Stability tests of 5-Aminosalicylic acid containing solutions and chitosan-Ca-alginate microparticles prepared by spray-drying

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Introduction

5-ASA is widely used for its local effects in the treatment of inflammatory bowel diseases (Loftus, 2004). Because of the 4-aminophenol structure and antioxidative properties it is considered as a light, temperature and oxygen sensitive drug and its major degradation products are well established (Jensen, 1992). As a zwitter ion, dominantly anionic, 5-ASA has low aqueous solubility and it is poorly absorbed and inactivated before reaching the lower intestine. The optimum oral 5-ASA delivery system requires technologies that protect the drug during microencapsulation and during the distribution in the stomach and small intestine. For these requirements, new chitosan-alginate microparticluated colon drug delivery system was prepared and characterized. Stability of 5-ASA in solutions at various pH intended for spray-drying to obtain 5-ASA loaded chitosan-Ca-alginate microparticles and in microparticles was studied.

Materials and methods

Chemicals

Sodium alginate (LF 10/60, fG 65-75%, viscosity, 20-70 *mPas* for a 1% *w/v* solution), Protanal FMC BioPolymers, Norway; chitosan (150 KDa, $[R^2_G]^{1/2}$ 44$\pm$5 nm, viscosity 20-100 *mPas* for a 1% *w/w* solution in acetic acid, 1% *w/w*, minimum 85% deacetylated), France Chitine, France; 5-ASA, Fluka Chemie AG, Switzerland. All other reagents were of analytical grade.

Stability tests of 5-ASA in solutions and in chitosan -Ca- alginate microparticles

The stability tests of 5-ASA were performed on 5-ASA solutions at various pHs: 3.0 (0.5% *w/w* in solution of 1% *w/w* acetic acid), 5.0 (aqueous solution of 5-ASA, 0.5% *w/w*), 7.0 (aqueous solution of 5-ASA 0.5% *w/w* with adjusted pH by 0.2 M NaOH) and 7.4 (solution of 5-ASA in PBS, 0.5% *w/w*). The solvents and pH range were chosen to simulate the preparation conditions. ^{1}HNMR spectra in H2O/D2O (90/10, Bruker Advance DRX 500, Germany) and UV/VIS spectra (UV/VIS spectrophotometer, Perkin Elmer Lambda 16, USA) of 5-ASA were recorded in different time intervals during one week. All the samples tested were light-protected and nitrogen was used in solutions to prevent the oxidative self-coupling of 5-ASA moieties. In order to evaluate the stability of 5-ASA in microparticles, ^{1}HNMR and UV/VIS spectra of 5-ASA were recorded after release from alginate and alginate-Ca-chitosan microparticles in PBS (pH 7.0-7.4), respectively. CO_2 in drug release medium was added in order to maintain an anaerobic environment.

Preparation of chitosan -Ca- alginate microparticles

Aqueous dispersion of alginate (3% *w/w*) and 5-ASA (0.5% *w/w*) adjusted to pH 7.0 by 0.2 M NaOH was infused into a spray dryer nozzle unit (Buchi Mini Spray Dryer B-191, Switzerland) and

sprayed into solution of chitosan (0.25% *w/w*) and $CaCl_2$ (2.5% w/v) in 1% *w/w* acetic acid under defined conditions (nozzle diameter 0.7 mm, aspirator pressure 100, atomizer pressure 600 Nih^{-1}, flow rate 10 ml, inlet temperature 140^0C, outlet temperature 100^0C). The microparticles were cured for 24 h, separated and freeze-dried.

Characterization of chitosan -Ca- alginate microparticles

The amount of drug entrapped was calculated from the difference between the total amount of drug added and non-loaded (299 nm, (UV/VIS spectrometer, Perkin Elmer Lambda 16, USA). The particle size was determined after suspending the particles in conducted liquid Isoton containing Tween 80, 1% w/w (Coulter Multisizer, Coultronic, France), while the suspension of particles in PBS (0.0001M, pH 6.8) was used as a medium to determine the zeta potential of the particles (Malvern Zeta Sizer 2000, UK). Surface morphology was investigated with a SEM (JEOL JSM-6301F, Japan), while the localization of both the polymers in the particles was determined using CLSM (Olympus, Fluoview FU 300, France) after fluorescent labelling of chitosan and alginate with FITC (green) and RBITC (red), respectively. The interaction between the polymers and 5-ASA into particles was also evaluated. X-ray diffraction pattern (at 200C, Bragg-Brentano geometry, Cu-Kα1, λ=1.5406 Å, slit width 10, Philips PW1830, Holland), FTIR spectra (KBr pellets, 4000-400 cm^{-1}, Bruker Vector 22) and DSC curves (10^0C/min, 0-325^0C, Mettler Toledo 30TC 15, Switzerland) for 5-ASA alone, in physical mixtures and in loaded particles were recorded.

Results and discussion

The formulation process resulted in negatively charged particles (-30.7$\pm$1.6 *mV*) with particle size of 6.2 μm, drug loading 72% and calcium content 3.6$\pm$0.2%. The yield was approximately 60%. Acceptable spherical morphology was observed, but also flattened, disk-shaped particles. The surface appears smooth with low porosity. By imaging with CLSM, the chitosan was localized dominantly in the particle wall, with a low quantity homogeneously distributed throughout the particle matrix. Homogeneous distribution of the alginate throughout the particle was observed with a heterogeneous deposition at the alginate/chitosan interface.

Formulation of the primary dispersion of alginate and 5-ASA for spray-drying in respect to pH is of crucial importance considering not only the affinity of the drug for the polymer, but also the 5-ASA solubility and stability in different pH solutions. Literature data (Zerrouk et al., 1998) indicate that 5-ASA solubility increase with increasing pH (alginate's one, as well), but it can be rapidly degraded especially in an alkaline medium. In this respect, in the actual stability studies, the formulation, preparation and 5-ASA release conditions were considered. In the UV absorption spectra of 5-ASA alone and with alginate in PBS (λ_{max} 328 nm, pH 7.0) and in HPLC water with pH 7.0 (adjusted with 0.2M NaOH) no additional peaks were observed during 3 days when solutions maintained at room temperature. However, from the third to the seventh day, although the samples were protected from the light, it was found that exposure to the neutral pH medium resulted in a change in a colour of the solution, which was considered as a qualitative indication for the degree of 5-ASA degradation. Furthermore, an additional peak in the spectrum at 420 nm was observed, which can be explained by formation of tetrameric species of 5-ASA (Jensen, 1992). In addition, the stability tests of 5-ASA in aqueous mediums with pH between 7.4-8.2 showed relatively fast and intensive coloration, making them unsuitable for microparticle preparation despite higher 5-ASA solubility. Considering that, the compromise between 5-ASA stability and solubility pointed to the optimal medium for spray-drying including aqueous solution with pH 7.0. The intensive coloration obtained under these conditions underlined the necessity of considering all the precaution measures in order to prevent 5-ASA degradation during preparation of 5-ASA-alginate solution for spray-drying. Besides light protection, they included freshly prepared solutions immediately before spray-drying and use of nitrogen as well.

When working in such conditions, no additional degradation products were detected, which was confirmed by ^{1}HNMR spectra of 5-ASA, also. No differences in the ^{1}HNMR spectra of 5-ASA in mediums with different pH values were observed during 4 days. The shift of proton signal (δ ppm) in the different pH solution originate from the ionisation processes of 5-ASA, only (Table 1, Fig. 1).

Table 1. ^{1}H-NMR spectra of 5-ASA in aqueous mediums with different pH values

Assignment	Proton signal δ ppm *(J, Hz)*			
5-ASA	pH 3.0[a]	pH 5.0[b]	pH 7.0[c]	pH 7.4[d]
H-6 H-2’	7.88 *(3999.58)*	7.55 *(3772.43)*	7.20 *(3597.57)*	7.19 *(3592.85)*
H-4 H-6’	7.36 *(3645.43)*	7.16 *(3587.46)*	6.89 *(3443.74)*	6.88 *(3440.81)*
H-3 H-5’	6.99 *(3.6031)*	6.85 *(3425.57)*	6.72 *(3359.68)*	6.71 *(3357.19)*

[a]*- solution 5-ASA in 1% (w/w) acetic acid (0.5 % w/w)*
[b]*- aqueous solution of 5-ASA (0.5 %, w/w)*
[c]*- aqueous solution of 5-ASA (0.5 % w/w) pH 7.0 (adjusted with 0.2M NaOH)*
[d]*- solution of 5-ASA in PBS (0.5 % w/w)*

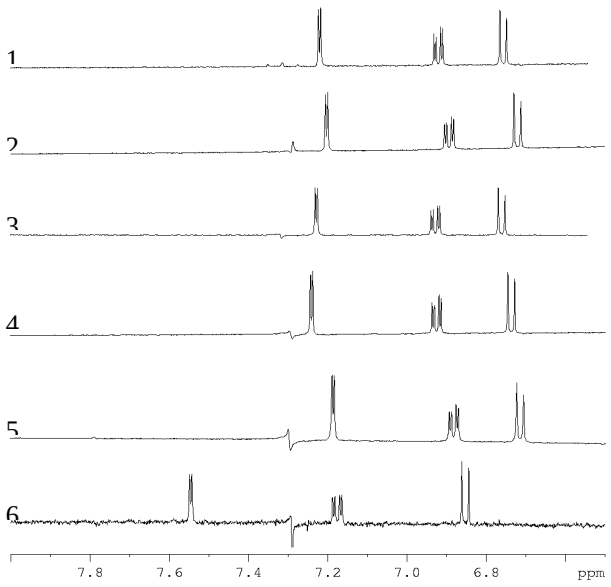

Fig. 1. ^{1}H-NMR spectra of 5-ASA in different pH mediums; 1: aqueous solution of 5-ASA (pH 7.0 adjusted with 0.2M NaOH); 2: aqueous solution of 5-ASA (adjusted pH 7.0) 4 days of preparation; 3: aqueous solution of 5-ASA and alginate (1 : 3 w/w), adjusted pH 7.0; 4: 5-ASA released from alginate and chitosan-Ca-alginate microparticles in PBS pH 7.0; 5: 5-ASA released from alginate and chitosan-Ca-alginate microparticles in PBS pH 7.4; 6: aqueous solution of 5-ASA (pH 5.0).

In order to evaluate the 5-ASA stability during spray-drying (under defined pressure and temperature) and cross-linking procedure, as well as polyelectrolyte complexation of oppositely charged polymers, ^{1}HNMR spectra of 5-ASA were registered after dissolution of 5-ASA loaded alginate microparticles in water and PBS (pH 7.0-7.4) and after 5-ASA release from chitosan-Ca-alginate microparticles in these mediums. This includes also the influence of freeze-drying process on 5-ASA stability. ^{1}HNMR spectra of 5-ASA in all samples corresponded to the ^{1}HNMR spectra of 5-ASA in the mediums with adequate pH (Fig. 1).

Considering X-ray diffraction studies, pure 5-ASA had peaks at 15.2 and 16.65^0. It remained in the mixture with other compounds. Both peaks disappeared in the particles, suggesting that the formation of 5-ASA crystals was hindered by spray-drying in the presence of alginate (Fig. 2). The same was confirmed by DSC studies. The thermograms of 5-ASA loaded Ca-alginate microparticles and 5-ASA loaded chitosan-Ca-alginate microparticles respectively are almost identical to those of empty particles, indicating molecularly dispersed drug within the particles during the spray-drying (Fig. 3). In the FTIR spectra of 5-ASA loaded Ca-alginate microparticles, the characteristic peaks of 5-ASA were not altered. However, the characteristic peaks of 5-ASA could not be observed in chitosan-Ca-alginate microparticles. Considering ^{1}HNMR, X-ray and DSC studies, which confirm 5-ASA stability and molecularly dispersed drug within the polymers, one can conclude that 5-ASA entrapment in polymer matrix increases vibration barrier for 5-ASA intramolecular bonds, so the characteristic peaks can not be identified.

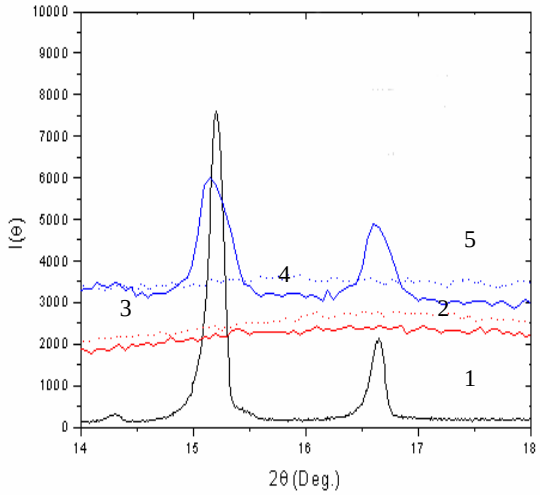

Fig. 2. X-ray diffractograms of 5-ASA (1), blank (2) and loaded chitosan-Ca-alginate microparticles (3), physical mixture of chitosan-alginate with (4) and without 5-ASA (5).

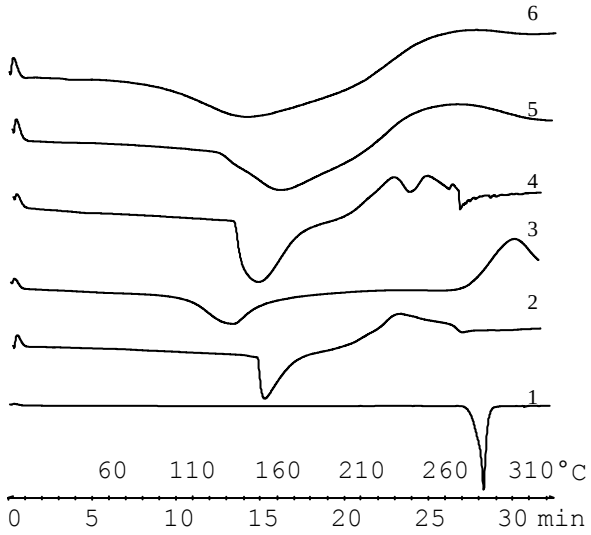

Fig. 3. DSC analyses of 5-ASA (1), alginate (2), chitosan (3), physical mixture of 5-ASA-alginate-chitosan (4), chitosan-Ca-alginate microparticles (5), 5-ASA loaded chitosan-Ca-alginate microparticles (6).

Conclusion

New microparticulated 5-ASA loaded chitosan-Ca-alginate was prepared and characterized. The formulation and the technology applied ensure 5-ASA stability during microencapsulation when working in adequate conditions, such as light protection, freshly prepared solution and use of nitrogen to prevent self-coupling of 5-ASA moieties. In the FTIR spectra, the characteristic peaks of 5-ASA were not altered indicating no covalent interaction between the drug and the polymers. X-ray and DSC studies indicate molecularly dispersed drug within the particles.

References

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