Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 338 (2007) 238–247
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Development of pH- and time-dependent oral microparticles to optimize budesonide delivery to ileum and colon

Yogita Krishnamachari, Parshotam Madan, Senshang Lin *

College of Pharmacy and Allied Health Professions, St. John's University, 8000 Utopia Parkway, Jamaica, NY 11439, United States

Received 3 November 2006; received in revised form 11 February 2007; accepted 12 February 2007
Available online 16 February 2007

Abstract

A microparticulate system consisting of non-enzymatically degrading poly(dl-lactide-*co*-glycolide) (PLGA) core and delivering budesonide site specifically to distal ileum and colon was developed. Budesonide-loaded microparticles were fabricated using solvent evaporation technique and formulation variables studied included different molecular weight grades of PLGA polymer as well as concentration of polymer, surfactant and drug. Eudragit® S-100, an enteric polymer, was then used to form a coating on the surface of budesonide-loaded PLGA microparticles for site specific delivery to the distal ileum and colon. Budesonide-loaded PLGA microparticles prepared from various formulation parameters showed mean encapsulation efficiencies ranging between 50% and 85% and mean particle size ranging between 10 and 35 μm. *In vitro* release kinetics studies showed a biphasic release pattern with an initial higher release followed by a slower drug release. Increasing polymer and surfactant concentrations exhibited sharply contrasting drug release profiles, with increasing polymer concentrations resulting in a lower drug release and vice versa. The budesonide-loaded PLGA microparticles coated with Eudragit® S-100 coating showed a decrease in entrapment efficiency with an accelerated *in vitro* drug release. Moreover, complete retardation of drug release in an acidic pH, and, once the coating layer of enteric polymer was dissolved at higher pH (7.4 and 6.8), a controlled release of the drug from the microparticles were observed. From the results of this investigation, the application of double microencapsulation technique employing PLGA matrix and Eudragit® S-100 coating shows promise for site specific and controlled delivery of budesonide in Crohn's disease.

Keywords: Budesonide; Microparticles; Poly(lactide-*co*-glycolide); Colon drug delivery; Crohn's disease

1. Introduction

Crohn's disease is a chronic inflammatory bowel disease of unknown origin primarily affecting the terminal ileum and proximal colon. Complications associated with Crohn's disease include deficiency of nutrients (like proteins and vitamins), arthritis, skin problems, and inflammation in the eyes or mouth. In recent times active Crohn's disease patients have failed to respond to treatment with aminosalicylate products. The National Cooperative Crohn's Disease Study (NCCDS) and the European Cooperative Crohn's Disease Study (ECCDS) trials have found that corticosteroids has demonstrated a statistically significant improvement and effectiveness for the treatment of the disease as compared to aminosalicylates (Baker, 2001; Feagan and Sandborn, 2002; Klotz and Schwab, 2005). The long transit time of a delivery system in the colon offers another potential advantage. Hence a controlled release formulation of a corticosteroid delivering the drug topically in the ileum and colon would be an ideal choice of treatment for moderate to active form of the disease.

Budesonide, a second generation glucocorticoid, exhibits high affinity to the corticosteroid receptors with a high ratio of topical to systemic anti-inflammatory activity. A previous study has reported a microsphere delivery system for budesonide, consisting of a hydrophobic core (cellulose acetate butyrate) and an enteric coating, subjects to degradation by the colonic microflora (Rodriguez et al., 1998). Haksworth et al. studied the composition of the normal intestinal and colonic microflora of

* Corresponding author. Tel.: +1 718 990 5344; fax: +1 718 990 6316.
E-mail address: linse@stjohns.edu (S. Lin).

0378-5173/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2007.02.015

human, rabbit, guinea pig, rats and mouse to account for interspecies variations in the composition and number of microflora (Haeberlin and Friend, 1992). Such studies become relevant while extrapolating drug release data obtained from animal models to humans. In all models studied, they found that the large intestine contains a complex bacterial flora composition. The number of anaerobic and aerobic bacteria found in mouse and rats was much larger in comparison to humans indicating a great interspecies variability. Additionally Keighley et al. investigated the influence of inflammatory bowel disease on the composition and count of colonic microflora (Haeberlin and Friend, 1992). There was a large variation in the ileal and colonic counts of microflora between normal subjects and patients with active Crohn's disease unlike ulcerative colitis conditions where the microflora count remained unaffected in most cases. Due to these large variations, the use of polymers that are subject to degradation by colonic bacteria may not be a prudent approach for colon specific drug release.

Additionally, in Crohn's disease, the lesions are found in most cases in the terminal ileum and proximal colon. John et al. measured the proximal colon transit time in a human volunteer with an enteric coated capsule (Mojaverian et al., 1989). The residence time of the dosage form was approximately 12–14 h. Hence, there is a need to fabricate a delivery system delivering the drug in a controlled manner through the proximal colon transit time. Poly(lactide-*co*-glycolide) (PLGA) has received great attention in recent times due to its biodegradable and biocompatible properties. PLGA is degraded by non-enzymatic acidic or alkaline hydrolysis of the ester backbone under the conditions of body fluids. By varying the polymer molecular weight and co-polymer composition, it is possible to achieve a desired drug release rate (Martin et al., 2002; Habib et al., 1999; Shameem et al., 1999).

In this investigation, pH- and time-dependent oral microparticles were developed to optimize budesonide delivery to ileum and throughout entire region of colon. The first objective of the current study was to fabricate budesonide-loaded microparticles as time-dependent controlled drug delivery system employing a controlled release polymer whose degradation is independent of the colonic microflora composition and count. The effect of formulation variables on particle size and entrapment efficiency as well as *in vitro* drug release kinetics of the budesonide-loaded microparticles was investigated. The formulation variables used for the fabrication of budesonide-loaded microparticles were polymer molecular weight, and concentration of polymer, surfactant and the drug. The second objective was to modify budesonide-loaded microparticles further to be pH-dependent for site specific delivery to the distal ileum and colon. The second objective was achieved by Eudragit® S-100 coating on budesonide-loaded microparticles. Solvent evaporation technique was employed at both stages of microparticles fabrication. Using this approach, the risk of the polymer undergoing erratic degradation and failing to deliver drug completely could be minimized. Although the two-step fabrication technique used in the investigation is long and complex process, if the formulation concept works, this process can be improved by spray drying technique.

2. Material and methods

2.1. Materials

Budesonide and poly(vinyl alcohol) (PVA, 87–89% hydrolyzed, M_W 13,000–23,000), poly(dl-lactide-*co*-glycolide) (PLGA, 50:50, M_W 5000 and 75,000) were supplied by Sigma–Aldrich (St. Louis, MO). Eudragit® S-100 was a kind gift from Rohm Pharma Polymers, Degussa Corp. (Piscataway, NJ). Acetone, dichloromethane, ethanol, liquid paraffin oil, methanol and *n*-hexane were purchased from VWR (West Chester, PA). All other chemicals and solvents used were of analytical grade.

2.2. Fabrication of budesonide-loaded PLGA microparticles

The fabrication of budesonide-loaded PLGA microparticles was based on a classical oil/water emulsification solvent evaporation method (Kompella et al., 2001). The polymer along with the drug was dissolved in 5 mL of dichloromethane. This solution was poured into 35 mL of water containing PVA (w/v) as an emulsion stabilizer. The emulsion was formed by high speed homogenization for 3 min at 7000 rpm using a homogenizer (Brinkmann Polytron PT 3000, Westbury, NY). The speed was then reduced to 2000 rpm and the emulsion was stirred until complete evaporation of the solvent. After evaporation of the solvent, the microparticles were recovered by centrifugation using a centrifuge (Sorvall Centrifuge, Thermo Electron Corporation, Ashville, NC) at 14,000 rpm for 30 min, and then the microparticles were washed with distilled water. The washing step was repeated twice and the microparticles obtained were lyophilized overnight in the presence of 4% mannitol as cryoprotector under a pressure of 130×10^{-3} mbar at $-40\,^{\circ}C$ using Labconco shell freeze dryer (Labconco, Kansas City, MO, USA). The lyophilized microparticles were stored at $4\,^{\circ}C$ until further use.

The effect of formulation variables, such as polymer molecular weight, and concentrations of polymer, surfactant, and the drug on the characteristics of budesonide-loaded microparticles were evaluated. The composition of microparticulate formulations of budesonide prepared using various formulation variables are summarized in Table 1.

2.3. Fabrication of Eudragit® S-100 coated budesonide-loaded PLGA microparticles

The method employed was a slightly modified version of W/O emulsion solvent evaporation method used for Eudragit® polymer coating (Rodriguez et al., 1998). Briefly, a 4% (w/v) solution of Eudragit® S-100 was prepared in a mixture of methanol and acetone (3 mL each). The solvent for Eudragit® S-100 was chosen such that it dissolves Eudragit® S-100 but maintains the integrity of the budesonide-loaded PLGA microparticles. The lyophilized budesonide-loaded PLGA microparticles were added to this polymeric solution. The resulting suspension was poured into 70 mL of liquid paraffin containing 1% (w/v) Span 85 as emulsifier and stirred at

Table 1
Composition of budesonide-loaded PLGA microparticles fabricated using various formulation variables

Formulation code	Budesonide (% (w/v))	PVA concentration (% (w/v))	PLGA concentration (% (w/v))	
			M_W, 5000	M_W, 75,000
Effect of polymer molecular weight				
A1	0.5	5	0	1
A2	0.5	5	0.25	0.75
A3	0.5	5	0.50	0.50
A4	0.5	5	0.75	0.25
A5	0.5	5	1	0
Effect of polymer concentration				
B1[a]	0.5	5	1	
B2	0.5	5	2	
B3	0.5	5	4	
B4	0.5	5	8	
Effect of surfactant concentration				
C1	0.5	1	1	
C2	0.5	3	1	
C3[a]	0.5	5	1	
Effect of drug concentration				
D1	0.25	5	1	
D2[a]	0.5	5	1	
D3	1	5	1	

PLGA: poly(dl-lactide-*co*-glycolide); PVA: poly(vinyl alcohol).
[a] Same composition as formulation A5.

800 rpm using a three blade propeller. The stirring was continued until complete solvent evaporation resulting in the formation of Eudragit® S-100 coated budesonide-loaded microparticles. The microparticles were filtered, washed with *n*-hexane, dried under vacuum and then stored in a desiccator until further use. Due to its low boiling point (68 °C), *n*-hexane can be efficiently removed by overnight vacuum drying.

To evaluate the polymer matrix impact between PLGA and Eudragit® S-100, budesonide-loaded Eudragit® S-100 microparticles was fabricated using the above method replacing PLGA with Eudragit® S-100 (4% (w/v)).

2.4. Particle size and entrapment efficiency of budesonide-loaded PLGA microparticles

All formulations of Eudragit® S-100 coated and uncoated budesonide-loaded PLGA microparticles were evaluated and characterized by their particle size and entrapment efficiency. Drug-loaded microparticles (10 mg) were suspended in 50 mL of deionized water by sonication. The particle size was measured using dynamic light scattering (Nicomp 780 DLS particle sizer; Nicomp, Santa Barbara, CA, USA).

Five milligrams of drug loaded microparticles were suspended in 5 mL of methanol. The suspension was shaken vigorously for 5 min to allow the drug to be extracted into methanol. The suspension was then intermittently shaken up to 1 h to ensure complete drug extraction. One milliliter of the suspension was withdrawn, filtered using a 0.45 μm membrane filter, and analyzed by high-performance liquid chromatography (HPLC) for the drug content. The entrapment efficiency was calculated as the ratio of the total amount of budesonide in the microparticles and the total amount of budesonide used, expressed as a percentage.

The entrapment efficiency measurement had been validated by periodic sampling of the extract until concentration remained constant in every successive measurement. A mass balance of 95% between the supernatant and drug content in microparticles was observed.

2.5. In vitro release kinetics of budesonide-loaded PLGA microparticles

To study the release kinetics of Eudragit® S-100 coated and uncoated budesonide-loaded PLGA microparticles, a method utilizing a water shaker bath (Forma Scientific, Thermo Forma, Marietta, OH, USA) was used. Briefly, the microparticles containing 1000 μg of the entrapped drug were suspended in 200 mL of the release medium maintained at 37 °C and shaken at 75 rpm for a predetermined time. To simulate the acidic conditions of the stomach and the gastric emptying time, 0.1N HCl (pH 1.2) was used as release medium and samples were withdrawn up to the end of 3 h. To simulate the environment of ileum (pH 6.8) and colon (pH > 7), two more release media were used: phosphate buffered saline (PBS) at pH 6.8 and 7.4, and studied for 24 h. One milliliter of the medium was withdrawn at predetermined time intervals and the same volume of fresh media was replenished. The samples collected were then centrifuged at 2000 × *g* for 5 min and the concentration of budesonide in the supernatant of each sample was analyzed by HPLC as outlined below.

2.6. HPLC analysis of budesonide

The HPLC system used for the analysis of budesonide was an HP 1100 series (HP, Palo Alto, CA), equipped with a quaternary pump, an autosampler processor, a UV detector, an integrator and a Novapak C_{18} column (5 μm, 150 mm × 3.9 mm ID). The UV detector wavelength was set at 254 nm and a combination of methanol and water (69:31) at a flow rate of 0.8 mL/min was used as the mobile phase. Under these conditions of analysis, upon injection of 10 μL of the sample, a well-separated peak was detected at a retention time of 5 min. The limit of detection was 0.1 μg/mL and the limit of quantification was 0.2 μg/mL.

2.7. Statistical analysis

A one way ANOVA test and student's *t*-test was performed to analyze the effect of formulation factors on characteristics of Eudragit® S-100 coated and uncoated budesonide-loaded PLGA microparticles. A *p*-value less than 0.05 was considered as representing a significant difference.

3. Results and discussion

In the current study, Eudragit® S-100 coated and uncoated budesonide-loaded PLGA microparticles were successfully fabricated by the solvent evaporation technique conducted at room temperature.

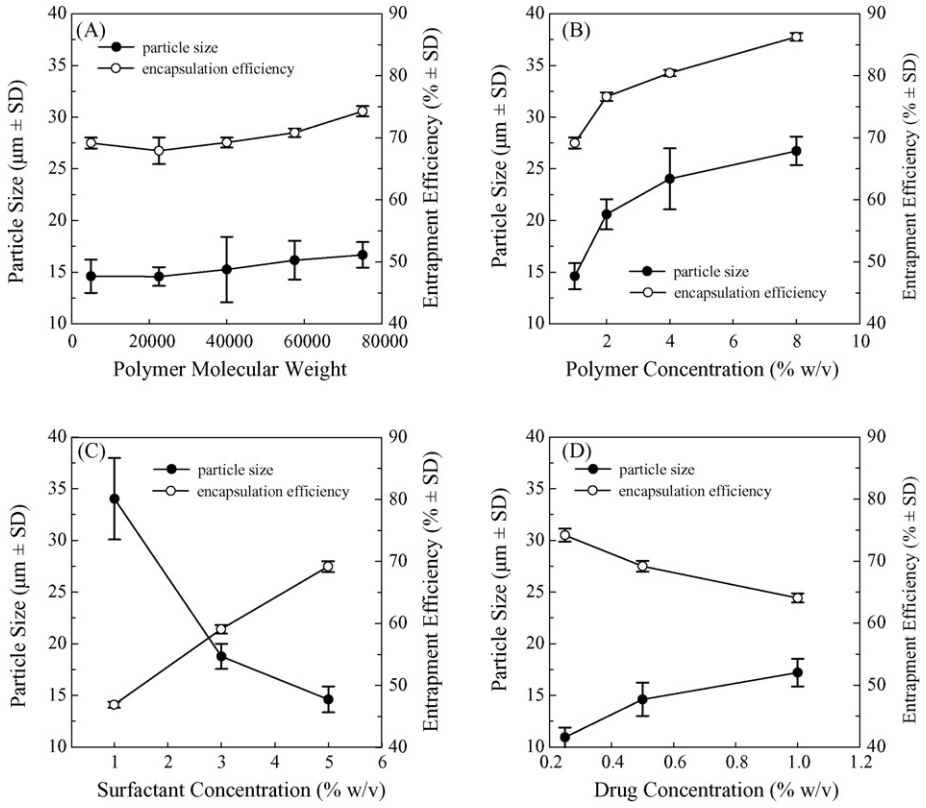

Fig. 1. Effect of various formulation variables on particle size and entrapment efficiency of budesonide-loaded PLGA microparticles (data shown as mean ± standard deviation, $n = 3$).

3.1. Particle size and entrapment efficiency of budesonide-loaded PLGA microparticles

The effect of the formulation variables on particle size and entrapment efficiency of budesonide-loaded PLGA microparticles is shown in Fig. 1. The results are detailed as follows.

3.1.1. Effect of polymer molecular weight

The PLGA polymers of varying molecular weights were prepared by mixing various proportions of low molecular weight PLGA ($M_W = 5000$) and high molecular weight PLGA ($M_W = 75{,}000$). The ratios are shown in Table 1. The particle size of the microparticles fabricated with the five different molecular weights of PLGA exhibited interesting results. Due to difference in the inherent viscosities of high and low molecular weight PLGA polymers (0.9 g/dL versus 0.3 g/dL, respectively), microparticles fabricated using higher molecular weight with larger inherent viscosity were expected to have a larger particle size than those fabricated using lower molecular weight polymers. The results in Fig. 1A indicate that although the particle size increased with increasing molecular weight of PLGA, increase in the particle size did not show a statistically significant difference among the molecular weights of the polymers used. This could be probably because of the low concentration of the polymer (1% (w/v)) used, which may not have exerted a significant difference in the particle size.

As shown in Fig. 1A, the entrapment efficiency was increased with increasing molecular weight of PLGA. A statistically significant difference ($P < 0.05$) in entrapment efficiency (69.17% versus 74.28%) was observed only between the lowest and the highest molecular weight polymer used, but not for other molecular weights. The entrapment efficiency of microparticles is known to be affected by the molecular weight grade of PLGA employed for fabrication (Fu et al., 2005). The high viscosity of the higher molecular weight PLGA may have restricted drug diffusion to the external phase during the formation of microparticles resulting in a higher entrapment efficiency. In this investigation, even though a statistically significant difference in entrapment efficiency was observed between the microparticles prepared using the lowest and highest molecular weight polymers, the difference was not as large as that observed in a study conducted by Gao and co-workers (2005). Again, this could be attributed to the relatively small polymer concentration (1% (w/v)) used in this study compared to the higher polymer concentration (40% (w/v)) used in the study reported by Gao and co-workers.

3.1.2. Effect of polymer concentration

The particle size as well as entrapment efficiency of budesonide-loaded PLGA microparticles increased with increase in the polymer concentration (Fig. 1B). It is interesting to note that the increase in particle size is comparable with the increase in entrapment efficiency. The viscosity of PLGA solution depends on the concentration of PLGA in the organic phase (Rodriguez et al., 1998). Additionally, increasing the polymer concentration and keeping the volume of the organic phase con-

stant at 5 mL; the viscosity of the organic phase would increase leading to the formation of large sized droplets, which would result in larger size of the microparticles. It is known that the entrapment efficiency of microparticles fabricated using an O/W emulsion solvent evaporation technique is dependant on the diffusion of the drug to the external phase during emulsification (Fu et al., 2005). The higher viscosity of the polymer solution at the highest polymer concentration would be expected to decrease diffusion of the drug into the external aqueous phase, which would result in higher entrapment efficiency. Therefore, the effect of polymer concentration would be expected to have a similar effect on both particle size as well as the entrapment efficiency of budesonide-loaded PLGA microparticles.

3.1.3. Effect of surfactant concentration

Increasing the surfactant (PVA) concentration from 1% to 5% (w/v) exhibited a reversal in trend between particle size and entrapment efficiency of budesonide-loaded PLGA microparticles (Fig. 1C). Microparticles fabricated using 1% (w/v) PVA had the largest particle size and the lowest entrapment efficiency while those fabricated with 5% PVA showed the lowest particle size and the highest entrapment efficiency. In addition, a linear relationship ($r^2 = 0.9998$) was observed between surfactant concentration (from 1% to 5% (w/v)) and entrapment efficiency of the microparticles. The decrease in the particle size and increase in the entrapment efficiency at increasing surfactant concentrations could be attributed to the decreased emulsion droplet size during the formation of microparticles (Castellanos et al., 2001). When added in a small concentration (e.g., 1%), the surfactant may not have been able to cover the entire organic droplet surface. Thus, some of the droplets would tend to aggregate till the surface area was decreased to such a point that the available amount of surfactant was able to coat the entire surface of the agglomerate and form a stable emulsion resulting in a larger microparticle size. This would also explain the smaller droplet size at higher surfactant concentration resulting in a greater surface area for rapid solvent evaporation and rapid hardening of microparticles, and further decreased drug diffusion to the external phase. As a result, the higher entrapment efficiency was obtained at higher surfactant concentrations.

The optimum surfactant concentration was evaluated as a measure of the final particle size. At a surfactant concentration of 0.5% no microparticles were formed. An increase in concentration from 1% to 3% (w/v) resulted in a two-fold decrease in particle size. A further increase to 5% resulted only in a small increase. Additionally, further increase greater than 5% (data not shown) did not result in any further decrease. Therefore, a surfactant concentration of 5% was considered optimum for coating the entire droplet surface for all other formulations.

3.1.4. Effect of drug concentration

Unlike the effect of polymer and surfactant concentrations on the particle size and entrapment efficiency, the effect of drug concentration was not very pronounced. From the results displayed in Fig. 1D, it is seen that the particle size increased with increase in drug concentration from 0.25% to 1% (w/v), whereas the entrapment efficiency decreased in the same drug concentration range. The increase in the particle size could be attributed to the increased drug content of the emulsion droplet at higher drug concentration. The decrease in the entrapment efficiency with increase in drug concentrations could be related to the increased extent of drug diffusion to the external phase due to greater flux at the higher drug content during the emulsification and microparticle formation process (Gabor et al., 1999).

3.2. Effect of Eudragit® S-100 coating on particle size and entrapment efficiency of budesonide-loaded PLGA microparticles

The effect of Eudragit® S-100 coating on the particle size and entrapment efficiency of budesonide-loaded PLGA microparticles prepared at various formulation variables is outlined in Table 2. The following discussion relates to this effect.

3.2.1. Effect of polymer molecular weight

The Eudragit® S-100 coated budesonide-loaded PLGA microparticles fabricated using various polymer molecular weights (ranging from 5000 to 75,000) exhibited an increase of about 30 μm in particle size. This increase was observed for all microparticles fabricated, despite the difference of particle size (from 43.82 to 48.38 μm) of microparticles resulted from polymers of the various molecular weights (Table 2). For the purpose of comparison, budesonide-loaded Eudragit® S-100 microparticles were fabricated and it is interesting to note that the mean particle size was observed to be 30.56 μm (±2.31). The results indicate that the increase in particle size of the Eudragit® S-100 coated PLGA microparticles by 30 μm is in agreement with the particle size obtained from the Eudragit® S-100 microparticles by employing the same concentration of Eudragit® S-100 (4% (w/v)). These findings suggest that a Eudragit® S-100 concentration at 4% (w/v) is efficiently able to coat microparticles having particle size up to 16.67 μm (formulation A1).

When the microparticles were coated with Eudragit® S-100, the entrapment efficiency of budesonide-loaded PLGA microparticles decreased for all the five formulations fabricated with different molecular weight of PLGA. The overall decrease in entrapment efficiency could be attributed to drug extraction occurring during the coating process. The solvents used for dissolving Eudragit® S-100 (methanol and acetone) are also good solvents for budesonide. While these solvents remain with the budesonide-loaded PLGA microparticles during the coating process, some amount of drug extraction and diffusion into the external phase takes place resulting in an overall decrease in entrapment efficiency. However, as shown in Table 2, the extent of decrease in entrapment efficiency was dependent on the molecular weight of PLGA. The decrease in entrapment efficiency was highest for the formulation fabricated with high molecular weight PLGA (9.92%), and entrapment efficiency further decreased as the proportion of high molecular weight PLGA was decreased. This decreasing trend (from 9.92% to 5.68%) was observed for formulations A1–A4 (Table 2). These findings were expected because formulation A1 exhibited the highest entrapment efficiency before coating and hence contained a higher content of drug. This higher amount of drug resulted in greater

Table 2
Effect of Eudragit® S-100 coating on particle size and entrapment efficiency of budesonide-loaded PLGA microparticles

Formulation code	Particle size[a] (μm)			Entrapment efficiency[a] (%)		
	Before coating	After coating	Difference	Before coating	After coating	Difference
Effect of polymer molecular weight						
A1	16.67 ± 1.24	48.38 ± 1.73	31.71	74.28 ± 0.83	64.36 ± 0.76	9.92
A2	16.16 ± 1.87	46.91 ± 1.57	30.75	70.83 ± 0.68	62.92 ± 1.72	7.91
A3	15.26 ± 3.17	43.82 ± 1.63	28.56	69.26 ± 0.83	63.05 ± 2.84	6.21
A4	14.58 ± 0.91	47.38 ± 3.92	32.80	67.92 ± 2.17	62.24 ± 1.47	5.68
A5	14.61 ± 1.61	45.43 ± 2.43	30.82	69.17 ± 0.88	60.04 ± 1.43	9.13
Effect of polymer concentration						
B1[b]	14.61 ± 1.26	45.29 ± 2.43	30.68	69.17 ± 0.88	60.04 ± 1.43	9.13
B2	20.60 ± 1.46	50.91 ± 0.49	30.31	76.65 ± 0.68	66.08 ± 2.81	10.57
B3	24.03 ± 2.95	54.70 ± 1.96	30.67	80.46 ± 0.51	69.67 ± 1.08	10.79
B4	26.73 ± 1.38	58.23 ± 0.91	31.50	86.29 ± 0.61	72.78 ± 1.71	13.51
Effect of surfactant concentration						
C1	34.03 ± 3.95	58.24 ± 1.54	24.21	46.89 ± 0.48	39.40 ± 1.52	7.49
C2	18.78 ± 1.22	50.16 ± 1.23	31.38	59.06 ± 0.68	49.28 ± 2.18	9.78
C3[b]	14.61 ± 1.26	45.29 ± 2.43	30.68	69.17 ± 0.88	60.04 ± 1.43	9.13
Effect of drug concentration						
D1	10.94 ± 0.95	36.73 ± 2.12	25.79	74.18 ± 1.04	64.21 ± 0.71	9.97
D2[b]	14.61 ± 1.61	45.23 ± 2.43	30.62	69.17 ± 0.88	60.04 ± 1.43	9.13
D3	17.21 ± 1.35	50.62 ± 1.94	33.41	64.05 ± 0.72	56.78 ± 1.92	7.27

[a] Data are presented as mean ± S.D., $n = 3$.
[b] Same composition as formulation A5.

diffusion during Eudragit® S-100 coating process thus contributing a greater decrease in the overall entrapment efficiency. Similarly, the formulations A2–A4, which showed lower initial entrapment efficiency, showed a lower decrease in entrapment efficiency after coating in the same order. However, formulation A5, fabricated with low molecular weight PLGA, was expected to show the least decrease in entrapment efficiency, and it showed a large decrease in entrapment efficiency, almost equivalent to formulation A1. One possible explanation for the greater loss of entrapment efficiency could be the effect of glass transition temperature (T_g) of the low molecular weight polymer. The coating process was carried out at room temperature (25 °C), which is close to the T_g of low molecular weight PLGA ($T_g = 24$ °C). The slightly increased mobility of the drug in the PLGA matrix might have resulted in a greater drug diffusion and loss into the external medium during the coating process. However, the difference in the entrapment efficiency was not very large, and therefore, this explanation cannot be conclusively stated.

3.2.2. *Effect of polymer concentration*

At 1% polymer concentration, the particle size of the microparticles increased from 14.61 (±1.26) μm for the uncoated microparticles to 45.29 (±2.43) μm after the microparticles were coated with Eudragit® S-100. This indicates an increase by about 30 μm after coating. A similar increase in particle size was observed at all concentrations of PLGA after Eudragit® S-100 coating (Table 2). It is interesting to note that the increase in particle size of the Eudragit® S-100 coated PLGA microparticles by 30 μm is in consensus with the particle size obtained from the Eudragit® S-100 coated microparticles when the same concentration of Eudragit® S-100 (4% (w/v)) was used. These findings suggest that a 4% (w/v) concentration of Eudragit® S-100 is efficiently able to coat microparticles ranging from a lower size (16.67 μm as described above) to a higher size (26.73 μm at 8% PLGA). As a result, an identical increase in the particle size (after coating) was observed in various particle sizes (ranging from 14.61 to 26.73 μm) of microparticles fabricated using different polymer concentrations.

After coating, the entrapment efficiency decreased from 69.17 (±0.88)% to 60.04 (±1.43)% at 1% (w/v) PLGA; from 76.65 (±0.68)% to 66.08 (±2.81)% at 2% (w/v) PLGA; from 80.46 (±0.51)% to 69.67 (±1.08)% at 4% (w/v) PLGA; and from 86.29 (±0.61)% to 72.78 (±1.71)% at 8% (w/v) PLGA, respectively (Table 2). The decreased overall entrapment efficiency (from 9.13% to 13.51%) upon coating for all formulations appears to be dependant on the concentration of PLGA employed. This could be attributed to drug loss occurring during the coating process. As mentioned earlier, when the budesonide-loaded PLGA microparticles were suspended in the polymeric solution during the process of coating with Eudragit® S-100, some extraction of the drug from drug-loaded PLGA microparticles may have occurred. The extent of decrease was dependant upon the concentration of PLGA, because the entrapment efficiency increased from 69.17% to 86.29% as the concentration of PLGA increased from 1% to 8% (w/v). The higher drug content in budesonide-loaded PLGA microparticles fabricated at PLGA concentration of 8% had a higher drug diffusion into the Eudragit® S-100 solvents as compared to that of the lower drug content from the lower PLGA concentration, and thus resulted in lower entrapment efficiency following the coating. However, the

difference in entrapment efficiency amongst the four formulations was not very large because the high PLGA concentration (8% (w/v)) would be expected to impede drug diffusion, thus partly counteracting the higher drug loss during coating.

3.2.3. Effect of surfactant concentration

The effect of Eudragit® S-100 coating on particle size and entrapment efficiency of budesonide-loaded PLGA formulations prepared at varying concentrations of PVA is also summarized in Table 2. These effects are comparable to the effects seen in budesonide-loaded PLGA microparticles fabricated using various concentrations of PLGA. The particle size and entrapment efficiency were affected by Eudragit® S-100 coating in the same manner as that observed at varying concentrations of PLGA. The increase in the particle size was by about 30 μm as well as an overall decrease in the entrapment efficiency. The decrease in entrapment efficiency was dependant on concentration of PVA in budesonide-loaded PLGA microparticles, except for the particle size prepared at 1% level of PVA concentration. The particle size of microparticles prepared at PVA concentrations of 3% and 5% PVA showed the expected increase of 30 μm. However, the formulation prepared with 1% PVA concentration showed an increase of only about 24 μm. It appears that a 4% (w/v) concentration of Eudragit® S-100 was unable to cover the entire surface of the largest budesonide-loaded PLGA microparticles, because the particles size at 1% PVA level was much bigger than that of PVA at 3% and 5% levels (34.03 μm versus 18.78 μm and 14.61 μm).

3.2.4. Effect of drug concentration

The effects of Eudragit® S-100 coating on particle size and entrapment efficiency of budesonide-loaded PLGA microparticles prepared at increasing concentrations of drug ranging from 0.25% to 1% (w/v) are also summarized in Table 2. The particle size of microparticles was affected by Eudragit® S-100 coating in the same manner as that observed at varying concentrations of PLGA. There was an increase in the particle size by about 30 μm, except in the case of microparticles containing 0.25% of drug concentration. Due to the low concentration of drug in the solution during the coating process, significant extraction and diffusion of drug from the drug-loaded in microparticles may have taken place resulting in the decease of particle size. Similarly, as was observed in the case of varying concentrations of PLGA, the microparticles exhibited an overall decrease in the entrapment efficiency, and the decrease was dependant on concentration of drug.

3.3. In vitro release kinetics of budesonide-loaded PLGA microparticles

The effect of various formulation factors on the release kinetics of budesonide-loaded PLGA microparticles is displayed in Figs. 2–5. Results from Figs. 2–5 indicate that all formulations showed a biphasic release profile. An initial fast release phase was observed mainly due to diffusion of the drug located at or near the surface of the microparticles. This was followed by a slow release phase, due to drug release occurring by matrix erosion and drug diffusion occurring from the inner core of the PLGA matrix.

The release rate of drug from all uncoated budesonide-load PLGA microparticles (open symbols) showed considerable drug release at pH 1.2, because PLGA is a non-enteric polymer. Hence it was imperative to coat the PLGA microparticles with an enteric polymer to retard drug release at lower pH regions of the gas-

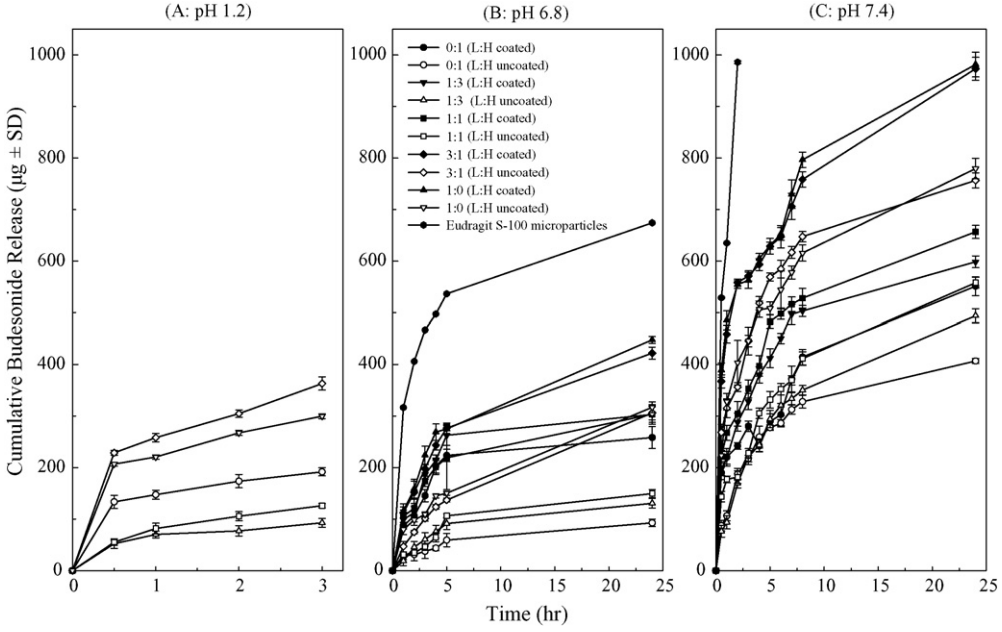

Fig. 2. Comparative *in vitro* drug release kinetics of Eudragit® S-100 coated (close symbols) and uncoated (open symbols) budesonide-loaded PLGA microparticles fabricated using various polymer molecular weights (H: 75,000; L: 5000) at (A) pH 1.2, (B) pH 6.8 and (C) pH 7.4 of the dissolution medium (data shown as mean ± standard deviation, $n = 3$).

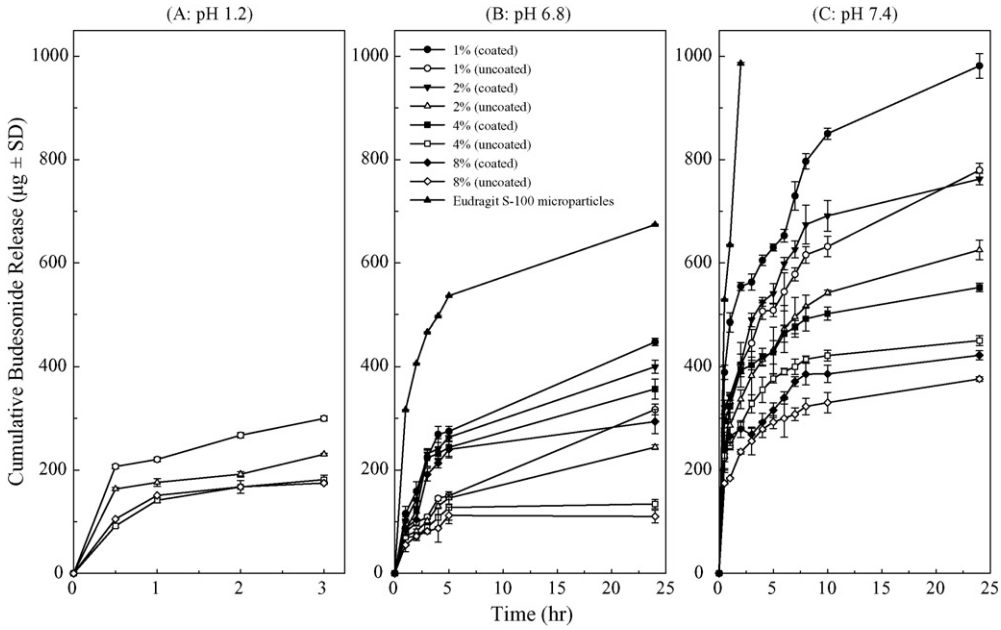

Fig. 3. Comparative *in vitro* drug release kinetics of Eudragit® S-100 coated (close symbols) and uncoated (open symbols) budesonide-loaded PLGA microparticles fabricated using various polymer concentrations at (A) pH 1.2, (B) pH 6.8 and (C) pH 7.4 of the dissolution medium (data shown as mean ± standard deviation, $n = 3$).

trointestinal tract as well as to optimize the site specific delivery of drug to the terminal ileum and colon.

A similar trend of release profile was observed at pH 6.8 for all uncoated budesonide-load PLGA microparticles (open symbols) prepared from various formulation variables as described in Table 1, but the release was considerably lower at pH 7.4. For all the formulations studied, a lower drug release was observed significantly at pH 6.8. This may have been either due to retardation of PLGA hydrolysis at the nearly neutral pH or due to lipophilic interaction of the unhydrolyzed PLGA and the drug.

3.3.1. Effect of polymer molecular weight

An interesting aspect of this study was the large variation in release profiles from microparticles fabricated at the same concentration of PLGA (1% (w/v)) but fabricated using two different molecular weight grades (M_W 75,000 and 5000) of PLGA. Because higher molecular weight polymers exhibit a

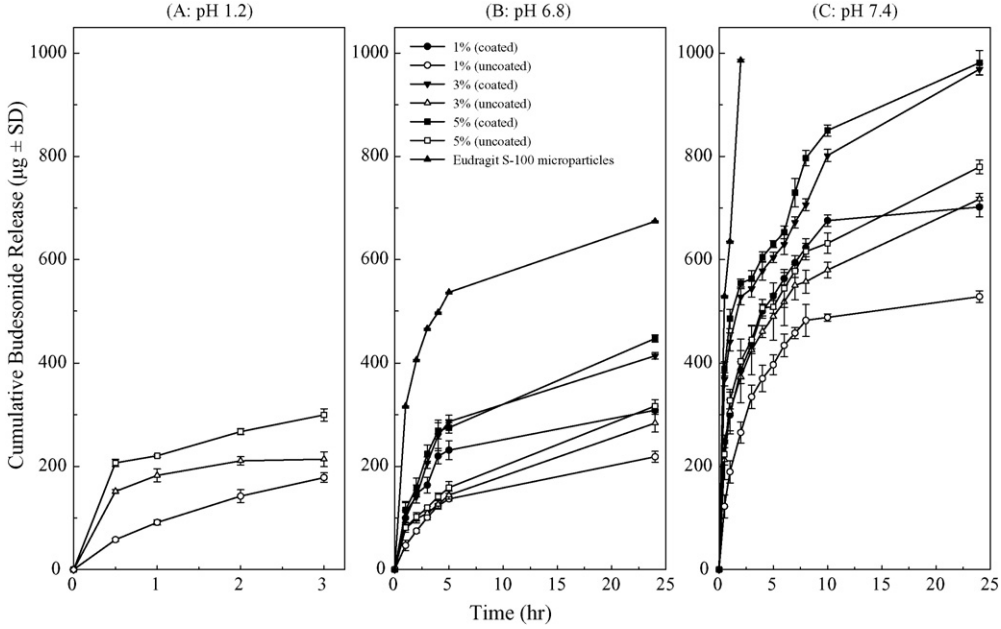

Fig. 4. Comparative *in vitro* drug release kinetics of Eudragit® S-100 coated (close symbols) and uncoated (open symbols) budesonide-loaded PLGA microparticles fabricated using various surfactant concentrations at (A) pH 1.2, (B) pH 6.8 and (C) pH 7.4 of the dissolution medium (data shown as mean ± standard deviation, $n = 3$).

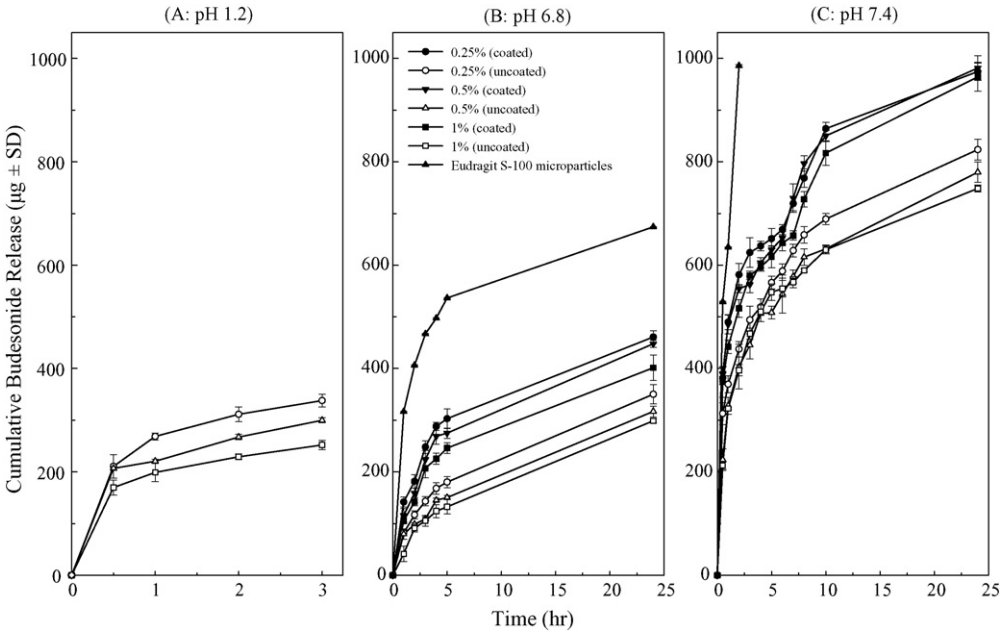

Fig. 5. Comparative *in vitro* drug release kinetics of Eudragit® S-100 coated (close symbols) and uncoated (open symbols) budesonide-loaded PLGA microparticles fabricated using various drug concentration at (A) pH 1.2, (B) pH 6.8 and (C) pH 7.4 of the dissolution medium (data shown as mean ± standard deviation, $n = 3$).

higher glass transition temperature (T_g), microparticles fabricated using PLGA M_W 75,000 ($T_g = 40\,^{\circ}C$) exhibited a lower release rate (Fig. 2C) than those fabricated using PLGA M_W 5000 ($T_g = 23\,^{\circ}C$). At temperatures above the T_g, the polymer undergoes transition from a glassy state to a rubbery state resulting in a greater mobility of the drug in the polymer matrix and faster diffusion (Shameem et al., 1999). Since the dissolution studies were carried out at 37 °C, which is much above the T_g of PLGA (M_W 5000) a greater release was observed at pH 7.4 of the dissolution medium.

Furthermore, budesonide-loaded PLGA microparticles fabricated with PLGA (M_W 75,000) released a very small amount of drug (about 40%) at the end of 24 h at pH 7.4. Since this formulation was not able to release nearly all the entrapped drug through the colon transit time, a lower polymer molecular weight was selected for the further development of budesonide-loaded PLGA microparticles.

3.3.2. *Effect of polymer concentration*

Fig. 3 shows the effect of PLGA concentration on drug release kinetics from the uncoated budesonide-load PLGA microparticles (open symbols). The release of drug decreased with increase in polymer concentration from 1% to 8% (w/v) at all three pH values (1.2, 6.8 and 7.4) of the dissolution medium. The higher polymer concentration appears to have impeded drug diffusion and resulted in significantly lower release flux. Additionally, the larger particle size at higher polymer concentration also restricted the total surface area resulting in a slower release.

The release rate of drug (Fig. 3A and B) from all uncoated budesonide-load PLGA microparticles (open symbols) showed considerable drug release at pH 1.2 (15–30% in 3 h) as well as at pH 6.8 (10–30% in 24 h). Hence it was imperative to coat the PLGA microparticles with an enteric polymer to retard the drug release in lower pH regions of gastrointestinal track as well as to optimize the site specific delivery of drug to the terminal ileum and colon.

Due to a much higher diffusion flux and a greater amount of drug release (80% and 65% at the end of 24 h at pH 7.4) obtained at PLGA concentrations of 1% and 2% (w/v), respectively (Fig. 3C), it can be hypothesized that diffusion could possibly be the dominant mechanism of drug release through the entire duration of dissolution study. At higher PLGA concentrations of 4% and 8% (w/v), diffusion was greatly retarded and hence complete drug release was expected to occur very slowly, and be completed only upon complete erosion of the PLGA matrix.

3.3.3. *Effect of surfactant concentration*

As expected, due to the smaller particle size obtained at higher surfactant concentrations, a larger drug release rate was observed from uncoated budesonide-loaded PLGA microparticles prepared using higher surfactant concentrations at all three pH values of dissolution medium. The trend was similar at pH 1.2 (Fig. 4A) and at pH 6.8 (Fig. 4B) from all uncoated budesonide-load PLGA microparticles (open symbols) as well as from coated microparticles (closed symbols).

A remarkably higher amount of drug release at pH 7.4 from all uncoated budesonide-load PLGA microparticles (open symbols) fabricated using various surfactant concentrations (Fig. 4C) was observed. All formulations containing the lowest concentration (1% (w/v)) of PLGA, the release of drug appeared to be governed mainly by diffusion of drug from the microparticles. The release rate decreased with decrease in surfactant concentrations. However, the release from microparticles fabricated at different concentrations of surfactant was still greater than that observed at PLGA concentrations of 4% and 8% (w/v) (Fig. 3C).

3.3.4. Effect of drug concentration

Drug concentration did not seem to influence the release kinetics of budesonide-loaded PLGA microparticles (Fig. 5). This kind of a release profile was expected as particle size and entrapment efficiency were comparable for all three formulations. Additionally, the surfactant concentrations was kept constant at 5% (w/v) in all formulations resulting in small particle size which was independent of the small increment in drug concentration from 0.25% to 1% (w/v).

3.4. In vitro release kinetics of Eudragit® S-100 coated budesonide-loaded PLGA microparticles

Eudragit® S-100 is an enteric polymer showing complete dissolution at pH greater than 7. None of the Eudragit® S-100 coated budesonide-loaded PLGA showed drug release at pH 1.2 which indicates that upon Eudragit® S-100 coating, the release of drug can be retarded successfully in an acidic environment.

Ideally, drug release is not expected at pH 6.8 (pH of jejunum). However, it can be seen from Figs. 2B, 3B, 4B, and 5B that a small proportion of the drug (12% or less) was released within 1 h (jejunum transit time) from all coated formulations. Hence, the loss of drug at pH 6.8 from Eudragit® S-100 coated budesonide-loaded microparticles can be minimized.

It can be seen from Figs. 2–5 that upon Eudragit® S-100 coating, all coated budesonide-loaded PLGA microparticle formulations (close symbols) exhibited an accelerated release with an increase in the cumulative amount of drug release. This is possibly due to the fact that there is considerable drug extraction from PLGA microparticles during the coating process described earlier. Apparently, the extracted drug gets entrapped in between the PLGA coat and Eudragit® S-100 coat, and the entrapped drug releases as the Eudragit® S-100 coating dissolves, while the release from PLGA microparticles continues in a controlled manner.

Results in Fig. 2B indicate that Eudragit® S-100 microparticles (solid hexagon) did not release the drug completely at pH 6.8 even at the end of 24 h of release study. This was probably due to the incomplete dissolution of the enteric polymer at this pH and hence drug release at this pH would be expected to be governed by both PLGA matrix and Eudragit® S-100 membrane.

Upon observing the release profiles shown in Fig. 2C, it can be seen that drug release from Eudragit® S-100 microparticles (solid hexagon) was complete within 2 h at pH 7.4. Therefore, following the first 2 h of release, the release profile of drug from Eudragit S-100 coated as well as uncoated budesonide-loaded PLGA microparticles would be expected to be similar. The presence of PLGA provided a control release property to the formulation.

In all coated microparticles evaluated, formulations A4, A5, B1, C2, C3, D1, D2 and D3 exhibited a nearly complete release (80–88%) within 10 h of incubation in dissolution medium at pH 7.4. This was a desired release profile considering the desired site of drug release is the terminal ileum and proximal colon of the gastrointestinal tract, and the total transit time through these regions appears between 12 and 14 h.

4. Conclusions

The results of this investigation indicate that solvent evaporation method can be successfully employed to fabricate budesonide-loaded PLGA microparticles with particle size in the range of 10–35 μm. The entrapment efficiency and *in vitro* drug release kinetics were found to be greatly influenced by such formulation variables such as molecular weight of PLGA, concentration of PLGA, concentration of surfactant and concentration of drug. These findings indicate that these variables can be suitably altered to achieve the desired controlled release profile of budesonide. Furthermore, attaining site-specific delivery in the terminal ileum and proximal colon can be achieved by coating the budesonide-loaded PLGA microparticles by Eudragit® S-100, an enteric polymer. The release studies showed complete retardation of drug release in an acidic pH while a controlled release of the drug from the microparticles was observed, once the coating layer of enteric polymer dissolved at a higher pH (pH 7.4 and pH 6.8). This double microencapsulation technique employing PLGA matrix and Eudragit® S-100 coating shows promise for site specific and controlled delivery of budesonide in Crohn's disease. Further investigation with animal studies should be carried out to establish the effectiveness and efficiency of this approach to the formulation.

References

Baker, D.E., 2001. Budesonide modified release capsules. Rev. Gastroenterol. Disord. 1, 147–155.

Castellanos, I.J., Carrasquillo, K.G., de Jesus Lopez, J., Alvarez, M., Griebenow, K., 2001. Encapsulation of bovine serum albumin in poly(lactide-*co*-glycolide) micropsheres by the solid-in-oil-in-water technique. J. Pharm. Pharmacol. 53, 167–178.

Feagan, B.G., Sandborn, W.J., 2002. Initial therapy for mild to moderate Crohn's disease: mesalmaine or budesonide? Rev. Gastroenterol. Disord. 2, s9–s15.

Fu, X., Ping, Q., Gao, Y., 2005. Effects of formulation factors on encapsulation efficiency and release behavior *in vitro* of huperzine A-PLGA microspheres. J. Microencapsul. 22, 705–714.

Gabor, F., Ertl, B., Wirth, M., Mallinger, R., 1999. Ketoprofen-poly(D,L-lactic-*co*-glycolic acid) microspheres: influence of manufacturing parameters and type of polymer on the release characteristics. J. Microencapsul. 16, 1–12.

Habib, M., Onyilofor, S., Ebube, N.K., Owusu-Ababio, G., 1999. Preparation and characterization of ofloxacin microspheres for the eradication of bone associated bacterial biofilm. J. Microencapsul. 16, 27–37.

Haeberlin, B., Friend, D., 1992. Anatomy and physiology of the gastrointestinal tract: implications for colonic drug delivery. In: Friend, D. (Ed.), Oral Colon-specific Drug Delivery. CRC Press Inc., Florida, pp. 20–30.

Klotz, U., Schwab, M., 2005. Topical delivery of therapeutic agents in the treatment of inflammatory bowel disease. Adv. Drug Deliv. Rev. 57, 267–279.

Kompella, U.B., Bandi, N., Ayalasomayajula, S.P., 2001. Poly(lactic acid) nanoparticles for sustained release of budesonide. Drug Deliv. Technol. 1, 28–34.

Martin, T.M., Bandi, N., Shulz, R., Roberts, C.B., Kompella, U.B., 2002. Preparation of budesonide and budesonide-PLA microparticles using supercritical fluid precipitation technology. AAPS Pharm. Sci. Technol. 3, E18.

Mojaverian, P., Chan, K., Desai, A., John, V., 1989. Gastrointestinal transit of a solid indigestible capsule as measured by radiotelemetry and dual gamma scintigraphy. Pharm. Res. 6, 719–724.

Rodriguez, M., Vila-Jato, J.L., Torres, D., 1998. Design of new multiparticulate system for potential site specific and controlled drug delivery to the colonic region. J. Control. Rel. 55, 67–77.

Shameem, M., Lee, H., Deluca, P.P., 1999. A short-term (accelerated release) approach to evaluate peptide release from PLGA depot-formulations. AAPS Pharmsci. 1 (article 7).