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In vitro study of *N*-succinyl chitosan for targeted delivery of 5-aminosalicylic acid to colon

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ABSTRACT

In vitro study on *N*-succinyl chitosan and chitosan (control) matrices for selective colon delivery of 5-aminosalicylic acid is described. Matrices containing β-cyclodextrin were also prepared. As shown by DSC analyses, the drug was successfully loaded into the matrices reaching up to 95% entrapment efficiency. Swelling and drug release were studied at pH 1.2, pH 7.4, and in a pH gradient medium to simulate the gastro-intestinal transit. Main result of this study was a higher capability of *N*-succinyl chitosan alone to better control drug release in the simulated gastro-intestinal transit: *N*-succinyl chitosan gave the lowest release in acid medium (≅15%) and the highest in alkaline environment (≅92%). The cyclodextrin did not provide any interesting role since they enhanced drug release from the unswollen *N*-succinyl chitosan (pH 1.2, ≅50% drug release). Kinetic studies showed a non-Fickian transport from all the matrices, which also showed good mucoadhesive properties (work of adhesion ≅1.5 mN mm).

1. Introduction

Chitosan is a hydrophilic, biocompatible and biodegradable, non-toxic and bioadhesive cationic polysaccharide. Because of these properties, chitosan was widely used to develop conventional and novel delivery systems for drugs and vaccines, and it has been extensively explored for colon specific drug delivery (Hejazi & Amiji, 2003; Park, Saravanakumar, Kim, & Kwon, 2010). Chitosan is soluble only in acidic solution below pH 6.5 and, therefore, several derivatives were synthesized to improve polymer solubility at both neutral and alkaline pH. In particular, *N*-succinyl-chitosan (NS-chitosan) was introduced and studied (Hou et al., 2010; Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004).

Chitosan and *N*-succinyl-chitosan have been explored for the development of colon specific delivery systems containing 5-aminosalicylic acid (5-ASA) (Dubey, Dubey, Omrey, Vyas, & Jain, 2010; Jain et al., 2008; Kumar et al., 2004). 5-ASA is an anti-inflammatory drug used for the long term therapy of inflammatory bowel disease, a group of inflammatory conditions that affect colon and small intestine. However, when orally administered, 5-ASA is mostly absorbed in the stomach and in the small intestine, thus causing systemic side effects, while therapeutic doses do not reach the colon.

5-ASA is slightly soluble in water and is light and oxygen sensitive (Zerrouk, Gines Dorado, Arnaud, & Chemtob, 1998). Drug solubility is a key factor in the drug release from inert as well as swellable delivery systems where diffusion, preceded by the drug dissolution, controls the release. For poorly soluble drugs, their low dissolution rate is the actual limiting factor of drug delivery (Sangalli et al., 2001). One approach to increase 5-ASA solubility and stability consists in its inclusion in cyclodextrins (Zerrouk et al., 1998). As well known, cyclodextrins are oligosaccharides that are largely employed in pharmaceutical formulations for their capability to form inclusion complexes with poorly soluble drugs to improve their water solubility and, therefore, their bioavailability (Bertacche, Lorenzi, Nava, Pini, & Sinico, 2006; Caddeo, Manconi, Valenti, Pini, & Sinico, 2007). In the last decade, cyclodextrins have also been proposed as modulators of drug release from different polymeric delivery systems that include hydrogels, gels, and erodible hydrophilic matrices as well as biodegradable microspheres (Miro et al., 2009).

Different approaches have been evaluated to achieve colon specific 5-ASA release. They consist of polymeric prodrugs, selectively degradable by the local microflora, pH-dependent systems, and formulations based on time-dependent/delayed drug release (Casadei, Pitarresi, Calabrese, Paolicelli, & Giammona, 2008; Cassano et al., 2010; Iruin, Fernandez-Arevalo, Alvarez-Fuentes, Fini, & Holgado, 2005; Nunthanid et al., 2008; Tozaki et al., 2002; Zou et al., 2005). In

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colon drug targeting, large attention has been paid to formulation of pH-dependent systems using Eudragit® or other polymers capable of delaying drug release to the colon. In particular, polysaccharide microspheres were coated with Eudragit®-enteric materials to reduce drug release in the gastric fluid, thus showing an improved colon drug targeting (EudThakral, Ray, & Majumdar, 2010; Iruin et al., 2005; Paharia et al., 2007).

In this research, aiming at developing new colon specific delivery systems for 5-ASA, three-dimensional amorphous matrices made with NS-chitosan, were prepared and characterized by using several methods (SEM; DSC; FTIR, swelling behaviour, and drug release). Moreover, in this first part of our research, we have particularly focused on the influence of β-cyclodextrin on the 5-ASA release. Therefore, matrices including these cyclic oligosaccharides were also prepared, characterized and their properties compared to those of the free-cyclodextrin formulations. As a comparison, chitosan matrices were also prepared and tested.

2. Experimental

2.1. Materials

Chitosan of average molecular weight (75,000 Da), succinic anhydride and 5-aminosalicylic acid (5-ASA) were obtained from Sigma–Aldrich, (Milan, Italy). β-Cyclodextrin (CD) was kindly supplied by Roquette Co. (Lestrem, France). All the products and solvents were of analytical grade. Spectra-Por® dialysis membrane (cut-off 12,000–14,000 Da, regenerated cellulose) was purchased from Spectrum Lab Inc. (CA, USA).

2.2. Preparation and characterization of N-succinyl-chitosan

Chitosan was succinylated according to method reported previously (Yamaguchi, Araj, Itoh, & Hirano, 1981) with some modifications. Briefly 0.64 g of chitosan was dissolved under stirring in a 5%, v/v aqueous acetic acid solution (50 ml), and the resulting solution was slowly diluted with 50 ml of methanol. Then 4.22 g of succinic anhydride, previously dissolved in a minimum amount of acetone (30 ml), was added dropwise to the polymer solution. The reaction was maintained under stirring overnight at room temperature. The obtained viscous gel was diluted with NaOH 2 M solution, which was added dropwise until pH 10 was reached and a clear solution was formed. The solution was concentrated by a rotary evaporator (Rotavapor Büchi R110, Switzerland) and immediately dialyzed for three days against distilled water. Then, the solution was freeze-dried to obtain a white cotton-like material. The product was characterized by elemental analysis (Fisons model EA 1108 Elemental Microanalyser), FTIR (Bruker Equinox 55), and DSC spectra (Toledo model 821e).

IR (KBr mull) 3319 cm^{-1} ($-NH_2$ and –OH stretching); 1731 cm^{-1} (carboxylic acid C=O stretching); 1668 cm^{-1} (amide I); 1585 cm^{-1} (amide II); 1162 cm^{-1}, 1072 cm^{-1} and 1035 cm^{-1} (sugar structure).

Elemental analysis: found C: 36.96; H: 6.62; N: 4.30; calculated from $[C_6H_{10}O_4N(HCOCH_3)_{0.15}(H_2)_{0.17}(HCOC_2H_4COONa)_{0.68}]$ C: 37.91; H: 6.11; N: 4.88.

The degree of succinylation, defined as the average number of succinyl groups per repeating units of glucosamine, was calculated from the elemental analysis data and chemically determined also using 2,4,6-trinitrobenzenesulfonic method (Snyder & Sobocinski, 1975).

2.3. Preparation of chitosan or N-succinyl-chitosan matrices

Chitosan and NS-chitosan were dispersed in distilled water (0.5%, w/v). 5-ASA or 5-ASA and CDs (4:1 molar ratio) were added to the dispersion, which was homogenized using an ultraturrax, then frozen at −20 °C and freeze-dried for 24 h at −70 °C and 60 mmHg, using a Freeze-Dryer Criotecnica, (MMCOTA, Rome, Italy).

2.4. Determination of 5-aminosalycilic acid content in matrices

The amount of 5-ASA loaded into the freeze-dried matrices was determined by a dissolution method. Briefly 10 mg of dried powder was dissolved in a hydroalcoholic solution (methanol-pH 1.2 buffer, 1:1, v/v for chitosan matrices; methanol-pH 7.4 buffer, 1:1, v/v for NS-chitosan matrices). The mixture was vigorously shaken for 2 h in order to dissolve the matrix into the solution. After centrifuging, the supernatant was withdrawn and the 5-ASA content was analyzed by HPLC (see below). The drug loading capacity was expressed as the ratio of actual-theoretical 5-ASA content.

2.5. Quantitative determination of 5-aminosalycilic acid

5-ASA content was quantified at 300 nm by HPLC using a chromatograph Alliance 2695 (Waters, Italy) equipped with a photodiode array detector 996 and a computer integrating apparatus (Empower 2). The column was an X bridge-Waters C18 column (60 Å, 5 μm, 4.8 mm × 150 mm, Waters). The mobile phase was a mixture of acetonitrile, water, and acetic acid (72:20:8, v/v/v), which was filtered through a 0.45 μm membrane filter before use, and was delivered at a flow rate of 1.0 ml/min. The injected sample volume was 10 μl. Sample preparation and analyses were performed at room temperature. A standard calibration curve (peak area of 5-ASA versus known drug concentration) was built up by using working, standard solutions (0.5–0.005 mg/ml). Calibration graphs were plotted according to the linear regression analysis, which gave a correlation coefficient value (R^2) of 0.9996. The 5-ASA retention time (t_r) was 3.0 min and the minimum detectable amount was 25 μg/ml.

2.6. Characterization of 5-aminosalycilic acid-loaded matrices

FTIR measurements were carried out at room temperature using a Bruker Equinox 55 instrument. About 2 mg of the samples were ground thoroughly with KBr and pellets were formed under a hydraulic pressure of 600 kg/cm^2. Spectra of 5-ASA, chitosan, NS-chitosan, 5-ASA loaded polymer matrices were recorded.

DSC studies were performed using a DSC Mettler Toledo model 821e. The samples (2–5 mg) were scanned in sealed aluminium pans under nitrogen atmosphere. DSC thermograms were scanned in the first heating run at a constant rate of 10 °C/min and a temperature range of 0–325 °C. DSC thermograms of pure substances and drug loaded polymer matrices were recorded.

The surface morphology of the polymer matrices was examined using a scanning electron microscope (SEM), Hitachi S4100 (Madrid, Spain). Powder samples were dispersed on an aluminium stub with a self-adhered carbon film. The samples were made electrically conductive by coating with gold/palladium under vacuum. SEM images were taken at an excitation voltage of 20 kV.

2.7. In vitro swelling studies

The study was performed in a membrane dialysis bag that contained 100 mg of each systems; the membrane bag was placed in a closed flat bottom tube with 40 ml of a buffer solution that was maintained at 37 °C for 24 h. The test was carried out in different buffered aqueous media at: pH 1.2, pH 7.4, and in a pH gradient medium by placing the matrices for 2 h at pH 1.2 and then replacing the acid medium with the alkaline buffer (pH 7.4) to simulate the gastro-intestinal transit. At specific time intervals, samples were removed, blotted with a piece of paper for 5 s to absorb excess water on surface and then weighted.

The swelling ratio (S_w) was calculated using the following equation:

$$S_w = \frac{W_t - W_{t0}}{W_{t0}} \quad (1)$$

where W_t represents the weight of the system at a certain time and W_{t0} represents the original dry weight.

2.8. In vitro release studies

Each matrix was placed in a dialysis bag and in a closed flat bottom tube, 40 ml of pH 1.2 or pH 7.4 solutions were loaded. The release study was carried out for 24 h under magnetic stirring in a thermostatic bath at 37 °C. In order to simulate the passage through the stomach and the intestine, tests were performed also using a pH gradient medium as seen for the swelling studies. During the experiments, at regular time intervals, 20 ml of the medium were withdrawn and replaced with the same amount of fresh solution to ensure sink conditions. The withdrawn samples were analyzed for 5-ASA content by HPLC as described above (see Section 3.6). Cumulative drug release was expressed as a percentage of the actual loaded 5-ASA content.

2.9. Mathematical modelling of release kinetics

In order to describe the drug release mechanism, the in vitro drug release mean data (cumulative drug release up to 60%) were fitted to the power law equation (Eq. (2))

$$\frac{M_t}{M_\infty} = K \times t^n \quad (2)$$

where M_t and M_∞ are the absolute amount of drug released at t and infinite time, respectively; K is a constant reflecting structural and geometric characteristic of the device, and n is the release exponent characterizing the diffusion mechanism. According to the criteria for release kinetics from swellable systems, release exponent values $n = 0.45$, $0.45 < n < 0.89$ and 0.89 indicate, respectively, Fickian (Case I) diffusion, non-Fickian (anomalous) transport, and diffusion and zero-order (Case II) transport (Ritger & Peppas, 1987; Zhang et al., 2009).

2.10. Preparation of GI tissues and mucoadhesive test

Male Wistar rats (13-weeks old) had been fasted for 24 h. The fasted conditions were set to minimize the contents in the gastro-intestinal tract, which disturbed the washing process for the following use. The intestine tissues (i.e., duodenum, jejunum, ileum and colon) were excised from sacrificed rats. Each tissue section was slowly washed with a large amount of normal saline solution (0.9% NaCl, w/v) and immediately used for this study. Swelling of samples was simulated putting the matrices in a flat bottom tube with buffer solution (pH 7.4) and thermostated at 37 °C. At scheduled time intervals buffer solution was withdrawn and mucoadhesion studies were performed using the different intestine tissue sections. The study was carried out using a universal tensile tester (Lloyd Instruments, LR 50K model, UK). The stainless steel plate (L-shape) was fitted by one of its side into the upper and lower jaws of the instrument so as the other surfaces of the plates were facing each other. The rat intestine tissue was stuck at the upper plate surface with the glue, while matrix was placed on the lower plate. PBS, pH 7.4, was used as a medium and 20 μl were spread on the contact surface between matrix and tissue. Then the upper jaw with tissue stuck on the plate was lowered slowly so that it just touched the matrix surface. No external force was applied. The matrix was kept in contact with the tissue for 5 min and then the upper jaw was slowly moved upward at the speed of 10 mm/min.

All the experiments were done in triplicate. The maximum detachment force (F_{MAX}), i.e., the force required for separating the system from the tissue surface was obtained directly from NimaST518.vi software (Nima Technology Ltd., Coventry, England) and the total amount of forces involved in the probe withdrawal from the tissue (work of adhesion, W_{ad}) was then calculated from the area under the force versus distance curve. These parameters were used to compare the different prepared matrices.

2.11. Statistical analysis of data

Data analysis was carried out with the software package R, version 2.10.1. Results are expressed as the mean ± standard deviation. Multiple comparisons of means (Tukey test) were used to substantiate statistical differences between groups, while Student's t-test was applied for comparison between two samples. Significance was tested at the 0.05 level of probability (p).

3. Results and discussion

3.1. Preparation of N-succinyl chitosan

Following methods reported in the literature, *N*-succinyl chitosan was successfully synthesized as confirmed by spectroscopic and analytical methods (see Section 2). In particular, FTIR analysis gave evidence of amide linkage formation and presence of carboxyl groups (Aiping, Tian, Lanhua, & Hao, 2006; Dai, Li, Zhang, Wang, & Wie, 2008). The degree of succinylation, defined as the molar ratio between the *N*-succinyl groups and the repeating units of glucosamine, was 0.61.

3.2. Preparation of chitosan and N-succinyl-chitosan matrices

The rational of this work was to exploit the promising properties of NS-chitosan to formulate freeze-dried matrices as 5-ASA targeting systems to colon as well as to evaluate its possible combination with β-cyclodextrin. As a comparison, corresponding chitosan formulations were also prepared and characterized.

Freeze-drying was used to prepare chitosan or NS-chitosan matrices including either 5-ASA or 5-ASA and cyclodextrin. This procedure gave rise to highly fluffy cotton-like powders. The obtained drug loading capacity was high for all the samples with a mean 5-ASA content ranging from ≅89% (matrix **1**: 5-ASA/chitosan) to ≅95% (matrix **4**: 5-ASA/cyclodextrin/NS-chitosan). No statistical differences in drug entrapment efficiency could be found between matrices with or without cyclodextrin.

3.3. Characterization of 5-aminosalycilic acid loaded matrices

Drug loaded matrices were characterized by different methods. FTIR spectra of the 5-ASA loaded matrices showed characteristic peaks of both drug and polymers, thus indicating that 5-ASA was filled in the polymeric networks. Moreover, little peak shifts and reduced intensity of bands confirmed the drug loading into the polymer matrices (data not shown). However, more information regarding drug/polymer interaction was obtained from DSC. In the calorimetric study, thermal curve of the pure drug exhibited an endothermic peak at 277 °C and an enthalpy of 24 mW mg^{-1}, corresponding to its melting point (Fig. 1). The chitosan and NS-chitosan thermograms showed typical polysaccharide behaviour with two degradation steps: a wide endotherm around 100 °C and an exotherm at 315 °C that is less evident in NS-chitosan. The first peak in the polymer thermograms corresponds to water evaporation, while the second one refers

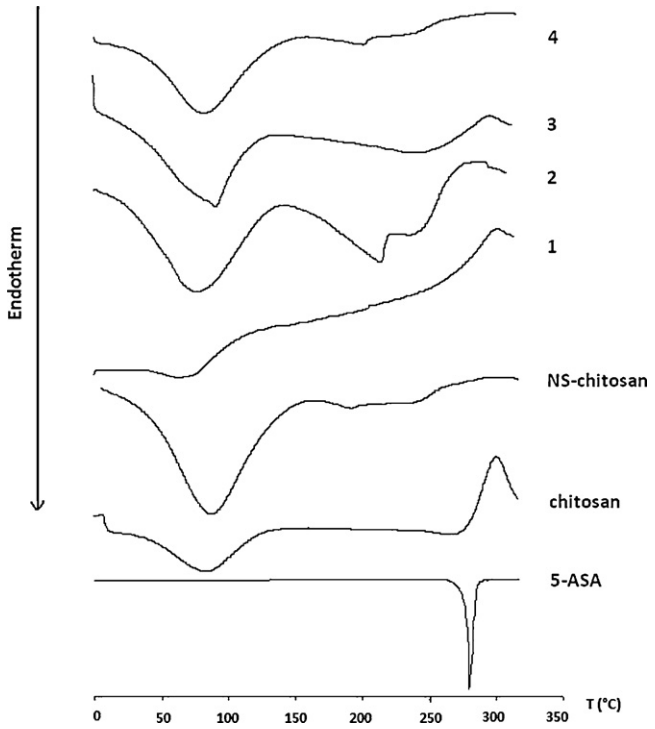

Fig. 1. DSC curves of pure samples (5-ASA, chitosan, NS-chitosan), and 5-ASA-loaded matrices (**1** = 5-ASA/chitosan; **2** = 5-ASA/NS-chitosan), and 5-ASA and cyclodextrin-loaded formulations (**3** = 5-ASA/cyclodextrin/chitosan; **4** = 5-ASA/cyclodextrin/NS-chitosan).

to polymer degradation. In thermograms of 5-ASA containing matrices, with and without cyclodextrin, 5-ASA fusion peak disappeared, thus, indicating solid-state interactions between the drug and both polymers. In addition, the thermogram of matrix **1** (5-ASA/NS-chitosan) showed exotherm–endotherm peaks around 210 °C, which denotes an interaction between anionic succinyl groups of the polymer and cationic amino groups of 5-ASA. These signals were not present in the thermograms of the pure NS-chitosan as well as in that of matrix **4** (5-ASA/cyclodextrin/NS-chitosan), where an inclusion complex between 5-ASA and cyclodextrin could have formed during the freeze-drying procedure.

SEM analyses showed no differences in morphology between matrices prepared with and without cyclodextrin and all the prepared polymeric systems showed a three-dimensional structure.

3.4. In vitro swelling studies

Swelling studies were carried out in conditions simulating gastric (buffer, pH 1.2), intestinal (buffer, pH 7.4), and gastro-intestinal (gradient pH) environments (Fig. 2a–c). For all formulations **1**–**4**, swelling was in accordance with the polymer properties. In fact, chitosan matrices swelled highly in acid environment while NS-chitosan matrices did in alkaline media.

Cyclodextrin had a different influence on the matrix swelling. Indeed S_w was reduced when the system was able to interact with the aqueous medium (i.e., at pH 1.2 for chitosan **2** and at pH 7.4 for NS-chitosan **4**) as a consequence of preferential water uptake by the hydrophilic but no-swellable oligosaccharides. On the contrary, no differences on S_w were found when the systems behaved as "inert" matrices (i.e., at pH 7.4 for chitosan **2** and at pH 1.2 for NS-chitosan **4**).

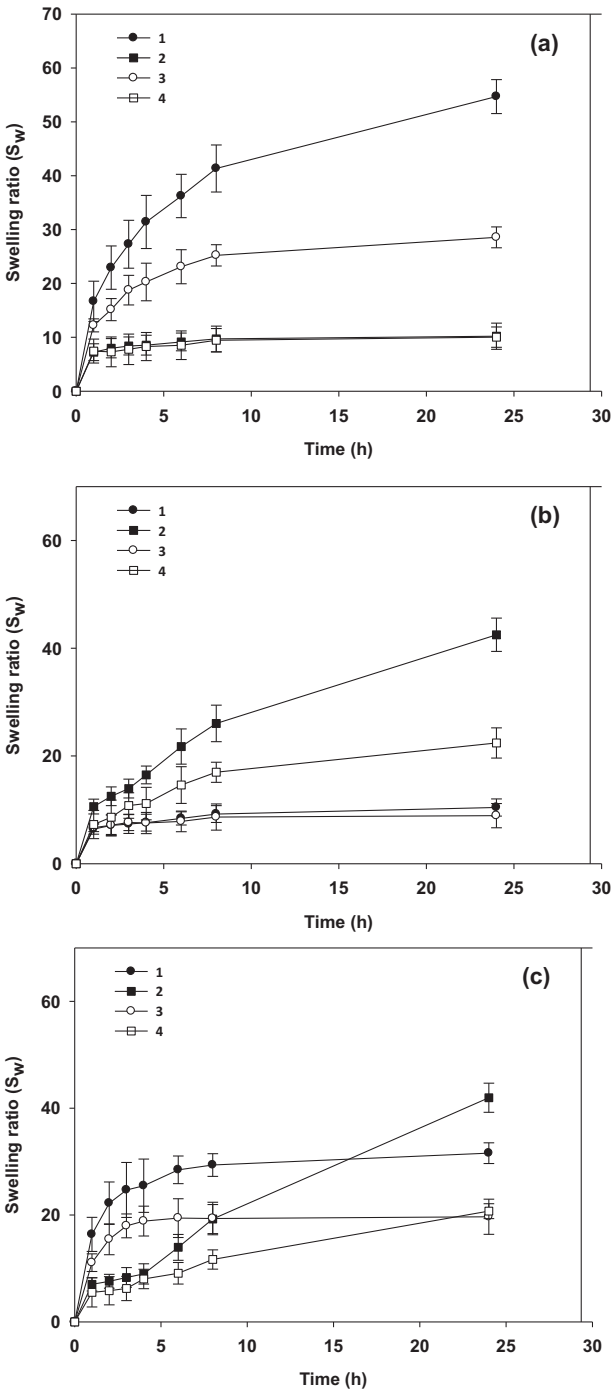

Fig. 2. Swelling ratio (S_w) of 5-ASA freeze-dried systems at: (a) pH 1.2, (b) pH 7.4, and (c) pH gradient medium (2 h at pH 1.2 and up to 24 h at pH 7.4). Error bars represent standard deviation, $n = 3$ (legend: **1** = 5-ASA/chitosan; **2** = 5-ASA/NS-chitosan; **3** = 5-ASA/cyclodextrin/chitosan; **4** = 5-ASA/cyclodextrin/NS-chitosan).

3.5. In vitro release studies

In vitro drug release was studied in the same experimental conditions tested for swelling experiments. As shown in Fig. 3, 5-ASA release was in accordance with swelling kinetics of matrices **1** and **3**. As soon as the matrices swelled, the drug was solubilised and released from the systems while 5-ASA release was strongly reduced from the unswollen matrices. Therefore, chitosan matrix **1** released 5-ASA quite fast and up to 82% of the loaded drug in acid medium while from NS-chitosan **3** the release was prompt

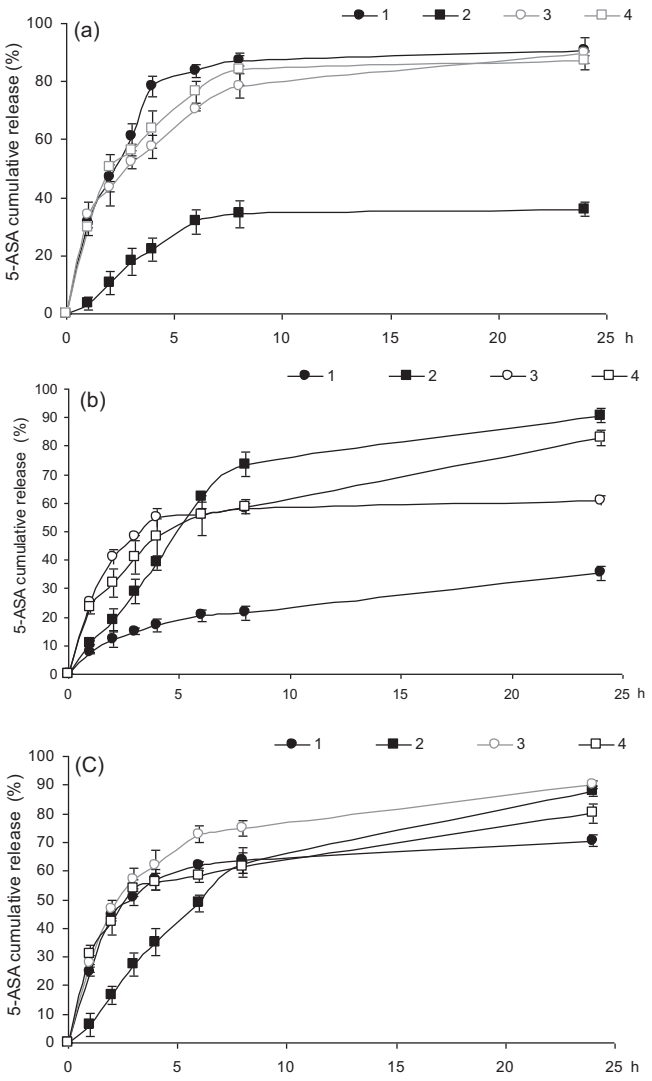

Fig. 3. 5-ASA cumulative release (%) from chitosan or *N*-succinyl-chitosan freeze-dried systems, with and without cyclodextrin, at: (a) pH 1.2; (b) pH 7.4 and (c) pH gradient medium (2 h at pH 1.2 and up to 24 h at pH 7.4). Error bars represent standard deviation, n =3 (legend: **1** = 5-ASA/chitosan; **2** = 5-ASA/NS-chitosan; **3** = 5-ASA/cyclodextrin/chitosan; **4** = 5-ASA/cyclodextrin/NS-chitosan).

and high (>90%of the loaded drug) in alkaline buffer. When the pH gradient medium was used, drug release was quite fast in the first 6 h from all the matrices, except from NS-chitosan system **3** (Fig. 3c) that showed a slow and constant drug release in the first 8 h to reach the highest cumulative 5-ASA release (≅92%) at the end of the experiments.

When cyclodextrin-containing matrices were tested, a faster drug release was obtained from the unswollen matrices, while it was slightly reduced from the swollen chitosan (pH 1.2) and NS-chitosan (pH 7.4). Therefore, unswollen matrices behaved as inert matrices that, according to literature, increase drug release rate most probably because of the cyclodextrin ability to form an inclusion complex with 5-ASA with a consequent enhanced drug solubility that improves drug delivery (Sangalli et al., 2001; Zerrouk et al., 1998). When cyclodextrin is dispersed in the swollen matrices, on the contrary, the complexing capability of the cyclodextrin is less effective in the release rate promotion probably because of a limited diffusivity of the formed drug/cyclodextrin inclusion complex through the swollen polymer (Sangalli et al., 2001).

At the end of the experiments in the pH gradient medium, the highest drug release was obtained from the NS-chitosan (matrix **3**) and the cyclodextrin-containing chitosan matrices (matrices **2** and **4**). However, NS-chitosan alone was capable of better controlling drug release that in acidic medium was lower (≅15%) than from the other formulations, all of which released more than 49% of 5-ASA (Fig. 3c). Therefore, results from the release experiments showed that NS-chitosan is a potential candidate for targeting 5-ASA to colon.

Table 1
Comparison of estimate parameters from curve fitting of drug release in mixed pH media to power law expression. Legend: **1** = 5-ASA/chitosan; **2** = 5-ASA/NS-chitosan; **3** = 5-ASA/cyclodextrin/chitosan; **4** = 5-ASA/cyclodextrin/NS-chitosan.

Systems	$n \pm$ SE	K (h^{-1}) ± SE	R^2
1	0.72 ± 0.05	0.38 ± 0.11	0.9954
2	0.91 ± 0.03	0.12 ± 0.01	0.9920
3	0.69 ± 0.05	0.30 ± 0.15	0.9948
4	0.70 ± 0.04	0.35 ± 0.09	0.9958

3.6. Mathematical modelling of release kinetics

To deduce the mechanism of drug release from the matrices, data were fitted to the general power law equation (2), usually used to describe drug release from swellable matrices (Ritger & Peppas, 1987). Results related to experiments in the pH gradient medium are shown in Table 1. As can be seen from the obtained correlations coefficient values ($R \geq 0.99$), the release data fit well to the empirical equation. The n exponent ranged from 0.69 to 0.91 (Table 1), thus indicating a non-Fickian (anomalous) transport ($0.45 < n < 0.89$) for all the tested samples (Ritger & Peppas, 1987; Zhang et al., 2009).

Mechanism of drug release from hydrophilic and erodible matrices is rather complex as a consequence of the several physical processes involved, especially when cyclodextrin is present together with a poor soluble drug. In fact, the involved processes regard: penetration of water into the matrix with consequent swelling and solubilisation/erosion of the matrix, dissolution of both the drug and cyclodextrin in the swollen matrix, cyclodextrin/drug complex formation, and counterdiffusion of drug, cyclodextrin, and complex in the swollen layer (Cappello et al., 2006). Therefore, all these processes are responsible of an anomalous non-Fickian diffusion mechanism.

Table 2
Ex vivo mucoadhesive performance of 5-ASA freeze-dried systems. Effect of gastro-intestinal mucosa on maximum detachment force (F_{MAX}) and work of adhesion (W_{ad}). Legend: **1** = 5-ASA/chitosan; **2** = 5-ASA/NS-chitosan; **3** = 5-ASA/cyclodextrin/chitosan; **4** = 5-ASA/cyclodextrin/NS-chitosan.

Systems		Duodenum	Jejunum	Ileum	Colon
1	F_{MAX} (mN)	2.2 ± 0.5	3.3 ± 0.3	3.3 ± 0.4	3.8 ± 0.5
	W_{ad} (mN mm)	0.9 ± 0.2	1.3 ± 0.2	1.3 ± 0.1	1.5 ± 0.2
2	F_{MAX} (mN)	2.7 ± 0.3	2.4 ± 0.2	2.7 ± 0.3	4.0 ± 0.5
	W_{ad} (mN mm)	1.1 ± 0.3	0.9 ± 0.2	1.1 ± 0.2	1.5 ± 0.3
3	F_{MAX} (mN)	2.0 ± 0.4	3.2 ± 0.4	3.5 ± 0.5	3.6 ± 0.5
	W_{ad} (mN mm)	0.8 ± 0.2	1.1 ± 0.2	1.4 ± 0.3	1.5 ± 0.2
4	F_{MAX} (mN)	2.9 ± 0.4	2.3 ± 0.3	2.8 ± 0.4	3.9 ± 0.5
	W_{ad} (mN mm)	1.1 ± 0.2	0.9 ± 0.1	1.1 ± 0.3	1.5 ± 0.4

3.7. Mucoadhesion study

Polymer ability to swell is known to be a prerequisite for mucoadhesion, since it concerns wetting uncoiling and spreading of the polymer over the mucus (Thirawong, Nunthanid, Puttipipatkhachorn, & Sriamornsak, 2007). In this study *ex vivo* mucoadhesive properties of the studied systems were evaluated using different part of rat intestine. The maximum detachment force (F_{MAX}), and work of adhesion (W_{ad}) on different GI mucosa are shown in Table 2. As can be seen, all the tested systems showed good mucoadhesion for the colon: W_{ad} mean value was higher for NS-chitosan matrix 3 but no statistical differences could be found among the matrices both with and without cyclodextrin. However, results of Table 2 show that the chitosan systems were as mucoadhesive as NS-chitosan matrices in the last portion of the gastro-intestinal tract. On the other hand, these results confirm the highest potential of the NS-chitosan matrix as delivery system for targeting 5-ASA to colon.

4. Conclusion

Overall obtained results showed that NS-chitosan matrix might be a good candidate for colon specific delivery of 5-ASA. Indeed, NS-chitosan matrix was able to better control drug release, which was quite low in acidic medium and almost complete in alkaline environment, in comparison with chitosan and cyclodextrin-containing polymeric matrices. Cyclodextrin showed a dual effect on drug release that was always reduced from the swollen polymeric matrices. On the contrary, cyclodextrin improved drug release from the unswollen polymers. Further studies are in progress to evaluate new delivery systems for 5-ASA by using NS-chitosan.

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