the
367 dissolution of enteric-coated dosage forms based on pH-dependent coatings. Several authors
368 have reported simulation of the pH changes in the intestine using *in vitro* dissolution set up
369 applying bicarbonate based media (Garbacz et al., 2014; Goyanes et al., 2015b; Merchant et
370 al., 2014b; Wulff et al., 2015). Recent studies by Karkossa et al. applied *in vitro* methods
371 mimicking the *in vivo* gastrointestinal transits and physiological conditions of individual
372 subjects (Karkossa and Klein, 2018, 2019). In comparison to the reported pH-regulation
373 methods by bubbling gases (Garbacz et al., 2014; Merchant et al., 2014a), the pH response of
374 the current method using the enclosure system in relation to gas supply was slower because of
375 the time needed for gas diffusion through the media surface. Whilst the method was effective
376 in stabilising the pH at a certain level and showed potential in providing pH increase simulating
377 that of the proximal to mid small intestine, its efficiency and flexibility in providing wider pH

changes and offering individualised *in vitro* bio-prediction need to be investigated in future studies.

The bicarbonate based dissolution method reported in this study enabled the comparison of drug release from enteric coated prednisolone tablets, pellets and microparticles. Prednisolone was used as a model drug in this study to demonstrate the effect of changes in dissolution media on drug release from enteric coated dosage forms. As a neutral compound, prednisolone would have less effect on the dissolution process of enteric polymers than ionisable compounds. Similar to previous reports, drug release rates from all three enteric coated dosage forms were rapid in phosphate buffers with no significant differences between the dissolution profiles (Amaral Silva et al., 2019; Merchant et al., 2014b; Shibata et al., 2016). In bicarbonate buffers, a significant drug release lag time was noted for enteric coated tablets and pellets, which was again in agreement with published studies (Amaral Silva et al., 2019; Merchant et al., 2014b; Shibata et al., 2016). It was well documented that the dissolution of enteric polymers containing carboxylic groups was dependent on the composition of the dissolution media including the buffer species, molarity and ionic strength (Amaral Silva et al., 2019; Boni et al., 2007; Karkossa and Klein, 2017; Ozturk et al., 1988; Spitael and Kinget, 1977). Being a much weaker buffer, the ability of bicarbonate buffer (apparent pKa ~ 6.04) to facilitate the polymer dissociation is lower than that of phosphate buffer (pKa = 7.19) (Boni et al., 2007). Recently Al-Gousous et al reported that the effective pKa of bicarbonate in the boundary layer between the dissolving polymer and water is lower than its reported apparent pKa (Al-Gousous et al., 2019). This could cause poor capability of bicarbonate buffer to remove the hydrogen ions and maintain the surface pH of the dissolving polymer for the dissolution to continue, which could further explain the slow dissolution of enteric polymers in bicarbonate buffers leading to long drug release lag times.

In contrast to enteric coated tablets and pellets, enteric coated microparticles displayed rapid drug release (short lag time and fast release rate) in bicarbonate buffers similar to that in the phosphate buffer. This accelerated drug release from microparticles in bicarbonate buffers may be explained by the large specific surface area available for polymer dissolution. The specific surface area of enteric coated microparticles used in this study were 6-7 times higher than that of pellets, which could lead to proportional increase in polymer dissolution rate according to Noyes and Whitney equation (Noyes and Whitney, 1897), and thus rapid onset of drug release. It needs to be pointed out that the enteric polymers used in the commercial tablet (polyvinyl acetate phthalate) was different from that used in the pellets and microparticles (Eudragit® L 30 D -55). It was reported previously that drug release from prednisolone tablets coated with different enteric polymers showed varied lag times and longer lag time was observed from polyvinyl acetate phthalate coated tablets than that from tablets coated with Eudragit® L 30 D -55 (Liu et al., 2011). However, all tablet formulations showed a minimum of 30 min delay before the onset of dissolution in bicarbonate buffers (Liu et al., 2011), in agreement with the findings of the current study. Furthermore, the dissolution processes from enteric coated tablets and multiparticuates were different. For enteric coated tablets, polymer dissolution in buffer led to tablet disintegration and subsequent drug release (Ozturk et al., 1988). The pellets and microparticles used in this study were based on microcrystalline cellulose cores that did not disintegrate. The onset of drug release could relate to the formation of cracks in the coating caused by polymer dissolution and thinning of the coating layer (Liu et al., 2009). Unlike the spherical shape of the multiparticulate cores, tablets are not geometrically spherical, resulting in variations in coating uniformity on different areas of the tablet. The coating at the edges of the tablet surface was observed to be thinner than that at the center of the tablet surface, contributing to faster polymer dissolution in these areas and tablet disintegration (Niwa et al., 2014). These differences in the tablet and multiparticulate formulations make direct

comparison of their dissolution behaviour a challenge; however, the marked acceleration of drug release from the microparticle formulation in comparison to pellets and tablets demonstrated its potential in improving *in vivo* performance of enteric coated products.

The delayed onset of drug release from enteric coated tablets and pellets in *in vitro* dissolution testing using bicarbonate buffers reflects the *in vivo* performance of these dosage forms. Up to 2 h delays were reported for enteric coated tablets and pellets to disintegrate post gastric emptying *in vivo* (Bogentoft et al., 1984; Ebel et al., 1993; HARDY et al., 1987; Liu and Basit, 2010). Clinically, this caused retarded absorption and onset of action of the active ingredient, or even ineffective therapy, as reported in the cases of enteric coated pancreatic enzymes and aspirin (Guarner et al., 1993; Jirmář and Widimský, 2018). Research has been carried out in speeding up the dissolution of enteric coated formulations in the proximal small intestine, for example the design of a double-coating system (DuoCoat^{TM}) that has a buffered inner coat to accelerate the dissolution of the outer enteric coating (Liu and Basit, 2010). The findings of this study showed that microparticles that provide large specific surface areas for dissolution could be another approach in effective delivery of drugs to the proximal small intestine.

Research has made significant progress in the application of physiological bicarbonate buffers for *in vitro* dissolution testing of pharmaceutical products, especially facilitated by the availability of automated pH regulation systems. Recent development in 3D printing for personalised and patient-centric medicines has broadened the application of bicarbonate-based systems on dissolution testing of 3D printed modified release formulations including tablets, Printlets, Miniprintlets and Caplets (Awad et al., 2019; Fina et al., 2018; Goyanes et al., 2015a; Goyanes et al., 2016; Goyanes et al., 2015c; Vithani et al., 2019; Wang et al., 2016). However, current pH-regulation of bicarbonate buffers by bubbling gases can potentially change hydrodynamics of the dissolution medium and affect the compatibility with bio-relevant media. The preparation of bicarbonate buffers for media change of the two-stage dissolution testing

452 can be tedious and impractical for testing multiparticulate formulations. In this study, we
453 designed the novel enclosure system that avoided gas bubbling into the medium and the
454 associated disruption to the hydrodynamics of the testing system, meeting the compendial
455 requirement. It was compatible with biorelevant media providing wider range of applications
456 for bicarbonate-based dissolution systems. The single-vessel media change method enabled the
457 use of bicarbonate buffers for two-stage dissolution testing of delayed release multiparticulate
458 formulations, which is timely considering the recent progress in developing patient-centric
459 formulations for paediatric and geriatric patients (Liu and Basit, 2010). Future study will apply
460 the dissolution system in testing a range of compounds and formulations to evaluate its
461 potential in providing bio-predictive dissolution for pharmaceutical products.

462 **5. Conclusions**

463 An innovative bicarbonate-buffer solution based dissolution system was successfully
464 developed which stabilised and regulated the pH of the medium through the novel design of an
465 enclosure system. The system eliminated the need of bubbling gases to the medium and thus
466 complied with compendial requirements and was compatible with biorelevant media. Media
467 change from stomach to intestinal conditions for testing delayed release products was achieved
468 by titration in a single vessel, facilitating the used of bicarbonate-based media in testing
469 multiparticulate dosage forms. The new dissolution system enabled the comparison of enteric
470 coated microparticles with pellets and tablets in bicarbonate buffers. The microparticles
471 showed much faster drug release (shorter onset time) in the physiological bicarbonate buffer
472 than tablets and pellets, indicating potential improvement in *in vivo* performance.

473 **6. Acknowledgement**

474

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605

606 **Figure legends**

607

608 **Fig. 1**. Schematic of enclosure and gas delivery

609 **Fig. 2**. pH-regulation through the enclosure system: (a) pH increase from pH 6.2 to 6.8 under
610 different N_2 gas pressures and outlet directions and b) pH stabilisation at pH 6.8 using N_2 (3-4
611 bar) and CO_2 (0.1 bar) gases.

612 **Fig. 3.** Scanning electron microscopy images of a coated a) microparticle and b) pellet

613 **Fig. 4.** Drug release from enteric coated prednisolone formulations in 0.1 M HCl and
614 subsequently in pH 6.8 phosphate buffer.

615 **Fig. 5.** Drug release from enteric coated prednisolone formulations in 0.1 M HCl and
616 subsequently in pH 6.8 bicarbonate buffer.

617 **Fig. 6**. Drug release from enteric coated prednisolone formulations in 0.1 M HCl and
618 subsequently pH 6.2-6.8 bicarbonate buffer.

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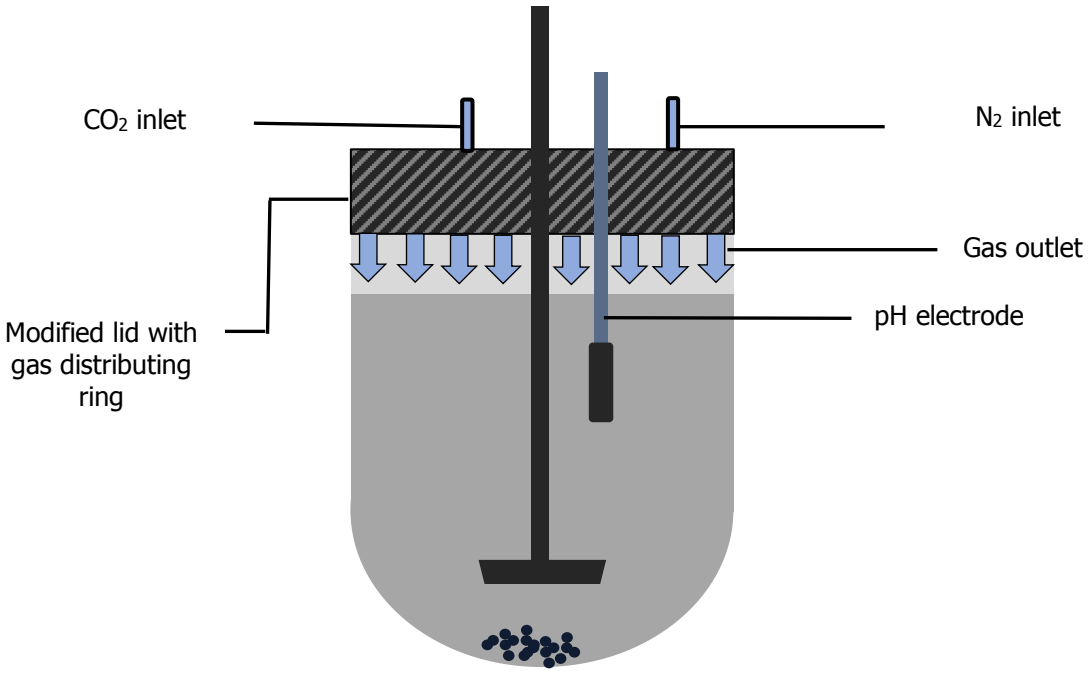

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621

622 Figure 1

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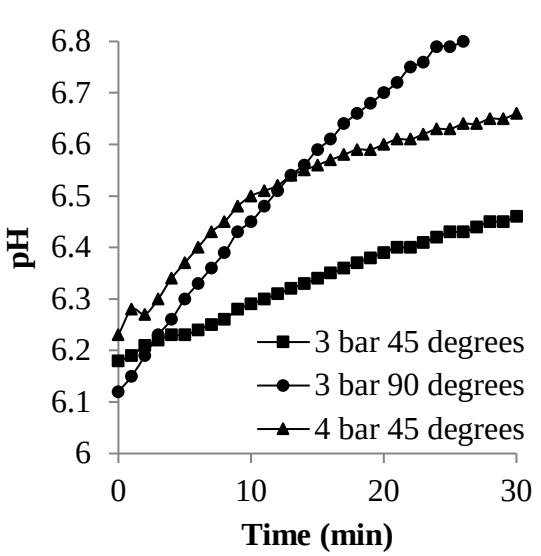
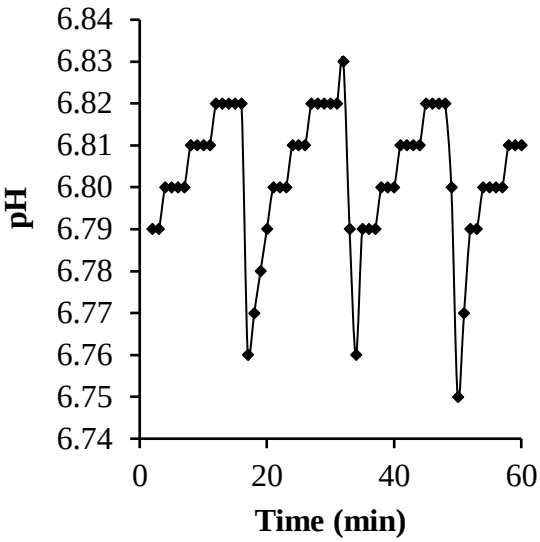
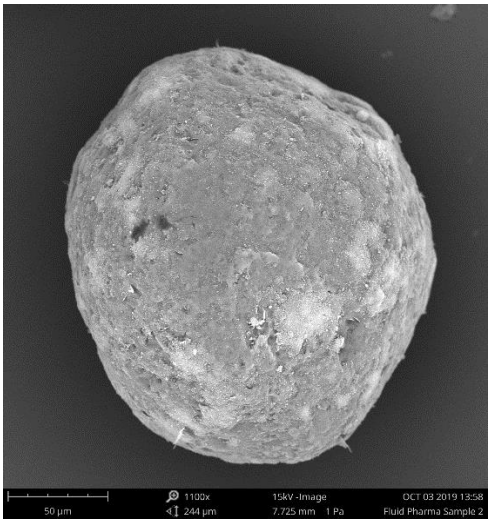

6.8
6.7
6.6
6.5
6.4
6.3
6.2
6.1
6
pH
0
10
20
30
Time (min)
3 bar 45 degrees
3 bar 90 degrees
4 bar 45 degrees

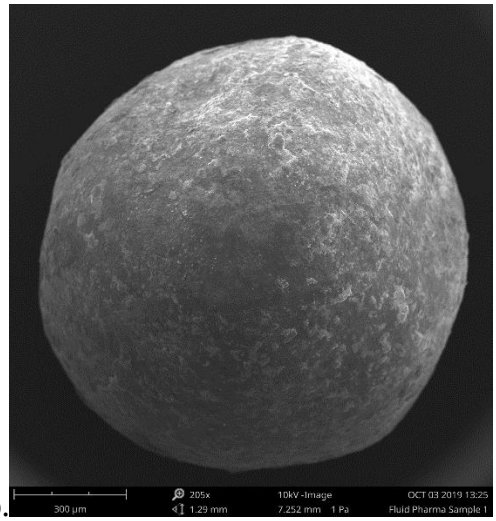
a.


6.84
6.83
6.82
6.81
6.80
6.79
6.78
6.77
6.76
6.75
6.74
pH
0
20
40
60
Time (min)

b.

Figure 2

a. 

b. 

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629 Figure 3

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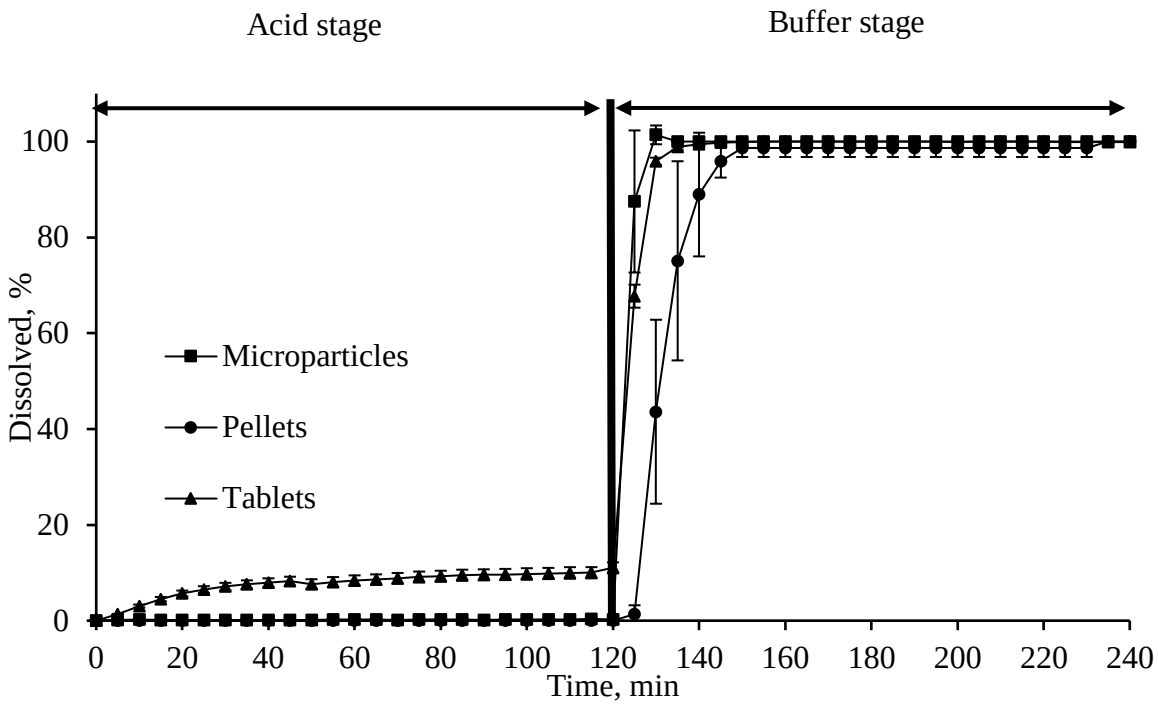

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634 Figure 4

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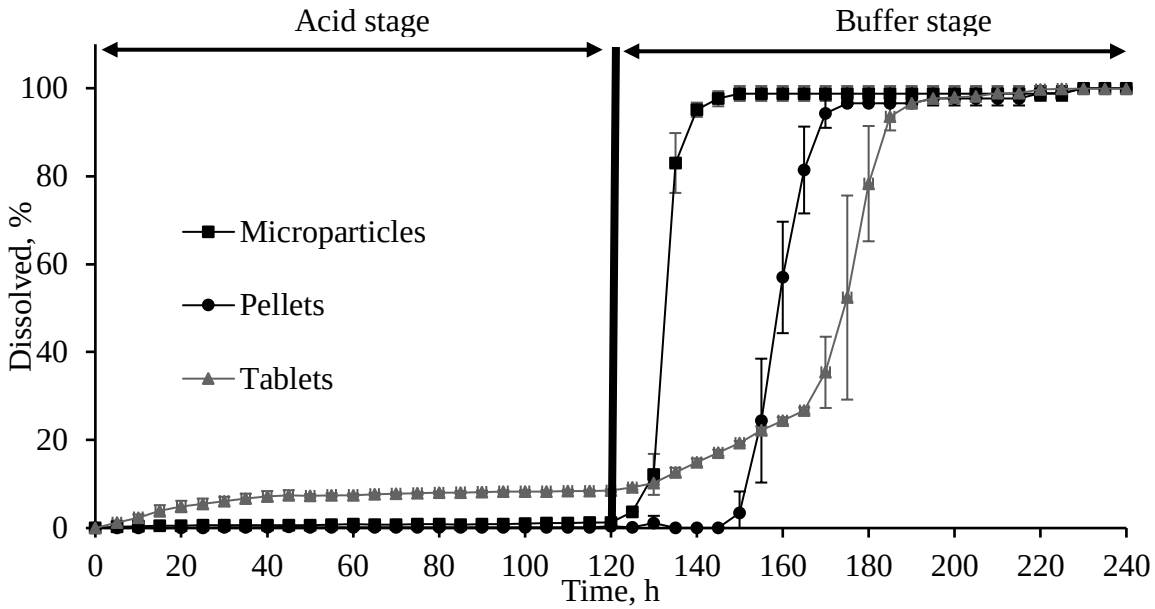

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639 Figure 5

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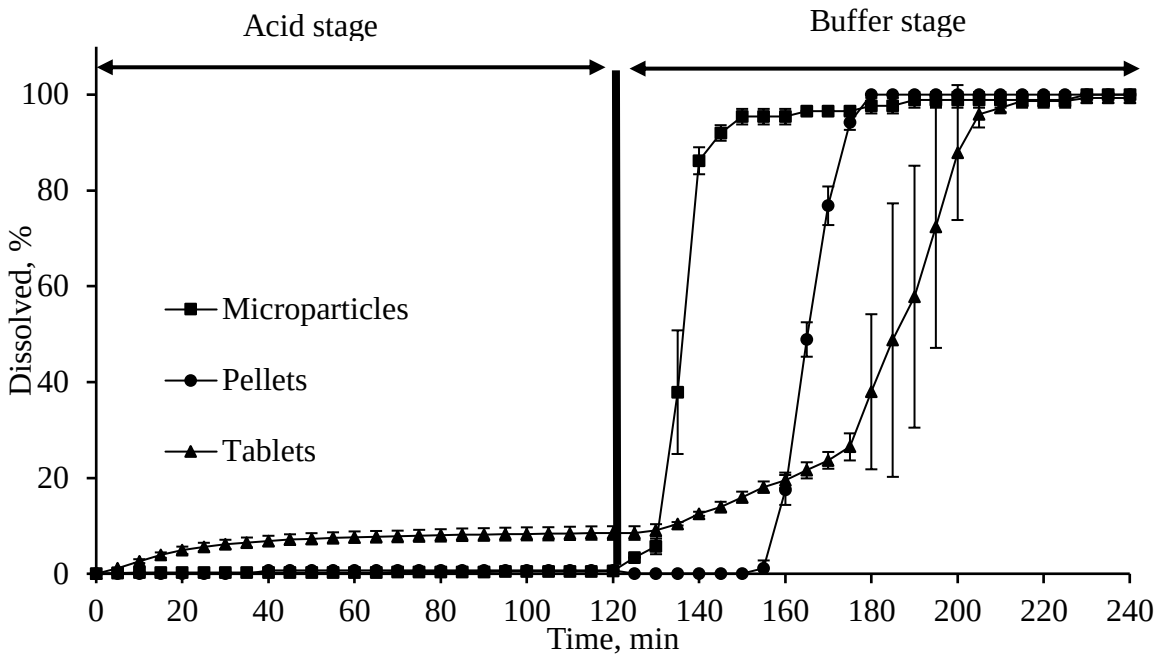

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643 Figure 6

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Tab. 1. Repeatability of intermediate and final pH adjustment during media change using 0.65 M NaOH for pH 6.2 bicarbonate buffer.

Repetition (n)	Starting pH (acid stage)	Intermediate pH (after NaOH addition)	Final pH (buffer stage)
1	0.76	2.12	5.99
2	0.76	2.17	6.11
3	0.76	2.23	6.16
4	0.69	2.25	6.28
5	0.64	2.20	6.28
6	0.64	2.22	6.30
7	0.83	2.09	6.11
8	0.67	2.21	6.17
Mean	**0.72**	**2.19**	**6.18**
SD	**0.06**	**0.05**	**0.10**

Tab. 2. pH measurements at different locations of the dissolution vessel.

Distance from bottom of vessel	1	2	3	Mean	SD
5 cm	6.79	6.81	6.81	6.80	0.01
9 cm	6.78	6.81	6.81	6.80	0.02
11 cm	6.78	6.8	6.81	6.80	0.02
Mean	6.78	6.81	6.81		
SD	0.01	0.01	0.00		

Tab. 3. The t_{lag} (min) and t_{85} (min) from enteric coated prednisolone microparticles, pellets and tablets in pH 6.8 phosphate buffer, pH 6.8 bicarbonate buffer and pH 6.2 - 6.8 bicarbonate buffer after acid treatment.

Buffer Solution	**Microparticles**		**Pellets**		**Tablets**	
	t_{lag}	t_{85}	t_{lag}	t_{85}	t_{lag}	t_{85}
pH 6.8 phosphate buffer	0.00	5.00	0.00	20.00	0.00	10.00
pH 6.8 bicarbonate buffer	2.35	15.00	24.08	45.00	37.09	65.00
pH 6.2-6.8 bicarbonate buffer	6.26	20.00	33.96	55.00	43.49	80.00