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Assessement of anti-inflammatory properties of microspheres prepared with chitosan and 5-amino salicylic acid over inflamed Caco-2 cells

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ABSTRACT

Microspheres based on the interaction between 5-amino salicylic acid (5-ASA) and chitosan were designed as a new colonic delivery system. Microspheres were prepared by a spray drying technique and characterised by physical properties, morphology and drug dissolution characteristics. Effects of the microspheres over inflamed Caco-2 cells were also evaluated, by determining cell viability and expression of mRNA levels of biomarkers involved in the pathogenesis of Inflammatory Bowel Diseases (IBD) (IL-1β and IL-8). Results showed that chitosan improved dissolution properties of 5-ASA. The amount of free drug released over 120 min did not exceed 9 mg/cm^2, while microspheres showed a high dissolution rate reaching approximately 50 mg/cm^2 of drug released. Contact of inflamed cells with free 5-ASA reduced mRNA levels of IL-1β and IL-8. Microspheres did not show cytotoxic activity and maintained ILs levels observed in non-inflamed cells, mimicking the anti-inflammatory effect of 5-ASA alone.

1. Introduction

Chitosan, the most important derivative of chitin, has gained increasing attention in colon targeting, because of its specific biodegradability by colonic bacteria (Zhang & Neau, 2002) in addition with well-documented biocompatibility, low toxicity and mucoadhesive properties (Muzzarelli & Muzzarelli, 2009; Ravi Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004). These properties make chitosan suitable for other many biomedical applications, such as tissue engineering, wound dressing and drug delivery (Jayakumara, Deepthy Menona, Manzoora, Naira, & Tamurab, 2010; Park, Saravanakumar, Kim, & Chan Kwon, 2010).

Many efforts have been made for colon specific delivery, including synthesis of prodrugs (Jung, Lee, & Kim, 2001), polymeric prodrugs (Zou et al., 2005), developing of time and/or pH-dependent formulations (Gupta, Beckert, & Price, 2001; Rudolph, Kleina, Beckertb, Petereitb, & Dressman, 2001) and bacterial degradable polymeric matrices (Davaran, Hanaee, & Khosravi, 1999). As for chitosan, colonic formulations include single-unit preparations and multiparticulate systems (Aguzzi et al., 2010; Hiorth, Tho, & Sande, 2003; Lorenzo-Lamosa, Remuñán-López, Vila-Jato, & Alonso, 1998; Ofori-Kwakye & Fell, 2001; Saboktakin, Tabatabaie, Maharramov, & Ramazanov, 2010; Shimono et al., 2002; Takeuchi, Yasuji, Yamamoto, & Kawashima, 2000; Tozaki et al., 2002; Zambito & Di Colo, 2003; Zhang, Alsarra, & Neau, 2002). Specifically, the use of micro- and nano-particulates can offer several advantages in colon delivery, including long retention in the ascending colon (Hardy, Wilson, & Wood, 1985) and accumulation in ulcerated regions of intestinal inflamed tissue (Lamprecht, Schäfer, & Lehr, 2001; Lamprecht, Yamamoto, Takeuchi, & Kawashima, 2005; Moulari, Pertuit, Pellequer, & Lamprecht, 2008; Pertuit et al., 2007). *In vitro* release of 5-ASA from chitosan microspheres with different formulations was investigated by Varshosaz, Jaffarian Dehkordi, and Golafshan (2006), who prepared the microspheres by a solvent evaporation technique and determined their encapsulation efficiency and bioadhesion. Enteric coated chitosan–pectin multiparticulate systems were recently formulated, showing great potential for site-specific delivery of triamcinolone through oral administration (Oliveira, Ferrari,

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Carvalho, Raul, & Evangelista, 2010). However, no information is available about biological effects of these systems in cell cultures.

Inflammatory bowel disease (IBD) is a group of chronic inflammations of the intestine, whose two major categories are ulcerative colitis (UC) and Crohn's disease (CD). Compared to not-inflamed mucosa the majority of active IBD tissues express significantly elevated transcript levels of interleukins (IL-1 and IL-8) whose immunoregulatory properties may contribute to pathological immune reactions in IBD (Stallmach et al., 2004).

Aminosalicylates (5-aminosalicylic acid and its derivatives) are among the primary classes of drugs used in the treatment of IBD. 5-ASA is a poorly soluble amphoteric compound with minimum solubility values between pH 2 and pH 5.5 (French & Mauger, 1993). The precise mechanism of action of 5-ASA is unknown, but is likely multifactorial, since the drug interacts with the biosynthesis or action of a variety of mediators possibly involved in the pathogenesis of IBD, including prostaglandins (PGE_2, PGD_2), leukotrienes (LTB_4), cytokines (IL-1β, IL-2, IL-6), interferon-γ, nuclear factor-κB, platelet-activating factor and TNF-α (Clemett & Markham, 2000). Moreover, 5-ASA was found to possess antioxidant properties within the inflamed gut (McKenzie, Doe, & Buffinton, 1999; Williams & Hallett, 1989) and to directly stimulate intestinal epithelial wound repair *in vitro* (Baumgart, Vierziger, Sturm, Wiedenmann, & Dignass, 2005).

5-ASA can be delivered orally or topically (via enemas or suppositories) to the colon, where the drug exerts a local therapeutic effect. However, 5-ASA cannot be simply given orally in the treatment of IBD because it is absorbed by the small intestine, so the amount reaching the colon is fairly reduced (Crotty & Jewel, 1992).

Given these premises, the aim of the present work was the design of chitosan microparticles for colonic delivery of 5-ASA in the treatment of IBD. Microparticulate systems based on the ionic interaction between 5-ASA and chitosan were prepared by a spray drying technique. Chitosan was chosen as drug carrier not only for its colonic biodegradability, but mainly to improve dissolution of 5-ASA at the release/action site, where physiological pH (6.4 in the right colon (Evans et al., 1988)) was substantially reduced in patients with ulcerative colitis (Nugent, Kumar, Rampton, & Evans, 2001). 5-ASA loaded chitosan microspheres were characterised for physicochemical and biopharmaceutical properties. Moreover the influence of 5-ASA-chitosan interaction and formulative parameters on anti-inflammatory effect was evaluated by using inflamed Caco-2 cell model. This model was based on the evaluation of markers (interleukins) that can be related to the inflammatory status of the cells. In particular the anti-inflammatory effect was evaluated for drug alone, for the polymeric solutions and for microspheres to verify that 5-ASA chitosan interaction did not impair the drug activity.

2. Materials and methods

2.1. Materials

The following base chitosans (CS) were purchased from Faravelli (I): low CSL: viscosity* 12 mPa s and deacetylation degree (DD) 98%; medium CSM: viscosity* 269 mPa s and DD 92%; high CSH: viscosity* 386 mPa s and DD 91%. (*Viscosity measurements were performed on 1% (w/v) solutions in HCl 0.1 M at 90 s^{-1} with rotational rheometer equipped with coaxial cylinders C14 (Bohlin CS, Bohlin Instrument Division, Metrics Group Ltd., Cirencester, UK). For cell experiments, each chitosan was previously dissolved (1%, w/v) in HCl 0.1 M and freeze dried (Edwards mod RV8) to obtain the corresponding water soluble hydrochloride form (HCSL, HCSM and HCSH, respectively).

2.2. Preparation of the 5-ASA/chitosan microspheres

The microspheres, hereafter referred to as 5-ASA/HCSL, 5-ASA/HCSM and 5-ASA/HCSH, were prepared dispersing known amounts of chitosan powder in 5-ASA solutions 0.25% (w/w) in HCl 0.1 M. The amounts of polymer were previously calculated in order to obtain a stoichiometric molar ratio (1:1 mol:mol) between the drug and the free $-NH_3^+$ groups of the chitosan. Dispersions were stirred until complete polymer dissolution (3 h approximately) at room temperature and then filtered through Millipore® membrane (1.2 μm porosity) to remove chitosan insoluble impurities. The obtained solutions were spray dried using a laboratory-scale spray-dryer (Buchi® Mini Spray Dryer, B-191, Buchi, CH), with a 0.7-mm nozzle at a feed rate of 4 ml/min. The nozzle air pressure was 6 bar and the inlet temperature 155 °C (resulting in outlet temperature of 145 °C). The airflow rate and the aspirator were 380 ml/h and 35 m^3/h (100%), respectively. The efficiency of the process was always ≥30%.

2.3. Determination of the loading efficiency

Microspheres (8 mg) were dispersed in 10 ml of bi-distilled water, stirred until complete dissolution (3 h approximately) and then filtered through 0.45 μm Millipore® membranes. The amount of drug in solution was analyzed by HPLC (series 200, Perkin Elmer, I) according to Cendrowska, Drewnowska, Grzeszkiewicz, and Butkierwicz (1990). The stationary phase was a Spherisorb® C18 column (10 μm, 250 mm × 4.6 mm) and the mobile phase was a mixture of H_2O/CH_3CN (78/22, v/v) and acetic acid 0.5% (v/v). The flow rate was set at 1 ml/min, the injection volume was 20 μl, the detector wavelength 300 nm and the run time 10 min. Data were recorded using TotalChrom WS 6.2 software package (Perkin Elmer, I). The loading efficiency (LE) was calculated from the ratio between the experimental and theoretical amount of drug. Each experiment was done in triplicate.

2.4. Characterisation of the microspheres

2.4.1. Instrumental analyses

DSC analysis was performed with a DSC FP800 apparatus, equipped with FP89 software package (Mettler-Toledo GMBH, CH), on 4 mg samples at 10 °C/min in the 30–350 °C temperature range.

Microsphere morphology was investigated by means of scanning electron microscopy (SEM) using a Zeiss DSM 950 (Zeiss Co., G) scanning electron microscope. Gold sputtering was performed before SEM analysis.

Particle size of the microspheres was determined by a Coulter Counter method (Coulter Multisizer II, Coulter electronics Ltd., UK) with a 100 μm orifice tube. Prior to the analysis, each sample was suspended in 5-ASA saturated physiological solution (NaCl 0.9%, w/v) (conductive medium) with Tween 80 (0.5%, w/v) to enhance sample wettability. Data were on line collected by means of Accu-Comp Windows Software for Z2 (Beckman Coulter, I) and particle diameters (d_{10}, d_{50}, d_{90}) were calculated.

Solubility measurements were performed by dispersing 20 mg of microspheres in 1 ml of distilled water under continuous stirring (25 °C for 24 h). Then, the amount of drug in solution in equilibrium with the solid phase was measured using a UV–Vis spectrophotometer (Perkin Elmer Lambda 25, I) at 300 nm wavelength.

2.4.2. Intrinsic dissolution studies

Experiments were carried out following the protocol described by Aguzzi et al. (2010). Briefly, 10 mm flat matrices were prepared with 50 mg of microspheres (or free drug), using a Perkin-Elmer hydraulic press at 5 tons for 1 min. Measurements were done for 2 h using an on line dissolution system (mod. AT7, Sotax, I),

Table 1
Cell treatments for inflammation studies.

Treatment	Cell inflammation	Sample
A: control	NO	–
B: blank	YES	–
C	YES	5-ASA
D	YES	HCSL
E	YES	5-ASA/HCSL
F	YES	HCSM
G	YES	5-ASA/HCSM
H	YES	HCSH
I	YES	5-ASA/HCSH

equipped with the USP apparatus 2, at 50 rpm, 37 °C and 300 ml of distilled water (previously degassed under vacuum). The amount of drug dissolved was assayed every 5 min and data were collected using a specific software package (DissLab, Perkin Elmer, I). Data (mg released/cm^2) were normalized according to the dose of drug in the microspheres. The drug release rate, expressed as mg released per unit of time and area (mg/min cm^2), was then calculated by linear fitting of the experimental points as a function of time during the first 30 min of the test (linear portion of the curves). Each experiment was done in triplicate.

2.4.3. Inflamed Caco-2 cell culture

Caco-2 cells (ATCC, USA) were incubated (37° C; 5% CO_2 atmosphere) in 6-well tissue culture plates (Corning, Spain) (1×10^5 cells/cm^2) with pH 7.4 complete Dulbecco's Modified Eagle's Medium (DMEM) for 24 h. The media was then replaced and inflammation was induced by adding lipolysaccharide (LPS) (1 μg/ml) (Sigma–Aldrich Química S.A., S), IL (interleukin)-1β (5 ng/ml) (Strathmann Biotec AG, G) and TNF-α (50 ng/ml) (Strathmann Biotec AG, G). Control cells were kept without the cocktail (A, control, non-inflamed cells). Twenty-four hours after, the inflammation cocktail was removed and cells were treated according to Table 1, by adding amounts of microspheres and pure components corresponding to 0.05 mmol/l of 5-ASA in the culture medium.

2.4.4. Cytotoxicity assays (LDH activity)

The cytotoxic effect was determined by measuring lactate dehydrogenase (LHD) activity in cell substrate supernatant from each well obtained according with the previous paragraph, at 340 nm (UV–Vis spectrophotometer, Beckman DU-70, S). 30 μl of centrifuged medium were incubated (in 96-well plate) with 80 μl of β-NADH (1.45 mM) for 5 min at 37 °C. The kinetic determination of LDH was initiated by adding 20 μl of sodium pyruvate (25 mM). The initial oxidation rate of NADH is proportional to the LDH catalytic activity. LDH activity in the sample was calculated by measuring the per time absorbance decrease.

2.4.5. RNA isolation and reverse transcriptase polymerase chain reaction (RT-PCR)

Total RNA was extracted from Caco-2 cells using RNeasy Total RNA Kit (Qiagen, IZASA S.A., S), according to the manufacturer protocol. Purified total RNA (decontaminated by genomic DNA using RQ1 RNase-Free DNase (Promega, Madison, WI)) was reversely transcribed using Super ScriptTM II RNase H- Reverse Transcriptase enzyme (Invitrogen, S) and oligo-dT primer 20 mM (Sigma–Aldrich Química S.A., S) to obtain cDNA. cDNA samples (3 μl) were amplified using primer pairs (1 μl) as indicated in Table 2. Amplified cDNA samples (10 μl) were subjected to electrophoresis and densitometric analysis was performed by using NIH Image Software (National Institutes of Health, Bethesda, MD). Results were standardized by the β-microglobulin (βMG) housekeeping gene signal to correct any variability in gel loading. Results are presented as fold change relative to the β-MG housekeeping gene.

2.4.6. Statistical analysis

One-way analysis of variance (ANOVA) with the post hoc Sheffé test for multiple comparisons was performed using the software Siphar 4.0 (Cedex, F). Differences between groups were considered to be significant at a level of P less than 0.05.

3. Results and discussion

3.1. Chemico-physical characterisation of the microspheres

Table 3 shows the loading efficiency (LE %) of each sample, expressed as a percentage of the amount of drug entrapped in the microspheres. As observed, satisfactory values (>90%) were found in all cases and no significant differences were observed depending on the chitosan used.

Calorimetric curves of the microspheres based on the three chitosan grades were very similar. Fig. 1 shows the DSC curves of pure free 5-ASA (a), the spray-dried drug (b), the polymer HCSH (c) and the drug loaded microspheres (5-ASA/HCSH) (d). 5-ASA shows a typical calorimetric profile of a crystalline anhydrous substance, with a melting point at around 270 °C, accompanied by an exothermic effect of decomposition. No significant changes in the thermal behaviour of the drug were produced by the spray drying process. Calorimetric curve of HCS is typical of an amorphous hydrated compound, with a broad endothermic peak in the range 60–150 °C, corresponding to the polymer dehydration, followed by an exothermic phenomenon (at about 220 °C) due to the decomposition of the polymer chain. In the case of microspheres, a shift of the melting peak of the drug is observed. The enthalpy of the peak is also significantly reduced in comparison with the free drug. These results can be directly correlated with an almost complete amorphization of the drug and subsequent loss of crystallinity. Based on the DSC profiles, the properties of amorphization of the three chitosans employed can be considered equivalent.

Table 2
Primers and expected products for RT-PCR analysis. Nucleotide sequences are reported for primer pairs used in RT-PCR analysis experiments in Caco-2 cells.

Gene	Forward primer	Reverse primer	Expected product bp
IL-1β	5′-CAGATGAAGTGCTCCTTCCA-3′	5′-GAGAACACCACTTGTTGCTC-3′	386
IL-8	5′-ATGACTTCCAAGCTGGCCGT-3′	5′-TCAGCCCTCTTCAAAAACTTCT-3′	290
β-MG	5′-CCAGCAGAGATGGAAAGTC-3′	5′-GATGCTCTACATGTCTCG-3′	249

Table 3
Drug amount and loading efficiency (LE) for the studied samples (mean values ± s.d.; $n = 3$).

Microspheres	Theoretical amount of drug (%)	Experimental amount of drug (%)	LE (%)
5-ASA/HCSL	48	43.67 ± 1.155	90.98 ± 2.406
5-ASA/HCSM	46	42.42 ± 0.764	92.21 ± 1.660
5-ASA/HCSH	46	42.57 ± 1.159	92.54 ± 2.520

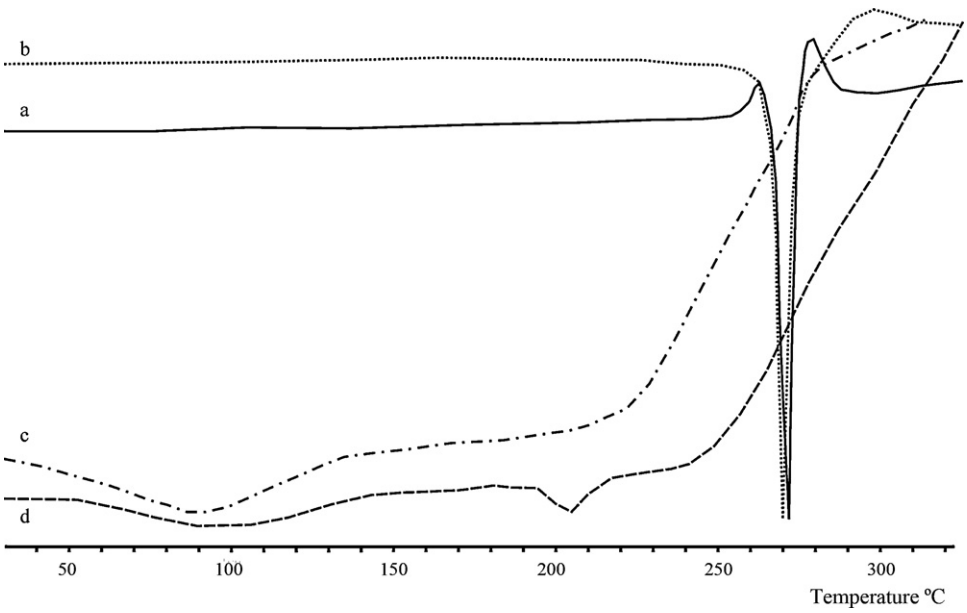

Fig. 1. DSC curves of the samples. Free drug (a), spray dried drug (b), HCSHV (c) and 5-ASA/HCSH microspheres (d).

Table 4
Granulometric parameters of the microspheres (mean values ± s.e.; n = 5–7).

Microspheres	d_{10} (%V)	d_{50} (%V)	d_{90} (%V)
5-ASA/HCSL	1.849 ± 0.426	4.030 ± 0.7465	13.292 ± 2.3288
5-ASA/HCSM	1.783 ± 0.0379	3.777 ± 0.2143	14.240 ± 2.5186
5-ASA/HCSH	2.199 ± 0.0553	5.893 ± 0.2552	23.149 ± 5.2016

SEM microphotographs showed that all the samples studied were constituted by spherical microparticles with diameters between 1 and 2 μm (Fig. 2). However, depending on the viscosity of the chitosan used, some differences were found in aggregation and surface appearance of the individual microspheres. Those prepared with low viscosity chitosan (5-ASA/HCSL) exhibited a uniform surface and low tendency to form aggregates (Fig. 2a, b). Some surface irregularities were observed with increasing viscosity of the chitosans, accompanied by an increase in the number and size of the aggregates (Fig. 2c, d). In those prepared with higher viscosity chitosan, almost complete aggregation and partial superficial exfoliation of the microspheres appeared (Fig. 2e, f).

Table 4 shows values of d_{10}, d_{50} and d_{90}, representing the diameters corresponding to cumulative percentage of particles <10%, <50% and <90%, respectively. Microspheres prepared with chitosan of low (5-ASA/HCSL) and medium (5-ASA/HCSM) MW have very similar values of d_{10} and d_{50} (respectively, equal to about 1.8 and 4 μm). The d_{90} for both samples did not exceed 14 μm. These values could be promising for adhesion of the microspheres to the colonic mucous. Other authors found a size-dependent deposition on inflamed colonic mucosa for particle ranging from 10 to 0.1 μm, with higher adhesion for particles lower than 10 μm (Lamprecht et al., 2001). In the case of microspheres prepared with high viscosity chitosan (5-ASA/HCSH) higher values of d_{50} and d_{90} were measured, being respectively equal to around 6 and 23 μm. These results are in agreement with the aggregation tendencies observed in the SEM microphotographs.

Solubility of microspheres was 2.31 ± 0.315 mg/ml (5-ASA/HCSL), 2.19 ± 0.449 mg/ml (5-ASA/HCSM) and 2.30 ± 0.292 mg/ml (5-ASA/HCSH). These values were significantly higher (P < 0.001, one-way ANOVA; Post hoc Scheffé test) than that of the free drug (0.82 ± 0.08 mg/ml). This effect can probably be attributed to the amorphization of the active substance by interaction with chitosan, as it was put in evidence in calorimetric studies. These results are in line with other papers, in which chitosan was able to increase dissolution of several poorly soluble drugs (Genta, Pavanetto, Conti, Giunchedi, & Conte, 1995; Maestrelli, Zerrouk, Chemtob, & Mura, 2004; Mura, Zerrouk, Mennini, Maestrelli, & Chemtob, 2003; Portero, Remuñán-López, & Vila-lato, 1998).

No significant differences were found on drug solubility depending on MW and DD of chitosan.

Fig. 3 shows the release curves (mg released/cm^2 *vs* time) obtained from tablets prepared with the microspheres and the free drug. 5-ASA exhibits a very slow dissolution profile, because of its low water solubility. At the end of the test (120 min) the amount released did not exceed 9 mg/cm^2. In the case of microspheres, dissolution profiles are almost superimposable and significantly higher than the free drug, with a high dissolution rate leading to approximately 50 mg/cm^2 of drug released after 120 min. The estimated values of intrinsic dissolution rate (IDR), expressed as mg released per unit of area and time (mg/cm^2 min), were in line with solubility measurements. Dissolution rate of the microspheres (1.009 ± 0.2158, 1.0320 ± 0.3650 and 1.1104 ± 0.1027 for 5-ASA/HCSL, 5-ASA/HCSM and 5-ASA/HCSH, respectively) was in fact noticeably higher in comparison with the free active ingredient (0.0512 ± 0.0130), indicating that dissolution properties of 5-ASA are significantly improved by interaction with chitosan. Moreover, no significant differences were found on dissolution rate depending on the viscosity grade of the polymer.

3.2. *In vitro anti-inflammatory effect of microspheres*

Cytotoxicity studies were performed according to LDH assays, and the results are reported in Fig. 4. LDH activity from inflamed Caco-2 cells treated with 5-ASA (column C), the three chitosans (columns D, F, H; for low, medium and high viscosity, respectively) and their corresponding microspheres (columns E, G, I) are given. Enzymatic activity from non-inflamed (control, column A) and inflamed, but not treated, cells (blank, column B) are also shown. As observed, LDH activity of blank was not significantly different from that of the control. This result revealed the absence of toxicity of the pro-inflammatory cocktail concentration used for

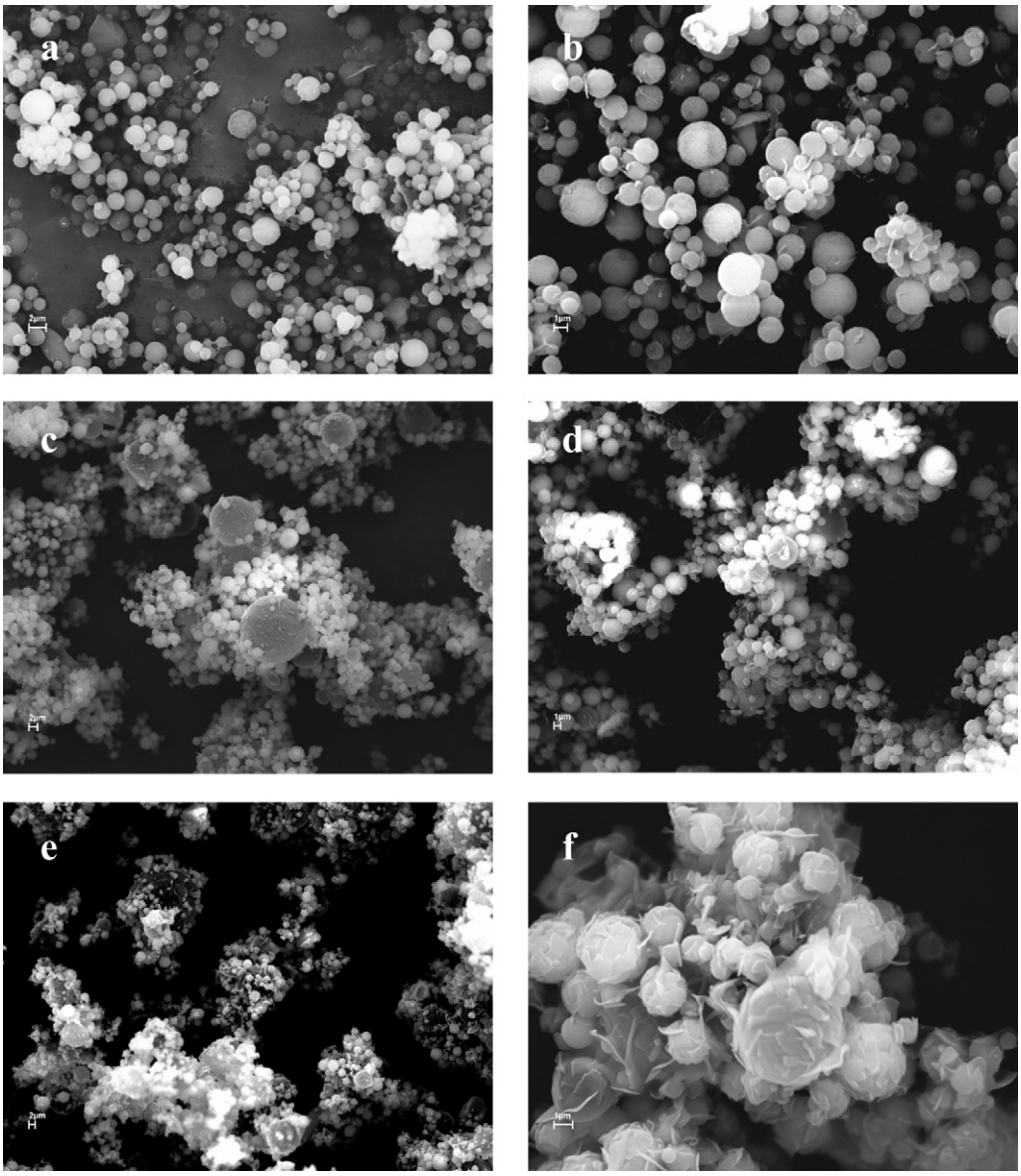

Fig. 2. SEM microphotographs of the microspheres: (a, b) 5-ASA/HCSL; (c, d) 5-ASA/HCSM; (e, f) 5-ASA/HCSH.

induction of inflammation. Similar behaviour was found when cells were treated with the samples, indicating that neither the drug, nor chitosans or microspheres showed cytotoxic effect on Caco-2 cells at the concentration studied.

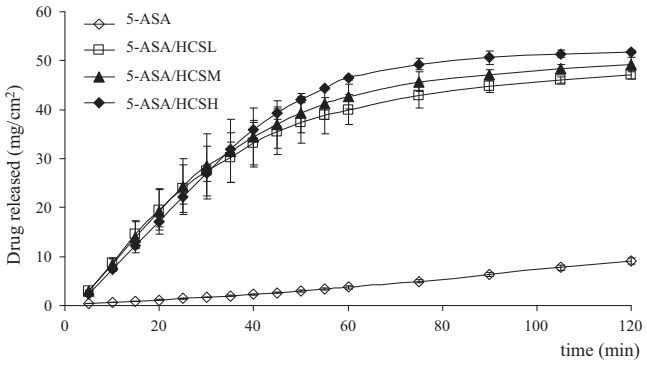

Fig. 3. Intrinsic dissolution profiles of the free drug and the microspheres (mean values ± s.d.; $n = 3$).

In Fig. 5 m-RNA levels of IL-1β and IL-8 are given. High levels of these cytokines were found in patients with ulcerative colitis (Murata, Ishiguro, Itoh, Munakata, & Yoshida, 1995). In particular, IL-8 possesses a potent chemotactic activity for neutrophiles and T limphocytes (Baggiolini, 2001) and its expression has to be controlled to avoid tissue injure (De Dooy, Mahieu, & Van Bever, 2001).

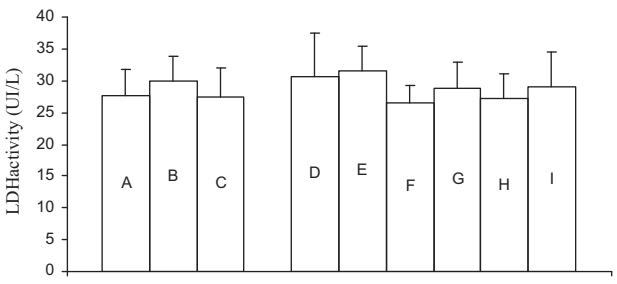

Fig. 4. Enzymatic activity (LDH) in Caco-2 cell cultures (see Table 1 for the meaning of the abbreviations) (mean values ± s.d.; $n = 6$).

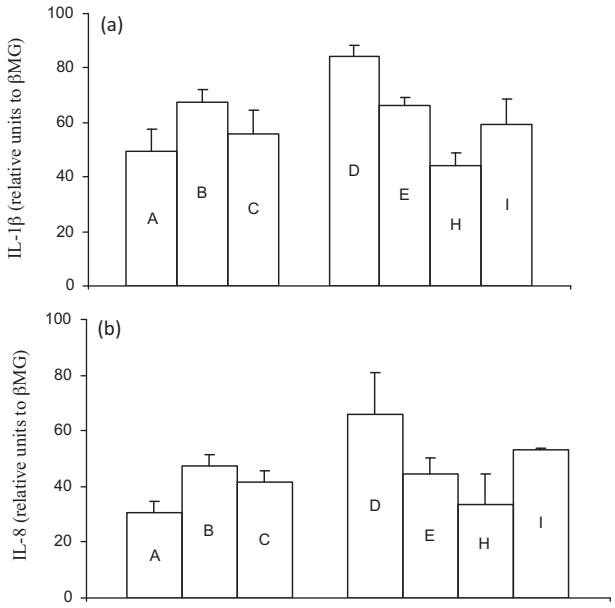

Fig. 5. IL-1β (a) and IL-8 (b) mRNA levels analyzed by RT-PCR A (see Table 1 for the meaning of the abbreviations) (mean values ± s.d.; $n = 6$).

The figure shows increased mRNA levels of IL-1β ($P < 0.05$) and IL-8 ($P < 0.01$) in inflamed cells (B) in comparison with the control. This indicates that inflammation was effectively induced by the applied protocol. Contact of inflamed cells with free 5-ASA reduced mRNA levels of IL-1β and IL-8 to those of control cells (C *vs* A). These results are in line with those found in the literature, showing that 5-ASA decreases the content, synthesis and release of IL-1/1β in colonic epithelial cells (Bruin et al., 1995; Mahida, Lamming, Gallagher, Hawthorne, & Hawkey, 1991; Rachmilewitz, Karmeli, Schwartz, & Simon, 1992). In the present study only the HCS low and high MW and the systems prepared with them are considered, while HCSM is not included because it has viscosity and DD values quite similar to those of HCSH, and its effects on LDH also resemble HCSH. HCSL (D) increased the ILs mRNA levels of inflamed cells ($P < 0.01$ for IL-1β). In contrast, HCSH (H) reduced the ILs mRNA levels to the values of non-inflamed cells, reverting the induction. The differences between the two grades of chitosan might be due to the different degree of deacetylation (higher for HCSL than HCSH). Prasitsilp, Jenwithisuk, Kongsuwan, Damrongchai, and Watts (2000) showed that the % of deacetylation has a very important effect on the biocompatibility of the chitosan, improving cell adhesion when high % DD samples are used. Other authors found weaker effects on inflammation mediators for chitin in comparison with chitosan (Mori et al., 1997). 5-ASA/HCSL microspheres reduced dramatically ILs mRNA levels when compared to the chitosan alone (E *vs* D; IL-1β, $P < 0.01$; IL-8, $P < 0.05$). In the case of 5-ASA/HCSH, levels of ILs returned to the values of 5-ASA treatment, suggesting that the anti-inflammatory effect of 5-ASA was not affected by complexation with the polymer.

4. Conclusions

Accordingly to the results, successful interaction between 5-ASA and chitosan was achieved by spray drying technique, leading to spherical microparticles with amorphous character and relatively high loading efficiencies. Microspheres had dimensions theoretically suitable to obtain deposition on inflamed colonic mucosa. The proposed formulations were all capable to clearly increase 5-ASA dissolution rate with respect to the drug alone. Chitosans and drug loaded microspheres did not show cytotoxic effects on Caco-2 cells. The cell culture model was suitable to assess the effect of the formulations on the inflammation markers. In all the formulations the interaction between chitosan and 5-ASA did not impair the anti-inflammatory effect of the drug in this *in vitro* model. When the effect of the polymer alone was considered differences were observed depending on the chitosan grade. These differences could be ascribed not only to molecular weight but also to deacetylation degree. A study specifically aimed to understand this aspect could be the subject of a further investigation.

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