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Synthesis and evaluation of dextran–budesonide conjugates as colon specific prodrugs for treatment of ulcerative colitis

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ABSTRACT

Budesonide is a potent glucocorticoid with high affinity for the glucocorticoid receptor, which is now used for the treatment of inflammatory bowel diseases. Current oral formulations of budesonide present low efficacy against ulcerative colitis because of the premature drug release in the upper part of the gastrointestinal tract. The objective of this study was to develop a colon specific delivery system for budesonide to increase the efficacy in the treatment of ulcerative colitis. Dextran–budesonide conjugates were prepared with different molecular weights (MW) of dextran (10,000, 70,000 and 500,000) in the presence of dimethylaminopyridine (DMAP) using succinate spacer. The conjugates were characterized by ^{1}H NMR and IR spectroscopy and elemental analysis. The degree of substitution, aqueous solubility and chemical stability of conjugates in HCl 0.1N, phosphate buffer solutions pH 6.8 and 7.4 were studied. Drug release characteristics of the conjugates were also studied in the presence of the luminal contents of different segments of the rat gastrointestinal tract. Degree of substitution (DS) was dependent on the polymer MW and was 19.33, 14.29 and 11.60 mg/100 mg conjugate for MW 10,000, 70,000 and 500,000, respectively. Solubility of the drug in conjugates of MW 10,000 and 70,000 was increased with respect to the free drug and was dependent on DS. The three conjugates were found to be stable in HCl 0.1N, phosphate buffer solutions pH 6.8 and 7.4 incubated at 37 °C within 6 h and the rate constants for degradation of conjugates to budesonide and budesonide hemisuccinate were less than 0.006 h^{-1}. Less than 10% of the drug was released in contents of the stomach and small intestine, while about two-fold increase was observed after incubating the conjugates with colonic luminal contents. Conjugate prepared by dextran 70,000 showed the most desirable solubility, stability and release properties and could therefore be evaluated in vivo, for potential clinical use in the treatment of ulcerative colitis.

1. Introduction

Inflammatory bowel disease (IBD) is a chronic relapsing inflammation afflicting any part of the entire bowel wall which can affect anywhere between the mouth to anus, e.g. oropharynx, esophagus, stomach and rectum. IBD is the result of a dysregulated, aberrant and even inappropriate overactivation of mucosal response in the intestinal wall due to the defects in the barrier function of the intestinal epithelium and mucosal immune system (Klotz and Schwab, 2005; Jung et al., 2006). Two specific IBD subtypes based on the site and extent of inflammation are Crohn's disease and ulcerative colitis. Crohn's disease was first described as a disease in the distal ileum, however, it may be found anywhere in the gastrointestinal (GI) tract from the mouth to the anus. Ulcerative colitis invariably affects the rectum and may extend proximally in a confluent pattern to involve a part of or the entire colon (Friend, 2005; Knigge, 2002). There is no complete cure for IBD (other than colectomy in ulcerative colitis) and the major goal is to decrease the relapse episodes and to increase the patient quality of life (Gionchetti et al., 2002). Aminosalicylates and glucocorticoids are the drugs of choice for the active phase of IBD and immunosuppressants are usually used to establish, and importantly, maintain remission of IBD. Systemic glucocorticoids are currently being used for the treatment of mild, moderate and severe ulcerative colitis, though their severe adverse effects limit their use (Rodriguez et al., 2001). Since IBD is characterized by local inflammation, targeting drugs directly to the site of injury has the benefit of lower adverse effects and more effective therapy. Different

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delivery systems have been developed for colon targeted therapy including time-dependent, pH-sensitive, pressure-controlled and microbially triggered systems (Friend, 2005). The prodrug approach is a microbially triggered system, in which the drug–carrier linkage is stable in the upper part of GI tract and is specifically hydrolyzed by colonic microflora (Sinha and Kumria, 2001). Dextran as a natural polysaccharide remains intact under mild acidic and basic conditions and is digested by colonic microbial dextranase. These unique properties along with the limited inter- and intra-species variations in the activity of dextranase make it a suitable carrier for colon specific drug delivery (Mehvar, 2000; Lee et al., 2001). Prodrugs of dextran with different drugs such as naproxen and ketoprofen have been prepared to target the drug to the colonic region of pig (Larsen et al., 1989; Larsen et al., 1991). Prodrugs of dexamethasone and methylprednisolone have also been synthesized (McLeod et al., 1993), which found to be more effective than free drug in the treatment of rat induced colitis (McLeod et al., 1994).

Budesonide is a potent glucocorticoid with high local anti-inflammatory effect and low systemic bioavailability due to the result of extensive first pass metabolism. Budesonide is available in two controlled-release oral dosage forms, Budenofalk® and Entocort® (Fedorak and Bistritz, 2005). These two formulations deliver the drug to the ileum and ascending colon and only a small fraction of the active molecule is released in transverse and descending colon and consequently they are less effective in the treatment of ulcerative colitis (Edsbacker et al., 2003). On the light of above considerations, designing and developing a system which could deliver budesonide to the colon seems imperative. Therefore, the aim of this study was to prepare dextran–budesonide conjugates for colon specific delivery. Dextran–budesonide conjugates were synthesized using succinate as the spacer. To investigate the effect of polymer molecular weight on drug release profile, dextrans with various molecular weights (10,000, 70,000 and 500,000 Da) were examined. Solubility and chemical stability of the conjugates in media with different pH and release of budesonide after incubation with the contents of different GI segments of rats were also assessed.

2. Materials and methods

2.1. Materials

Budesonide was a gift from AstraZeneca (UK). Other chemicals and solvents were purchased from commercial sources: dextran (weight-average molecular weights 10,000, 70,000 and 500,000 (Sigma, St. Louis, USA), 4-dimethylaminopyridine, 1,1′-carbonyldiimidazole, succinic anhydride (Merck, Germany), anhydrous DMSO (Fluka, Germany), HPLC grade acetonitrile and methanol (Caledon, Canada). All other solvents and chemicals were of analytical grade.

2.2. Synthesis of budesonide-21-hemisuccinate (BS)

Budesonide-21-hemisuccinate was synthesized based on the method reported for dexamethasone-21-hemisuucinate (Pang et al., 2002) with slight modification in the purification step. Briefly, 10.0 mmol of budesonide, 13.0 mmol DMAP and 13.0 mmol of succinic anhydride were dissolved in anhydrous acetone. The obtained solution was stirred at 25 °C for 15 h. Acetone was then evaporated in a rotary evaporator and the remaining residue was washed 5 times with 5.0 ml HCl 0.1N and extracted with ethyl acetate. After evaporation of the solvent, budesonide-21-hemisuccinate was obtained as a white powder. The product was characterized by ^{1}H NMR and IR spectroscopy. ^{1}H NMR spectra (DMSO-d_6) were recorded on a Bruker 300 MHz spectrometer. Chemical shifts (δ) were reported in ppm downfield from the internal standard tetramethylsilane (TMS). The IR spectra were recorded with a PerkinElmer 1420 Ratio Recording IR spectrometer as a KBr disc (cm^{-1}).

Table 1
Detailed explanation of the three synthesized conjugates

Conjugate code	Conjugate name	Dextran MW (Da)
BSD-10	Budesonide succinate–dextran 10	10,000
BSD-70	Budesonide succinate–dextran 70	70,000
BSD-500	Budesonide succinate–dextran 500	500,000

2.3. Synthesis of dextran conjugates

Dextran conjugates were synthesized according to the method reported for dexamethasone (Pang et al., 2002). 2.0 mmol of the budesonide hemiester and 3.5 mmol of 1,1′-carbonyldiimidazole were dissolved in 5.0 ml anhydrous DMSO and stirred at 25 °C for 30 min. Then 6.0 ml anhydrous triethylamine and 5% (w/v) solution of dextran in DMSO (60.0 ml) were added and the reaction mixture was stirred for 21 h at 25 °C. The reactions were carried out in completely dried and tight-closed glasswares. The product was precipitated by addition of ethanol:diethyl ether (1:1) while stirring. The resultant, a gummy polymer, was collected with filtration under reduced pressure and dispersed in methanol. The precipitate was washed with diethyl ether and dried in a vacuum oven. Budesonide–dextran conjugates of varying molecular weights were prepared with the same manner. The obtained products were characterized using ^{1}H NMR and IR spectroscopy and elemental analysis. Elemental analysis was performed on polymers before the reaction and on the final synthesized conjugates by an elemental analyzer system (Thermo finnigan flash EA 1112). A schematic presentation of the synthesis process is depicted in Fig. 1 and detailed explanation of the three synthesized conjugates is presented in Table 1.

2.4. Determination of drug content in conjugates

Drug content was determined following basic hydrolysis of the conjugates (Mehvar, 1999). 5.0 ml NaOH 0.1N and 3.0 ml methanol were added to 5.0 mg of each conjugate and vortexed. Since there is an acetal group at C_{16} and C_{17} position of budesonide, the drug is susceptible to basic hydrolysis and degrades under basic condition. To control the degradation process, solutions of budesonide in the concentration range of 10–200 μg/ml were prepared in methanol and undergone basic hydrolysis by adding 5.0 ml NaOH 0.1N. All mixtures were then left at room temperature overnight. A 100 μl sample of each solution was mixed with 100 μl HCl 0.1N and 50 μl of the resulting solution was injected into the HPLC. The concentration of the degradation product was determined and proportioned to the original concentration of the drug. Finally, a standard curve was constructed for determination of drug content in each conjugate.

2.5. HPLC analysis

A reversed-phase HPLC method was used for determination of budesonide. HPLC analysis was performed using a Waters 515 pump; Waters 2487 dual λ absorbance detector and data were integrated using Millennium® software for HPLC.

A C_{18} Waters μ-Bondapak HPLC column (250 mm × 4.6 mm) and a mobile phase consisting of acetonitrile:KH_2PO_4 0.025 M (55:45, pH 3.2) at a flow rate of 1 ml/min were applied. The eluent was detected at 244 nm. Injection volume was 50 μl and dexamethasone was applied as an internal standard. Quantitation was

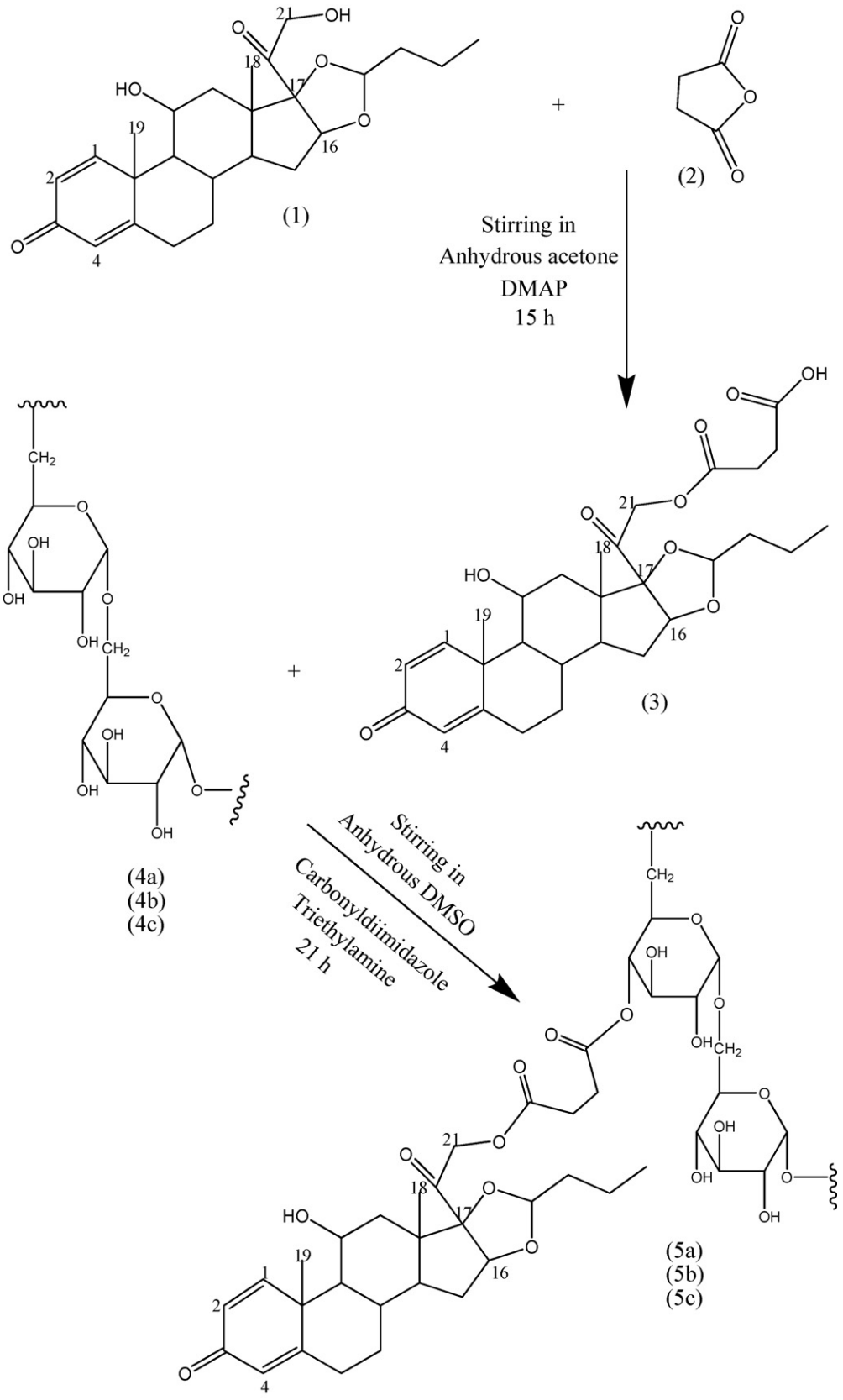

Fig. 1. Schematic presentation of the synthesis of dextran conjugates. (1) Budesonide, (2) succinic anhydride, (3) budesonide-21-hemisuccinate, (4a) dextran 10,000, (4b) dextran 70,000, (4c) dextran 500,000, (5a) BSD-10, (5b) BSD-70 and (5c) BSD-500.

achieved by measurement the peak area ratios of the drug to the internal standard. The mobile phase was prepared daily, filtered and degassed by ultrasonication before use.

2.6. Solubility and chemical stability studies

In order to compare the solubility of conjugates with that of untreated budesonide, 100 mg of each conjugate and budesonide powder were separately transferred to 1 ml distilled water and shaked at 25 °C for 6 h. After centrifugation, the supernatant was decanted and basic hydrolysis was performed on the supernatant according to the previously reported method (Mehvar, 1999). The drug concentration was measured by HPLC method to determine the solubility of parent drug as well as the conjugates.

For determination of chemical stability of the conjugates, 5 mg of each conjugate was incubated in different media including HCl 0.1N, phosphate buffer solutions pH 6.8 and 7.4 at 37 °C for 6 h. At 1, 2, 4 and 6 h, a 100 μl portion of the reaction mixture was removed, centrifuged at 5000 × *g* for 5 min and 50 μl of the supernatant was injected into the HPLC system to determine the percentage of the drug released. Stability data were fitted to zero order $W = W_0 - k_0 t$ and first order $\ln W = \ln W_0 - k_1 t$ kinetic models, where W is the amount of conjugate remained unchanged at time t and W_0 is the initial amount of drug. The goodness of fit was assessed by linear regression method and the most probable release kinetic was determined. The rate constants for hydrolysis of conjugates to budesonide (K_{BUD}) and budesonide hemisuccinate (K_{BS}) were calculated for each conjugate. A calibration curve was constructed for the determination of budesonide hemisuccinate in stability and dissolution samples. BS was synthesized, purified and its chemical structure and purity was examined using ^{1}H NMR and HPLC studies. Solutions of BS in the concentration range of 1–10 μg/ml were prepared and injected to HPLC in triplicate and the calibration curve was developed for determination of BS.

2.7. Release of budesonide after incubation with rat GI contents

Within one week prior to the start of the dissolution studies, male Wistar rats were maintained on normal diet and 1 ml (per day) of 2% (w/v) solution of dextran in water was administered directly into the stomach in order to induce enzymes specifically acting on dextran in the caecum and colon. The rats were then sacrificed by decapitation and after midline incision; luminal contents of stomach, small intestine, caecum and colon were removed and transferred to appropriate buffer solutions containing 0.5% sodium lauryl sulfate (SLS) to preserve the sink condition for budesonide. Contents were homogenated in acetate buffer pH 4.4, phosphate buffer solutions pH 7.4 and 6.8 to simulate the gastric, small intestinal and colonic pH of the rat, respectively. These are the reported normal pH values of rat GI tract (McLeod et al., 1993; Lee et al., 2001). The phosphate buffer solution used to dilute caecal and colonic contents was saturated with N_2. Drug release studies were performed in triplicate on each conjugate (an amount equivalent to 3 mg budesonide) in 50 ml appropriate buffer solution at 37 °C using an undersized, homely designed USP dissolution apparatus II (paddle method). Dissolution study was performed for 2 h in acetate buffer pH 4.4 containing contents of the rat stomach, 4 h in phosphate buffer pH 7.4 containing contents of rat intestinal segment and 18 h in phosphate buffer pH 6.8 containing caecal and colonic contents of rat in separate experiments (each buffer contained 4% of the appropriate contents of the GI tract). The dissolution process in the presence of colonic contents was carried out under nitrogen atmosphere to maintain the anaerobic condition. At predetermined time intervals, 100 μl sample of each medium was withdrawn and replaced with the same volume of fresh medium. After addition of methanolic solution of the internal standard, the samples were vortexed and centrifuged at 10,000 × *g* for 5 min and 50 μl of the supernatant was injected into the HPLC.

2.8. Statistical analysis

Cumulative percentage of budesonide (B_{24}%) and budesonide hemisuccinate (BS_{24}%) released after 24 h were used to compare the release profiles.

One-way ANOVA followed by Tukey's test was used to compare the results of solubility and stability studies, B_{24}% and BS_{24}% of different conjugates and the drug release profiles in different segments of the GI tract. P-value < 0.05 was considered significant.

3. Results

3.1. Synthesis of dextran conjugates

Budesonide-21-hemisuccinate was synthesized based on the methods reported for dexamethasone (McLeod et al., 1993; Pang et al., 2002) with modification. About 30% excess of succinic anhydride and DMAP was reacted with budesonide for 15 h to obtain high yield of the product (85%). Washing the reaction mixture with HCl and extracting with ethyl acetate was a very simple and efficient procedure for removing DMAP and purification of budesonide hemisuccinate. ^{1}H NMR ($CDCl_3$) results were as follows: δ (ppm): 0.960 (s, 3H, 18-CH_3), 1.468 (s, 3H, 19-CH_3), 2.194 (s, 4H, succinate C_2 and C_3), 6.057 (s, 1H, C_4—H), 6.318 (d, 1H, C_2—H), 7.295 (d, 1H, C_1—H).

IR results were as follows: 3500 (OH), 2950 (C—H, stretch, aliphatic and alkene), 1750 (C=O, ester), 1675 (C=O, carboxylic acid), 1620 (alkene, α,β-unsaturated ketone).

Dextran conjugates were produced and purified in high yields (>90%). NMR spectra of conjugates in DMSO-d_6 showed the unsaturated carbon protons as a doublet in δ 6.128 for C_2—H, a doublet in δ 7.280 for C_1—H and a singlet in δ 5.581 for C_4—H and large peaks of enormous numbers of CH_2 and CH groups of dextran.

Elemental analysis data for the conjugates were calculated based on the data found for the unreacted polymers and the degree of substitution of each conjugates. Results of elemental analysis are given in Table 2.

3.2. Determination of drug content in conjugates

Budesonide has an acetal group which is susceptible to basic hydrolysis. Fig. 2a is the HPLC chromatogram of a solution containing budesonide, its degradation product and budesonide hemisuccinate indicating that the method is capable of separating these three peaks. Fig. 2b–e shows the progressive degradation of budesonide after alkaline hydrolysis of BSD-70. HPLC chro-

Table 2
Results of degree of substitution and elemental analysis of the conjugates

Conjugate code	mg drug/100 mg of conjugate[a]	Elemental analysis (%)			
		Calculated[b]		Found	
		C	H	C	H
BSD-10	19.33 ± 0.84	45.67	6.34	45.52	6.20
BSD-70	14.29 ± 0.41	43.66	6.33	44.38	6.26
BSD-500	11.60 ± 0.37	44.00	6.31	45.28	6.62

[a] Results are mean ± S.D. (n = 3) calculated after basic hydrolysis of conjugates.
[b] Calculated based on the results of DS and elemental analysis data found for unreacted polymer: dextran 10,000 (C: 40.96, H: 6.25), dextran 70,000 (C: 40.79, H: 6.24), dextran 500,000 (C: 40.42, H: 6.17).

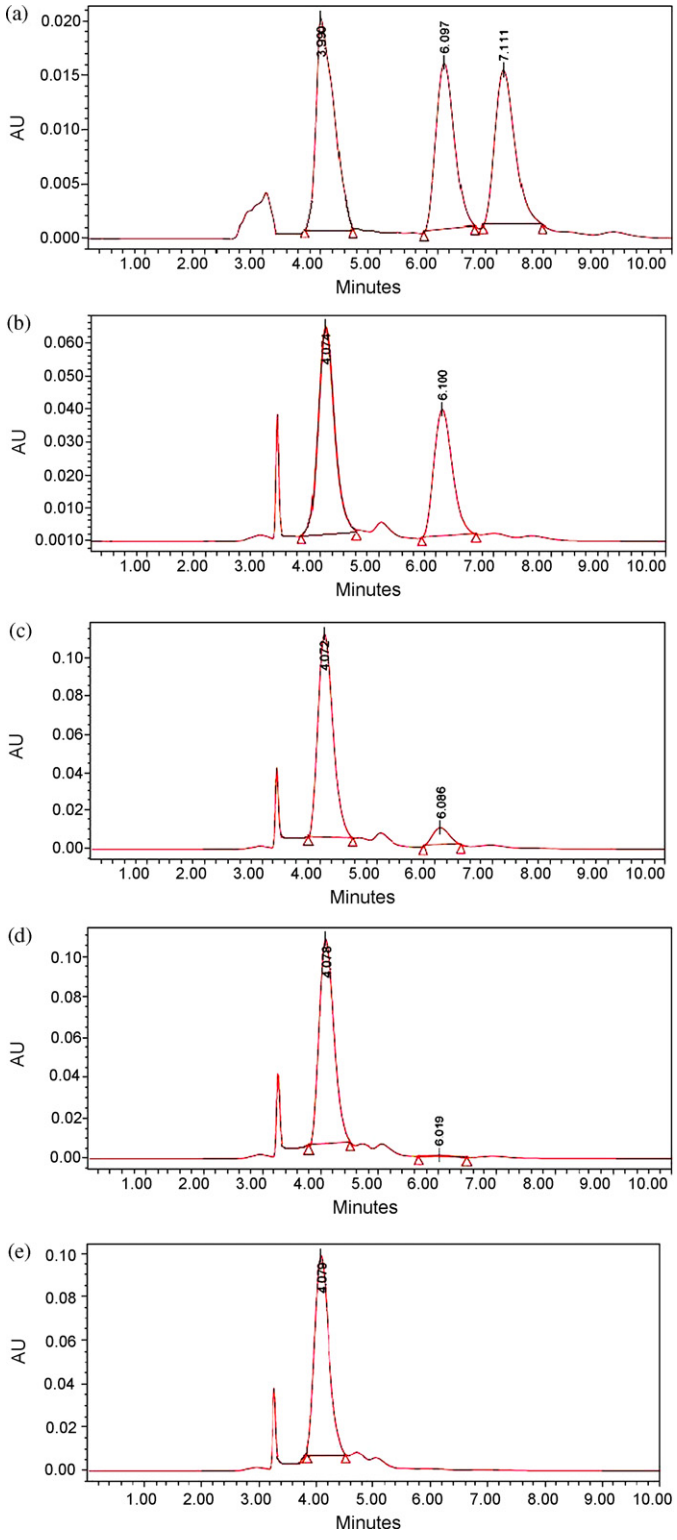

Fig. 2. HPLC chromatograms of (a) a solution containing budesonide, its degradation product and budesonide hemisuccinate and progressive degradation of budesonide (b) 0 min, (c) 10 min, (d) 30 min and (e) 60 min after alkaline hydrolysis of BSD-70. Retention times for degradation product of budesonide is 3.99–4.1 and for budesonide and budesonide hemisuccinate are 6–6.1 and 7–7.11 min, respectively.

Table 3
Solubility of conjugates at 25 °C in water ($n = 3$)

Conjugate code	Solubility	
	Budesonide–dextran conjugate (mg/ml)	Equivalent of budesonide (mg/ml)
BSD-10	25.247 ± 1.919	4.898 ± 0.372
BSD-70	53.552 ± 2.894	10.389 ± 0.561
BSD-500	0.170 ± 0.064	0.020 ± 0.007
Budesonide	–	0.037 ± 0.009

matograms indicate that the drug degradation was completed after 60 min. Therefore, all solutions were kept at room temperature overnight after alkaline hydrolysis to ensure completion of hydrolysis. A calibration curve was constructed based on the basic hydrolysis of the standard solutions of budesonide and the drug content in conjugates was determined using this calibration curve. Good linear relationships were found when the peak area of budesonide degradation product was plotted vs. budesonide concentration ranging from 10 to 200 μg/ml. The coefficient of the linear regression analysis (R^2) was 0.9992. The CV%, which is a criterion for evaluating precision of the method, for all the concentration studied, was less than 8% and %error which shows the accuracy of the method was less than 9%. Results of coefficient of variation and percent error indicate that method is reproducible and accurate. DS which is the amount of budesonide (mg) per 100 mg of each conjugate was calculated. DS was decreased as the molecular weights (MW) of the polymer used in the conjugate increased. DS values were 19.33 ± 0.84, 14.29 ± 0.41 and 11.60 ± 0.37 mg/100 mg of conjugate for BSD-10, BSD-70 and BSD-500, respectively. The results of DS are shown in Table 2.

3.3. Solubility and chemical stability studies

The results of solubility studies of conjugates are presented in Table 3. The data indicate an improvement in the solubility of the drug after binding to hydrophilic carrier. Solubility of BSD-10, BSD-70 and BSD-500 were 25.247 mg/ml (equivalent to 4.898 mg budesonide/ml), 53.552 mg/ml (equivalent to 10.389 mg budesonide/ml), and 0.170 mg/ml (equivalent to 0.020 mg budesonide/ml), respectively, and for budesonide was 0.04 mg/ml. Results show that solubility was also dependent on the MW of polymer used and DS of conjugates.

Based on the chemical structures of conjugates, both the esteric bond between the drug and the spacer and the bond between the spacer and the polymer are susceptible to hydrolysis. Therefore, upon incubation in different buffer solutions, the drug and the hemisuccinate ester could be released. The amount of the drug and hemiester released in HCl 0.1N and phosphate buffer solutions pH 6.8 and 7.4 were calculated to determine the concentration of conjugate in each sampling time. A calibration curve was constructed for determination of budesonide hemisuccinate which was linear ($R^2 = 0.9995$) in the concentration range of 1–10 μg/ml. Results of analysis of regression showed that the stability data followed first order kinetics ($P < 0.05$). K_{BUD} and K_{BS} were calculated based on the first order kinetics. The results of the rate constants for hydrolysis of conjugates calculated according to the first order kinetics are summarized in Table 4. K_{BUD} and K_{BS} values were small ranging from 0.0007 to 0.0058 h^{-1} for K_{BUD} and 0.0015 to 0.0035 h^{-1} for K_{BS} which indicates that conjugates are stable at pH of stomach and small intestine. Stability was also dependent on the MW of conjugates and the values of K_{BUD} and K_{BS} decreased as the MW of the conjugates increased ($P < 0.05$). Stability profiles were different ($P < 0.05$) in three buffer solutions and were more profound in acidic media.

Table 4
Rate constants for hydrolysis of conjugates in different buffers ($n = 3$)

Buffer type	K_{BUD} (h^{-1})			K_{BS} (h^{-1})		
	BSD-10	BSD-70	BSD-500	BSD-10	BSD-70	BSD-500
HCl 0.1N	0.0032 ± 0.0002	0.0058 ± 0.0003	0.0041 ± 0.0002	0.0022 ± 0.0002	0.0035 ± 0.0002	0.0026 ± 0.0002
Phosphate buffer pH 6.8	0.0041 ± 0.0003	0.0047 ± 0.0002	0.0007 ± 0.0002	0.0023 ± 0.0002	0.0029 ± 0.0003	0.0016 ± 0.0002
Phosphate buffer pH 7.4	0.0035 ± 0.0002	0.0028 ± 0.0003	0.0024 ± 0.0004	0.0015 ± 0.0002	0.0016 ± 0.0002	0.0030 ± 0.0003

3.4. Release of budesonide after incubation with GI contents of rat

Fig. 3a and b shows the cumulative release profiles of budesonide and budesonide hemisuccinate after incubation of conjugates with contents of different segments of the rat GI tract. Less than 10% of the drug was released in the presence of contents of the stomach and small intestine. When conjugates were incubated in the buffer containing rat caecal and colonic contents, a rapid increase (about two-fold) was observed in the release profile (sampling time 8 h in release profiles). Drug release was decreased as the MW of the polymer increased. B_{24}% was 22%, 11% and 4% and BS_{24}% was 10%, 18% and 11% for BSD-10, BSD-70 and BSD-500, respectively. Net amount of budesonide released in the presence of contents of each segment of the rat GI tract was calculated (Fig. 4). Only for BSD-70, the amount of drug released in the presence of caecal and colonic contents were different from that of released in the small intestinal contents ($P < 0.05$). In order to determine the effect of contents of colonic segment on drug release profile, dissolution study was conducted in the presence and absence of these contents. Fig. 5 shows the release profile of three conjugates in the presence and absence of rat caecal and colonic contents. B_{24}% for BSD-70 was 6% in the absence of colonic contents and increased to 11% in the presence of these contents which shows the significant role ($P < 0.05$) of the colonic microflora for hydrolysis of conjugates. For BSD-10 the B_{24}% was increased from 14% in the absence of colonic contents to 22% in the presence of colonic contents ($P < 0.05$). For BSD-500 the difference was small (3.08 vs. 3.8), but it was significant at $P < 0.05$.

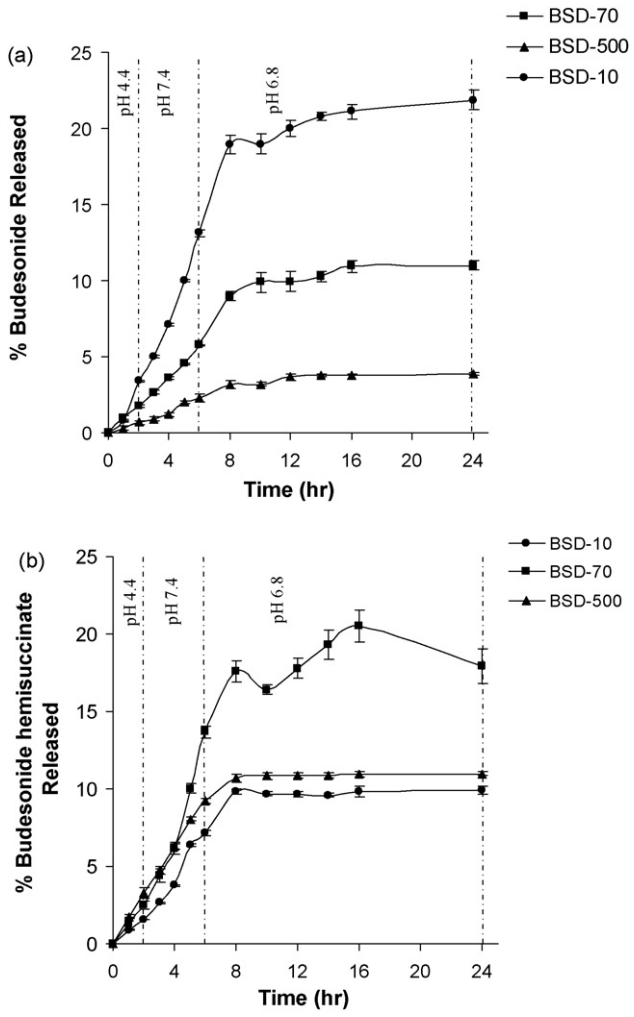

Fig. 3. Release profiles of (a) budesonide and (b) budesonide hemisuccinate, after incubation of each conjugate with contents of different segments of the rat GI tract ($n = 3$).

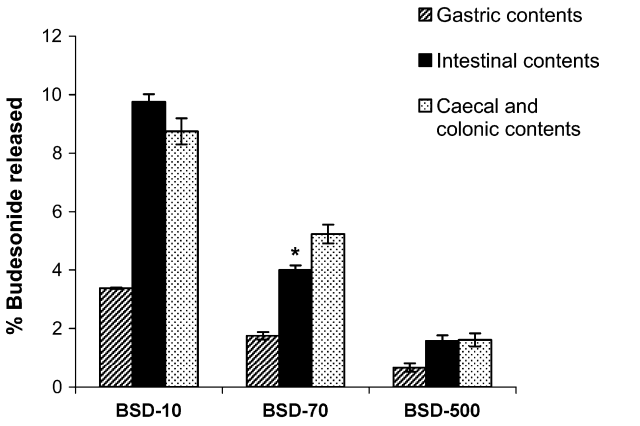

Fig. 4. Total amount of budesonide released in the presence of contents of each segment of the rat GI tract ($n = 3$). (*) The difference was significant ($P < 0.05$).

4. Discussion

Budesonide has received much attention for the treatment of inflammatory bowel diseases to the extent that its controlled-release formulations, Entocort® and Budenofalk®, are now being used to induce and maintain clinical remission of Crohn's disease (Fedorak and Bistritz, 2005). Enteric-coated formulations release the drug in terminal ileum and ascending colon and less amount of drug could be delivered to the transverse and descending colon (Edsbacker et al., 2003). Colon specific delivery of budesonide could be a promising approach for treatment of ulcerative colitis. Polysaccharide prodrugs are attractive choices for targeting drugs to the colonic region because of their stability in upper GI tract and specific release of the drug in the presence of enzymes secreted by the colonic microflora.

Dextran as a natural polysaccharide has enormous numbers of hydroxyl groups which could be esterified with carboxylic groups of different drugs. Since budesonide does not have any carboxylic acid group (Fig. 1), succinic anhydride was used to insert a carboxylic group at C_{21} of budesonide. This esterification process is catalyzed with DMAP. For removing DMAP, the reaction mixture

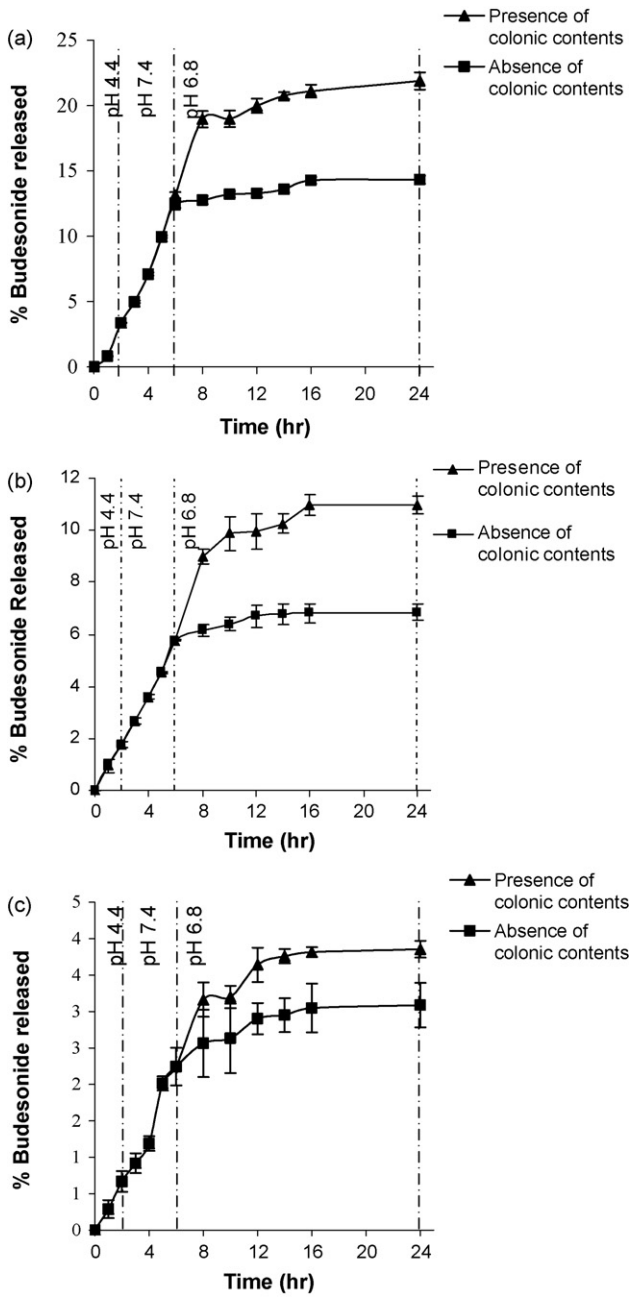

Fig. 5. Release profiles of (a) BSD-10, (b) BSD-70 and (c) BSD-500 in the presence and absence of rat caecal and colonic contents ($n = 3$).

was washed with HCl 0.1N and the product extracted in ethyl acetate (Ko et al., 2002). This purification process was more efficient than the reported method for dexamethasone (McLeod et al., 1993; Pang et al., 2002) for separation of DMAP and excess amount of succinic anhydride from the reaction product. By washing the reaction mixture with HCl, DMAP and succinic anhydride were converted to pyridinium and succinic acid, respectively and remained in the aqueous phase. In this process of esterification, maintaining the anhydrous media is an essential perquisite to produce the product in a high yield. In the next step, budesonide hemisuccinate was conjugated to dextran in DMSO. This was a straightforward reaction and produced a gummy polymer which was powdered and washed to remove any unreacted hemiester and dextran.

Basic hydrolysis was performed to release the drug from the conjugate. The results of drug content per 100 mg of conjugate in Table 2 show that increasing the MW of the polymer, resulted in a decrease in the amount of drug conjugated to the polymer ($P < 0.05$). This may be due to the steric hindrance of the bulky structure of dextran preventing the effective interaction between hydroxyl groups and carboxylic group of budesonide hemisuccinate. In addition, in a solvent like DMSO, the polymer is highly folded and only the surface hydroxyls could be available for the reaction. Degree of substitution is an important factor to be considered in formulating a prodrug in a dosage form and it would affect the solubility and drug release profile of the conjugates (Zou et al., 2005). Considering that budesonide is a low dose drug, 9 mg/day in mild to moderate Crohn's disease (PDR, 2007), DS obtained for BSD-10 and BSD-70 seems appropriate for the formulation of the dosage forms. Aqueous solubility of budesonide was drastically increased after conjugation with dextran 10,000 and 70,000. Comparison of BSD-10 and BSD-70 indicates that by increasing the DS, the solubility of the conjugate was decreased (25.25 mg/ml for BSD-10 against 53.55 mg/ml for BSD-70). This observation is in accordance with studies on dextran–nalidixic acid conjugates and polymeric prodrugs of 5-ASA (Lee et al., 2001; Zou et al., 2005). For BSD-500, although the DS is smaller than that of the two other conjugates, the solubility is much lower, even than untreated budesonide. This observation was also reported for prodrugs of 5-ASA with hydroxypropyl cellulose and chitosan (Zou et al., 2005). This may be due to the very high molecular weight of the polymer which limits the water solubility of the conjugate and the drug. It is reported that the water solubility of dextran is dependent on MW and the degree of branching (Mehvar, 2000).

Both the esteric bond between the drug and the spacer and the bond between the spacer and the polymer are susceptible to hydrolysis. The chemical stability studies of conjugates in different buffer solutions were studied to determine the amount of budesonide and budesonide hemisuccinate released in 6 h at 37 °C. About 4% of budesonide and less than 3% of budesonide hemisuccinate was detected after 6 h incubation in different buffer solutions (data not shown). The hydrolysis was more profound in acidic media and as the pH increased, the amount of drug released was decreased. This is attributed to the fact that ester hydrolysis is catalyzed in acidic media. By increasing the MW of the polymer, the amount of drug released in each medium diminished. This observation also indicates that by increasing the MW of the polymer, the bulky structure of the polymer hinders the hydrolysis reaction. Other studies performed on methylprednisolone– and dexamethasone–dextran conjugates have shown that hydrolysis of conjugates to drug and hemiester and hydrolysis of the hemiester to the drug is of first order kinetic (Anderson and Taphouse, 1981; McLeod et al., 1993). Linear regression analysis of the stability data showed that the values of regression coefficients for the first order kinetics were larger than those obtained for zero order model. This implies that the stability profile of the conjugates could be better described by first order kinetics. K_{BUD} and K_{BS} were calculated to determine the degradation half-life of the conjugates. K_{BUD} and K_{BS} are rate constants for hydrolysis of conjugates to budesonide and budesonide hemisuccinate, respectively. As presented in Table 4, the values of K_{BUD} and K_{BS} for three conjugates were significantly different in various buffer media ($P < 0.05$). This observation proves the pH dependency of conjugates and budesonide hemisuccinate hydrolysis. For BSD-10 and BSD-70 in three studied media, K_{BUD} was larger than K_{BS} ($P < 0.05$) showing that the rate-limiting step in the hydrolysis of conjugates is the hydrolysis of conjugates to budesonide hemisuccinate.

It is expected that esteric bonds between the drug and the spacer and between the spacer and the polymer, could be hydrolyzed by the esterases of the GI tract. Although for polysaccharide esters, there is a hindering effect of the polymer which prevents the effective action of the esterases. When the prodrug reaches the caecal and colonic region, polysaccharide chain is degraded by the action of enzymes released from the colonic microflora and esteric bonds become accessible to esterases. Release of the drug from the conjugates was studied in contents of different segments of the rat GI tract. Cumulative release profiles of three conjugates in Fig. 3 indicated that less than 10% of budesonide and budesonide hemisuccinate were released in the contents of stomach and small intestine, while there is a significant increase in release of budesonide and budesonide hemisuccinate in the presence of the contents of caecum and colon. After 10 h, no significant change in the drug release percentage was observed. This may be due to the inadequacy of anaerobic condition for a long period which is necessary for the function of microflora to produce liberating enzymes. In all three profiles, a decrease point in release profiles of budesonide hemisuccinate was observed which was coincident with an increase in the release profile of budesonide. Such a release pattern has also been reported for dexamethasone–dextran conjugates (McLeod et al., 1993). This means that there is a possibility of hydrolysis of budesonide hemisuccinate to budesonide and fluctuations in the release profiles of budesonide hemisuccinate is originated from this hydrolysis process. There was a significant difference ($P < 0.05$) between the B_{24}% of budesonide of three conjugates and by increasing the polymer MW the percentage of drug released was decreased. In the case of budesonide hemisuccinate, only BS_{24}% of the BSD-70 was different from the two other conjugates ($P < 0.05$). This may be due to the higher DS and lower solubility of BSD-10 which lowers the amount of drug released. Similar finding was obtained for the polymeric prodrugs of 5-ASA (Zou et al., 2005). Fig. 4 shows that there is a significant difference between the amounts of drug released in different segments of the rat GI tract for BSD-70. For BSD-10, the amount released in intestinal and colonic contents was not statistically different ($P > 0.05$). It shows that dextran 10,000 is not efficient to protect the esteric bond against hydrolysis. For BSD-500, the very low solubility is the reason for low release in all segments of the GI tract. Cumulative release profile of three conjugates in the presence and absence of the colonic contents (Fig. 5) showed a significant difference ($P < 0.05$) between two profiles which confirms the importance of colonic contents for releasing the drug from the conjugate.

In all cases, budesonide hemisuccinate release profiles were also studied, since a major part of the drug in conjugates is released in this form and for glucocorticoids, the 21-esters have the good affinity toward the receptor and could trigger anti-inflammatory effects (Williams and Lemke, 2002).

In summary, amongst synthesized conjugates, BSD-70 showed promising results as a prodrug for colon specific delivery of budesonide. Further pathophysiologic studies on animals are being performed in our laboratory to prove its effectiveness as a new formulation for treatment of ulcerative colitis.

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