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Influence of different chitosan salts on the release of sodium diclofenac in colon-specific delivery

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Abstract

Chitosan (CH) was dissolved in aqueous solutions containing aspartic, glutamic, hydrochloric, lactic and citric acids to obtain different chitosan salts. Chitosan salts were collected from the solutions by spray-drying and the powders obtained were mixed with Sodium Diclofenac (SD), taken as a model anti-inflammatory drug. This study evaluated in vitro the influence of acid type on the release behaviour of SD from the physical mixture during gastrointestinal transit. The physical mixture of the chitosan salts with SD provided slower drug release than the pure drug both in acidic and alkaline pHs. In addition, the interaction with β-glucosidase at pH 7.0 enhanced the release rate. Among the CH salts used, glutamic and aspartic salts provided the best control of release. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Chitosan salts; Colon-drug delivery; Spray-drying; Diclofenac sodium

1. Introduction

Colon-specific drug delivery by oral route avoids the inactivation of peptide drugs in the gastrointestinal tract and side effects due to gastric irritation after anti-inflammatory administration, but allows local treatment of bowel diseases (Mrsny, 1992; Tozer et al., 1995; Yeh et al., 1995; Hosny and Gouda, 1998; Marvola et al., 1999; Orienti and Zecchi, 2000). Strategies for specific drug delivery to the various regions of the gastrointestinal tract and, in particular, to the colon, have been reviewed by Rubinstein (Rubinstein, 1995). At present, one of the most realistic possibilities of obtaining colon-specific release, uses the ecosystem of the specific microflora in the large intestine (Gorbach, 1971; Moore and Holdeman, 1975; Simon and Gorbach, 1986; Lorenzo-Lamosa et al., 1998). There are two main classes of bacterial enzymes, azoreductases and polysaccharidases, which are in a sufficient quantity to be exploited in colonic drug targeting. Based on this idea, different natural and synthetic polymers have been evaluated for their susceptibility to cleavage by these bacterial enzymes (Friend, 1991) and, thus, their use as major constituents of colon-specific drug delivery systems (Brondsted and Kopecek, 1992). Promising alternative polymers are natural polysaccharides whose glycosidic

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bonds are hydrolysed in the colon. These polysaccharides (Rubinstein et al., 1992; Ashford et al., 1994; Leopold, 1999) include chitosan (Tozaki et al., 1997), pectin (Werch and Ivy, 1941; Salyers et al., 1977), guar–gum (Baylis and Houston, 1986; Tomolin et al., 1989; Macfarlane et al., 1990), dextrans (Larsen et al., 1989, 1991; Harboe et al., 1989a,b), amylose (Englyst and MacFarlane, 1986; Ring et al., 1988) and chondroitin sulphate (Salyers, 1979; Salyers and Brien, 1980) Chitosan (Paul and Sharma, 2000; Ravi Kumar, 2000), in particular, is considered a good candidate as it is biocompatible (Sawayanagi et al., 1983; Hirano et al., 1990; Paul and Sharma, 2000), widely available at low cost and may be hydrolyzed by the β-glucosidase present in the colon. The major drawback to its use in colon-specific release is its insolubility at the alkaline pH of the colon thus hindering enzyme activity. However, chitosan salification may significantly modify its solubility characteristics and different chitosan salts display different physical–chemical properties depending on the nature of the counter ion. This work investigated the suitability of chitosan salified with different acids in colon-specific drug delivery based on the possibility of minimising release during transit in the upper part of the gastrointestinal tract and enhancing polymer hydrolysis by β-glucosidase once in the colon. Sodium Diclofenac (SD) was used as a model anti-inflammatory drug. We expected that this drug would benefit from the proposed systems, first, because SD is particularly well absorbed in the colon (Aiedeh and Taha, 1999) and, second, because its release in the gastric cavity is avoided eliminating local side effects.

2. Materials and methods

2.1. Materials

High-molecular-weight chitosan (MW 60 0000, viscosity 400 mPa s (1% solution in 1% acetic acid) degree of deacetylation 80%) was purchased from Fluka (Buchs, Switzerland). Aspartic acid, glutamic acid, hydrochloric acid, lactic acid, citric acid, β-glucosidase from almonds and diclofenac sodium were all purchased from Fluka–Sigma–Aldrich.

2.2. Preparation of the chitosan salts

0.25 g chitosan (1.55 mmoles glucosamine) were dissolved in 50 ml water containing different acids (aspartic, glutamic, hydrochloric, citric and lactic) in different molar ratios: 1:1 moles monomer:moles acid (1:1 CH monomer/Asp, 1:1 CH monomer/Glu, 1:1 CH monomer/Lac and 1:1 CH monomer/HCl) and 1:2 moles monomer:moles acid (1:2 CH monomer/Asp, 1:2 CH monomer/Glu, 1:2 CH monomer/Lac and 1:2 CH monomer/HCl) at room temperature. The solutions were spray-dried (Büchi Mini Spray Dryed, B-191, Switzerland) with an inlet temperature of 105 °C and the products obtained were collected. In particular, the spray-dried citric acid CH solution could not be characterized because we obtained a single sticky aggregated particle.

2.3. Chitosan salt characterization by Fourier transform infrared spectrometry

Infrared (IR) spectra were recorded with a Jasco FT-IR-410 spectrophotometer. The samples were prepared by processing compressed KBr disks.

2.4. Preparation of the drug–polymer physical mixture

1.55 mmoles of chitosan salts (0.46 g of 1:1 CH monomer/Asp, 0.50 g of 1:1 CH monomer/Glu, 0.39 g of 1:1 CH monomer/Lac, 0.43 g of 1:1 CH monomer/HCl, 0.67 g of 1:2 CH monomer/Asp, 0.75 g of 1:2 CH monomer/Glu, 0.53 g of 1:2 CH monomer/Lac and 0.62 g of 1:2 CH monomer/HCl) and 1.55 mmoles diclofenac sodium (0.49 g) were weighed and mixed in a mortar until homogeneity.

2.5. Water uptake of the drug–polymer mixtures

In order to quantify the swelling of the drug–polymer mixtures in acidic and alkaline environments, disks approximately 20 mg in weight were

prepared by a punch press working at 7 ton/cm^2. The disks were immersed in 10 cm^3 volume pH 2.0 or pH 7.0 aqueous buffers at 37 °C and weighed after 24 and 48 h. The water uptake was determined as the ratio between the weight of the hydrated disks at each time and the initial weight of the dry disks. As a comparison, the water uptake was also determined for the disks prepared by the chitosan salts without the drug.

2.6. DLS studies of the polymer–enzyme interactions in pH 7.0 aqueous buffer

In order to predict the possible hydrolysis of the chitosan salts by the β-glucosidase present in the colon, we analyzed the polymer–enzyme interactions in pH 7.0 aqueous buffer by measuring the size of the chitosan salts in solution and comparison with those obtained in the presence of the enzyme. The measurements were carried out by an instrument equipped with a 50 mW He–Ne laser (532 nm) and thermostated at 37 °C (90 Plus Particle Sizer Analyzer, Brookhaven). The solutions of the chitosan salts in pH 7.0 aqueous buffer were prepared concentrating 1 mg/ml, β-glucosidase was added to the polymer solutions at the concentration of 1 mg/ml. The solutions were filtrated by a 0.22 micron filter (Millex-HV, Millipore) before measurement. Measurements were carried out by fixing the scattering angle at 90°. Results were the combination of 3, 5 min runs for a total correlation function (ACF) accumulation time of 15 min. The diffusion coefficient was evaluated from the time autocorrelation function, g^2 (τ) using the forced single-exponential fit (Eq. (1))(Chu, 1974; Berne and Pecora, 1976):

$$g^2(\tau) = A\,e^{-2\Gamma\tau} + B \quad (1)$$

$$\Gamma = Dq^2 \quad (2)$$

$$q = (4\pi n/\lambda_0)\sin(\theta/2) \quad (3)$$

where τ is the delay time, both A and B are constant, D is the translational diffusion coefficient, q is the scattering vector, n is the refractive index of pure solvent, λ_0 is the wavelength of incident light in vacuo, and θ is the scattering angle. The hydrodynamic radius, R_H, was calculated using Stokes–Einstein equation:

$$R_H = k_B T/6\pi\eta D_0 \quad (4)$$

where k_B, T, and η are the Boltzmann constant, the absolute temperature, and the solvent viscosity, respectively.

2.7. In vitro release studies

To detect the amount of free drug available from the drug–polymer mixtures, the solid mixtures (50 mg) were introduced in a donor cell containing 3 ml of pH 2.0 separated by a dialysis membrane (MW cut off = 10.000) from a receiving compartment containing 10 ml of the same aqueous buffer, which was replaced after time intervals suitable to guarantee sink conditions throughout the runs. In order to simulate gastrointestinal conditions, the donor and receiving compartment pH was maintained at pH 2.0 for 3 h, at 5.5 for 2 h, at pH 7.4 for 4 h and pH 7.0 up to 48 h. The release at pH 7.0 was evaluated also in the presence of β-glucosidase, enzyme present in the colon and able to hydrolyse chitosan. β-glucosidase was added to the pH 7.0 aqueous buffer at the concentration of 1 mg/ml. The system was thermostated at 37 °C and the drug was spectrophotometrically detected in the receiving phase.

3. Results and discussion

3.1. Fourier transform infrared spectrometry

Fig. 1 shows the FT–IR spectra of the samples under study. The FT–IR spectra of CH depict characteristic absorption bands at 3436, 2916 and 2850/cm, which represent the presence of OH group, CH_2 and CH_3 groups (aliphatic groups). The amino group has a characteristic absorption band in the region of 3400–3500/cm, which must have been masked by the absorption band due to OH group (Shanmugasundaram et al., 2001). CH showed the characteristic band of the amino group at 1669/cm. In the spectrum of spray-dried CH–HCl the characteristic absorp-

tion band of CS at about 1669 cm^{-1} ($–NH_2$) disappears giving rise to two new bands located at 1631 and 1522/cm. This behaviour reflects the interaction between the amino groups and the HCl. Moreover, the spectrum of spray-dried CH–Lat, CH–Asp, CH–Glu shows a large peak ($–NH_2$) at 1582, 1623, 1631/cm, respectively. The large shift of these vibrations to higher wavenumbers compared with the usual wavenumbers of the amino groups proves the formation of a carboxylate between the $–COO^-$ groups of the acids and the $–NH_3^+$ groups of CH (Lorenzo-Lamosa et al., 1998). Consequently, it seems reasonable to conclude that CH was ionically crosslinked with the acids.

3.2. Water uptake of the drug–polymer mixtures

The disks obtained by the chitosan salts without the drug strongly swelled at acidic pH (2.0) losing their shape, while they did not solubilize or swell at alkaline pH (7.0) according to the basicity of the aminoglucoside polymer. The drug–polymer mixtures had a completely different behaviour: at acidic pH they maintained their integrity and swelled, at alkaline pH swelled slightly. This was attributed to the presence of the ionizable drug with the polymer influencing the solubility characteristics of the mixtures. At acidic pH, the formation of the unsoluble unionized form of the drug provided hydrophobicity to the

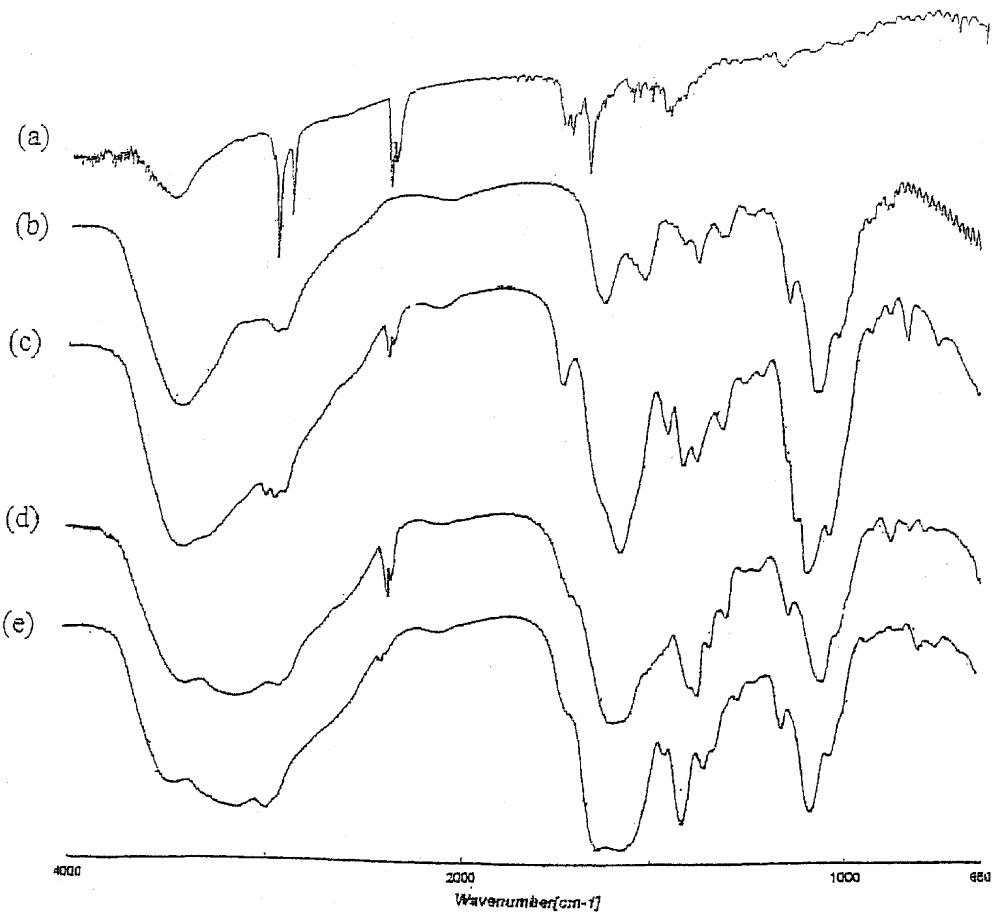

Fig. 1. IR spectra of: (a) CH; (b) CH–HCl; (c) CH–Lat; (d) CH–Asp; (e) CH–Glut.

Table 1
Water uptake (weight of the hydrated drug–polymer disks/weight of the dry disks) after 24 and 48 h in the presence of the aqueous buffers at 37 °C

Chitosan salts	pH 2.0		pH 7.0	
	24 h	48 h	24 h	48 h
1:1 CH monomer/Asp	1.082 ± 0.005	1.351 ± 0.006	1.042 ± 0.003	1.091 ± 0.002
1:2 CH monomer/Asp	1.059 ± 0.004	1.343 ± 0.002	1.059 ± 0.002	1.117 ± 0.006
1:1 CH monomer/Glu	1.053 ± 0.002	1.308 ± 0.002	1.029 ± 0.002	1.084 ± 0.001
1:2 CH monomer/Glu	1.037 ± 0.008	1.298 ± 0.004	1.054 ± 0.006	1.113 ± 0.009
1:1 CH monomer/Lat	1.150 ± 0.009	1.426 ± 0.009	1.030 ± 0.001	1.046 ± 0.001
1:2 CH monomer/Lat	1.199 ± 0.001	1.480 ± 0.007	1.042 ± 0.003	1.058 ± 0.008
1:1 CH monomer/HCl	1.112 ± 0.005	1.375 ± 0.005	1.001 ± 0.005	1.032 ± 0.002
1:2 CH monomer/HCl	1.131 ± 0.002	1.396 ± 0.006	1.012 ± 0.004	1.039 ± 0.005

Data shown are the mean ± standard deviations, $n = 3$.

drug–polymer mixture while at alkaline pH the presence of the diclofenac salts provided hydrophilicity. The water uptake was more evident at acidic than alkaline pH, indicating the prevalence of the polymer with respect to the drug in determining the solubility characteristics of the mixture. At acidic pH the water uptake was high in the presence of hydrochloric and lactic salts and increased on increasing the acid:monomer ratio. In the presence of aspartate and glutamate salts, the water uptake was reduced and decreased on increasing the acid:monomer ratio. At alkaline pH the water uptake was more evident in the presence of aspartate and glutamate salts and in the presence of the higher acid:monomer ratio (Table 1). This indicated that aspartic and glutamic acids provide chitosan salts less hydrophilic than the other salts examined at acidic pH and more hydrophilic at alkaline pH. This may be attributed to the physico-chemical characteristics of these two aminoacids characterized by improved solubility at alkaline pH than acidic pH.

3.3. DLS studies of the enzyme–polymer interactions in alkalyne aqueous solution

The mean size of aspartate and glutamate salts in pH 7.0 aqueous buffer was higher than the other salts examined and the size increased at the higher acid:monomer ratio (Table 2). This was in accordance with the improved hydrophilicity of these two salts in alkaline environment. The presence of β-glucosidase in solution increased the polymer size indicating the establishment of polymer–enzyme interactions (Fig. 2). The polymer–enzyme size increased over time indicating that the enzyme catalyzed hydrolysis of the polymer took place providing lower molecular weight chains interacting with the enzyme. The size increasing over time was more evident in the presence of aspartate and glutamate salts and the higher acid:monomer ratio (Fig. 2) according to the higher hydrophilicity of these systems favouring the interactions between the chitosan salts and the soluble β-glucosidase.

Table 2
Mean size of chitosan salts in pH 7.0 aqueous buffer at 37 °C without β-glucosidase

Chitosan salts	Mean diameter (nm)
1:1 CH monomer/Asp	16.2 ± 0.3
1:2 CH monomer/Asp	18.6 ± 0.5
1:1 CH monomer/Glu	11.3 ± 0.3
1:2 CH monomer/Glu	13.0 ± 0.8
1:1 CH monomer/Lat	7.2 ± 0.6
1:2 CH monomer/Lat	8.9 ± 0.2
1:1 CH monomer/HCl	5.6 ± 0.6
1:2 CH monomer/HCl	7.0 ± 0.1
β-glucosidase	68.4 ± 0.3

The data were obtained by DLS measurements. Data shown are the mean ± standard deviations, $n = 3$.

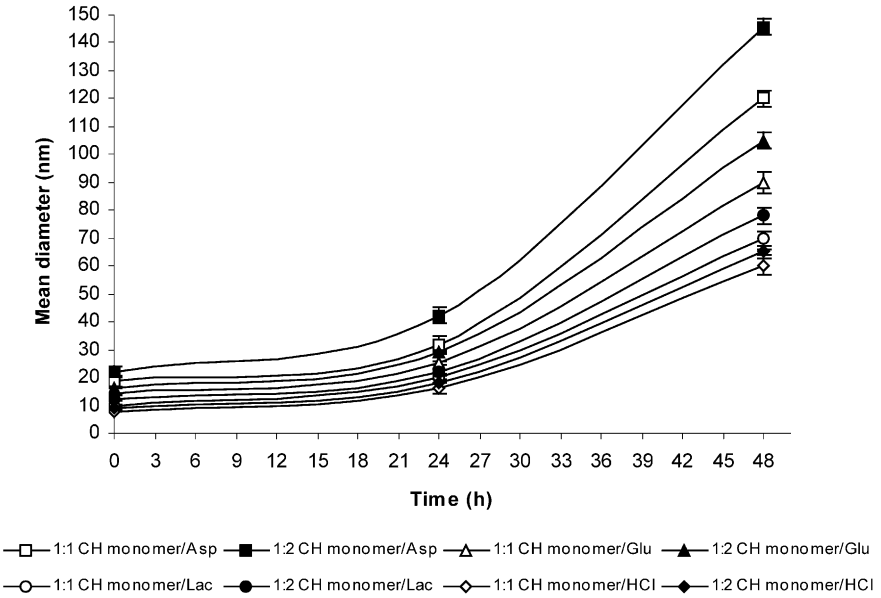

Fig. 2. Mean size of chitosan salts in pH 7.0 aqueous buffer at 37 °C as a function of time in the presence of β-glucosidase. The data were obtained by DLS measurements and each data represents the average of three determinations ± standard deviation.

3.4. In vitro release studies

Drug availability, expressed as fractional release over time (Fig. 3), was lower from the drug–polymer mixtures than the pure drug at each pH analyzed. This may be attributed to the presence of the gelled polymer in the mixtures, hindering the diffusion of the dissolved, poorly soluble non ionized form of the drug at acidic pH, highly soluble ionized form of the drug at alkaline pH, toward the external environment. This behaviour indicated the ability of these systems to slow down the release in the gastrointestinal tract, but to consider these systems suitable for colon-specific drug delivery the fractional release after 9 h (mean transit time in the stomach and small intestine) and after 48 h (mean residence time in the gastrointestinal tract) (Table 3), must be carefully analyzed. An ideal colon-specific system should be characterized by a low release at 9 h, thereby carrying most of its drug content to the colon, and a high release at 48 h, providing a high availability of the drug in the colon (Chien, 1992). As regards the 9 h fractional release, the different polymer–drug mixtures did not provide significant differences as to be expected by a counterbalance of their different solubility characteristics in acidic and alkaline pH which represented the external medium of release for different times (pH 2.0 for 3h, pH 5.5 for 2h, pH 7.4 for 4h).

The 48 h fractional release was higher from the aspartate and glutamate salts and increased on increasing the acid:monomer ratio according to their enhanced hydrophilicity at alkaline pH. The presence of β-glucosidase further increased the release at 48 h due to the favourable effect of the polymer hydrolysis on the hydrophilic characteristics of the polymer–drug mixture.

4. Conclusions

Chitosan salified with aspartic, glutamic, hydrochloric and lactic acids is a suitable supporting material for the preparation of colon-specific drug-delivery systems. Physical mixtures of the chitosan salts with diclofenac sodium (1:1, w:w) provided slower drug release with respect to the pure drug both at acidic and alkaline pHs. Their interactions with β-glucosidase at pH 7.0 en-

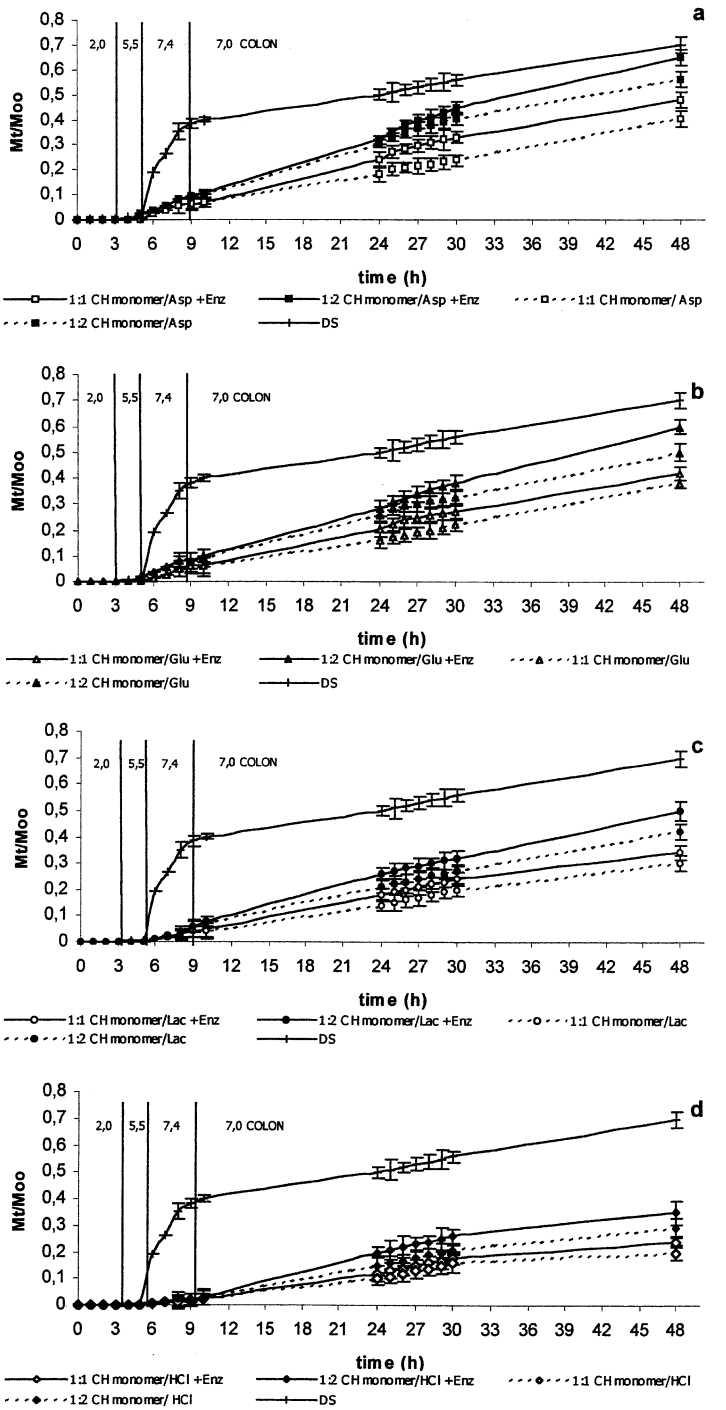

Fig. 3. Fractional release of diclofenac sodium from the mixtures with chitosan salts (a, aspartic; b, glutamic; c, lactic; d, hydrochloric) at different pHs in the presence of β-glucosidase or without β-glucosidase. Each data represents the average of three determinations ± standard deviation.

Table 3
Fractional amount of diclofenac sodium released from mixtures of chitosan salts (1:1; w:w) after 9 h (M9/M∞) and 48 h (M48/M∞)

Chitosan salts	Without enzyme		With enzyme	
	M9/M∞	M48/M∞	M9/M∞	M48/M∞
1:1 CH monomer/Asp	0.063 ± 0.002	0.403 ± 0.011	0.063 ± 0.005	0.480 ± 0.025
1:2 CH monomer/Asp	0.094 ± 0.005	0.559 ± 0.009	0.094 ± 0.001	0.660 ± 0.006
1:1 CH monomer/Glu	0.058 ± 0.003	0.380 ± 0.003	0.058 ± 0.006	0.420 ± 0.009
1:2 CH monomer/Glu	0.080 ± 0.001	0.500 ± 0.009	0.080 ± 0.002	0.600 ± 0.004
1:1 CH monomer/Lat	0.040 ± 0.004	0.300 ± 0.012	0.040 ± 0.004	0.340 ± 0.006
1:2 CH monomer/Lat	0.060 ± 0.006	0.420 ± 0.020	0.060 ± 0.003	0.500 ± 0.006
1:1 CH monomer/HCl	0.020 ± 0.004	0.200 ± 0.006	0.020 ± 0.009	0.240 ± 0.011
1:2 CH monomer/HCl	0.026 ± 0.002	0.290 ± 0.005	0.026 ± 0.003	0.350 ± 0.006

Data shown are the mean ± standard deviations, $n = 3$.

hanced the release rate due to the enzyme catalysed hydrolysis of chitosan. Among the different salts analyzed aspartate and glutamate provided the lowest release at the acidic pHs and the highest at alkaline pHs. Moreover, in the presence of β-glucosidase the release rate increase was more pronounced due to their solubility characteristics favouring enzyme–polymer interactions.

References

Aiedeh, K., Taha, M.O., 1999. Synthesis of chitosan succinate and chitosan phathalate and their evaluation as suggested matrices in orally administered, colon-specific drug delivery systems. Arch. Pharm. Pharm. Med. Chem. 332, 103–107.

Ashford, M., Fell, J., Atwood, D., Sharma, H., Woodhead, P., 1994. Studies on pectin formulations for colonic drug delivery. J. Control. Release 30, 225–232.

Baylis, C.E., Houston, A.P., 1986. Degradation of guar gum by faecal bacteria. Appl. Environ. Microbiol. 48, 626–632.

Berne, B., Pecora, R., 1976. Dynamic light scattering with application to chemistry, biology and physics. Wiley-Interscience, New York.

Brondsted, H., Kopecek, J., 1992. Hydrogels for site-specific drug delivery to the colon: in vitro and in vivo degradation. Pharm. Res. 9, 1540–1545.

Chien, Y.W., 1992. Oral drug delivery and delivery systems. In: Novel Drug Delivery Systems, vol. 50, second ed. Dekker, New York, pp. 139–196.

Chu, B., 1974. Laser Light Scattering. Academic Press, New York.

Englyst, H.N., MacFarlane, G.T., 1986. Breakdown of resistant and readily digestible starch of human gut bacteria. J. Sci. Food Agric. 37, 699–706.

Friend, D.R., 1991. Colon-specific drug delivery. Adv. Drug Deliv. Rev. 7, 149–199.

Gorbach, S.L., 1971. Intest. Microflora. Gastroenterology 60, 1110–1129.

Harboe, E., Larsen, C., Johansen, M., Olesen, H.P., 1989a. Macromolecular Prodrugs XIV. Absorption Characteristic of naproxen after oral administration of a dextran T-70-naproxen ester prodrug in pigs. Int. J. Pharm. 53, 157–165.

Harboe, E., Larsen, C., Johansen, M., Olesen, H.P., 1989b. Macromolecular Prodrugs XV. Colon targeted delivery—bioavailability of naproxen from orally administered dextran–naproxen prodrugs varying in molecular size in the pig. Pharm. Res. 6, 919–923.

Hirano, S., Seino, H., Akiyama, Y., Nonaka, I., 1990. In: Gebelein, C.G., Dunn, R.L. (Eds.), Progress in Biomedical Polymers. Plenum Press, New York, p. 283.

Hosny, E.A., EL-Mahrouk, G.M., Gouda, M.W., 1998. Formulation and in vitro and in vivo availability of diclofenac sodium enteric-coated beads. Drug Dev. Ind. Pharm. 24 (7), 661–666.

Larsen, C., Harboe, E., Johansen, M., Olesen, H.P., 1989. Macromolecular Prodrugs XVI. Colon targeted delivery—a comparison of rate of release of naproxen from dextran ester prodrugs in homogenates of various segments of the pig gastrointestinal tract. Pharm. Res. 6, 995–999.

Larsen, C., Jensen, B.H., Olesen, H.P., 1991. Bioavailability of ketoprofen from orally administered ketoprofen dextran ester prodrug in the pig. Acta Pharm. Nord. 3, 71–76.

Leopold, C.S., 1999. Coated dosage forms for colon-specific drug delivery. Pharm. Sci. Technol.To. 2, 197–204.

Lorenzo-Lamosa, M.L., Remuñàn, C., Vila-Jato, J.L., Alonso, M.J., 1998. Design of microencapsulated chitosan michrospheres for colonic drug delivery. J. Control. Release 52, 109–118.

Macfarlane, G.T., Hay, S., Macfarlane, S., Gibson, G.R., 1990. Effect of ifferent carbohydrates on growth, polysaccharidase and glycosidase production of *Bacteroides ovatus* in batch and continuos culture. J. Appl. Bacteriol. 68, 179–187.

Marvola, M., Nykanen, P., Rautio, S., Isonen, N., Autere, A., 1999. Enteric polymers as binders and coating materials in

multiple-unit site-specific drug delivery systems. Eur. J. Pharm. Sci. 7 (3), 259–267.

Moore, W.E.C., Holdeman, L.V., 1975. Discussion of current bacteriological invastigation of the relationship between intestinal flora, diet and colon cancer. Cancer Res. 35, 3418–3420.

Mrsny, R.J., 1992. The colon as a site for drug delivery. J. Control. Release 22, 15–34.

Orienti, I., Zecchi, V., 2000. Substituted chitosan as excipients for oral dosage forms. In: Muzzarelli, R.A.A. (Ed.), Chitosan per os. Atec, Ancona, pp. 165–186.

Paul, W., Sharma, C.P., 2000. Chitosan, a drug carrier for the 21st century: a review. S.T.P. Pharma Sci. 10 (1), 5–22.

Ravi Kumar, M.N.V., 2000. A review of chitin and chitosan applications. React. Funct. Pol. 46, 1–27.

Ring, S.G., Jee, J.M., Whittam, M., Orford, P., Johnson, I.T., 1988. Resistant starch. Its chemical form in food stuffs and effects on digestibility in vitro. Food Chem. 28, 97–109.

Rubinstein, A., 1995. Approaches and opportunities in colon-specific drug delivery. Crit. Rev. Ther. Drug Carrier Syst. 12 (12 and 3), 101–149.

Rubinstein, R., Nakar, D., Sintov, A., 1992. Chondroitin sulfate: a potential biodegradable carrier for colon-specific drug delivery. Int. J. Pharm. 84, 141–150.

Salyers, A.A., 1979. Energy sources of major intestinal fermentative anaerobes. Am. J. Clin. Nutr. 32, 158–163.

Salyers, A.A., Brien, M., 1980. Cellular location of enzymes involved in chordroitin sulfate breakdown by *Bacteroides thetaiotaomicron*. J. Bacteriol. 143, 772–780.

Salyers, A.A., Vercellotti, J.R., West, S.E.H., Wilkins, T.D., 1977. Fermentation of mucin and plant polysaccharides by strains of *Bacteroides* from the human colon. Appl. Environ. Microbiol. 33, 319–322.

Sawayanagi, Y., Nambu, N., Nagai, T., 1983. Dissolution properties and bioavailability of phenytoin from ground mixtures with chitin and chitosan. Chem. Pharm. Bull. 31, 2064–2068.

Shanmugasundaram, N., Ravichandran, P., Neelakanta Reddy, P., Nalini, R., Subrata, P., Panduranga Rao, K., 2001. Collagen-chitosan polymeric scaffolds for the in vitro culture of human epidermoid carcinoma cells. Biomaterials 22, 1943–1951.

Simon, G.I., Gorbach, S.L., 1986. The human intestinal microflora. Dig. Dis. Sci. 31, 147s–162s.

Tomolin, J., Taylor, J.F., Read, N.W., 1989. The effect of mixed faecal bacteria on a selection of viscous polysaccharide in vitro. Nutr. Rep. Int. 39, 121–135.

Tozaki, H., Komoike, J., Tada, C., Maruyama, T., Terabe, A., Suzuki, T., Yamamoto, A., Muranishi, S., 1997. Chitosan capsules for colon specific drug delivery: improvement of insulin absorption from the rat colon. J. Pharm. Sci. 86, 1016–1021.

Tozer, T.N., Friend, D.R., Mcleod, A.D., 1995. Kinetic perspectives on colonic delivery. S.T.P. Pharma. Sci. 5, 5–12.

Yeh, P.-Y., Berenson, M.M., Samowitz, W.S., Kopeckova, P., Kopecek, J., 1995. Site specific drug delivery and penetration enhancement in the gastrointestinal tract. J. Control. Release 36, 109–124.

Werch, S.C., Ivy, A.C., 1941. On the fate of ingested pectin. Am. J. Digest. Dis. 8, 101–105.