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Research paper

Chitosan coated Ca–alginate microparticles loaded with budesonide for delivery to the inflamed colonic mucosa

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Abstract

Using a novel one-step spray-drying process uncoated and Eudragit® S 100 coated chitosan–Ca–alginate microparticles efficiently loaded with budesonide (BDS), with bioadhesive and controlled release properties in GIT, were prepared. Microparticles were spherical with mean particle size of 4.05–5.36 μm, narrow unimodal distribution and positive surface charge. A greater extent of calcium chloride limited the swelling ratio of beads, while swelling behaviour of coated beads was mainly determined by properties of enteric coating. Comparing the release profiles of formulations, under different pH conditions, influence of polymer properties and concentration of cross-linker on the rate and extent of drug release was evident. Coating has successfully sustained release of BDS in buffers at pH 2.0 and 6.8, while providing potential for efficient release of BDS at pH 7.4. Release data kinetics indicated influence of erosion and biodegradation of polymer matrix on drug release from microparticles. Prepared formulations were stable for 12 months period at controlled ambient conditions.

In conclusion coated microparticles prepared by one-step spray-drying procedure could be suitable candidates for oral delivery of BDS with controlled release properties for local treatment of inflammatory bowel diseases.

Keywords: Alginate; Chitosan; Budesonide; Spray-drying; Controlled release

1. Introduction

Dosage forms that deliver drugs into the colon rather than upper GIT proffer number of advantages. Oral delivery of drugs to the colon is valuable in the treatment of diseases of colon (ulcerative colitis, Chron's disease) whereby high local concentration can be achieved while minimizing side effects that occur because of drug release in the upper GIT or unnecessary systemic absorption [1]. At the forefront of newer agents in treatment of inflammatory bowel diseases (IBD) are biological therapy (anti-TNF agents), than Budesonide (BDS), a second generation glucocorticoid, Azathioprine, immunosuppressant, topical and oral aminosalicylates [2]. BDS is one of the most used drug substances in the treatment of active IBD [3].

In order to achieve specific colonic drug delivery, different approaches have been reported over the last few years [4]. Most techniques used to deliver drugs to the colon, rely on the variation of the pH value through the GIT [5], enzymatic degradation by colonic bacteria [2,6] and even the relatively constant small intestine transit time [7]. Enteric formulations have long been in use clinically for the treatment of this pathology, but with limited success as they rely on pH-dependent drug release in GIT. A novel formulation that will offer efficient treatment of colon diseases should combine biopolymer colloidal drug carriers that offer prolonged residence time and controlled release at the site of action [8]. By modifying drug residence time and drug release rate, increased therapeutic concentration at the site of inflammation and increased therapeutic activity might be achieved.

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Having in mind bio/mucoadhesive properties of natural biopolymers, chitosan-alginate microparticulated systems should have potential for colon targeting. In order to achieve localization and prolonged residence time in the colon, matrices should have optimal particle size, between 4 and 15 μm [9–13]. Carrier systems in that size range are able to attach more efficiently to the mucus layer and accumulate in the inflamed region without the need for macrophage uptake [10,11].

Mucoadhesiveness of the polymers depends on the flexibility of polymer backbone structure and its polar functional groups, which is however reduced during the cross-linking procedure used in order to prepare microparticulated carriers with controlled drug release [14,15]. So, care should be taken for preserving mucoadhesiveness and obtaining desired biopharmaceutical properties during formulation and preparation of the microparticles. Because, disadvantage of the mucoadhesive colloidal drug carrier systems is adherence to the substrate by non-specific interactions i.e. polymers cannot distinguish between the adherence to intestinal mucus or to the surfaces of other gut parts or contents, coating of chitosan and alginate-based microparticles by polymer with pH-dependent properties such as Eudragit is desired. At this point, we might obtain direct targeting of the microparticles to the colon region whereby increased drug control release will be present.

The aim of this work was to prepare microparticulated carrier system with physical and biopharmaceutical properties that will be in favour of efficient treatment of inflammatory bowel diseases. Chitosan–Ca–alginate microparticles loaded with BDS were produced using novel one-step spray-drying procedure [16]. Prepared microparticles were further enteric coated by Eudragit® S 100 using the same procedure. Particle size and morphology, the swelling behaviour, surface charge, drug content were determined and drug release studies were performed.

2. Materials and methods

2.1. Materials

Budesonide (BDS) was purchased from Crystal Pharma, Spain, and Calcium chloride ($CaCl_2$) from Alkaloid, Macedonia. Chitosan, low viscous, was supplied by Fluka, Switzerland. Sodium alginate (Protanal® LF 10/60) was kindly donated by FMC BioPolymer, Norway. The core coating material, copolymer of methacrylic acid and methacrylic acid ester, Eudragit® S 100, was a generous gift from Rohm Pharma, Germany. The rest of the used chemicals and reagents were of analytical grade.

2.2. Preparation procedure

Types of polymers used in the formulation were chosen according to their physical–chemical properties. Sodium alginate LF 10/60 consists of 65–75% of guluronic acid (G) and 25–35% of manuronic acid (M). Having in mind that MG types compared with MM and GG types of sodium alginate have best flexibility [17], and that polymer gels formed from alginate with high percentage of guluronic acid (>70%) have highest mechanical strength and stability towards monovalent ions [18], Sodium alginate LF 10/60 was chosen for preparation of microparticles. Chitosan with low viscosity, highly deacetylated was chosen for polyelectrolyte complexation with sodium alginate. The fact that the deacetylated chains are fully stretched by the electrostatic repulsion among the $-NH_3^+$ groups (and the acetylated blocks are micelle-like agglomerates because of the hydrophobic forces), leads to a conclusion that higher degree of deacetylation might contribute to the efficient process of coating. The goal was to prepare MP with sufficient mechanical strength, defined swelling properties, controlled release of active substance and certain surface properties as factors important for the efficacy of the system and its prolonged residence time at the site of action due to muco/bioadhesivity of the alginate/calcium/chitosan microparticulated system [19,20].

In order to establish the process conditions for production of positively charged chitosan coated Ca–alginate microparticles by one-step spray-drying procedure [16], with approximate diameter around 4 μm showing narrow particle distribution and to evaluate their influence on microparticle characteristics, different drug free batches were prepared and evaluated for process yield, particle size and distribution, shape and surface morphology, zeta potential, DSC studies and swelling behavioufr. Eliminating one factor at a time concentrations for chitosan and alginate solution as well as process conditions were established.

Three formulations with same proportions of polymers, produced under identical operating conditions but with varying concentration of $CaCl_2$ (MP1, MP2 and MP3; Table 1 and Fig. 1), were selected for preparation of BDS loaded microparticles (MPB1, MPB2 and MPB3) and Eudragit coated microparticles containing BDS (E-MPB1, E-MPB2 and E-MPB3), which were further evaluated for process yield, particle size and distribution, shape and surface morphology, zeta potential, DSC studies, swelling behaviour and drug release.

Six batches ($n = 6$) for each formulation were prepared and separately characterized to study batch-to-batch reproducibility of the tested parameters. Six measurements for each batch were performed (only the dissolution tests were performed in triplicate) to calculate the mean values and standard deviations.

Schematic presentation of the preparation procedure for BDS loaded microparticles (Buchi 290, Mini Spray Dryer, Switzerland) and the composition of the formulations are presented in Fig. 1 and Table 1, respectively.

BDS loaded Chitosan–Ca–alginate microparticles were further coated using Eudragit® S 100 (formulation E-MPB1, E-MPB2 and E-MPB3) by spray-drying proce-

Table 1
Composition and production yield of spray-dried (mean ± SD; $n = 6$ batches), empty and BDS loaded chitosan–Ca–alginate microparticles

	Chitosan, low viscous, highly deacetilated (g)/ sodium alginate (LF 10/60) (g)	$CaCl_2$ (g)/ sodium alginate (LF 10/60) (g)	BDS (g)/ total polymers (g)	Production yield (%) ± SD
MP1	0.5	0.325	–	52.5 ± 0.6
MP2	0.5	0.625	–	53.3 ± 0.3
MP3	0.5	1.250	–	49.8 ± 1.4
MPB1	0.5	0.325	0.02	53.5 ± 0.4
MPB2	0.5	0.625	0.02	52.6 ± 1.2
MPB3	0.5	1.250	0.02	51.4 ± 0.9

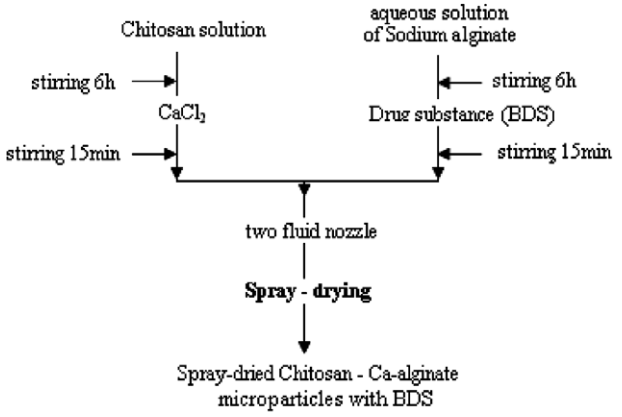

Fig. 1. Schematic presentation of preparation of BDS loaded chitosan–Ca–alginate microparticles using one-step spray-drying process.

dure (Buchi 190, Mini Spray Dryer, Switzerland). Composition of the coating formulation, production yield and schematic presentation of coating procedure are given in Table 2 and Fig. 2, respectively.

Production yields (expressed as the weight percentage of the beads obtained with respect to the initial amount of polymers and drug used for the preparation [21]) were around 50%, which can be explained by the relatively low volumes of feed solutions sprayed for the preparations of each batch of MPs, the structure of the apparatus that is not equipped with a trap to recover the smaller and lighter particles exhausted by the aspirator and the loss of material mostly due to powder adhering to the cyclone walls.

Table 2
Composition of the coating formulation and production yield (mean ± SD; $n = 6$ batches) of Eudragit coated chitosan–Ca–alginate microparticles containing BDS

	Eudragit®S 100 (g)/total polymers (g)	Acetone (g)/Eudragit® S 100 (g)	Propylene glycol in coating solution (%)	Production yield (%) ± SD
E-MPB1	2.47	65	0.37	49.2 ± 0.9
E-MPB2	2.87	65	0.37	48.3 ± 0.7
E-MPB3	3.71	65	0.37	50.8 ± 1.1

2.3. Characterization of microparticles

2.3.1. Study of morphology

Shape and surface morphology of the microparticles were examined by scanning electron microscopy (SEM) (Jeol-SEM 6400, Japan). Samples were gold coated using a sputter coater.

2.3.2. Differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and X-ray diffraction

DSC scans were recorded using a differential scanning calorimeter Mettler Toledo DSC-882 (Mettler, Switzerland). Samples were accurately weighed into 40 μL aluminium pans and heated from 25 to 350 °C at a heating rate of 10 °C/min under a nitrogen flow rate of 50 ml/min.

Thermogravimetric measurements were performed with Mettler Toledo TGA-851 (Mettler, Switzerland). Samples were accurately weighed into 70 μL aluminium pans and heated from 25 to 350 °C at a heating rate of 5 °C/min under a nitrogen flow rate of 50 ml/min.

X-ray diffractograms of empty, empty coated, drug loaded and drug loaded Eudragit S 100 coated microparticles, polymers, drug substance and their physical mixtures were recorded using Philips PW 1820, Netherlands. Samples were irradiated with monochromatized Cu-Kα radiation and analysed between 2 and 60 °C. The range and the chart speed were 2×10^4 cps and 10 mm/2θ, respectively.

2.3.3. Particle size analysis

Spray-dried, uncoated, empty and BDS loaded, as well as Eudragit coated chitosan–Ca–alginate microparticles containing BDS, were characterized in terms of particle size and particle size distribution determined by laser diffractometry using Mastersizer 2000, Malvern Instruments Ltd., UK equipped with Hydro 2000S, Malvern Instruments Ltd., UK, for wet dispersions and Scirocco 2000, Malvern Instruments Ltd., UK, for dry powder. Six measurements for each batch were performed. Wet dispersions for particle size analysis were prepared as follows: samples (40 mg) were dispersed in 2 ml distilled water and sonicated in ultrasonic bath for 5 min. A small aliquot was removed and transferred to the optical measurement cell containing the blank dispersing medium, distilled water. Measurements were performed while the sample was in the cell under stirring (2520 rpm/stirrer rate) and ultrasound (50%), previously applied for 1 min. The obscuration was set between 10% and 12%.

For dry powder measurements the conditions were: vacuum – 2 bar and feed rate – 50%, while obscuration was set between 0.2% and 4%.

The particle size distribution was also expressed in terms of SPAN factor determined as:

$$\text{SPAN} = (d_{90} - d_{10})/d_{50}$$

where d_{10}, d_{50} and d_{90} are the diameter sizes and the given percentage value is the percentage of particles smaller

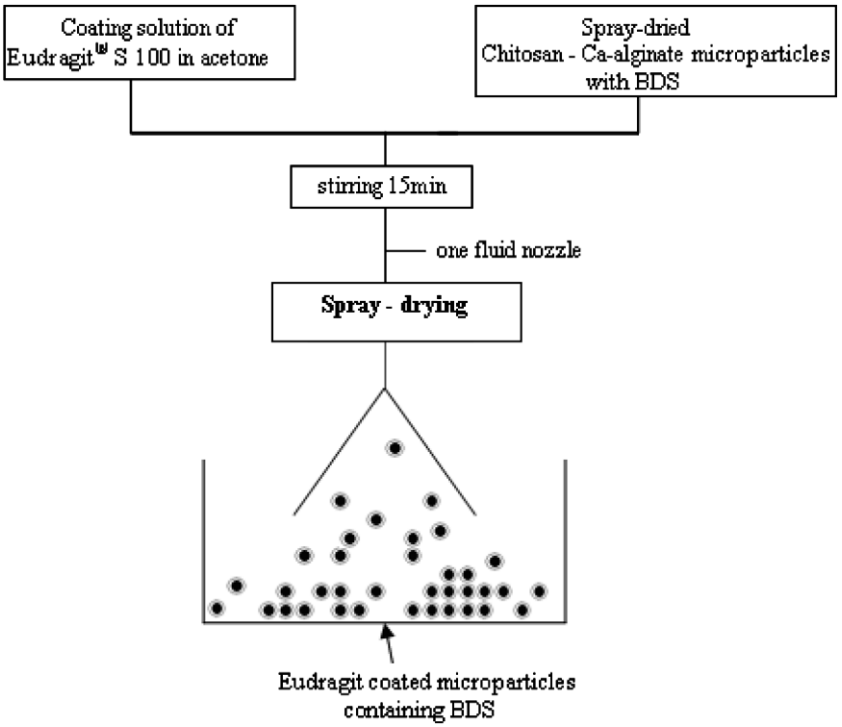

Fig. 2. Schematic presentation of coating procedure of BDS loaded chitosan–Ca–alginate microparticles.

than that size. A high SPAN value indicates a wide size distribution [22].

2.3.4. Swelling studies

Dry ionically cross-linked beads increase their volume after minutes in water or in buffers with different pH and composition, due to matrix rehydration and in accordance with the degree of cross-linking. The maintenance of the polyelectrolyte complexes depends considerably on the ion exchange in the tested buffer solution.

The swelling properties of the uncoated, empty and BDS loaded, and Eudragit coated chitosan–Ca–alginate microparticles containing BDS were determined in acidic buffer pH 2.0 and phosphate buffer pH 6.8 and 7.4, respectively. Samples of beads of known weight (40 mg) were placed in a glass vial containing 5 ml of swelling solution, continuously mixing on magnetic stirrer (300 rpm stirring rate), and allowed to swell at room temperature. Measurements were performed, in predetermined time interval (1, 3 and 24 h), by laser diffractometry using Mastersizer 2000, Malvern Instruments Ltd., UK, equipped with Hydro 2000S, Malvern Instruments Ltd., UK, for wet dispersions. Six measurements for each batch were performed while the sample was in the cell under stirring (2520 rpm/stirrer rate) and ultrasound (50%), previously applied for 1 min. The obscuration was set between 10% and 12%.

Particle size increase, in predetermined time interval, is calculated as ratio between d_{50} (μm) of the swollen beads and d_{50} (μm) of the beads measured as dry powder, and presented as volume increase ratio (VIR).

2.3.5. Zeta potential measurements

The zeta potential (ζ) of uncoated, empty and BDS loaded, as well as Eudragit coated, chitosan–Ca–alginate microparticles containing BDS was measured using Zetasizer Nano Series, Nano-ZS, Malvern Instruments Ltd., UK. Measurements were done after suspending the samples in water and different buffer solutions at pH 2.0, 5.0 and 6.8. Prepared buffer solutions were of low molarities and ionic strength. The composition, molarities and ionic strength were as follows: acid buffer pH 2.0 (Eur. Pharm. IV; 9×10^{-7} M, ionic strength 2.7×10^{-3} M), acetic buffer pH 5.0 (0.00645 g CH_3COONa, 0.04945 g CH_3COOH in 1000 ml of water; 9×10^{-4} M, ionic strength 9×10^{-4} M) and phosphate buffer pH 6.8 (0.0622 g Na_2HPO_4, 0.0639 g NaH_2PO_4 in 1000 ml of water; 9.7×10^{-4}, ionic strength 2×10^{-3} M). Six measurements for each batch were performed.

2.3.6. Drug content determination

In order to determine BDS content in the microparticles, accurately weighted microparticles (10 mg) were dispersed in phosphate buffer pH 7.4 (10 ml) and shaken using horizontal shaker (Unitronic OR, Selecta, Barcelona, Spain) (100 rpm) at 37 ± 0.5 °C for 24 h, to ensure complete dissolution of the particles. Samples were centrifuged (Tehtnika Centric 322B, Slovenia) at 4000 rpm for 15 min. Centrifugates were withdrawn, filtered through 0.45 μm membrane filter (Ministar RC 25, Sartorius, Germany) and assayed by HPLC.

Commercial BDS is an epimeric mixture of two isomers which have the same pharmacological activity patterns.

The two epimers can be separated under the conditions described below.

Analyses were performed on Agilent 1100 Series HPLC system, equipped with 1100 Quaternary Pump and Agilent 1100 DAD detector. The column used was LiChroCART® 150-4.6, Purospher® STAR RP-18 endcapped (5 μm) (Merck KGaA, Darmstadt, Germany) at 242 nm UV detection. The mobile phase was acetonitrile/water (35:65). Chromatographic conditions for this method were: flow rate 1 ml/min, column temperature 25 °C, injection volume 50 μl.

Results were calculated from linear regression of the external standard of BDS.

The drug encapsulation efficiency was calculated from the following equation [23]:

EE (%) = (Actual drug loading/Theoretical drug loading) × 100.

Six measurements for each batch were performed.

2.3.7. In vitro drug release studies

Dissolution tests were performed using microparticles suspension (5–6 mg for E-MPB1, E-MPB2, E-MPB3 and 1–2 mg for MPB1, MPB2 and MPB3) in 10 ml of buffer solutions with pH 2.0, 6.8 and 7.4, placed in closed glass tubes, on horizontal shaker (100 rpm, 37 °C, for 24 h). At appropriate intervals (1, 2, 3, 4, 5, 7, 9 and 24 h), 1-ml samples were withdrawn, replaced by 1 ml of fresh buffer, and assayed by HPLC method described above.

The dissolution tests were performed under sink conditions and in triplicate for each batch/formulation to calculate the mean values and standard deviations.

The in vitro release pattern was evaluated to check the goodness of fit to the Higuchi's square root of time equation [24] (Eq. (1)), Korsmeyer–Peppas power law equation [25,26] (Eqs. (2) and (3)) and fitting the data to the heuristic model proposed by Peppas and Sahlin [27] (Eq. (4)) for quantifying the phenomena controlling the release from swellable matrix, in which the contribution of the relaxation or erosion mechanism and of the diffusive mechanism can be quantified. The goodness of fit was evaluated using the r (correlation coefficient) values.

$$M = Kt^{1/2} \tag{1}$$

where M, is the amount of drug dissolved in time t, K is the Higuchi dissolution constant, and t is the release time.

$$M_t/M_\infty = Kt^n \tag{2}$$

This in logarithmic form is:

$$\log(M_t/M_\infty) = \log K + n \log t \tag{3}$$

where M_t is the amount of drug dissolved in time t, M_∞ is the amount of drug dissolved after infinite time (all the drug content in the formulation), M_t/M_∞ is the fractional release of the drug in time t, K is a constant incorporating the structural and geometric characteristics of the dosage form, n is the release (diffusion) exponent, which depends on the release mechanism and the shape of the matrix tested and t is the release time. Exponent n for polymeric controlled delivery systems of spherical geometry has values of $n = 0.43$ for Fickian diffusion, $0.43 < n > 0.85$ for anomalous transport and $n > 0.85$ for Case-II transport [28].

$$M_t/M_\infty = K_1 t_{1/2} + K_2 t \tag{4}$$

where M_t is the amount of drug dissolved in time t, M_∞ is the amount of drug dissolved after infinite time (all the drug content in the formulation), M_t/M_∞ is the fractional release of the drug in time t, K_1 and K_2 are, respectively, the diffusion and erosion terms. According to this equation, if the diffusion to erosion ratio $K_1/K_2 = 1$, then the release mechanism involves diffusion and erosion equally. If $K_1/K_2 > 1$, then diffusion prevails, while erosion predominates when $K_1/K_2 < 1$ [29].

2.4. Stability studies

The prepared empty, drug loaded and Eudragit coated microparticles containing BDS were sealed in glass vials and were stored under controlled ambient conditions (26 ± 0.5 °C, 65% RH). Periodical testing of different parameters (particle size, particle size distribution, swelling behaviour, zeta potential and dissolution rate studies) during 12 months real time stability studies was performed. X-ray and DSC studies were performed at the start of the stability period evaluation and after 12 months period at normal storage conditions.

3. Results and discussion

Microparticles were prepared according to the above-stated formulation and subjected to characterization.

3.1. Study of morphology

Surface morphology of six different formulations of chitosan–Ca–alginate microparticles is presented in Fig. 3. Differences in the formulations are based on the presence of Eudragit® S 100, as coating material, and $CaCl_2$ concentration. There are some differences in surface morphology of microparticles, but generally spherical morphology with presence of spherical disks with a collapsed centre can be noticed. Surface appearance is smooth with low porosity. Insufficiency of ideal spherical morphology probably developed during the drying process.

3.2. Differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and X-ray diffraction

DSC scans of sodium alginate showed wide endothermic peak at 81.52 °C that has been attributed to the evaporation of water, and appearance of an exothermic behaviour was detected starting around 200 °C, with its maximum at 250.65 °C, coinciding with the exothermic behaviour of the

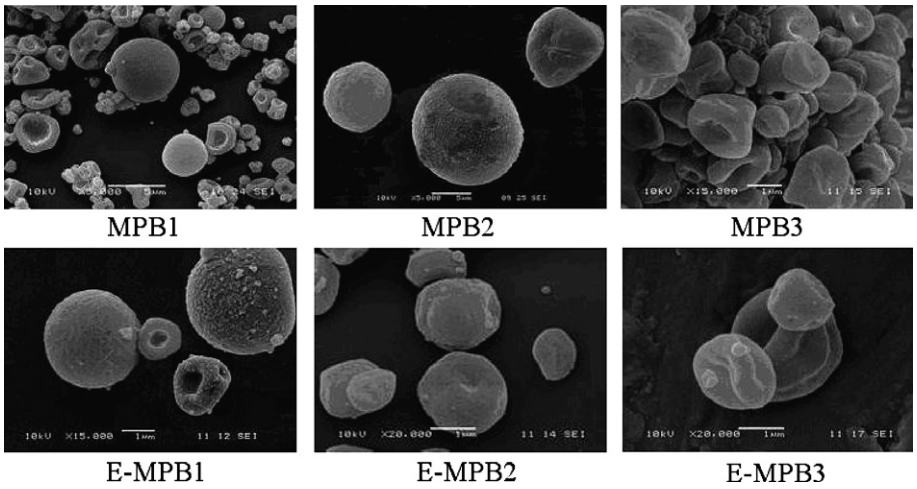

Fig. 3. SEM of uncoated (MPB1, MPB2, MPB3) and Eudragit coated (E-MPB1, E-MPB2, E-MPB3) chitosan–Ca–alginate microparticles containing BDS.

sodium alginate as referred to in several publications [30] as the decomposition of the polymer.

DSC scans of the chitosan polymer exhibited an endothermic peak at 88.7 °C that has been attributed to the evaporation of absorbed water. The exothermic baseline deviation beginning around 250 °C and gaining its maximum at 302.36 °C indicates the onset of chitosan degradation [30,31].

Sharp endotherm was observed for BDS at 257 °C, corresponding to its melting transition point. Exotherm at 270 °C refers to the onset of BDS degradation [32].

DSC scans of the $CaCl_2$ exhibited two endothermic peaks at 145.45 and 169.52 °C.

DSC scans of the Eudragit® S 100 showed three endothermic peaks at 76.38, 212.93 and 253.14 °C.

DSC scans of sodium alginate, chitosan, BDS, $CaCl_2$and Eudragit® S 100 are presented in Fig. 4.

DSC scans of physical mixture of sodium alginate/chitosan/$CaCl_2$, sodium alginate/chitosan/$CaCl_2$/BDS, sodium alginate/chitosan/$CaCl_2$/Eudragit® S 100 and sodium alginate/chitosan/$CaCl_2$/Eudragit®S 100/BDS are presented in Fig. 5.

Non-specific electrostatic interaction between chitosan and alginate is also present in their physical mixtures. Exothermic peak between 210 and 230 °C in physical mixtures is related to the breakdown of their weak unspecific interactions [33]. In physical mixtures where Eudragit is present interaction between Eudragit and chitosan (disappearance of chitosan degradation peak at 302.36 °C) can be proposed. Absence of BDS's endotherm at 257 °C in physical mixtures with Eudragit might be result of its low percentage in the physical mixtures (2%) or BDS probable interaction with Eudragit. Possibility for interactions between BDS and excipients used in the formulations was further analysed by DSC analysis of physical mixtures of BDS with each excipient prepared in 1:1 ratio. Thermal analyses of physical mixtures of BDS with each excipient are presented in Fig. 6.

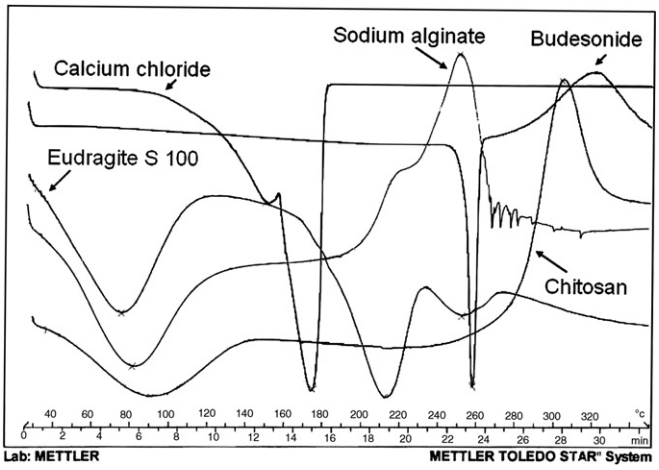

Fig. 4. DSC scans of sodium alginate, chitosan, calcium chloride, Eudragit® S 100 and BDS.

Physical mixtures of BDS/sodium alginate and BDS/$CaCl_2$ exhibited shifting of BDS endotherm, corresponding to its melting transition point, for 9–10 °C lower compared to BDS alone. Smaller changes in BDS melting transition point were observed in BDS/chitosan physical mixture, where endotherm appeared at 254 °C. Thermogram of BDS/Eudragit® S 100 physical mixture did not show BDS endotherm at all.

DSC scans of uncoated and Eudragit coated chitosan–Ca–alginate microparticles and corresponding physical mixtures sodium alginate/chitosan/$CaCl_2$/BDS and sodium alginate/chitosan/$CaCl_2$/Eudragit® S 100/BDS are presented in Fig. 7.

The broad endothermic peak around 100 °C in the DSC thermograms of microparticles (MPB1 – 96.92 °C, MPB2 – 99.7 °C, MPB3 – 116.45 °C) can be attributable to polyelectrolyte interaction between chitosan and the alginate [34–37]. Also, the chitosan–alginate reaction can be charac-

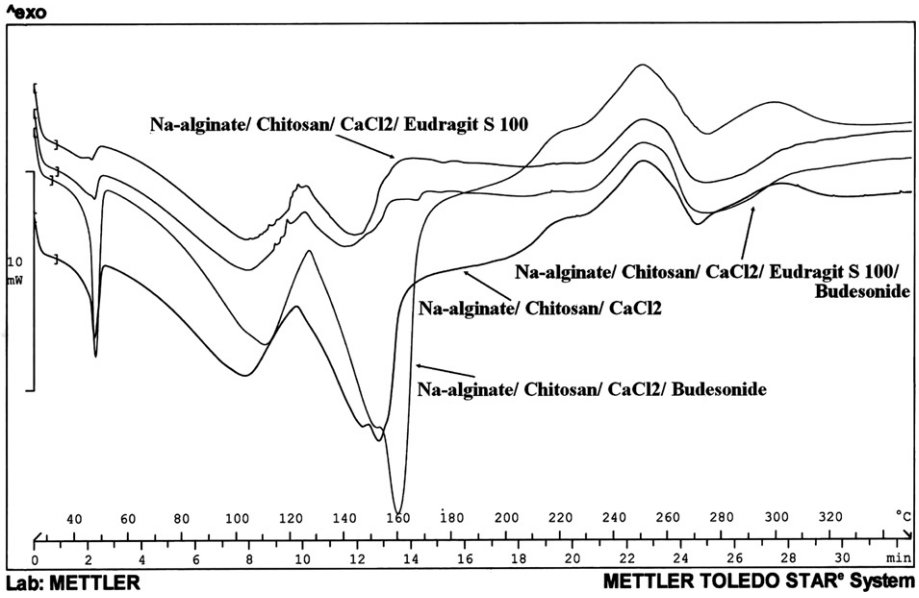

Fig. 5. DSC scans of physical mixtures of sodium alginate/chitosan/$CaCl_2$, sodium alginate/chitosan/$CaCl_2$/BDS, sodium alginate/chitosan/$CaCl_2$/Eudragit S® 100 and sodium alginate/chitosan/$CaCl_2$/Eudragit S® 100/BDS.

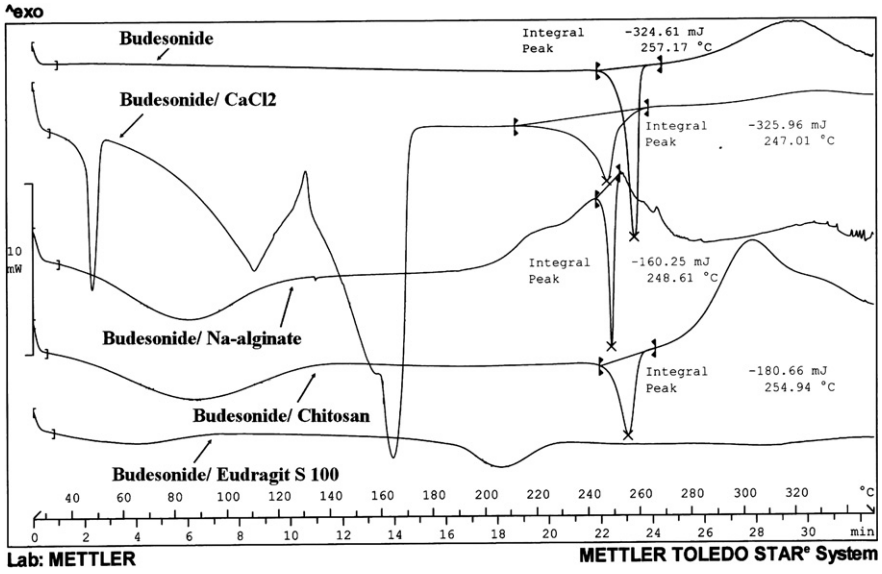

Fig. 6. DSC scans of physical mixtures of BDS/excipients (sodium alginate, chitosan, $CaCl_2$and Eudragit® S 100) (ratio 1:1) compared with BDS.

terized by the disappearance of the degradation exothermic peaks of chitosan at 302.36 °C and of alginate at 250.65 °C [33]. Entire absence of BDS endotherm in microparticle thermograms suggests that BDS is completely entrapped in the polymer matrix and also points to possibility of transformation of its crystal to amorphous structure, which probably occurs during preparation of microparticles using spray-drying technique [38].

DSC thermograms of Eudragit coated microparticles exhibited the endotherm which refers to chitosan–alginate interaction which is shifted towards lower temperatures when compared with uncoated microparticles (MPB2 – 99.7 °C and E-MPB2 – 88.29 °C). No other endothermic or exothermic peak assigned with polymers or drug substance was observed.

The thermal properties of the systems were investigated by thermogravimetry. The thermogravimetric curves (not presented) (sample weight% as a function of temperature), for physical mixtures of sodium alginate/chitosan/$CaCl_2$, sodium alginate/chitosan/$CaCl_2$/Eudragit® S 100 and Eudragit coated microparticles showed weight loss, 9–15% at 160 °C, 20–30% at 180 °C, continuing to complete degradation at 350 °C. For physical mixtures, degradation took place in three stages. The first stage could be attributed to the loss of bound water (around 100 °C), second stage corresponds to interactions between components

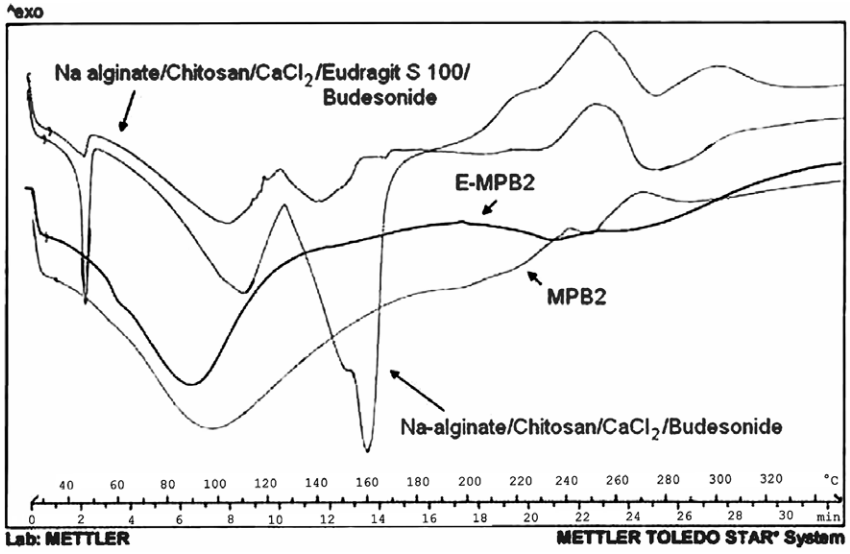

Fig. 7. DSC scans of uncoated (MPB2) and Eudragit coated (E-MPB2) chitosan–Ca–alginate microparticles and physical mixtures of sodium alginate/chitosan/$CaCl_2$/BDS and sodium alginate/chitosan/$CaCl_2$/Eudragit® S 100/BDS.

(100–160 °C) and the third stage represents the thermal degradation (after 180 °C).

Having in mind, all stated above, alterations in thermal behaviour of components in microparticles, compared with pure substances, appeared, likely, as a result of interactions during preparation process. Entire absence of BDS endotherm in microparticle thermograms suggests that BDS is completely entrapped in the polymer matrix and also points to possibility of significant reduction in drug crystallinity in the polymer matrix. These results were further confirmed by X-ray diffraction studies. The diffraction patterns of BDS loaded and BDS loaded Eudragit coated microparticles did not contain any peaks associated with the crystalline molecule of the drug substance.

3.3. Particle size analysis

Particle size of empty (MP1, MP2, MP3) and BDS loaded (MPB1, MPB2, MPB3) uncoated chitosan–Ca–alginate microparticles, as well as, Eudragit coated chitosan–Ca–alginate microparticles containing BDS (E-MPB1, E-MPB2, E-MPB3) expressed as volume diameter (d_{10},d_{50} and d_{90}) ± SD, measured as aqueous dispersion and dry powder, are presented in Table 3.

Due to matrix rehydration for empty (MP1, MP2, MP3) and BDS loaded (MPB1, MPB2, MPB3) uncoated chitosan–Ca–alginate microparticles, there is statistical difference between d_{50}, (t-test, $p < .05$) for aqueous dispersion and dry powder, while for Eudragit coated chitosan–Ca–alginate microparticles containing BDS (E-MPB1, E-MPB2, E-MPB3) there is no statistical difference between d_{50} (t-test, $p < .05$) for aqueous dispersion and dry powder and their particle size could be determined in aqueous medium [39].

Particle size distribution of prepared BDS loaded microparticles, uncoated (MPB1, MPB2, MPB3) and Eudragit coated (E-MPB1, E-MPB2, E-MPB3), is presented in Fig. 8.

The particle size distribution, for empty (MP1, MP2, MP3) and BDS loaded (MPB1, MPB2, MPB3) uncoated chitosan–Ca–alginate microparticles, as well as, Eudragit coated chitosan–Ca–alginate microparticles containing

Table 3
Mean particle size of chitosan–Ca–alginate microparticles, uncoated, empty and BDS loaded, as well as Eudragit coated microparticles containing BDS (mean ± SD, $n = 6$ measurements for each batch)

	d_{10} (μm) ± SD		d_{50} (μm) ± SD		d_{90} (μm) ± SD	
	Aqueous dispersion	Dry powder	Aqueous dispersion	Dry powder	Aqueous dispersion	Dry powder
MP1	2.061 ± 0.07	1.502 ± 0.01	5.847 ± 0.02	$4.621 \pm 9 \times 10^{-3}$	23.694 ± 0.12	13.837 ± 0.11
MP2	$2.143 \pm 3 \times 10^{-3}$	1.735 ± 0.04	6.853 ± 0.01	$5.225 \pm 6 \times 10^{-3}$	22.919 ± 0.35	12.879 ± 0.15
MP3	$2.211 \pm 8 \times 10^{-3}$	1.320 ± 0.03	$5.982 \pm 7 \times 10^{-3}$	$4.354 \pm 4 \times 10^{-3}$	13.688 ± 0.09	11.616 ± 0.02
MPB1	2.156 ± 0.05	1.563 ± 0.02	5.987 ± 0.08	$4.691 \pm 8 \times 10^{-3}$	23.674 ± 0.30	13.885 ± 0.10
MPB2	$2.266 \pm 4 \times 10^{-3}$	1.865 ± 0.02	6.935 ± 0.03	5.361 ± 0.05	23.191 ± 0.42	12.889 ± 0.13
MPB3	$2.281 \pm 5 \times 10^{-4}$	$1.470 \pm 7 \times 10^{-3}$	$6.002 \pm 3 \times 10^{-3}$	$4.454 \pm 9 \times 10^{-3}$	13.738 ± 0.01	11.623 ± 0.01
E-MPB1	1.960 ± 0.02	1.975 ± 0.02	$4.374 \pm 9 \times 10^{-3}$	4.602 ± 0.01	9.011 ± 0.15	14.809 ± 0.12
E-MPB2	$1.522 \pm 5 \times 10^{-3}$	$1.333 \pm 8 \times 10^{-3}$	4.110 ± 0.06	4.045 ± 0.02	16.131 ± 0.39	13.998 ± 0.14
E-MPB3	$1.831 \pm 6 \times 10^{-3}$	1.348 ± 0.02	4.254 ± 0.03	4.261 ± 0.03	14.926 ± 0.14	15.088 ± 0.21

BDS (E-MPB1, E-MPB2, E-MPB3) expressed in terms of SPAN factor is presented in Table 4.

A high SPAN value indicates a wide size distribution [21]. According to Gottlieb and Schwartzbach [40], SPAN values lower than 2, for spray dried particles with d_{50} under 10 μm, indicate very narrow distribution. SPAN factor values for analysed microparticles indicate narrow unimodal distribution.

Drug carrier systems with particle size $\geqslant$ 200 μm have decreased gastrointestinal transit time, and therefore distinct decrease of efficiency. However, systems smaller than 200 μm show a more prolonged passage time, and so possibilities for controlled release of the encapsulated drug substance [11,32,41]. In fact, optimal particle size for localization of microparticles and their prolonged residence time in the colon is between 4 and 15 μm [9–13,16]. Nevertheless, particles with a size bigger than 4 μm, which have been reported to be taken up by macrophages less effectively [42], have a remarkable deposition in the inflamed tissue. Carrier systems in that size range are able to attach more efficiently to the mucus layer and accumulate in the inflamed region without the need for macrophage uptake [10,11].

Determined particle size favours the formulation of dosage form with prolonged and controlled release properties for treatment of IBD.

3.4. Swelling studies

Figs. 9 and 10 illustrate the swelling behaviour of uncoated (empty and BDS loaded) and Eudragit coated chitosan–Ca–alginate beads containing BDS in different buffers with pH 2.0, pH 6.8 and pH 7.4.

Table 4
The particle size distribution for uncoated, empty and BDS loaded, and Eudragit coated chitosan–Ca–alginate microparticles containing BDS expressed in terms of SPAN factor (mean ± SD, $n = 6$ measurements for each batch)

	SPAN factor ± SD	
	Aqueous dispersion	Dry powder
MP1	3.699 ± 0.03	2.669 ± 0.02
MP2	3.032 ± 0.01	2.133 ± 0.05
MP3	$1.918 \pm 5 \times 10^{-3}$	2.365 ± 0.02
MPB1	3.349 ± 0.05	$2.627 \pm 2 \times 10^{-3}$
MPB2	3.017 ± 0.04	2.056 ± 0.02
MPB3	$1.909 \pm 9 \times 10^{-4}$	$2.280 \pm 3 \times 10^{-3}$
E-MPB1	1.612 ± 0.01	2.789 ± 0.01
E-MPB2	3.554 ± 0.04	3.131 ± 0.03
E-MPB3	3.078 ± 0.01	3.091 ± 0.05

Comparing volume increase ratio between uncoated, empty and BDS loaded, chitosan–Ca–alginate beads, in all tested buffers it can be clearly seen that there isn't any difference.

Volume increase ratio in acidic media for uncoated chitosan–Ca–alginate microparticles (VIR between 1.15 and 1.78) is higher when compared with Eudragit coated microparticles (VIR 0.9–1.7) for 24 h. The swelling behaviour can be well justified due to the fact that wet beads tend to absorb water (free or bulk water) in order to fill the void regions of the polymer network within the beads that remain dehydrated, until they reach the equilibrium state [43]. The phenomenon is provoked by the relaxation of the polymer network [44]. This phenomenon may be attributed to protonation of primary amino groups ($-NH_3^+$) on chitosan and thus creating an expulsive force within the test hydrogel, but also there are the hydrogen-bond formations

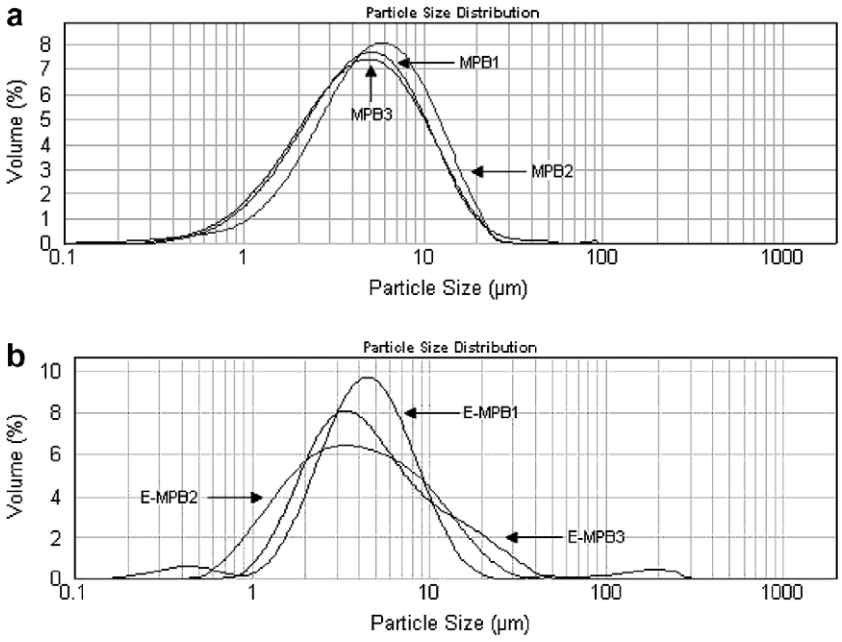

Fig. 8. Particle size distribution of uncoated (a) MPB1, MPB2, MPB3 and Eudragit coated; (b) E-MPB1, E-MPB2, E-MPB3, chitosan–Ca–alginate microparticles containing BDS.

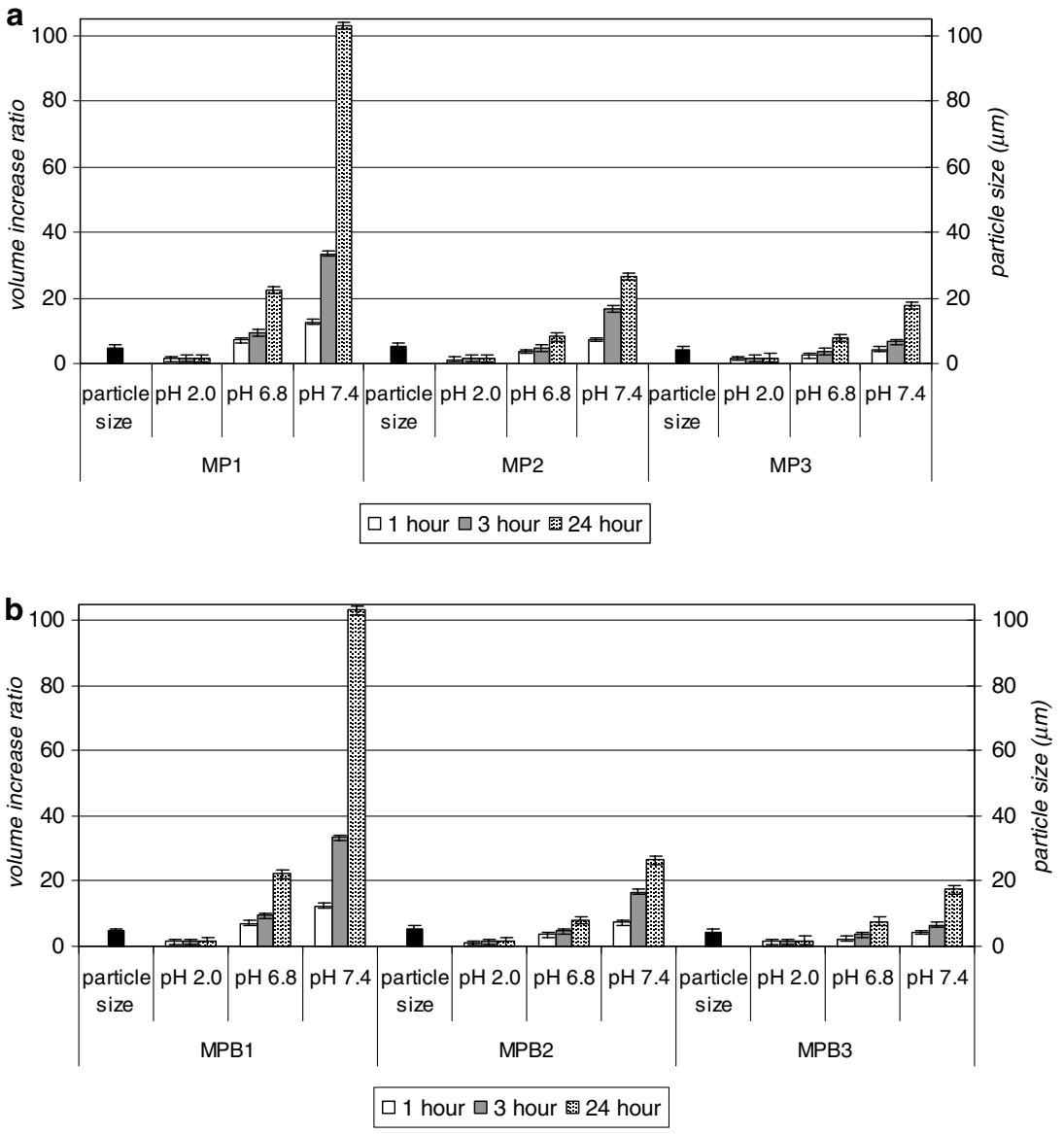

Fig. 9. Swelling behaviour of uncoated (a) empty (MP1, MP2, MP3) and (b) BDS loaded (MPB1, MPB2, MPB3) chitosan–Ca–alginate microparticles in different buffers at pH 2.0; 6.8 and 7.4 (mean ± SD, $n = 6$ measurements for each batch).

between –COOH and –OH groups and the ionic cross-links by Ca^{2+} between the carboxylate ions on alginate and chitosan maintain the structure of the polymer network stable [44,45]. In fact, not only pH but also buffer composition influenced the degree of swelling. For the medium where ion exchange was not expected the microparticles showed relatively small degree of swelling, because of high degree of cross-linking. In buffers where ion interchange is expected dramatic matrix destabilization can be expected.

Formulations tested in phosphate buffer with pH 6.8 and 7.4 exhibited rapid swelling. VIR of uncoated chitosan–Ca–alginate microparticles in pH 6.8 for MPB1 was 7.1–22.25; MPB2, 3.61–8.03; MPB3, 2.32–7.59 for 24 h, compared with Eudragit coated formulations where VIR values of 6.49–14.49 for E-MPB1, 3.58–7.97 E-MPB2 and 2.03–6.78 E-MPB3 were determined for the same period. In phosphate buffer with pH 7.4 VIR for MPB1 was 12.52–103.32; MPB2 7.27–26.54; MPB3 4.34–17.43 while for Eudragit coated microparticles VIR values were between 10.79 and 27.83 for E-MPB1, 4.65 and 16.56 E-MPB2 and 3.73 and 9.99 E-MPB3 for time period of 24 h. The swelling behaviour of uncoated beads in the case is related with the exchange of Ca^{2+} ions from the polymer matrix with Na^{+} ions from the phosphate buffers [46] and formation of calcium phosphate [47]. Also, low binding of chitosan with alginate due to the lower cationic nature of chitosan at these conditions (pK_a of chitosan is 6.2–7.0) [48] influences the swelling. So, lower cross-linking and destabilization of polymer matrix determine swelling properties of microparticles in tested phosphate buffers.

In the case of coated beads swelling behaviour is mainly determined by properties of enteric coated polymer, but after its dissolution, swelling is influenced by the properties of chitosan–Ca–alginate matrix.

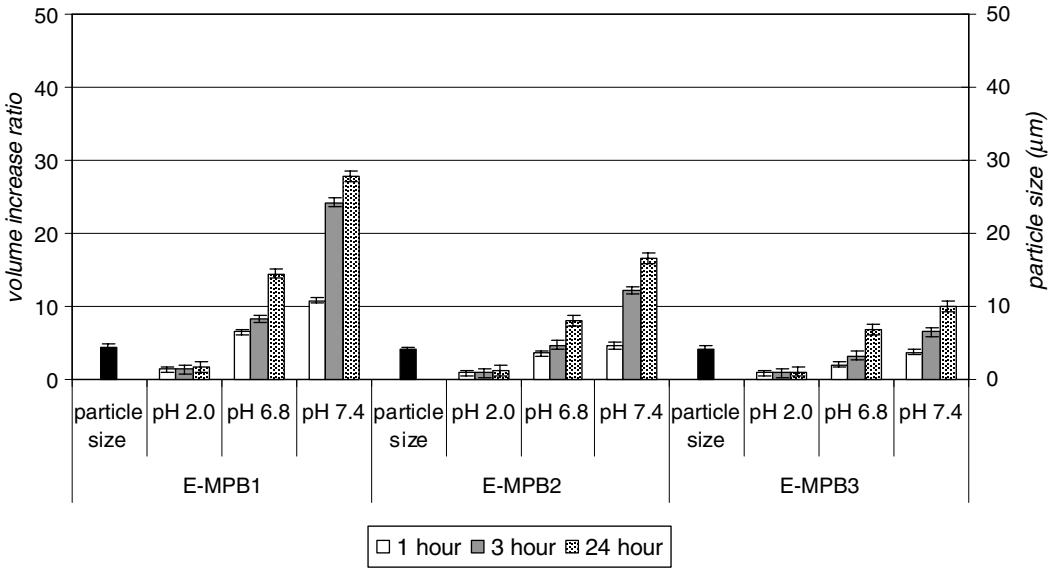

Fig. 10. Swelling behaviour of Eudragit coated (E-MPB1, E-MPB2, E-MPB3) chitosan–Ca–alginate microparticles containing BDS in different buffers at pH 2.0; 6.8 and 7.4 (mean ± SD, $n = 6$ measurements for each batch).

A greater extent of cross-linking ($CaCl_2$) of test beads results in a greater restriction of the mobility of the polymer chains and thus limits the swelling ratio of test beads [45].

3.5. Zeta potential measurements

The stability of many colloidal systems is directly related to the magnitude of their zeta potential. In general, if the value of the particle zeta potential is large, the colloidal system will be stable. Conversely, if the particle zeta potential is relatively small, the colloid system will agglomerate [31].

Table 5 presents measured zeta potential values for uncoated and coated beads, in water and different buffer solutions with low molarity and ionic strength.

A surface charge of the Ca–alginate particles was also determined, and was negative in all measured media (data not presented). Considering, that chitosan–Ca–alginate microparticles showed a positive value of the zeta potential, in all measured media, a preposition of the presence of chitosan on the surface of the particles could be made [31]. Since cell membranes are negatively charged, positively charged microparticles are expected to be more easily associated and subsequently taken up by the membranes than negatively charged microparticles [49]. These findings are in favour of expected mucoadhesiveness of prepared microparticles using one-step procedure. In fact, during one-step procedure both chitosan molecules and calcium ions are competing with each other at the same time with the negatively charged groups of the alginate molecules and this competition may result in that chitosan molecules are only slightly bound and hence keep their flexibility when the particles are suspended in aqueous milieu. As a result of this, they are able to interact with the mucus chains and show good mucoadhesiveness.

Table 5
Values of zeta potential (mV) for uncoated, empty and BDS loaded, and Eudragit coated, chitosan–Ca–alginate microparticles containing BDS in water and different buffer solutions (mean ± SD, $n = 6$ measurements for each batch)

	ζ (mV) ± SD			
	Water	pH 2.0	pH 5.0	pH 6.8
MP1	38.73 ± 1.36	32.53 ± 2.01	42.50 ± 0.37	30.70 ± 0.88
MP2	39.38 ± 1.29	33.36 ± 1.16	41.00 ± 1.83	41.36 ± 1.68
MP3	40.40 ± 0.71	32.70 ± 0.69	39.01 ± 0.40	30.81 ± 1.43
MPB1	40.03 ± 1.13	32.36 ± 0.16	42.76 ± 0.75	10.22 ± 0.69
MPB2	44.77 ± 0.85	33.37 ± 1.19	41.64 ± 0.39	10.31 ± 0.53
MPB3	40.65 ± 1.24	27.50 ± 1.27	42.60 ± 0.96	12.64 ± 0.22
E-MPB1	39.03 ± 0.29	30.24 ± 1.16	40.20 ± 0.71	8.78 ± 0.46
E-MPB2	54.03 ± 1.17	31.31 ± 0.54	40.86 ± 0.78	9.81 ± 0.16
E-MPB3	39.75 ± 1.11	32.28 ± 1.28	40.09 ± 0.51	9.60 ± 0.30

3.6. Determination of drug content

BDS content in all prepared formulation, determined by HPLC method, presented as BDS (g)/total polymers (g) was 0.02. Table 6 presents determined BDS content in microparticles (BDS (mg)/microparticles (g)), as well as encapsulation efficiency EE (%).

Amount of $CaCl_2$ used in the stage of preparation of particles with spray drying did not show effect on the BDS content in the final preparation in uncoated and Eudragit coated chitosan–Ca–alginate microparticles containing BDS.

3.7. Drug release studies

The effect of the pH value on the release of BDS from uncoated and Eudragit coated chitosan–Ca–alginate beads in different buffers (pH 2.0, 6.8, and 7.4) simulating the human gastrointestinal tract is shown in Figs. 11–13.

Table 6
BDS content in uncoated and Eudragit coated chitosan–Ca–alginate microparticles (BDS (mg)/microparticles (g)), and encapsulation efficiency EE (%) (mean ± SD, $n = 6$ measurements for each batch)

	BDS (mg)/microparticles (g) ± SD	EE (%) ± SD
MPB1	16.17 ± 0.04	99.98 ± 0.23
MPB2	13.90 ± 0.04	99.86 ± 0.31
MPB3	10.78 ± 0.03	99.92 ± 0.29
E-MPB1	5.39 ± 0.04	99.94 ± 0.77
E-MPB2	4.64 ± 0.04	99.96 ± 0.86
E-MPB3	3.59 ± 0.03	99.78 ± 0.73

In previous studies, chitosan–alginate microparticles were able to decrease the gel erosion [50] and allow a drug sustained release in acidic media [34,51]. However, in our study, uncoated chitosan–Ca–alginate microparticles did not have such properties. These contradictory results may be related to differences in the preparation procedures, the composition of chitosan and the composition and structure of the alginate gels [52–54].

When comparing drug release from Eudragit coated formulations to uncoated, it can be clearly seen that Eudragit coating has successfully sustained the release of BDS in acidic media and pH 6.8 which is due to pH dependent solubility of Eudragit® S 100. In addition, these findings might be associated not only with physical coating of microparticles with Eudragit, but also with possibility of certain interactions between chitosan and Eudragit [39]. During the coating procedure (spray drying) one might suspect that redistribution of drug substance (BDS) in the hydrophilic matrix will be triggered by Eudragit solvent (acetone) what might influence the drug release rate at pH 7.4, the pH where the Eudragit coating is soluble. So, at pH 7.4, BDS was released faster from coated beads than the uncoated ones.

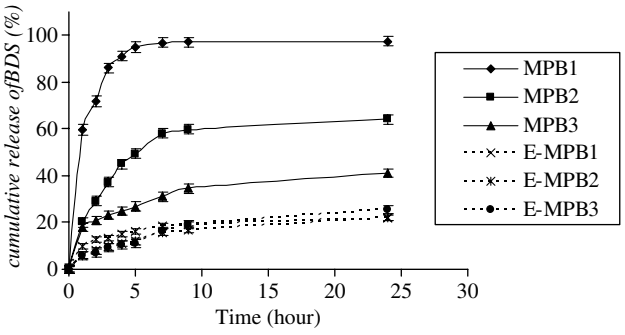

Fig. 11. In vitro release profile of uncoated (MPB1, MPB2, MPB3) and Eudragit coated (E-MPB1, E-MPB2, E-MPB3) chitosan–Ca–alginate microparticles containing BDS in buffer pH 2.0 at 37 ± 0.5°C (mean ± SD, $n = 3$ measurements for each batch).

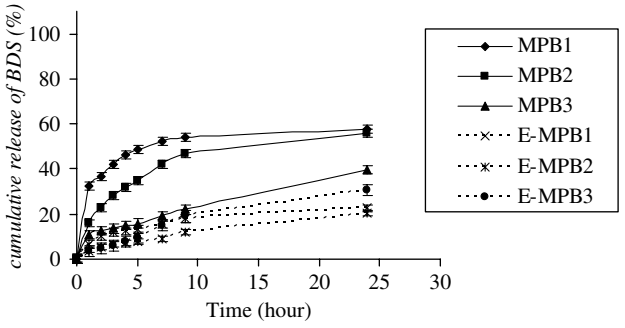

Fig. 12. In vitro release profile of uncoated (MPB1, MPB2, MPB3) and Eudragit coated (E-MPB1, E-MPB2, E-MPB3) chitosan–Ca–alginate microparticles containing BDS in buffer pH 6.8 at 37 ± 0.5°C (mean ± SD, $n = 3$ measurements for each batch).

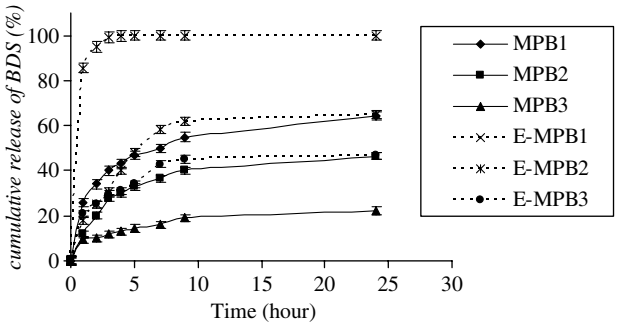

Fig. 13. In vitro release profile of uncoated (MPB1, MPB2, MPB3) and Eudragit coated (E-MPB1, E-MPB2, E-MPB3) chitosan–Ca–alginate microparticles containing BDS in buffer pH 7.4 at 37 ± 0.5°C (mean ± SD, $n = 3$ measurements for each batch).

From Figs. 11–13 we can see an overall positive effect of calcium ions on the release of BDS. Comparing the release profiles of different formulations it is evident that the rate and extent of drug release decreased significantly with increasing the concentration of $CaCl_2$. The lower rate of release could be a result of the increased rigidity of the polymeric chains due to the formation of the thicker junction zones and the contraction of the microvoids.

In order to understand the release mechanism, the release data were fitted to empirical equations proposed by Higuchi (Eq. (1)), Korsmeyer–Peppas (Eq. (3)) and Peppas and Sahlin (Eq. (4)). Values of estimated parameters are given in Table 7.

Comparing the values of the correlation coefficient (r) of different kinetics models it can be clearly seen that release data showed best fitting to the heuristic model proposed by Peppas and Sahlin [27] for quantifying the phenomena controlling the release from swellable matrix, in which the contribution of the relaxation or erosion mechanism and of the diffusive mechanism can be quantified. Since $K_1/K_2 < 1$, erosion dominantly controls drug release from matrix. Having in mind hydrophobicity of BDS, we can conclude that sustained release is due to the stabilization of the hydrophilic matrix. The polymer portion dissolute out from the hydrogel would control the release of BDS and as the hydrogel becomes stabilized, the release of the BDS would also be stabilized [55].

3.8. Stability studies

After 12 months storage at controlled ambient conditions no significant differences ($p < .05$) in particle size, par-

Table 7
Comparison of different dissolution kinetics models in pH 7.4

Batches	Higuchi		Korsmeyer–Peppas			Peppas and Sahlin			
	K (%h$^{-1/2}$)	r	K	n	r	K_1	K_2	K_1/K_2	r
MPB1	8.589	0.967	1.142	0.233	0.991	0.198	−0.019	−10.220	0.996
MPB2	7.955	0.922	1.922	0.418	0.941	0.247	−0.027	−9.067	0.994
MPB3	3.266	0.996	2.384	0.274	0.995	0.045	−0.002	−21.969	0.998
E-MPB1	12.158	0.932	0.147	0.103	0.963	0.440	−0.097	−4.501	0.993
E-MPB2	11.767	0.930	1.621	0.428	0.951	0.338	−0.036	−9.420	0.989
E-MPB3	6.322	0.898	1.507	0.264	0.958	0.210	−0.023	−9.104	0.983

ticle size distribution, swelling behaviour, zeta potential and drug release rate of the prepared formulations were observed.

On the basis of X-ray powder diffraction and DSC analysis during the stability study period it was confirmed that BDS is completely entrapped in the polymer matrix and stability of the structure of the microparticles and amorphous state of the drug substance were preserved.

4. Conclusions

In this work, a new dosage form, based on chitosan and alginate, loaded with BDS, with bioadhesive and controlled release properties in GIT is described. Microparticulate system consists of hydrophilic (chitosan–Ca–alginate) matrix coated with pH sensitive (Eudragit® S 100) polymer. Chosen preparation procedure (novel one-step spray-drying process) offers simplicity and reproducibility. Results from physical characterization of prepared microparticles are in favour of their localization and prolonged presence time in colon. Influence of polymer properties and concentration of cross-linker on the rate and extent of drug release is evident. Eudragit coating has successfully sustained release of BDS in the upper GIT (pH 2.0 and 6.8), while providing potential for efficient release of BDS in colon (pH 7.4). Based on all experimental results it can be concluded that Eudragit coated chitosan–Ca–alginate microparticles loaded with BDS could be suitable candidates for oral delivery of BDS with controlled release properties, opening a new therapeutic potential for this carriers for local treatment of inflammatory bowel disease. Prepared formulations were stable during 12 months storage period at controlled room temperature.

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