Accepted Manuscript

Title: Colonic Delivery of Indometacin Loaded PGA-co-PDL Microparticles Coated with Eudragit L100-55 from Fast Disintegrating Tablets

Authors: Hesham M. Tawfeek, Ahmed A.H. Abdellatif, Thomas J. Dennison, Afzal R. Mohmmed, Younis Sadiq, Imran Y. Saleem

PII: S0378-5173(17)30777-9
DOI: http://dx.doi.org/doi:10.1016/j.ijpharm.2017.08.069
Reference: IJP 16922

To appear in: *International Journal of Pharmaceutics*

Received date: 31-5-2017
Revised date: 2-8-2017
Accepted date: 8-8-2017

Please cite this article as: Tawfeek, Hesham M., Abdellatif, Ahmed A.H., Dennison, Thomas J., Mohmmed, Afzal R., Sadiq, Younis, Saleem, Imran Y., Colonic Delivery of Indometacin Loaded PGA-co-PDL Microparticles Coated with Eudragit L100-55 from Fast Disintegrating Tablets.International Journal of Pharmaceutics http://dx.doi.org/10.1016/j.ijpharm.2017.08.069

Colonic Delivery of Indometacin Loaded PGA-co-PDL Microparticles Coated with Eudragit L100-55 from Fast Disintegrating Tablets

Hesham M. Tawfeek[1#], Ahmed A. H. Abdellatif [2,5], Thomas J. Dennison[3], Afzal R. Mohmmed[3], Younis Sadiq[4] and Imran Y. Saleem[4]*

[1]Department of Industrial Pharmacy, Faculty of Pharmacy, Assiut University, Assiut, Egypt.

[2]Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Al-Azhar University, Assiut, Egypt.

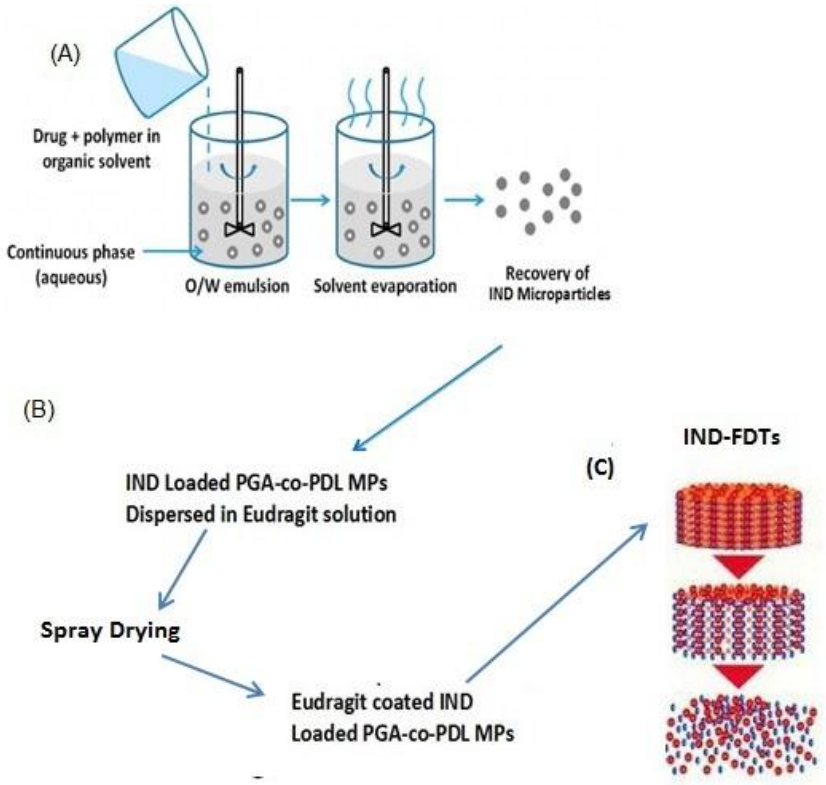
[3]Aston School of Pharmacy, Aston University, Birmingham, UK

[4]School of Pharmacy and Biomolecular Science, Liverpool John Moores University, Liverpool, UK.

[5]Department of Pharmaceutics, Faculty of Pharmacy, Qassim University, Buraydah, Kingdom of Saudi Arabia.

[*]*Corresponding Authors*: Dr Imran Saleem (Liverpool John Moores University, School of Pharmacy & Biomolecular Sciences, Byrom Street, Liverpool L3 3AF, UK; Telephone: +44-151-231-2265; Fax: +44-151-231-2170; E-mail: i.saleem@ljmu.ac.uk
[#]Dr. Hesham M. Tawfeek (Assiut University, Faculty of Pharmacy, Department of Industrial Pharmacy, Assiut, Egypt; Telephone: +2-088-241-1290; Fax: +2-088-2080774; E-mail: hesham.hassan@pharm.au.edu.eg

Graphical Abstract



Abstract:

The aim of this work was to investigate the efficient targeting and delivery of indometacin (IND), as a model anti-inflammatory drug to the colon for treatment of inflammatory bowel disease. We prepared fast disintegrating tablets (FDT) containing IND encapsulated within poly(glycerol-adipate-co-ω-pentadecalactone), PGA-co-PDL, microparticles and coated with Eudragit L100-55 at different ratios (1:1.5, 1:1, 1:0.5). Microparticles encapsulated with IND were prepared using an o/w single emulsion solvent evaporation technique and coated with Eudragit L-100-55 via spray drying. The produced coated microparticles (PGA-co-PDL-IND/Eudragit) were formulated into optimised FTD using a single station press. The loading, *in vitro* release, permeability and transport of IND from PGA-co-PDL-IND/Eudragit microparticles was studied in Caco-2 cell lines. IND was efficiently encapsulated (570.15±4.2 µg/mg) within the PGA-co-PDL microparticles. *In vitro* release of PGA-co-PDL-IND/Eudragit microparticles (1:1.5) showed significantly ($p<0.05$, ANOVA/Tukey) lower release of IND 13.70±1.6 and 56.46±3.8 % compared with 1:1 (89.61±2.5, 80.13±2.6 %) and 1:0.5 (39.46±0.9 & 43.38±3.12) after 3 and 43 h at pH 5.5 and 6.8, respectively. The permeability and transport studies indicated IND released from PGA-co-PDL-IND/Eudragit microparticles had a lower permeability coefficient of $13.95\pm0.68\times10^{-6}$cm/s compared to free IND $23.06\pm3.56\times10^{-6}$cm/s.

These results indicate the possibility of targeting anti-inflammatory drugs to the colon using FDTs containing microparticles coated with Eudragit.

Key words: Eudragit L100-55, PGA-co-PDL, fast disintegrating tablets, indometacin, microparticles, spray-drying, colon targeting

1 Introduction

Drug targeting to specific regions of the gastrointestinal tract is still under focus of extensive research to achieve an effective and patient attractive formulation with an enhanced therapeutic effect. Moreover, it is challenging to design a drug delivery system, DDS, able to deliver drugs to the specific site of action and at the right period of time (Mishra et al., 2010). One of the most important diseases which affect the gastro-intestinal tract, GIT, is the inflammatory bowel disease (IBD), which is an inflammatory condition of the colon and small intestine such as Crohn's disease (CD) and ulcerative colitis (UC) (Cosnes et al., 2011). People suffering from IBD require the frequent intake of anti-inflammatory drugs at high doses, which are associated with significant adverse effects (Abdellatif et al., 2016; Santino et al., 2017). Therefore, different methods and approaches have been studied to develop oral colon DDS, among them series of enteric soluble cellulose polymers and methacrylic acid co-polymers have been investigated (Donini et al., 2002; Silva et al., 2009; Wang et al., 2010). However, targeting to the colon using enteric coated formulations has many drawbacks such as dose dumping in the small intestine leading to intestinal damage as well as the frequent administration which reduces patient compliance (Hao et al., 2014). Moreover, in many cases there is a lower efficiency due to diarrhoea, a symptom of IBD that enhances drug elimination

and affects its release time (Lamprecht et al., 2004). DDS, incorporating microparticles and nanoparticles could enhance the drug bioavailability compared to matrix systems, and thus reduce the drug dose and side effects as well as the potential of any systemic toxicity. Previous attempts to produce microparticles with enteric and extended release pattern using emulsion solvent evaporation have been demonstrated (Alhnan et al., 2011). In addition, they showed better *in vivo* performance, less irritation and reproducible drug release compared with single unit systems (Beloqui et al., 2016; Duan et al., 2016; Rashid et al., 2016; Youshia and Lamprecht, 2016). Furthermore, these systems are capable of passing through the GIT easily due to their small particle size, leading to less inter-intra-subject variability.

The techniques used to deliver drugs to the colon are reliant on the variation of the pH through the GIT (Makhlof et al., 2009; McConnell et al., 2008). One of the most commonly used pH-dependent polymers for colon delivery is the methacrylic acid co-polymers, Eudragit L100-55, which is widely used for different oral colon drug delivery targeted dosage forms (tablet coating, tablet matrix, microspheres and nanoparticles) (Chickpetty et al., 2011; Jelvehgari et al., 2010a; Moustafine et al., 2006; Oosegi et al., 2008; Zakeri-Milani et al., 2009). Furthermore, it is used to control drug release within the pH 4 to 6 range, hence can be used to deliver drugs in the treatment of IBD due to the extremely acidic colon environment (Friend, 2005; Sasaki et al., 1997). In addition it has mucoadhesive properties (Wang and Zhang, 2012; Yamanaka and Leong, 2008), which ensures the coated particles remain at the site of action and are not affected by diarrhoea associated with IBD.

Poly(glycerol-adipate-co-ω-pentadecalactone), PGA-co-PDL, is biodegradable polyester studied as a carrier for small and large molecular weight compounds ((Tawfeek et al., 2014; Alfagih et al., 2015; Kunda et al., 2015a; Kunda et al., 2015b; Kunda et al., 2015c; Tawfeek et al., 2011a; Tawfeek et al., 2013)). Fast disintegrating tablets, FDTs, or orodispersible tablets are intended for administration to patients with difficulty in swallowing, such as the elderly and paediatric patients. Hence, FDTs have a rapid disintegration, drug release, achieve improved bioavailability and fast absorption (El Maghraby and Elsergany, 2014). Moreover, FDTs containing multiparticulate drug delivery systems, such as nanoparticles or microparticles have been studied for oral delivery of drugs, such as acetazolamide and scopolamine (Li et al., 2011; Preeti et al., 2014) and prednisolone (Chen et al., 2015).

The aim of the current work was to study the applicability of novel formulation design to deliver indometacin (IND) as a model anti-inflammatory drug for the treatment of IBD. This was performed through encapsulating IND within PGA-co-PDL microparticles (MPs) coated

with Eudragit L100-55 (PGA-co-PDL/Eudragit MPs). The Eudragit L100-55 will begin to dissolve at pH ≥ 5.5, and the PGA-co-PDL acts as a controlling matrix for sustained release of IND. Such design will control and extend the IND release at the site of inflammation. Finally, the prepared PGA-co-PDL/Eudragit MPs were incorporated into fast disintegrating tablets to facilitate their administration to patients i.e. elderly and paediatrics.

2 Materials and Methods

2.1 Materials

Indometacin (γ form) was purchased from Sigma-Aldrich (Cairo, Egypt). Sodium starch glycolate, SSG, magnesium stearate, cross-linked sodium carboxymethyl cellulose, Ac-Di-Sol, Transwell (Corning) semi-permeable membrane supports (12 well, 1.12 cm^2, 0.4 μm pore size) were obtained from Fisher Scientific, UK. PGA-co-PDL (15 KDa) was synthetized in our lab (LJMU, UK). Eudragit L100-55 was obtained from Evonik, GmbH, Germany. Anhydrous lactose was obtained from DFE pharma. All other chemicals and reagents were used as received.

2.2 Methods

2.2.1 Preparation of Eudragit coated PGA-co-PDL microparticles

PGA-co-PDL MPs encapsulated with IND non-coated and coated with Eudragit L100-55 (PGA-co-PDL/Eudragit) were prepared by single emulsion/solvent evaporation technique followed by spray drying. PGA-co-PDL MPs were prepared as previously reported (Tawfeek et al., 2011a). Briefly, 50 mg IND and 450 mg PGA-co-PDL (15 KDa) was dissolved in 5 ml dichloromethane (DCM). The organic layer was emulsified in 10 ml water containing 0.1% polyvinyl alcohol (PVA) using a IKA yellow line DI 25 basic homogeniser at 8000 rpm for 40-50 s. This o/w emulsion was left to mix with a Silverson L4 RT mixer at 2000 rpm for 3 h to allow for DCM evaporation under room temperature. The particles obtained were collected by centrifugation (EBA 20, Hettich) at 6000 x g for 10 min at room temperature and washed with PBS buffer and centrifuged again. PGA-co-PDL/Eudragit MPs were prepared by dissolving the calculated amounts of Eudragit L100-55 (1:0.5, 1:1 and 1:1.5 PGA-co-PDL: Eudragit, wt/wt ratio) in 20 ml PBS solution with pH value of 7.0±0.2. PGA-co-PDL MPs were suspended in Eudragit solution, mixed for 2 mins, and spray dried utilizing a mini-spray dryer (Büchi, B-290 Flawil, Switzerland) according to previous method (Tawfeek, 2013). The dry particles (PGA-co-PDL/Eudragit) were separated from the air stream using a high-performance

cyclone (Büchi Labortechnik), and the dry particles were collected and stored in desiccator until further use. Spray dried particles were also prepared using PGA-co-PDL MPs alone.

2.3 Characterisation of PGA-co-PDL/Eudragit microparticles

2.3.1 Yield, Encapsulation efficiency and IND loading

Spray dried PGA-co-PDL/Eudragit MPs yields were quantified as the percentage mass obtained compared with the anticipated total powder yields (n=3). The encapsulation efficiency of IND loaded PGA-co-PDL/Eudragit MPs (n=3) were obtained by dissolving the particles in 2 ml DCM under mixing for 3 h followed by HPLC analysis. The chromatographic conditions were as follows: HPLC system Agilent 1100 series (Santa Clara, CA, USA) equipped with a column (Aeris 3.6 µm C4 200A Wide Pore 4.6 mm i.d. × 150 mm length), security cartridge of the same material (Phenomenex, UK); mobile phase was composed methanol: water 75:25% containing 0.2% ortho-phosphoric acid. The flow rate was adjusted to 1.5 ml/min; injection volume of 100 µl; temperature 23 °C; UV detection at λ_{max} of 372 nm. IND calibration curve was prepared by accurate dilution of a previously prepared stock solution (1 mg/ml) in HPLC water and PBS (pH 7.4) to obtain the following concentrations: 0.5, 1, 2.5, 5, 7.5, 10, 15, 20, 25, 50, 70 and 100 µg/ml of IND (n=9, R2=0.998). PGA-co-PDL and Eudragit L100-55 did not interfere with the UV absorption of IND. Moreover, IND loading (µg/mg PGA-co-PDL) was also determined using equation 2.

$$\text{Encapsulation efficiency (\%)} = \frac{Actual\ weight\ of\ IND\ in\ sample}{Theoritical\ weight\ of\ IND\ added} \times 100 \qquad (1)$$

$$\text{IND loading (µg/mg polymer)} = \frac{Total\ amount\ of\ encapsulated\ IND\ (\mu g)}{Total\ amount\ of\ PGA{-}co{-}PDL\ (mg)} \qquad (2)$$

2.3.2 Powder X-ray diffraction

Powder X-ray diffraction (PXRD) patterns were determined using a RigakuMiniflex X-ray diffractometer. Samples were finely ground and packed into an aluminium sample holder. Patterns were collected between 5° and 50° 2θ, at increments of 0.02° 2θ, scanning speed 2°min^{-1}, voltage 30 KV, current 15 mA using CuKα (1.54 Å) radiation.

2.3.3 Differential scanning calorimetry

Differential scanning calorimetry thermograms were obtained using a Perkin Elmer DSC 8000 with Intracooler 2 cooling accessory and Pyris v. 10.1.0.0420 software (Seer Green, Buckinghamshire, UK). The furnace temperature was calibrated using the Perkin Elmer

supplied standard reference materials Indium (m.p. = 156.60 °C) and zinc (m.p. = 419.47 °C). Samples, 3–5 mg, were accurately weighed onto aluminium pans and sealed prior to heating at a constant heat rate of 20 $^{\circ}$C min^{-1} in a nitrogen atmosphere over a temperature range of 25 $^{\circ}$C–250 $^{\circ}$C.

2.3.4 Particles Morphology

PGA-co-PDL and PGA-co-PDL/Eudragit MPs were visualised by scanning electron microscopy (FEI–Inspect S Low VAC Scanning Electron Microscope). A suspension of particles in water was deposited on 13 mm aluminium stubs layered with a sticky conductive carbon tab and air-dried. An atomic layer of gold was deposited onto the particle containing stubs using an EmiTech K 550X Gold Sputter Coater, 25 mA for 3 min.

2.3.5 *In vitro* release

In vitro release of IND from PGA-co-PDL and PGA-co-PDL/Eudragit MPs at different weight ratios was performed in 0.1 N HCl, (pH 1.2) for 2 h, pH 5.5 for 3 h followed by pH 6.8 for the remainder of 48 h at 37 ± 0.5 °C using an automated Varian VK 7000 dissolution tester. The *in vitro* release was done according to the pH shift method to mimic the *in vivo* conditions using 0.2 M tribasic sodium phosphate (Hosny, 1996). Samples (5 mL) were removed from the dissolution vessels at specified time points and replaced with fresh buffer. The IND contents in the collected samples were measured using a UV spectrophotometer (Perkin Elmer) at λ_{max} of 372 nm.

2.4 Preparation and optimization of FDTs

FDTs were prepared using two different superdisintegrants namely; cros-carmellose, Ac-Di-Sol and sodium starch glycolate, SSG, at three different concentrations (5, 8 and 12% weight per total tablet weight). Briefly, the superdisintegrant and the diluent, lactose, were mixed for 15 min using a turbula mixer (W.A. Bachofen, Switzerland) and subsequently with 1% (w/w) lubricant, magnesium stearate, for another 5 min. Tablets were compressed using a single-station tablet Riva minipress at different compression forces 5, 10 and 15 KN and superdisintegrant concentrations as indicated in Table 1. The composition of the different prepared blank FDTs tablets is shown in Table 2.

The prepared FDTs were characterised and optimised in terms of weight uniformity and *in vitro* disintegration (USP pharmacopeia 2008). Moreover, the crushing strengths F (KN) were determined using a model 6D tablet tester (Dr.Schleuniger, Germany) and the wettability test was performed as previously reported (Jung et al., 2012). In addition, the effect of addition of

PGA-co-PDL and PGA-co-PDL/Eudragit MPs (10 %w/w of total tablet weight; 20 mg MPs containing 11.4 mg IND/tablet) on the crushing strength, wettability and the *in vitro* disintegration time of FDTs containing the optimum concentrations of SSG, 8 and 12% and compressed at 5 KN was also investigated and compared with blank FDTs.

2.5 Permeability and transport study

The permeability and transport study of IND from the prepared PGA-co-PDL/Eudragit MPs were studied in Caco-2 cell lines, grown on 12 well transwell semi-permeable membrane supports (1.12 cm^2, 0.4 µm pore size) for 21 days, using DMEM media. Permeability study was performed over 6 h at different time points of 30, 60, 120, 180, 240 and 360 mins (n=3). The transepithelial electrical resistance, TEER, values for each well were taken at t=0 and t=360 mins to monitor cell integrity over the experimental period. A positive control (IND alone), treatment 1 (IND loaded PGA-co-PDL/Eudragit, 1:1.5 wt ratio, MPs suspension) and treatment 4 (IND loaded PGA-co-PDL MPs emulsion) were applied to the apical (donor) side of the cells (500 µL) and samples taken at the time points aforementioned. In addition, negative control treatment 2 (PGA-co-PDL /Eudragit, 1:1.5 wt ratio, MPs suspension) and treatment 3 (PGA-co-PDL MPs emulsion) were sampled at t=360 and TEER values measured at t=0 and t=360 (n=3). 1.5 ml DMEM was added to the basolateral (acceptor) side of each well. IND alone was analysed and found to be 26.5 µg/ml IND, and treatment 1 and 4 were adjusted to give same concentration, based on the drug loading. The apical samples (100 µl) from each well were taken at t=0 to give the initial apical concentration and a final apical sample was taken at t=360 (100 µl). Basolateral samples (200 µl) were taken at the time points stated and immediately replaced with fresh DMEM to simulate sink conditions. Cumulative drug concentration was calculated from the data to account for this. Between sampling times points the cells were maintained in the incubator at 37 °C, 95% relative humidity and 5% CO_2.

Apparent permeability P_{app} (cm/s) was calculated according to the following equation:

$$P_{app} = \frac{dQ}{dt} \cdot \frac{1}{AC0} \qquad (3)$$

Where; d_Q/d_t is the rate of appearance of the drug on the basolateral side, C_0 is the initial concentration on the apical side and A is the surface area of the monolayer (cm^2).

2.6 Statistical analysis

All statistical analysis was performed using Minitab® 16 Statistical Software. One-way analysis of variance (ANOVA) with the Tukey's comparison was employed for comparing the formulations with each other. Statistically significant differences were assumed when $p<0.05$. All values are expressed as their mean ± standard deviation.

3 Results and Discussions

PGA-co-PDL/Eudragit MPs were produced to passively target the site of inflammation. This type of targeting based on the change in the physiological conditions of GIT will control the release of IND to the affected site especially with IBD patients. Moreover, the ulcerogenic and toxicity effects of IND will be minimised due to the encapsulation and coating processes and lower doses of IND used.

3.1. PGA-co-PDL Microparticle preparations

IND loaded PGA-co-PDL MPs were prepared via single emulsion (o/w) solvent evaporation technique. The prepared particles had an encapsulation efficiency and IND loading of 63.16±3.5 % and 570.15±4.2 µg/mg particle; respectively. Therefore, IND dose in the final prepared tablets was 11.40 mg/tablet (20 mg particles per tablet). Similar studies have reported low IND loading in microspheres (5.7 µg/mg particles) used for colon targeting prepared via spray drying (Jain et al., 2014; Lee et al., 2004) and an electrospray technique with encapsulation efficiency of 35.39±1.63 %, whereas in our study we achieved an encapsulation efficiency of 63.16±3.5 %. (Arvind K Jain et al., 2014). However, by increasing the amount of MPs in the FDTs a higher dose of IND would be obtained. For example, 40 mg MPs would contain approximately 24.0 mg IND that is similar to the conventional oral dose of IND (25 mg /Tablet). Corrigan *et al*., showed that IND had an encapsulation efficiency of 65.2±0.6 % during preparation of PLGA nanoparticles by emulsion solvent evaporation technique due to leaking to the external aqueous medium (Corrigan and Li, 2009). Moreover, the degree of polymer crystallinity affects the encapsulation efficiency, hence drugs will be encapsulated in the amorphous region of the semi crystalline PGA-co-PDL (Kim et al., 2005; Tawfeek et al., 2014). PGA-co-PDL/Eudragit MPs were efficiently prepared via spray drying with yields of (40.74±5.5, 43.33±4.3 and 55.7±6.7 % for 1:0.5; 1:1 and 1:1.5 PGA-co-PDL to Eudragit L100-55 weight ratios; respectively), which was similar to our previous reports (Tawfeek et al., 2011a).

3.2. PGA-co-PDL Microparticle/Eudragit characterisation

PXRD and Differential thermal analysis, DSC, techniques are used to study the crystal and thermal behaviour during processing and manufacturing. Furthermore, they provide information about the efficiency of coating of MPs as well as the degree of drug entrapment and crystallinity. The latter is an important consideration as the formation of drug crystals during microencapsulation process can have major impact on the property of final products (Alhnan and Basit, 2011; Nilkumhang et al., 2009). Furthermore, IND has 4 different polymorphs, γ-form (I), α-form (II), β-form (III) and δ-form (IV) (Dubbini et al., 2014). Hence, the polymorphic transitions should be considered during processing because different solid forms affect the physical properties e.g., solubility, physical stability, dissolution and bioavailability (Raina et al., 2014; Yoshinari et al., 2003; Zhang et al., 2004). PXRD showed characteristic peaks at 21.5° and 24.2°ɵ for PGA-co-PDL co-polymer (Fig.1; Trace A). Eudragit L100-55 has a characteristic amorphous structure (Shen et al., 2011), (Fig.1; Trace B). However, the non-coated PGA-co-PDL MPs loaded IND (Fig.1; Trace C) did not show the characteristic crystalline peaks of the IND γ-form ((Basavoju et al., 2008)), hence indicating efficient encapsulation of IND inside PGA-co-PDL MPs. However, the appearance of small peak at 27.28° ɵ was attributed to the formation of α-form of IND (Alhnan et al., 2010), which was confirmed using DSC (Fig. 2; Trace C). In addition, the reduced PGA-co-PDL peaks intensities was attributed to the processing steps and was also reported in our previous study using PGA-co-PDL encapsulated α-chymotrypsin and with PLGA nanoparticles entrapped cyclosporine (Tawfeek et al., 2014; Wagh and Apar, 2014). PGA-co-PDL/Eudragit MPs loaded IND (1:1.5 wt/wt ratio) showed complete disappearance of PGA-co-PDL characteristic peaks (Fig.1; Trace D) which was attributed to the efficient coating of Eudragit L-100-55 around PGA-co-PDL particles. Similar behaviour was also observed by (Nadal et al., 2016) who used Eudragit L100 to coat MPs containing ferulic acid using spray drying method.

DSC was performed to obtain more evidence about the coating and the thermal changes after spray drying and particles formulation (Fig. 2). The DSC results were in accordance with PXRD data. IND alone showed an endothermic crystalline peak at 160 °C due to drug melting which is characteristic for γ-form of IND (Basavoju et al., 2008) (Fig. 2; Trace A). PGA-co-PDL polymer showed the characteristic melting broad peak at 57.12 °C, indicating the outlet temperature of spray dryer did not exceed the low melting of the polymer (Tawfeek et al., 2011b). PGA-co-PDL showed an area under the endothermic peak of 269.04 mJ and heat of fusion (ΔH) of 60.59 J/gas as previously reported (Tawfeek et al., 2014). Moreover, Eudragit L100-55 (Fig 2; Trace B) showed two very broad endothermic peaks at 70.14 and 209.84 °C.

The first was due to side chain mobility (β-relaxation) and the second was due to the anhydride formation resulting from water evaporation during the heating process of the DSC scan leading to polymer decomposition. In addition, the glass transition temperature Tg which appeared at 120 °C represented the main polymeric linear chain mobility (α-relaxation) (Assaf et al., 2013). Non-coated PGA-co-PDL MPs loaded IND showed similar melting peak as PGA-co-PDL polymer but with a lower area under the peak 50.04 mJ and ΔH of 27.80 J/g. In addition, it was noticed the complete disappearance of IND melting endotherm which possibly attributed to the complete entrapment of IND inside the PGA-co-PDL MPs (Fig. 2; Trace C). Meanwhile, the appearance of the small DSC hump at 155 °C was most likely attributed to the formation of the α-form of IND, which is in accordance with the PXRD results (Fig. 1; Trace C). Spray drying process reflects the thermal changes during the particles formation and processing (Tawfeek et al., 2011a). This could be observed with the PGA-co-PDL melting endotherm which became broader and coupled with decrease in both the area under the endothermic peak and the ΔH values (Fig. 2; Traces, D-F). Hence, increasing the amounts of Eudragit led to a higher reduction in the melting behaviour of PGA-co-PDL.

3.3. Morphology and *in vitro* release of IND from PGA-co-PDL/Eudragit coated microparticles

Scanning electron microscope (Fig. 3A) showed spherical, large and smooth nature of spray dried blank Eudragit particles. Spray dried IND loaded PGA-co-PDL MPs appeared spherical, small, aggregated with smooth surface showing corrugations (Fig. 3B) due to excessive build up of vapour pressure during spray drying process as previously reported (Tawfeek, 2013). A similar morphology was obtained with IND loaded PGA-co-PDL/Eudragit MPs (Fig. 3C-E). Furthermore, it was found that by increasing the amount of Eudragit, larger particles were observed with the surface morphology becoming smooth (Fig. 3E), indicating efficient coating of Eudragit around the PGA-co-PDL MPs.

The *in vitro* release pattern (Fig. 4) was characterised by three phases, the lowest release phase was considered at pH 1.2. Non-coated PGA-co-PDL MPs released 31.91±1.7 % of IND after 2 h, whereas, PGA-co-PDL/Eudragit MPs with different Eudragit ratios, 1:0.5; 1:1 and 1:1.5 wt ratios, released 11.57±1.8, 4.53±0.85 and 2.43±0.75 % of IND at pH 1.2; respectively after the same period. Moreover, the burst release characterised by many biodegradable polymeric particles were not observed in the release of IND from Eudragit coated MPs, and this provided indication about the efficient coating and entrapment of IND inside the PGA-co-PDL/Eudragit MPs. Eudragit L100-55 a methacrylic acid – ethyl (1:1 ratio) acrylate co-polymer is widely

used in the oral formulations for colon targeting either alone or in combination with Eudragit S100 (Asghar et al., 2009; Cetin et al., 2010; Jelvehgari et al., 2010b; Shen et al., 2011). Eudragit L100-55 prevented the premature release of IND in upper GIT, since it is a pH sensitive polymer having threshold pH value above 6.0. Increasing the pH from 1.2 to 5.5 and lastly to 6.8 led to 94.12±3.6 % release of IND from non-coated PGA-co-PDL MPs. The higher release of IND from non-coated MPs at acidic medium was attributed to the release of the α-form of IND, which showed a reported higher dissolution rate compared to γ-form (Aceves-Hernandez et al., 2009; Hulse et al., 2012). However, at higher pH values, IND diffused from the polymer matrix through the developed pores and channels showing a higher dissolution as reported from its acidic nature (Ali et al., 2016). PGA-co-PDL/Eudragit MPs 1:1.5 wt. ratio showed significantly ($p<0.05$, ANOVA/Tukey) lower release of IND 13.70±1.6 and 56.46±3.8 % compared with 1:0.5 and 1:1 wt. ratios, 39.46±0.9 & 43.38±3.12 and 89.61±2.5, 80.13±2.6 % after 3 and 43 h at pH 5.5 and 6.8, respectively. Furthermore, once the Eudragit dissolved, IND still needs to be released from PGA-co-PDL MPs and hence the formulation was able to maintain a sustained drug release. Similar results occurred from domperidone ODTs solid dispersions with Eudragit L100-55 when released in 0.1N HCL and pH 6.8 (Assaf et al., 2013). Hence, IND had a sustained release pattern from PGA-co-PDL/Eudragit MPs. As the pH increased the polymer solubility increased (Khan et al., 2000) resulting in an increase in IND release, meanwhile the release was greatly reduced at acidic pH.

3.4. Optimizing the FDTs

Optimum blank FDTs, without MPs, were selected based on investigating the effect of different concentrations of SSG or Ac-Di-Sol on the mechanical properties of FDTs. Blank FDTs, were prepared by direct compression technique at different compression forces ranging from 5 to 15 KN. The prepared tablets had an average weight of 201±1.2, 198.5±0.7 and 202±2.5 mg for tablets containing 5, 8 and 10 % w/w of Ac-di-Sol and SSG, respectively. Fig. 5A & B showed that increasing the concentration of superdisintegrant, Ac-di-Sol or SSG, at the same force of compression led to a non-significant reduction in the crushing strength. This could be attributed to the interference of SSG or Ac-Di-Sol with the bonding property and/or the tablet porosity especially at higher superdisintegrant concentration. Also, higher superdisintegrant concentration led to a high hygroscopicity and porosity which facilitate water absorption resulting in a decreased tablet crushing strength (Fu et al., 2004).

The FDTs compressed at 5 KN and containing Ac-Di-Sol showed significantly ($p<0.05$; ANOVA/Tukey) faster disintegration of 60.0±5.1, 65.0±7.5 and 50.0±6.5 s for 5, 8 and 12 %

Ac-Di-Sol compared to the other compression forces (Fig. 5C). Meanwhile, a significantly ($p<0.05$, ANOVA/Tukey's) faster disintegration time of 16.5±2.7 s was recorded from tablets containing 12% SSG compared to 5 and 8% SSG when compressed at 5 KN (Fig. 5C). Similar results have been reported by Tawfeek *et al*. (Tawfeek et al., 2014), using high concentrations of SSG in FDTs containing itopride HCl significantly decreased the *in vitro* disintegration time (Tawfeek et al., 2015). Hence, it could be concluded that formulation 12SSG-5CF was considered an optimum formulation in terms of *in vitro* disintegration time as stated in the optimum time for disintegration for orodispersible tablets in European Pharmacopeia (pharmacopoeia, 2002).

Wetting is an important criterion in FDTs and is responsible for rapid disintegration (Abed et al., 2010; Sunada and Bi, 2002). The higher compression force produced tablets with stronger bonding of particles leading to a longer wetting time as shown with both Ac-Di-Sol and SSG tablets compressed at 15 KN. Hence, the fastest wetting time of 12.26±1.2 s was recorded in batch 12SSG-5CF, whereas the highest wetting time of 95.5±8.8 and 99.5±5.5 s which was observed in batches 8AC-15CF and 5SSG-15CF; respectively (Fig. 5D). These results were in accordance with the *in vitro* disintegration time data observed in above section.

3.5. The effect of MPs addition to FDTs characteristics

Evidently, from the data above, batch 12SSG-5CF (formulation 8) was considered an optimum formulation in terms of crushing strength, *in vitro* disintegration and wetting time followed by 8SSG-5CF (formulation 7). These batches were selected for further evaluation which gives finally 4 formulations after incorporation of 10 %w/w non-coated and PGA-co-PDL/Eudragit MPs loaded IND in tablets as shown in Table 3.

Crushing strength, wetting time, *in vitro* disintegration

Crushing Strength: It was observed that by increasing the concentration of SSG from 8 to 12 %w/w a non-significant ($p> 0.05$, ANOVA/ Tukey's) higher increase in crushing force was required to break the tablets containing both non-coated and PGA-co-PDL/Eudragit MPs loaded IND. FDTs containing PGA-co-PDL/Eudragit MPs loaded IND compressed at 5 CF and containing 8 %w/w SSG (formulation 9) had a crushing force at 88.0±6.5 KN which was significantly ($p<0.05$. ANOVA/Tukey's) higher than non-coated PGA-co-PDL MPs loaded IND (formulation 7) at 35.5±4.5 KN. Similar behaviour was also found with FDTs containing non-coated and PGA-co-PDL/Eudragit MPs and 12 %SSG as shown in Table 4. Formulations

of MPs into tablets increased tablets tensile strength as compaction of smaller particles resulted larger surface area available for binding (Dolenc et al., 2009).

Wetting Time: Addition of non-coated PGA-co-PDL MPs to FDTs did not significantly affect the wetting time compared to FDTs without MPs. However; FDTs containing PGA-co-PDL/Eudragit MPs with 12 %SSG (Formulation 10) showed significantly ($p<0.05$, ANOVA/Tukey's) higher wetting time (32.0 s) compared to non-coated PGA-co-PDL MPs (17.5 s, Formulation 8) compressed at the same compression force of 5 KN and containing 12 %SSG. It was also found that formulation 9 showed the highest wetting time of 139.7±5.8 s as shown in Table 4. The delay in wetting for PGA-co-PDL/Eudragit particles could be attributed to the adherent properties of Eudragit and the high bonding of the produced tablets presented with a higher crushing force as mentioned earlier, which hinders liquid penetration into tablet especially at high compression force which (Marais et al., 2003).

Disintegration Time: It was observed that PGA-co-PDL/Eudragit MPs (Formulations 9 & 10) increased significantly ($p<0.05$, ANOVA/Tukey's) the disintegration time with both 8 and 12 %SSG compared to non-coated PGA-co-PDL MPs (formulations 7 & 8). In addition tablets containing non-coated PGA-co-PDL MPs loaded IND (Formulation 8) showed significantly ($p<0.05$, ANOVA/Tukey's) low disintegration time of 7.2±2.1 s using 12 %SSG compared to 40.5±5.5 s with PGA-co-PDL/Eudragit MPs loaded IND (Formulation 10) as depicted in Table 4. Similar trend was also found with Formulations 7 & 9 containing 8 %SSG. The European Pharmacopeia states that orodispersable tablet are required to disintegrate in less than 3 minutes, hence all formulation pass this requirement (pharmacopoeia, 2002). The delay in the disintegration time for PGA-co-PDL/Eudragit MPs loaded IND could be possibly attributed to the adherent property of Eudragit which causing the particles to bind stronger therefore resulting in a slower disintegration time as well as the higher wetting time as mentioned previously (Chang and Hsiao, 1989). Moreover, they disintegrate in relatively short period of time capable of releasing the IND loaded PGA-co-PDL/Eudragit MPs in the mouth cavity to be readily swallowed into the GIT.

3.6. Permeability and transport study

Caco-2 cells were used in this study as a model of human intestinal absorption of drugs and other compounds ((van Breemen and Li, 2005)). Permeability of IND across Caco-2 monolayers were tested in the apical to basolateral direction, and apparent permeability coefficients (P_{app}) were used to compare IND permeability between an IND control and treatments 1 and 4 (Table 5). Controls showed the greatest P_{app} value of 23.06± 3.56 x 10^{-6}

cm/s. P_{app} values for treatment 1 were 13.95± 0.68 x 10^{-6}cm/s and treatment 4 were 13.27± 0.58 x 10^{-6} cm/s, a drop of almost half. Reduction in P_{app} for treatments 1 and 4 were significant compared to IND control ($p<0.05$, ANOVA/Tukey's), whilst differences in P_{app} between the two treatments were insignificant ($p>0.05$, ANOVA/Tukey's). P_{app} values indicated medium to high permeability of IND and were intermediate of those previously reported for IND using Caco-2 models (ElShaer et al., 2014; Jin et al., 2014; Jung et al., 2006). Also, these results are in agreement with a previous report for famotidine loaded MPs and dexamethasone loaded NPs coated MPs transport across Caco-2 cell monolayers (Beck et al., 2007; Degim et al., 2005).

As observed in the *in vitro* drug release studies, we could demonstrate the influence of coating on the absorption of IND across Caco-2 cell monolayers. IND loaded PGA-co-PDL/Eudragit MPs at ratio of 1:1:5 presented the lowest percentage of IND release after 360 mins (24.78±2.26 %). Another possible explanation could be attributed to the effect of pH and acidity of the apical and basolateral layers across Caco-2 cell monolayers. In the case of IND, both the apical and basolateral layers had a pH value of 7.4 (DMEM, medium), which will facilitate the transport of free IND via passive diffusion in the apical to basolateral direction. However, non-coated and PGA-co-PDL/Eudragit MPs, the pH of the apical layer was measured and shifted to a slightly acidic value of 5.3. Also, Eudragit L100-55 is a methacrylic acid derivative which has the potential to decrease the pH of the apical layer while the basolateral pH was maintained at 7.3. This led to a reduction in the permeability of IND and conversion of the IND transport mechanism to active carrier mediated transport, which demonstrated saturation and competitive inhibition phenomena. Similarly, the effect of salicylic acid on the transport of IND across Caco-2 cell monolayers has previously been reported, with authors concluding that when the concentration of salicylic acid was increased from 25 µM to 33 µM, the IND P_{app} was reduced by 80% (Neuhoff et al., 2005).

It must be emphasized that such a delay in IND absorption could further increase the advantages of encapsulation of IND in these PGA-co-PDL/Eudragit MPs. Among the major problems in the treatment of IBD is the necessity for administration of high doses of anti-inflammatory drugs and the low residence time of these drugs in the gut due to rapid absorption and diarrhoea, a frequent symptom of IBD (Kawashima et al., 1993; Lamprecht et al., 2001; Lamprecht et al., 2004). Thus, the fabricated PGA-co-PDL/Eudragit MPs could combine controlled drug release and prolonged residence time of MPs at the site of action, thus decreasing the frequency of drug administration. Furthermore, the lowering in P_{app} could increase their biological importance through increased protection of the GIT mucosa, as in the case of diclofenac loaded nanocapsules coated MPs (Beck et al., 2006). In addition, no drop in

cell integrity was noticed ($p>0.05$, ANOVA/Tukey's) over the experimental period (from t=0 to t=360 mins), with mean TEER values of 1822±68 and 1767±79 $\Omega.cm^2$ at the beginning and end of testing, respectively. TEER values for negative controls (treatments 2 and 3) were similarly maintained, suggesting that the MPs are non-toxic to the cells. Furthermore, it indicates non-paracellular route for drug absorption. It was reported by Borchard G et al. (Borchard et al., 1996), that a reduction of TEER value more than 50 % from the initial value could be indicative for tight junctions relaxations and establishment of paracellular transport.

4. Conclusions

From the data obtained in this study it can be concluded that IND loaded PGA-co-PDL/Eudragit MPs delivered in the form of FDTs could be considered as a promising formulation for targeting IND to the colon. The prepared PGA-co-PDL/Eudragit MPs showed good encapsulation efficiency and IND loading, moreover, they were efficiently coated with Eudragit L100-55 via spray drying technique as demonstrated from DSC and PXRD studies. Moreover, PGA-co-PDL/Eudragit MPs showed a sustained release profile specifically at higher GIT pH. Furthermore, the optimum FDTs containing IND loaded PGA-co-PDL/Eudragit MPs and 12 %SSG showed disintegration time and wetting of 40.5±5.5 s and 32.0 s; respectively. In addition, the permeability of PGA-co-PDL/Eudragit MPs favoured transcellular pathway over paracellular route without affecting cell integrity, as indicated by little change in TEER value at end of permeability study. Moreover, they had a lower permeability compared with free IND, which could be beneficial in treatment of IBD.

Spray-drying and tablet manufacture are techniques employed for large scale-production in the pharmaceutical industry. However, despite the promising results reported, the method of fabricating MPs is via the solvent emulsion evaporation technique, and there is possibility of the MPs size and encapsulation efficiency changing during scale-up. An alternative is to use microfluidic strategies, which allows control of process parameters enabling the fabrication of MPs of desired shape, size, and morphology and controlling encapsulation efficiency at the bench-scale to reproducible larger-scale production (Choi et al., 2017; Duncanson et al., 2012; Seo et al., 2015; Zhao, 2013). Furthermore, the advances in microfluidic systems such as multiple modules or parallelisation allows for scale-up and as technology improves, the cost will also be reduced ensuring more economical viable products for pharmaceutical companies and patients.

Acknowledgements

Dr Hesham Tawfeek would like to thank the Egyptian Ministry of Higher Education for the financial support to visit and work with Dr Imran Saleem, School of Pharmacy & Biomolecular Sciences, Liverpool John Moores University, UK.

References

Abdellatif, A., El Hamd, M., Saleh, K., 2016. A Formulation, Optimization and Evaluation of Controlled Released Alginate Beads Loaded-Flurbiprofen. J. Nanomed. Nanotechnol. 7, 357.

Abed, K.K., Hussein, A.A., Ghareeb, M.M., Abdulrasool, A.A., 2010. Formulation and optimization of orodispersible tablets of diazepam. AAPS PharmSciTech. 11, 356-361.

Aceves-Hernandez, J.M., Nicolas-Vazquez, I., Aceves, F.J., Hinojosa-Torres, J., Paz, M., Castano, V.M., 2009. Indomethacin polymorphs: Experimental and conformational analysis. J. Pharm. Sci. 98, 2448-2463.

Alfagih, I., Kunda, N., Alanazi, F., Dennison, S.R., Somavarapu, S., Hutcheon, G.A., Saleem, I.Y., 2015. Pulmonary Delivery of Proteins Using Nanocomposite Microcarriers. J. Pharm. Sci. 104, 4386-4398.

Alhnan, M.A., Basit, A.W., 2011. In-process crystallization of acidic drugs in acrylic microparticle systems: influence of physical factors and drug-polymer interactions. J. Pharm. Sci. 100, 3284-3293.

Alhnan, M.A., Cosi, D., Murdan, S., Basit, A.W., 2010. Inhibiting the gastric burst release of drugs from enteric microparticles: the influence of drug molecular mass and solubility. J. Pharm. Sci. 99, 4576-4583.

Alhnan, M.A., Kidia, E., Basit, A.W., 2011. Spray-drying enteric polymers from aqueous solutions: a novel, economic, and environmentally friendly approach to produce pH-responsive microparticles. Eur. J. Pharm. Biopharm. 79, 432-439.

Ali, H.R.H., Saleem, I.Y., Tawfeek, H.M., 2016. Insight into inclusion complexation of indomethacin nicotinamide cocrystals. J. Incl. Phenom. Macrocycl Chem. 84, 179-188.

Asghar, L.F., Chure, C.B., Chandran, S., 2009. Colon specific delivery of indomethacin: effect of incorporating pH sensitive polymers in xanthan gum matrix bases. AAPS PharmSciTech. 10, 418-429.

Assaf, S.M., Qandil, A.M., Al-Ani, E.A., 2013. Fast and pH-dependent release of domperidone from orally disintegrating tablets. Pharm. Dev. Technol. 18, 897-905.

Basavoju, S., Bostrom, D., Velaga, S.P., 2008. Indomethacin-saccharin cocrystal: design, synthesis and preliminary pharmaceutical characterization. Pharm. Res. 25, 530-541.

Beck, R., Pohlmann, A., Hoffmeister, C., Gallas, M., Collnot, E., Schaefer, U., Guterres, S., Lehr, C., 2007. Dexamethasone-loaded nanoparticle-coated microparticles: correlation between in vitro drug release and drug transport across Caco-2 cell monolayers. Eur. J. Pharm. Biopharm. 67, 18-30.

Beck, R.C.R., Haas, S.E., Guterres, S.S., Ré, M.I., Benvenutti, E.V., Pohlmann, A.R., 2006. Nanoparticle-coated organic-inorganic microparticles: experimental design and gastrointestinal tolerance evaluation. Quimica Nova. 29, 990-996.

Beloqui, A., Coco, R., Preat, V., 2016. Targeting Inflammatory Bowel Diseases by Nanocarriers Loaded with Small and Biopharmaceutical Anti-Inflammatory Drugs. Curr. Pharm. Des. 22, 6192-6206.

Borchard, G., Lueβen, H.L., de Boer, A.G., Verhoef, J.C., Lehr, C.-M., Junginger, H.E., 1996. The potential of mucoadhesive polymers in enhancing intestinal peptide drug absorption. III: Effects of chitosan-glutamate and carbomer on epithelial tight junctions in vitro. J. Control. Release. 39, 131-138.

Cetin, M., Atila, A., Kadioglu, Y., 2010. Formulation and in vitro characterization of Eudragit(R) L100 and Eudragit(R) L100-PLGA nanoparticles containing diclofenac sodium. AAPS PharmSciTech. 11, 1250-1256.

Chang, R.-K., Hsiao, C., 1989. Eudragit RL and RS pseudolatices: properties and performance in pharmaceutical coating as a controlled release membrane for theophylline pellets. Drug Dev. Ind. Pharm. 15, 187-196.

Chen, Y.D., Liang, Z.Y., Cen, Y.Y., Zhang, H., Han, M.G., Tian, Y.Q., Zhang, J., Li, S.J., Yang, D.S., 2015. Development of oral dispersible tablets containing prednisolone nanoparticles for the management of pediatric asthma. Drug. Des. Devel. Ther. 9, 5815-5825.

Chickpetty, S.M., Baswaraj, R., Kumar, G.S., 2011. Development of novel combined time and pH-dependent based drug delivery systems for targeting 5-fluorouracil to the colon. Curr. Drug. Deliv. 8, 566-574.

Choi, A., Seo, K.D., Kim, D.W., Kim, B.C., Kim, D.S., 2017. Recent advances in engineering microparticles and their nascent utilization in biomedical delivery and diagnostic applications. Lab Chip. 17, 591-613.

Corrigan, O.I., Li, X., 2009. Quantifying drug release from PLGA nanoparticulates. Eur. J. Pharm. Sci. 37, 477-485.

Cosnes, J., Gower-Rousseau, C., Seksik, P., Cortot, A., 2011. Epidemiology and natural history of inflammatory bowel diseases. Gastroenterology. 140, 1785-1794.

Degim, Z., Unal, N., Essiz, D., Abbasoglu, U., 2005. Caco-2 cell culture as a model for famotidine absorption. Drug. Deliv. 12, 27-33.

Dolenc, A., Kristl, J., Baumgartner, S., Planinsek, O., 2009. Advantages of celecoxib nanosuspension formulation and transformation into tablets. Int. J. Pharm. 376, 204-212.

Donini, C., Robinson, D.N., Colombo, P., Giordano, F., Peppas, N.A., 2002. Preparation of poly(methacrylic acid-g-poly(ethylene glycol)) nanospheres from methacrylic monomers for pharmaceutical applications. Int. J. Pharm. 245, 83-91.

Duan, H., Lu, S., Gao, C., Bai, X., Qin, H., Wei, Y., Wu, X., Liu, M., 2016. Mucoadhesive microparticulates based on polysaccharide for target dual drug delivery of 5-aminosalicylic acid and curcumin to inflamed colon. Colloids Surf. B. Biointerfaces. 145, 510-519.

Dubbini, A., Censi, R., Martena, V., Hoti, E., Ricciutelli, M., Malaj, L., Di Martino, P., 2014. Influence of pH and method of crystallization on the solid physical form of indomethacin. Int. J. Pharm. 473, 536-544.

Duncanson, W.J., Lin, T., Abate, A.R., Seiffert, S., Shah, R.K., Weitz, D.A., 2012. Microfluidic synthesis of advanced microparticles for encapsulation and controlled release. Lab Chip 12, 2135-2145.

El Maghraby, G.M., Elsergany, R.N., 2014. Fast disintegrating tablets of nisoldipine for intra-oral administration. Pharm. Dev. Technol. 19, 641-650.

ElShaer, A., Hanson, P., Mohammed, A.R., 2014. A novel concentration dependent amino acid ion pair strategy to mediate drug permeation using indomethacin as a model insoluble drug. Eur. J. Pharm. Sci. 62, 124-131.

Friend, D.R., 2005. New oral delivery systems for treatment of inflammatory bowel disease. Adv. Drug. Deliv. Rev. 57, 247-265.

Fu, Y., Yang, S., Jeong, S.H., Kimura, S., Park, K., 2004. Orally fast disintegrating tablets: developments, technologies, taste-masking and clinical studies. Crit. Rev. Ther. Drug Carrier Syst. 21, 433-476.

Hao, S., Wang, B., Wang, Y., Xu, Y., 2014. Enteric-coated sustained-release nanoparticles by coaxial electrospray: preparation, characterization, and in vitro evaluation. J. Nanopart. Res. 16, 1-11.

Hosny, E.A., 1996. Formulation and comparative evaluation of bioadhesive containing diclofenac sodium and commercial enteric coated tablets in-vitro and in dogs. Int. J. Pharm. 133, 149-153.

Hulse, W.L., Gray, J., Forbes, R.T., 2012. A discriminatory intrinsic dissolution study using UV area imaging analysis to gain additional insights into the dissolution behaviour of active pharmaceutical ingredients. Int. J. Pharm. 434, 133-139.

Jain, A.K., Sood, V., Bora, M., Vasita, R., Katti, D.S., 2014. Electrosprayed inulin microparticles for microbiota triggered targeting of colon. Carbohydr. Polym. 112, 225-234.

Jelvehgari, M., Zakeri-Milani, P., Siahi-Shadbad, M.R., Loveymi, B.D., Nokhodchi, A., Azari, Z., Valizadeh, H., 2010a. Development of pH-sensitive insulin nanoparticles using Eudragit L100-55 and chitosan with different molecular weights. AAPS PharmSciTech. 11, 1237-1242.

Jin, X., Luong, T.-L., Reese, N., Gaona, H., Collazo-Velez, V., Vuong, C., Potter, B., Sousa, J.C., Olmeda, R., Li, Q., 2014. Comparison of MDCK-MDR1 and Caco-2 cell based permeability assays for anti-malarial drug screening and drug investigations. J. Pharmacol. Toxicol Methods. 70, 188-194.

Jung, S.J., Choi, S.O., Um, S.Y., Kim, J.I., Choo, H.Y.P., Choi, S.Y., Chung, S.Y., 2006. Prediction of the permeability of drugs through study on quantitative structure–permeability relationship. J. Pharm. Biomed. Anal. 41, 469-475.

Jung, S.Y., Kim, D.W., Seo, Y.G., Woo, J.S., Yong, C.S., Choi, H.G., 2012. Development of sildenafil-loaded orally disintegrating tablet with new lactate salt. Drug Dev. Ind. Pharm. 38, 635-641.

Kawashima, Y., Iwamoto, T., Niwa, T., Takeuchi, H., Hino, T., 1993. Uniform and improved bioavailability of newly developed rapid and sustained release suspensions of ibuprofen microspheres. Int. J. Pharm. 89, 9-17.

Khan, M.Z.I., Štedul, H.P., Kurjaković, N., 2000. A pH-dependent colon-targeted oral drug delivery system using methacrylic acid copolymers. II. Manipulation of drug release using Eudragit® L100 and Eudragit S100 combinations. Drug Dev. Ind. Pharm. 26, 549-554.

Kim, B., Hwang, S., Park, J., Park, H.J., 2005. Characteristics of felodipine-located poly (ε-caprolactone) microspheres. J. Microencapsul. 22, 193-203.

Kunda, N.K., Alfagih, I.M., Dennison, S.R., Somavarapu, S., Merchant, Z., Hutcheon, G.A., Saleem, I.Y., 2015a. Dry powder pulmonary delivery of cationic PGA-co-PDL nanoparticles with surface adsorbed model protein. Int. J. Pharm. 492, 213-222.

Kunda, N.K., Alfagih, I.M., Dennison, S.R., Tawfeek, H.M., Somavarapu, S., Hutcheon, G.A., Saleem, I.Y., 2015b. Bovine serum albumin adsorbed PGA-co-PDL nanocarriers for vaccine delivery via dry powder inhalation. Pharm. Res. 32, 1341-1353.

Kunda, N.K., Alfagih, I.M., Miyaji, E.N., Figueiredo, D.B., Goncalves, V.M., Ferreira, D.M., Dennison, S.R., Somavarapu, S., Hutcheon, G.A., Saleem, I.Y., 2015c. Pulmonary dry powder vaccine of pneumococcal antigen loaded nanoparticles. Int. J. Pharm. 495, 903-912.

Lamprecht, A., Schafer, U., Lehr, C.M., 2001. Size-dependent bioadhesion of micro- and nanoparticulate carriers to the inflamed colonic mucosa. Pharm. Res. 18, 788-793.

Lamprecht, A., Yamamoto, H., Takeuchi, H., Kawashima, Y., 2004. Design of pH-sensitive microspheres for the colonic delivery of the immunosuppressive drug tacrolimus. Eur. J. Pharm. Biopharm. 58, 37-43.

Lee, C.-M., Kim, D.-W., Lee, H.-C., Lee, K.-Y., 2004. Pectin microspheres for oral colon delivery: Preparation using spray drying method and in vitro release of indomethacin. Biotechnol. Bioprocess Eng. 9, 191-195.

Li, F.-Q., Yan, C., Bi, J., Lv, W.-L., Ji, R.-R., Chen, X., Su, J.-C., Hu, J.-H., 2011. A novel spray-dried nanoparticles-in-microparticles system for formulating scopolamine hydrobromide into orally disintegrating tablets. Int. J Nanomedicine. 6, 897.

Makhlof, A., Tozuka, Y., Takeuchi, H., 2009. pH-Sensitive nanospheres for colon-specific drug delivery in experimentally induced colitis rat model. Eur. J. Pharm. Biopharm. 72, 1-8.

Marais, A.F., Song, M., de Villiers, M.M., 2003. Effect of compression force, humidity and disintegrant concentration on the disintegration and dissolution of directly compressed furosemide tablets using croscarmellose sodium as disintegrant. Trop. J. Pharm. Res. 2, 125-135.

McConnell, E.L., Fadda, H.M., Basit, A.W., 2008. Gut instincts: explorations in intestinal physiology and drug delivery. Int. J. Pharm. 364, 213-226.

Mishra, B., Patel, B.B., Tiwari, S., 2010. Colloidal nanocarriers: a review on formulation technology, types and applications toward targeted drug delivery. Nanomedicine (Lond). 6, 9-24.

Moustafine, R.I., Zaharov, I.M., Kemenova, V.A., 2006. Physicochemical characterization and drug release properties of Eudragit® E PO/Eudragit® L 100-55 interpolyelectrolyte complexes. Eur. J. Pharm. Biopharm. 63, 26-36.

Nadal, J.M., Gomes, M.L., Borsato, D.M., Almeida, M.A., Barboza, F.M., Zawadzki, S.F., Kanunfre, C.C., Farago, P.V., Zanin, S.M., 2016. Spray-dried Eudragit(R) L100 microparticles containing ferulic acid: Formulation, in vitro cytoprotection and in vivo anti-platelet effect. Mater. Sci. Eng. C. Mater. Biol. Appl. 64, 318-328.

Neuhoff, S., Ungell, A.L., Zamora, I., Artursson, P., 2005. pH-Dependent passive and active transport of acidic drugs across Caco-2 cell monolayers. Eur. J. Pharm. Sci. 25, 211-220.

Nilkumhang, S., Alhnan, M.A., McConnell, E.L., Basit, A.W., 2009. Drug distribution in enteric microparticles. Int. J. Pharm. 379, 1-8.

Oosegi, T., Onishi, H., Machida, Y., 2008. Gastrointestinal distribution and absorption behavior of Eudragit-coated chitosan-prednisolone conjugate microspheres in rats with TNBS-induced colitis. Int. J. Pharm. 348, 80-88.

pharmacopoeia, E., 2002. 4th Edition, Supplement 4.2.

Preeti, G., Parijat, P., Praveen, R., Kumar, M.R., Harish, D., 2014. Box-Behnken Designed Nanoparticles-in-Microparticles System (NiMS) for Formulating Mouth Dissolving Tablets of Acetazolamide. J. Pharm. Res. 13, 74-79.

Raina, S.A., Alonzo, D.E., Zhang, G.G., Gao, Y., Taylor, L.S., 2014. Impact of polymers on the crystallization and phase transition kinetics of amorphous nifedipine during dissolution in aqueous media. Mol. Pharm. 11, 3565-3576.

Rashid, M., Kaur, V., Hallan, S.S., Sharma, S., Mishra, N., 2016. Microparticles as controlled drug delivery carrier for the treatment of ulcerative colitis: a brief review. Saudi Pharm. J. 24, 458-472.

Santino, A., Scarano, A., Santis, S., Benedictis, M., Giovinazzo, G., Chieppa, M., 2017. Gut microbiota modulation and anti-inflammatory properties of dietary polyphenols in IBD: new and consolidated perspectives. Curr. Pharm. Des. 23, 2344-2351.

Sasaki, Y., Hada, R., Nakajima, H., Fukuda, S., Munakata, A., 1997. Improved localizing method of radiopill in measurement of entire gastrointestinal pH profiles: colonic luminal pH in normal subjects and patients with Crohn's disease. Am. J. Gastroenterol. 92, 114-118.

Seo, K.D., Kim, D.S., Sanchez, S., 2015. Fabrication and applications of complex-shaped microparticles via microfluidics. Lab Chip. 15, 3622-3626.

Shen, X., Yu, D., Zhu, L., Branford-White, C., White, K., Chatterton, N.P., 2011. Electrospun diclofenac sodium loaded Eudragit(R) L 100-55 nanofibers for colon-targeted drug delivery. Int. J. Pharm. 408, 200-207.

Silva, I., Gurruchaga, M., Goñi, I., 2009. Physical blends of starch graft copolymers as matrices for colon targeting drug delivery systems. Carbohydr. Polym. 76, 593-601.

Sunada, H., Bi, Y., 2002. Preparation, evaluation and optimization of rapidly disintegrating tablets. Powder Technol. 122, 188-198.

Tawfeek, H., Khidr, S., Samy, E., Ahmed, S., Murphy, M., Mohammed, A., Shabir, A., Hutcheon, G., Saleem, I., 2011a. Poly (glycerol adipate-co-ω-pentadecalactone) spray-dried microparticles as sustained release carriers for pulmonary delivery. Pharm. Res. 28, 2086-2097.

Tawfeek, H., Khidr, S., Samy, E., Ahmed, S., Murphy, M., Mohammed, A., Shabir, A., Hutcheon, G., Saleem, I., 2011b. Poly(glycerol adipate-co-omega-pentadecalactone) spray-dried microparticles as sustained release carriers for pulmonary delivery. Pharm. Res. 28, 2086-2097.

Tawfeek, H.M., 2013. Evaluation of PEG and mPEG-co-(PGA-co-PDL) microparticles loaded with sodium diclofenac. Saudi Pharm. J. 21, 387-397.

Tawfeek, H.M., Abdel-Aleem, J.A., Ahmed, M.M., 2015. Development and optimization of itopride hydrochloride fast disintegrating tablets using factorial design and response surface methodology. Int. J. Pharm. Sci. Res. 6, 1661.

Tawfeek, H.M., Evans, A.R., Iftikhar, A., Mohammed, A.R., Shabir, A., Somavarapu, S., Hutcheon, G.A., Saleem, I.Y., 2013. Dry powder inhalation of macromolecules using novel PEG-co-polyester microparticle carriers. Int. J. Pharm. 441, 611-619.

Tawfeek, H.M., Khidr, S.H., Samy, E.M., Ahmed, S.M., Gaskell, E.E., Hutcheon, G.A., 2014. Evaluation of biodegradable polyester-co-lactone microparticles for protein delivery. Drug Dev. Ind. Pharm. 40, 1213-1222.

van Breemen, R.B., Li, Y., 2005. Caco-2 cell permeability assays to measure drug absorption. Expert Opin. Drug Metab. Toxicol. 1, 175-185.

Wagh, V.D., Apar, D.U., 2014. Cyclosporine a loaded PLGA nanoparticles for dry eye disease: in vitro characterization studies. J. Nanotechnol. 2014.

Wang, K., Xu, X., Wang, Y., Yan, X., Guo, G., Huang, M., Luo, F., Zhao, X., Wei, Y., Qian, Z., 2010. Synthesis and characterization of poly(methoxyl ethylene glycol-caprolactone-co-methacrylic acid-co-poly(ethylene glycol) methyl ether methacrylate) pH-sensitive hydrogel for delivery of dexamethasone. Int. J. Pharm. 389, 130-138.

Wang, X.Q., Zhang, Q., 2012. pH-sensitive polymeric nanoparticles to improve oral bioavailability of peptide/protein drugs and poorly water-soluble drugs. Eur. J. Pharm. Biopharm. 82, 219-229.

Yamanaka, Y.J., Leong, K.W., 2008. Engineering strategies to enhance nanoparticle-mediated oral delivery. J. Biomater. Sci. Polym. Ed. 19, 1549-1570.

Yoshinari, T., Forbes, R.T., York, P., Kawashima, Y., 2003. The improved compaction properties of mannitol after a moisture-induced polymorphic transition. Int. J. Pharm. 258, 121-131.

Youshia, J., Lamprecht, A., 2016. Size-dependent nanoparticulate drug delivery in inflammatory bowel diseases. Expert Opin. Drug Deliv. 13, 281-294.

Zakeri-Milani, P., Barzegar-Jalali, M., Azimi, M., Valizadeh, H., 2009. Biopharmaceutical classification of drugs using intrinsic dissolution rate (IDR) and rat intestinal permeability. Eur. J. Pharm. Biopharm. 73, 102-106.

Zhang, G.G., Law, D., Schmitt, E.A., Qiu, Y., 2004. Phase transformation considerations during process development and manufacture of solid oral dosage forms. Adv. Drug Deliv. Rev. 56, 371-390.

Zhao, C.X., 2013. Multiphase flow microfluidics for the production of single or multiple emulsions for drug delivery. Adv. Drug Deliv. Rev. 65, 1420-1446.

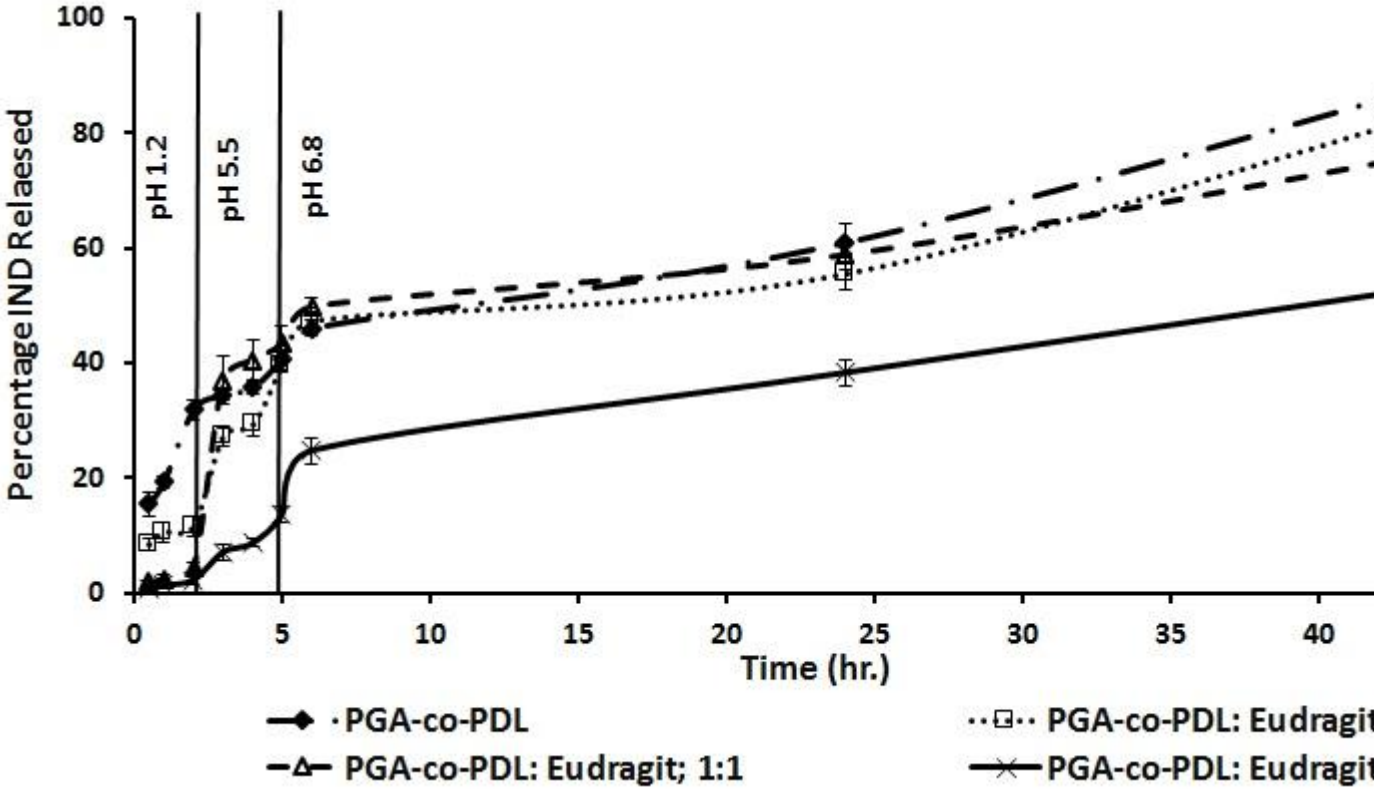
Fig. 1: The XRD pattern of (A) PGA-co-PDL; (B) Eudragit L100-55; (C) non-coated IND loaded PGA-co-PDL microparticles and (D) Eudragit coated IND loaded PGA-co-PDL microparticles (1:1.5 wt ratio).

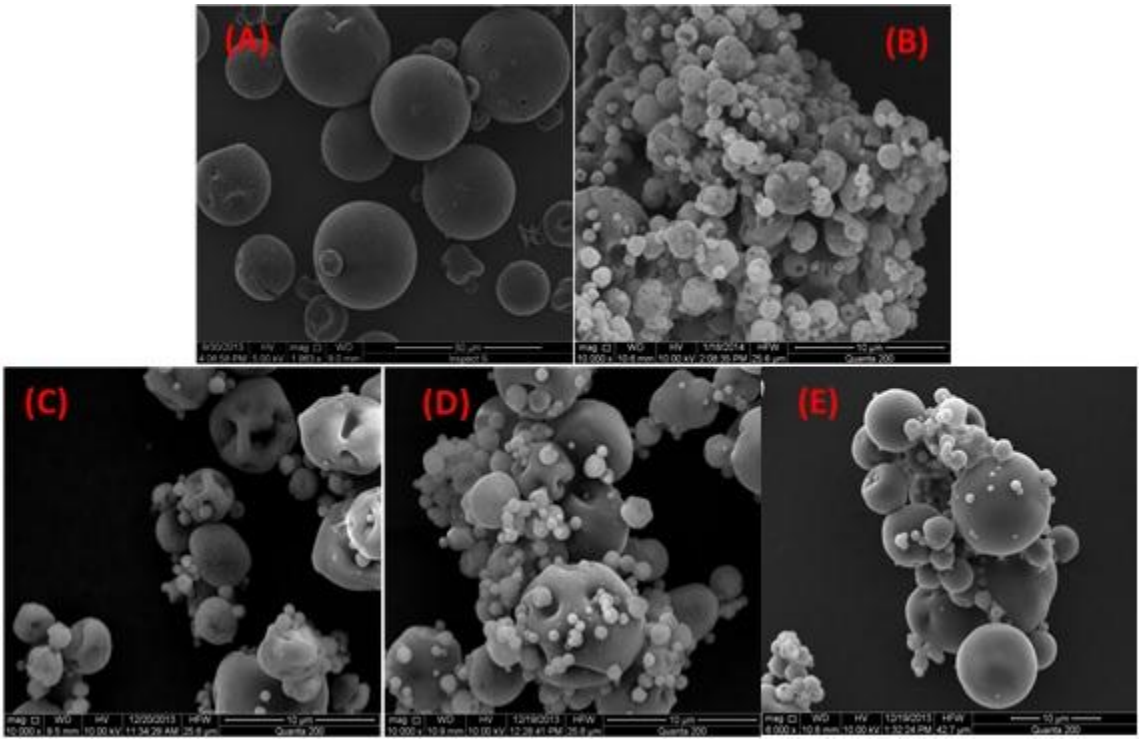
Fig. 2: DSC thermograms of (A) Indomethacin; (B) Eudragit L100-55; (C) IND loaded PGA-co-PDL micrparticles; (D) Eudragit coated IND loaded PGA-co-PDL microparticles (1:0.5wt. ratio); (E) Eudragit coated IND loaded PGA-co-PDL microparticles (1:1wt. ratio) and (F) Eudragit coated IND loaded PGA-co-PDL microparticles (1:1.5wt ratio).

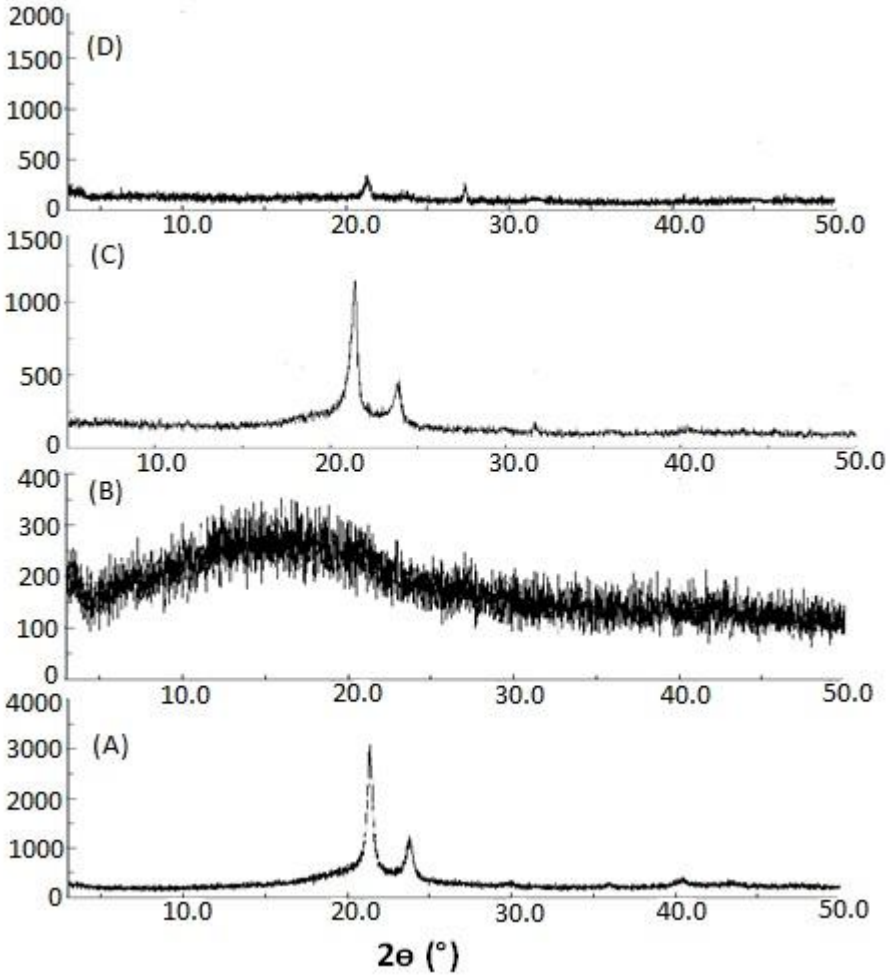
Fig. 3: Scanning electron microscope images of (A) spray dried blank Eudragit L100-55 particles; (B) spray dried blank PGA-co-PDL particles; (C) spray dried Eudragit coated IND loaded PGA-co-PDL microparticles (1:0.5wt. ratio); (D) spray dried Eudragit coated IND loaded PGA-co-PDL microparticles (1:1wt. ratio) and (E) spray dried Eudragit coated IND loaded PGA-co-PDL microparticles (1:1.5wt ratio). Scale bar represents 50 µm for image (A) and 10 µm for images (B-E).

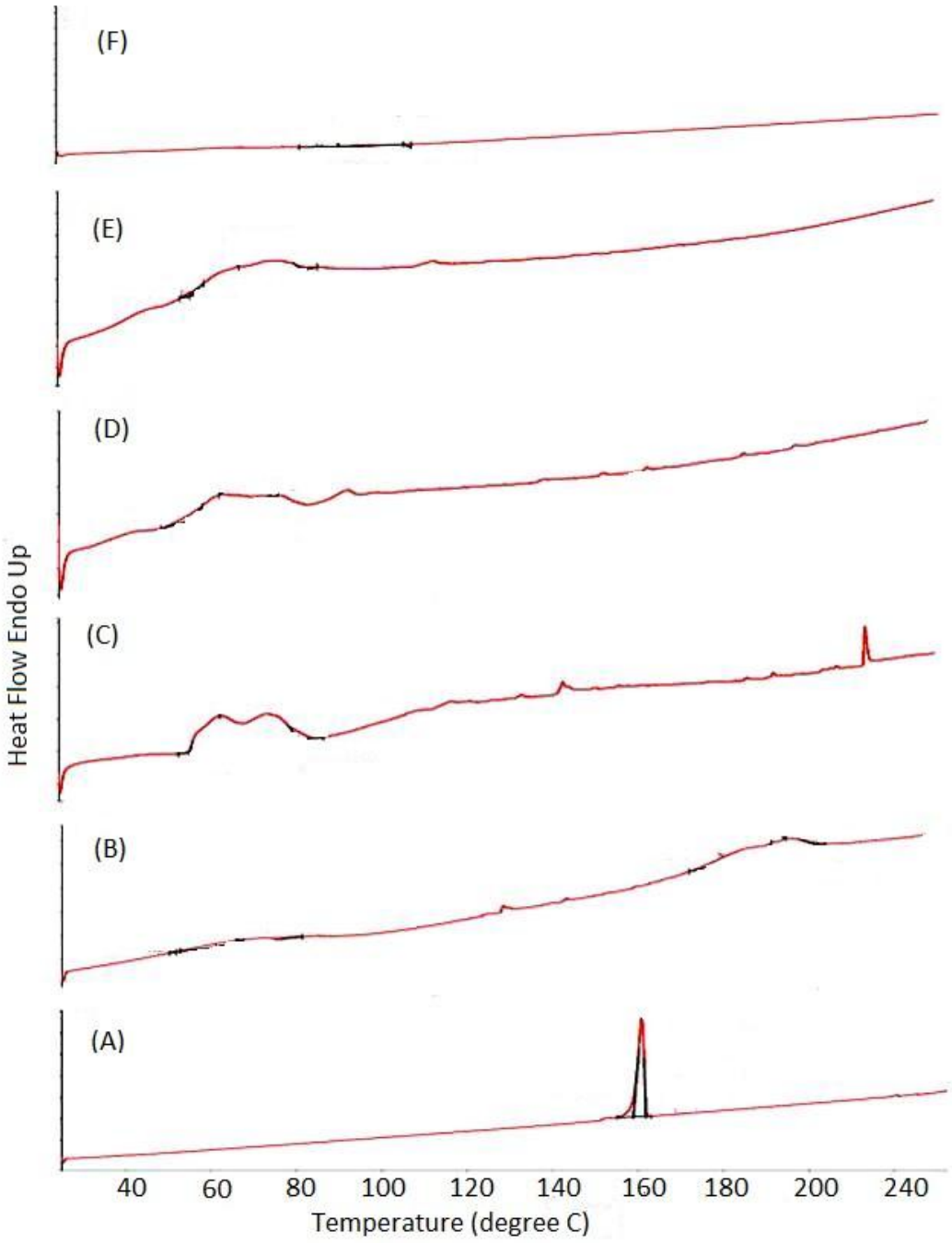
Fig. 4: *In vitro* release study of IND from IND loaded PGA-co-PDL microparticles and spray dried Eudragit coated IND loaded PGA-co-PDL microparticles (1:0.5; 1:1 and 1:1.5 wt. ratios at pH 1.2, 5.5 and 6.8 for 2, 3 and 43 hrs, respectively, (n=3).

Fig. 5: (A) The crushing strength (KN) of blank FDTs using different compression forces and Ac-Di-Sol as superdisintegrant, (n=10). (B) The crushing strength (KN) of blank FDTs using different compression forces and SSG as superdisintegrant, (n=10). (C) *In vitro* disintegration time (seconds) of blank FDTs containing either Ac-Di-Sol or SSG different concentrations and compressed at 5, 10 and 15 KN, (n=6). (D) The wetting time (seconds) for blank FDTs containing either Ac-Di-Sol or SSG different concentrations and compressed at 5, 10 and 15 KN, (n=6) * Significantly different ($p<0.05$, ANOVA/Tukey's) at 5 KN compared to 10 and 15 KN for FDTs containing Ac-Di-Sol, ** significantly different($p<0.05$, ANOVA/Tukey's) at 12% SSG compared to 5 and 8% at 5 KN compression force. # Significantly different ($p<0.05$, ANOVA/Tukey's) at 5 KN compared to 10 and 15 KN for FDTs tablets containing different concentrations of either Ac-Di-Sol or SSG.









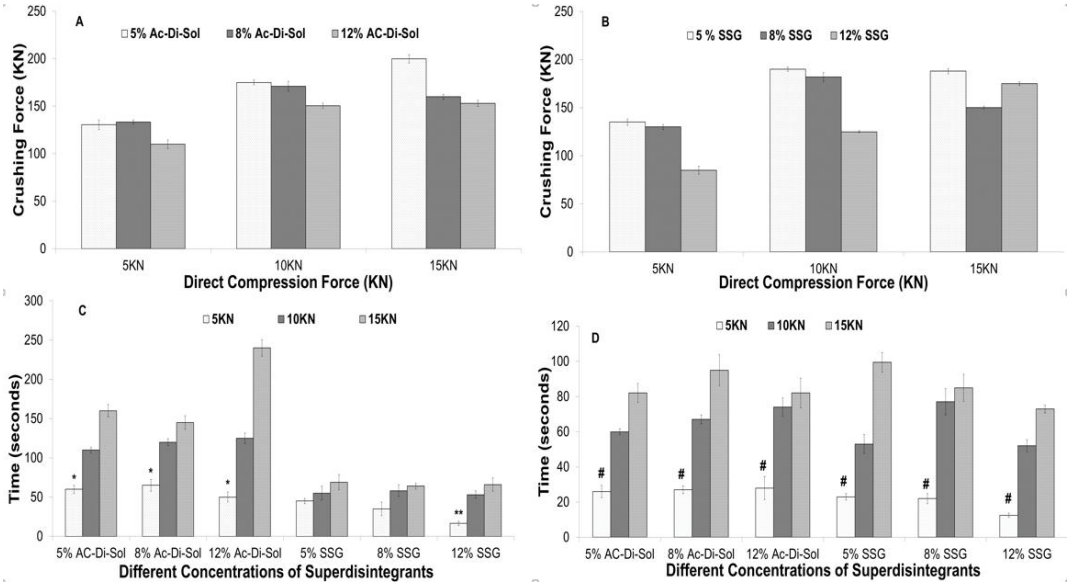


Table 1. The Superdisintegrant type and concentrations and the corresponding compression force

Compression Force (KN)	**Superdisintegrant type and Concentration (w/w)**					
	Ac-Di-Sol 5%	**Ac-Di-Sol 8%**	**Ac-Di-Sol 12%**	**SSG 5%**	**SSG 8%**	**SSG 12%**
5KN	5AC-5CF	8AC-5CF	12AC-5CF	5SSG-5CF	8SSG-5CF	12SSG-5CF
10KN	5AC-10CF	8AC-10CF	12AC-10CF	5SSG-10CF	8SSG-10CF	12SSG-10CF
15KN	5AC-15CF	8AC-15CF	12AC-15CF	5SSG-15CF	8SSG-15CF	12SSG-15CF

KN, Kilonetwon; CF, Compression force; AC, Ac-Di-Sol; SSG, Sodium starch glycolate

Table 2. The composition of the different prepared blank FDTs

Excipients (mg)	Formulation 1 (SSG)	Formulation 2 (SSG)	Formulation 3 (SSG)	Formulation 4 (Ac-Di-Sol)	Formulation 5 (Ac-Di-Sol)	Formulation 6 (Ac-Di-Sol)
Superdisintegrant	10	16	24	10	16	24
Diluent	188	182	174	188	182	174
Lubricant	2	2	2	2	2	2
Total	200	200	200	200	200	200

Diluent, lactose anhydrous; Lubricant, magnesium stearate

Table 3. The composition of FDTs containing non-coated and PGA-co-PDL-IND/Eudragit MPs and compressed at 5 KN compression force.

Excipients (mg)	**Formulation 7**	**Formulation 8**	**Formulation 9**	**Formulation 10**
Superdisintegrant, SSG	16	24	16	24
MPs	20 PGA-co-PDL	20 PGA-co-PDL	20 PGA-co-PDL Eudragit/ (1:1.5 wt/wt)	20 PGA-co-PDL Eudragit/ (1:1.5 wt/wt)
Diluent	162	154	162	154
Lubricant	2	2	2	2
Total	200	200	200	200

Diluent, lactose anhydrous; Lubricant, magnesium stearate

Table 4. The crushing strength, wetting time and *in vitro* disintegration time for FDTs after MPs addition (mean ± SD, n=6)

Formulation	**Crushing strength (KN)**	**Wetting time (s)**	***In vitro* disintegration time (s)**
Formulation 7	35.5±4.5	16.3±1.2	19±3.2***
Formulation 8	42.0±3.2	17.5	7.2±2.1***
Formulation 9	88.0±6.5*	139.7±5.8	151±8.9
Formulation 10	96.3±5.3	32.0±1.7**	40.5±5.5

*Significantly higher crushing strength Vs Formulation 7;** significantly higher wetting time Vs Formulation 8; *** significantly lower disintegration time Vs Formulations 9 & 10.

Table 5. P_{app} vales for IND control and treatments 1 and 4 in the A-B direction across Caco-2 monolayers at pH 7.4 in both compartments (mean ± SD, n=3)

Treatment	**P_{app} (10^{-6} cm s^{-1})**
Control	23.06± 3.56
1	13.95± 0.68*
4	13.27± 0.58*

*Significant reduction in Papp for treatments 1 and 4 Vs IND control